SHORT COMMUNICATION

Transfer of RP4::Mu to *Salmonella typhimurium*

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Restriction-proficient strains of *Salmonella typhimurium* are shown to be ineffective as recipients of normal RP4::Mu_{cts62} due to the operation of two restriction systems (*hsdSA* and *hsdLT*) on the Mu moiety of this plasmid. Strains mutant in both these *hsd* loci are excellent recipients.

INTRODUCTION

RP4::Mu hybrid plasmids have been demonstrated to be of great potential significance for the in vivo genetic engineering of a variety of bacterial genera (Dénarié *et al.*, 1977; Faelen *et al.*, 1977). This is due to the transposable nature of Mu and to the exceptionally broad host range of the P group plasmid RP4. Thus bacteriophage Mu DNA which has a very limited host range can be introduced into a variety of hosts as RP4::Mu. However, the properties of this plasmid vary in different hosts.

Salmonella typhimurium is resistant to Mu infection (Taylor, 1963). In this report we describe the transfer of RP4::Mu_{cts62} from *Escherichia coli* to the closely related *Salmonella typhimurium* and the expression of the plasmid in this new host.

METHODS

Bacterial strains and plasmids. Relevant genotypes are given in Tables 1 and 2. *Escherichia coli* strain 4K was originally obtained from Dr S. W. Glover (Bannister & Glover, 1970). *Salmonella typhimurium* strain JC5466 carrying RP4 or pGM14 (=RP4::Mu_{cts62}) are described by Dénarié *et al.* (1977), and were obtained via Dr J. Pemberton. *Salmonella typhimurium* strains 4233, 4296, 4423 and 4253 were obtained from Dr S. Hattman (see Hattman *et al.*, 1976) and Dr K. Sanderson, and strain SL1654 (=CL4419) from Dr B. A. D. Stocker. The latter strain is *hsdM*⁺ at both the *hsdLT* and *hsdSA* loci (see Ornellas & Stocker, 1974).

Media. R broth (Beacham & Yagil, 1976) was used for all conjugation experiments and for assays of Mu. Selective medium was appropriately supplemented M9 minimal medium (Miller, 1972) with 0.2% (w/v) glucose as carbon source.

Conjugation. For quantification of transfer frequency, crosses were performed using filter matings. Equal cell numbers of donor and recipient were deposited on a Millipore filter (0.45 µm pore size) and the filter was then placed on solidified R broth medium for 2–4 h. Patch matings (Holloway, 1978) were sometimes used to ascertain that transfer frequencies were high or to construct particular strains. Selection for simultaneous ampicillin resistance (Ap^R) and tetracycline resistance (Tc^R) was used to detect transfer of RP4 markers. Transconjugants were checked for appropriate genetic markers.

Induction and assay of Mu. Mu was assayed by the soft agar overlay method. Heat induction of Mu_{cts62} was performed as described by Bukhari & Ljungquist (1977). Immunity to Mu was determined by spotting a drop of Mu_{cts62} lysate on a lawn of cells in soft agar and incubating at 28 °C.

RESULTS AND DISCUSSION

Crosses between an *E. coli* strain [JC5466(pGMI4)] carrying RP4::Mu_{cts}62 and an *S. typhimurium* recipient (B96 or SA1475) revealed an extremely low level (1×10^{-7} to 5×10^{-7} , or less, per donor) of Ap^RTc^R transconjugants (Table 1). Overnight matings (patch or filter) were usually necessary to obtain even a few transconjugants. These putative RP4::Mu-containing *S. typhimurium* recipients transferred the drug resistance markers at high frequency to secondary *S. typhimurium* and *E. coli* recipients in patch matings. However, most of the original transconjugants and the secondary *E. coli* recipients were (a) not temperature sensitive for growth and (b) did not produce Mu particles after heat induction. One secondary *E. coli* recipient was tested and was additionally found to be not immune. A minority (about 10%) of the secondary *E. coli* recipients were slightly temperature-sensitive in that smaller irregular-shaped colonies were obtained at the high temperature and these were also immune to Mu. However, they did not produce Mu after heat induction. Crosses with an RP4 donor [strain JC5466(RP4)] showed that this plasmid was transferred at high frequency to *S. typhimurium* (Table 2). We conclude that only those RP4 plasmids which have lost Mu DNA, or plasmids in which Mu is defective, are transferable to *S. typhimurium*. Whilst restriction or zygotic induction could simply explain a low frequency of transfer of RP4::Mu to *S. typhimurium*, the reason for a total absence of detectable non-defective plasmid transfer was less apparent. Zygotic induction seemed particularly unlikely since *E. coli* recipients which are not lysogenic for Mu are good recipients of RP4::Mu_{cts}62. However, use of an *S. typhimurium* recipient deficient in two out of the three known restriction systems (Colson *et al.*, 1970; Colson & Van Pel, 1979) resulted in a high frequency of transconjugants (Tables 1 and 2). *S. typhimurium* recipients of pGMI4 in turn transferred drug resistance at high frequency back to *E. coli* (Table 2) and to

Table 1. *Transfer frequency of RP4::Mu_{cts}*

Donor <i>E. coli</i>	Recipient <i>S. typhimurium</i>	Restriction genotype of recipient	Transfer frequency (per initial donor)
JC5466(pGMI4)	SL1654	<i>hsdLT</i> ⁻ <i>hsdSA</i> ⁻	2.3×10^{-3}
JC5466(pGMI4)	SA1475	<i>hsdLT</i> ⁺ <i>hsdSA</i> ⁺	$< 5 \times 10^{-8}$
JC5466(pGMI4)	B96	<i>hsdLT</i> ⁺ <i>hsdSA</i> ⁺	5×10^{-7}

Table 2. *Transfer frequencies of RP4 and RP4::Mu_{cts}*

Donor <i>E. coli</i>	Recipient <i>S. typhimurium</i>	Restriction genotype of recipient	Transfer frequency (per initial donor)	
			RP4	RP4::Mu _{cts}
JC5466(RP4)	4233	<i>hsdLT</i> ⁺ <i>hsdSA</i> ⁺	1.7×10^{-4}	
JC5466(pGMI4)	4233	<i>hsdLT</i> ⁺ <i>hsdSA</i> ⁺		1.3×10^{-7}
JC5466(pGMI4)	4296	<i>hsdLT</i> ⁻ <i>hsdSA</i> ⁻		1.3×10^{-4}
JC5466(pGMI4)	4423	<i>hsdLT</i> ⁺ <i>hsdSA</i> ⁻		4.6×10^{-7}
JC5466(pGMI4)	4253	<i>hsdLT</i> ⁻ <i>hsdSA</i> ⁺		2.4×10^{-6}
<i>S. typhimurium</i>	<i>E. coli</i>			
B96(pGMI4)*	4K	<i>hsdK</i> ⁻		7.3×10^{-3}
B96(pGMI4)*	C600	<i>hsdK</i> ⁺		4.7×10^{-3}

* This strain was derived from a mating between B96 and SL1654(pGMI4). The latter was derived from a cross with JC5466(pGMI4) such as that shown in Table 1.

S. typhimurium (data not shown). *Salmonella typhimurium* recipients of RP4::Mu_{cts62}, from *E. coli* or from *S. typhimurium*, produced approximately the same amount of Mu after heat induction of liquid cultures as *E. coli* (10^7 to 3×10^8 plaque-forming units ml⁻¹), and obvious lysis of the culture was observed. However, temperature sensitivity of single colonies was not observed; this is in contrast to *E. coli* (RP4::Mu_{cts62}) which at 41 °C gave rise to only very small 'nibbled' colonies.

To ascertain the relative importance of the LT and SA restriction systems, the frequency of transfer to strains mutant in either the *hsdLT* or *hsdSA* loci was determined. (The role of the third restriction locus, *hsdSB*, was not tested.) It is evident that both the *hsdLT* and *hsdSA* systems are involved in the restriction of Mu since transfer to such strains was greatly reduced relative to transfer to an *hsdLT*⁻*hsdSA*⁻ recipient (Table 2). In these crosses the nature of the transconjugants with restricting recipients was not tested. It may also be noted (Table 2) that no restriction by *E. coli* from an *S. typhimurium* donor was observed.

Given the relative ease with which RP4::Mu_{cts} (or a related hybrid plasmid) has been transferred to quite unrelated genera such as *Rhizobium*, *Pseudomonas*, *Klebsiella*, *Rhodopseudomonas* and *Erwinia* (Boucher *et al.*, 1977; Dénarié *et al.*, 1977; Tucker & Pemberton, 1979; Coplin, 1979), the complete lack of transfer to *S. typhimurium* was initially surprising; however, the operation of (at least) two restriction systems would seem to offer an explanation. Evidently only the Mu portion of the plasmid is significantly restricted (Table 2); the defective RP4::Mu plasmids have perhaps escaped restriction by virtue of loss of restriction sites (in Mu genes) for the LT or SA restriction enzymes. In fact, this would seem to be a useful system with which to isolate mutants of Mu in essential functions. Such reasoning also leads to the expectation that *hsdLT*⁻ or *hsdSA*⁻ mutant recipients in the population would be found with normal RP4::Mu_{cts}, though this was not found. Low frequency transfer of RP4::Mu to restriction proficient *S. typhimurium* also offers an alternative to *Rhizobium meliloti* (Boucher *et al.*, 1977) for detecting insertion of Mu into RP4.

Transfer to *R. meliloti* is similar to that reported here in that, firstly, restriction also seems to reduce transfer to this organism as judged by the effect of heat treatment of the recipients. Secondly, transfer to *R. meliloti* is reduced about 10⁴-fold due solely to the Mu phage moiety of the hybrid plasmids; however, in this case the reason may be partly related to the expression of Mu genes in this organism, rather than solely restriction, since a 'small colony' phenotype results.

In summary, it appears that transfer of RP4::Mu_{cts62} to *S. typhimurium* LT2 is unusual in that restriction seems to be a complete barrier to transfer of the normal hybrid plasmid, but, as expected, once this barrier is surmounted the expression of Mu appears normal.

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