Interspecific Crosses between *Rhizobium leguminosarum* and *R. meliloti*: Formation of Haploid Recombinants and of R-primes

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Using R68.45 as a conjugative plasmid in crosses between strains of *Rhizobium leguminosarum* and *R. meliloti*, transfer of chromosomal genes occurred. Haploid recombinants were formed in which *rif* and *str* alleles were transferred from *R. leguminosarum* to *R. meliloti*. In crosses in the reverse direction haploid recombinants arose at barely detectable frequencies and progeny were isolated which were high frequency donors of the selected allele. Genetic evidence showed that such progeny harboured R-primes in which sections of the *R. meliloti* chromosome were inserted into R68.45. Four different classes of R68.45-prime, each carrying a different prototrophic marker, were obtained.

INTRODUCTION

Some plasmids can incorporate sections of bacterial chromosomes *in vivo* to form primes. These have been of great value in the study of gene function and organization. Best known are the F-primes of *Escherichia coli* (Low, 1972), but more recently primes of the sex plasmid SCP1 have been found in *Streptomyces coelicolor* (Hopwood & Wright, 1973, 1976a). Chromosomal genes have been inserted into P1 group R plasmids in *E. coli* (Olsen & Gonzalez, 1974), *Klebsiella pneumoniae* (Dixon, Cannon & Kondorosi, 1976) and *Pseudomonas aeruginosa* (Hedges, Jacob & Crawford, 1977) and these primes are of particular interest because P1 plasmids have a very wide host range among Gram-negative bacteria (Olsen & Shipley, 1973). This allows the functions of genes on R-primes to be examined in different species and genera. For example, the expression of the *K. pneumoniae* *nif* genes has been studied in several genera (Dixon *et al.*, 1976; Cannon & Postgate, 1976), and the function of the *P. aeruginosa* *trp* genes has been studied in *E. coli* (Hedges *et al.*, 1977) as have the *E. coli* *trp* genes in *P. aeruginosa* (Nagahari, Sano & Sakaguchi, 1977).

Another property of P1 group R plasmids is their ability to mobilize chromosomal genes in different genera: *P. aeruginosa* (Haas & Holloway, 1976), *E. coli* (Beringer & Hopwood, 1976), *Rhizobium* spp. (Johnston & Beringer, 1977; Meade & Signer, 1977; Kondorosi *et al.*, 1977) and *Acinetobacter calcoaceticus* (Towner & Vivian, 1976).

We show here that R68.45 can mediate recombination between *Rhizobium meliloti* and *R. leguminosarum*. We have isolated R-primes carrying segments of the *R. meliloti* chromosome and found that genes on the inserted regions were expressed in *R. leguminosarum*.

METHODS

Bacterial strains and plasmids are shown in Table 1.

Media. The complete (TY) and minimal (Y) media for *Rhizobium* spp. were described by Beringer (1974). The complete (NA) and minimal media for *E. coli* and *P. aeruginosa* were described by Beringer, Hoggan & Johnston (1978). Lennox Broth Agar (LBA) was described by Miller (1972).

Table 1. *Bacterial strains and plasmids*

	Relevant characteristics	Source
Bacteria		
<i>Rhizobium leguminosarum</i> : all strains derived from parental strain 300 (Johnston & Beringer, 1975)		
1155	<i>phe-1 trp-18 rif-84</i>	This paper*
1370	<i>ura-14 cys-8 rif-69</i> (R68.45)	This paper*
1379	<i>phe-1 ade-27 rib-2 str-69</i>	This paper*
6640	<i>ura-14 met-14 str-84 rif-283</i>	This paper*
6643	<i>ura-14 met-14 str-84 rif-283</i> (R68.45)	This paper*
<i>Rhizobium meliloti</i> : all strains derived from parental strain 6646		
6646	prototrophic	Strain 2011 from Rothamsted Culture Collection, Harpenden, England
6685	<i>rif-263</i>	This paper*
6686	<i>str-161</i>	This paper*
6693	<i>rif-263</i> (R68.45)	This paper*
<i>Escherichia coli</i>		
1163	JS3.2 (R1033)	R. W. Hedges
Plasmids		
RP4	Ap Km Tc†	Datta <i>et al.</i> (1971)
R68.44	Ap Km Tc	Haas & Holloway (1976)
R68.45	Ap Km Tc	Haas & Holloway (1976)
R1033	Ap Km Tc Cm Ge Sm Su	Smith <i>et al.</i> (1975)

* Auxotrophic mutations in *Rhizobium* strains were induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; antibiotic resistant mutations were spontaneous (Beringer, 1974). R plasmids were introduced into *Rhizobium* strains by conjugal transfer from appropriate R⁺ strains of *E. coli*, *P. aeruginosa* or *R. leguminosarum*.

† Abbreviation of antibiotic resistance markers: Ap, ampicillin; Cm, chloramphenicol; Ge, gentamicin; Km, kanamycin; Sm, streptomycin; Su, sulphonamides; Tc, tetracycline.

Growth of bacteria was as described by Beringer *et al.* (1978).

Bacterial mating. Transfer and recombination frequencies were estimated quantitatively by the method of Jacob *et al.* (1976). Recombinants were analysed as described by Beringer & Hopwood (1976).

For patch crosses, loopfuls of each parental culture were mixed on the surface of a TY plate which was incubated for 48 h before being replicated to an appropriate selective medium. The selective plates were incubated at 28 °C for 3 d. High frequency transfer of a marker was indicated by confluent growth on the selective plates, while 'normal' levels of transfer seldom gave more than one or two colonies per patch.

Plant inoculation tests. Peas (*Pisum sativum* var. Dark Skin Perfection) were inoculated and scored for nodulation as described by Beringer (1974). Seedlings of lucerne (*Medicago sativa* var. du Puits) were grown on agar slants in test tubes as described for clover by Johnston & Beringer (1977). Acetylene reduction assays on root systems were as described by Johnston & Beringer (1975). Peas and lucerne are the normal hosts for *R. leguminosarum* and *R. meliloti* respectively.

RESULTS

Transfer of chromosomal genes between R. leguminosarum and R. meliloti

Transfer of rif and str alleles from R. leguminosarum to R. meliloti. In *R. leguminosarum*, *rif* and *str* genes are closely linked (Beringer & Hopwood, 1976). We made use of this fact to determine whether R68.45 could promote the co-transfer of *rif* and *str* from *R. leguminosarum* to *R. meliloti* (Table 2). When each antibiotic resistance was selected alone, and when the donor was R⁺, two sizes of *R. meliloti* colonies were observed: large colonies of bacteria that were resistant to only one antibiotic and were probably spontaneous resistant mutants, and small colonies of bacteria that were usually (about 90%) doubly resistant and were probably recombinants. When the donor was R⁻, there were no small colonies on either medium used for selecting singly-resistant colonies and no colonies of any sort on plates containing both antibiotics. This indicates that the presence of R68.45 in the donor was required for the generation of the small-colony recombinants. The frequency of the

Table 2. Frequency of colony types in crosses between *R. leguminosarum* 6640 (*ura-14 met-14 rif-283 str-84*) and *R. meliloti* 6646, and between 6643 (6640 R68.45) and 6646

(a) Initial selective frequencies

Donor strain	Antibiotic(s) in selective medium					
	Rifampicin		Streptomycin		Rifampicin + Streptomycin	
	Large*	Small*	Large	Small	Large	Small
6640	2×10^{-7}	$< 5 \times 10^{-9}$	2×10^{-7}	$< 5 \times 10^{-9}$	$< 5 \times 10^{-9}$	$< 5 \times 10^{-9}$
6643	3×10^{-7}	10^{-7}	3×10^{-7}	10^{-7}	$< 5 \times 10^{-9}$	10^{-7}

(b) Percentages of colonies which were both *rif* and *str*

Donor strain	Antibiotic in selective medium			
	Rifampicin		Streptomycin	
	Large	Small	Large	Small
6640	$< 2\%$	—	$< 2\%$	—
6643	10%	96%	$< 3\%$	93%

* Large and small colonies represent a subjective assessment of colony size based on the observation that there was not a continuum of colony size.

recombinants was about 10^{-7} , which is about 10-fold lower than in analogous crosses between strains of *R. leguminosarum* (Beringer *et al.*, 1978).

In these crosses the frequency of R68.45 transfer to *R. meliloti* was about 1%, the same as in crosses within *R. leguminosarum* (Beringer *et al.*, 1978). However, whereas about 80% of the recombinants from crosses between strains of *R. leguminosarum* were R^+ (Beringer *et al.*, 1978), there was no enrichment for R^+ transconjugants in *R. leguminosarum* $\times$ *R. meliloti* crosses. We have no explanation for this difference.

Twenty of the small-colony *rif str* recombinants were grown on agar slopes without antibiotics and were re-plated on agar with and without antibiotics. Growth in the absence of rifampicin and streptomycin was normal but in the presence of either antibiotic the small-colony character was retained. This indicates that the small colony size was due to the poor expression of the antibiotic resistance rather than to a non-specific debilitation of *R. meliloti* due to the insertion of segments of *R. leguminosarum* chromosome.

These same 20 recombinants all effectively nodulated lucerne but failed to nodulate peas. Thus symbiotic proficiency of *R. meliloti* was not affected by the presence of *R. leguminosarum rif* or *str* alleles.

A cross between *R. leguminosarum* 1370 (*ura-14 cys-8 rif-69* R68.45) and *R. meliloti* strain 6686 (*str-161*), with selection for the transfer of *rif*, again yielded a mixture of large and small colonies. All of a sample of 16 large colonies were *rif str* and all of 121 small ones were *rif*. The latter presumably arose by co-transfer of *rif* and *str*⁺. The fact that both *str* and *str*⁺ of *R. leguminosarum* could be transferred to and expressed in *R. meliloti* indicates that recombinants rather than partial diploids for this region were formed.

Transfer of rif and str alleles from R. meliloti to R. leguminosarum. Crosses were also done to see if R68.45 could mobilize *rif* or *str* alleles in the reverse direction, from derivatives of *R. meliloti* strain 6646 to *R. leguminosarum*. Four crosses of this type failed to generate any unambiguous recombinants but from one cross five colonies appeared to be due to co-transfer of *rif* and *str*⁺ to *R. leguminosarum* at a frequency of about 4×10^{-9} . It is not known whether the barely detectable frequencies of transfer were due to a greatly decreased recombination frequency or to the inability of *R. meliloti rif* or *str* alleles to be expressed in *R. leguminosarum*. The limited expression of *R. leguminosarum rif* and *str* alleles in

Table 3. *Phenotypes of progeny in a cross between R. meliloti strain 6693 (rif-263 R68.45) and R. leguminosarum strain 1379 (phe-1 rib-2 ade-27 str-69)*

Selective minimal (Y) medium containing:	Selected allele	No. of colonies tested	Relevant phenotype	No. in class	Frequency of transfer of prototrophic allele
Phenylalanine + riboflavin + streptomycin	<i>ade-27</i> ⁺	99	Phe ⁻ Rib ⁻ Str-r R68.45 Str-r Rif-r R68.45	74 25*	10 ⁻⁸
Adenine + riboflavin + streptomycin	<i>phe-1</i> ⁺	109	Ade ⁻ Rib ⁻ Str-r R68.45 Str-r Rif-r R68.45	2 107*	4 × 10 ⁻⁹
Phenylalanine + adenine + streptomycin	<i>rib-2</i> ⁺	100	Phe ⁻ Ade ⁻ Str-r Str-r Rif-r R68.45	2 98*	≤ 10 ⁻⁹

* These classes probably represent *str* mutants of the donor. They resembled *R. meliloti* in being less mucoid than *R. leguminosarum* and grew on LBA; *R. meliloti* grows on this medium (Meade & Signer, 1977) but *R. leguminosarum* does not.

Table 4. *Phenotypes of progeny in which a prototrophic allele was transferred with R68.45 from R. meliloti to R. leguminosarum*

Strains	Phenotype	Markers transferred from <i>R. meliloti</i>
ML1 to ML20	Phe ⁻ Rib ⁻ Str-r R68.45	<i>ade-27</i> ⁺ and R68.45
ML21 and ML22	Rib ⁻ Ade ⁻ Str-r R68.45	<i>phe-1</i> ⁺ and R68.45
ML23 to ML30	Ura ⁻ Str-r Spc-r R68.45	<i>trp-16</i> ⁺ and R68.45

R. meliloti does imply that there may be problems in the normal expression of these alleles in different species.

Transfer of other alleles from R. meliloti to R. leguminosarum. In order to see if prototrophic alleles could be transferred from *R. meliloti* to *R. leguminosarum*, *R. meliloti* strain 6693 (*rif-263* R68.45) was used as a donor to a number of multiply auxotrophic *str* strains of *R. leguminosarum* with selection for the transfer of prototrophy for one of the auxotrophic alleles (see Table 3 for an example). Many of the colonies on the selective plates were *str* mutants of the donor but some resembled *R. leguminosarum* and carried the appropriate non-selected markers. The *rib-2*⁺ colonies could have been due to reversion because they were R⁻. However, all the *ade-27*⁺ and *phe-1*⁺ colonies were R⁺ although the overall frequency of R68.45 transfer was only about 2%, suggesting that these colonies were not revertants but recombinants. In another cross, eight out of nine colonies selected for the transfer of *trp-16*⁺ received R68.45, although in seven other crosses, in which colonies were selected for transfer of various other alleles, there was no evidence of co-transfer of R68.45 with the selected prototrophic allele (frequencies < 10⁻⁹). All the colonies which appeared to have arisen by co-transfer of R68.45 with *ade-27*⁺, *phe-1*⁺ or *trp-16*⁺ were purified, grown on TY slants and numbered as in Table 4. Since each of the strains in a particular class came from a single cross they may have been clonal.

Characterization of R. leguminosarum 'recombinants'

Because the transfer frequency of selected alleles in the crosses between *R. meliloti* donors and *R. leguminosarum* was so low the possibility was examined that the presumptive recombinants represented a special class with altered donor properties.

Donor properties. Each 'recombinant' was patch-crossed to a strain of *R. leguminosarum* carrying the same allele that was originally selected. For example, ML21 and ML22 were

Table 5. Segregation of markers among colonies of ML21 and ML22

Cultures of ML21 and ML22 were grown non-selectively; 150 colonies from each were tested for segregation of relevant markers.

Strain	Relevant phenotype	No. in class
ML21	Str-r Ade ⁻ Rib ⁻ Km Tc*	144
	Str-r Phe ⁻ Ade ⁻ Rib ⁻ Km Tc	1
	Str-r Phe ⁻ Ade ⁻ Rib ⁻	1
	Str-r Ade ⁻ Rib ⁻ Tc	1
	Str-r Ade ⁻ Rib ⁻ Km	3
ML22	Str-r Ade ⁻ Rib ⁻ Km Tc*	147
	Str-r Phe ⁻ Ade ⁻ Rib ⁻ Km	3

* The initial phenotype.

crossed to strain 1155 (*phe-1 trp-18 rif-84*) with selection for *phe-1*⁺. The selective replica plates gave confluent growth, indicating high frequency transfer. In analogous crosses, strains ML1 to 20 and ML23 to 30 donated *ade-27*⁺ and *trp-16*⁺ respectively at high frequency.

Quantitative estimates of transfer frequencies for these alleles were made using two ML strains of each class as donors in crosses to appropriately marked strains of *R. leguminosarum*, with selection for the transfer of R68.45 and of *ade-27*⁺ (ML7 and ML18 as donors), *phe-1*⁺ (ML21 and ML22) or *trp-16*⁺ (ML24 and ML27). The frequencies of transfer of R68.45 and the prototrophic markers were about 1%. Fifty prototrophic colonies from each cross were picked; all were found to be resistant to kanamycin, indicating the presence of R68.45. Conversely 90 to 95% of colonies selected for R68.45 transfer were prototrophic. This very high frequency of co-transfer of R68.45 and prototrophy suggested that regions of the *R. meliloti* chromosome had been inserted into R68.45 to form R68.45-primers. This possibility was investigated in a number of ways.

Stability of R-primers. One of the features of plasmid primers is instability (Jacob & Wollman, 1961; Hopwood & Wright, 1973, 1976a; Dixon *et al.*, 1976), so the stability of some of the putative R68.45-primers was tested. The strains were grown non-selectively on TY and plated for single colonies on TY. For each strain 150 colonies were tested for prototrophy and for the tetracycline and kanamycin resistance markers carried on R68.45 [carbenicillin resistance was not tested since this marker is not expressed well in *R. leguminosarum* strains (Beringer, 1974)]. The strains tested were those used in the crosses above, together with ML2 and ML25 which were much more unstable than the other strains.

Of the ML2 colonies 99% were Ade⁻, and of the ML25 colonies 77% were Trp⁻, although all the colonies still carried both R plasmid resistances. The other six strains were more stable: 95% or more of the colonies were *ade-27*⁺, *phe-1*⁺ or *trp-16*⁺ as appropriate. With the exception of ML21 and ML22 all colonies were resistant to both antibiotics, even when the prototrophic marker was lost, although in most cases the resistance to tetracycline was poorly expressed. For ML21 and ML22 there was segregation not only of *phe-1* but also of sensitivity to one or other antibiotic; one colony was sensitive to both antibiotics and may represent loss of the whole plasmid (Table 5). Segregation of antibiotic sensitivity in R-prime strains has been reported (Dixon *et al.*, 1976).

Elimination of R-primers. If indeed R68.45-primers had been formed, the ML strains should have tended to lose their prototrophic marker if a plasmid of the same incompatibility group were introduced into them to replace R68.45. This was found to be so. When R1033 was transferred from *E. coli* strain 1163 into ML7, ML18, ML21, ML22, ML24 and ML27 by selecting for gentamicin resistance, 90 to 95% of the transconjugants were Ade⁻, Phe⁻ or Trp⁻ as appropriate. The nature of the minority of R1033 transconjugants which had not lost their prototrophic marker is not clear. Since R1033 determines resistance to the same antibiotics as R68.45 as well as to gentamicin, it was not possible to determine whether or not the resident

Fig. 1. Positions of relevant alleles on the chromosome of *R. leguminosarum* (from Beringer *et al.*, 1978, and unpublished). Numbers above the markers are allele numbers, and numbers below the line are co-inheritance percentages of pairs of alleles.

plasmid had been eliminated. The similarity in the antibiotic resistance markers and presumably transfer genes of R68.45 and R1033 may allow recombination to occur between them.

The genetic evidence so far presented indicated that the ML strains harboured R-primes in which sections of the *R. meliloti* chromosome had been inserted into R68.45. Accordingly these plasmids in the ML strains 7, 18, 21, 22, 24 and 27 were designated pAJ7, 18, 21, 22, 24 and 27 respectively.

How far do the R-primes extend? Figure 1 gives the map positions of relevant alleles on the *R. leguminosarum* chromosome (Beringer *et al.*, 1978). Crosses were made between certain ML strains and appropriately marked *R. leguminosarum* recipients to see if the R68.45-primes extended to cover a neighbouring allele as well as the selected marker and also to see if they mobilized neighbouring genes at higher frequencies than did R68.45. Other plasmid primes are known to enhance gene transfer of the chromosome in the region of the inserted segment (Pittard, Loutit & Adelberg, 1963; Hopwood & Wright, 1976*b*). However, it was found that pAJ7 and pAJ18 failed to cover *rib-2* and *met-12*, pAJ21 and pAJ22 did not cover *met-12* or *trp-16* and pAJ24 and pAJ27 did not cover *phe-1* or *met-14*. No enhancement of the transfer of any of the flanking markers was detected. The possibility that some of the inserted segments in R68.45 did extend as far as one of the flanking markers but were unable to express in *R. leguminosarum* cannot be eliminated. However, in the region *phe-1*—*trp-16* this cannot apply since it is clear that *R. meliloti* genes equivalent to those defined by these alleles can work in *R. leguminosarum*. It should also be mentioned that the identity of gene order in these two species remains to be established even though preliminary genetic maps for both species exist (Meade & Signer, 1977; Kondorosi *et al.*, 1977; Beringer *et al.*, 1978). If, for example, the genes defined by *phe-1* and *trp-16* are not as closely linked in *R. meliloti* there would be no chance for the *phe-1* and *trp-16* alleles to be covered by the same R68.45-prime.

Transfer of pAJ18 and pAJ24 to R. leguminosarum strains with different ade and trp alleles. Strains ML18 and ML24 were patch-crossed to *R. leguminosarum* strains carrying different *ade* and *trp* alleles respectively. Of eight *ade* alleles, ML18 (the *ade-27*⁺ donor) donated only *ade-73*⁺ at high frequency; *ade-73* maps near *ade-27* (Fig. 1). Three *ade* alleles mapping elsewhere on the chromosome and four unmapped alleles failed to be covered. The mapped *trp* alleles of *R. leguminosarum* occur in two regions (Beringer *et al.*, 1978): *trp-15*, -16, -18 and -19 lie between *phe-1* and *met-14* (Fig. 1) while *trp-12* and -14 map together at another region. When ML24 (the *trp-16*⁺ donor) was patch-mated to strains carrying *trp* alleles there was high frequency donation of *trp-15*⁺, -18⁺ and -19⁺ and of the wild-type alleles of three other unmapped *trp* mutations but not of *trp-12*⁺ and -14⁺. This agrees with the mapping data and suggests a similar arrangement of *trp* alleles in *R. leguminosarum* 300 and *R. meliloti* 6646. The colony size of the *trp-15*⁺, -16⁺ and -18⁺ transconjugants was normal but the colonies selected for *trp-19*⁺ transfer grew slowly, suggesting poor expression in *R. leguminosarum* of the *R. meliloti* gene equivalent to that defined by *trp-19*. Also, when an *R. trifolii* strain carrying a *trp* allele (*trp-47*) which mapped in the same region as *trp-16* of *R. leguminosarum* (A. W. B. Johnston, unpublished results) was used as a recipient in a cross with ML24, transfer of *trp-47*⁺ occurred at high frequency.

Table 6. Screening of recombinants formed in *R. leguminosarum* × *R. leguminosarum* crosses to determine the frequency of R-prime formation

Marker	No. of recombinants tested	R plasmid*	No. of R-primes†
<i>cys-9</i> ⁺	44	RP4	0
<i>phe-1</i> ⁺	28	RP4	0
<i>phe-1</i> ⁺	65	R68.44	2 (probably clonal)‡
<i>met-11</i> ⁺	22	R68.44	0
<i>rib-2</i> ⁺	16	R68.44	0
<i>ade-26</i> ⁺	17	R68.44	0
<i>liv-6</i> ⁺	9	R68.44	0
<i>ura-14</i> ⁺	305	R68.45	0
<i>met-14</i> ⁺	63	R68.45	0
<i>phe-1</i> ⁺	102	R68.45	1

* The RP4 and R68.44 recombinants tested here arose at frequencies of 10^{-8} ; with R68.45 the frequencies were about 10^{-6} .

† R⁺ recombinants for each marker were collected from different crosses involving the same R plasmid and patch-mated to a suitable *R. leguminosarum* recipient, as described in Methods.

‡ These two *phe-1*⁺ R-primes were obtained from recombinants arising in the same cross.

Although pAJ24 failed to mobilize *phe-1*⁺ and *met-14*⁺ at higher frequencies than did R68.45, the closely linked *trp-15*, -18 and -19 alleles might have been transferred by high frequency mobilization rather than by virtue of the presence on the plasmid of the wild-type genes corresponding to the mutations. However, *trp-15*⁺, -18⁺ and -19⁺ were transferred at similar frequencies to *trp-16*⁺: 100% of Trp⁺ transconjugants received R68.45 and more than 90% of the R⁺ transconjugants were Trp⁺. These results suggest that pAJ24 covered *trp-15*, -18 and -19 rather than that these alleles were mobilized from the chromosome.

Isolation of R-primes in *R. leguminosarum*

R68.45-primes might have been isolated fairly readily in crosses between *R. meliloti* and *R. leguminosarum* because crossing-over was greatly reduced, giving enrichment for the transfer of alleles via R68.45-primes. The use of interspecific crosses to produce R plasmid primes in other bacteria has been reported (Hedges *et al.*, 1977); this method is analogous to the original use of Rec⁻ recipients in plasmid prime selection (Low, 1968).

In this study the frequencies of R68.45-prime formation ranged from 10^{-8} to $< 10^{-10}$. This implies that in crosses between strains of *R. leguminosarum* where the recombination frequency is about 10^{-6} (Beringer & Hopwood, 1976) as many as 1% of 'recombinants' might in fact be due to the formation of R-primes. This possibility was tested by screening R⁺ recombinants of *R. leguminosarum* which had inherited a single marker from an *R. leguminosarum* donor for their ability to donate that marker at high frequency in patch crosses. The presence of an R-prime was then confirmed by testing the frequency of transfer of the marker relative to the R plasmid and by looking for its elimination after replacing the R plasmid by another P1 plasmid. Both procedures were performed as described above for the *R. meliloti* R-primes. The data in Table 6 show that R-primes were found at low frequency among recombinants isolated from crosses involving the plasmids R68.44 and R68.45.

DISCUSSION

In crosses between strains of *R. leguminosarum* and *R. meliloti* two types of R68.45-mediated chromosomal gene transfer were observed: the formation of true haploid recombinants and the production of R-primes.

The transfer of *rif* and *str* alleles from *R. leguminosarum* to *R. meliloti* appeared to involve the production of haploid recombinants since both *str* and *str*⁺ alleles could be transferred

and expressed and were co-inherited at high frequency with *rif*. The frequency of inheritance was about 10-fold lower than in similar crosses between strains of *R. leguminosarum* (Beringer *et al.*, 1978) or in interspecific crosses between *R. leguminosarum*, *R. trifolii* and *R. phaseoli* (Johnston & Beringer, 1977). This may reflect the fact that these last three species are very closely related and are taxonomically more distant from *R. meliloti* (Graham, 1964). The expression of the *rif* and *str* alleles in the *R. meliloti* recombinants was poor, as judged by their slow growth rate in the presence of either antibiotic.

In crosses in which *R. meliloti* was the donor to *R. leguminosarum*, transfer of *rif* and *str* alleles was barely detectable (frequency $\leq 10^{-9}$). We do not know why the frequency of transfer of such alleles is some 100-fold lower in this direction than from *R. leguminosarum* to *R. meliloti*. It could be due to a lowered recombination or transfer frequency, or to the inability of *R. meliloti* *rif* or *str* alleles to be expressed in *R. leguminosarum*. Another explanation could be that in *R. leguminosarum* there are essential genes closely linked to *rif/str* which cannot be functionally replaced by corresponding genes of *R. meliloti*.

When selection was made for the transfer of various prototrophic alleles from *R. meliloti* to *R. leguminosarum* the frequency was low and for some alleles no transfer was detected. Crosses in which selection was made for the transfer of *phe-1*⁺, *ade-27*⁺ and *trp-16*⁺ from *R. meliloti* to *R. leguminosarum* yielded progeny which could be interpreted as carrying R68.45-primers. These were unstable, yielding auxotrophic segregants at frequencies of 5 to 99%. It is not known whether the loss of the prototrophic marker was due to loop-out of the inserted region or to conversion to a homogenote by some type of recombination event with the chromosome (Jacob & Wollman, 1961; Hopwood & Wright, 1973, 1976*a*). The apparently low recombination frequency between *R. meliloti* and *R. leguminosarum* and the fact that in some cases loss of the prototrophic allele was accompanied by the loss of one of the antibiotic resistance markers on R68.45 possibly favours the former general explanation.

In *E. coli*, F-primes mobilize chromosomal genes close to the region inserted in the plasmid at much higher frequencies (up to 1000-fold) than does F (Pittard *et al.*, 1963). A lesser enhancement was also found in *S. coelicolor* with some SCP1-primers (Hopwood & Wright, 1976*b*). The enhanced donor ability of F-primes is due to an interaction with the chromosome through a recombination event between the chromosome and the homologous region on the plasmid (Pittard & Adelberg, 1963). None of the R68.45-primers carrying sections of the *R. meliloti* chromosome mobilized adjacent markers at high frequency. The DNA sequences on the plasmid and the chromosome of *R. leguminosarum* may be sufficiently different to prevent or reduce recombination between the two. Jacob *et al.* (1976) constructed *in vitro* a derivative of the P group plasmid RP4 which contained a region of the *R. leguminosarum* (strain 300) chromosome but it was no better at mobilizing the chromosome of *R. leguminosarum* even though there was complete homology between the inserted region and the chromosome. The failure of R-primers to act as better sex factors may reflect the apparently poor recombination ability of *R. leguminosarum* (Beringer *et al.*, 1978).

The expression of some *R. meliloti* genes in *R. leguminosarum* was not always complete. The pAJ24 plasmid donated prototrophy to an *R. leguminosarum* *trp-19* strain at high frequency but the transconjugants grew slowly in the absence of tryptophan. In addition, in a cross between *R. meliloti* strain 6693 and an *R. leguminosarum* strain carrying *cys-8*, slow growing progeny were isolated. These were found to be high frequency donors of *cys-8*⁺ and they proved to be R68.45*cys-8*⁺ primers. However, the poor growth rate of these strains made the scoring procedures less clear than for the other primers and data on them were not included. Nevertheless there appear to be at least two prototrophic alleles of *R. meliloti* which fail to express properly in a member of the same genus.

The fact that the R-primers described here were derivatives of a plasmid with a broad host range means that the expression of Rhizobium genes in other bacterial genera can be tested. We have found (unpublished observations) that the pAJ24 prime in strain ML24 donated

Trp⁺ at high frequency to a strain of *P. aeruginosa* which carried a *trpF* mutation. Out of 100 transconjugants selected for transfer of carbenicillin resistance 89 were Trp⁺, and of 100 Trp⁺ transconjugants all were carbenicillin-resistant. This indicated that pAJ24 contained a gene corresponding to *P. aeruginosa trpF* (which specifies phosphoribosyl anthranilate isomerase) and that the *Rhizobium* gene can express in the new host. Such crosses using other *Rhizobium* primes should facilitate the allocation of genes concerned with particular biosynthetic steps to regions of the *Rhizobium* chromosomes.

The isolation of R-primes in *Rhizobium* should assist in its genetic analysis since it offers the potential for dominance and complementation studies. It will be of particular interest if, in the future, R-primes can be isolated which contain genes concerned with symbiotic characters since these could be of help in elucidating the expression and organization of such genes.

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