Physical Identification of Bacteriocinogenic, Nodulation and Other Plasmids in Strains of *Rhizobium leguminosarum*

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Plasmids obtained from four field isolates of *Rhizobium leguminosarum* were visualized following electrophoresis on agarose gels. The relative mobilities of the bands observed corresponded to plasmids with molecular weights of about 100×10^6 and greater. Each field isolate examined had a different pattern of plasmids.

Lysates from *R. leguminosarum* strain 300 normally produced three plasmid bands, although in some preparations two much larger plasmids were also visible. The smallest plasmid band seen in strain 300 probably contains two co-migrating plasmids, because in one derivative of strain 300 it was replaced by a doublet, presumably as a result of the presence of a small deletion in one of the co-migrating plasmids. No apparent symbiotic defects were associated with the presence of this deletion. However, a non-nodulating derivative of strain 300 (strain 6015) was found to have suffered a deletion in the third-smallest plasmid of this strain.

Transfer of the determinant of *medium*-sized bacteriocin production pRL1JI (from isolate 248) was correlated with the appearance of an extra plasmid with a molecular weight of about 130×10^6 . Another determinant of *medium* bacteriocinogenicity, pRL4JI (from isolate 309), was correlated with the presence of an extra plasmid of the same size (about 160×10^6) as one seen in the donor strain 309. The third determinant, pRL3JI (from isolate 306), could not be correlated with the presence of an extra plasmid of the same size as any in strain 306, and it appears that recombination occurred between pRL3JI and plasmids resident in strain 300.

INTRODUCTION

There is recent evidence that plasmids play an important role in determining the ability of *Rhizobium* to induce nitrogen-fixing nodules on legume roots. Large plasmids (molecular weights greater than 10^6) have been found in several *Rhizobium* species (Nuti *et al.*, 1977; Casse *et al.*, 1979; Prakash *et al.*, 1980). Loss of one large plasmid from a strain of *R. leguminosarum* was associated with loss of the ability to nodulate peas (Casse *et al.*, 1979; Prakash *et al.*, 1980). Evidence that at least some of the structural genes for nitrogenase (*nif* genes) are present on *Rhizobium* plasmids came from the demonstration that cloned *nif* DNA isolated from *Klebsiella pneumoniae* hybridized specifically to *Rhizobium* plasmid DNA (Nuti *et al.*, 1979; Ruvkun & Ausubel, 1980).

Hirsch (1979) reported high frequency conjugal transfer of the ability to produce *medium*-sized bacteriocins from three different *R. leguminosarum* field isolates to other strains of

R. leguminosarum. This transfer was associated with the transfer of chromosomal markers at much lower frequencies. The three presumptive bacteriocinogenic plasmids found in field isolates 248, 306 and 309 were named pRL1JI, pRL3JI and pRL4JI, respectively.

When pRL1JI, or a derivative into which the transposon Tn5 had been inserted, was transferred to a number of symbiotically defective derivatives of *R. leguminosarum* strain 300, the transconjugants gained the ability to form effective nodules on peas (Johnston *et al.*, 1978; Brewin *et al.*, 1980). The mutants used included strains that failed to nodulate (Nod⁻) or which induced nodules that did not fix nitrogen (Fix⁻), suggesting that pRL1JI carries more than one gene important in the symbiosis.

The transposon Tn5 was inserted into pRL1JI by the method of Beringer *et al.* (1978a) and when this plasmid (pJB5JI) was introduced into *R. trifolii* and *R. phaseoli*, two species that do not normally nodulate peas, the transconjugants acquired the ability to nodulate this host (Johnston *et al.*, 1978). However, when pRL3JI or pRL4JI was transferred to the symbiotically defective mutants that were suppressed by pRL1JI, no individual transconjugants were restored to symbiotic proficiency (Brewin *et al.*, 1980).

In this paper we report the physical demonstration of these bacteriocinogenic plasmids and of the plasmids present in the genetically well-characterized *R. leguminosarum* strain 300 (Beringer *et al.*, 1978b) and some of its derivatives.

METHODS

Bacterial strains and plasmids. These are shown in Table 1.

Bacterial manipulations. Culture conditions, bacterial crosses, plant nodulation tests and screening for bacteriocin production were described by Beringer (1974) and by Brewin *et al.* (1980).

Growth media. Liquid TY medium contained 0.5 % (w/v) Difco Bacto-Tryptone, 0.3 % (w/v) Difco Bacto-Yeast Extract and 7 mM-CaCl₂. Liquid PA medium contained 0.4 % (w/v) Difco Bacto-Peptone and 2 mM-MgSO₄. *Rhizobium leguminosarum* strains grew poorly in PA medium, never giving very turbid cultures, but this was found to be an advantage in obtaining good lysis and plasmid preparations.

Buffers. TE buffer was 50 mM-Tris/HCl with 20 mM-EDTA, pH 8.0. Dialysis buffer was 10 mM-Tris/HCl with 1 mM-EDTA, pH 8.0. Tris/borate electrophoresis buffer contained (per litre); 10.8 g Tris, 0.93 g EDTA and 5.5 g boric acid, pH 8.3.

Isolation of large plasmids. The protocol for small-scale crude plasmid preparation was as follows: 200 ml PA medium was inoculated with 2 ml of a stationary phase stock culture of *R. leguminosarum* (in TY) or *A. tumefaciens* (in PA) and shaken at 28 °C for about 36 h. Bacteria were harvested at a final concentration of about 10⁸ viable bacteria ml⁻¹, washed in TE buffer and resuspended in 16 ml TE buffer. Then 2 ml Pronase (Sigma Pronase P, 5 mg ml⁻¹ in TE buffer, pre-digested 1 h at 37 °C) and 2 ml sodium dodecyl sulphate (SDS; 10 %, w/v, in TE buffer) were added and the mixture was incubated at 37 °C with gentle shaking for 1 h or until lysis was complete. The clear, viscous lysate was adjusted to pH 12.4 by addition of 3 M-NaOH (about 0.5 ml) with gentle but thorough stirring, using a plastic rod or pipette. After standing at room temperature for 30 min, the lysate was adjusted to pH 8.5 with 2 M-Tris/HCl, pH 7.0 (about 1.5 ml) and transferred to a 40 ml centrifuge tube. Then 5 M-NaCl was added to give a final concentration of 1 M; the contents of the tube were mixed by gentle inversion and left on ice for at least 4 h. The SDS/NaCl complex was precipitated by centrifugation at 10000 rev. min⁻¹ for 20 min at 4 °C. The supernatant was carefully decanted to a fresh tube, and 50 % (w/v) polyethyleneglycol (PEG) 6000 was added to give a final concentration of 10 %; the contents of the tube were mixed by gentle inversion and left on ice overnight. The DNA was precipitated by centrifuging at 7000 rev. min⁻¹ at 4 °C for 15 min. The supernatant was discarded and the pellet was resuspended in 0.5 ml TE buffer containing 0.1 % (v/v) diethylpyrocatecholate to inhibit nuclease activity. The samples were stored at 4 °C.

For large-scale plasmid preparations, the volumes were increased 10-fold and 4.5 ml of the DNA solution was added to a solution of 30 g CsCl dissolved in 25 ml TE buffer, followed by 2.6 ml ethidium bromide (10 mg ml⁻¹). The remaining crude plasmid preparation was retained to test directly for the presence of plasmids on gels. The solution was mixed by gentle inversion, then centrifuged in a swinging bucket rotor for 10 min at 5000 rev. min⁻¹ at 4 °C and the resultant skin (of residual PEG 6000) was removed before transferring the solution to a polycarbonate ultracentrifuge tube (40 ml capacity) and centrifuging in a Beckman VTi50 rotor for 18 h at 40000 rev. min⁻¹ at 15 °C. The gradient was illuminated with u.v. light (366 nm) and the lower band corresponding to plasmid DNA was removed from the side of the tube with a

Table 1. *Bacterial strains and plasmids*

Strain	Relevant characteristics			Reference
<i>Agrobacterium tumefaciens</i> C58	Harbours the Ti plasmid pTiC58			Watson <i>et al.</i> (1975)
<i>Rhizobium leguminosarum</i>	Bacteriocin produced	Symbiotic properties*	Chromosomal genetic markers	
Field isolates				
248	medium	Nod ⁺ Fix ⁺		Josey <i>et al.</i> (1979)
306	medium	Nod ⁺ Fix ⁺		Josey <i>et al.</i> (1979)
309	medium	Nod ⁺ Fix ⁺		Josey <i>et al.</i> (1979)
300	small	Nod ⁺ Fix ⁺		Johnston & Beringer (1975)
Derivatives of strain 300				
897	small	Nod ⁺ Fix ⁺	<i>phe-1 trp-12 str-37</i>	Johnston & Beringer (1976)
1027†	small	Nod ⁻	<i>phe-1 ade-27 rif-45</i>	Brewin <i>et al.</i> (1980)
1062	small	Nod ⁺ Fix ⁺	<i>ura-14 trp-16 str-86</i>	Johnston <i>et al.</i> (1978)
6015	small	Nod ⁻	<i>phe-1 trp-12 str-37 rif-392 nod-6007</i>	Johnston <i>et al.</i> (1978)

Properties determined

Plasmid‡	Derivation	Production of			Nod ⁺	
		medium bacteriocin	Kanamycin resistance			
pRL1JI	Strain 248	+	—	+		Johnston <i>et al.</i> (1978)
pRL3JI	Strain 306	+	—	—		Hirsch (1979)
pRL4JI	Strain 309	+	—	—		Hirsch (1979)
pJB5JI	pRL1JI	—	+	+		Johnston <i>et al.</i> (1978)

* Nod⁺, strain induces nodules on peas; Fix⁺, strain induces nodules on peas which fix nitrogen.

† Nod⁻ phenotype caused by *ade* mutation (Pain, 1979).

‡ Defined by genetic criteria, i.e. linked transfer of the properties determined.

syringe. Ethidium bromide was removed from the sample by five extractions with 2 vol. of cold 2-propanol saturated with CsCl/TE buffer, and the sample was dialysed against four changes of dialysis buffer at 4 °C (1 l each time, minimum 4 h between changes) to remove CsCl.

All buffers, solutions, glassware, etc. were sterilized by autoclaving or rinsing with 75 % ethanol to reduce the risk of contamination with nucleases.

Agarose gel electrophoresis. A horizontal Perspex apparatus giving a gel slab 13.3 cm wide, 14 cm long and 0.6 cm thick was used. Sample wells were formed using a Perspex comb with nine teeth. Samples of crude plasmid preparations were centrifuged for 2 min in an Eppendorf 5412 centrifuge and 40 µl of the supernatant (or 40 µl plasmid preparations purified on CsCl gradients) was added to 10 µl loading dye [20 % (w/v) Ficoll 400, 0.125 % (w/v) bromophenol blue, 50 mM-EDTA] and loaded on the gel. Electrophoresis was performed on 0.7 % (w/v) Agarose (Seakem) in Tris/borate buffer, at 45 mA (180 V) and 4 °C for 6 h. The gel slab was then removed from the apparatus and stained for 20 min in Tris/borate buffer containing ethidium bromide (0.5 µg ml⁻¹), removed and stored at 4 °C for 4 h before visualizing the plasmid bands (storage was found to enhance the visualization of faint bands and reduce the background fluorescence). The gel was illuminated under u.v. light (366 nm) to visualize the plasmid bands, and photographed using a Polaroid Land camera with Polaroid type 55 film.

RESULTS

Plasmid isolation

Several methods for the isolation of large plasmids with molecular weights greater than 10⁸ have been reported (Zaenen *et al.*, 1974; Ledebøer *et al.*, 1976; Currier & Nester, 1976; Nuti *et al.*, 1977; Hansen & Olsen, 1978; Casse *et al.*, 1979). The method used here was a modification of a protocol developed at the Rijksuniversiteit, Gent by R. Villaroel and

Fig. 1. *Rhizobium leguminosarum* plasmids and reference *Agrobacterium tumefaciens* plasmid after agarose gel electrophoresis. Track 1, strain C58; 2, 248; 3, 306; 4, 309; 5, 897; 6, 1027; 7, 1062; 8, 6015.

J. Ellis (personal communication). It was simple and reproducible, giving discrete fluorescent bands when the gels were stained with ethidium bromide and examined under u.v. light (Fig. 1).

The discrete bands observed after electrophoresis of crude plasmid preparations were assumed to represent covalently closed circular (ccc) plasmid DNA of various sizes and the diffuse fluorescing band, which migrated much further, to represent linear DNA fragments (Fig. 1). This was confirmed by scaling up the crude preparation protocol 10-fold for strains C58, 248, 897 and 6015 and subjecting the resuspended DNA following PEG 6000 precipitation to centrifugation on CsCl/ethidium bromide density gradients. The lower band, corresponding to ccc DNA, was recovered from each gradient and, after removal of ethidium bromide and CsCl, the DNA was run on agarose gels. The bands (not shown) corresponded to those obtained from crude preparations although the slower-migrating bands were relatively faint compared with the fastest-migrating band for samples recovered from CsCl gradients, indicating a greater loss of the larger plasmids during density gradient centrifugation and subsequent purification. The fast-migrating diffuse zone of linear DNA was absent or very faint in samples recovered from CsCl gradients (not shown). The fluorescence observed under u.v. light at and just beyond the wells (Fig. 1) may be due to open circular forms of the large plasmids.

Comparison of plasmids in derivatives of R. leguminosarum strain 300 with the Ti plasmid from A. tumefaciens strain C58

Strain 897 (a derivative of strain 300) has been reported to have three plasmids with molecular weights of about 100×10^6 , 165×10^6 and 204×10^6 (Prakash *et al.*, 1980). The Ti plasmid of strain C58, pTiC58, has a molecular weight of about 130×10^6 (Holsters

et al., 1978). The relative mobilities of the bands of strains 897 and C58 (Fig. 1, tracks 5 and 1) are compatible with plasmids of these sizes migrating at rates approximately inversely proportional to the logarithms of their molecular weights. Casse *et al.* (1979) showed a linear relationship between the logarithms of the molecular weights and relative mobilities of ccc plasmid DNA in the range of 91×10^6 to 140×10^6 molecular weight.

The field isolate from which strain 897 was obtained, strain 300 (not shown), and a different auxotrophic derivative, strain 1027 (Fig. 1, track 6), gave identical band patterns to strain 897. However, another auxotrophic derivative of strain 300, strain 1062, had an extra band that migrated faster than the fastest plasmid band of strain 897, implying that strain 1062 has acquired a new plasmid species with a molecular weight of less than 100×10^6 (Fig. 1, track 7). It is possible that strains 300, 897 and 1027 have two different plasmids of molecular weights about 100×10^6 and that strain 1062 has suffered a small deletion in one of them, although phenotypically it appears to differ from the other strains only in auxotrophic requirements and antibiotic resistances that map to the chromosome (with the exception of strain 1027, which has an adenine requirement, these strains induced nodules on peas which fixed nitrogen). Consistent with this hypothesis is the observation that the fastest-migrating band in strains 897, 1027 and 6015 was consistently brighter (relative to their slower-migrating bands) than either of the two fast-migrating bands of strain 1062 (Fig. 1).

In addition to the plasmid bands seen in Fig. 1, we have also seen, in a minority of preparations, one, and sometimes two, additional bands moving more slowly than the slowest-migrating band described here.

Strain 6015 is a mutant of strain 300 which has lost the ability to form nodules, and it has never been found to revert (frequency $< 10^{-9}$). The slowest-migrating band of strain 6015 was found to migrate faster than that of strains 897, 1027 or 1062, implying a deletion in the largest plasmid (molecular weight 204×10^6) of a region containing genes involved in nodulation (Fig. 1, track 8).

Prakash *et al.* (1980) named the plasmids that they isolated from a derivative of strain 300: pRle-LPR115a, pRle-LPR115b and pRle-LPR115c in order of increasing size. Since we have observed variations in the sizes and numbers of plasmid species present in different derivatives of strain 300, we have deliberately omitted to name them until more is known of the relationships between the plasmids.

Identification of bacteriocinogenic plasmids

In attempts to identify the plasmid bands corresponding to pRL1JI, pRL3JI and pRL4JI, the plasmids found in the field isolates 248, 306 and 309 were compared with those of transconjugants of various strain 300 derivatives which had received one of the bacteriocin determinants. It can be seen that the plasmid bands of strains 248, 306 and 309 (Fig. 1, tracks 2, 3 and 4, respectively) differ from each other and from strain 300 or some of its derivatives (e.g. tracks 5 and 6).

pRL1JI

When pRL1JI-determined bacteriocin production was transferred to strain 300 derivatives (strains 1062, 1027 and 6015) the transconjugants were found to have gained a band corresponding in size to the second smallest plasmid in strain 248 (Fig. 2, track 2) whether bacteriocinogenicity had been transferred either directly from strain 248 (Fig. 2, tracks 3 and 9) or from a derivative of strain 300 that had acquired the plasmid (Fig. 2, tracks 5 and 6). This extra band corresponding to pRL1JI showed the same relative mobility as pTiC58 which has a molecular weight of 130×10^6 (Holsters *et al.*, 1978). Derivatives of strain 6015 which had received pRL1JI from strain 248 or strain 1062(pRL1JI) gained the

Fig. 2. Plasmids of *R. leguminosarum* pRL1JI transconjugants after agarose gel electrophoresis. Track 1, strain 1062; 2, 248; 3, 1062(pRL1JI), donor 248; 4, 1027; 5, 1027(pRL1JI), donor 1062(pRL1JI); 6, 6015(pRL1JI), donor 1062(pRL1JI); 7, 6015; 8, 248; 9, 6015(pRL1JI), donor 248.

ability to nodulate peas (Brewin *et al.*, 1980) and we therefore conclude that pRL1JI, defined as the conjugative plasmid determining *medium*-sized bacteriocin production linked to genes involved in symbiosis, has a molecular weight of about 130×10^6 .

Strains carrying pJB5JI, a plasmid obtained by the insertion of Tn5 into pRL1JI (Johnston *et al.*, 1978), were found to have a plasmid band with the same relative mobility as pRL1JI (data not shown). This is not unexpected since Tn5 which has a molecular weight of about 3.8×10^6 (Berg *et al.*, 1975), would cause only a very slight proportional increase in the size of pRL1JI.

One derivative of strain 6015 which had received pRL1JI from strain 1062(pRL1JI) had, in addition, an extra band corresponding to the smallest plasmid in strain 1062 (Fig. 2, track 6). This could have been due to mobilization of the strain 1062 plasmid by pRL1JI, a phenomenon observed in crosses to other strains of *R. leguminosarum* (N. J. Brewin, unpublished observations) and to *R. phaseoli* (J. L. Beynon, personal communication).

It was noted that in the transconjugant that had apparently received the smallest strain 1062 plasmid (Fig. 2, track 6), the second smallest band stained less intensely than did the band of corresponding size in the strain 6015 parent (Fig. 2, track 7). This is consistent with strain 6015 having received a plasmid that is incompatible with the one of the two plasmids in the strain 1062 doublet (see above).

Fig. 3

Fig. 4

Fig. 3. Plasmids of *R. leguminosarum* pRL3JI transconjugants after agarose gel electrophoresis. Track 1, strain 1062; 2, 306; 3, 1062(pRL3JI), donor 306.

Fig. 4. Plasmids of *R. leguminosarum* pRL4JI transconjugants after agarose gel electrophoresis. Track 1, strain 1062; 2, 309; 3, 1062(pRL4JI), donor 309.

pRL3JI

The bacteriocinogenic determinant transferred from field isolate 306 was designated pRL3JI (Hirsch, 1979). Strain 306 appears to have at least four large plasmids, of which the smallest is larger than pTiC58 (Fig. 1, tracks 1 and 3). Strain 6015(pRL3JI), which had received the determinant of *medium* bacteriocin production from strain 306, had acquired a new plasmid species slightly smaller than the second smallest plasmid in strain 6015 (data not shown) which corresponded in size to the second smallest plasmid in strain 306. However, strain 1062(pRL3JI) which had received the determinant of *medium* bacteriocin production from strain 306 showed no bands corresponding to any of those in strain 306 (Fig. 3, tracks 2 and 3). Two changes were apparent in this transconjugant: a new plasmid appeared with a molecular weight of about 130×10^6 (its relative mobility was the same as that of pTiC58 and pRL1JI in several gels, which are not shown); and an even larger plasmid replaced the largest one usually visible in strain 1062 (Fig. 3, tracks 1 and 3). This could be explained by unequal recombination of pRL3JI with a strain 1062 plasmid. Because recombination between these plasmids occurs it is not yet possible to correlate the genetic determinant of bacteriocinogenicity, pRL3JI, with a distinct plasmid species.

pRL4JI

Strain 309 is another field isolate which can donate the ability to produce a *medium* bacteriocin (Hirsch, 1979) and this strain appeared to have at least four plasmids (Fig. 1, track 4). When the determinant of *medium* bacteriocinogenicity, pRL4JI, was transferred from strain 309 to strain 1062, a new plasmid band was observed corresponding in size to the third-smallest plasmid of strain 309 (Fig. 4, tracks 2 and 3); this was also present in derivatives of strains 6015 and 1027 which had received pRL4JI from strain 1062 (pRL4JI) (data not shown). The mobility of this plasmid was very similar to that of the third-smallest plasmid species in strain 1062 (or the second-smallest plasmid species in strains 1027 and 6015). However, a derivative of strain 6015 which had received the determinant of *medium* bacteriocinogenicity directly from strain 309 appeared to have a new plasmid species smaller than the smallest in strain 309 and larger than the smallest in strain 6015 (data not shown). This could be a deleted derivative, which retained the region determining production of the *medium* bacteriocin, of the plasmid observed in other strains carrying pRL4JI. Only one transconjugant clone was examined from each of the crosses described above.

DISCUSSION

We have demonstrated, by physical means, the presence of some plasmids in *R. leguminosarum* which had previously been defined only by genetic criteria. We have shown that strain 6015, a derivative of strain 300 which does not nodulate, appears to have suffered a deletion in the largest of the three plasmids which are usually visible when plasmid preparations are subjected to agarose gel electrophoresis. Strain 6015 also appears to have lost the ability to fix nitrogen, and genetic evidence supports the view that strain 6015 has lost some genes involved in symbiosis (Buchanan-Wollaston *et al.*, 1980).

Surprisingly, strain 1062, a separate derivative of strain 300 which did not differ symbiotically from its parent, had a band of molecular weight smaller than any of those in strain 300. Possibly, the fastest-migrating band of strain 300 actually comprises two plasmid species with indistinguishable mobilities (this band was relatively brighter than the corresponding band in strain 1062) and one of these plasmids has suffered a spontaneous deletion in strain 1062.

When the determinant of *medium* bacteriocin production pRL1JI was transferred between strains an extra plasmid with a molecular weight of about 130×10^6 was inherited. Similarly, strains carrying pRL4JI had acquired in most cases a single extra band of molecular weight about 160×10^6 .

Plasmid rearrangement was observed in a strain 1062 transconjugant which had received the determinant of *medium* bacteriocin production pRL3JI from field isolate 306. The new plasmid band in this transconjugant did not correspond to any in strain 306 and, furthermore, one of the plasmids of strain 1062 appeared to have been replaced by a new, larger species. Without more detailed analysis we can only speculate on the nature of these apparently recombinational events.

When the determinants of *medium* bacteriocin production pRL1JI, pRL3JI or pRL4JI were transferred to derivatives of strain 300, the transconjugants no longer made the *small* bacteriocin produced by strain 300 (Hirsch, 1979; Brewin *et al.*, 1980). Since there was no evidence for the loss of a plasmid from transconjugants, it is more likely that the loss of the ability to produce *small* bacteriocin is due to the repression of this property by the incoming plasmids, rather than to the elimination of its determinants (although the possibility of replacement of one plasmid species by another of the same size cannot be ruled out by the evidence presented here).

The three bacteriocinogenic plasmids studied here – pRL1JI, pRL3JI and pRL4JI – have previously been shown to have many properties in common (Hirsch, 1979; Brewin

et al., 1980). They specify the production of *medium* bacteriocins that are apparently similar; they repress (or eliminate) the production of *small* bacteriocin by strains that contain them; they transfer between strains at high frequencies; they mobilize chromosomal genes at low frequencies; they cannot co-exist within the same cell without apparently undergoing interplasmid recombination. However, an important difference between these three plasmids is that, unlike the other two, the plasmid pRL1JI carries a number of symbiotic gene functions capable of restoring several mutations of strain 300 derivatives (Nod⁻ and Fix⁻) to a wild-type phenotype (Brewin *et al.*, 1980). Despite these extra symbiotic gene functions specified by pRL1JI, this plasmid appears to be smaller than either pRL3JI or pRL4JI. However, it is not known what fraction of pRL1JI is concerned with the specification of symbiotic gene functions not present on pRL3JI or pRL4JI, nor indeed what other gene functions (apart from bacteriocin production) might be specified by any of these large plasmids.

Although the plasmids pRL3JI and pRL4JI do not themselves specify genes for nodulation and fixation that are absent from the Nod⁻ strain 6015, they were found to mobilize these alleles at relatively high frequency in crosses from a wild-type donor (Brewin *et al.*, 1980). Presumably this transfer was the result of selected inter-plasmid recombination. In the present physical study, we have shown that, at least in the case of pRL3JI, plasmid rearrangements occurred after transfer of the bacteriocinogenic plasmid into a new host. Perhaps these phenomena are to be expected if one or more plasmids contain several similar DNA sequences; however, such interactions are likely to make the physical and genetic analysis of *Rhizobium* plasmids rather more complicated than it otherwise might be.

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