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Differential Expression of the Pea Symbiotic Plasmid pJB5JI in Genetically Dissimilar Backgrounds

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Abstract

The *Rhizobium leguminosarum* host-range (Sym) plasmid, pJB5JI, is a 130 Mdal conjugative plasmid which carries the genes for host-specificity (i.e., for peas) and some of the genes for nitrogen-fixation. We have transferred plasmid pJB5JI into different species of *Rhizobium* and examined the symbiotic properties of the resulting transconjugants. The donor strain *R. leguminosarum* 6015 (pJB5JI), was only moderately effective on peas. Transconjugants of a Nod$^-$ mutant of *R. trifolii, #2,* became highly effective on peas, but did not regain their ability to nodulate clover. Transconjugants of a Nod$^+$ strain of *R. trifolii,* 0403, could nodulate peas, but only ineffectively, and retained the ability to nodulate clover effectively. All reisolates of #2 transconjugants from pea nodules had gained the ability to form ineffective nodules on clover. Reisolates of 0403 transconjugants from both clover and pea nodules exhibited three different symbiotic phenotypes: They could n odulate (1) both peas and clover effectively; (2) clover effectively but peas ineffectively; or (3) only clover effectively. Transconjugants made with a fastgrowing soybean- *Rhizobium* were unable to nodulate peas, but retained the ability to nodulate soybeans. The presence of the plasmid in these transconjugants was ascertained by backcrossing to a Nod$^-$ of *R. leguminosarum.*.In all of the studies, the identity of the donors, the recipients and the transconju gants were confirmed by their antibiotic and auxotrophic markers, as well as by immunofluorescence and immunodiffusion using strain specific antisera. In this study, we have shown that plasmid pJB5JI is readily transferable to different species of *Rhizobium.* However, its symbiotic expression varies depending on the genetic background it resides in.

Keywords: Symbiotic plasmid, fast-growing *Rhizobium japonicum, R. leguminosarum, R. trifolii,* soybeans, peas, clover.

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1. Introduction

Recent advances in the genetics of the legume- *Rhizobium* symbiosis have been achieved mostly with the "fast-growing" species of *Rhizobium: R. leguminosarum, R. meliloti, R. phaseoli,* and *R. trifolii.* This may in part be due to the fact that symbiosis-related genes have been shown to be plasmid borne in many of the fast-growing rhizobia (Nuti *et al.*, 1979; Beynon *et al.*, 1980; Hirsch *et al.*, 1980; Hombrecher *et al.*, 1981; Haaykaas, *et al.*, 1981, Masterson *et al.*, 1983, Sadowsky and Bohlool, 1983). The genetics of the root-nodule bacteria of such an important crop as soybean has been neglected due to difficulties in consistently demonstrating plasmids in all the slow-growing *R. japonicum* strains and identifying the location of symbiosis-related genes in these organisms. Several researchers have reported the transfer of plasmids to slow-growing rhizobia (Kuykendall, L.D., 1979; Kennedy *et al.*, 1981; Pilacinski and Schmidt, 1981), but in all instances, the plasmids used belonged to the IncP-1 group of R plasmids: R1822, R68.45, pRD1, and RP4 (originally from *Pseudomonas aeruginosa).* Keyser *et al.* (1982) have reported the isolation of a unique group of fast-growing, acid producing, rhizobia from soybean nodules collected in the People's Republic of China. Masterson *et al.* (1982) have shown that the structural genes for nitrogen fixation (nif DHK) are located on large plasmids in several of these fast *R. japonicum.* Recently, Sadowsky and Bohlool (1983) have shown that a large plasmid in at least one strain of this group, might be involved in nodulation.

The *Rhizobium leguminosarum* plasmid, pJB5JI, is a pea Sym (host-range) plasmid with a Tn5 (Kanamycin) insertion in the genes for bacteriocin production (Johnston *et al.*, 1978). It has been used by several researchers (Johnston *et al.*, 1978; Beynon *et al.*, 1980; Brewin *et al.*, 1980a; Brewin *et al.*, 1980b; Hirsch *et al.*, 1980; Djordjevic *et al.*, 1982; Johnston *et al.*, 1982) to transfer pea nodulation genes into *R. leguminosarum, R. phaseoh,* and *R. trifolii* strains. This 130 megadalton (Mdal) plasmid carries some of the nitrogen fixation (nif) as well as the pea nodulation (nod) genes.

Djordjevic *et al.* (1982) and Christensen and Schubert (1983) have recently transferred pJB5JI into strains of *R. trifolii* and studied the plasmid profile and nodulation characteristics of the resulting transconjugants. In this study we found that pJB5JI can be readily transferred to two different strains of *R. trifolii* and a strain of the fast-growing *R. japonicum* group recently reported by Keyser *et al.*, (1982). The expression of pea nodulation ability of the pJB5JI in the different recipient strains varied considerably.

Table 1. Bacteria used and their sources

Organism	Relevant characteristic	Source
Rhizobium japonicum[a]		
USDA 192	Wild-type	USDA, Beltsville
USDA 193		
USDA 194		
USDA 201		
USDA 205		
USDA 206		
USDA 208		
Transconjugants		
201-8	*Kan*^{r}	This study
201-11	201(pJB5JI), *Kan*^{r}	
201-13	*Kan*^{r}	
201-14	201(pJB5JI), *Kan*^{r}	
Rhizobium leguminosarum		
6015(pJB5JI)[b]	Nod$^+$:strain 300 *phe-1*, *trp-12*, *rif*-392, *str*-37, *inf*-6007, *Nod*$^+$, *Kan*^{r}	B. Rolfe
6015	Nod$^-$; strain 300 *phe*-1, *trp*-12, *rif*-392, *str*-37, *inf*-6007, *Nod*$^+$ *Kan*^{r}	P. Hirsch
Transconjugants		
6015-11-7	6015(pJB5JI), derived from back-cross of 6015 to 201-11	This study
6015-14-8	6015(pJB5JI), derived from back-cross of 6015 to 201-14	
Rhizobium trifolii		
#2	*Nod*$^-$	W. Broughton
0403	*Nod*$^+$, Wild-type	
Transconjugants		
2-33	2(pJB5JI) *Nod*$^+$, *Fix*$^+$	This study
2-54	2(pJB5JI) *Nod*$^+$, *Fix*$^-$	
403-33	403(pJB5JI) *Nod*$^+$, *Fix*$^-$	

[a] Keyser et al. (1982).

[b] Plasmid pJB5JI (pRL1JI::Tn5) is a self-transmissible plasmid that carries genes for pea nodulation (Brewin 1980a)

2. Materials and Methods

Cultures

The *Rhizobium* strains used and their sources are listed in Table 1. Cultures were grown at 28°C on yeast extract mannitol, YEM (Bohlool and Schmidt, 1970) agar slants. Isolates containing plasmid pJB5JI were maintained on tryptone yeast extract (TY) agar slants (Beringer, 1974) containing 50µg/ml kanamycin.

Bacterial crosses

The sym plasmid pJB5JI was transferred from *R. leguminosarum* strain 6015(pJB5JI) to a fast-growing acid-producing strain of *R. japonicum,* USDA 201. The transconjugants thus obtained were back-crossed to a nonnodulating (Nod^-) *R. leguminosarum,* strain 6015, in order to confirm the presence of pJB5JI in the *R. japonicum* transconjugants. The plasmid was also transferred to a wild-type isolate of *R. trifolii,* 0403, and to a nodulation deficient (Nod^-) isolate of *R. trifolii,* #2. Bacterial crosses were done according to the membrane-filter method of Buchanan-Wollaston *et al.* (1980. Aliquots from serial dilutions of the bacterial mixtures, harvested from the membrane filters, were spread-plated onto the appropriate selective media. To select for *R. japonicum* and *R. trifolii* transconjugants receiving the Sym plasmid (Kanr) minimal Y-medium of Beringer (1974) supplemented with 50µg/ml kanamycin was used. In backcrosses to the Nod^- mutant 6015 (Rif-, Strr), the Y-medium was supplemented (Y-supp) with kanamycin (50µg/ml), rifampicin (20µg/ml), streptomycin (100µg/ml), phenylalanine (50µg/ml) and tryptophan (50µg/ml). All mixtures were also spread-plated onto nonselective TY medium to obtain total viable cell counts.

Before proceeding with plasmid analyses and plant infections, culture purity was ascertained for all donors, recipients, and transconjugants in the following manner: each culture was streaked two consecutive times on the appropriate selective medium. Isolated colonies were transferred to TY liquid medium and after 2 days of growth restreaked on plates of the same selective medium. Colonies were transferred to slants of TY or TY supplemented with 50µg/ml kanamycin, as appropriate.

Each pure culture thus obtained was further identified by immunofluorescence (Schmidt *et al.,* 1968) and immunodiffusion (Vincent, 1970) using strain-specific antiserum against R. *japonicum* USDA 201, *R. trifolii* strains 0403 and #2, or *R. leguminosarum* 6015(pJB5JI), prepared according to Schmidt *et al.,* (1968).

Plasmid screenings

Plasmid DNA isolation and visualization were essentially by the method described by Hirsch *et al.* (1980). Cultures were grown for 48 hr in 200 ml of PA medium (Hirsch et al., 1980) supplemented with 0.2 g/l of K_2HP0_4. The tracking dye mixture used had the following composition: 50% (w/v) glycerol, 0.125% (w/v) bromphenol blue, and 50mM Na-EDTA, pH 8.0. For molecular weight estimations, the electrophoretic mobilities of plasmid bands were compared to the reference strain *R. leguminosarum* 6015 (pJB5JI) (Hirsch *et al.*, 1980; Prakash *et al*., 1980).

Plant infection assays

All pea and soybean nodulation tests were performed in modified Leonard Jars (Leonard, 1943) with quarter-strength Hoagland's plant nutrient solution (Hoagland, 1938). Clover nodulation was tested in screw-capped test tubes (2.5 by 20 cm) containing 25 ml of quarter-strength Hoagland's plant nutrient solution supplemented with 1.0% agar.

Pea (Pisum sativum var. Wisconsin Perfection) and soybean (*Glycine max* vars. Peking, Chippewa 64, and Lee) seeds were surface sterilized by immersion in a 4.0% (w/v) calcium hypochlorite solution for 20 min followed by exhaustive washings in sterile distilled water. Plants were inoculated with one ml aliquots of 2 day-old YEM cultures and the plant vessels were topped off with approximately 2 cm of sterilized Perlite and 2-3 cm of sterilized paraffin coated sand [1.0% paraffin in chloroform mixed with silica sand (1:10) and evaporated to dryness]. Clover seeds (*Trifolium repens* var. landino) were surface sterilized in 2.0% (v/v) sodium hypochlorite for 10 min followed by exhaustive washings in sterile distilled water. Seedlings on Hoagland's agar slants were inoculated with 0.1 ml of 2 day-old YEM cultures. Each culture was inoculated onto three separate plants. In all plant infection assays, 25% of the vessels were used as uninoculated controls.

After four weeks of growth, plants were examined for nitrogenase activity by the acetylene reduction method of Hardy *et al.* (1968).

For the recovery of isolates from nodules, root sections with attached nodules were excised from the plants and surfaced sterilized for 5 min in a solution of 75% ethanol and 8% H_20_2. After exhaustive washings in sterile distilled water, nodules were macerated in 10% glycerol (peas and clover) or in distilled water (soybeans) and streaked onto YEM agar containing 0.26 mg/1 bromthymol blue. Macerates were also spread onto slides and nodule occupants examined by direct immunofluorescence using strain-specific fluorescent antibodies.

3. Results

Before examining the ability of fast-growing, acid-producing *R.* japonicum and *R.* trifolii isolates to receive plasmid pJB5JI from *R.* leguminosarum 6015(pJB5JI), it was necessary to determine the recipient's frequency of spontaneous mutation for kanamycin resistance. Two of the isolates, *R. japonicum* USDA 192 and *R. trifolii* 0403 had high frequencies of spontaneous resistant mutants up to concentrations of 100µg/m1 (frequencies of 3.2 x 10-6 and 3.4 x 10^{-8}) but the other isolates produced no colonies at 100µg/ml or higher.

Table 2. Frequency of transfer of plasmid marker in crosses between *Rhizobium leguminosarum*, *R. japonicum*, *R. trifolii* and their transconjugants.

Crosses[a]		Transfer frequency[b]
Donor	Recipient	
A. Crosses		
1. *R. leguminosarum* 6015(pJB5JI)	*R. japonicum* USDA 201	4×10^{-4}
2. " " "	" USDA 205	$<4 \times 10^{-9}$
3. " " "	*R. trifolii* #2	4×10^{-4}
4. " " "	" 0403	2×10^{-4}
B. Backcrosses		
1. *R. japonicum* 201-11 and 201-14	*R. leguminosarum* 6015	2×10^{-3}
2. *R. trifolii* #2(pJB5JI)	" "	7×10^{-3}
3. " 0403(pJB5JI)	" "	1×10^{-2}

[a] Transconjugants from crosses A1 and 3 were selected on Y minimal medium containing 50µg/ml kanamycin; A2 and 4 on Y medium with 100µg/ml kanamycin; B3, 6, and 7 on Y supp (Y medium supplemented with phe, trp, Str, Rif, and Kan).

[b] Transfer frequency is defined as the number of colonies of the recipients formed on selective media divided by those formed on non-selective media.

R. trifolii strains 0403 and #2 and soybean strains USDA 201 and USDA 205 were examined for their ability to receive, maintain and express plasmid pJB5JI from *R. leguminosarum* 6015(pJB5JI). Transfer frequencies for the kanamycin marker varied widely (Table 2). Transfer to *R. trifolii* strains 0403 and #2 occurred at frequencies of 2.0 x 40^{-4} and 4.4 x 10^{-4}, respectively. The plasmid profiles in Fig. 1C, lanes 3,4, and 6, respectively, show the appearance of a new plasmid in the transconjugants corresponding in size to pJB5JI.

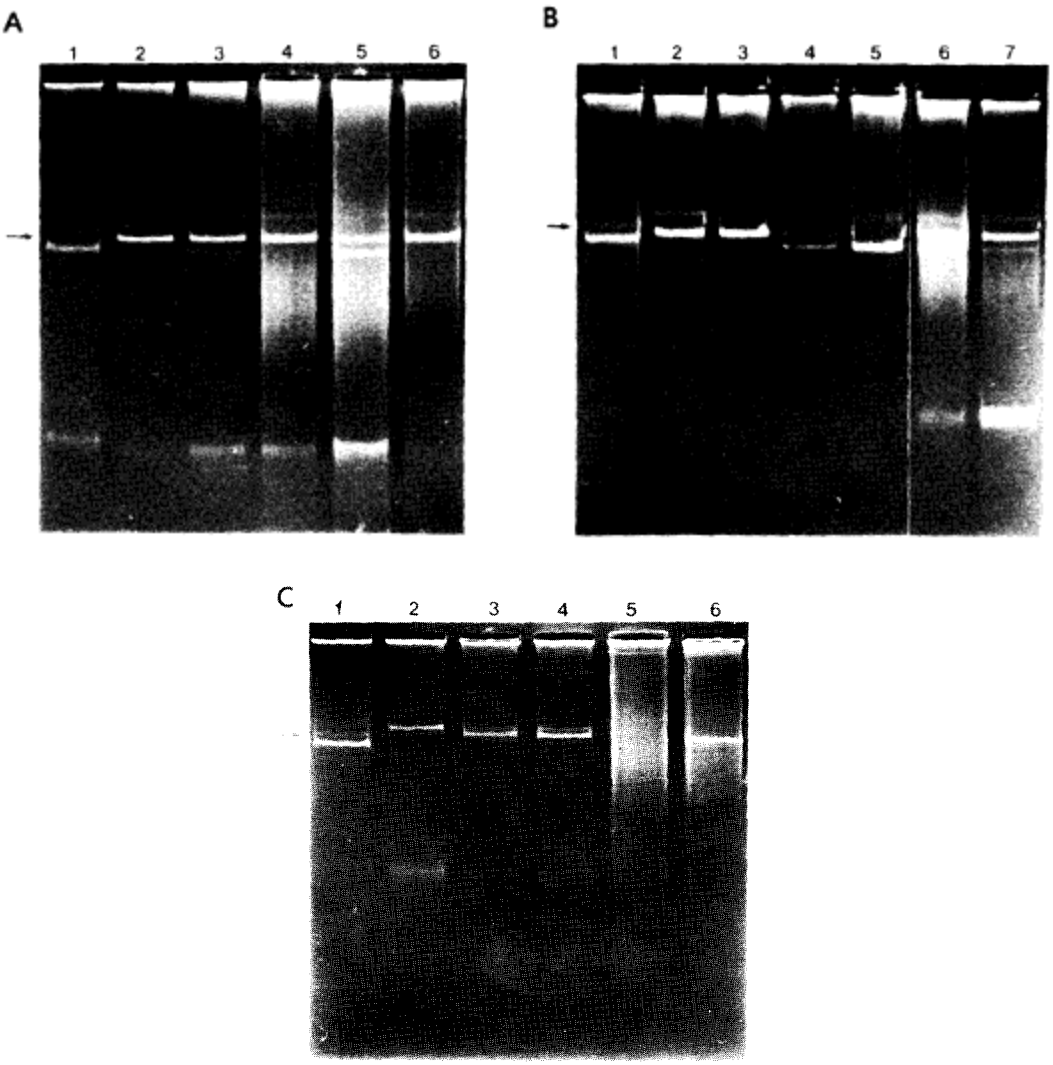

Figure 1. Plasmid profile of plasmid donors, recipients and the resulting transconjugants. Arrow indicates the position of plasmid pJB5JI.
A. 1, donor strain, *R. leguminosarum* 6015(pJB5JI); 2, *R. japonicum* USDA 201; 3, 201-11(pJB5JI); 4, 201-8 Kan^r; 5, 201-14(pJB5JI); 6, 201–13 Kan^r.
B. 1, donor strain, 6015(pJB5JI); 2, USDA 201; 3, 201-11(pJB5JI); 4, 201-14(pJB5JI); 5, Nod$^-$ *R. leguminosarum* 6015; 6, backcrosses 6015-14(pJB5JI); 7, backcross 6015-11(pJB5JI).
C. 1, donor strain 6015(pJB5JI); 2, *R. trifolii* #2; 3, #2-33(pJB5JI); 4, #2-54(pJB5JI); 5, *R. trifolii* 0403; 6, 0403-33(pJB5JI).

Transfer of pJB5JI to *R. japonicum* strain USDA 201 occurred at a frequency comparable to the *R. trifolii* strains (4.0×10^{-4}). But, the low frequency of transfer to USDA 205 ($< 1 \times 10^{-9}$) precluded the use of this organism in further studies. Strain USDA 201 has an indigenous plasmid similar in size to pJB5JI (Figs. 1A, lane 2; and 1B, lane 2). Plasmid profile of the pJB5JI transconjugants of this strain, given in Fig. 1A, lanes 3,4,5, and 6, show three different patterns: some transconjugants (Fig. 1A, lanes 4 and 6) had a pattern identical to the parent strain USDA 201 (Fig. IA, lane 2). Another group of transconjugants represented by 201-11 in Fig. IA, lane 3, had in addition to the two parental plasmids, a smaller plasmid of approximately 100 Mdal in size. The third group, represented by 201-14 in Fig. 1A, lane 5, appeared to have lost the largest of the parental plasmids, but gained a plasmid of 100 Mdal in size.

Transconjugants 201-11, 201-14, and several from #2 and 0403 crosses were back-crossed to the Nod$^-$ *R. leguminosarum* 6015. The results of the frequencies of transfer, and plasmid profiles, are also given in Table 2 and Fig. 1B, respectively. Although pJB5JI in USDA 201 transconjugants cannot be visualized easily due to the presence of an indigenous plasmid of similar size, it can be seen (Table 2 and Fig. 1B, lanes 6 and 7) that the plasmid can be readily back-transferred to the Nod$^-$ *R. leguminosarum*.

The symbiotic properties of the parental strains, transconjugants and the back-crosses are given in Table 3. The transconjugants of *R. japonicum* USDA 201 were unable to nodulate peas. Their symbiotic properties on three cultivars of soybeans, however, remained unaffected. Mating of these transconjugants with a Nod$^-$ *R. leguminosarum* resulted in isolates capable of nodulating peas.

Presence of pJB5JI in *R. trifolii* 0403, produced transconjugants that could nodulate both clover and peas. Nodulation on peas, however, was ineffective. The transconjugants of the clover Nod$^-$ R. *trifolii* #2 on the other hand, formed highly effective nodules on peas, but remained incapable of nodulating clover (Table 3).

4. Discussion

The *R. leguminosarum* conjugative host-range (Sym) plasmid, pJB5JI, has been used by several workers (Beynon *et al.*, 1980; Brewin et al., 1980a, b; Christensen and Schubert, 1983; Djordjevic *et al.*, 1982; Ruiz-Saint *et al.*, 1984) to transfer pea-nodulating ability to non-nodulating mutants of *R. leguminosarum* and to other species of *Rhimbium.* In this study we show that the hybrids of different rhizobia bearing this plasmid differ substantially in nodulation and nitrogen fixation characteristics. The synopsis of all the results is given in Table 4.

Table 3. Symbiotic properties of *R. japonicum*, *R. leguminosarum* and *R. trifolii* and their pJB5JI transconjugants.

	Symbiotic properties[a]				
	Soybean variety				
Organism	Peking	Chippewa	Lee	Pea	Clover
R. japonicum					
USDA 201	HE	IE	IE	Nod$^-$	Nod$^-$
Transconjugants 201-11 & 201-14	HE	IE	IE	Nod$^-$	
R. leguminosarum					
6015(pJB5JI)	Nod$^-$			PE	Nod$^-$
6015	Nod$^-$			Nod$^-$	Nod$^-$
Transconjugants[b]	Nod$^-$			PE	Nod$^-$
R. trifolii					
#2				Nod$^-$	Nod$^-$
#2Transconjugants				HE	Nod$^-$
0403				Nod$^-$	HE
Transconjugants				IE[c]	HE

[a] Based on plant and nodule color (and for peas also top dry weight) after 4 weeks of growth: HE=Highly-Effective: dark-green plant tops (571±150 mg/plant), dark-pink nodules; PE=Partially-Effective: yellow-green plants (250±14 mg/plant), light-pink nodules; IE=Ineffective: yellow-plants (155±18 mg/plant), small white ineffective nodules; Nod$^-$=no nodules.

[b] Includes primary transconjugants, and backcrosses with other pJB5JI containing transconjugants: 12 isolates from 201-11 and 201-14, 3 from #2- and 3 from 0403-transconjugants.

[c] All 18 plants had ineffective nodules; but one plant also had one large effective nodule which contained rhizobia that were typed as 0403 by immunofluorescence and isolated for further study.

In order to determine the stability of plasmid pJB5JI in transconjugants after passage through plants, several #2 and 0403 transconjugants were re-isolated from clover and/or pea nodules and, after purification and immunofluorescence identification, were reinoculated onto peas and clover plants. The results shown in Table 4 indicate that all 6015(pJB5JI) nodule re-isolates retained their symbiotic phenotype. Nodule re-isolates of #2 and 0403 transconjugants produced a variety of nodulation and nitrogen fixation patterns on peas and clover plants. All of the #2 transconjugants retained the ability to nodulate peas highly effectively, and gained the ability to ineffectively nodulate clover, after passage through the pea nodules. Re-isolates of 0403 from pea and clover nodules produced three different patterns of symbiotic phenotypes (Table 4).

Table 4. Synopsis of results of bacterial crosses

Plasmid donor		Recipients Culture	Symbiotic[c] phenotype on: Peas	Clover		Transconjugants[a] Symbiotic[c] phenotype on: Peas	Clover		Nodule Reisolates[b] From	Symbiotic[c] phenotype on: Peas	Clover
		1. *R. leg.* 6015	–	–	→	+/+	–	→	Peas	+/+	–
		2. *R. trif.*									
		a. #2	–	–	→	+/+	–	→	Peas	+/+	+/–
		b. 0403	–	+/+	→	+/–	+/+	→	a. Peas	1. +/–	+/+*
										2. +/±	+/+*
									b. Clover	1. –*	+/+
										2. +/–	+/+
										3. +/±	+/+*
R. leg. (with pJB5JI)	→	3. *R. jap.* PRC 201	–	–	→	–	–	→		n.t.	n.t.
R. jap. (201 with pJB5JI)	→	4. *R. leg.* 6015	–	–	→	+/+	–	→		n.t.	n.t.

[a] Six transconjugant clones from each cross were tested in triplicate on each host.

[b] Isolates were obtained from 5 nodules on different plants and tested in triplicate on each host.

[c] Symbiotic Properties: – = No nodules; +/+ = effective nodules; +/– = ineffective nodules: +/± = approximately 150–250 small ineffective nodules, and from 1–15 large effective nodules on each plant; +/+* = most plants +/+, but 1–2 plants out of 8–9, ineffectively nodulated; –* = only 1 culture out of the 5 tested did not form nodules; n.t = not tested.

Presence of pJB5JI in a fast-growing isolate of *R.* japonicum did not cause the transconjugants to nodulate peas but neither did it alter their symbiotic characteristics on their own host, soybeans. As was reported by Keyser *et al.* (1982, the fast-growing *R. japonicum* form only ineffective nodules on most New World commercial varieties of soybeans. It is interesting to note that the acquisition of pJB5JI, and its complement of Nif genes, did not improve the symbiotic performance of these strains on the soybean commercial varieties, Chippewa and Lee. While *R. japonicum* USDA 201 transconjugants that received pJB5JI do not themselves nodulate peas, they are capable of transferring this property to a non-nodulating strain of *R. leguminosarum* cured of its Sym plasmid.

The physicai presence of pJB5JI in; the fast-growing soybean strain 201 was difficult to ascertain on gels due partly to the presence of an indigenous plasmid similar in size and partly to the fact that there was considerable rearrangement of the plasmid profile of some of the transconjugants. Our most convincing evidence for actual transfer lies in the fact that the Nod6015 is rendered nodulating, and shows pJB5JI in gels, after mating with 201-11 or 201-14. The smallest plasmid which appeared in 201-11 and 20114 transconjugants may be due to either the cotransfer of another plasmid from *R. leguminosarum* 6015(pJB5JI), or a deletion in an incoming or resident plasmid. Since USDA 201 contains a plasmid which has a similar electrophoretic mobility as pJB5JI, deletions in, or recombinational events between this, and other plasmids and the major replicon would not be detected. However, back-crosses of strain 6015 with strain 201 transconjugants,

6015-11 and 6015-14, appear to have received pJB5JI intact as judged by the appearance of a new plasmid band in 6015 (Fig. 113, lanes 6, 7) with an electrophoretic mobility similar to pJB5JI.

In a nodulation-deficient isolate of *R. trifolii,* #2, nitrogen-fixation and nodulation genes were expressed so that the resulting transconjugants nodulated peas effectively. The presence of this plasmid in *R. trifolii* #2 transconjugants did not, however, correct nodulation-deficiency on clover. This indicates that the information encoded by this plasmid was not sufficient to overcome the genetic deficiencies for nodulation of clover. However, after passage through pea plants, all of the #2 transconjugants gained the ability to nodulate clover, albeit ineffectively. When pJB5JI was transferred to a clover nodulation-competent *R. trifolii* strain 0403, the resulting transconjugants were capable of nodulating both clover and peas. However, peas were only nodulated ineffectively (Nod+, Fix-), indicating a possible negative control of the Nif-genes on pJB5JI by this strain. The same transconjugants could still nodulate clover and express the Nif genes in this symbiosis.

The variable stability of pJB5JI in different strains of *R. trifolii is* illustrated by the fact that re-isolates of 0403 transconjugants from pea and clover nodules exhibit a range of symbiotic properties on peas (Table 4). Beynon *et al.* (1980, Djordjevic *et al.* (1982 and Christensen and Schubert (1983 have also noted that while some host range plasmids could coexist in culture, they may be incompatible in the host nodule resulting in elimination of one or the other.

It is important to note that although the pJB5JI transconjugants of strains #2 by themselves are incapable of nodulating clover, they could do so after passage through pea nodules. It is not known whether the parental strain *#2* is Nod^- on clover due to loss of genetic information (i.e., plasmids), or because of repression of genes involved in nodulation. It is therefore, difficult to postulate a plausible mechanism for the phenomenon observed with nodule re-isolates of *#2* transconjugants. It is not possible, based on the results presented, to indicate whether the transconjugant gains new information after passage through pea nodules, or its existing genetic constitution is somehow modified (i.e., derepressed) by the host.

The purity of all donor, recipient and transconjugant cultures were ascertained by immunofluorescence and immunodiffusion using strain-specific fluorescent antibodies (Schmidt *et al.,* 1968) before and after each cross. Nodule re-isolates were also tested by immunofluorescence to preclude the possibility of contamination.

In conclusion, our results indicate that although the pJB5JI plasmid is transferrable to different species of *Rhizobium,* it is differentially expressed in different genetic backgrounds. Also, although the *R. leguminosarum* pea host-range plasmid, pJB5JI, can effectively function in closely-related rhizobia, our results suggest that it does not by itself carry all the genetic

information necessary for the nodulation of peas in a dissimilar environment such as the fast-growing soybean rhizobia from China.

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