1-1-1986

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Transposon Mutagenesis and Excision of R' Plasmids by Conjugative, Chimeric Plasmid pUW942 in Extra-Slow-Growing *Rhzobium japonicum* Strains†

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Received 18 June 1985/Accepted 7 October 1985

Transposons Tn5O1 (specifying mercury resistance) and Tn7 (specifying resistance to trimethoprim and streptomycin) were introduced into extra-slow-growing *Rhzobium japonicum* by conjugal transfer of the 82 kilobase chimeric plasmid pUW942. Mercury-resistant transconjugants were obtained at a frequency of $10^{-7}$ to $10^{-9}$. The transfer frequency of streptomycin resistance was lower than that of mercury resistance, and Tn7 was relatively unstable. pUW942 was not maintained as an autonomously replicating plasmid in *R. japonicum* strains. However, some of the Hg' transconjugants from the RJ19FY, RJ17W, and RJ12S strains acquired antibiotic markers of the vector plasmid pUW942. Southern hybridization of plasmid and chromosomal DNA of *R. japonicum* strains with $^{32}$P-labeled pUW942 and pAS8Rep-1, the same plasmid as pUW942 except that it does not contain Tn5O1, revealed the formation of cointegrates between pUW942 and the chromosome of *R. japonicum*. More transconjugants with only Tn5O1 insertions in plasmids or the chromosome were obtained in crosses with strains RJ19FY and RJ17W than with RJ12S. These retained stable Hg' both in plant nodules and under nonselective in vitro growth conditions. One of the RJ19FY and two of the RJ12S Hg' transconjugants with vector plasmid-chromosome cointegrates conjugally transferred plasmids of 82, 84 or 86, and 90 kilobases, respectively, into plasmidless *Escherichia coli* C. These plasmids strongly hybridized to pUW942 and EcoRI digests of total DNA of each respective *R. japonicum* strain but not to indigenous plasmid DNA of the *R. japonicum* strains. These R' plasmids consisted of pUW942-specific EcoRI fragments and an additional one or two new fragments derived from the *R. japonicum* chromosome.

The genetic analysis of slow-growing rhizobia has been hampered by the lack of suitable gene transfer systems and apparent difficulties in the isolation of auxotrophic mutants. The genetics of these rhizobia are further complicated by spontaneous independent rearrangements between the chromosome and plasmid DNA (2). We have been interested in characterizing a biotype of extra-slow-growing (ESG) strains (doubling times of $\geq 14$ h) of *Rhzobium japonicum* (9) from alkaline soils which show a distinct plasmid profile. They contain from two to four plasmids ranging from 74 to 200 kilobases (kb), and they have 140- and 182-kb plasmids in common (9). A correlation between one or more of these plasmids and the ability of the strains to survive in soils with high pH, high conductivity, high sodium content, and low levels of iron was suggested. However, under in vitro conditions, ESG strains neither tolerated high NaCl concentrations nor could they survive on media deleted specifically for iron by ethylenediamine dioxidohydroxyphenyl)leucine when compared with common *R. japonicum* strains (unpublished data). There also was no evidence of any correlation between the indigenous plasmids of the ESG strains and intrinsic antibiotic resistance, efficiency of nitrogen fixation, and sensitivity or resistance to 32 heavy metals (unpublished data). Curing of one or two plasmids from strains RJ19FY and RJ14C by elevated temperatures did not result in any phenotypic changes (unpublished data; S. G. Puenkpe, personal communications). Thus, other approaches were required to characterize the plasmids.

Transposons have been used to characterize the genomes of many bacteria. In several fast-growing rhizobia, nodulation and nitrogen fixation genes have been characterized by using transposon elements such as Tn5, which specifies resistance to kanamycin, neomycin, and streptomycin (8, 15), and Tn7, which specifies resistance to trimethoprim and streptomycin (3). Tn5 mutagenesis of four strains of *R. japonicum* was reported by Haugland (R. A. Haugland, 2nd International Symposium on the Molecular Genetics of the Bacteria-Plant Interaction, 1984, abstract no. 11) and was successfully used in the genetic characterization of the *nif* region of the slow-growing *R. japonicum* 311b110 by Hahn and Hennecke (10). However, Tn7 transfer either did not occur in this strain or transposition occurred at undetectable levels (3). Tn501 which specifies mercury resistance was inserted into the genome of *R. japonicum* 311b110 at a frequency of $1.9 \times 10^{-8}$ via plasmid pMD100 (G. S. Bullerjahn, R. H. Benzinger, and M. Davidson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, I130, p. 143). Tn501 has been shown to facilitate the integration of R91-5 into the *Pseudomonas putida* PPN chromosome and yield Hfr donors (6) and also enhanced the donor ability in *Bordetella pertussis* (21). In this study we show transposon mutagenesis of the genome of ESG strains of *R. japonicum* with Tn501 and Tn7 obtained by conjugal transfer of the chimeric plasmid pUW942 (21). The ability of Hg' transconjugants to transfer Hg' to different homologous (*R. japonicum*) and heterologous bacteria (*Escherichia coli*, *Pseudomonas syringae*, and *Agrobacterium spp.*) also was studied.

MATERIALS AND METHODS

**Bacterial strains.** *R. japonicum* 311b110 was obtained from D. Weber (U.S. Department of Agriculture, Beltsville, Md.). Strains RJ19FY, RJ17W, RJ12S, and RJ23A have been

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† Paper number 7828, Journal Series, Nebraska Agricultural Experiment Station.
previously described (9). *E. coli* UW937 containing pUW942 was provided by A. A. Weiss (Stanford University, Stanford, Calif.). *E. coli* J53(pJ1) and J53(R27) were provided by R. Olsen (University of Michigan, Ann Arbor). Plasmids RP1 and R27 were used as standards in molecular mass determinations of R, plasmids. *E. coli* C and *E. coli* AB2463 with pAS8Rep-1 were obtained from T. L. Thompson (University of Nebraska-Lincoln). The plasmids of these strains and pertinent markers are listed in Table 1.

**Media and culture conditions.** *E. coli* strains were grown on nutrient broth-yeast extract (NBY) agar (18) supplemented with one or more of the following: 50 μg of mercuric chloride per ml, 25 μg of kanamycin per ml, 50 μg of trimethoprim per ml, 50 μg of ampicillin per ml, and 10 μg of tetracycline per ml. Gentamicin (10 μg/ml), neomycin (75 μg/ml), kanamycin (75 μg/ml), and streptomycin (10 or 20 μg/ml) were used with L-arabinose agar (19). All the antibiotics were obtained from Sigma Chemical Co. (St. Louis, Mo.).

*R. japonicum* strains were maintained on L-arabinose agar slants at 25°C. For broth cultures, L-arabinose broth (10 ml in 250-ml flasks) was inoculated from slants and incubated at 25°C on a rotary shaker at 250 rpm. Mercury-resistant mutants of *R. japonicum* were maintained on L-arabinose plates with HgCl₂ (12 μg/ml). Mercury-containing plates were used within 1 week after preparation. The MIC of antibiotics was determined by the agar dilution method (20) with L-arabinose or NBY agar. Auxotrophy of *R. japonicum* mutants was tested on AIHM agar plates with 0.1% L-arabinose (4).

**Identification of *R. japonicum* strains.** In addition to distinct morphological properties, the identities of *R. japonicum* strains were confirmed by using indigenous resistance to gentamicin (10 μg/ml), kanamycin (20 μg/ml), and tetracycline (20 μg/ml) on L-arabinose agar.

**Conjugation experiments.** Bacterial matings to transfer Tn501 from *E. coli* UW937 and *E. coli* C with recombinant plasmids were performed by mixing 1 ml of donor cells (*E. coli* UW937) at approximately 10^8 CFU/ml from late log phase cultures and 4 ml of recipient cells (ESG *R. japonicum*) in late log phase at approximately 10^8 CFU/ml. The mating mixture was concentrated onto Millipore (0.45-μm-pore-size) filters. Filters were incubated on L-arabinose agar for 48 to 96 h. Mercury-resistant transconjugants were selected on L-arabinose with HgCl₂ (12 μg/ml) and gentamicin (10 μg/ml). Streptomycin-resistant transconjugants from a different cross were selected on L-arabinose agar with streptomycin (10 μg/ml) and gentamicin (10 μg/ml). In both cases the plates were incubated at 25°C for 30 days. Control plates with Hg, gentamicin-streptomycin, and gentamicin were maintained for both the parents. CFU of donors and recipients were counted by plating on NBY agar and L-arabinose with gentamicin (10 μg/ml) plates, respectively. The frequency of transconjugants was far less when recipient *R. japonicum* cells were used from early or mid-log phase in all the different crosses.

To transfer Tn501 from *R. japonicum* into *E. coli* C, a 20-μl mixture of donor (10^10 CFU/ml) and recipient (10^6 CFU/ml) cells, at a ratio of 1:4, was spot inoculated onto L-arabinose agar and incubated for 48 h at 25°C. The cell mixtures were suspended in 1 ml of phosphate buffer (pH 7.1), and 100 μl was plated directly on NBY with HgCl₂ (50 μg/ml). Plates were incubated at 37°C for 48 h.

**DNA isolation.** Total DNA from *R. japonicum* was isolated by the procedure of Hahn and Hennecke (10). Plasmid DNA from *E. coli* was isolated by the method of Kado and Liu (12). Plasmid DNA for restriction enzyme analysis and molecular mass determinations was isolated from *E. coli* by the alkaline lysis method of Ish-Horowicz and Burke (11). pAS8Rep-1 and pUW942 were isolated for use as labeled probes by the cleared lysate technique of Davis et al. (5) and purified by centrifugation with ethidium bromide-cesium chloride. Plasmids from *R. japonicum* were isolated by a modified procedure of Rosenberg et al. (14). Early log phase cells were taken from L-arabinose agar and washed twice with 0.1% Sarkosyl with TE buffer (0.05 M Tris, 0.02 M EDTA [pH 8])-3% NaCl and once with 0.1% Sarkosyl-TE buffer before the cells were suspended in 120 μl of lysozyme mixture (14).

The mixture was incubated at 37°C at 200 rpm. After 20 min, 300 μl of 1% Sarkosyl in Tris-borate buffer and 1.5 μl of 10 mg of pronase (self-digested at 37°C for 1 h) per ml were added and incubated at 37°C at 200 rpm for 30 min. Lysates were sheared at low speed by vortexing for 30 s.

**Gel electrophoresis.** Horizontal 0.7% agarose gels (14 by 11 cm) in Tris-borate buffer (89 mM Tris base, 2.5 mM disodium EDTA, 89 mM boric acid [pH 8.2]) were prepared, and 35 μl of samples was loaded into each well. Plasmids were separated by electrophoresis for 25 min at 50 V without submerging the gel. Buffer was then added to submerge the gel, and electrophoresis was continued for 50 V for 17 h at room temperature to separate *R. japonicum* plasmids. For restriction enzyme digestes, gels (14 by 11 cm) were run at 100 V for 3 h in Tris-borate buffer. *E. coli* plasmids were separated by electrophoresis at 75 V for 3 hr in E buffer (12) or in 0.5% agarose–Tris-borate buffer for molecular mass determinations. Gels were stained in 0.5 μg of ethidium bromide solution per ml for 30 min.

**DNA hybridization.** Plasmids and restriction enzyme digests were blotted onto nitrocellulose filters and hybridized by nick translation (Nicktranslotion Kit; Bethesda Research Laboratories, Gaithersburg, Md.) by the directions of the manufacturer. Southern hybridization (17) was conducted at 65°C, under stringent conditions, in the presence of 50% formamide.

**Nodulation tests.** Soybean (*Glycine max* Cv. *Amsoy 71*) seeds were surface sterilized (10) and germinated on water agar for 3 days in the dark. Seedlings were planted in 500-ml bottles with sterile vermiculite and moistened with 50 ml of nitrogen-free nutrient solution (19). Plants were inoculated with 6 ml of mid-log phase cells (10^8 CFU/ml) suspended in sterile phosphate buffer (pH 7.1). Sterile conditions were maintained for 10 days, until seedlings emerged from the containers. Plants were grown at 25°C for 5 weeks with a 16 h-8 h light-dark regime. Roots were thoroughly washed in sterile distilled water, and the nodules were surface sterilized (10). Bacteria were isolated on L-arabinose plates with or without gentamicin (10 μg/ml), and their identity was determined as described above.

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**TABLE 1. E. coli strains used in this study**

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>Plasmid</th>
<th>Molecular mass (kb)</th>
<th>Resistance markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>J53</td>
<td>R27</td>
<td>172</td>
<td>Tc'</td>
</tr>
<tr>
<td>J53</td>
<td>RP1</td>
<td>40</td>
<td>Ap' Km' Tm' Te'</td>
</tr>
<tr>
<td>UW937</td>
<td>pUW942</td>
<td>82</td>
<td>Ap' Km' Sm' Tp' Hg'</td>
</tr>
<tr>
<td>AB2463</td>
<td>pAS8Rep-1</td>
<td>74</td>
<td>Ap' Km' Sm' Tp'</td>
</tr>
<tr>
<td>C</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>pAK1</td>
<td>86</td>
<td>Ap' Km' Sm' Tp' Hg'</td>
</tr>
<tr>
<td>C</td>
<td>pAK2</td>
<td>84</td>
<td>Ap' Km' Sm' Tp' Hg'</td>
</tr>
<tr>
<td>C</td>
<td>pAK3</td>
<td>90</td>
<td>Ap' Km' Sm' Tp' Hg'</td>
</tr>
</tbody>
</table>

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**Vol. 51, 1986**

**R. JAPONICUM Tn501 AND Tn7 MUTAGENESIS AND R' PLASMIDS**

7
RESULTS

Transfer of pUW942 to *R. japonicum* ESG strains. Plasmid pUW942 contains Tn501 (Hg'), Tn7 (Tp' and Sm'), and Tn1 (Ap'). *R. japonicum* RJ23A, RJ19FY, RJ17W, and RJ12S acquired mercury resistance by mating with *E. coli* UW937 carrying pUW942. The frequency of Hg' transconjugants ranged from $8 \times 10^{-7}$ in strain RJ17W to $2 \times 10^{-9}$ in strain RJ12S per recipient (Table 2). RJ23A Hg' transconjugants were very unstable and lost Hg' in two or three subcultivations on the selective medium. Auxotrophs were detected on AIHM agar (4) and AIHM agar with calcium pantothenate in the case of RJ17W. A total of 2 of 37, 3 of 29, and 3 of 12 of RJ19FY, RJ17W, and RJ12S transconjugants, respectively, were auxotrophic. Auxotrophs were not further characterized. Some of the prototrophic revertants of RJ17W were not Hg', suggesting that such auxotrophy was due to 'Tn501 insertion.

The presence of pUW942 sequences in addition to Tn501 in the Hg' transconjugants was revealed by growing them on kanamycin-neomycin (75 μg/ml), to which wild-type *R. japonicum* was sensitive. In addition, hybridization of Southern blots with $^{32}$P-labeled pUW942 and pAS8Rep-1, which is pUW942 without Tn501, was used to test for the presence of pUW942 sequences in excess of Tn501. Figure 1 shows examples of transconjugants, the DNA of which hybridized with pUW942 but not with pAS8Rep-1 (data not shown), indicating that they have only Tn501. In three of these transconjugants, Tn501 was inserted into indigenous plasmids, and in the other three transconjugants it was inserted into chromosomal DNA. Vector plasmid pUW942 or pAS8Rep-1 did not hybridize with chromosome or indigenous plasmids of any of the wild-type *R. japonicum* strains.

Many transconjugants (9 of 47 of RJ19FY, 2 of 29 of RJ17W, and 11 of 14 of RJ12S) retained Kmr' and Nm'. Plasmid cointegrates in strain RJ12S (Fig. 2) were relatively more stable than in RJ17W and RJ19FY (11 of 11 of RJ12S derivatives retained Kmr' after they were passed through 10 subcultures on L-arabinose medium). Hybridization results (Fig. 2) show retention of pUW942 sequences in indigenous plasmids and chromosomal DNA. Up to 80% of individual colonies of the 11 RJ12S transconjugants retained their Kmr' marker after they were passed through 10 series of subcultures on L-arabinose agar.

The presence of Tn7, transposed from pUW942, in the transconjugants was determined by growing them on streptomycin (10 μg/ml) to which wild-type RJ19FY, RJ17W, and RJ12S were sensitive. All the wild-type strains were resistant to high levels of trimethoprim (50 μg/ml); hence, this marker could not be used. In all the *R. japonicum* Hg' transconjugants, the presence of Tn7 was comparatively less stable than that of Tn501. Several of the transconjugants

![FIG. 1. Southern analysis of Hg' transconjugants of different *R. japonicum* strains by hybridization with $^{32}$P-labeled pUW942 DNA. (A) Lanes 1 and 3, plasmid profiles of two Hg' transconjugants of *R. japonicum* RJ19FY (the 76-kb plasmid shows faintly); lanes 2 and 4, autoradiograph of DNA from lanes 1 and 3, respectively. Lane 2 shows hybridization of pUW942 DNA with the 182-kb plasmid and lane 4 shows the chromosome hybridized with pUW942. (B) Lanes 1 and 3, plasmid profiles of two Hg' transconjugants of *R. japonicum* RJ17W; lanes 2 and 4, autoradiographs of DNA from lanes 1 and 3, respectively. DNA in lane 2 shows the hybridization of the 182-kb plasmid to pUW942, and DNA in lane 4 shows the chromosome hybridized to pUW942. (C) Lanes 1 and 3, plasmid profiles of two transconjugants of *R. japonicum* RJ12S; lanes 2 and 4, autoradiographs of DNA from lanes 1 and 3, respectively. Lane 2 shows the 114-kb plasmid of RJ12S hybridized with pUW942, and lane 4 shows the chromosomal DNA hybridized with pUW942. Conditions for electrophoresis and Southern analysis are given in the text. The molecular mass (in kilobases) of the indigenous plasmids of *R. japonicum* strains is given on the left side of the figure; ch indicates chromosomal DNA.

![FIG. 2. Southern hybridization of DNA of RJ12S Hg' transconjugants with $^{32}$P-labeled pAS8Rep-1 DNA. Lanes A, C, E, G, and I show the plasmid profiles of five Hg' transconjugants of RJ12S. Lanes B, D, F, H, J are the respective autoradiographs of lanes A, C, E, G, and I. Lane B, a Hg' transconjugant with the 182-kb plasmid DNA and chromosomal DNA hybridized with pAS8Rep-1; lanes D, F, and H, a 114-kb plasmid from three different Hg' transconjugants hybridized with pAS8Rep-1; lane J hybridization of chromosomal DNA of an Hg' transconjugant with pAS8Rep-1. ch indicates chromosomal DNA. The molecular mass (in kilobases) of indigenous plasmids of RJ12S is given on the right.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Frequency of Hg' per recipient</th>
<th>Frequency of Sm' per recipient</th>
<th>Proportion of Km' Nm' colonies among Hg' transconjugants</th>
</tr>
</thead>
<tbody>
<tr>
<td>RJ19FY</td>
<td>$7.5 \times 10^{-8}$</td>
<td>$3.4 \times 10^{-8}$</td>
<td>9/47</td>
</tr>
<tr>
<td>FJ17W</td>
<td>$8.5 \times 10^{-9}$</td>
<td>$2.0 \times 10^{-9}$</td>
<td>2/29</td>
</tr>
<tr>
<td>RJ12S</td>
<td>$2.3 \times 10^{-9}$</td>
<td>$2.0 \times 10^{-9}$</td>
<td>11/14</td>
</tr>
<tr>
<td>RJ23A</td>
<td>$6.8 \times 10^{-8}$</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

* Reflects incorporation of transposon Tn501.
* Reflects incorporation of transposon Tn7.
* Presence of pUW942.
* RJ23A Hg' transconjugants were unstable and lost Hg' at high frequency.
with vector plasmid cointegrates lost Tn7 from their genome after a few transfers on nonselective medium (RJ19FY, 6 of 9; RJ17W, 8 of 9; RJ12S, 5 of 11).

Transposition of Tn7 occurred at a low frequency into all the strains of *R. japonicum* via pUW942. In strains RJ12S and RJ19FY, Tn7 was transferred at a frequency of $2 \times 10^{-9}$ and $3.4 \times 10^{-8}$ per recipient, respectively. In strain RJ23A, Tn7 transfer was undetectable (Table 2).

**Plant infection tests.** Three Hg\(^+\) transconjugants of each strain of *R. japonicum* with Tn501 on one of the resident plasmids and two transconjugants with chromosomal-pUW942 cointegrates were tested for nodulation ability on soybeans. Uninoculated plants did not nodulate. Nodulation by Hg\(^+\) transconjugants was almost equal to that by wild-type strains. None of the Hg\(^+\) transconjugants was nonnodulating. All the Hg\(^+\) transconjugants were reisolated from nodules and tested for their ability to grow on HgCl\(_2\)-containing medium. Transconjugants with Tn7 insertions on plasmids retained Hg\(^+\) in all cases. However, in RJ19FY and RJ17W, Hg\(^+\) transconjugants with pUW942-chromosomal cointegrates lost Hg\(^+\) in 2 to 8% of the colonies isolated from single nodules, suggesting the instability of cointegrates. Further subjection of the Hg\(^+\) transconjugants to 37°C for 5 days eliminated the resistance to 75 μg of kanamycin per ml in 92 to 96% of surviving colonies in all the three strains.

**Transfer of Hg\(^+\) from *R. japonicum* to recipients.** Transconjugants with Tn501 on plasmids or in the chromosome or with pUW942 in the chromosome could not transfer Hg\(^+\) to *Agrobacterium tumefaciens* G106, *A. rhizogenes* A1 or AB11; *P. syringae* 5D4132, *R. japonicum* RJ23A, or *R. japonicum* 31b110. None of the RJ17W Hg\(^+\) transconjugants with Tn501 or pUW942 could transfer Hg\(^+\) into a plasmidless *E. coli* C strain. However, RJ19FY Hg\(^+\) transconjugants with pUW942 insertions in the chromosome transferred Hg\(^+\) to *E. coli* C at a frequency ranging from $10^{-3}$ to $10^{-7}$ per recipient. None of the indigenous ESG *R. japonicum* plasmids were mobilizable into *E. coli* C along with Hg\(^+\). One of the Hg\(^+\) transconjugants of RJ19FY, RJ19FY29, transferred Hg\(^+\) into *E. coli* C at a frequency of $2.6 \times 10^{-7}$ along with the transfer of plasmids of 83, 84, and 86 kb (Fig. 3). Subsequently all these Hg\(^+\) transconjugants were found to be Ap\(^\prime\) Km\(^\prime\) Sm\(^\prime\) Tp\(^\prime\) and Hg\(^+\) (Table 3), indicating the transfer of pUW942.

RJ12S transconjugants with Tn501 on plasmids did not transfer Hg\(^+\) to *E. coli*. However, RJ12S68 and RJ12S79, with pUW942 in their chromosomes, transferred Hg\(^+\) along with a 90-kb plasmid at a frequency of $4.2 \times 10^{-5}$ and $1.5 \times 10^{-4}$, respectively. *E. coli* C Hg\(^+\) transconjugants with 90-kb plasmids acquired all the resistance markers of pUW942.

**EcoRI digests of R\(^+\) plasmids.** The 86-kb R\(^+\) plasmids transferred from RJ19FY29 to *E. coli* C did not hybridize with indigenous plasmids of RJ19FY (data not shown) but strongly hybridized to 16.8- and 4.9-kb fragments of EcoRI digests of total DNA of wild-type RJ19FY (Fig. 4). The 82-kb plasmid had the same restriction pattern as that of pUW942, did not hybridize to either plasmids or chromosomal DNA of wild-type RJ19FY, and appeared to be the same as the original pUW942. Similarly, an R\(^+\) plasmid transferred from RJ12S68 hybridized with a 10.8-kb fragment of an EcoRI digest of wild-type RJ12S total DNA (Fig. 4), as well as the vector plasmid.

**EcoRI digests of the 86- and 84-kb plasmids transferred from RJ19FY29 to *E. coli* C consisted of two additional fragments of 2 and 1.7 kb (pAK\(_1\)) or 2.1-kb (pAK\(_2\)), respectively, in addition to pUW942-specific fragments (Fig. 5). EcoRI digests of the 90-kb plasmid pAK\(_3\) transferred from RJ12S68 or RJ12S79 consisted of an additional 8.3-kb fragment (Fig. 5).

Transfer of Hg\(^+\) from R\(^+\) plasmids from *E. coli* C into the parent wild-type *R. japonicum* occurred at approximately a 10 times higher frequency when compared with that of pUW942 (Table 4). In addition, the transfer frequency of Hg\(^+\) into RJ12S was increased when pAK\(_1\) was the vector plasmid for Tn501.

**DISCUSSION**

The chimeric plasmid pUW942 with RP4 transfer genes and the ColE1 replication region was successfully used to introduce transposons Tn501 and Tn7 into ESG strains of *R. japonicum*. pUW942 was not maintained in an autonomously replicating state. Instead, all or part of it either integrated into the chromosome or plasmid(s) of *R. japonicum*. Integration of pUW942 into the chromosome or resident plasmids of *R. japonicum* may be the result of the ColE1 region, since R plasmids transferred by conjugation to *R. japonicum* do not usually integrate (2).

**TABLE 3. Transfer of Hg\(^+\) from *R. japonicum* containing chromosomally integrated pUW942 to *E. coli* C**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Frequency of Hg(^+) transfer per recipient</th>
<th>Plasmid transfer (kb)</th>
<th>Coinheritance of Ap(^\prime) Km(^\prime) Sm(^\prime) Tp(^\prime) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RJ19FY29</td>
<td>$2.6 \times 10^{-7}$</td>
<td>82, 84, or 86</td>
<td>99</td>
</tr>
<tr>
<td>RJ19FY41</td>
<td>$4.3 \times 10^{-6}$</td>
<td>None</td>
<td>92</td>
</tr>
<tr>
<td>RJ19FY53</td>
<td>$3.8 \times 10^{-6}$</td>
<td>None</td>
<td>78</td>
</tr>
<tr>
<td>RJ19FY54</td>
<td>$1.2 \times 10^{-6}$</td>
<td>None</td>
<td>67</td>
</tr>
<tr>
<td>RJ12S67</td>
<td>$3.7 \times 10^{-6}$</td>
<td>None</td>
<td>95</td>
</tr>
<tr>
<td>RJ12S68</td>
<td>$4.2 \times 10^{-5}$</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>RJ12S79</td>
<td>$1.5 \times 10^{-4}$</td>
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<td>100</td>
</tr>
<tr>
<td>RJ12S85</td>
<td>$5.9 \times 10^{-6}$</td>
<td>None</td>
<td>76</td>
</tr>
</tbody>
</table>

* E. coli C is F\(^\prime\) and lacks host restriction and modification activity (13).
Tn501 insertions were stable in RJ17W, RJ19FY, and RJ12S, whereas RJ23A exhibited high instability. Tn501 insertions into the chromosome of strain RJ17W resulted in auxotrophy; revertants became Hg sensitive. Tn501 insertions were stable, because Hg' was retained by the transconjugant strains after nodulation of soybeans or by maintaining them on nonselective medium for several generations.

Loss of Tn7 from some of the transconjugants that retained other resistance factors of the vector plasmid suggested its high instability. The low transfer frequency of Tn7 into ESG R. japonicum is in contrast to that which occurs in fast-growing Rhizobium meliloti (3). Only strains RJ19FY, RJ17W, and RJ12S acquired Tn7. The low frequency of transfer and inability of RJ23A to acquire Tn7 is not due to the lack of transfer of pUW942, since its Hg' (Tn501) transfer was relatively high. However, transposition of Tn7 is extremely site specific in E. coli (1), Caulobacter crescentus (7), and R. meliloti (3), so that a similar situation may occur in R. japonicum strains and account for the low number of Tn7 transpositions.

R. japonicum Hg' transconjugants of RJ19FY, RJ17W, and RJ12S with Tn501 on plasmids did not transfer Hg' to E. coli C, P. syringae 5D4132, or Agrobacterium spp. or to the same serogroup strain RJ23A. Excision of pUW942, along with chromosome fragments of one of the RJ19FY and two of the RJ12S Hg' transconjugants, resulted in the formation of R' plasmids that could be transferred to and stably maintained in E. coli C. Such plasmids, comprised of pUW942 and chromosome fragments of RJ19FY or RJ12S, were unstable in P. syringae recipients, presumably because of the inability of the ColEl replication region to maintain itself in a nonintegrated state in Pseudomonas spp. (16). The majority of Hg' transconjugants of RJ19FY and RJ12S transferred Hg' along with the other pUW942 markers at a frequency ranging from 10⁻⁵ to 10⁻⁶ to E. coli C, but this was not accompanied by any plasmid transfer. Hg' E. coli C also retained other plasmid pUW942 markers in both cases in which Hg' was transferred from R. japonicum transconju-

![FIG. 4. Southern hybridization of EcoRI digests of total DNA of R. japonicum with 32P-labeled R' plasmids. Lanes A and C, EcoRI digests of total DNA of wild-type RJ19FY and RJ12S, respectively; lane b, hybridization of 32P-labeled pAK1 to 16.8- and 4.9-kb DNA fragments of RJ19FY; lane d, hybridization of 32P-labeled pAK1 to a 10.8-kb DNA fragment of RJ12S. The molecular mass (in kilobases) of fragments of λ DNA digested with HindIII is shown on the left.](image)

![FIG. 5. EcoRI digests of pUW942 and R' plasmids from E. coli C Hg' transconjugants. Lane a, digest of plasmid pAK1; lane b, digest of plasmid pAK2; lane c, digest of plasmid pUW942; lane d, digest of plasmid pAK3. The molecular mass (in kilobases) of fragments of λ DNA digested with HindIII is shown on the left.](image)

### TABLE 4. Transfer of Hg' from E. coli C with pUW942 and R' plasmids to R. japonicum strains

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Frequency of transfer per recipient</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli C(pUW942)</td>
<td>RJ19FY</td>
<td>4.5 × 10⁻⁸</td>
</tr>
<tr>
<td></td>
<td>RJ17W</td>
<td>4.5 × 10⁻⁸</td>
</tr>
<tr>
<td></td>
<td>RJ12S</td>
<td>1.9 × 10⁻⁹</td>
</tr>
<tr>
<td>E. coli C(pAK1)</td>
<td>RJ19FY</td>
<td>5.4 × 10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>RJ17W</td>
<td>7.3 × 10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>RJ12S</td>
<td>1.5 × 10⁻⁸</td>
</tr>
<tr>
<td>E. coli C(pAK3)</td>
<td>RJ19FY</td>
<td>7.3 × 10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>RJ17W</td>
<td>6.5 × 10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>RJ12S</td>
<td>3.8 × 10⁻⁹</td>
</tr>
</tbody>
</table>

* 82-kb plasmid transferred from RJ19FY29; same as pUW942.
* 86-kb plasmid transferred from RJ19FY29.
* 90-kb plasmid transferred from RJ12S68.
gants with pUW942 integrated into the chromosome. Formation of R' plasmids, resulting from the excision of integrated pUW942, could be detected physically only in E. coli C. Integration may have occurred at specific sites in the R. japonicum chromosome, but we could not detect any chromosomal markers of these R' plasmids because of the inability to obtain suitable auxotrophic mutants of R. japonicum. Nor were intergeneric crosses with a few E. coli auxotrophs successful in complementation. A search to detect chromosome mobilization by integrated pUW942 in the case of strains RJ19FY and RJ12S Hg' transconjugants between genetically marked sublines of Sm' and rifampcin-resistant donors and erythromycin-resistant recipients was unsuccessful. Transfer frequencies were too low to detect any enhancement in donor ability of the Hg' transconjugants.

Where R' plasmids were used to transfer Hg' from E. coli C into R. japonicum, there were noticeable increases in transfer efficiency not only to the R. japonicum strain from which the R' plasmid was derived but also to other strains, thereby suggesting some genetic homology among these strains. The results reported here show the genetic manipulability of very slow growing R. japonicum strains, as well as the complexity of transposon mutagenesis.

ACKNOWLEDGMENTS

We thank Lois Girton and William Haskins for assistance with DNA hybridization experiments, A. Summers for advice, and D. Kuczmaszki for photography. This work was supported by grant 59-2311-0-1-528-0 from the U.S. Department of Agriculture Science and Education Administration and the Nebraska Soybean Development and Utilization Marketing Board.

LITERATURE CITED


