JOURNAL OF BACTERIOLOGY, Apr. 1982, p. 395–397
0021-9193/82/040395-03$02.00/0

Vol. 150, No. 1

Phenotypic Expression of Mutations in a Wide-Host-Range R Plasmid in *Escherichia coli* and *Rhizobium meliloti*

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Eight different derivatives of R plasmid RP1 with thermosensitive mutations affecting maintenance in *Escherichia coli* and *Pseudomonas aeruginosa* were introduced into *Rhizobium meliloti*. None of the plasmids showed a thermosensitive character in *R. meliloti*. On the other hand, a certain deletion mutation in RP1 was found to cause plasmid instability in rhizobia and agrobacteria, but not in *E. coli*.

R plasmids of incompatibility class P-1 have a wide host range among gram-negative bacteria (6). Plasmids of this Inc group have been used to bring about the exchange of genetic material, of chromosomal [reviewed recently by Holloway (8)] or plasmid origin (e.g., reference 9) or both, between different strains in such diverse organisms as *Acinetobacter calcoaceticus*, *Agrobacterium tumefaciens*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Rhizobium leguminosarum*, and *Rhodopseudomonas capsulata*. Variants of these R plasmids that are thermosensitive for replication would be very useful, e.g. for the isolation of transposon-insertion mutants, for site-specific mutagenesis, and for the isolation of Hfr strains derived from other organisms than *E. coli*. A number of groups (4, 7, 11–13) recently described the isolation of mutants of R plasmid RP1 that are thermosensitive in *E. coli* and *P. aeruginosa*. For some of the thermosensitive R plasmids it has been shown that they have a lethal effect on their bacterial host, if this is being incubated at a high temperature (5, 7). Under these circumstances bacterial cell division is blocked, resulting in the formation of filaments (7; this paper). In the present paper we describe the behavior of thermosensitive R plasmid mutants in the bacterium *Rhizobium meliloti*, which fixes nitrogen in symbiotic association with alfalfa.

To be able to test the behavior of the RP1 derivatives (Table 1), these were transferred from *E. coli* donor strains to rifampin-resistant *R. meliloti* strains LPR2120 and LPR2136 by conjugation as described previously (9). Transfer occurred at a frequency of about 10^{-2} per recipient, which is a value comparable with that of wild-type RP1 transfer. From each cross transconjugants were purified on YMB medium (10) into which rifampin and tetracycline had been incorporated at 20 and 2 mg/liter, respectively. Thereafter, the transconjugants were checked for kanamycin resistance (250 mg/liter) and for sensitivity to the *R. meliloti*-specific bacteriophages LPB64 and LPB70, as described before (10). They were then tested for growth and R plasmid loss at 29 and 40°C. The *E. coli* donor strains were treated in a similar way as a control.

All *R. meliloti* R^+ strains [including *R. meliloti*(pRL186)] grew at 29°C as well as at 40°C. In contrast, cultures of *E. coli*(pRL186) did not show any growth at the higher temperature. An explanation for this may be that *E. coli*(pRL186) cells after incubation at 40°C form long filaments, whereas the *R. meliloti*(pRL186) cells do not do so. As can be seen from Table 2, less than 0.005% of the *E. coli* cells, with pME301, pMR5, pTH10, or pTI2, remained R^+ after growth at 40°C, whereas no R plasmid loss was observed from *R. meliloti* cells with the same plasmids after growth at 40°C. In *E. coli* as well as in *R. meliloti* these latter four plasmids were stable at 29°C.

Plasmid pRL180 (P. J. J. Hooykaas, Thesis, University of Leiden, Leiden, The Netherlands, 1979) is a derivative of RP1::*Tn*904 (RP1::*Tn*904 was supplied by R. Olsen, Ann Arbor, Mich.) with a deletion in the kanamycin resistance (Km^r) region, and confers resistance to carbenicillin (Cb), streptomycin (Sm), and tetracycline (Tc). After NTG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) mutagenesis of *E. coli*(pRL180), two Tc^s derivatives (pRL182 and pRL183) were obtained by replica plating. The Tc^s locus of plasmid pRL183 was found to revert to Tc^r after a second treatment with NTG. Therefore, *E. coli*(pRL183) was used for localized mutagenesis with NTG. Among 50 Tc^r revertants 2 were found to carry plasmids (pRL187 and pRL188) that were more unstable than the parental plasmid at 40°C (Table 2), and one (pRL186) was found to cause inhibition of host growth at 40°C. However, in *R. meliloti* none of plasmids

TABLE 1. Plasmids and their origin

Plasmid	Markers[a]	Thermosensitive in[b]:		Origin
		E. coli	*P. aeruginosa*	
pME301	Cb Km Tc IncP-1	+	+	J. Watson and D. Haas (Zurich)
pMR5	Cb Km Tc IncP-1	+	+	M. Robinson (Bristol) (12)
pRL180[c]	Cb Sm Tc IncP-1	−	?	Our isolate
pTH10	Cb Km Tc IncP-1	+	?	S. Harayama (Tokyo) (7)
pTI2	Cb Cm Km Tc IncP-1	+	?	T. S. Ilyina (Moscow) (11)

[a] Cb, Carbenicillin; Km, kanamycin; Tc, tetracycline; IncP-1, incompatibility class P-1.
[b] Data provided by the people who kindly have sent us the plasmid-carrying strains.
[c] pRL186, pRL187, and pRL188 have the same markers as pRL180 (this paper).

pRL186, pRL187, or pRL188 either showed a thermosensitive behavior or caused an inhibition of the growth of the host at 40°C. Unexpectedly, however, plasmid pRL180 as well as pRL186 through pRL188 were found to be somewhat unstable in *R. meliloti* during incubation at 29°C as well as at 40°C. The instability of these plasmids was also found in other hosts of the *Rhizobiaceae* family, namely, *Agrobacterium tumefaciens*, *A. rhizogenes*, *Rhizobium trifolii*, and *R. leguminosarum*.

To exclude the possibility that the R plasmids in the purified *R. meliloti* transconjugants were thermoresistant revertants, all of them were reintroduced into *E. coli* KMBL1164 (K-12 *pro*$^-$ *thi*$^-$) from the *R. meliloti* R$^+$ donors by conjugation. As expected, the newly constructed *E. coli* R$^+$ strains showed the same behavior as the original *E. coli* R$^+$ strains.

None of the thermosensitive R plasmids tested in our studies showed a thermosensitive behavior in *R. meliloti*. The mechanism underlying the thermosensitive behavior in *E. coli* and *P. aeruginosa* is largely unknown. Others (5, 7) and we (this paper, regarding pRL186) have shown that the presence of thermosensitive plasmids may cause an inhibition of normal cell division during incubation at a high temperature. It was recently found that the responsible plasmid mutation (for plasmid pEG1) is located in a region of the plasmid not involved in plasmid replication (3). Our finding that the R plasmids are stable in *R. meliloti* suggests that the plasmid locus involved in thermosensitivity in *E. coli* and *P. aeruginosa* encodes a function that either is not expressed in *R. meliloti* or does not affect cell division in this organism.

The unstable character of plasmid pRL180 and its derivatives in *R. meliloti* was in contrast with its stable character in *E. coli*. Recently, an R plasmid (pRP301) was reported to be stable in *E. coli*, but unstable in *Methylophilus methylotrophus* (14). Plasmids pRL180 (Hooykaas, thesis) and pRP301 (1) are both derivatives of RP1 lacking the Km^r determinant by deletion. Together, these findings suggest the possibility that a region near or overlapping with the Km^r locus is essential for R plasmid stability in bacteria that are distant from *E. coli*.

Our data clearly show that certain mutations in wide-host-range R plasmids are phenotypically not expressed in organisms distant from the bacterium in which the mutations were first discovered. It should be realized that this strongly limits the usefulness of such wide-host-range R plasmid mutants.

TABLE 2. Stability of R plasmid mutants in *R. meliloti* and *E. coli*[a]

Plasmid	% R$^+$ cells after growth at:			
	E. coli		*R. meliloti*	
	29°C	40°C	29°C	40°C
RP4	100	100	100	100
pME301, pMR5, pTH10, pTI2	100	<0.005	100	100
pRL180	100	100	15–99	15–99
pRL186	100	—[b]	15–99	15–99
pRL187, pRL188	100	22–47	15–99	15–99

[a] Cells with R plasmids were grown for 20 generations on nonselective medium TY (2) and then plated to selective plates (TY + Tc) and nonselective plates (TY). Colonies from the TY plates were replica-plated to TY + Tc. The numbers in the table denote the % R$^+$ cells after the treatment.
[b] For pRL186 no value is known, because cultures of *E. coli*(pRL186) do not grow at 40°C.

We thank S. Harayama (Tokyo), T. S. Ilyina (Moscow), M. K. Robinson (Bristol, U.K.), and J. Watson and D. Haas (Zurich) for kindly providing *E. coli* strains with thermosensitive R plasmids. We also thank J. Hille for critically reading the manuscript.

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