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Behavior of *Inc-Q* Plasmids in *Agrobacterium tumefaciens*

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Inc-Q plasmids were introduced into *Agrobacterium tumefaciens*, by mobilization from *Escherichia coli* with an *Inc-P* plasmid, or by transformation with purified plasmid DNA. It was found that they were stably maintained. The presence of an *Inc-Q* plasmid did not influence tumorigenicity. These results suggest that these plasmids may be used in genetic complementation studies of Ti plasmid mutants in *A. tumefaciens*.

The Ti plasmid of *Agrobacterium tumefaciens* is essential for crown gall tumor formation (1-3). Part of the Ti plasmid, the T-DNA region, is transferred to the plant cell during tumor induction (4,5). Another segment of the Ti plasmid, distinct from the T-DNA region, was also found to be essential for tumorigenicity (6,7). This segment, the so-called Vir-region, was shown to be complementable *in trans* by R prime plasmids (8; J. Hille, I. Klasen, and R. Schilperoort, submitted for publication).

Detailed complementation studies of such Ti plasmid mutants might give information on the mechanism of tumor induction by the bacterium. To this end, we sought possible cloning vehicles that: (i) can replicate in *A. tumefaciens*, (ii) do not themselves influence tumorigenicity, (iii) have a suitable size and can be used for cloning with insertional inactivation, and (iv) most preferably can also replicate in *Escherichia coli*. A vector that fits these four conditions can be used in *E. coli* for cloning and can then subsequently be introduced in *A. tumefaciens* for complementation studies.

The wide host range *Inc-Q* plasmid pKT214, derived from RSF1010 and encoding resistance against tetracycline, streptomycin, and chloramphenicol (9), was chosen as a possible candidate, since it agrees well with points (iii) and (iv). It has a molecular weight of 10.5 Mdalton and contains unique

sites for *Bgl*II and *Xba*I in the tetracycline resistance locus. This plasmid was transferred from *E. coli* to *A. tumefaciens* by mobilization with the *Inc-P* plasmid R772 (encoding kanamycin resistance). Transconjugants were obtained which carried R772 and pKT214, as well as transconjugants which carried pKT214 only (transfer of pKT214: 10^{-3} per recipient). With the same frequency pKT214 was mobilized within *A. tumefaciens* C58 strains, and from *A. tumefaciens* to *E. coli*.

Purified pKT214 DNA, isolated from *E. coli*, was used to transform *A. tumefaciens* strain C58 by a freeze/thaw method (10). About 2000 $Tc^rSm^rCm^r$ transformants were obtained with 1 μ g plasmid DNA and 10^9 bacteria. The transformation frequency was not influenced when the same plasmid, isolated from *Agrobacterium*, was used. This value is comparable to the frequency of transformation of small Ti plasmid derivatives (11), which indicates that pKT214 is not sensitive to any restriction/modification system of *A. tumefaciens* strain C58.

In order to obtain an extra selectable marker on pKT214, this plasmid was made linear with *Bam*H1. A small Ti plasmid derivative, pAL2832 (11), was also digested with *Bam*H1, which gives fragments of 0.9 and 1.6 Mdalton. The 0.9-Mdalton fragment carries the *Cb* resistance marker of transposon Tn1. These fragments were mixed,

FIG. 1. Map of the *Inc-Q* plasmid pRL220. In the circle *Pst*I sites are shown; the dark part represents the largest *Pst*I fragment of RSF1010, from which this plasmid is derived. Abbreviations: Cb, resistance to carbenicillin; Cm, resistance to chloramphenicol; Sm, resistance to streptomycin; Tc, resistance to tetracycline.

ligated, and used to transform *A. tumefaciens* to Cb resistance. One out of forty transformants turned out to carry pKT214-Cb, the plasmid of which was named pRL220 (see Fig. 1).

To test for possible influence of an *Inc-Q* plasmid on the tumorigenicity of *Agrobacterium*, several different strains were transformed with pRL220 and thereafter tested for tumor-inducing capacity on two different plants (see Table 1). No influence on on-

cogenicity was observed. This is in contrast to *Inc-W* plasmids. It was reported that, in their presence, *Agrobacterium* strains failed to induce tumors (12).

We have shown that *Inc-Q* plasmids do conform to the conditions stated. They replicate in *E. coli* as well as in *A. tumefaciens*, they can be transferred reciprocally between these species by mobilization and transformation, and they do not influence the tumor-inducing capacity of *A. tumefaciens*. Furthermore, cloning with the use of insertional inactivation is possible in the unique sites for *Bgl*II and *Xba*I in the Tc resistance locus of pKT214 and pRL220.

These plasmids can be utilized for site-specific mutagenesis of Ti plasmids in the manner described by Ruvkun and Ausubel (13). Ti plasmid fragments, cloned on pKT214, can, e.g., be transposon mutagenized in *E. coli*. Mutagenized recombinant plasmids can be introduced into an *Agrobacterium* strain, carrying a Ti plasmid. Upon introduction of pRL220, the described recombinant plasmid is lost because of incompatibility. With a certain frequency the transposon is rescued by homologous recombination with the corresponding region in the Ti plasmid.

The unique *Bgl*II site of pRL220 can be applied for cloning the *cos* site of phage λ , which is conveniently located between *Bgl*II sites on the plasmid pHCT9 (14). This per-

TABLE 1
TUMOR-INDUCING CAPACITY OF STRAINS CARRYING pRL220

Strain	Plasmid(s)	Tested on tomato		Tested on sunflower	
		Without pRL220	In the presence of pRL220 ^a	Without pRL220	In the presence of pRL220 ^a
LBA288	Cryptic ^b	— ^c	—	—	—
LBA677	Cryptic, pTi	+	+	+	+
LBA937	Cryptic, pTi, R772	+	+	+	+
LBA973	Cryptic, pAL969 ^d	+	+	+	+
LBA958	Cryptic, pTi-C58	+	+	+	+

^a Plasmid pRL220 was introduced into these strains by transformation.

^b The cryptic plasmid in *A. tumefaciens* is described by Casse *et al.* (1979).

^c (—) No tumor induction; (+) tumor induction.

^d Plasmid pAL969 is an R772::Ti cointegrate plasmid.

mits the use of this plasmid for cosmid cloning in *E. coli*. Fragments of about 15 Mdalt can be cloned on such a plasmid. The possibility to do so will contribute to complementation studies with Ti plasmid mutants.

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