JOURNAL OF BACTERIOLOGY, Mar. 1983, p. 1535–1542
0021-9193/83/031535-08$02.00/0

Vol. 153, No. 3

Comparison of Ti Plasmids from Three Different Biotypes of *Agrobacterium tumefaciens* Isolated from Grapevines

VIC C. KNAUF,[1] CHRISTOS G. PANAGOPOULOS,[2] AND EUGENE W. NESTER[1]*

Department of Microbiology and Immunology SC-42, University of Washington, Seattle, Washington 98195[1] and Benaki Phytopathologic Institute, Kiphissia, Athens, Greece[2]

Received 15 July 1982/Accepted 28 December 1982

Twenty-six plasmids from grapevine isolates of *Agrobacterium tumefaciens* were analyzed by *Sma*I fingerprinting and by hybridization of nick-translated DNA to DNA of another plasmid. These experiments established that octopine Ti plasmids are not highly conserved, although octopine Ti plasmids from biotype 1 *A. tumefaciens* strains appeared to be very similar. Octopine Ti plasmids from biotype 3 strains are more variable in terms of host range and *Sma*I fingerprints, but share extensive DNA homology. Fingerprints of nopaline Ti plasmids from strains of a given biotype resemble each other but not fingerprints of Ti plasmids from strains of the other two biotypes. The wide host range octopine Ti plasmid from the biotype 3 strain Ag86 shares more DNA homology with narrow host range Ti plasmids, nopaline Ti plasmids, and octopine catabolism plasmids than with the wide host range octopine Ti plasmid from biotype 1 strain 20/1. pTiAg86 does share homology with the portion of pTi20/1 integrated and expressed in plant tumor cells. Since all wide host range Ti plasmids studied contain these sequences, we suggest that natural selection for a wide host range resulted in the presence of the common sequences in distantly related plasmids. The lack of homology between this "common DNA" and limited host range Ti plasmids shows that the DNA sequences per se are not required for tumorigenesis.

Crown galls are tumorous growths of plant tissue induced by *Agrobacterium tumefaciens* strains containing a Ti plasmid (31, 35) of about 200 kilobases. In all cases so far examined (2, 22, 28, 29, 34), plant tumor cells contain a specific region (10 to 20 kb) of the bacterial Ti plasmid integrated within the nuclear DNA. This piece of DNA (T-DNA) is responsible for tumorous phenotypes (10, 11) and also for the synthesis of tumor-specific compounds termed opines.

The opine concept (12; see reference 22 for review) suggests that the Ti plasmid includes (i) genes that will function in the eucaryotic milieu of a tumor to allow synthesis of a specific set of unusual compounds (opines) and (ii) genes that are expressed in *Agrobacterium* sp. that allow the pathogen to catabolize that particular set of opines. This has led to the classification of Ti plasmids as octopine, nopaline, or agropine types (12, 25). For example, an *A. tumefaciens* strain with an octopine Ti plasmid will induce plant tumors that synthesize octopine. That octopine Ti plasmid also codes for the ability of the strain to utilize octopine as a sole carbon and nitrogen source.

Analysis of *Sma*I fingerprints of Ti plasmids (25) indicated that octopine Ti plasmids were highly conserved and formed a homogeneous group of plasmids, whereas nopaline-type Ti plasmids had more variable *Sma*I patterns. Thomashow et al. (30) subsequently described four similar octopine Ti plasmids from biotype 3 *A. tumefaciens* strains, all of which exhibited an unusually narrow host range on plants. They appeared to be only distantly related to the wide host range octopine Ti plasmids previously studied (27).

Since Ti plasmids from other biotype 3 *A. tumefaciens* strains had not been analyzed, it was not clear whether these limited host range Ti plasmids formed a distinct group of Ti plasmids, whether octopine Ti plasmids were in fact a heterogeneous group, whether biotype 3 *A. tumefaciens* strains harbor unique Ti plasmids, or a combination of these possibilities.

In this report, we describe the plasmids of 26 strains of *A. tumefaciens* isolated from grapevines. These strains include members of each of the three biotypes of *Agrobacterium* (17), strains with narrow host range (20, 30), strains with wide host ranges (20), and strains containing plasmids that code for octopine catabolism but not virulence.

MATERIALS AND METHODS

Strains. The first column of Table 1 lists grapevine isolates of *A. tumefaciens*. The bases for host range and biotype assignments are described elsewhere (20). All strains were maintained on nutrient agar.

Strain A136 (25) is a biotype 1 of *Agrobacterium* that utilizes neither octopine, nopaline, nor arginine as a sole carbon source (8). Each transformant strain listed in Table 1 represents strain A136 transformed with plasmid DNA (13) isolated from a grapevine isolate of *A. tumefaciens*. Transformants were selected by their ability to form orange colonies on BTB medium (16) supplemented with 100 μg of octopine (Sigma Chemical Co., St. Louis, Mo.) or 100 μg of octopine with 10 μg of nopaline (gift of R. Jensen) per ml of medium. Arginine utilization medium consisted of 90 mg of K_2HPO_4, 150 mg of NaCl, 500 mg of $MgSO_4 \cdot 7H_2O$, 100 mg of $CaCl_2 \cdot 6H_2O$, and 500 mg of arginine per liter, adjusted to pH 7.5 with NaOH and made 1.6% agar by weight. As indicated below, this medium sometimes contained, in addition, 10 μg of either octopine or nopaline per ml.

DNA analysis. Plasmid DNA was isolated from *A. tumefaciens* by the method of Currier and Nester (4); the plasmid content of *A. tumefaciens* strains was screened by the technique of White and Nester (32). *Sma*I was obtained from Boehringer Mannheim Corp., Indianapolis, Ind., and *Bst*I was the kind gift of Richard Meagher. Nick-translation of ^{32}P-labeled DNA, Southern blotting protocols, and the recombinant plasmid pNW-31C-8,29-1 were described by Thomashow et al. (28).

RESULTS

Transfer of Ti plasmids to a common strain of *A. tumefaciens*. The cryptic plasmids found in many strains complicate analysis of coresident Ti plasmids. Therefore, we introduced each Ti plasmid into the avirulent strain A136 (25). Although strain A136 and derivatives of strain A136 described here contain a 430-kilobase plasmid, pAtC58, the size of this cryptic plasmid mitigates against its isolation by the procedure employed. Previous work (24, 30) and these data suggest that pAtC58 sequences do not contribute to restriction enzyme patterns or affect the outcome of Southern blotting experiments. The plasmid screening technique (32) detects pAtC58.

Since in planta crosses (18) are time consuming and mobilization of Ti plasmids by RP4 may result in transposition events (15), we introduced Ti plasmids into strain A136 by transformation. Transformants were initially selected by their ability to form bright yellow colonies on BTB medium (16) containing 10 μg of nopaline and 100 μg of octopine per ml.

Each transformant was compared to the wild-type isolate from which the transforming DNA had been isolated. Plasmid screens showed that each transformant contained pAtC58 and also one plasmid with a mobility similar to one of the

TABLE 1. Grapevine isolates of *A. tumefaciens*

Isolate	Biotype	Origin	Transformant	Plasmid	Opine type	Host range of transformant
NCPPB1001	1	Romania	A503	pTiNCPPB1001	Octopine	Wide
20/1	1	Hungary	A871	pTi20/1	Octopine	Wide
S-8	1	Hungary	A870	pTiS-8	Octopine	Wide
Ag19	1	Greece	A890	pAtAg19	Octopine	Avirulent
Ag34	1	Greece	A868	pAtAg34	Octopine	Avirulent
Ag125	1	Greece	A842	pAtAg125	Octopine	Avirulent
ATV	1	Spain	A877	pTiATV	Nopaline	Wide
ATB	1	Spain	A878	pTiATB	Nopaline	Wide
1 D 1109	1	California		pAt1 D 1109		
19/5	2	Hungary	A872	pTi19/5	Nopaline	Wide
M-A5	2	South Africa	A873	pTiM-A5	Nopaline	Wide
PPI-1	2	Bulgaria	A880	pTiPPI-1	Nopaline	Wide
PPI-6	2	Bulgaria	A881	pTiPPI-6	Nopaline	Wide
CG8	3	New York	A882	pTiCG8	Nopaline	Wide
CG54	3	New York	A884	pTiCG54	Nopaline	Wide
2/6	3	Hungary	A851	pTi2/6	Nopaline	Wide
15/5	3	Hungary	A852	pTi15/5	Nopaline	Wide
Ag57	3	Greece	A853	pTiAg57	Octopine	Narrow
Ag63	3	Greece	A854	pTiAg63	Octopine	Narrow
Ag83	3	Yugoslavia	A857	pTiAg83	Octopine	Wide
Ag86	3	Yugloslavia	A858	pTiAg86	Octopine	Wide
Ag105	3	Greece	A859	pTiAg105	Octopine	Narrow
Ag123	3	Greece	A862	pTiAg123	Octopine	Narrow
Ag158	3	U.S.S.R.	A855	pTiAg158	Octopine	Narrow
Ag162	3	U.S.S.R.	A856	pTiAg162	Octopine	Narrow
K305	3	Australia	A867	pTiK305	Octopine	Wide

plasmids of the wild-type isolate. Plasmid DNA from the transformant was isolated and restricted by *Sma*I or *Bst*I. The digested sample was separated by gel electrophoresis, and the resulting pattern (fingerprint) was compared with that of a similar digest of plasmid DNA from the natural isolate. In each case, the pattern of transformant plasmid DNA either matched or was a subset of the plasmid DNA pattern for the wild-type strain. Each transformant was tested for virulence on a variety of plant hosts to ascertain whether the transferred plasmid conferred virulence. These host range data are described elsewhere (20).

Transformants A842, A868, and A890 (containing plasmids from strains Ag125, Ag34, and Ag19, respectively) catabolized octopine but did not induce crown galls on any plant host tested. All other transformants were virulent on *Nicotiana glauca* and other hosts (20), indicating that the Ti plasmid had been transferred.

Identification of octopine- and nopaline-type plasmids. Strain A136, like strain NTI (8), will not grow if octopine, nopaline, or arginine is present as the sole carbon source, the sole nitrogen source, or both in the case of octopine and nopaline. If an A136 derivative containing an octopine Ti plasmid is plated on minimal medium containing arginine as sole carbon source, the strain will not grow unless (i) octopine is present at inducing levels (10 μg/ml or greater) or (ii) the Ti plasmid operon governing octopine and arginine catabolism is expressed constitutively. The situation is slightly different for an A136 strain with a nopaline Ti plasmid in that inducing levels of nopaline allow growth on nopaline, arginine, or octopine.

No nopaline Ti plasmids were found that expressed the nopaline catabolism operon constitutively, i.e., no transformants of strain A136 that catabolized nopaline could grow on minimal medium with arginine unless nopaline was also present at 10 μg/ml. One transformant, A503, could grow on arginine as the sole carbon and nitrogen source. This strain contains pTiNCPPB1001, a Ti plasmid previously identified as an octopine Ti plasmid (25). All other plasmids introduced into strain A136 could be identified as to whether they were of the octopine or nopaline type by the requirement of the transformant for low levels of octopine or low levels of nopaline to grow on arginine.

Nopaline Ti plasmids. Gel electrophoresis of Ti plasmid DNA after restriction with the enzyme *Sma*I often gives fingerprints characteristic of certain Ti plasmid classes (25). We compared the *Sma*I fingerprints of the 25 plasmids transferred to strain A136. Examples of these fingerprint patterns are shown in Fig. 1.

The nopaline Ti plasmids from ten grapevine isolates could be divided into three groups by their *Sma*I fingerprints. The Ti plasmids from biotype 1 isolates ATV and ATB have identical *Sma*I fingerprints. The Ti plasmids from biotype 2 strains PPI-1 (Bulgaria), PPI-6 (Bulgaria), and M-A5 (South Africa) have identical *Sma*I fingerprints which are almost identical to the *Sma*I fingerprint of the biotype 2 isolate 19/5 (Hungary). The Ti plasmids from biotype 3 isolates (two from Hungary, two from the United States) had *Sma*I fingerprints resembling each other but not resembling *Sam*I fingerprints of other Ti plasmids. Unlike other nopaline Ti plasmids (9, 18), the nopaline Ti plasmids from biotype 3 strains do not code for sensitivity to Agrocin 84 (data not shown).

When Ti plasmid DNA from biotype 3 strain 2/6 was nick-translated and used as a hybridization probe against blotted Ti plasmid DNA, extensive DNA homology was detected for pTi2/6 with all 10 nopaline Ti plasmids (Fig. 2). Under the conditions employed (27), the stringency of the hybridization protocol corresponded to melting temperature (T_m) − 17°C. These results indicate that all of these nopaline Ti plasmids are closely related.

Octopine plasmids. Although Sciaky et al. (25) looked at the *Sma*I fingerprints of octopine Ti plasmids from nine strains, these Ti plasmids could be traced back to five natural isolates. All of the isolates were biotype 1, and one of them, NCPPB1001, was isolated from grapevine. We have studied two other biotype 1 strains from grapevine with octopine Ti plasmids. The plasmids pTi20/1 and pTiS-8 give *Sma*I fingerprints identical to the pTiB6 pattern described by Sciaky et al. (25) and therefore, like pTiNCPPB1001, belong to the same octopine Ti plasmid class.

Biotype 1 strains Ag19 (25), Ag34, and Ag125 also contain large plasmids coding for octopine catabolism. However, these plasmids do not code for virulence on any host we have tested, including grapevines. Each of the three *Sma*I fingerprints of these plasmids was different (Fig. 1), indicating a diverse family of plasmids exists. These plasmids do not appear to be virulence plasmids; therefore, all of the octopine Ti plasmids found in biotype 1 isolates so far all belong to the group typified by pTiB6 (25).

No biotype 2 strains in this study contained an octopine Ti plasmid, and we do not know of any natural biotype 2 isolates of *A. tumefaciens* which contain an octopine Ti plasmid.

Nine biotype 3 strains considered here contained octopine Ti plasmids. The plasmids pTiAg57, pTiAg63, pTiAg158, and pTiAg162 gave *Sma*I fingerprints similar to each other but different from the pTiB6 pattern. Thomashow et al. (30) suggested that these Ti plasmids formed

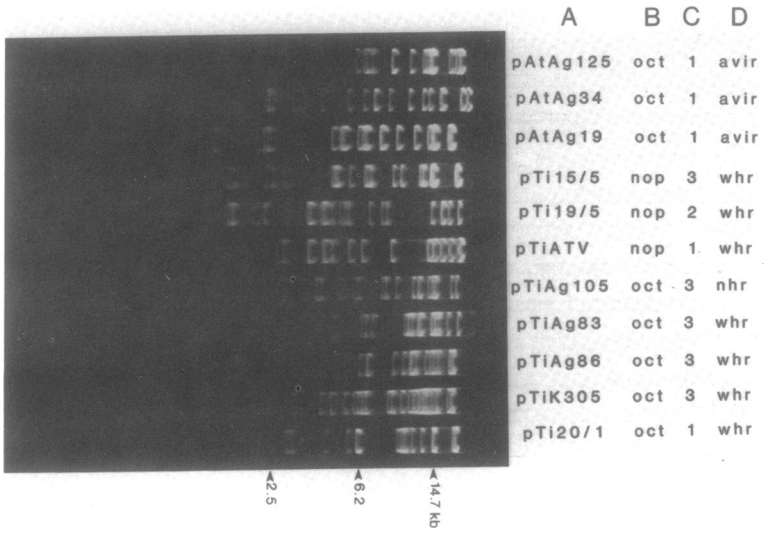

FIG. 1. *Sma*I fingerprints of plasmids from grapevine isolates of *Agrobacterium*. (A) Plasmid designation. (B) Opine catabolism coded by plasmid: oct, octopine; nop, nopaline. (C) Biotype of the *Agrobacterium* strain in which the plasmid was originally found. (D) Virulence properties coded by plasmid: avir, avirulent; whr, wide host range; nhr, narrow host range. A 0.5-μg sample of DNA of each plasmid was digested with *Sma*I and subjected to horizontal gel electrophoresis in 0.7% agarose at 50 V for 7 h.

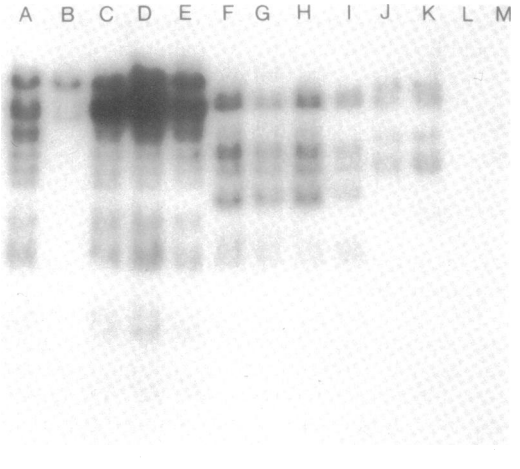

FIG. 2. Homology between pTi2/6 and other *Agrobacterium* plasmids. A 0.5-μg sample of each plasmid DNA was digested with *Sma*I, subjected to vertical gel electrophoresis in 0.7% agarose at 50 V for 4 h, and blotted according to Thomashow et al. (27). The hybridization probe was nick-translated pTi2/6 DNA. pTi2/6 was originally isolated from a biotype 3 *Agrobacterium* strain and codes for nopaline utilization and a wide host range. Stringency of hybridization corresponded to T_m − 17°C (27). Plasmid samples were (A) total plasmid from strain CG54 (pTiCG54, pAtCG54); (B) pTiAg162; (C) pTi15/5; (D) pTi2/6; (E) pTiCG8; (F) pTiM-A5; (G) pTiPPI-1; (H) pTiPPI-6; (I) pTi19/5; (J) pTiATV; (K) pTiATB; (L) pAt1 D 1109; and (M) pTiNCPPB 1001.

a second class of Ti plasmids distinguished primarily by their unusually limited host range. The plasmids pTiAg105 and pTiAg123 also code for this narrow host range (20) and have *Sma*I fingerprints identical to that of pTiAg162.

The plasmids pTiAg83, pTiAg86, and pTiK305 isolated from biotype 3 strains all code for a wide host range (20) and octopine utilization, but their *Sma*I restriction patterns differ from each other and from those of the pTiB6 and pTiAg162 classes (Fig. 1). These data suggest that octopine Ti plasmids are evolutionarily diverse and encompass more than one (25) or two classes (30).

Relationship between pTiAg86 and other plasmids. Since the octopine Ti plasmids from biotype 1 *Agrobacterium* and the limited host range octopine Ti plasmids from biotype 3 strains are significantly different (29), it was of interest to investigate the octopine Ti plasmids coding for a wide host range capability (20) but isolated from biotype 3 *Agrobacterium*. pTiAg86 DNA was nick-translated and used as a hybridization probe for DNA of a variety of plasmids blotted by the technique of Southern (26). When these hybridizations were done under conditions of moderately high stringency (T_m − 17°C), the plasmids pTiAg83, pTiK305, pTiAg105, and pTiAg123 exhibited extensive homology with the pTiAg86 probe. Specific bands of pAtAg19, pAtAg34, pAtAg125, and pTi15/5 showed significant, but limited, hybridization, whereas

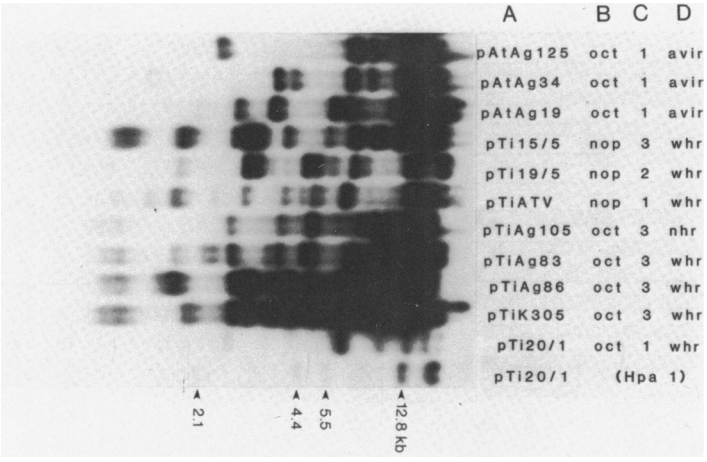

FIG. 3. Homology between pTiAg86 and other *Agrobacterium* plasmids. A 0.5-μg sample of each plasmid DNA was digested with *Sma*I, subjected to horizontal gel electrophoresis in 0.7% agarose at 50 V for 7 h, and blotted according to Thomashow et al. (27). One lane represents a *Hpa*I digest as indicated. The hybridization probe was pTiAg86 DNA. pTiAg86 was originally isolated from a biotype 3 strain and codes for octopine utilization and a wide host range. The stringency of hybridization corresponded to T_m − 41°C (27). (A) Plasmid designation. (B) Opine utilization coded by plasmid: oct, octopine; nop, nopaline. (C) Biotype of the *Agrobacterium* strain in which the plasmid was originally found. (D) Virulence properties coded by plasmid: avir, avirulent; whr, wide host range, nhr, narrow host range.

pTiATV, pTi19/5, and pTi20/1 appeared to have DNA sequences only slightly related. These experiments indicated that the octopine Ti plasmids from biotype 3 isolates are highly related, regardless of the host range capability coded by the Ti plasmids. The octopine plasmids pAtAg19, pAtAg34, and pAtAg125 share regions of homology with pTiAg86, possibly related to the trait of octopine catabolism (see references 7 and 19). The nopaline Ti plasmid pTi2/6 from a biotype 3 strain also shared strongly conserved regions with pTiAg86, and these regions were absent or more divergent in the nopaline Ti plasmids pTi19/5 and pTiATV and the octopine Ti plasmid pTi20/1.

When the stringency of hybridization was lower (T_m − 41°C), much more homology could be detected between blotted plasmids and pTiAg86. The autoradiogram in Fig. 3 was overexposed to bring up the hybridization patterns for the *Sma*I and *Hpa*I digests of pTi20/1. The hybridization patterns in Fig. 3 also indicate that the homology between pTiAg86 and other plasmids is comparatively much greater than the homology seen between the two wide host range octopine Ti plasmids pTiAg86 and pTi20/1.

By blotting both *Sma*I and *Hpa*I digests of pTi20/1 (Fig. 3), we were able to map some of the homology shared between pTiAg86 and pTi20/1. This is possible because the *Sma*I and *Hpa*I digests of pTi20/1 are identical to the *Sma*I and *Hpa*I digests of $pTiB_6$-806 (data not shown). Since the restriction enzyme maps of $pTiB_6$-806 are known (3), it is reasonable to assume that the relative positions of pTi20/1 restriction fragments are the same. Thus, the physical map of pTi20/1 can also be extended to include a functional map (reviewed in reference 22). The hybridization patterns in Fig. 3 indicate that pTiAg86 and pTi20/1 share DNA homology in regions of pTi20/1 that are associated with replication, octopine catabolism, and the T-DNA. It is interesting that even at this low stringency we do not detect homology between pTiAg86 and the region of pTi20/1 spanning *Hpa*I fragments 5-9-10-16-12-15-6. This region had previously been found conserved among octopine, nopaline, agropine, and rhizogenic virulence plasmids (7, 23) and includes loci required for virulence of pTiA6 (10).

Common DNA in Ti plasmids. Thomashow et al. (27) reported that the plasmids pTiAg57, pTiAg63, pTiAg158, and pTiAg162 do not have a highly conserved common DNA region once thought to be carried by all Ti plasmids. Therefore, it was of interest to see whether other Ti plasmids from biotype 3 strains or limited host range strains also lacked the highly conserved common DNA.

Blotted *Sma*I digests of plasmid DNA were hybridized to ^{32}P-labeled pNW31C-8,29-1 DNA (Fig. 4). This probe, a pBR322 clone of *Bam*HI

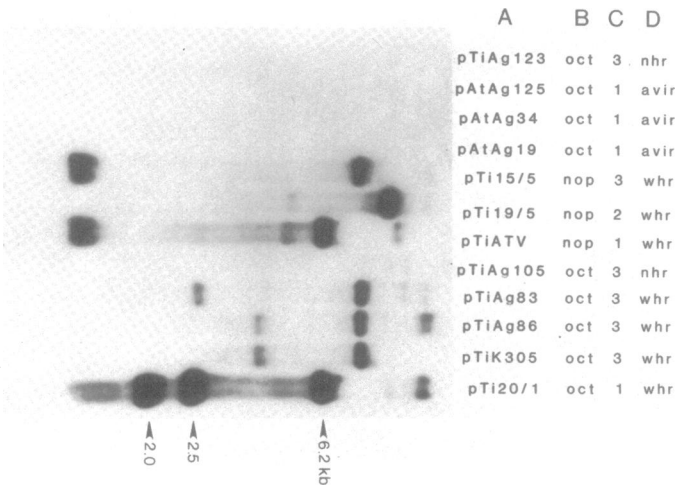

FIG. 4. Presence of common DNA sequences in *Agrobacterium* plasmids. A 0.5-μg sample of each plasmid DNA was digested with *Sma*I, subjected to horizontal gel electrophoresis in 0.7% agarose at 50 V for 7 h, and blotted according to Thomashow et al. (27). The hybridization probe was pNW-31C-8,29-1 DNA. The plasmid was a pBR322 clone of the *Bam*HI fragments 8 and 29 at the T-DNA of the octopine Ti plasmid pTiA6. Hybridization conditions corresponded to a stringency of T_m − 41°C (27). (A) Plasmid designation. (B) Opine utilization coded by plasmid: oct, octopine; nop, nopaline. (C) Biotype of the *Agrobacterium* strain in which the plasmid was originally found. (D) Virulence properties coded by plasmid: avir, avirulent; whr, wide host range; nhr, narrow host range.

bands 8 and 29 of the octopine plasmid pTiA6 (28), includes the highly conserved region found in most Ti plasmids that is integrated into plant DNA (1, 5). As a negative control, ^{32}P-labeled pBR322 DNA was also hybridized to similar blots. No hybridization was detected between pBR322 and these Ti plasmids, even after long exposures of autoradiographs.

Since the plasmid pTi20/1 has an *Sma*I fingerprint similar to that of pTiA6, it is not surprising that it has fragments corresponding to *Sma*I 10c, 16, and 17 that hybridize to the probe which contains *Sma*I fragment 10c, 16, and 17 sequences. Also, the three nopaline Ti plasmids tested, pTiATV, pTi19/5, and pTi15/5, all showed homology with the probe of common DNA. Hybridization to this probe is notably lacking for the plasmid pTiAg105 and pTiAg123, as might be predicted from the similarity between the *Sma*I fingerprints of these Ti plasmids and those studied by Thomashow et al. (27). Other octopine Ti plasmids from biotype 3 strains pTiAg83, pTiAg86, and pTiK305 all showed homology to the probe DNA. If the DNA of pTiAg83, pTiAg86, and pTiK305 which shares homology with the T-DNA of pTiA6 is the T-DNA of those Ti plasmids from biotype 3 strains, then an *Sma*I fragment appears to be conserved among the T-DNA of those Ti plasmids. Like pAtAg19 (7), the avirulent octopine plasmids pAtAg34 and pAtAg125 do not show homology to the common DNA.

***Agrobacterium* strain 1 D 1109.** The *Agrobacterium* strain 1 D 1109 was of special interest since it was reported (21) to have a nopaline Ti plasmid that coded for a very narrow host range (weakly virulent on grapevine). We have been unable to induce tumors on any plant host, including grapevines, with this strain. Since the ability of strain 1 D 1109 to catabolize nopaline was lost after the introduction of an octopine Ti plasmid, Loper and Kado (21) concluded that a nopaline Ti plasmid had been excluded by incompatibility with the octopine Ti plasmid. However, we have shown that the resident plasmid of strain 1 D 1109 is compatible with octopine Ti plasmids (V. Knauf, manuscript in preparation). We detected no DNA homology, even after long exposure of autoradiographs, between strain 1 D 1109 and either the common DNA probe or the nopaline Ti plasmid pTi2/6 (Fig. 2). Unless the plasmid in strain 1 D 1109 can be shown to code for virulence by transferring to another strain, there remains some doubt as to whether this plasmid is a Ti plasmid.

DISCUSSION

Although it is commonly stated that octopine Ti plasmids are highly conserved (12, 14, 25), the data presented in this paper clearly indicate that octopine Ti plasmids from biotype 3 strains of *Agrobacterium* are widely divergent from the

highly conserved octopine Ti plasmids found in the biotype 1 strains examined. Moreover, the concept that octopine Ti plasmids have developed more recently than nopaline Ti plasmids (14) should be reevaluated since the 10 nopaline Ti plasmids in this study appear more closely related to each other than the octopine plasmid pTi20/1 appears related to the octopine plasmid pTiAg86.

Even though the biotype of a strain of *A. tumefaciens* is chromosomally coded (17) and presumably independent of Ti plasmids, there is a correlation between the plasmid and the biotype of the natural isolate originally containing that plasmid. Nine out of nine octopine Ti plasmids from biotype 3 strains share extensive homology with pTiAg86. No octopine Ti plasmids have been found in biotype 2 strains, and the octopine Ti plasmids from biotype 1 isolates appeared to be very distantly related to pTiAg86. Considering the plasmids pTi20/1 and pTiS-8 together with the five octopine Ti plasmids described by Sciaky et al. (25) and pTiAch5 (6), eight out of eight octopine Ti plasmids from biotype 1 isolates share essentially the same *Sma*I fingerprint pattern. Of the 10 nopaline Ti plasmids considered here, the three different *Sma*I fingerprint types coincide exactly with the biotype groupings of the original parent strains.

Previous work (10, 23) indicates that two main regions of the octopine Ti plasmid pTiA6 are directly associated with the ability to induce tumors. One region, termed the oncogenic region and represented by the *Bam*HI 8,29 clone of pTiA6 DNA, is part of the DNA integrated into plant DNA and is responsible for tumor morphology (10, 11, 23). The second, the virulence region, does not appear in plant DNA but nonetheless is required for tumor formation (10, 23). White and Nester (33) have shown that the *Agrobacterium rhizogenes* virulence plasmid shares with pTiB6 group plasmids a wide host range and a conserved portion of the virulence region, but has a highly divergent oncogenic region. Thomashow et al. (27) have shown that plasmids such as pTiAg162 share little homology with either the virulence or oncogenic regions of pTiB6 group plasmids and express very limited host ranges. The data in this study indicate that the wide host range pTiAg83 and pTiAg86 plasmids share homology with pTiB6 group plasmids in the oncogenic region but not in the virulence region. As had been observed earlier for octopine and nopaline Ti plasmids (1, 5), it seems remarkable that Ti plasmids pTiAg86 and pTi20/1 have homologous DNA sequences in the oncogenic regions, whereas DNA sequences in other regions have diverged considerably more. Since pTiAg105 and pTiAg86 appear closely related by DNA homology, it is interesting that the plasmid coding for the wider host range (20) is the plasmid with some homology to the common DNA probe.

We suggest that the region of T-DNA termed the common DNA is found in many Ti plasmids because certain DNA sequences (the common DNA) are required for the expression of a wide host range. Tumor formation by *A. tumefaciens* requires plant host wounding by some other agent. Thus, *A. tumefaciens* is an opportunist which cannot "choose" its next host plant. Strains of *Agrobacterium* which continue over time to induce crown galls would be those which induce crown galls often enough to outcompete less pathogenic variants. For most strains, this would require a wide host range, and therefore the common DNA, to maximize opportunities. Since cultivated grapevines are often subject to wounding, biotype 3 strains such as Ag105 which are associated only with grapevines may remain virulent (or increase their virulence) on grapevines while allowing divergence of the common DNA at the expense of host range.

LITERATURE CITED

1. **Chilton, M.-D., M. H. Drummond, D. J. Merlo, and D. Sciaky.** 1978. Highly conserved DNA of Ti-plasmids overlaps T-DNA maintained in plant tumors. Nature (London) **275**:147–149.
2. **Chilton, M.-D., M. H. Drummond, D. J. Merlo, D. Sciaky, A. L. Montoya, M. P. Gordon, and E. W. Nester.** 1977. Stable incorporation of plasmid DNA into higher plant cells; the molecular basis of crown gall tumorigenesis. Cell **11**:362–271.
3. **Chilton, M.-D., A. L. Montoya, D. J. Merlo, M. H. Drummond, R. Nutter, M. P. Gordon, and E. W. Nester.** 1978. Restriction endonuclease mapping of a plasmid that confers oncogenicity upon *Agrobacterium tumefaciens* strain B6-806. Plasmid **1**:254–269.
4. **Currier, T. C., and E. W. Nester.** 1976. Isolation of covalently closed circular DNA of high molecular weight from bacteria. Anal. Biochem. **66**:431–441.
5. **Depicker, A., M. Van Montagu, and J. Schell.** 1978. Homologous DNA sequences in different Ti-plasmids are essential for oncogenicity. Nature (London) **275**:150–153.
6. **De Vos, G., M. De Beuckeleer, M. Van Montagu, and J. Schell.** 1981. Restriction endonuclease mapping of the octopine tumor-inducing plasmid pTiAch5 of *Agrobacterium tumefaciens*. Plasmid **6**:249–253.
7. **Drummond, M. H., and M.-D. Chilton.** 1978. Tumor-inducing (Ti) plasmids of *Agrobacterium* share extensive regions of DNA homology. J. Bacteriol. **136**:1178–1183.
8. **Ellis, J. G., A. Kerr. J. Tempé, and A. Petit.** 1979. Arginine catabolism: a new function of both octopine and nopaline Ti-plasmids of *Agrobacterium*. Mol. Gen. Genet. **173**:263–269.
9. **Ellis, J. G., and P. J. Murphy.** 1981. Four new opines from crown gall tumors—their detection and properties. Mol. Gen. Genet. **181**:36–43.
10. **Garfinkel, D. J., and E. W. Nester.** 1980. *Agrobacterium tumefaciens* mutants affected in crown gall tumorigenesis and octopine catabolism. J. Bacteriol. **144**:732–743.
11. **Garfinkel, D. J., R. B. Simpson, L. W. Ream, F. F. White, M. P. Gordon, and E. W. Nester.** 1981. Genetic analysis of crown gall: fine structure map of the T-DNA by site-directed mutagenesis. Cell **27**:143–153.
12. **Guyon, P., M.-D. Chilton, A. Petit, and J. Tempé.** 1980. Agropine in "null-type" crown gall tumors: evidence for

generality of the opine concept. Proc. Natl. Acad. Sci. U.S.A. 77:2693–2697.
13. **Holsters, M., D. de Waele, A. Depicker, E. Messens, M. van Montagu, and J. Schell.** 1978. Transfection and transformation of *Agrobacterium tumefaciens*. Mol. Gen. Genet. **163**:181–187.
14. **Hooykaas, P. J. J., H. Den Dulk-Ras, G. Ooms, and R. A. Schilperoort.** 1980. Interactions between octopine and nopaline plasmids in *Agrobacterium tumefaciens*. J. Bacteriol. **143**:1295–1306.
15. **Hooykaas, P. J. J., H. den Dulk-Ras, and R. A. Schilperoort.** 1980. Molecular mechanisms of Ti-plasmid mobilization by R plasmids: isolation of Ti-plasmids with transposon-insertions in *Agrobacterium tumefaciens*. Plasmid **4**:64–75.
16. **Hooykaas, P. J. J., C. Roobol, and R. A. Schilperoort.** 1979. Regulation of the transfer of Ti-plasmids of *Agrobacterium tumefaciens*. J. Gen. Microbiol. **110**:99–109.
17. **Kerr, A., and C. G. Panagopoulos.** 1977. Biotypes of *Agrobacterium radiobacter* var. *tumefaciens* and their biological control. Phytopathol. Z. **90**:172–179.
18. **Kerr, A., and W. P. Roberts.** 1976. *Agrobacterium*: correlations between and transfer of pathogenicity, octopine and nopaline metabolism and bacteriocin 84 sensitivity. Physiol. Plant Pathol. **9**:205–211.
19. **Klapwijk, P. M., T. Scheulderman, and R. A. Schilperoort.** 1978. Coordinated regulation of octopine degradation and conjugative transfer of Ti plasmids in *Agrobacterium tumefaciens*: evidence for a common regulatory gene and separate operons. J. Bacteriol. **136**:775–785.
20. **Knauf, V. C., C. G. Panagopoulos, and E. W. Nester.** 1982. Genetic factors controlling host range of *Agrobacterium tumefaciens*. Phytopathology **72**:1545–1549.
21. **Loper, J. E., and C. I. Kado.** 1979. Host range conferred by the virulence-specifying plasmid of *Agrobacterium tumefaciens*. J. Bacteriol. **139**:591–596.
22. **Nester, E. W., and T. Kosuge.** 1981. Plasmids specifying plant hyperplasias. Annu. Rev. Microbiol. **35**:531–565.
23. **Ooms, G., P. M. Klapwijk, J. A. Poulis, and R. A. Schilperoort.** 1980. Characterization of Tn*904* insertions in octopine Ti plasmid mutants of *Agrobacterium tumefaciens*. J. Bacteriol. **144**:82–91.
24. **Prakash, R. K., R. A. Schilperoort, and M. P. Nuti.** 1981. Large plasmids of fast-growing Rhizobia: homology studies and location of structural nitrogen fixation (*nif*) genes. J. Bacteriol. **145**:1129–1136.
25. **Sciaky, D., A. L. Montoya, and M.-D. Chilton.** 1978. Fingerprints of *Agrobacterium* Ti-plasmids. Plasmid **1**:238–253.
26. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
27. **Thomashow, M. F., V. C. Knauf, and E. W. Nester.** 1981. Relationship between the limited and wide host range octopine-type Ti plasmids of *Agrobacterium tumefaciens*. J. Bacteriol. **146**:484–493.
28. **Thomashow, M. F., R. Nutter, A. L. Montoya, M. P. Gordon, and E. W. Nester.** 1980. Integration and organization of Ti-plasmid sequences in crown gall tumors. Cell **19**:729–739.
29. **Thomashow, M. F., R. Nutter, K. Postle, M.-D. Chilton, F. R. Blattner, A. Powell, M. P. Gordon, and E. W. Nester.** 1980. Recombination between higher plant DNA and the Ti-plasmid of *Agrobacterium tumefaciens*. Proc. Natl. Acad. Sci. U.S.A. **77**:6448–6452.
30. **Thomashow, M. F., C. G. Panagopoulos, M. P. Gordon, and E. W. Nester.** 1980. Host range of *Agrobacterium tumefaciens* is determined by the Ti-plasmid. Nature (London) **283**:794–796.
31. **Watson, B., T. C. Currier, M. P. Gordon, M.-D. Chilton, and E. W. Nester.** 1975. Plasmid required for virulence of *Agrobacterium tumefaciens*. J. Bacteriol. **123**:255–264.
32. **White, F. F., and E. W. Nester.** 1980. Hairy root: plasmid encodes virulence traits in *Agrobacterium rhizogenes*. J. Bacteriol. **141**:1134–1141.
33. **White, F. F., and E. W. Nester.** 1980. Relationship of plasmids responsible for hairy root and crown gall tumorigenicity. J. Bacteriol. **144**:710–720.
34. **Yadav, N. S., K. Postle, R. K. Saiki, M. F. Thomashow, and M.-D. Chilton.** 1980. T-DNA of a crown gall teratoma is covalently joined to host plant DNA. Nature (London) **287**:458–461.
35. **Zaenen, I., N. Van Larebeke, H. Teuchy, M. Van Montagu, and J. Schell.** 1974. Supercoiled circular DNA in crown gall inducing *Agrobacterium* strains. J. Mol. Biol. **86**:109–127.