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Diversity Among B6 Strains of *Agrobacterium tumefaciens*

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A total of 20 laboratory substrains of *Agrobacterium tumefaciens* strain B6 were compared with respect to six characteristics, including 3-ketolactose production, lysogeny, octopine catabolism, tumorigenic host range, and plasmid content. Within this group of strains diversity was found for all characteristics except 3-ketolactose production. Six substrains were lysogenized with an omega-type phage, whereas one substrain appeared neither sensitive to nor lysogenized with this bacteriophage. All but two substrains catabolized octopine and induced tumors on carrot disks. These 18 substrains harbor deoxyribonucleic acid sequences homologous to pTiB6-806. The two substrains unable to catabolize octopine were nontumorigenic and lacked detectable Ti plasmid sequences. Of the 20 substrains, 13 also contained sequences homologous to the cryptic plasmid pAtB6-806; 2 of the 18 substrains tumorigenic on carrots failed to induce tumors on *Kalanchoe* leaves. Their inability to induced tumors on this host could not be correlated with lysogeny, with the presence or absence of pAtB6-806, or with the very large cryptic plasmid recently described. The Ti plasmids from these two strains were indistinguishable from pTiB6-806 by restriction enzyme analysis and could genetically convert a cured *A. tumefaciens* strain to tumorigenicity on both plant species. The results with these two strains suggest that parameters of tumorigenicity, such as host range, may be controlled by the bacterial chromosome.

Agrobacterium tumefaciens is the etiological agent of crown gall, a neoplastic disease of plants (29). Within the last several years, considerable progress has been made toward an understanding of factors involved in gall formation by this bacterium (7, 12, 18, 31, 32). However, the basic mechanism of tumorigenesis remains to be determined.

Recent genetic and physical studies with *A. tumefaciens* have involved the use of only a few standard strains. These include the nopaline utilizer strain C58 (10, 15, 30–32) and the octopine-utilizing strains A6 (10, 27), 15955 (14), and B6 (7, 13, 22).

Strain B6 has received particular attention over the years. It has been used to investigate lysogeny (2, 3, 26, 33), the relationship of agrobacteriophages to tumor induction (19), the effect of physical (16) and chemical (17) agents on the capacity for tumor induction and the initial events involved in infection and attachment (21, 22). Also, it was with strain B6-806 that Chilton et al. (7) unequivocally showed the presence of Ti plasmid DNA sequences in DNA from transformed plant cells. The Ti plasmid from strain B6-806 has come to represent the prototype octopine-specifying extrachromosomal element (9, 28).

With the dispersal of B6 strains to various laboratories came the possibility of strain divergence. In fact, it is already clear that B6 strains from various laboratories differ from one another in several respects. For example, strains may be either lysogenized with or sensitive to bacteriophage PS8 (2, 26, 33). Second, although strain B6-806 contains three genetically distinct large plasmids (5, 28, 31), at least one other strain, B6-Miller, appears to lack one of the cryptic elements, pAtB6-806 (A. Matthysee, personal communication). Sciaky et al. (28) observed that the Ti plasmids from strains B6-806 and B6-T differed in that endonuclease *Sma*I fragment 2 from pTiB6-T was approximately 1.1 $\times 10^6$ daltons larger than the same fragment from digests of pTiB6-806. Finally, in preliminary experiments, we noted that two B6 strains, although tumorigenic on a number of plant species, failed to induce tumors when inoculated onto *Kalanchoe* leaves. Strain B6-806 produces large tumors on this host.

The design of experiments utilizing strain B6 and the interpretation and comparison of results from such experiments may be contingent upon characteristics of the particular strain being used. For this reason we considered it of impor-

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tance to assess strain divergence by characterizing B6 strains acquired from a number of different laboratories. Results from this study show that B6 strains collected from various laboratories differ with respect to a number of characteristics, including lysogeny, plasmid complement, and tumorigenicity.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study, their characteristics, and their origins are listed in Table 1. Strain TR1WS deserves special mention. This strain was grown from a culture labeled *Agrobacterium radiobacter* TR1W, a derivative of *A. radiobacter* strain TR1 showing a wrinkled colonial morphology (T. C. Currier, personal communication). Strain TR1WS showed a smooth colonial morphology, tumorigenicity on tobacco and sunflower plants, and the presence of plasmid DNA homologous to pTiB6-806. Hybridization of total TR1WS DNA with radiolabeled B6-806 chromosomal DNA showed essentially 100% homology (Farrand, unpublished data). Thus, although the origin of strain TR1WS is unclear, these reasons and others presented below indicate that it is undoubtedly a B6 strain.

Media. All strains were routinely grown in L-broth (20). Basal medium was described by Kerr et al. (18), and AB minimal medium was described by Chilton et al. (6). Minimal medium contained either 0.5% glucose or 500 μg of octopine (Sigma Chemical Co.) per ml as carbon source. Soft nutrient agar contained 8.0 g of nutrient broth (Difco Laboratories) per liter and 7.0 g of agar per liter.

Buffers. TES buffer was composed of 0.05 M Tris (Sigma), 0.05 M sodium chloride, and 0.005 M EDTA (Sigma), pH 8.0. TE buffer contained 0.05 M Tris and 0.02 M EDTA, pH 8.0. Standard saline citrate (1× SSC) consisted of 0.15 M sodium chloride and 0.015 M trisodium citrate. Phosphate buffer, which was used in the DNA-DNA hybridization studies, was an equimolar mixture of dibasic sodium phosphate and monobasic sodium phosphate.

Biochemical tests. Production of 3-ketolactose was assayed by the method of Bernaerts and De Ley (4). The ability of the bacteria to utilize octopine was determined by assessing growth in basal medium supplemented with 500 μg of octopine per ml as sole carbon and nitrogen source. Growth, as estimated by turbidity, was scored after 24, 48, and 72 h of incubation at 30°C.

Bacteriophage studies. Strains were tested for lysogeny with omega-type bacteriophage as follows. Each strain was grown overnight in 5 ml of L-broth. The cells were pelleted by centrifugation, and the culture supernatant was passed through a membrane filter (pore size, 0.45 μm; Millepore Corp.). The supernatant was serially diluted in L-broth, and the dilutions were tested for the presence of bacteriophage by soft agar assay, using strain B6-806 as the indicator. A 0.1-ml amount of each supernatant dilution, 3 drops of the indicator bacterial suspension grown in L-broth to

TABLE 1. *Sources and characteristics of Agrobacterium strains used*

Strain	Characteristics and/or reference[a]	Source	Obtained from:
B6-806	PS8^s; pTiB6-806, pAtB6-806 (28)	J. A. Lippincott	E. W. Nester
B6-806 (PS8)	PS8 lysogen of B6-806 (6)	E. W. Nester	
B6-Braun		A. C. Braun	
B6-W		J. Kemp	
B6-M	Progenitor of B6-806 (26) and B6-S (R. M. Klein, personal communication)	C. Pootjes	
B6-S	PS8^s; isolated by R. M. Klein (26)	C. Pootjes	
B6-Heb		G. Heberlein	
B6-Moore		L. W. Moore	
B6-Schil		R. A. Schilperoort	
B6-T		J. Tourneur	
TR1WS		E. W. Nester	
A217	Derivative of B6-806; PS8^s; pAtB6-806; lacks pTiB6-806 (28)	E. W. Nester	
A277	Derivative of C58 harboring pTiB6-806 (28)	E. W. Nester	
SA110	Strain A136 (32) made resistant to neomycin	This laboratory	
B6-Miller		A. Miller	A. Matthysee
23308	ATCC strain of B6	A. C. Braun	
B6		T. Stonier	J. A. Lippincott
B6-6		R. E. Beardsley	
B6-Man		P. Manigault	
11156[b]	ATCC strain of B6	W. C. Price	
11157[b]	ATCC strain of B6	W. C. Price	
11158[b]	ATCC strain of B6	W. C. Price	

[a] PS8^s, Sensitive to bacteriophage PS8; pTiB6-806 and pAtB6-806, harbors the Ti plasmid or cryptic plasmid of strain B6-806, respectively; ATCC, American Type Culture Collection.

[b] These strains represent what were described as smooth (11156), intermediate (11157), and rough (11158) colonial morphology variants of strain B6 (C. Gainor and W. C. Price, Phytopathology 42:8-9, 1952). We observed no differences in the growth characteristics of these three isolates.

a turbidity of 50 Klett units (no. 66 red filter), and 3 ml of soft agar were gently mixed and spread onto a nutrient agar plate. After overnight incubation at 30°C, each set of plates was examined for plaques.

All strains were tested for sensitivity to authentic bacteriophage PS8 by routine soft agar plaque assay. Stocks of bacteriophage PS8 were prepared as follows. A 10-ml L-broth culture of strain B6-806(PS8) was grown to late exponential phase. The cells were pelleted by centrifugation, and the culture supernatant was filtered through a membrane filter (pore size, 0.45 μm). Soft agar assays were performed as described above, using each *Agrobacterium* strain as an indicator. Each culture was grown to a turbidity of 50 Klett units and plated with approximately 200 plaque-forming units of phage. The plates were examined for plaques after overnight incubation at 30°C.

Tumorigenicity assays. The ability of each strain to induce the formation of tumors was tested on *Kalanchoe* leaves (32) and carrot disks (1).

Isolation of plasmid and total DNA. Plasmid DNA was isolated by a modification of the procedure of Currier and Nester (11). After chloroform extraction, the lysate was adjusted to 0.3 M sodium acetate, and the solution was mixed with 2 volumes of cold 95% ethanol (24). After overnight storage at −20°C, the precipitated DNA was collected by centrifugation (11,000 × *g*, 30 min, −4°C) and redissolved in TES buffer. Plasmid DNA was purified by cesium chloride-ethidium bromide density equilibrium gradient centrifugation as described by Currier and Nester (11). Alternatively, plasmid DNA was partially purified by the procedure of Casse et al. (5) and analyzed by electrophoresis in 0.7% horizontal agarose gels as previously described (24).

Total bacterial DNA was isolated essentially as described by Marmur (23). The cells were lysed as described by Currier and Nester (11). The DNA was dissolved and stored in 0.1× SSC.

Restriction endonuclease analysis. Plasmid DNA samples, which were purified by two equilibrium centrifugations in cesium chloride-ethidium bromide gradients, were digested with restriction endonucleases under conditions recommended by the supplier (New England Biolabs). Fragments were separated by electrophoresis in 0.7% agarose gels, stained with ethidium bromide (0.5 μg/ml), and photographed on Polaroid type 57 film under near-UV transillumination.

Preparation of radiolabeled plasmid DNA. Radioisotope labeling with [^{3}H]thymidine (20 to 40 Ci/mmol; New England Nuclear Corp.) was carried out as described by Chilton et al. (6). Radiolabeled pAtB6-806 was isolated from strain A217, and radiolabeled pTiB6-806 was isolated from strain A277 (28).

DNA solution hybridization. Solution hybridizations were performed essentially as described by Currier and Nester (10), except that total bacterial DNA was used as driver. Sheared (6) driver DNA in distilled water was evaporated to dryness at 37°C under a stream of filtered air. The dried DNA samples were redissolved to a final driver DNA concentration of 1 mg/ml in the hybridization reaction mixture. This mixture contained the radiolabeled plasmid probe DNA in 0.15 M phosphate buffer. The reaction mixtures were drawn up in a series of capillary tubes, and the tube ends were flame sealed. The samples were denatured by boiling at 105°C for 7 min in a salt water bath, and the hybridization reactions were initiated by transferring the capillary tubes to a water bath maintained at 67°C. Capillaries from each reaction mixture were removed from the water bath at timed intervals and chilled in ice water to stop the renaturation. The extent of duplex formation was assayed by hydroxyapatite chromatography as described by Chilton et al. (6).

Conjugation. Strains B6-806, B6-Braun, and TR1WS were mated with strain SA110 as described by Kerr et al. (18). Transconjugants were selected on basal medium containing octopine (500 μg/ml) as sole source of carbon and nitrogen and either rifampin (10 μg/ml) or neomycin (50 μg/ml) to counterselect against the donor.

RESULTS

Biochemical characterizations. All 20 B6 strains produced 3-ketoglycosides from lactose (Table 2). This was true even of strain A217, which is known to lack pTiB6-806 (28). Strain A277, a derivative of strain C58 harboring pTiB6-806 (28), also produced a positive 3-ketolactose reaction.

Of the 20 B6 strains, 18 were able to grow with octopine as sole source of carbon and nitrogen (Table 2). Strain A217, originally selected for its inability to catabolize octopine (28), showed no growth with this substrate. Surprisingly, strain B6-Man also failed to grow in basal medium with octopine. Both strains grew well in basal medium supplemented with inorganic nitrogen and glucose.

Bacteriophage analysis. Lysogeny in strain B6 was first described by Beardsley (3). The bacteriophage which he isolated is known as omega phage (33) or PS8. Theoretically, B6 strains should exhibit either lysogeny with or sensitivity to this bacteriophage. When tested, 12 of the B6 strains were found to be sensitive to infection with authentic bacteriophage PS8 (Table 2). Furthermore, each sensitive strain plaqued PS8 with essentially the same efficiency (data not shown). Seven of the eight strains resistant to PS8 produced a bacteriophage which formed plaques on the standard PS8 indicator strain (25), B6-806. The eighth strain, B6-Heb, failed to plaque authentic PS8, even when challenged with a high titer (ca. 10^8 plaque-forming units) of the bacteriophage, nor did the supernatant from this strain show any lytic activity when tested against strain B6-806.

Tumorigenicity. Strain B6 has been used extensively because of its capacity to initiate large, fast growing tumors on a number of plant species (21, 22); 18 of the 20 B6 strains and strain A277 produced rapidly proliferating tumors

TABLE 2. *Characteristics of Agrobacterium strains*[a]

Strain	3-Ketolactose	Bacteriophage study		Octopine utilization	Tumorigenicity on:[b]		% Sequence homology with:[c]	
		Sensitivity	Lysogeny		Carrot	Kalanchoe	pAtB6-806	pTiB6-806
B6-806	+	+	−	+	+	+	100	100
11156	+	+	−	+	+	+	100	100
11157	+	+	−	+	+	+	100	100
11158	+	+	−	+	+	+	100	100
23308	+	+	−	+	+	+	100	100
B6-Moore	+	+	−	+	+	+	100	100
B6-806 (PS8)	+	−	+	+	+	+	100	100
B6-Schil	+	−	+	+	+	+	100	100
B6-W	+	−	+	+	+	+	100	100
B6-Tourneur	+	−	+	+	+	+	100	100
B6-Heb	+	−	−	+	+	+	100	100
B6-Braun	+	+	−	+	+(10/10)	−(0/10)	<5	100
B6	+	+	−	+	+	+	<5	100
B6-Miller	+	+	−	+	+	+	<5	100
B6-S	+	+	−	+	+	+	<5	100
B6-6	+	+	−	+	+	+	<5	100
B6-M	+	−	+	+	+	+	<5	100
TR1WS	+	−	+	+	+(10/10)	−(0/10)	<5	100
A277	+	−	−	+	+	+	<5	100
A217	+	+	−	−	−(0/10)	−(0/10)	100	<5
B6-Man	+	−	+	−	−(0/10)	−(0/10)	100	<5

[a] +, Positive reaction; −, negative reaction.
[b] The numbers in parentheses indicate the number of tumorigenic subclones/number of subclones tested.
[c] Determined as described in the text.

when inoculated onto carrot disks (Table 2). When tested on *Kalanchoe* leaves, however, only 16 of these 18 strains proved tumorigenic. Two strains, B6-Braun and TR1WS, failed to produce tumors on this host (Fig. 1 and Table 2). Strains B6-Man and A217, the two strains which failed to utilize octopine (see above), were nontumorigenic on both plant species (Table 2).

Plasmid analysis. Sciaky et al. (28) and Genetello et al. (13) showed that strain B6-806 harbors two genetically distinct plasmids of approximately the same mass. One, the Ti plasmid, codes for the ability to catabolize octopine (7, 13, 25) and is essential for tumorigenicity (7, 13). The other plasmid, pAtB6-806 (28), is genetically cryptic. All 20 B6 strains and strain A277 were assayed for the presence of each element by renaturation kinetic analysis, as described above. The ratio of probe DNA to driver DNA was adjusted such that the presence of one copy of pAtB6-806 per bacterial genome in the driver DNA would accelerate the renaturation of the probe DNA by a factor of 10. As Fig. 2 shows, when complementary sequences are present as in strains B6-806 and 23308, the entire renaturation curve of the probe DNA is shifted to the left. This indicates that virtually all pAtB6-806 sequences are present in these strains. When homologous sequences are absent, the curves approximate the curve of probe DNA renatured in the presence of the completely heterologous *Escherichia coli* DNA. The data show that 11 of the 20 B6 strains examined harbored nucleotide sequences homologous to both plasmids (Table 2). Seven B6 strains appear to harbor only the Ti plasmid and do not accelerate the renaturation rate of pAtB6-806. However, under the conditions employed, specific sequences representing 5% or less of this plasmid would escape detection. Two strains, A217 and B6-Man, contain sequences homologous only to pAtB6-806,

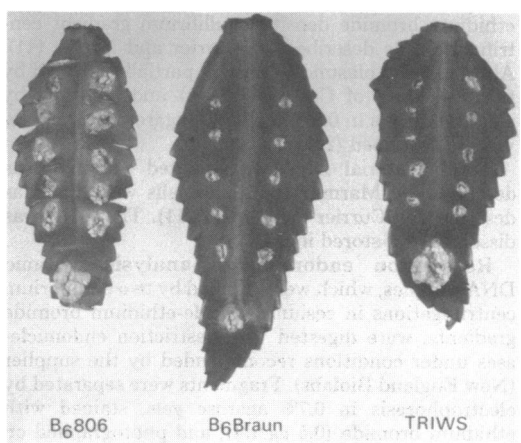

FIG. 1. *Tumor induction by B6 strains on Kalanchoe leaves. Each leaf was inoculated with 10 subclones of a B6 strain. In addition, each was inoculated at its most distal portion with a culture of the tumorigenic strain 15955. Plants were maintained under ambient conditions of light and temperature. Tumorous tissue began to appear 12 days postinfection.*

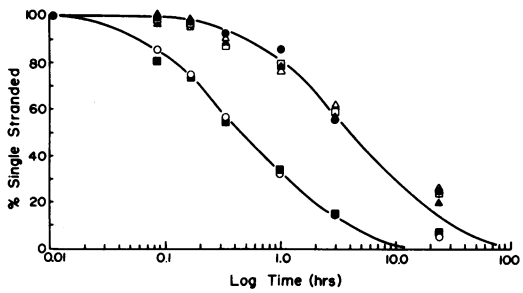

FIG. 2. *Renaturation kinetics of pAtB6-806 plasmid DNA in the presence of bacterial DNA from various B6 strains. The renaturation of tritiated pAtB6-806 DNA was measured as described in the text. Lines connecting the points depict ideal second-order kinetics curves. Symbols represent renaturation in the presence of 1 mg of total bacterial DNA per ml; the DNA was from E. coli strain J53 (●), strain B6-806 (■), strain 23308 (○), strain B6-Braun (▲), strain TR1WS (△), or strain B6-Miller (□).*

whereas DNA from strain A277 showed homology only with pTiB6-806. No strain examined lacked both plasmids.

Analysis of strains B6-Braun and TR1WS. It is clear from the data presented above that the inability of strains B6-Braun and TR1WS to induce tumors on *Kalanchoe* leaves cannot be attributed to the absence of either the Ti plasmid or the 120-megadalton cryptic plasmid. Nor could this trait be correlated with the presence or absence of the large (>300-megadalton) cryptic plasmid recently described by Casse et al. (5). When strains were analyzed by the technique of these authors, we observed that such a plasmid was present in strain B6-806 and also in strains B6-Braun and TR1WS (data not shown). Furthermore, it is unlikely that the inability to induce tumors on *Kalanchoe* is due to any major alterations in the Ti plasmids of these two strains. Restriction endonuclease cleavage patterns of pTiA277, pTiB6-Braun, and pTiTR1WS generated by *Sma*I, *Bam*HI, and *Hpa*I were indistinguishable (data not shown).

To determine the genetic location of the defect blocking tumorigenicity on *Kalanchoe*, the Ti plasmids from the representative strains were transferred by conjugation to strain SA110. With strains B6-Braun and TR1WS as donors, transconjugants were obtained at frequencies of approximately 10^{-6} (Table 3). However, with strain B6-806 as the donor, transfer frequencies were impossible to calculate because of the large numbers of rifampin- or neomycin-resistant donors appearing on the selective medium. Only three bonafide transconjugants were obtained from this latter cross. In all cases transconjugants were resistant to neomycin, rifampin, and nalidixic acid, failed to plaque bacteriophage PS8, and did not appear to be lysogenized with a bacteriophage which would plaque on strain B6-806. These traits are all consistent with the transconjugants being derived from strain SA110. All transconjugants tested were able to catabolize octopine, and the three transconjugants derived from strain B6-806 were all tumorigenic on both carrot disks and *Kalanchoe* leaves (Table 3). From the other two crosses, 16 of the 20 transconjugants derived from strain B6-Braun and 18 of the 20 derived from strain TR1WS were tumorigenic on carrot disks. These tumorigenic transconjugants, unlike their B6 parents, were also able to induce tumors on *Kalanchoe* leaves (Table 3).

DISCUSSION

It is clear from this study that there is diversity among the B6 strains of *A. tumefaciens*. Of the six criteria used for comparison, there was only one (3-ketolactose production) in which all 20 B6 strains examined gave identical reactions. Within the five remaining categories strain differences were observed.

With one exception, all B6 strains were either sensitive to or lysogenized by an omega-type bacteriophage (Table 2). The exception, strain B6-Heb, may have sustained a mutation leading to phage resistance. Alternatively, this strain may harbor a replication-defective prophage which continues to confer superinfection immunity. This strain is most likely a B6 derivative since it harbors sequences homologous to both pTiB6-806 and pAtB6-806. To our knowledge, strains B6-806 (2) and B6-S (26) are the only members of this collection specifically derived to be sensitive to bacteriophage PS8. The remaining sensitive strains may represent subcul-

TABLE 3. *Characteristics of B6 strains and their transconjugants*

Strain	Transfer frequency[a]	Octopine utilization[b]	Tumorigenicity on:[c]	
			Carrot	*Kalanchoe*
B6-806		20/20	20/20	20/20
SA110 (pTiB6-806)		3/3	3/3	3/3
B6-Braun	10^{-6}	20/20	20/20	0/20
SA110 (pTiB6-Braun)		20/20	16/20	16/20
TR1WS	10^{-6}	20/20	20/20	0/20
SA110 (pTiTR1WS)		20/20	18/20	18/20
SA110		0/20	0/20	0/20

[a] Expressed as the number of Oct$^+$ transconjugants per milliliter divided by the input donor titer.

[b] Expressed as the number of Oct$^+$ clones/number of clones tested.

[c] Expressed as the number of clones able to induce tumors/number of clones tested.

tures of either of these two strains acquired by other workers. For this reason, our data do not prove that PS8 can be lost by passage in culture.

As described by other workers (8, 18, 25, 31), the ability to utilize octopine correlates with the presence of the Ti plasmid (Table 2). The two strains unable to catabolize this arginine derivative were found to lack sequences homologous to pTiB6-806. It is known that strain A217 lacks pTiB6-806 (28). However, strain B6-Man was sent to us as a tumorigenic isolate and should therefore harbor a Ti plasmid (J. Lippincott, personal communication). This strain is most probably of B6 lineage, as indicated by its lysogeny with an omega-type bacteriophage and the presence of sequences complementary to pAtB6-806.

Consistent with the lack of a Ti plasmid, strains A217 and B6-Man failed to induce tumors on the two plant species tested. Sixteen of the remaining 18 strains proved highly tumorigenic, whereas two B6 strains, B6-Braun and TR1WS, induced tumors on carrot disks but not on *Kalanchoe* leaves (Table 2 and Fig. 1). These two strains produce well-formed unorganized tumors on tomato, tobacco, and sunflower plants (unpublished data). This apparent host range limitation could not be correlated with any other characteristic examined. Although both strains lack pAtB6-806, other isolates lacking this plasmid are tumorigenic on *Kalanchoe* (Table 2). Nor could such avirulence be attributed to the presence or absence of the large cryptic plasmid recently described by Casse et al. (5; data not shown). In addition, strain A277 appears to be fully tumorigenic, indicating that pTiB6-806 is sufficient to impart tumorigenicity in a background other than that of B6. Since strains B6-Braun and TR1WS differ in lysogeny, it is considered unlikely that this parameter influences host range.

It is clear from these results that some divergence has occurred among B6 strains from various laboratories. If one considers only three traits, lysogeny, tumorigenicity (i.e., presence of pTiB6-806), and the presence of pAtB6-806, the isolates fall into six of the eight possible groups. Variability with respect to these three traits cannot be readily explained. As described above, the bacteriophage-sensitive strains may all have been derived from strain B6-806 (2) or B6-S (25); these two differ from each other with respect to the cryptic plasmid pAtB6-806 (Table 2). These two isolates were independently derived from the same lysogenic strain, most probably B6-M (25; R. M. Klein, personal communication). Our data are consistent with such a derivation for strain B6-S. This isolate, like B6-M, lacks sequences homologous to pAtB6-806. On the other hand, strain B6-806 differs from both B6-S and B6-M in that it harbors the cryptic plasmid (Table 2) (28).

Further variability was found with respect to plasmid content. Strain 23308 contains sequences homologous to pAtB6-806. This strain, which can be obtained from the American Type Culture Collection, can be traced back to the laboratory of A. C. Braun. A strain (B6-Braun) obtained more recently from Braun lacks the cryptic plasmid (Table 2). Both are sensitive to bacteriophage PS8, but only strain 23308 appears to be able to induce tumors on *Kalanchoe* leaves.

Variability with respect to pAtB6-806 appears to be rather common. Of the 20 strains examined, 7 lacked detectable sequences homologous to this plasmid (Table 2 and Fig. 2). Whether this element was originally present in B6 or introduced after its dissemination to other laboratories is uncertain. However, the presence or absence of sequences homologous to this cryptic plasmid could not be correlated with any observable phenotype.

Finally, our data suggest that the lesions rendering strains B6-Braun and TR1WS nontumorigenic on *Kalanchoe* do not reside on their Ti plasmids. First, these plasmids, within the limitations of each technique, are physically identical to pTiB6-806, as judged by electrophoretic mobility in agarose gels (data not shown), DNA-DNA hybridization, and restriction endonuclease analysis (data not shown). Second, the Ti plasmids from both strains are genetically indistinguishable from pTiB6-806 in that when transferred into strain SA110, they confer octopine utilization and the ability to induce tumors on both carrot disks and *Kalanchoe* leaves. It is tempting to speculate that these two B6 derivatives harbor chromosomal defects rendering them nontumorigenic on the latter host plant. We are continuing to examine these two strains and their transconjugants to determine why not all progeny become tumorigenic upon inheritance of the Ti plasmid (Table 3). Preliminary results suggest that the Ti plasmids inherited by these transconjugants are structurally different from the Ti plasmid of the donor. Further investigation with these strains may provide information concerning the initial events and the specific role of the host plant in crown gall induction.

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ADDENDUM IN PROOF

Our most recent analysis shows that all 20 B6 strains

harbor a very large cryptic plasmid similar to that described by Casse et al. (5).

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