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# Characteristics of Ti Plasmids from Broad-Host-Range and Ecologically Specific Biotype 2 and 3 Strains of *Agrobacterium tumefaciens*

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*Agrobacterium tumefaciens* strains isolated from crown gall tumors on grapevines in California were consistently of the biotype 3 group. All 11 of these strains were limited in their host range and harbored Ti plasmids with molecular masses between 119 and 142 megadaltons (Mdal) as well as a larger cryptic plasmid of >200 Mdal; occasionally a smaller cryptic plasmid of 65 Mdal was also present. Ti plasmids of these strains have DNA sequences in common with Ti plasmids of octopine and nopaline strains belonging to the biotype 1 group and exhibited sequence homologies with the conserved region of the T-DNA. Ten of the 11 strains utilized octopine as a sole source of carbon and nitrogen and 3 strains catabolized both octopine and nopaline, whereas 1 strain catabolized only nopaline. All of these strains were resistant to the bacteriocin agrocin-84, except one grapevine strain that belonged to the biotype 1 group and was agrocin sensitive; it also differed in its plasmid and virulence characteristics. Isolations from *Rubus ursinus* ollalieberry galls yielded exclusively biotype 2 strains. These strains were insensitive to agrocin-84, utilized nopaline as a sole carbon and nitrogen source, and were highly virulent on all host plants tested. They contained Ti plasmids ranging between 100 and 130 Mdal and occasionally a cryptic plasmid of 69 Mdal. Their Ti plasmids have DNA sequences in common with Ti plasmids of biotype 1 strains and with the conserved region of the T-DNA.

Phenotypic variations among *Agrobacterium tumefaciens* strains are conferred by genes on the Ti plasmid, which determine traits such as opine utilization (3, 24), opine production in crown gall tumors (3, 10, 24), sensitivity to the bacteriocin agrocin-84 (9, 16, 34), and host range specificity (21, 31, 37). Differences in these features reflect variations among Ti plasmids. Of particular interest in our laboratory are those genes that determine host specificity. Certain strains, identified as biotype 3 organisms, appear to be unique in that they have been reported to be pathogens almost exclusively of grapevines (15, 26, 33). These limited-host-range strains have been shown not to contain the conserved region of the T-DNA (35), a specific segment of Ti plasmid DNA reported in all other Ti plasmids examined (4, 8).

*A. tumefaciens* strains, obtained from worldwide culture collections, showed variability in gall size and frequency of tumor formation, but had broad host ranges (K. L. Perry, S. Süle, and C. I. Kado, unpublished data). We reasoned that most strains in these collections were those selected on the basis of broad and stable pathogenic features and for their ability to produce 3-ketolactose. On the other hand, strains freshly isolated from nature might show greater host range diversities and variations in pathogenic features. The present communication demonstrates that *A. tumefaciens* strains, isolated from crown gall tumors on grapevines and *Rubus* sp. in California, exhibited relatively broad host ranges with minor variations. These studies also revealed the predominance of biotype 3 strains in grapevine and biotype 2 strains in *Rubus* sp., suggesting that these biotypes are ecologically host specific. Comparative analyses of their Ti plasmids revealed size variations and similar DNA sequence homologies, including those DNA sequences of the conserved region of the T-DNA. Portions of this work appeared in a preliminary report (K. L. Perry and C. I. Kado, Phytopathology 71:249, 1981).

## MATERIALS AND METHODS

**Media.** The following media allow growth of all biotypes: medium 928 (23 g of nutrient agar [Difco Laboratories, Detroit, Mich.], 5 g of mannitol, and 1 g of yeast extract [Difco] in 1 liter of distilled water); medium 523 (13); minimal medium 925 (17) supplemented with 1 g of yeast extract per liter. Selective media for given biotypes were: Schroth et al. medium (28); medium D1M (5.0 g of cellobiose, 1.0 g of $NH_4Cl$, 0.3 g of $MgSO_4 \cdot 7H_2O$, 3.0 g of $K_2HPO_4$, 1.0 g of

$NaH_2PO_4$, 0.01 g of malachite green, and 15.0 g of agar [Difco] in 1.0 liter of distilled water). Basal medium for opine studies consisted of filter-sterilized octopine (2 g; Sigma Chemical Co., St. Louis, Mo.) or nopaline (chemically synthesized as per Jensen et al. [12]; 3.0 g of $K_2HPO_4$, 1.0 g of $NaH_2PO_4$, 0.3 g of $MgSO_4 \cdot 7H_2O$, and 20.0 g of Noble agar in 1.0 liter of distilled water). Medium for biotyping consisted of 1% D-(+)-melezitose, *meso*-erythritol, D-(+)-cellobiose, or ethanol (200 proof; IMC Chemical Group, Inc., Agnew, Calif.), 1.5 g of $K_2HPO_4$, 0.5 g of $NaH_2PO_4$, 1.0 g of $NH_4Cl$, 0.3 g of $MgSO_4 \cdot 7H_2O$, 2.0 g of NaCl, 0.1 g of yeast extract, 10.5 mg of bromocresol purple, and 20 g of agar (Difco) per liter of distilled water. The medium was adjusted to pH 7.0 after autoclaving. Sodium malonate, sodium tartrate, and mucic acid (Sigma) were filter sterilized and added to a final concentration of 0.3% in the above basal medium. The indicator bromothymol blue (25 mg/liter) was used. KL medium consisted of 0.1% yeast extract, 1% lactose, and 1.5% agar (Difco) and was used to test for 3-ketolactose formation as described by Bernaerts and DeLey (2).

**Bacterial strains.** *A. tumefaciens* strains used in these studies are listed in Table 1. Strain NT1RE carried resistance to rifampin (100 μg/ml) and erythromycin (200 μg/ml). *Escherichia coli* RR1 isolates harboring pBR322-derived recombinant plasmids as described by Thomashow et al. (36) were graciously provided by E. W. Nester.

**Opine utilization.** Octopine [*N*-2-(D-1-carboxyethyl)-L-arginine] and nopaline [*N*-2-(D-1,3-dicarboxypropyl)-L-arginine] were tested for purity by paper electrophoresis (20) and used as a sole source of both carbon and nitrogen. Growth at 28°C was scored at 3 and 7 days.

**Biotyping.** The criteria used for grouping strains into a particular biotype were those of Süle (33) and Panagopoulos et al. (26).

**Pathogenicity.** Tests were conducted on at least four plants of each host and on at least two different inoculation dates. Young seedlings (when secondary leaves began to show) were grown under standard greenhouse conditions (17) and were inoculated in their stems. Inoculations were made at three sites per plant as described previously (17, 21). Crown gall tumor formation was scored 30 days after inoculation. Hosts with no tumors were held for an additional 2 to 3 months to ascertain the nonpathogenic response. Strains giving nonpathogenic responses were retested at least three times for verification.

**Host plants.** The host plants used and their sources are: *Lycopersicum esculentum* Mill., Earlipak tomato (Ferry Morse Seed Co.); *Kalanchöe daigremontiana* Hamet and Perrier (Plant Pathology, University of California, Davis); *Helianthus annuus* L., Russian Mammoth sunflower (W. Atlee Burpee Co.); *Vitis vinifera* L. Mission grapevine from a virus-free stock (Foundation Plant Materials, University of California, Davis); *Nicotiana tabacum* L., cv. Turkish tobacco (Plant Pathology, University of California, Davis); *Raphanus sativus* L., White Icicle radish (W. Atlee Burpee Co.); *Vicia faba* L., Windsor broadbean (W. H. Perron Ltd.); *Chenopodium amaranticolor* Coste and Reyn., goosefoot (Plant Pathology, University of California, Davis); *Tagetes patula* L., Senator Dirkson marigold (W. Atlee Burpee Co.).

**Agrocin-84 sensitivity.** Agrocin-84 was isolated and purified from *A. radiobacter* strain K84, and sensitivity to the bacteriocin was evaluated according to the methods of Süle and Kado (34). Alternatively, *A. radiobacter* strain K84 was spread over a 1-cm-diameter area in the center of a petri dish (100 by 15 mm) containing 20 ml of medium 925 agar without $NH_4Cl$ and supplemented with 0.6% mannitol, 0.2% glutamic acid, and 0.01% yeast extract. After 36 to 64 h of incubation at 22°C, the cells along with the 1.5-cm-diameter plug of agar underneath were removed. Immediately thereafter, 100 μl of log-phase cells of the test strain was spread over the agar surface. Inhibition zones were scored after 1, 2, and 3 days.

**Plasmid isolation and size estimation.** All plasmids were obtained by a rapid isolation procedure (14). Plasmid preparations were concentrated when necessary in vacuo at 690 mmHg (ca. 92 kPa) to reduce the volume by 50 to 75%. The plasmid preparations from *E. coli* were extracted twice with phenol-chloroform and then with ether, followed by precipitation with ethanol and resuspension in distilled water. Between 100 and 500 ng of plasmid DNA was recovered from 1 ml of *E. coli* cells in the late log phase of growth. All mixing and manipulations were minimized to preserve the integrity of very high-molecular-weight plasmids. Electrophoresis was performed at 12 to 16 V/cm on a horizontal gel apparatus, equipped with a cooling plate (14). The migration of plasmid samples was followed by observing the bromophenol purple tracking dye. $M_r$ estimations were made with the following plasmids of known molecular masses (in megadaltons [Mdal]) as standards: pTiC58, 120 ± 4.5 (39); pTR6, 154 ± 6 (29, 30); pTi27, 127 ± 5 and 153 ± 10 (6); pSW2, 69.8, 51.6, and 49.2 (5); pR6-5, 66 (1). Relative molecular masses were obtained from log-log plots of molecular mass versus migration (11, 23).

**In planta plasmid transfer.** Conjugation between virulent strains and an avirulent recipient, NT1RE, was performed on carrot root disks as described by Liao and Heberlein (18). Transconjugants harboring Ti plasmids were selected on the basis of opine utilization and antibiotic resistance and were verified by pathogenicity tests and agarose gel electrophoresis.

**Blot hybridization.** DNA-DNA reassociation analyses were performed according to Southern (32) as modified by Wahl et al. (38), except that the final washings of the DNA-bound B6 nitrocellulose membrane filter (Schleicher & Schuell, New York, N.Y.) were done for 12 to 16 h at 55°C in 0.1× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate–5 mM sodium pyrophosphate. Plasmid DNAs were labeled in vitro to $>25 \times 10^6$ cpm/μg of DNA with [$\alpha$-$^{32}$P]dATP, -dTTP, and -dCTP (Amersham Corp., Arlington Heights, Ill.) by nick translation (27). Hybridizations were performed for 12 to 16 h at 46°C for Ti plasmid probes or at 42°C for *E. coli* recombinant plasmid probes. The midpoint melting temperature was lowered by performing hybridizations in 50% formamide in 5× SSC–5× Denhardt reagent (7)–20 mM sodium phosphate (pH 6.5)–100 μg of sonicated, denatured salmon sperm DNA per ml–10% sodium dextran sulfate 500 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) at 46 and 42°C.

## RESULTS

**Virulence and host range.** New strains isolated from galls collected from the field proved to be

TABLE 1. Strains of *A. tumefaciens* used

| Strain | Original host and location | Source |
|---|---|---|
| Biotype 1 | | |
| Lodi 5 | Grapevine gall, San Joaquin Co., Calif. | This study |
| C58 | Wild type from cherry gall, Sodus Co., N.Y. | R. Dickey, Cornell University, Ithaca, N.Y. |
| NT1 | pTi plasmidless derivative of C58 | Watson et al., (39) |
| NT1RE | Rifampin- and erythromycin-resistant derivative of NT1 | This study |
| 15955 | Tomato gall, Davis, Calif. | J. E. DeVay, University of California, Davis |
| 1D135 | Soil under peach galls, Davis, Calif. | This laboratory |
| B6 | Tomato, Ames, Iowa | J. A. Lippincott, Northwestern University, Evanston, Ill.; via A. C. Braun (formerly from G. C. Kent [strain B901]) |
| Biotype 2 | | |
| 59A1 | *R. ursinus* ollalieberry gall, Santa Cruz Co., Calif. | This study |
| 60-6 | *R. ursinus* ollalieberry gall, Santa Cruz Co., Calif. | This study |
| 78B12 | *R. ursinus* ollalieberry gall, Santa Cruz Co., Calif. | This study |
| K84 | Soil around peach gall, South Australia | A. Kerr, Waite Agricultural Research Institute, Glen Osmond, South Australia |
| Biotype 3 | | |
| 9-3 | Grapevine var. Merlot gall, San Luis Obispo Co., Calif. | This study |
| 14-1 | Grapevine var. Merlot gall, San Luis Obispo Co., Calif. | This study |
| 23-4 | Grapevine var. Zinfandel gall, San Luis Obispo Co., Calif. | This study |
| 24-2 | Grapevine var. Zinfandel gall, San Luis Obispo Co., Calif. | This study |
| 37G4 | Grapevine var. Chenin Blanc gall, San Joaquin Co., Calif. | This study |
| 52BA1 | Grapevine var. Cabernet Sauvignon gall, Monterey Co., Calif. | This study |
| 56A2 | Grapevine var. Johannisberg Riesling gall, Monterey Co., Calif. | This study |
| 58-1 | Grapevine var. Cabernet Sauvignon gall, Monterey Co., Calif. | This study |
| 70D4 | Grapevine var. Sauvignon Blanc gall, Monterey Co., Calif. | This study |
| 73B4 | Grapevine var. Sauvignon Blanc gall, Monterey Co., Calif. | This study |
| 77B2 | Grapevine var. Sauvignon Blanc gall, Monterey Co., Calif. | This study |
| 15/1 | Grapevine gall, Hungary | S. Süle, Research Institute for Plant Protection, Budapest |

broad-host-range pathogens (Table 2). Strains were scored as pathogenic if galls were induced reproducibly, regardless of the size of tumors and the efficiency of tumor induction. Differences were noted in gall formation (e.g., gall size and the time required for gall development) depending on the host used. Large tumors were induced on commonly used test plants such as tomato, sunflower, *Kalanchöe*, and tobacco. On the other hand, radish and broadbean were relatively refractory to infection by biotype 3 strains, whereas strong gall formation was induced with biotype 2 strains. The single biotype 1 grapevine strain, Lodi 5, differed from all other strains by inducing small tumors on positively responding hosts.

**Biotype identification.** All but one (Lodi 5) of the grapevine strains were biotype 3, as characterized by the utilization of tartrate and malonate, a negative 3-ketolactose reaction, the inability to utilize erythritol, melezitose, ethanol, and mucic acid, and lack of growth on medium D1M and Schroth et al. medium. By contrast, biotype 2 strains catabolized erythritol, mucic acid, tar-

TABLE 2. Host range of *A. tumefaciens* biotype 1, 2, and 3 strains

| Biotype | Strain | Gall formation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sunflower | Tomato | *Kalanchöe* | Marigold | *Chenopodium* | Tobacco | Grape | Radish | Broadbean |
| 1 | Lodi 5 | + | ±[a] | + | ± | − | + | + | − | − |
| 2 | 59A1 | + | + | + | + | + | + | + | + | + |
| | 60-6 | + | + | + | + | + | + | + | + | + |
| | 78B12 | + | + | + | + | + | + | + | + | + |
| 3 | 9-3 | + | + | + | + | + | + | + | − | + |
| | 14-1 | + | + | + | + | + | + | + | − | + |
| | 23-4 | + | + | + | + | + | + | + | − | + |
| | 24-2 | + | + | + | + | + | + | + | ± | − |
| | 37G4 | + | + | + | + | + | + | + | − | − |
| | 52BA1 | + | + | + | + | + | + | + | ± | + |
| | 56A2 | + | + | + | + | + | + | + | − | − |
| | 58-1 | + | + | + | + | + | + | + | ± | + |
| | 70D4 | + | + | + | + | + | + | + | − | + |
| | 73B4 | + | + | + | + | + | + | + | − | + |
| | 77B2 | + | + | + | + | + | + | + | ± | + |

[a] Indicates very weak or inconsistent (or both) gall formation.

trate, and malonate and grew on medium D1M. The biotype 1 strain Lodi 5 differed from either group by a positive 3-ketolactose reaction, but utilized tartrate and malonate, which are characteristics of biotype 3 strains. According to the biotyping scheme of Süle (33), Lodi 5 might be classified as a biotype 3 strain.

**Opine catabolism.** Biotype 3 strains were typically octopine catabolizers (except 70D4). Three of 12 grapevine strains (9-3, 14-1, and 52BA1) catabolized octopine and nopaline, and 1 (70D4) utilized nopaline but not octopine.

Ti plasmids were transferred by mating with recipient strain NT1RE. Transconjugants receiving biotype 2 and 3 Ti plasmids grew on nopaline and octopine, respectively, indicating that these opine utilization functions were plasmid coded (see below).

**Bacteriocin sensitivity.** Only biotype 1 strains (Lodi 5, C58, and 1D135) were sensitive to agrocin-84. Biotype 2 strains from *Rubus* and the biotype 3 strains from grapevine were completely insensitive to purified agrocin-84 and to agrocin-84 released in agar medium by *A. radiobacter* strain 84. Biotype 1 and 2 strains can be either sensitive or resistant to agrocin-84 (15, 16). In contrast, all biotype 3 strains previously reported (26) and those studied here were resistant to agrocin-84.

**Plasmid profiles.** Plasmids in the new isolates were heterogeneous in size and number (Fig. 1 and 2). At least one large plasmid of ≥100 Mdal was observed in all strains (Table 3). All biotype 3 strains harbored very large ($M_r \geq 200 \times 10^6$) plasmids that were not observed in the biotype 2 strains. Only four strains harbored smaller plasmids. Linear extrapolations above 140 Mdal underestimate the size of plasmids (11); therefore, no specific estimates were attempted for the large plasmids. Ti plasmids, identified by hybridization analyses (see below), in biotype 3 strains ranged from 102 to 142 Mdal, a range that is not strikingly different from those in strains of biotypes 1 and 2.

**Biotype 2 and 3 Ti plasmids transfer in planta.** In planta matings showed that biotype 2 and 3 Ti plasmids were readily transferred to NT1RE. These plasmids, pTi60-6 and pTi58-1 from strains 60-6 and 58-1, conferred pathogenicity on NT1RE. Agarose gel electrophoresis showed that only the 100- and 135-Mdal Ti plasmids were transferred into NT1RE. Both pathogenicity on sunflowers (data not shown) and octopine utilization for 58-1 and nopaline utilization for 60-6, known functions of Ti plasmids, were expressed by the transconjugants.

**Homologies between Ti plasmids.** Nopaline Ti plasmid pTiC58 and octopine Ti plasmid pTi15955 DNAs, radioactively labeled as probes, hybridized to plasmids of biotype 2 and 3 strains (Fig. 1b and c). The pTi DNA probes did not bind to chromosomal or cryptic plasmid DNAs of either group. The Ti plasmid in strains harboring several plasmids was also distinguished, since the probes bound specifically to only one plasmid (Fig. 1b and c, Fig. 2b and c). These autoradiographs indicated that Ti plasmids of biotype 3 strains have DNA sequences in common with both octopine and nopaline Ti plasmids of biotype 1 strains. These data also revealed that Ti plasmid DNA sequences of biotype 2 and 3 strains are not distributed on any of the cryptic plasmids, but essentially are conserved on the Ti plasmids.

**T-DNA is conserved in biotype 2 and 3 Ti plasmids.** Blot hybridizations, using hybrid plasmid pNW31C-8, 29-1, which comprises *Bam*HI fragments 8 and 29 containing the highly conserved region of the T-DNA of octopine Ti plasmid of strain $B_6$806 (36), revealed that these sequences were present in Ti plasmids of biotype 2 and 3 strains (Fig. 3c). The binding of this probe to pTi58-1 was weaker than to other Ti plasmids (Fig. 3c, lane 8), suggesting that the T-DNA may be less conserved in this strain. This experiment furthers the notion that these sequences are conserved on broad-host-range Ti plasmids (4, 8, 35) independent of the biotype or the chromosomal background. Conversely, using hybrid plasmid pNW31C-2,19-1 (a cloned T-DNA *Bam*HI fragments 2 and 19 from the right-hand side of the T-DNA of pTi$B_6$806 [36]), little homology was observed with octopine and nopaline Ti plasmids of our biotype 3 or biotype 2 strains under the relatively stringent hybridization conditions used (i.e., midpoint melting temperature of 21°C), although some binding could be observed (Fig. 3b). This limited amount of homology (based on intensities of the blots relative to controls) with other octopine strains supports the premise (35) that octopine-type plasmids may be a heterogeneous group.

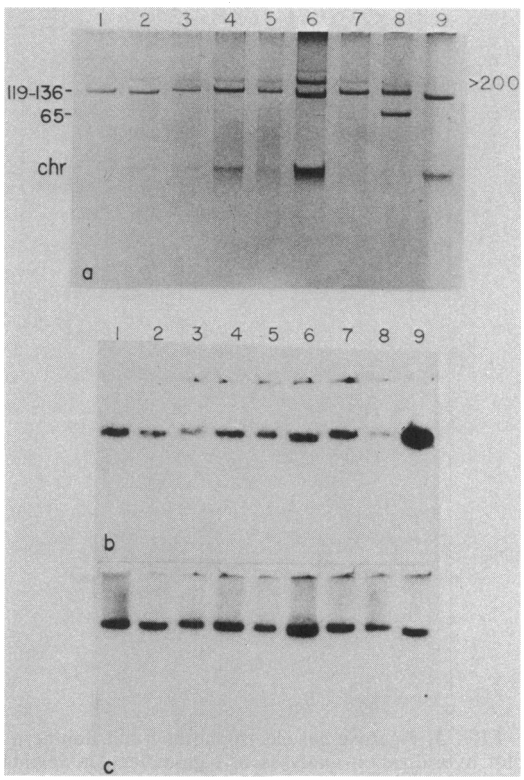


FIG. 1. Agarose gel electrophoretic and Southern blot hybridization analyses of high-molecular-weight plasmids of *A. tumefaciens* biotype 3 strains. (a) Agarose gel electrophoretogram stained with ethidium bromide; (b) and (c) modified Southern blot hybridizations with $^{32}$P-labeled C58 (b) and 15955 (c) Ti plasmid DNAs as probes. Numbers at sides indicate megadaltons; chr, chromosomal and linear DNA. Lanes 1 to 9 are biotype 3 strains 9-3, 14-1, 23-4, 24-2, 37G4, 52BA1, 56A2, 58-1, and C58, respectively.

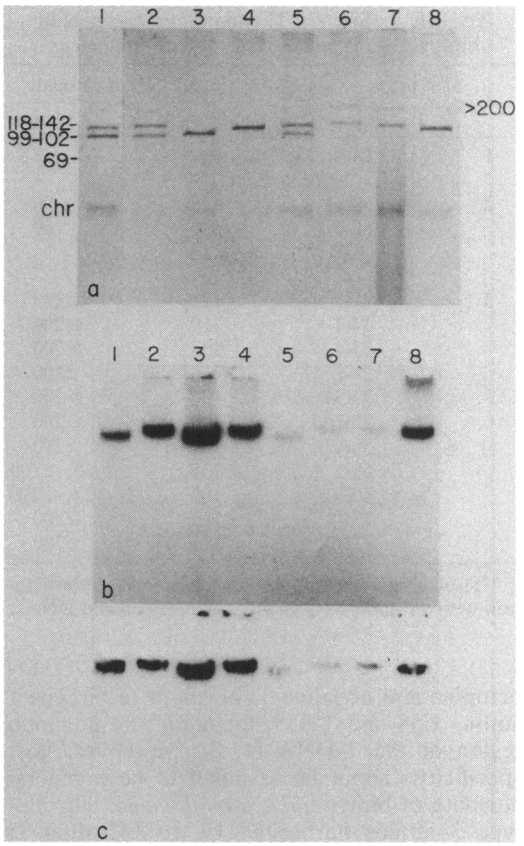


FIG. 2. Agarose gel electrophoretic and Southern blot hybridization analyses of high-molecular-weight plasmids of *A. tumefaciens* strains. (a) Agarose gel electrophoretogram; (b and c) modified Southern blot hybridizations with $^{32}$P-labeled C58 (b) and 15955 (c) Ti plasmids DNAs as probes. Numbers at sides indicate megadaltons; chr, chromosomal and linear DNA. For strains with multiple plasmids, see Table 3 for the pTi molecular weights. Lanes 1 and 8 are biotype 1 strains Lodi 5 and C58; lanes 2 to 4 are biotype 2 strains 59A1, 60-6, and 78B12; and lanes 5 to 7 are biotype 3 strains 70D4, 73B4, and 77B2, respectively.

## DISCUSSION

We have shown that biotype 3 strains from grapevine galls, collected in California, are not host specific or limited in host range. Their Ti plasmids share DNA sequences in common with

TABLE 3. Sizes of Ti and cryptic plasmids in biotype 1, 2, and 3 strains of *A. tumefaciens*

| Biotype | Strain | $M_r$ ($10^6$) of plasmid: | |
|---|---|---|---|
| | | pTi[a] | Cryptic |
| 1 | Lodi 5 | 99 | 123 |
| 2 | 59A1 | 128 | 99 |
| | 60-6 | 100 | 69 |
| | 78B12 | 118 | |
| 3 | 9-3 | 124 | ≥200 |
| | 14-1 | 119 | ≥200 |
| | 23-4 | 137 | ≥200 |
| | 24-2 | 136 | ≥200 |
| | 37G4 | 136 | ≥200 |
| | 52BA1 | 120 | ≥200 |
| | 56A2 | 136 | ≥200 |
| | 58-1 | 135 | 65, ≥200 |
| | 70D4 | 102 | 127, ≥200 |
| | 73B4 | 142 | ≥200 |
| | 77B2 | 131 | ≥200 |

[a] Ti plasmids determined by DNA-DNA hybridization with $^{32}$P-labeled C58 and 15955 Ti plasmid DNAs.

octopine and nopaline Ti plasmids of biotype 1 strains C58 and 15955, including the common region of the T-DNA (4, 8). Moreover, host specificity cannot be assumed to be a general attribute of biotype 3 *A. tumefaciens*. Our biotype 3 strains harbor a 119- to 142-Mdal Ti plasmid (Table 3). They also carry a large (≥200 Mdal) plasmid, and strain 58-1 has a third 65-Mdal cryptic plasmid. The plasmid profiles of our isolates differ from those of biotype 3 strains isolated in Greece and the USSR (37) in that the latter have two, not one, 130- to 140-Mdal plasmids and apparently lack the larger-molecular-weight (≥200 Mdal) plasmid. Thus, no generalizations can be made about plasmid profiles of the biotype 3 strains as a group.

Thomashow et al. (37) reported that Ti plasmids of biotype 3 strains, mainly limited to grapevine, do not contain the common DNA sequences found on all other Ti plasmids studied to date. This unique property is correlated with host range rather than biotype because our biotype 3 Ti plasmids contained these sequences (Fig. 3c) and were not limited to grapevines. Analysis of the T-DNA showed limited hybrid formation between these wild-type Ti plasmids and cloned DNA sequences representing the right side of the T-DNA of pTiB$_6$806. Although hybridizations to pTiC58 and pTi60-6 were slightly greater than to the rest of the wild-type strains (Fig. 3b, lanes 1 and 3), we conclude that this region is not well conserved in our strains.

The lack of sensitivity to agrocin-84 of our biotype 3 strains was also observed in grapevine strains in Europe (26) and New York (T. Burr and B. Hurwitz, Phytopathology 71:163, 1981). Insensitivity to agrocin-84 seems to be a general characteristic of biotype 3 Ti plasmids. Also, octopine catabolism is a general property of our biotype 3 strains and those reported elsewhere (26). That some strains also catabolize nopaline and that one strain, 70D4, utilizes nopaline but not octopine indicate a greater degree of opine variability among biotype 3 grape strains than suggested by studies of European, Australian, Greek, and Russian strains.

These variations among *A. tumefaciens* strains suggest that a continuum of phenotypes exists which span biotypes 1 and 2. The major significance of biotype 3 strains is not in their physiological or biochemical characteristics per se, but rather in the biological specificity or association with grapevines. The reports of bio-

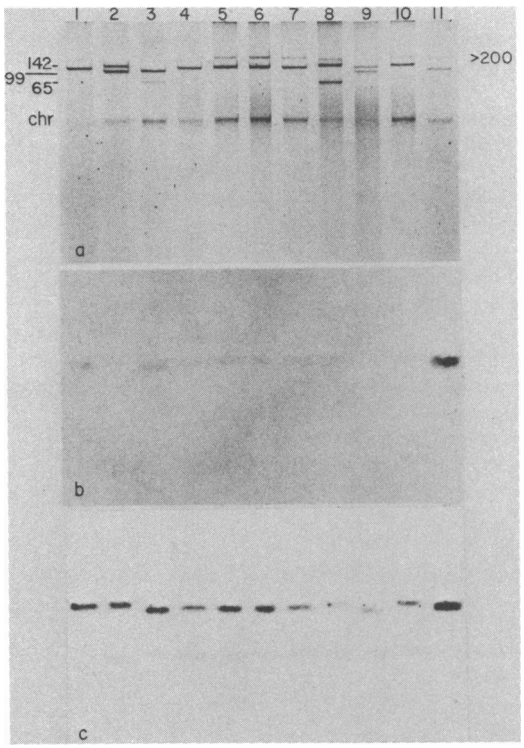


FIG. 3. Agarose gel electrophoretic and Southern blot hybridization analyses of high-molecular-weight plasmids of *A. tumefaciens* strains for T-DNA sequence homologies. (a) Agarose gel electrophoretogram stained with ethidium bromide; (b and c) blot hybridizations with $^{32}$P-labeled cloned T-DNA hybrid plasmids pNW31C-2, 19-1 (b), and pNW31C-8, 29-1 (c) (36), as probes. Numbers at sides indicate megadaltons; chr, chromosomal and linear DNA. Lanes 1 and 11 are well-characterized biotype 1 strains C58 and B6; lanes 2 to 4 are biotype 2 strains 59A1, 60-6, and 78B12; and lanes 5 to 10 are biotype 3 strains 9-3, 14-1, 52BA1, 58-1, 70D4, and 77B2, respectively.

type 3 strains from grapevine galls in Greece (15, 26), Europe, Australia, the USSR (26, 33), South Africa (22), New York (T. Burr, personal communication), and now California suggest that these strains may be specifically selected by the host. Previous reports of host specificity of grapevine strains (21, 26, 33), support this hypothesis, although some European grapevine strains (26; S. Süle, personal communication) and the California strains reported here are not host limited. To date, all reports (with a single exception [33]) indicate grapevine to be the original host. It should be noted that biotype 1 and 2 strains also naturally infect grapevines (26, 33). The question remains as to why biotype 3 strains are isolated almost exclusively from grapevine. The association of a specific biotype with a specific host is also observed with our biotype 2 strains, which were all isolated from *Rubus ursinus* (ollalieberry). These strains were highly virulent, inducing galls consistently larger than those induced by biotype 3 strains and at least as large as galls induced by biotype 1 strains C58 and 15955. Crown gall formations in radish and broadbean were particularly distinctive when compared with the hypovirulence of biotype 3 strains (Table 2).

Despite the broad-host-range trait of our isolates, the recovery of a specific biotype on a particular type of host suggests that selective pressures for a given ecological niche is operating in nature. The nature of this natural selection is probably dictated by the host plant.

## ACKNOWLEDGMENTS

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