Characterization of *Agrobacterium vitis* Strains Isolated from Turkish Grape Cultivars in the Central Anatolia Region

N. Argun, Department of Plant Protection, Agricultural Faculty of Ankara, 06110, Ankara, Turkey; **M. T. Momol,** Department of Plant Pathology, NFREC, IFAS, University of Florida, Quincy 32351; **S. Maden,** Department of Plant Protection, Agricultural Faculty of Ankara; **E. A. Momol** and **C. L. Reid,** Department of Plant Pathology, NYSAES, Cornell University, Geneva NY 14456; **H. Çelik,** Department of Horticulture, Agricultural Faculty of Ankara; and **T. J. Burr,** Department of Plant Pathology, NYSAES, Cornell University

ABSTRACT

Argun, N., Momol, M. T., Maden, S., Momol, E. A., Reid, C. L., Çelik, H., and Burr, T. J. 2002. Characterization of *Agrobacterium vitis* strains isolated from Turkish grape cultivars in the Central Anatolia Region. Plant Dis. 86:162-166.

Crown gall was detected in several vineyards in the Central Anatolia region of Turkey. Vineyards were planted to cultivars of grape that originated in Turkey and that were not grafted. The predominant species isolated from galls consisted of tumorigenic strains of *Agrobacterium vitis*. They were identified based on reactions to standard biochemical and physiological tests, by polymerase chain reaction amplification of specific Ti plasmid and chromosomal sequences, and by reaction to a species-specific monoclonal antibody. All strains utilized octopine, suggesting that they may carry similar types of Ti plasmids. Some of the strains exhibited a differential host range compared with others and were less virulent based on the numbers of galls that they induced on grape. When grapevines were treated with nontumorigenic *A. vitis* strain F2/5 prior to inoculation with the Turkish *A. vitis* strains, crown gall was effectively controlled. The genetic diversity of strains was evaluated by comparing DNA fingerprints that were generated by restriction enzyme digestion of the intergenic spacer region that lies between 16S and 23S rRNA genes. They segregated into two main groups, one that is similar to previously identified *A. vitis* strains carrying octopine type Ti plasmids and one that was more similar to strains carrying nopaline and vitopine Ti plasmids. The strains of *A. vitis* from Turkey may represent ancestral forms of the pathogen that will provide insight into the evolution of the bacterium.

Additional keywords: *Agrobacterium tumefaciens*, evolution, phylogeny

Crown gall is one of the most important bacterial diseases of grapevines worldwide and is especially debilitating on cultivars of *Vitis vinifera* (3). *Agrobacterium vitis* is the predominant species that causes the disease, although *A. tumefaciens* is occasionally isolated from infected vines. Typically, infections are initiated at wound sites on trunks and canes that are caused by freezing temperatures. Wounds release various chemical signals that are perceived by the bacterium and result in the induction of virulence (*vir*) genes on the Ti plasmid. Products of *vir* genes are responsible for the packaging and transport of another part of the plasmid, the T-DNA, from the bacterium to the plant cell where it is integrated into the plant genome and expressed. Infections occur following expression of various T-DNA genes which result in production of plant hormones that stimulate growth of gall tissue (25).

Four major T-DNA structures have been characterized in strains of *A. vitis*. They differ by their numbers of T-DNAs (delineated by characteristic border sequences) and by gene composition (6). Strains are often referred to by the type of opine synthase gene or genes they carry on their Ti plasmids (i.e., nopaline [N], vitopine [V], or octopine and cuccumopine [OC]). The OC strains are further grouped depending on whether their T-DNA TA region is large (OL) or small (OS). Phylogenetic models of *A. vitis* Ti plasmids have been developed by Otten and coworkers that are based on T-DNA structures, homology of oncogenes to those on other Ti plasmids, and the pattern of insertion by various insertion sequence (IS) elements (21).

It was discovered that the type of Ti plasmid that is carried by an *A. vitis* strain is highly correlated with the restriction fragment fingerprints derived from its intergenic spacer region (ITS) that lies between the 16s and 23s rRNA genes (22). Similar correlation was shown between Ti plasmid type and restriction fragment fingerprints generated from the 5′ region of the 23s gene (14).

In this article, we characterized a group of *Agrobacterium* strains that were isolated from crown gall–infected grapevines in Turkey. Samples were collected from vineyards in the Central Anatolia region that were planted to cultivars of *V. vinifera* that originated in Turkey. High incidences of crown gall were found in several vineyards. Because Turkey is considered an origin of *V. vinifera* (18), strains of *A. vitis* that were isolated from the region may provide clues as to the ancestral origin of this bacterium.

MATERIAL AND METHODS

Isolation of *A. vitis*. Samples were collected from vineyards located in the provinces of Nevşehir, Ankara, and Kırıkkale in the Central Anatolia region of Turkey in November and December 1999. Vineyards were generally less than 1 ha in size and consisted of *V. vinifera* cultivars that originated in Turkey, including Ereğli Çavuşu, Dimrit, Emir, Hasandede, Kalecik, Karası, and Narince. Trunks and canes with typical crown galls were collected, placed in plastic bags, and transported to the laboratory. To isolate *Agrobacterium* spp., surfaces of the galls were removed with a scalpel and sections of live gall tissue were triturated in 1 to 2 ml of sterile distilled water (SDW). Loopfuls of the gall suspensions were streaked on a semiselective medium for *A. vitis* (RS; 5). After 5 to 6 days at 28°C, colonies typical of *A. vitis* (smooth, translucent with red-pigmented centers) were streaked on potato dextrose agar (PDA). Isolates still resembling *A. vitis* were saved for further characterization. All isolates were maintained on PDA.

Characterization of strains. Isolates that produced typical colony types on RS and PDA were evaluated for their reaction to an *A. vitis*-specific monoclonal antibody (2). Tumorigenicity was determined on tobacco, tomato, and sunflower plants by puncturing stems with a needle that was dipped in a 48 h-old PDA culture. Rooted grape cuttings of *V. vinifera* cv. Chardonnay also were inoculated as previously described (7). Strains that reacted with the antibody and produced galls on any of the indicator plants were further compared with a standard set of biochemical and physiological tests that differentiate tumorigenic *Agrobacterium* spp. (16). These

Corresponding author: T. J. Burr
E-mail: tjb1@cornell.edu

Accepted for publication 11 October 2001.

Publication no. D-2001-1210-02R

include evaluation of 3-ketolactose production, alkali in litmus milk, growth on 2 and 4% NaCl, growth at 37°C, acid from erythritol, acid from melezitose, alkali from malonic acid, and alkali from L-tartaric acid. Strains were also evaluated for production of polygalacturonase (23) and endoglucanase (20) and for their ability to utilize octopine and nopaline (11). Reactions of *A. vitis* strain CG49, *A. tumefaciens* strain CG632, and A. *rhizogenes* strain K84 were compared with those of strains recovered in this study. All experiments were repeated at least once.

The ability of strains to cause necrosis (4) was determined on grape shoot explants. Actively growing shoots from potted vines in the greenhouse were harvested, then surface disinfected by submersion in 10% bleach for 10 min followed by rinsing thoroughly with sterile distilled water. Internodal sections (about 7 mm in length) were cut and supported vertically in 1% water agar in petri dishes. The exposed ends of the explants were inoculated with 2 µl of aqueous bacterial suspensions made to optical density $(OD)_{600}$ = 0.1 (about 10^8 CFU/ml). The appearance of necrosis was recorded after 72 h. Assays were repeated at least once.

Comparison of strains by polymerase chain reaction. Bacterial growth, DNA isolation, and polymerase chain reaction (PCR) procedures were done as previously reported (10) with annealing temperature for all reactions of 60°C. PCR was conducted with three different primer pairs that amplify characteristic fragment sizes from genes from *A. vitis*. Primers derived from *A. vitis pehA* (a chromosomal gene) amplify a 199-bp product from all tumorigenic and nontumorigenic *A. vitis* strains but not from *A. tumefaciens*. Primers derived from the *virA* gene of *A. tumefaciens* (denotes presence of a Ti plasmid) amplify a 480-bp product from *A. vitis* and *A. tumefaciens* strains. The *virE2* primers, derived from *A. tumefaciens* strain C58, were used because they amplify a product of about 1-kb from *A. vitis* strains carrying V type Ti plasmids but not from those carrying N or O type Ti plasmids (15). Sequences of *pehA* and *virA* primers (10) have also been published.

Sensitivity of strains to biological control. Nontumorigenic *A. vitis* strain F2/5 inhibits crown gall infection by an unknown mechanism that is specific to grape (9). F2/5 is generally most effective when applied to the grape wounds prior to inoculating with the tumorigenic strain. We wished to determine whether F2/5 is effective for preventing infection by the isolates from Turkey. Inoculations on potted grapevines (cv. Chardonnay) were done as previously described (7). The woody stems of rooted cuttings were inoculated by drilling holes and then applying 50 µl of bacterial suspensions adjusted to OD_{600} = 0.1. F2/5 was applied to the wounds at the same time that tumorigenic strains were applied in one experiment and 24 h prior to the pathogens in another. Water was applied as a negative control. Tumorigenic strain CG49 (known to be sensitive to F2/5) was compared to the Turkish strains. Three inoculations were made per vine and three vines were used per strain. The experiments were repeated once.

Analysis of restriction fragments from the ITS between 16S and 23S rRNA genes. The DNA sequence of the *A. vitis* ITS region that lies between the 16S and 23S rRNA genes is highly variable and therefore suitable for measuring diversity among bacterial strains (1). We previously grouped *A. vitis* strains based on ITS fingerprints and determined that the fingerprint of a strain correlated positively with the type of Ti plasmid it carried (22). Groups identified by fingerprinting the 5′ region of the 23S rRNA gene were almost identical to those identified by ITS fingerprints, strengthening the concept that these methods are reliable for identifying genetic groups of the bacterium (14). For analysis of the Turkish strains, bacterial culture, DNA isolation, and restriction analysis of PCR-amplified ITS regions were done as reported previously (22). PCR amplifications were carried out in 100-µl volumes and amplification products were purified with Millipore regenerated cellulose columns. Amplicons were digested with *Rsa*I, *Taq*I, *Cfo*I, *Hae*III, and *Ava*I (Promega Corp., Madison, WI) and the digests were analyzed by 2% agarose gel electrophoresis in Tris-borate-EDTA buffer. Gels were stained with ethidium bromide at 0.5 µg/ml.

Similarities between strains were computed based on the presence or absence of restriction fragments employing previously described methods (22). To determine the relationships between strains, calculated similarity coefficients were used in an

Table 1. Bacterial strains of *Agrobacterium* spp. used in this study

Strain	Origin (reference)	Identity/Ti type[a]
Tr1, Tr2, Tr3, Tr4, Tr6, Tr7	Turkey, Nevşehir	*A. vitis* / O
Tr5, Tr15	Turkey, Ankara	*A. vitis* / O
Tr8	Turkey, Ankara	*A. tumefaciens*
CG49	United States, NY (22)	*A. vitis* / N
CG470	United States, WA (22)	*A. vitis* / N
CG78	United States, NY (22)	*A. vitis* / V
CG450	United States, NY (22)	*A. vitis* / V
CG475	United States, NM (22)	*A. vitis* / OS
K309	Australia (19)	*A. vitis* / OL
CG632	United States, NY	*A. tumefaciens*
K84	Australia (12)	*A. rhizogenes* nontumorigenic

[a] Strains of *A. vitis* carry Ti plasmids with genes for utilization of octopine (O), nopaline (N), or vitopine (V). OL and OS = octopine type Ti plasmids with large and small TA regions, respectively.

Table 2. Characterization of strains from Turkey as compared to known strains of *Agrobacterium vitis*, *A. tumefaciens*, and *A. rhizogenes*[a]

	Strain										
Test	Tr1	Tr2	Tr7	Tr6	Tr4	Tr3	Tr5	Tr15	CG49	CG632	K84
Alkali in litmus milk	−	−	+	−	−	−	−	−	+	−	−
Growth on 2% NaCl	+	+	+	+	+	+	+	+	+	+	−
Growth on 4% NaCl	−	−	−	−	−	−	−	−	−	−	−
Growth at 37°C	−	−	−	−	−	−	−	−	−	−	−
3-Ketolactose	−	−	−	−	−	−	−	−	−	+	−
Acid from erythritol	−	−	−	−	−	−	−	−	−	−	+
Acid from melezitose	−	−	−	−	−	−	−	−	−	+	−
Alkali from malonic acid	+	+	+	+	+	+	+	+	+	−	+
Alkali from L-tartaric acid	+	+	+	+	+	+	+	NT	+	−	+
Utilization of octopine	+	+	+	+	+	+	+	+	−	NT	NT
Utilization of nopaline	−	−	−	−	−	−	−	−	+	NT	NT
Endogluconase production	+	+	+	+	+	+	+	+	+	−	−
Polygalacturonase production	+	+	+	+	+	+	+	+	+	−	−
Reaction with *A. vitis* antibody	+	+	+	+	+	+	+	+	+	NT	NT

[a] NT = not tested.

unweighted pair group method using an arithmetic means cluster analysis procedure (24) that is an option of the software program NTSYS-pc (17).

RESULTS AND DISCUSSION

Isolation and characterization. Crown gall was observed in the majority of the vineyards examined in the Central Anatolia region. We generally sampled from small vineyards on the outskirts of villages. Vines sampled in Nevşehir were not grafted or trellised and were pruned to have several fruiting canes. Tumorigenic strains of *A. vitis* were isolated from crown galls collected in both the provinces of Nevşehir and Ankara (Table 1). The strains produced typical colonies on RS medium, reacted positively with the *A. vitis* antibody, produced polygalacturonase and endoglucanase, and induced necrosis on grape explants. They also conformed closely to the established set of biochemical and physiological tests that are used to differentiate *A. vitis* from other *Agrobacterium* spp. (Table 2). The Turkish strains all utilized octopine, indicating that they may harbor similar types of Ti plasmids (22).

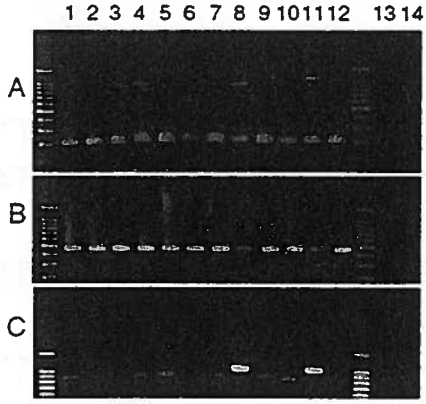

Fig. 1. Analysis of Turkish strains by polymerase chain reaction. **A,** Primers derived from *pehA* of *Agrobacterium vitis* amplify a 200-bp product from all strains; **B,** *virA* primers amplify a 480-bp product from all strains; **C,** *virE2* primers amplify a product of about 1 kb from strains CG78 and CG450, both of which carry V type Ti plasmids. Lane numbers correspond to the following strains: 1, Tr1; 2, Tr2; 3, Tr6; 4, Tr4; 5, Tr3; 6, Tr7; 7, Tr5; 8, CG450; 9, CG470; 10, CG475; 11, CG78; 12, K309; 13, K84; 14, H_2O. Bands in size-marker lanes represent increments of 100 bp.

In PCR reactions, all of the Turkish strains amplified a product of appropriate 200 bp with *pehA* primers, thus supporting further their identity as *A. vitis* (Fig. 1A). They all also yielded a characteristic 480-bp product from the *virA* primers, indicating that they carry Ti plasmids (Fig. 1B). In this case, the V strains, CG78 and CG450, produced a weaker signal than the other strains, which supports our previous findings that V strains produce a weak or no signal with *virA* primers depending on annealing temperature (T. J. Burr, *unpublished data*). The *virE2* primers gave a strong product of about 1 kb from V strains CG450 and CG78 but no product of the same size from any of the other strains (Fig. 1C). This also supports previous findings (15) and indicates that Turkish strains probably do not carry V type Ti plasmids.

All of the strains except Tr15 were found to be tumorigenic on grape, tobacco, and sunflower. All except Tr2 ,Tr5, Tr7, and Tr15 produced galls on tomato (Table 3). This difference in host range is interesting and is one indication of the diversity among the strains. Host range differences between *A. vitis* strains have been previously reported and are known to be associated with Ti plasmid structures. For example, strains carrying the OS type of Ti plasmid (with small TA region) have a limited host range compared with those with large TA regions (13). Further characterization of the Ti plasmids from the Turkish strains is needed to determine whether Ti structure among these strains is associated with host range differences.

Eight *A. tumefaciens* strains also were isolated from grape galls as determined by colony type on RS and PDA (colonies are more mucoid that *A. vitis*), a positive reaction for 3-keto-lactose production and a negative response to the *A. vitis*-specific antibody (*data not shown*). One of these strains caused gall formation when inoculated to tobacco, utilized octopine, and did not produce polygalacturonase. This again suggests that *A. tumefaciens* may be associated with grape crown galls but is less predominant than *A. vitis*.

Sensitivity of strains to biological control. The Turkish strains varied with regard to the severity of crown gall that they caused on grape (Table 3). For example, Tr1 produced fewer and smaller galls that Tr2. Such findings are common when evaluating groups of *A. vitis* (T. J. Burr, *unpublished data*) and may be related to differences in oncogene or *vir* gene makeup or to any of the several processes that are known to play a role in the infection process (25). When F2/5 and pathogens were applied to grape plants simultaneously, galling was only suppressed for strains Tr1, Tr4, and Tr6, strains that were less virulent than the other Turkish strains (Table 4). As reported previously, gall induction by strain CG49 is also significantly reduced when it is inoculated simultaneously with F2/5 (7). When F2/5 was applied 24 h prior to pathogenic strains, disease was prevented or strongly inhibited for all of the Turkish strains. It is encouraging that Turkish strains respond to F2/5 in a fashion similar to strains from other regions of the world; therefore, F2/5 may provide an effective means of disease control in Turkey.

The mechanism by which F2/5 inhibits grape crown gall is still unknown. We have demonstrated that it is probably not associated with antibiotic production or with competition for binding sites on grape cells (9). One of the most interesting findings is that the biological control response is specific to grape (i.e., F2/5 does not inhibit galling by *A. vitis* on several plant indicator hosts). Although other nontumorigenic *A. vitis* strains have been shown to have some biological control activity against *A. vitis* on grape (8), none of them (including nontumorigenic Turkish strains) have been found to be as effective as F2/5 when evaluated against a range of strains carrying different types of Ti plasmids (T. J. Burr, *unpublished data*).

Restriction analysis of ITS region. The restriction fragment fingerprint patterns of the ITS regions that were generated by the Turkish strains are shown in Figure 2. Analysis of the fingerprints revealed two main groups (Fig. 3). Strains Tr1, Tr4, Tr6, and Tr7 compose one group that is closest in similarity to *A. vitis* reference strains

Table 3. Tumorigenicity and necrosis determinations for *Agrobacterium vitis* strains[a]

	Strain								
Test, host	**Tr1**	**Tr2**	**Tr7**	**Tr6**	**Tr4**	**Tr3**	**Tr5**	**Tr15**	**CG49**
Gall formation									
Grape	+	+	+	+	+	+	+	NT	+
Tobacco	+	+	+	+	+	+	+	+	+
Tomato	+	−	−	+	+	+	−	−	+
Sunflower	+	+	+	+	+	+	+	−	+
Grape necrosis									
Vitis vinifera	+	+	+	+	+	+	+	+	+
V. riparia	+	+	+	+	+	+	+	+	+
V. labrusca	+	+	+	+	+	+	+	+	+

[a] Tests for gall formation and for induction of grape necrosis were done as described in the text. Galls were visible on tobacco (*Nicotiana glauca*), tomato, and sunflower within 10 to 14 days. The presence of necrosis on grape explants was recorded after 72 h. NT = not tested.

CG78 and CG470, which carry V and N type Ti plasmids, respectively (22). The second group (less than 0.50 similarity to group one) is made up of Tr2, Tr3, and Tr5. Tr3 is most similar to reference strains K309 and CG475, which carry OL and OS type Ti plasmids, respectively. These results further demonstrate the diversity among the Turkish strains and raise interesting questions regarding their position in the phylogeny of this bacterium.

It was previously found that fingerprints generated from ITS restriction fragments of *A. vitis* strains are highly correlated with the type of Ti plasmid they carry (22). For example, all but one of 31 strains with N type Ti plasmids that were collected from the United States and Europe had identical ITS fingerprints. The strains with O Ti plasmids clustered independently of N and V strains and were divided into subgroups based on whether they carried an OL or OS type T-DNA. Ti plasmids, therefore, appear to have preferable chromosomal hosts and do not transfer randomly between *A. vitis* in nature. All of the Turkish strains utilized octopine; therefore, we predicted that their fingerprints would be similar to the previously studied OL or OS strains. However, our results did not support this prediction and further analysis of T-DNAs from these strains is warranted.

The research of Otten and coworkers has provided a wealth of information concerning *A. vitis* T-DNA structure, oncogene function, and phylogenetic relationships (21). *A. vitis* OL and OS Ti plasmids have TA and TB T-DNAs and the composition of oncogenes found in their TA regions differ. In OL strains, the TA region is composed of oncogenes *iaaH* (which is disrupted by insertion element IS866), *iaaM*, *ipt*, and *6b*, whereas the TA region of OS strains is missing *iaaH* and *iaaM*. The TB regions of both types carry another functional set of *iaaH* and *iaaM* genes. Phylogenetic studies revealed that the T-DNAs of *A. vitis* are composed of regions of DNA that share a high degree of sequence homology with T-DNAs found in *A. tumefaciens*. Such findings provide strong evidence that T-DNAs in *A. vitis* have evolved through horizontal transfer of

Table 4. Effectiveness of F2/5 for inhibiting grape crown gall caused by Turkish strains[a]

Strain[b]	Without F2/5	F2/5, co-inoculate[c]	F2/5, 24 h prior[d]
Tr 1	3 (1.3)	4 (2.3)	0
Tr 2	9 (3)	9 (3)	0
Tr 3	9 (3)	9 (3)	0
Tr 4	7 (1.6)	1 (1)	1 (1)
Tr 5	9 (3)	8 (2.1)	2 (1)
Tr 6	7 (1.7)	3 (2)	0
Tr 7	9 (3)	9 (2)	0
CG49	9 (2.9)	4 (1.3)	0

[a] Number of inoculation sites that developed galls and average gall size rating. Ratings are based on gall diameter: 1 > 0.5 cm, 2 = 0.5 to 0.99 cm, and 3 ≥ 1.0 cm.

[b] Vines were inoculated at nine wound sites with the different strains as described in the text.

[c] Equal concentrations of F2/5 and pathogenic strain were applied simultaneously to the grape wounds.

[d] F2/5 was applied to the grape wound 24 h prior to the pathogenic strain. It was determined that wounds made 24 h prior to inoculation are as sensitive to infection as are fresh wounds (*data not shown*).

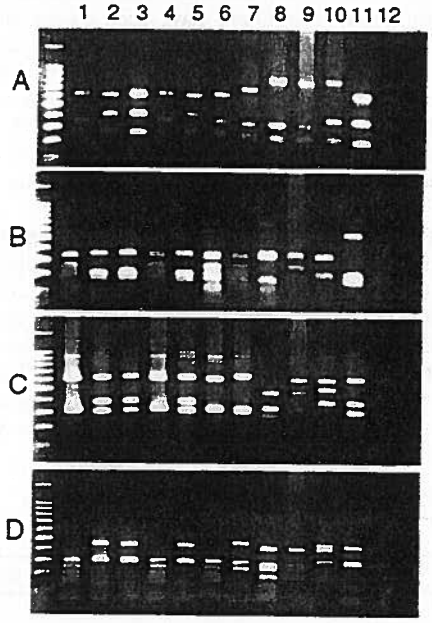

Fig. 2. Restriction fragment patterns generated from *A. vitis* strains. Fragments were generated as described in text. Genomic DNA was cut with **A**, *Rsa*I, **B**, *Cfo*I, **C**, *Ava*I, and **D**, *Taq*I as described in text. Banding patterns from digestion with *Hae*III are not shown. Lane numbers correspond to the following strains: 1, Tr1; 2, Tr2; 3, Tr3; 4, Tr4; 5, Tr5; 6, Tr6; 7, Tr7; 8, CG78; 9, CG470; 10, CG450; 11, K309; 12, CG475.

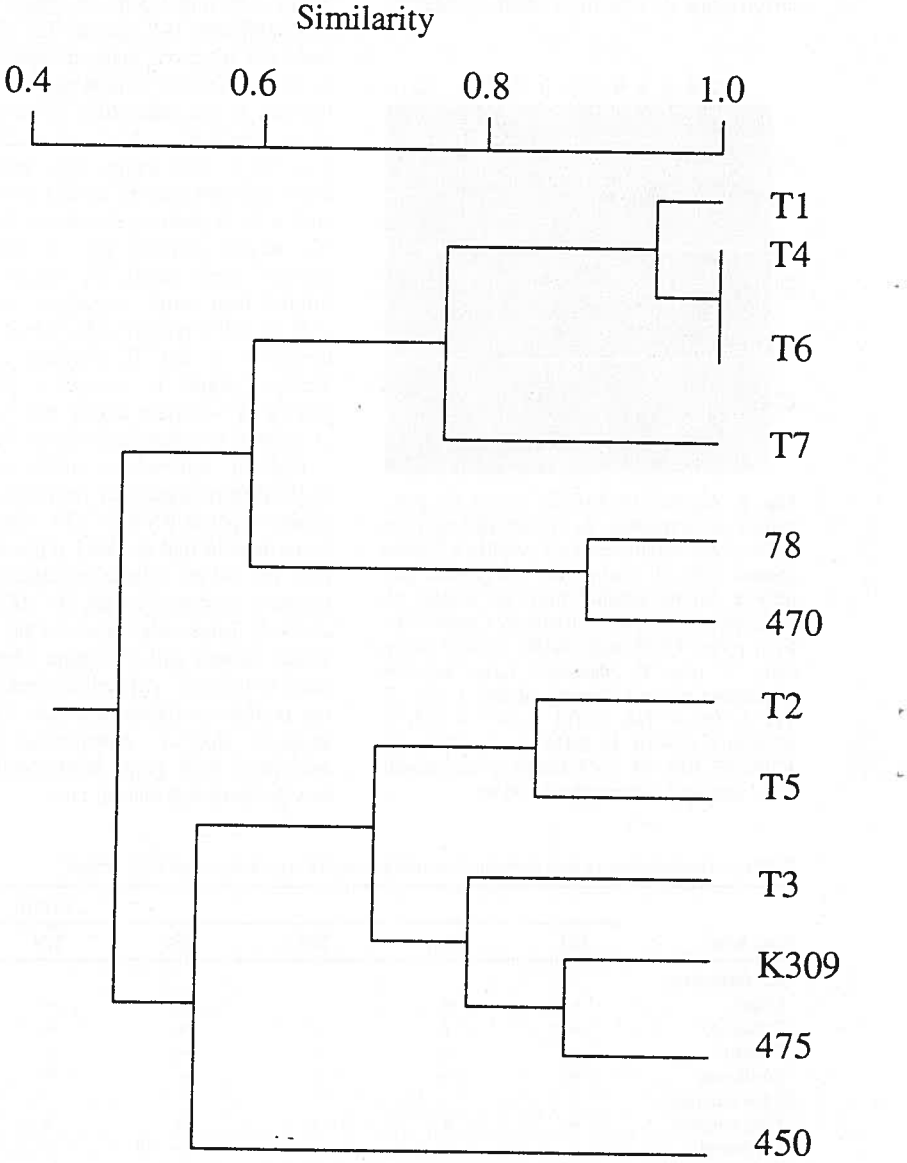

Fig. 3. Phenogram from unweighted pair group method using an arithmetic means cluster analysis of strains based on Nei-Li similarity coefficients that were determined from the DNA fingerprints of the intergenic spacer region.

relatively large DNA fragments. T-DNAs and other regions of Ti plasmids have further diverged through disruption of genes by IS elements. Within the OL and OS strains, for example, insertions have created different structural groups, although nucleotide sequence homology outside of the IS elements is greater than 90% (22). Factors such as the introduction of vine grafting and the worldwide dissemination of high-quality scion clones are also likely to have impacted the evolution of *A. vitis*.

To date, the phylogenetic development in *A. vitis* has been based on strains from Europe and the United States. Analysis of collections of these strains show that they are made up of strains carrying identical categories of Ti plasmids and, therefore, provide limited insight as to how ancestral strains of the bacterium may appear. It was hypothesized that wild *Vitis* spp. may harbor ancestral *A. vitis* strains. We recently characterized a group of strains that were isolated from wild-growing *V. riparia* (8). All of the strains were nontumorigenic and generated ITS patterns that were distinct from any of the tumorigenic strains that had been previously studied. Further studies with these strains may provide clues as to the evolution of the *A. vitis* chromosome.

The Turkish strains are especially intriguing because they may represent ancestral tumorigenic strains of *A. vitis*. Turkey is known as an origin for *V. vinifera;* therefore, the bacterium may have evolved there in close relation to its host. Also, the cultivars that we examined originated in Turkey. Together with the fact that cultivars in Nevşehir were not grafted, this precludes the possibility that *A. vitis* was spread to the vineyards on propagation material from Europe. All of the strains utilize octopine and, by ITS fingerprinting, only one group appears related to OS or OL strains; therefore, it may be that their T-DNAs are different from those previously studied. Further molecular characterization of the strains will be necessary to clarify this point.

Continued exploration of cultivated and wild *Vitis* spp. in Turkey could provide benefits such as the preservation of microbial germplasm that could be lost in the future if wild plants are eradicated. Research with such germplasm may provide key insights into how microorganisms evolve and how evolutionary events impact bacteria–plant interactions. By examining additional strains, we can also determine if strains that carry Ti plasmids encoding the utilization of opines other than octopine also exist in the region. Although, to date, the nontumorigenic strains of *A. vitis* from Turkey are not superior to F2/5 as crown gall biological controls, further analysis of strains from the area should be done.

ACKNOWLEDGMENTS

This material is based upon work supported by National Science Foundation Grant No. 9810332.

LITERATURE CITED

1. Barry, T., Colleran, G., Glennon, M., Dunican, L. K., and Gannon, F. 1991. The 16S/23S ribosomal s pacer as a target for DNA probes to identify eubacteria. PCR Methods Appl. 1:51-56.
2. Bishop, A. L., Burr, T. J., Mittak, V. L., and Katz, B. H. 1989. A monoclonal antibody specific to *Agrobacterium tumefaciens* biovar 3 and its utilization for indexing grapevine propagation material. Phytopathology 79:995-98.
3. Burr, T. J., Bazzi, C., Süle, S., and Otten, L. 1998. Crown gall of grape: Biology of *Agrobacterium vitis* and the development of disease control strategies. Plant Dis. 82:1288-97.
4. Burr, T. J., Bishop, A. L., Katz,, B. H., Blanchard, L. M., and Bazzi, C. 1987. A root-specific decay of grapevine caused by *Agrobacterium tumefaciens* and *A. radiobacter* biovar 3. Phytopathology 77:1424-27.
5. Burr, T. J., and Katz, B. H. 1984. Grapevine cuttings as potential sites of survival and means of dissemination of *Agrobacterium tumefaciens*. Plant Dis. 68:976-78.
6. Burr, T. J., and Otten, L. 1999. Crown gall of grape: Biology and disease management. Annu. Rev. Phytopathol. 37:53-80.
7. Burr, T. J., and Reid, C. L. 1993. Biological control of grape crown gall with nontumorigenic *Agrobacterium vitis* strain F2/5. Am. J. Enol. Vitic. 45:213-219.
8. Burr, T. J., Reid, C. L., Adams, C. E., and Momol, E. A. 1999. Characterization of *Agrobacterium vitis* strains isolated from feral *Vitis riparia*. Plant Dis. 83:102-107.
9. Burr, T. J., Reid, C. L., Tagliati, E., Bazzi, C., and Sule, S. 1997. Biological control of grape crown gall by strain F2-5 is not associated with agrocin production or competition for attachment sites on grape cells. Phytopathology 87:705-11.
10. Eastwell, K. C., Willis, L. G., and Cavileer, T. D. 1995. A rapid and sensitive method to detect *Agrobacterium vitis* in grapevine cuttings using the polymerase chain reaction. Plant Dis. 79:822-27.
11. Hooykaas, P. J. J., Roobol, C., and Schilperoort, R. A. 1979. Regulation of the transfer of Ti plasmids of *Agrobacterium tumefaciens*. J. Gen. Microbiol. 110:99-109.
12. Kerr, A. 1972. Biological control of crown gall: Seed inoculation. J. Appl. Bacteriol. 35:493-497.
13. Knauf, V. C., Yanofsky, M., Montoya, A., and Nester, E. W. 1984. Physical and functional map of an *Agrobacterium tumefaciens* plasmid that confers a narrow host range. J. Bacteriol. 160:564-68.
14. Momol, E. A., Burr, T. J., Reid, C. L., Momol, T., and Otten, L. 1998. Genetic diversity of *Agrobacterium vitis* as determined by DNA fingerprints of the 5′-end of the 23S rRNA gene and random amplified polymorphic DNA. J. Appl. Microbiol. 85:685-92.
15. Momol, E. A., Herlache, T. C., and Burr, T. J. 1998. Detection of *Agrobacterium vitis* in grape by PCR. (Abstr.) Phytopathology 88:S64.
16. Moore, L. W., Bouzar, H., and Burr, T. J. 2001. Agrobacterium. In: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, J. B. Jones, and W. Chun, eds. American Phytopathological Society Press, St. Paul, MN.
17. Nei, M., and Li, W.-H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Nat. Acad. Sci. USA. 76:5267-5273.
18. Nunez, D. R., and Walker, M. J. 1989. A review of palaeobotanical findings of early Vitis in the Mediterranean and of the origins of cultivated grapevines, with special reference to new pointers to prehistoric exploitation in the western Mediterranean. Rev. Palaeobot. Palynol. 61:205-237.
19. Ophel, K., and Kerr, A. 1990. *Agrobacterium vitis*, new species for strains of Agrobacterium biovar 3 from grapevines. Int. J. Syst. Bacteriol. 40:236-41.
20. Ophel, K. M., Jones, D. A., and Kerr, A. 1990. Molecular cloning of cell wall degrading enzymes from *Agrobacterium tumefaciens* biovar 3. (Abstr.) Phytopathology 80:982.
21. Otten, L., Canaday, J., Gerard, J.-C., Fournier, P., Crouzet, P., and Paulus, F. 1992. Evolution of Agrobacteria and their Ti plasmids—A review. Mol Plant-Microbe Interact. 5:279-287.
22. Otten, L., De Ruffray, P., Momol, E. A., Momol, M. T., and Burr, T. J. 1996. Phylogenetic relationships between *Agrobacterium vitis* isolates and their Ti plasmids. Mol. Plant-Microbe Interact. 9:782-86.
23. Rodriguez, Palenzuela- P., Burr, T. J., and Collmer, A. 1991. Polygalacturonase is a virulence factor in *Agrobacterium tumefaciens* biovar 3. J. Bacteriol. 173:6547-52.
24. Rohlf, F. J. 1988. NTSYS-pc. Applied Biostatistics. Setauket, New York.
25. Zupan, J., Muth, T. R., Draper, O., and Zambryski, P. 2000. The transfer of DNA from *Agrobacterium tumefaciens* into plants: a feast of fundamental insights. Plant J. 23:11-28.