Genetic Factors Controlling the Host Range of *Agrobacterium tumefaciens*

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This research was supported by The American Cancer Society Grant NP336, National Cancer Institute Public Health Service Grant CA13015, and National Science Foundation Grant PCM-8104310. V. C. Knauf was supported by National Research Service Award GM07270.

The authors acknowledge helpful discussions with Alice Montoya and Michael F. Thomashow, and thank Ramona Finical for typing the manuscript.

Accepted for publication 29 April 1982.

ABSTRACT

Knauf, V. C., Panagopoulos, C. G., and Nester, E. W. 1982. Genetic factors controlling the host range of *Agrobacterium tumefaciens*. Phytopathology 72:1545-1549.

Host range differences were observed among 34 isolates of *Agrobacterium tumefaciens* from grapevine by virulence testing on eight different plant hosts. Six different host range patterns were evident. In 20 of 22 cases, a laboratory-derived strain of *Agrobacterium* carrying the virulence (Ti) plasmid from a natural isolate expressed the same host range as the natural isolate of *A. tumefaciens*. Thus, the Ti plasmid is the primary determinant of host range whether the host range is wide or narrow. Two wild-type strains were virulent on two plant hosts on which other strains containing the same or similar Ti plasmids were avirulent. These data indicate that the bacterial chromosome can affect some host range properties. Moreover, the response on grapevine depends on the cultivar of grapevine tested; a given strain of *A. tumefaciens* may induce tumors on some, but not all, cultivars of *Vitis vinifera*.

Agrobacterium tumefaciens is a Gram-negative soil organism that can induce tumorous growths (crown galls) on dicotyledonous plants (17). Assignment of *Agrobacterium* strains to different species on the basis of pathogenicity as indicated in Bergey's Manual (1) does not accurately represent taxonomic relations (3,11,16). The studies of Keane et al (11) and DeLey and colleagues (5,13) separated most isolates of *Agrobacterium* into two groups, or biotypes, on the basis of biochemical tests, serotyping, electrophoretic protein patterns, and DNA homology. More recent work indicates that a third biotype can be distinguished by biochemical tests and that these strains are found almost exclusively in association with grapevines.

In every well-characterized case to date, the ability of *A. tumefaciens* to induce crown galls on plants resides on a plasmid (24,26). If this Ti (Ti = tumor inducing) plasmid is eliminated, the resulting strain is completely avirulent and phenotypically the same as the avirulent soil organism *Agrobacterium radiobacter*. Conversely, if the Ti plasmid is transferred into a cured strain or into *A. radiobacter*, the recipient strain becomes virulent (24).

The host range of *A. tumefaciens* is remarkably wide. DeCleene and DeLey (4) reported that at least 643 host plants from 331 genera were susceptible to crown galling. Although most of these plants were dicotyledonous, some gymnosperms are also susceptible, whereas very few, if any, monocotyledonous plants developed crown galls after inoculation with *A. tumefaciens*.

The host range of a given strain of *A. tumefaciens* is a specific character of that strain (2). Although most isolates induce crown galls on a wide range of common test plants, some strains exhibit very high host specificity and an unusually limited host range (2,18,23). Thomashow et al (23) found strains that differed genetically only in the Ti plasmid and expressed very different host ranges. Similarly, pairs of different strains containing the same Ti plasmid expressed the same host range. Loper and Kado (15) generated a transconjugant strain of *Agrobacterium* that expressed the wide host range associated with the donor strain rather than the virulence pattern of the recipient. Both studies indicated that host range is determined by the Ti plasmid.

We collected a variety of isolates of *A. tumefaciens* from grapevine in order to study host range variation among strains of *A. tumefaciens* isolated from one host species, the grapevine. This host was selected for several reasons: the host range is known to vary considerably among grapevine isolates of *A. tumefaciens* (15,19); crown gall has been and continues to be an economic problem for grape growers (8,18); agricultural methods for viticulture include grafting, pruning, and mechanical harvesting, all of which cause plant wounding, a prerequisite for crown gall formation; since grapevines are grown in many countries, strains of *Agrobacterium* from geographically diverse origins can be studied; and grapevine is the only known host on which all of the three described biotypes of *A. tumefaciens* (19,21) can be found naturally.

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0031-949X/82/12154505/$03.00/0

MATERIALS AND METHODS

Strains. The wild-type strains of *A. tumefaciens* isolated from grapevine are described in Table 1. Strain A729 is strain A136 (24), which has received the RP4-pTiB653 cointegrate plasmid by conjugation with strain B653::RP4, kindly provided by J. Schell. Strain A276, isolated by A. Montoya, represents a streptomycin and spectinomycin-resistant derivative of strain Ag63, which was then mated with strain B653::RP4 and received the RP4-pTiB653 cointegrate plasmid. The derivation of other strains is described in Results.

TABLE 1. List of strains (isolates) of *Agrobacterium tumefaciens* from grapevine that were used to study genetic control of host range

Biotype	Strain designation	Number of plasmid species[a]	Origin	Source
I	1 D 1109	One	USA (CA)	C. Kado
	Ag19	Two	Greece	C.G. Panagopoulos
	Ag34	One	Greece	C.G. Panagopoulos
	Ag125	Two	Greece	C.G. Panagopoulos
	S-8	One	Hungary	S. Süle
	20/1	Two	Hungary	S. Süle
	ATV	One	Spain	J. DeLey
	ATB	Two	Spain	J. DeLey
	NCPPB 1001	Two	Romania	J. DeLey
II	Ag110	None	Greece	C.G. Panagopoulos
	19/5	Two	Hungary	S. Süle
	PPI-1	One	Bulgaria	J. DeLey
	PPI-6	One	Bulgaria	J. DeLey
	M-A5	Two	South Africa	F. Matthee
III	Ag57	Two	Greece	C.G. Panagopoulos
	Ag63	Three	Greence	C.G. Panagopoulos
	Ag105	Two	Greece	C.G. Panagopoulos
	Ag119	Four	Greece	C.G. Panagopoulos
	Ag122	One	Greece	C.G. Panagopoulos
	Ag123	One	Greece	C.G. Panagopoulos
	Ag127	Two	Greece	C.G. Panagopoulos
	Ag164	N.D.	Greece	C.G. Panagopoulos
	Ag165	Two	Greece	C.G. Panagopoulos
	Ag158	Three	U.S.S.R.	C.G. Panagopoulos
	Ag162	Four	U.S.S.R.	C.G. Panagopoulos
	Ag83	Three	Yugoslavia	C.G. Panagopoulos
	Ag86	Four	Yugoslavia	C.G. Panagopoulos
	2/6	N.D.	Hungary	S. Süle
	15/5	Four	Hungary	S. Süle
	19/8	Three	Hungary	S. Süle
	K305	One	Australia	A. Kerr
	CG8	Two	USA (NY)	T. Burr
	CG48	Two	USA (NY)	T. Burr
	CG54	Three	USA (NY)	T. Burr

[a] Number of plasmids estimated by gel electrophoresis of DNA prepared by the method of White and Nester (25). N.D. = not determined.

Stock cultures established from single colonies were isolated and maintained on nutrient agar. Biotype determinations were based on the tests described by Kerr and Panagopoulos (12).

Pathogenicity assays and host plants. Green, tender stems of host plants were wounded with a sterile toothpick or needle, and freshly cultured bacteria were placed in the wound. Similar plants were inoculated with avirulent strain A136 and another strain, known to be virulent on the host plant, on the same day as the test inoculations to serve as negative and positive virulence controls. Virulence results were scored every 2 wk for up to 3 mo. Certain pathogen-plant host combinations consistently developed small knoblike growths significantly different from negative controls. These appeared to be only minimal responses when compared with the large galls incited by virulent strains (19). Such attenuated responses were considered avirulent, but are annotated in tabulations of virulence data. These knoblike growths did not achieve diameters equal to one-fourth the stem thickness, whereas true crown galls were usually much thicker than the diameter of the stem where the host was inoculated.

The plant species tested were grapevine (*Vitis vinifera* 'Sultanina,' 'Savatiano,' 'Razaki,' 'Roditis,' and an unnamed local Greek selection), sunflower (*Helianthus annuus* 'Mammoth'), tomato (*Lycopersicon esculentum* 'Red Cherry' and 'San Pietro'), tobacco (*Nicotiana tabacum* 'Xanthi' and 'Turkish'), tree tobacco (*Nicotiana glauca*), *Nicotiana glutinosa,* jimsonweed (*Datura stramonium*), and *Kalanchöe daigremontiana.* Plants of the latter were inoculated on the leaves as described by Garfinkel and Nester (6).

RESULTS

Table 1 lists strains of *A. tumefaciens* isolated from galls on grapevine. Although most were isolated in Europe, strains from North America, Australia, and South Africa are also included. The biotype of each strain was determined from the biochemical tests outlined in Table 2. These diagnostic criteria did not appear to be plasmid-coded since plasmidless strains such as ACH5 C3 (14) also respond characteristically to these tests.

The results in Table 3 indicate that the 34 strains of *A. tumefaciens* express at least six different host ranges; more differences might be detected if more test hosts were employed. Since all of the bacterial strains were isolated from grapevine, we consider those strains virulent on tobacco, tomato, and sunflower to have a "wide host range." The Biotype I strain Ag125 and 10 Biotype III isolates have limited host ranges. Another group appears to be avirulent by these data. None of the Biotype III strains expressed a host range identical to the host range of either a Biotype I or II strain.

Strain I D 1109 was reported to have attenuated virulence on the Mission cultivar of grapevine. We have not found this strain to be virulent on any host, but plants of cultivar Mission were not available for testing. Other data (V. Knauf, *unpublished*) suggest that this strain may not contain a Ti plasmid.

The grapevine isolates of *A. tumefaciens* described above

TABLE 2. Biotyping of strains (isolates) of *Agrobacterium tumefaciens* from grapevine

Test	Proportion of positive responses			Strains[a]
	Biotype I	Biotype II	Biotype III	
3-Ketolactose production	9/9	0/5	0/20	···
Erythritol utilization	0/9	5/5	0/20	···
Growth at 37 C	8[a]/9	0/5	2[a]/20	ATV, Ag162, Ag164
Propionate	8[a]/9	0/5	0/20	Ag125
Litmus milk (alkaline = +)	9/9	0/5	20/20	···
2% NaCl	9/9	1[a]/5	20/20	Ag110
L-tartrate	4[a]/9	5/5	18[a]/20	Ag19, Ag34, Ag125, S-8, Ag164, CG54
Ethanol	9/9	0/5	1[a]/20	Ag57
Malonate	0/9	4[a]/5	19[a]/20	Ag110, CG54
Melezitose utilization	9/9	0/5	3[a]/20	CG8, CG48, CG54
Mucic acid	0/9	5/5	3[a]/20	Ag158, Ag164, Ag165
DL-homoserine	9/9	2[a]/5	0/20	Ag110, PPI-1

[a] Strains that gave the indicated less common result in respective test and biotype are shown in the last column.

expressed several distinct host ranges. The genetic bases for these host range differences could be due to different Ti plasmids or due to factors coded by the chromosome or cryptic plasmids. To distinguish between these possibilities, we sought to vary the Ti plasmid content and hold all other factors constant. The strains in Table 4 were obtained by transforming *Agrobacterium* strain A136 with plasmid DNA from the grapevine isolates and selecting for octopine or nopaline catabolism (V. Knauf, *unpublished*). The parent of avirulent strain A136 was derived by curing the Biotype I wide-host-range strain C58 of its resident Ti plasmid by heat treatment.

The data in Table 4 indicate that the A136 type chromosome is compatible with both wide (eg, strain A503) and narrow (eg, strain A856) host ranges. It is also possible to compare the host range of a given strain in Table 4 with the host range of the wild type isolate containing the same Ti plasmid (Table 3). Thomashow et al (23) used this approach to show that the limited host ranges of strains Ag57, Ag63, Ag158, and Ag162 were plasmid coded since strain A136 with the Ti plasmid from any of those Biotype III strains expressed host ranges similar to the wild type isolates (22).

TABLE 3. Virulence of grapevine isolates of *Agrobacterium tumefaciens* on selected plant hosts

Strains	Virluence on[a]						
	Kalanchöe	Jimsonweed	Tomato	Tobacco	Sunflower	Tree tobacco[b]	Grapevine[c]
NCPPB 1001, S-8, 20/1, ATV, ATB, 19/5, M-A5, PPI-1, and PPI-6	+	+	+	+	+	+	–[d]
2/6, 15/5, CG8, CG48, and CG54	–	+	+	+	+	+	–
Ag83, Ag86, K305, Ag105, and Ag123	–	–	+	+	+	+	+
Ag57, Ag63, Ag119, Ag122, Ag127, Ag158, Ag162, Ag164, Ag165, and 19/8	–	–	–	–	–[d]	+	+
Ag125[e]	–	–	–	–	–	–	+
Ag19, Ag34, Ag110, 1 D 1109	–	–	–	–	–	–	–

[a] Genus and species of plant hosts are given in Materials and Methods.
[b] *Nicotiana glutinosa* gave responses similar to *Nicotiana glauca* (tree tobacco).
[c] Sultanina cultivar of grapevine *Vitis vinifera*.
[d] Reaction more positive than negative control, but it was only slight compared to similar inoculations with virulent strains.
[e] Ag125 occasionally induced very slight responses on sunflower and *N. glutinosa*.

TABLE 4. Virulence of *Agrobacterium tumefaciens* strain A136 derivatives on selected plant hosts

A. tumefaciens derivatives		Virulence on[a]						
Strain	Plasmid[b]	Kalanchöe	Jimsonweed	Tomato	Tobacco	Sunflower	Tree tobacco[c]	Grapevine[d]
A503	pTiNCPPB1001							
A870	pTiS-8							
A871	pTi20/1							
A877	pTiATV							
A878	pTiATB							
A872	pTi19/5							
A873	pTiM-A5							
A880	pTiPPI-1							
A881	PTiPPI-6	+	+	+	+	+	+	–[e]
A851	pTi2/6							
A852	pTi15/5							
A882	pTiCG8							
A884	pTiCG54	–	+	+	+	+	+	–
A857	pTiAg83							
A858	pTiAg86							
A867	pTiK305	–	–	+	+	+	+	+
A853[f]	pTiAg57							
A854[f]	pTiAg63							
A855[f]	pTiAg158							
A856[f]	pTiAg162							
A859	pTiAg105							
A862	pTiAg123	–	–	–	–	–[e]	+	+
A136	...[b]							
A890	pAtAg19							
A868	pAtAg34							
A842	pAtAg125	–	–	–	–	–	–	–

[a] Genus and species of host plants are given in Materials and Methods.
[b] All strains were derived indirectly from strain C58 and therefore contain pAtC58.
[c] *Nicotiana glutinosa* gave responses similar to *Nicotiana glauca* (tree tobacco).
[d] Sultanina cultivar of grapevine *Vitis vinifera*.
[e] Reaction more positive than negative controls, but only slight when compared with positive controls.
[f] Strains A853, A854, A855, and A856 have been previously referred to as Ag57tr, Ag63tr, Ag158tr, and Ag162tr, respectively (23).

With only three exceptions, the host range of each transformant agrees with the host range of the wild-type strain with the same Ti plasmid. Thus, five of the six host range patterns observed in Table 3 are also seen in Table 4. The pattern present in Table 3 but not in Table 4 corresponds to strain Ag125; the plasmid transformed into strain A136 to create strain A842 was not a Ti plasmid since this latter strain was avirulent. If the other plasmid of strain Ag125 had been transformed into strain A136, the pattern specific for grapevine only may have been generated.

The Biotype III strain Ag105 and Ag123 have the ability to induce tumors on tobacco and tomato plants, but the Biotype I strains containing the same Ti plasmids, A859 and A862, respectively, do not have this ability. Since plasmid preparations from strain Ag123 do not contain cryptic plasmids, these host range differences appear to be due to chromosomal differences between strain A136 and the wild-type isolates. However, it does not appear to be a specific property of Biotype III strains since other Biotype III strains such as Ag162 do not cause tumors on tobacco or tomato.

Since the chromosome could influence the expression of host range, it seemed possible that the chromosomal background of the grapevine-specific Biotype III strains may have features particularly adapted to tumorigenesis on grapevines. Thus, Ti plasmids such as pTi20/1 might express virulence on Sultanina grapevine in a Biotype III background, but not the Biotype I background of strain A136 (derived from the cherry isolate C58).

However, all attempts to transform Biotype III strains with Ti plasmid DNA were unsuccessful. This may have been due to incompatibility functions of resident Ti plasmids or low transformation frequencies of Biotype III strains for plasmids as large as the Ti plasmid (about 200 kb). To overcome these difficulties, the pTiB6S3::RP4 cointegrate plasmid was transferred by conjugation into the Biotype III strain Ag63. This cointegrate plasmid (9) consists of the promiscuous resistance factor RP4 and a Ti plasmid highly homologous to the octopine Ti plasmids pTiNCPPB1001, pTi20/1, and pTiS-8 (20; V. Knauf, *unpublished*). The transconjugant strain, A726, was isolated by selecting for the drug resistance markers on RP4 and on the Ag63 chromosome (A. Montoya, *personal communication*). Strain A726 lacks the plasmid band corresponding to pTiAg63, which presumably was lost due to incompatibility functions expressed by the cointegrate plasmid (10). Strain A726 gave the same biotype reactions as the parental strain Ag63; the strain also contains the two cryptic plasmids of Ag63. As a control, the cointegrate plasmid was also similarly introduced into the avirulent strain A136 to generate strain A729.

The cointegrate plasmid pTiB6S3::RP4 did not code for virulence on Sultanina grapevine in either the Ag63 or A136 chromosomal background. The Ti plasmid pTiAg63 coded for virulence on grapevine both in the A136 background (strain A854) and in the wild-type isolate Ag63. Thus, the Ag63 chromosome associated with grapevines in nature could not compensate for the avirulence of pTiB6S3::RP4 on Sultanina grapevine. Although the Ti plasmid pTiB6S3 codes for virulence on *K. daigremontiana* in its parental background, the formation of cointegrates with RP4 often results in strains avirulent on *Kalanchöe* (10). Curiously, strain A726 is virulent on *Kalanchöe* whereas strain A729 is not. It appears, then, that the chromosomal background can affect whether or not the RP4 cointegration with pTiB6S3 suppresses virulence on *Kalanchöe*.

In the course of the experiments with strains A726 and A729, we became aware that other cultivars of grapevine cultivated in Greece were susceptible to strains A726 and A729. These results (Table 5) indicate that different cultivars of a single host species can respond differently to *A. tumefaciens*. These differences in response correspond to virulence factors coded by the Ti plasmid, since strains A854 and Ag63 are virulent on all cultivars tested and strains A726 and A729 are virulent only on plants of cultivar Savatiano (Table 5).

DISCUSSION

These data show that genetic control of host range is a complex and interesting property of individual strains of *A. tumefaciens*. An inspection of the differences for strains in Tables 3 and 4 reveals that although host range is primarily a property of the Ti plasmid, the chromosomal background also plays a role.

Other work has suggested that chromosomal characters might affect host range. Garfinkel and Nester (6) described strains generated by transposon insertion mutagenesis that had lost part of their wide parental host range, even though the Ti plasmids appeared intact. Hamada and Farrand (7) found that the Ti plasmids from two strain B6 subcultures avirulent on *Kalanchöe* could be mated into another strain in which the natural virulence of pTiB6 for *Kalanchöe* was restored. Those data involve laboratory-generated variants of wild-type strains that may not exist in nature.

Several factors point to a special relationship between the grapevine and *A. tumefaciens*. One kind of *Agrobacterium* (Biotype III) appears to be limited to the grapevine ecosystem. Some cultivars of grapevine (eg, Sultanina) appear to be resistant to the wide-host-range Ti plasmids found in most strains. Some isolates of *A. tumefaciens* from grapevine seem specialized in the sense that they have limited virulence for other plant hosts. It may be that a natural association of Biotype III strains with grapevines has removed selective pressures that maintain wide host ranges in other strains of *Agrobacterium*. Conversely, limited host range Ti plasmids like pTiAg162 may resemble limited-host-range ancestral Ti plasmids that have served as evolutionary precursors to wide host range Ti plasmids.

In summary, it appears that more than one kind of factor may determine the outcome of an interaction between *Agrobacterium* and a potential host. The type of Ti plasmid, chromosomally coded characters, and variation within and among plant host genera all contribute to differences observed in the host ranges of strains of *A. tumefaciens*. Since even limited-host-range strains like Ag162 can induce tumors on plants as distantly related as *N. glauca* and *V. vinifera*, the wide host range of *A. tumefaciens* in general suggests that this pathogen exploits very fundamental properties of higher plant organization, presumably related to plant hormone activities (6). On the other hand, A277 (A136 with pTiB6-806), a strain with

TABLE 5. Cultivar-specific resistance of grapevine (*Vitis vinifera*) to crown galling by selected strains of *Agrobacterium tumefaciens* with identified chromosome and plasmids

	Presence of tumors after inoculation with				
Grapevine cultivars	strain: A136 chromosome: A136 plasmid: pAtC58	A729 A136 pTiB6S3::RP4 pAtC58	A845 A136 pTiAg63 pAtC58	Ag63 Ag63 pTiAg63 pAtAg63a,b	A726 A136 pTiB6S3::RP4 pAtAg63a,b
Sultanina	–	–[a]	+	+	–[a]
Savatiano	–	+	+	+	–[a]
Razaki	–	–[a]	+	+	–[a]
Roditis	–	–[a]	+	+	–[a]
Local Greek grape selection (unnamed)	–	–[a]	+	+	–[a]

[a] Host response differed from that caused by negative control (strain A136) but it was very minimal compared with that resulting from inoculations with strain Ag63. See Panagopoulos et al (19).

a wide host range that includes Douglas fir and tobacco plants, can infect some grapevine cultivars but not others. The species-specific and sometimes cultivar-specific host susceptibility implies that host range determinants carried on the Ti plasmid are sensitive to factors in the host that can vary among closely related plants. The ability of wide-host-range strains to interact with variable factors in a manner to induce tumors on such a diverse set of host plants makes host range studies of *A. tumefaciens* an excellent model to study host parasite interactions between plants and bacteria. Our current efforts are directed towards identifying the specific genetic loci responsible for the wide and narrow host ranges coded by pTiA6 (6) and pTiAg162, respectively.

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