Use of Polymerase Chain Reaction to Detect Pathogenic Strains of *Agrobacterium*

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ABSTRACT

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The polymerase chain reaction (PCR), a sensitive tool for the identification of specific regions of DNA present in small quantities, was used to detect the presence of T-DNA in 34 *Agrobacterium* strains from *Vitis* spp. Oligonucleotide primers, homologous to T-DNA regions from a wide and a narrow host range strain of *Agrobacterium tumefaciens*, were used to amplify portions of the T-DNA. In most cases the PCR results confirmed pathogenicity tests using detached leaves from *Agrobacterium*-free muscadine plants and DNA slot blot hybridization.

Additional keywords: crown gall, nucleic acid hybridization, *Vitis rotundifolia*.

Crown gall, caused by *Agrobacterium tumefaciens*, frequently occurs on *Vitis vinifera* L. and other *Vitis* spp. This disease in grape has historically been attributed primarily to *A. tumefaciens* biovar 3, although *A. tumefaciens* biovar 1 also has been implicated (3,4,29). The pathogen may reside in the vascular system of symptomless plants and, therefore, may unknowingly be transmitted by vegetative propagation (4,14,15,28). Gall development on systemically infested plants has been associated with certain predisposing factors, most notably early spring sap rise, followed by freezing conditions (15,28). This rapid temperature fluctuation causes wounding in the cane and hence the initiation of the disease.

Crown gall disease has been detected on muscadine grapes (*Vitis rotundifolia* Michx.) grown in several regions of Mississippi (7,8) and could become a serious problem. Surveys have suggested that most, if not all, commercially grown muscadines are systemically infested with agrobacteria (9–11). Even though biovar 3 strains associated with crown gall of grape are normally considered as having a narrow host range, the muscadine strains appear to have an even more restricted host range (11). In inoculation studies, galls were produced by muscadine strains only in meristem-cultured, *Agrobacterium*-free muscadine and Orlando Seedless grape. No galls were formed when eight other commonly used indicator hosts for crown gall were inoculated (11).

The objective of this research was to develop a rapid and sensitive method for determining the potential pathogenicity of *Agrobacterium* strains from muscadine. Conventional microbiological tests for pathogenicity are time-consuming and expensive, requiring the use of *Agrobacterium*-free test plants. In situ colony hybridization using cloned T-DNA regions of the Ti plasmid as a probe was unsuccessful because of high levels of background hybridization (C.-W. Sun and D. S. Luthe, *unpublished data*). Subsequent work indicated that DNA slot blot hybridization could be used to determine potential pathogenicity (26,27). However, this technique had two drawbacks: it was not as sensitive as expected, and the probes hybridized weakly to DNA from *A. tumefaciens* strains isolated from muscadine. Preliminary work (26) indicated that the polymerase chain reaction (PCR), a sensitive DNA amplification assay (23), may be useful for identifying pathogenic forms of *Agrobacterium*. Using DNA sequence data, oligonucleotide primers were prepared and used to amplify a portion of T-DNA in the PCR. If an unknown *Agrobacterium* strain harbors the Ti plasmid, the primers will hybridize with the T-DNA region and a small segment of the T-DNA will be amplified during the PCR. For the results presented here, total DNA was isolated from bacterial strains and used as target DNA in the PCR. Because culture of bacteria and DNA isolations are time-consuming, in the future we would like to develop a method that directly uses the xylem exudates as a source of target DNA. This work represents the first step in this process, i.e., testing the feasibility of the PCR using DNA isolated from muscadine strains of agrobacteria. Preliminary results of this research were previously published (6).

MATERIALS AND METHODS

***Agrobacterium* strains.** *Agrobacterium* strains were randomly selected from a collection of over 500 cryopreserved strains obtained from various muscadine cultivars in vineyards throughout Mississippi. These included strains from roots and vascular fluids of shoots of symptomless plants and galled tissues (see Table 1 for listing).

Agrobacterium strains were isolated from the vascular fluids of current season muscadine shoots using methods described by Tarbah and Goodman (28), i.e., vascular fluids were extracted using the water pressure method of Bennett et al (1). Agrobacteria also were isolated from roots by surface-sterilizing in 1% sodium hypochlorite for 15 min, rinsing in sterile distilled water, and cutting longitudinally with a sterile scalpel as described by Burr et al (5). The cut surface was streaked on modified New and Kerr selective (NKS) medium (3,20) and Roy and Sasser (RS) (22) selective medium. Additionally, root samples were assayed by placing surface-sterilized, rinsed, and chopped roots in sterile distilled water in a Waring Blendor (New Hartford, CT) and blending at high speed for 1 min (5). Platings of resultant water samples were made on NKS and RS media. Strains were obtained from galls by dissecting each gall from the trunk or cordon, surface-sterilizing, rinsing in sterile water, and grinding the gall tissue in a sterile Waring Blendor for 5 min (1 min bursts) or with a mortar and pestle. The ground tissue was diluted and plated on selective media (NKS and RS).

After streaking for purity, all cultures were stored in nutrient broth plus 10% glycerol at −80 C. These cryopreserved cultures were recovered by scraping cell suspensions with a sterile loop and inoculating nutrient glucose (2.5%) agar (NGA) plates. All cultures were incubated at 28 C.

A. tumefaciens strains used as positive controls included CG49 and Ag57 (biovar 3) from *V. vinifera* obtained from T. J. Burr, New York State Agricultural Experiment Station, Cornell Uni-

versity (Geneva, NY), Ag63 (biovar 3) and FACH (biovar 1) from *V. vinifera* supplied by R. N. Goodman, University of Missouri (Columbia), and B6 (biovar 1) from *Malus sylvestris* Mill. purchased from Carolina Biological Supply Company, Burlington, NC. *A. radiobacter* strain ATCC 31700 acquired from the American Type Culture Collection (Rockville, MD) was chosen as negative control for the study.

Biovar classification. Biovar classification of agrobacteria isolated from muscadine plants was determined putatively by growth of the strains on RS, New and Kerr (NK) (20), NKS, and Schroth's (SCH) (25) media. Additional diagnostic tests, as described by Moore et al (18), included growth in litmus milk, sodium chloride tolerance, production of 3-ketolactose, acid production from erythritol or melezitose, alkali production from malonate, and growth on nutrient agar at 35 C. Classifications were repeated for all strains using selective media. Strains remaining in question as to biovar classification were repeated further using the above tests and the mucic acid test (18).

Target DNA isolation. DNA was isolated from *A. tumefaciens* strains cultured in 500 ml of YSTA medium (5 g of Bacto-tryptone, 5 g of Bacto-yeast extract, 50 g of sucrose, and 2 g of K_2HPO_4 in 1 L of double-distilled water, pH 6.8) for 12 h. Before DNA isolation the cultures were chilled on ice and centrifuged at 8,000 *g* for 5 min. Total DNA was isolated from the bacterial pellet using a modification (26) of the method by Maniatis et al (17). The pellet was resuspended in 6 ml of sterile distilled water, then 3 ml of phenol saturated with TE buffer (10 mM Tris-HCl, pH 7.6, and 1 mM EDTA, pH 8.0) was added. After incubation at 4 C for 1 h to lyse the bacterial cells, the samples were centrifuged at 3,600 *g* for 8 min. The supernatant was extracted with chloroform three times. DNA was precipitated by the addition of two volumes of ethanol and 0.1 volume of 7.5 M ammonium acetate followed by incubation at −20 C overnight. The DNA pellet was collected by centrifugation at 15,000 *g* for 10 min, washed with 70% ethanol, dried, and resuspended in TE buffer. If excess RNA was present, samples were treated with RNase (DNase-free, 50

TABLE 1. Comparison of DNA slot blot hybridization, detached leaf pathogenicity test, and the polymerase chain reaction for determining the potential pathogenicity of *Agrobacterium* strains

Strain code	Biovar classification[a]	Slot blot		DLP tests[c]		PCR[d]	
		NHR	WHR[b]	Summit	Carlos	NHR	WHR
Strains from galled muscadine tissues							
B21c	1	−	−	+	+	+	NT[e]
B22	1	−	−	+	+	+	NT
B24A	1	−	−	+	+	+	+
372	1	+	+	+	+	+	+
504	1	+	+	+	+	+	+
513	1	−	−	+	+	+	+
459C	3	+	+	+	+	+	+
461	3	−	−	+	+	+	NT
518	3	+	+	+	+	+	NT
495	3	−	+	+	+	+	+
500	3/2	−	−	+	+	+	NT
497	2	NT	NT	+	−	+	NT
460	3	−	+	+	−	+	−
499	3/2	−	−	+	−	+	+
506	2	−	−	−	−	−	NT
464	U	NT	NT	−	−	−	NT
509	1	−	−	−	−	−	NT
266	3	−	−	+	+	−	NT
B25A	1	−	−	+	+	−	−
459	3	−	−	−	−	−	+
B32	1	−	−	−	−	+	NT
Strains from vascular fluids of asymptomatic plants							
452	3	+	+	+	+	+	+
453	3	−	+	NT	+	+	+
473	3	+	+	+	+	+	+
494	3	+	+	+	+	+	+
16	3/2	−	−	+	+	+	+
394	3	NT	NT	+	+	+	NT
160	3	−	−	+	−	+	+
152	3	−	−	−	+	+	NT
Strains from root tissues of asymptomatic plants							
B31rb	3/2	−	−	−	−	−	−
B21r	3/2	NT	NT	−	−	−	NT
Positive controls							
FACH	1	+	+	+	+	+	+
B6	1	+	+	+	+	+	+
CG49	3	+	+	+	+	+	+
Ag57	3	+	+	+	+	+	+
Ag63	3	+	+	+	+	+	+
Negative control							
ATCC 31700	1	−	−	−	−	−	−

[a]Not all *Agrobacterium* strains from muscadine were readily classified as biovar 1, 2, or 3. Some were classified as 3/2 because they had positive characteristics for biovar 3 but some attributes of biovar 2, such as growth on erythritol and New and Kerr medium. Some were listed as unclassified (U) if a positive result was obtained in at least one of each of the three tests for biovar classification.

[b]NHR indicates that the DNA primer was from the narrow host range strain of *A. tumefaciens*, biovar 3, Ag63. WHR indicates that the DNA primer was from the wide host range strain of *A. tumefaciens*, biovar 1, A6.

[c]Two muscadine cultivars, Summit and Carlos, were employed for detached leaf pathogenicity (DLP) tests.

[d]Each polymerase chain reaction (PCR) contained 200 ng of target DNA.

[e]Not tested.

μg/ml) and re-extracted with phenol/chloroform. The concentration of DNA was determined by measuring the absorbance at 260 nm.

Preparation of oligonucleotide probes. Two recombinant plasmids containing portions of T-DNA were used for this work. The plasmid Bam 8/29, which contains a subcloned portion of T-DNA of the plasmid pTiA6 from strain A6 (biovar 1), was used as a wide host range control. The plasmid Sal 9.3, which contains T-DNA from the Ti plasmid of the narrow host range strain, Ag63 (biovar 3), was selected because this strain was originally isolated from grapevine (2). The presence of the Ti plasmid from Ag63 appears to limit the host range of *A. tumefaciens* to grape (30). At the time this study was initiated no cloned probe from a muscadine strain was available. Both plasmids were the generous gift of Mike Thomashow, Michigan State University, East Lansing. A 1.1-kb DNA *Eco*RI fragment of Bam 8/29 and 1.0-kb *Bam*HI DNA fragment of Sal 9.3 were sequenced (26) using the dideoxynucleotide method (24). Two oligonucleotides (20-mers) for each plasmid were designed to be used as primers for the amplification of DNA in the PCR (26). The primers were synthesized by Biotix (Danbury, CT) or by Genosys Biotechnologies, Inc. (Woodlands, TX) and were purified following the procedure provided by Biotix. The primers from the plasmid 8/29, designated wide host primers, amplify a 180-bp DNA fragment. The sequences of the wide host range primers were 1) 5′-AAGTCGTCGAGATACTGTTT-3′ and 2) 5′-TATGATACCTTATGCTGATG-3′. The primers from Sal 9.3, designated narrow host range primers, amplify a 150-bp DNA fragment. The sequences of the narrow host range primers were 1) 5′-AATCCTTCGCCTAGTCGTTA-3′ and 2) 5′-TCAATCGCCTTAACTTGAAC-3′.

PCR. The PCR procedure used was a modification of that described by Krawetz et al (12) and Krogan et al (13). Each PCR reaction (50 μl) contained 6.7 mM Tris-HCl, pH 8.8; 6.7 mM $MgCl_2$; 10 mM β-mercaptoethanol; 1.66 mM $(NH_4)_2SO_4$; 0.5% Triton X-100; 1.5 mM each of dATP, dCTP, dGTP, and dTTP; 250 μM of each oligonucleotide primer (either wide or narrow host range); target DNA isolated from the bacterial strains; and 2.5 units of DNA polymerase from *Thermus aquaticus* (*Taq* polymerase, Promega, Madison, WI). The amount of target DNA added to the PCR varied depending on the experiment.

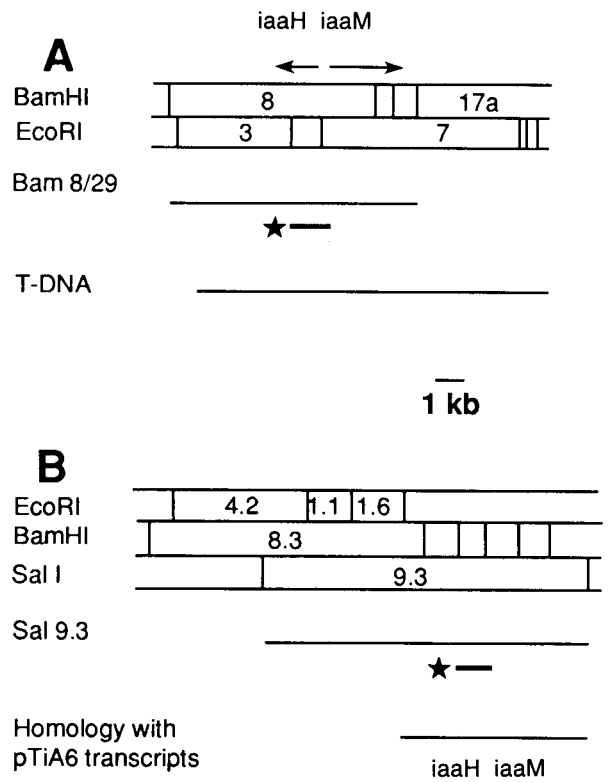

Fig. 1. Restriction maps of **A**, wide host range plasmid Bam 8/29, and **B**, narrow host range plasmid Sal 9.3. *iaa*H and *iaa*M designate pathogenicity genes present on the T-DNA. The heavy lines marked with a star indicate regions that were sequenced. Regions within these sequences were amplified in the polymerase chain reaction.

The temperature cycling for the PCR was conducted in a Coy TempCycler (Ann Arbor, MI) or Precision GTC-1 Thermal Cycler (Chicago, IL). For the first PCR cycle the reaction mixture (without *Taq* polymerase) was incubated at 95 C for 7 min followed by a 5-min incubation at 50 C. At this time *Taq* polymerase was added to the reaction, 35 μl of mineral oil was layered on top of the reaction mixture, and the sample was incubated at 72 C for 5 min. The first cycle was followed by 29 cycles as follows: 90 C for 2 min, 50 C for 2 min, and 72 C for 3 min. The reaction was stopped by chilling the mixture on ice.

Analysis of PCR products. Samples (15-μl aliquots) from the PCR were analyzed by polyacrylamide gel electrophoresis (16). Electrophoresis was conducted at 100 V for 1 h. After electrophoresis, the gel was stained with ethidium bromide (0.8 μg/ml) for 10 min. Bands were visualized with a UV transilluminator and gels were photographed using a Polaroid camera with a Kodak 22A Wratten (red) filter. DNA markers were the 1-kb ladder purchased from Bethesda Research Labs, Gaithersburg, MD.

Detached leaf pathogenicity assay procedure. PCR findings were compared with conventional assays employing a detached leaf pathogenicity (DLP) assay procedure as described by Thies et al (29). Muscadine leaves for DLP were collected from greenhouse-grown *Agrobacterium*-free plants produced by meristem tissue culture, transported to the laboratory, and stored at 4 C until processed. At least three leaves per strain per cultivar (cvs. Carlos and Summit) were inoculated in each study. Strains were classified as pathogenic or nonpathogenic based on the presence or absence of gall formation at the site of inoculation. Confirmatory reisolations were made.

Controls for each experiment included inoculation with sterile nutrient glucose broth in the same manner as with bacterial suspensions.

PCR comparisons. PCR analyses were compared with DNA slot blot hybridizations (26) and DLP tests using strains FACH, CG49, Ag57, and Ag63 from *V. vinifera;* B24A and 459C from muscadine; and ATCC 31700 (Table 1). Slot blots were probed with a 2-kb *Bam*HI-*Eco*RI fragment from Bam 8/29 (wide host range) or a 4.3-kb *Sal* 9.3-*Eco*RI fragment from Sal 9.3 (narrow host range). Both probes included the *iaa*H and *iaa*M regions of the T-DNA (26). Since these comparisons yielded identical results, both the PCR and the DLP test were subsequently used to screen a larger number of muscadine strains. All tests were repeated at least three times.

RESULTS AND DISCUSSION

Restriction maps of the two plasmids, Bam 8/29 and Sal 9.3, are shown in Figure 1. DNA slot blot hybridization (26) indicated that DNA inserts from both plasmids hybridized to DNA isolated from *A. tumefaciens* strains that were known to be pathogenic such as Ag57, FACH, and CG49, but that there was no hybridization to DNA isolated from *A. radiobacter* strains. These two probes also hybridized to DNA from several muscadine strains of known pathogenicity (459C, 504, and 460), but the hybridization was less intense, suggesting that there was less homology between the probes and the muscadine strains than from strains isolated from *V. vinifera* (26). For example, when Sal 9.3 was used as a probe an intense signal was obtained with 5–10 ng of Ag57 DNA, but about 500 ng of DNA from 459C or 504 was needed to obtain a clear positive hybridization (26). To improve the sensitivity of detection, we decided to test the applicability of the PCR.

Investigation of PCR sensitivity. The DNA sequence of a portion of Bam 8/29 and Sal 9.3 was determined (26) and was used to design oligonucleotides that could be used as primers to amplify specific regions of target DNA in the PCR. The primers are from the *iaa*H and *iaa*M regions of the T-DNA sequences, but they

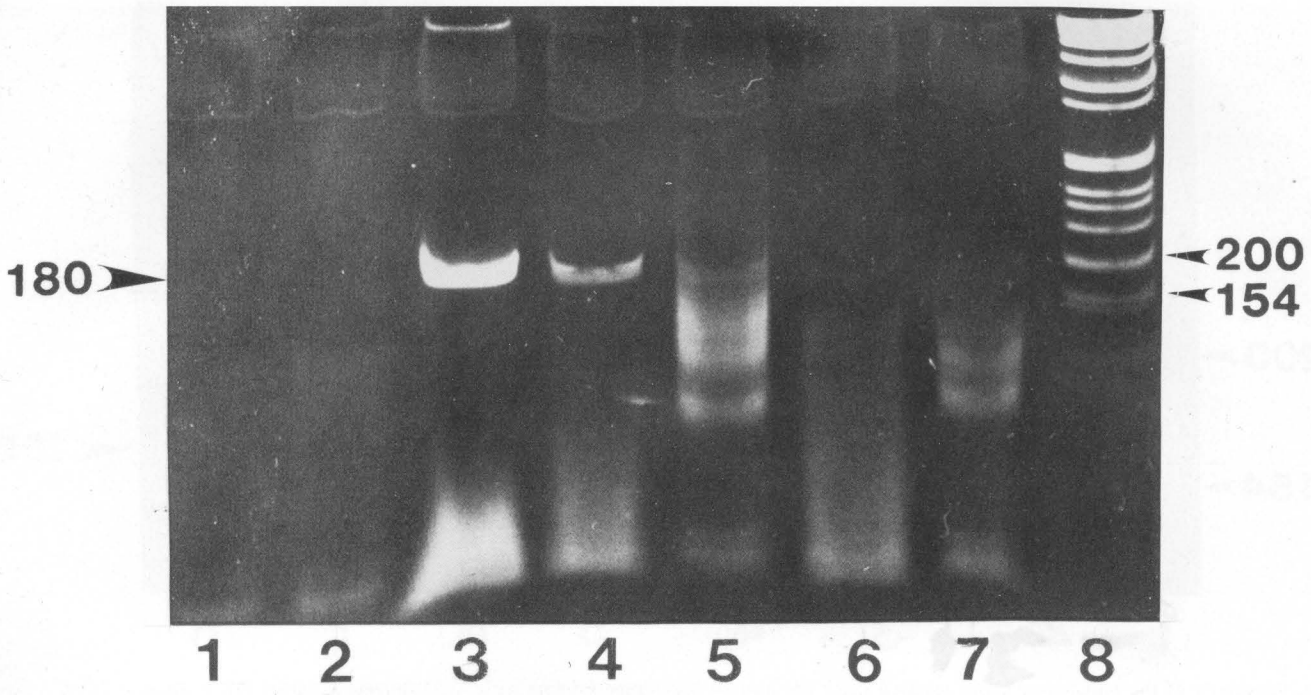

Fig. 2. Results of the polymerase chain reaction (PCR) using primers from the wide host range plasmid. Lane 1, control containing all of the PCR components but no target DNA. Lanes 2–7 contain 200 ng of target DNA from the following strains: 2, ATCC 31700 (*Agrobacterium radiobacter*); 3, B24A; 4, FACH; 5, CG49; 6, Ag63; 7, Ag57. Lane 8, DNA marker. The arrow on the left marks the amplified product of 180 bp. Numbers on the right margin refer to the size (in base pairs) of several of the DNA marker bands.

do not amplify overlapping sequences. *iaa*M and *iaa*H encode tryptophan monooxygenase and indoleacetamide hydrolase, respectively (19). The primers were selected from regions that contained less than 50% C-G pairs.

Results of the PCR, using the wide host range primers and target DNA from the several *Agrobacterium* strains, are given in Figure 2. A negative result was obtained with *A. radiobacter* strain ATCC 31700. Intense bands of 180 bp were obtained when DNA from biovar 1 strains (B24A and FACH) were used as target DNA. Very faint bands of 180 bp were present when DNA from biovar 3 strains CG49, Ag63, and Ag57 was used in the PCR. In addition to these bands, numerous smaller bands were present, which may be due to nonspecific priming. All of these strains, both biovar 1 and biovar 3, tested positive in the DLP test (Table 1). When the same *A. tumefaciens* strains were tested with the narrow host range primers, positives were obtained with Ag57, Ag63, CG49, B24A, and FACH (Fig. 3).

The sensitivity of the PCR was tested using the narrow host range primers with target DNA from Ag57 (Fig. 4). This strain was selected as a representative of biovar 3 isolated from *V. vinifera* (21). In this case, 0.01 ng of target DNA gave a weak positive result and 0.1 ng resulted in a strong positive (Fig. 4). This was about two orders of magnitude more sensitive than DNA slot blot hybridization, which gave positive results with 5–10 ng of Ag57 DNA when probed with Sal 9.3 (26). This degree of sensitivity was probably due to the high degree of homology between the target DNA and the primer. Both Ag63 (source of Sal 9.3) and Ag57 are closely related biovar 3 strains from Greece (21), and previous work (26) indicated that there was strong hybridization between Ag57 DNA and Sal 9.3 at the highest washing stringency.

The PCR was not as sensitive when the wide and narrow host range primers were used with FACH target DNA. In these cases the lowest amount of target DNA detected was 10 ng (Table 2). When the wide host range primers were used with target DNA from Ag57, the minimum amount of target DNA needed for a positive result was 150 ng (Fig. 5 and Table 2). The differences in sensitivity between these experiments and those using narrow host range primers and Ag57 target DNA are probably due to

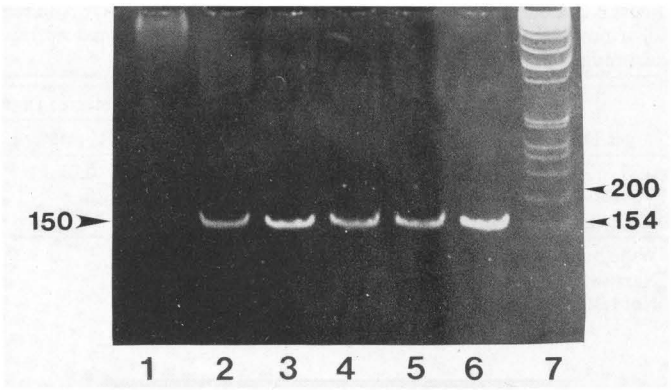

Fig. 3. Results of the polymerase chain reaction using primers from the narrow host range plasmid. Lanes 1–6 contain 200 ng of target DNA from the following strains: 1, ATCC 31700 (*Agrobacterium radiobacter*); 2, B24A; 3, FACH; 4, CG49; 5, Ag63; 6, Ag57. Lane 7, DNA marker. The arrow on the left marks the amplified product of 150 bp. Numbers in the right margin refer to the size (in base pairs) of several of the DNA marker bands.

the degree of sequence homology between the probe and the target DNA. DNA slot blot hybridizations (26) indicated that homology between the wide and narrow host range probes and FACH DNA, and the wide host range probe and Ag57 DNA, was less than the homology between Sal 9.3 and Ag57. If PCR primers had been selected from a more highly conserved regions of the *iaa*H and *iaa*M genes, the differences in sensitivity might have been less.

The sensitivity of the PCR also was determined using the muscadine strain 459C, which is known to cause galls on *Agrobacterium*-free muscadines, and the narrow host range primers. Positive results were obtained with 25 ng of target DNA, but the strongest band was obtained with 200 ng of DNA (Fig. 6). To ensure that sufficient target DNA was in the PCR, this amount of DNA was used in subsequent experiments. It is possible that the sensitivity would have been greater if primers had been

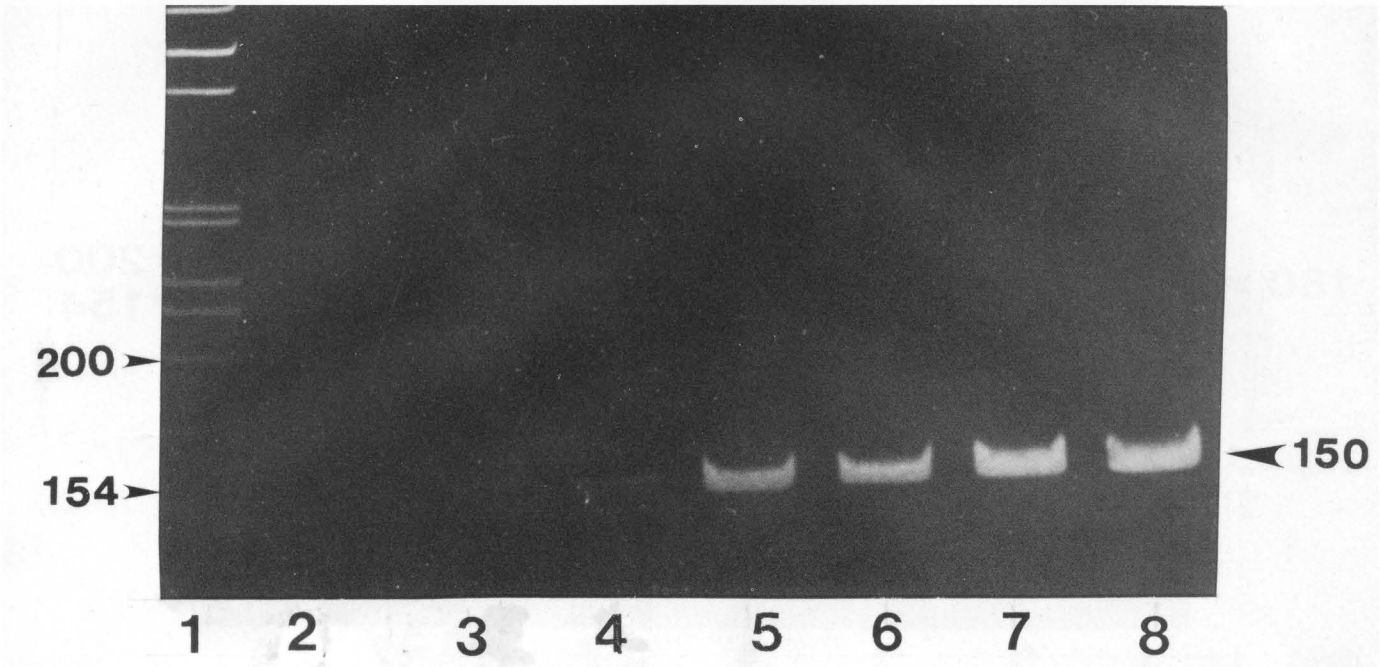

Fig. 4. Sensitivity of the polymerase chain reaction using the narrow host range primers with Ag57 (biovar 3) target DNA. Lane 1, DNA marker. Lanes 2–8 contain the following amounts of Ag57 DNA: Lane 2, 0 ng; lane 3, 0.001 ng; lane 4, 0.01 ng; lane 5, 0.10 ng; lane 6, 1.0 ng; lane 7, 10 ng; lane 8, 100 ng. The arrow on the right marks the amplified product of 150 bp. Numbers in the left margin refer to the size (in base pairs) of several of the DNA marker bands.

TABLE 2. Comparison of the minimum amount of target DNA required for a positive polymerase chain reaction result using wide and narrow host range primers

	Minimum amount of target DNA detected (ng)	
Target DNA	WHR[a] primers	NHR[b] primers
Ag57	150	0.01
FACH	10	10
459C	NT[c]	25

[a] Wide host range.
[b] Narrow host range.
[c] Not tested.

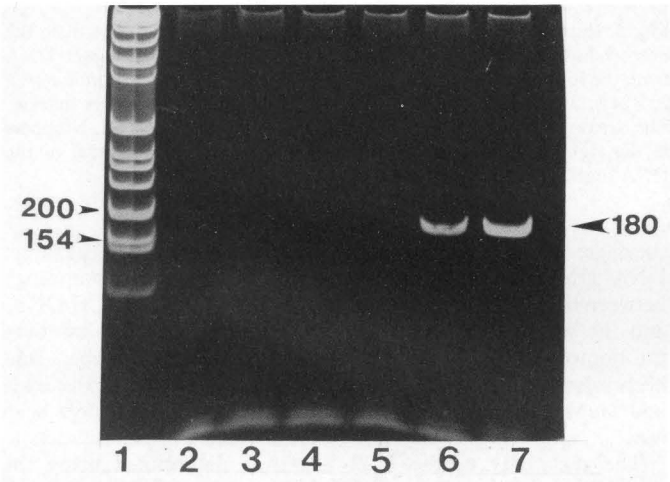

Fig. 5. Sensitivity of the polymerase chain reaction using the wide host range primers with Ag57 (biovar 3) target DNA. Lane 1, DNA markers. Lanes 2–7 contain the following amounts of Ag57 DNA: lane 2, 25 ng; lane 3, 50 ng; lane 4, 75 ng; lane 5, 100 ng; lane 6, 150 ng; lane 7, 200 ng. The arrow on the right marks the amplified product of 180 bp. Numbers in the left margin refer to the size (in base pairs) of several of the DNA marker bands.

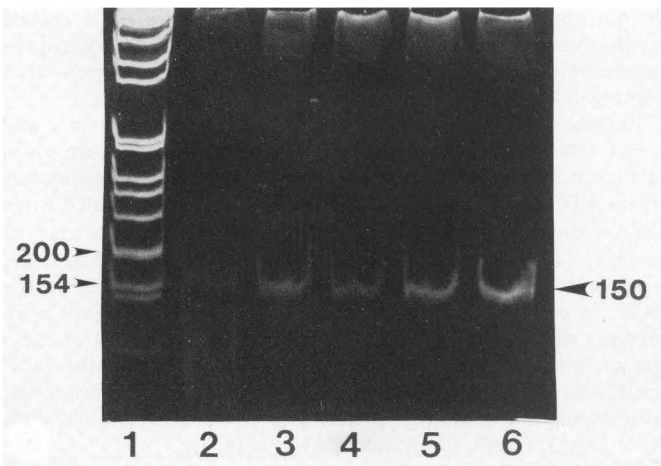

Fig. 6. Sensitivity of the polymerase chain reaction using the narrow host range primers with target DNA from muscadine strain 459C (biovar 3). Lane 1, DNA markers. Lanes 2–6 contain the following amounts of 459C DNA: lane 2, 25 ng; lane 3, 50 ng; lane 4, 100 ng; lane 5, 150 ng; lane 6, 200 ng. The arrow on the right marks the amplified product of 150 bp. Numbers in the left margin refer to the size (in base pairs) of several of the DNA marker bands.

derived from a muscadine strain of *A. tumefaciens*. Work is now underway to obtain a muscadine-specific primer.

Comparison of PCR, DLP tests, and DNA slot blot hybridizations. Since the PCR analyses (Figs. 2–6), DNA hybridizations (26), and DLP tests (Table 1) with strains 459C, FACH, CG49, Ag57, Ag63, and ATCC 31700 yielded identical results, both the PCR and the DLP test were subsequently used to screen a larger number of strains. The results of these, as well as biotype determinations, are shown in Table 1. In most cases the results of the PCR, DNA hybridizations, and DLP tests were similar.

Of 31 muscadine strains tested, agreement between PCR and DLP results was obtained with 27 (22 positive, five negative). Among those with disagreement were two strains with a positive DLP and a negative PCR, and two with a positive PCR and

a negative DLP. The 13% level of disagreement between the two tests is not surprising considering the accuracy level of the DLP tests alone. There is presently no absolute method for assessing the accuracy of the DLP test, but repetitive tests suggest an accuracy level of perhaps no more than 80% per three-leaf test as described above (C. H. Graves, *unpublished*). Strains providing positive readings can reasonably be assumed to be *A. tumefaciens,* but the reciprocal is not always true. The accuracy of the PCR is yet to be determined.

In two cases (strains B32 and 459) both the DLP and DNA hybridization tests (26) were negative but the PCR tests were positive, suggesting that perhaps the PCR gave false positives. In two cases (strains B25A and 266) the PCR was negative and the DLP tests were positive. Both of these strains were also negative in DNA hybridization tests (26). The reasons for such discrepancies are unknown, but it is possible that there was insufficient sequence homology between the primers and the target DNA. In nine cases the PCR and DLP were positive, but the slot blot hybridization was negative (B21c, 022, 024A, 513, 461, 500, 16, 160, and 152). The lack of hybridization is probably due to the limited homology between the probes and DNA from the muscadine strains (26). This was apparently overcome by using the PCR.

The PCR appears to be a promising technique for determining potential pathogenicity of *Agrobacterium* strains from muscadine. In our hands the PCR was typically one to two orders of magnitude more sensitive than slot blot hybridization. In most cases the results of the PCR and the DLP tests were similar. At present there is no absolute standard for easy evaluation of accuracy. However, the results of this study suggest that accuracy of PCR should equal, or exceed, the DLP method without excessive repetition, and it offers the advantages of a more rapid and less costly procedure when compared with the DLP. It may be possible to improve the sensitivity and reliability of the PCR for detecting pathogenic strains of *Agrobacterium* from muscadine by developing primers from the T-DNA region of muscadine strains of *A. tumefaciens.* Such primers should be more accurate with muscadine strains by virtue of the greater homology.

The long-range goals of this study include the development of systems for propagating muscadines free of *A. tumefaciens* for vineyard establishment and systems for efficient and rapid monitoring of plants of commerce for the presence of systemic *A. tumefaciens.*

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