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Attachment, Chemotaxis, and Multiplication of *Agrobacterium tumefaciens* Biovar 1 and Biovar 3 on Grapevine and Pea

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Tumorigenic (CG49) and nontumorigenic (CG484) strains of *Agrobacterium tumefaciens* bv. 3 attached to grape roots at a higher level than did a nonpectinolytic mutant of CG49 (CG50) or a tumorigenic strain of *A. tumefaciens* bv. 1 (CG628). Strains attached equally well to wounded and unwounded grape roots. Strains responded differently to pea plants in that biovar 3 strains consistently attached to unwounded roots at a lower level than they did to wounded roots, whereas CG628 attached equally well regardless of wounding. The lowest levels of attachment to pea roots were consistently observed for CG50. Population curves were calculated for the strains inoculated into wound sites on grape and pea roots. *A. tumefaciens* bv. 3 wild-type strains developed greater populations at wound sites on grape roots after 100 h (resulting in root decay) than did CG50 or CG628. Population curves for strains at wound sites on pea roots were different from those on grape roots. There were no significant differences in populations after 100 h, and no strains caused root decay. No differences in the chemotaxis of wild-type and mutant *A. tumefaciens* bv. 3 strains towards grape roots, crown pieces, or root extracts were observed, but the biovar 1 strain, CG628, always migrated the greatest distance towards all substrates. Polygalacturonase production may affect attachment to grape roots and multiplication of *A. tumefaciens* bv. 3 at wound sites and thus be associated with the specificity of the bacterium for grape.

Agrobacterium tumefaciens (Smith and Townsend) Conn causes crown gall disease of several plant species (7). Three biovars of *A. tumefaciens* were described by Kerr and Panagopoulos (9). Biovar 3, which is the predominant type isolated from grape plants (*Vitis vinifera* L.), has not been isolated from other plants. Ophel and Kerr recently proposed that *A. tumefaciens* bv. 3 be renamed *A. vitis* (18).

This host specialization of *A. tumefaciens* bv. 3 for grape plants has been reported by several researchers (4, 12, 20, 26). The bacterium survives systemically in grape plants and incites a decay of grape roots that is associated with the production of a chromosomally encoded polygalacturonase (PG) (3, 15, 19). PG has not been detected for other biovars and is associated with the ability of biovar 3 to cause a decay of grape roots (23). It may also play a role in other specific interactions related to the specificity of biovar 3 to grape roots.

Host-pathogen interactions prior to transformation, such as attachment, are known to be important in the infection process of *A. tumefaciens* (2, 10, 14, 16) and may be associated with host specificity. In this paper, we measure the effects of the type of biovar, its tumorigenicity, and PG production on the attachment of the bacterium to roots, bacterium chemotaxis towards root exudates, and bacterium multiplication at wound sites of grape and pea (*Pisum sativum* L.) roots.

MATERIALS AND METHODS

Plant material. Grape (cultivar Riesling) seeds were germinated in the greenhouse in humidified perlite and were used in experiments 1 week after seed emergence. Dormant cuttings of grape canes (cultivar Riesling) were planted in perlite for rooting, and roots were harvested for experiments

after 1 month. Roots from seedlings or cuttings were treated by immersion for 1 min in 10% Clorox and then four rinses in sterile distilled water. Pea seeds (cultivar Bonneville and Ranger) were treated by immersion for 15 min in 95% ethanol, 30 min in full-strength Clorox, and 15 min in half-strength Clorox and then four rinses in sterile distilled water, after which they were immersed for 1 h in a captan solution (1 g of active ingredient per liter). Seeds were then germinated in petri dishes on 0.7% water agar overlaid with sterile filter paper and used 2 or 3 days after germination.

Bacteria. Bacteria are listed in Table 1. Transposon mutagenesis with strain CG49 was performed by using pSUP2021 (24) as a Tn5 delivery system. Over 8,000 transconjugants were screened for pectinolytic activity by using thin agarose gels amended with polygalacturonic acid (15), and one PG-negative mutant (CG50) was identified. The nature of the mutation in CG50 has been characterized further (23). Strains were cultured on PDA medium (Difco), which was supplemented with kanamycin (50 ppm) for the mutant CG50. Cells were harvested from plates after growing for 24 h at 28°C.

Presence of Ti plasmid assay. Plasmid DNA was isolated as described by Slota and Farrand (25). Undigested plasmids were electrophoresed in 0.7% agarose in TBE buffer (13) at 5 V/cm. DNA was Southern transferred to a GeneScreen Plus-Hybridization Transfer Membrane (Dupont, NEN Research Products) by alkaline transfer (22) and was hybridized with a ³²P-labeled T-DNA probe, pTHE17 (6).

Tumorigenicity assays. Bacteria were collected on a sterile toothpick and placed onto stems of 2-week-old grape and pea seedlings in the greenhouse. A single needle puncture was then made through the inoculum into the stem. At least three grapevine and three pea seedlings were inoculated with each bacterial strain, and the test was repeated once. Tumorigenicity was assessed for up to 1 month after inoculation.

Root decay assays. One-week-old seedlings were placed in

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TABLE 1. Characteristics of *Agrobacterium* strains

Strain	Biovar	Source	Tumorigenicity ^a		Presence of pTi ^b	Grape root decay ^c		Pectinolytic activity ^d
			Grape	Pea		Wounded	Unwounded	
CG49	3	Grape gall (N.Y.)	+	—	+	+	—	+
CG50	3	Mutant of CG49 ^e	+	—	+	—	—	—
CG484	3	Grape roots (Wash.)	—	—	—	+	—	+
CG628	1	Grape callus (N.Y.)	+	+	+	—	—	—

^a Tumorigenicity was assayed as described in Materials and Methods.

^b Presence of Ti plasmid was determined as described in Materials and Methods.

^c Root decay was assayed as described in Materials and Methods.

^d Pectinolytic activity was determined as described by McGuire (15).

^e PG-deficient mutant obtained by transposon mutagenesis as described in Materials and Methods.

petri dishes on humidified perlite. Half of the seedlings were wounded by making a single needle puncture into the area of the root crown where lateral roots form. All the seedlings were then inoculated by depositing a 2- μ l drop (about 10^9 CFU ml⁻¹) of a bacterial suspension (or distilled sterile water for the controls) on the wounded or unwounded crown region. They were incubated at 25°C in the dark, and the appearance of necrosis was assessed 2 to 4 days after inoculation. Three wounded and unwounded grape seedlings were inoculated for each strain, and this experiment was repeated once.

Chemotaxis assays. Chemotaxis towards root tips, crown tissue, and root extracts from pea and grape seedlings was measured. One root tip or one crown piece (5 mm) of a seedling was excised and placed at the edge of a 5-cm (diameter) petri dish containing a semisolid medium (5 ml of 10 mM K₂HPO₄ solidified with 0.2% agar and adjusted to pH 6 before autoclaving) (8). Alternatively, a 10- μ l filter-sterilized droplet of extract from triturated pieces (50 mg, fresh weight, triturated in 1 ml of distilled water) of roots or crowns of seedlings was deposited at the edge of the plate. A 10- μ l drop of bacteria containing about 10^9 CFU ml⁻¹ was placed into the center of the plate. After 48 h of incubation at 28°C, the distance of migration of bacteria towards the root or crown pieces or the tissue extract was measured. Three petri dishes were prepared for each strain, and experiments were repeated once.

Root attachment assays. Treatments consisted of submersing wounded or unwounded roots that were harvested from grape cuttings or pea seedlings in a bacterial suspension adjusted to about 10^7 and 10^8 CFU ml⁻¹ for grape roots and 10^7 CFU ml⁻¹ for pea roots. Roots were selected for uniformity in size, and only healthy-appearing roots were used. Wounded roots were prepared by excising 3 mm of the root tip. About 2-cm lengths of wounded and unwounded roots were submersed in the bacterial suspensions for 1 h at 28°C. After removing roots from the bacteria, a 5-mm section adjacent to the previous cut for wounded roots was excised. For unwounded roots, the 3-mm tip section was removed and then a 5-mm section was excised for assay. The root pieces were placed individually in 10 ml of sterile distilled water and vigorously stirred on a vortex stirrer for 5 s, placed in a second 10 ml of water, vortexed again, removed from the water, and finally triturated in 500 μ l of 10 mM HEPES, pH 7.5 (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid). Triturates were dilution plated on PDA medium supplemented with cycloheximide (100 mg/liter) for strains CG49, CG484, and CG628 or with kanamycin (50 mg/liter) for CG50. Three wounded and unwounded root tips of grapevine and pea plants were assayed for each strain and for each concentration of bacterial suspension, and the

experiment was repeated once. An analysis of variance was determined by using Statworks (Cricket Software, Inc., Philadelphia, Pa.), and the least significant difference was calculated for separation of means (*P*, 0.05).

Bacterial growth assays. Grape and pea seedlings were placed in petri dishes on humidified perlite, and their crowns were wounded by making a single needle puncture. A 2- μ l droplet of a bacterial suspension containing at least 10^9 CFU ml⁻¹ was then deposited on the wound, and seedlings were incubated at 25°C in the dark. At various times, 2-cm-long pieces surrounding the inoculated wound were triturated in 500 μ l of 10 mM HEPES, pH 7.5, and triturates were dilution plated as described for the root attachment assays. Three grapevine and pea seedlings were inoculated for each strain, and the experiment was repeated once. Results were statistically analyzed as described above.

RESULTS

Chemotaxis assays. *A. tumefaciens* bv. 1 strain CG628 consistently migrated a much greater distance towards root and crown tissues and extracts from grape and pea plants than did strains of *A. tumefaciens* bv. 3 (Fig. 1). Biovar 3 strains migrated from 6 to 9 mm towards grape and pea extracts, whereas CG628 migrated from 16 to 23 mm. There were no apparent differences between the migration of wild-type and mutant biovar 3 strains towards any of the

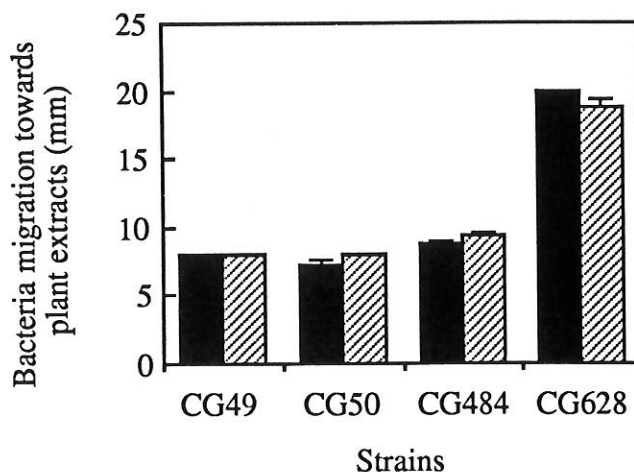


FIG. 1. Chemotaxis towards grape (■) and pea (▨) crown extracts by strains of *A. tumefaciens* bv. 1 and bv. 3. Bars are the means of three replicates. Also shown are the standard errors of the means.

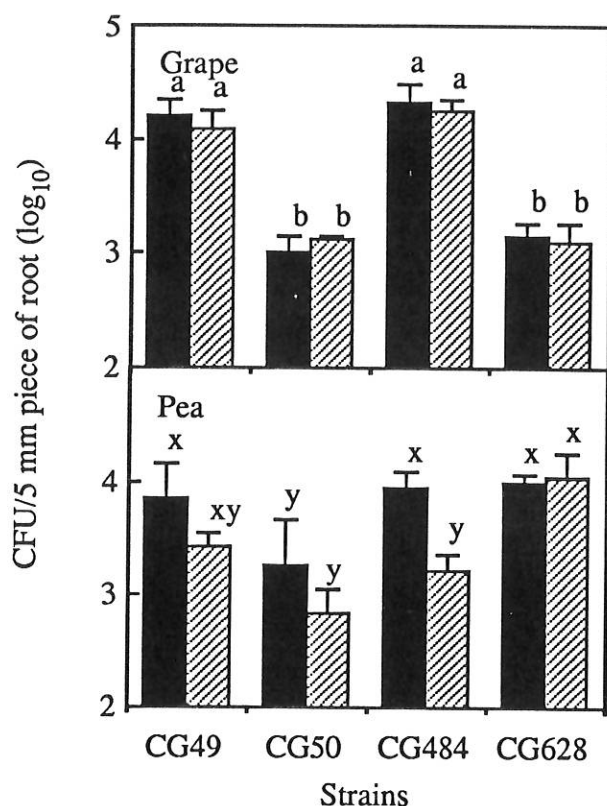


FIG. 2. Attachment of *A. tumefaciens* bv. 1 and bv. 3 to wounded (■) and unwounded (▨) grape and pea roots. Bars are the means of three replicates. Also shown are the standard errors of the means. Bars with the same letters represent numbers of bacteria that are not significantly different ($P = 0.05$).

tissues or extracts. Controls, consisting of media with no extracts or root pieces, showed no specific directional pattern of migration for any of the strains.

Root attachment assays. Differences in the level of attachment of strains to grape and pea roots were observed when a bacterial suspension containing 10^7 CFU ml^{-1} was used (Fig. 2). About 2×10^4 CFU/5-mm grape root was detected for strains CG49 and CG484, but only about 10^3 CFU/5-mm root was detected for CG50 and CG628. When a bacterial suspension of 10^8 CFU ml^{-1} was used, an equal number of cells (about 2×10^4 CFU/5-mm root) was detected for all strains. PG production by *A. tumefaciens* bv. 3 but not tumorigenicity affected attachment. CG50 (a nonpectinolytic mutant of CG49) attached to grape roots at about the same level as CG628, and there were no significant differences between attachment to wounded and unwounded grape roots for any of the strains. On wounded pea roots (only bacterial suspensions of 10^7 CFU ml^{-1} were tested), no significant differences in attachment were detected for strains CG49, CG484, and CG628. On unwounded pea roots, the level of attachment for biovar 3 strains was decreased, but this was not the case for biovar 1 strain CG628. A relatively lower level of attachment was consistently detected for CG50 on wounded and unwounded pea roots.

Bacterial growth assays. Only the PG-producing strains of *A. tumefaciens* bv. 3, CG49 and CG484, were able to multiply exponentially for at least 100 h at wound sites on grape roots (Fig. 3). For these strains, root decay symptoms were visible within 48 h of inoculation, and the progressive

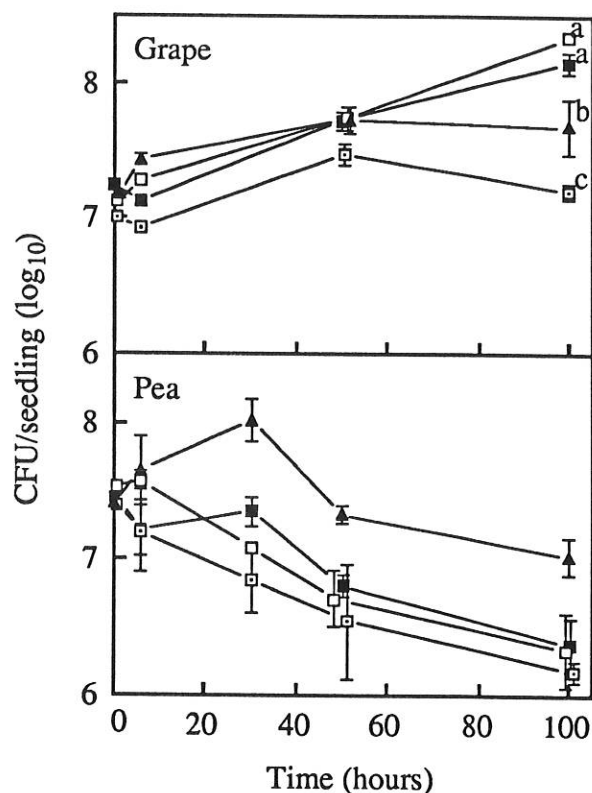


FIG. 3. Bacterial growth of CG49 (■), CG484 (□), CG50 (□), and CG628 (▲) at wound sites on grape and pea seedlings. Datum points are the means of three replicates; vertical bars represent standard errors of the means. Points at 100 h followed by the same letters represent numbers of bacteria that are not significantly different ($P = 0.05$). There were no significant differences in populations on pea seedlings.

necrosis extended from 0.5 to 1 cm in length after 4 days. Strain CG628 established the greatest populations at the wound sites in the first 48 h after inoculation; however, growth then tended to stabilize and decrease. CG50 produced the lowest population in the first 48 h and then produced a population curve similar to that of CG628. Neither CG50 nor CG628 caused root decay. On pea roots, biovar 3 strains generally showed a decrease in population over time. Strain CG628, however, increased in the first 48 h, and then the bacterial population began to decrease up to 4 days after inoculation. After 100 h, there were no significant differences between populations of any of the strains on pea roots. No necrosis was noticed on any of the pea seedlings that were inoculated with the different strains.

DISCUSSION

We have demonstrated that the capacity of *A. tumefaciens* bv. 3 to attach to grapevine roots, multiply at root wound sites, and produce PG may be associated with the specificity of this bacterium for grape. Chemotaxis, however, appeared to be nonspecific, since the biovar 1 strain migrated the greatest distance towards grape tissues.

We showed that wild-type biovar 3 strains attached at a higher level to grape roots than a nonpectinolytic mutant or *A. tumefaciens* bv. 1. Attachment of *Agrobacterium* spp. to host cells is known to be an important early step in tumori-

genesis (2, 10, 11, 17). Since biovar 3 does not generally incite tumors on grape roots but rather causes root decay, specific attachment to roots may provide other ecological advantages for the bacterium, i.e., a mechanism by which it can competitively colonize the grape rhizoplane, cause decay, and systemically invade the plant. Previously, it was demonstrated that strains of *A. tumefaciens* bv. 1 and 2 can be detected in vineyard soil (4, 5), yet biovar 3 is by far the predominant biovar isolated systemically from vines and from grape tumors. Although only one strain of biovar 1 was tested in these experiments, it will be interesting to determine whether preferential attachment of *A. tumefaciens* bv. 3 to grape roots can be observed when comparisons are made with other strains as well.

Wounding of grape roots did not affect the level of attachment by any of the strains, suggesting that specific exudates from wounded roots do not enhance the attachment process. Even the biovar 1 strain CG628 attached equally well to wounded and unwounded grape roots. However, it should be noted that CG628 was initially isolated from a grape tumor, is tumorigenic on grape roots, and therefore must be considered a grape pathogen. In contrast, on pea roots (nonhost for biovar 3), wounding affected the attachment of biovar 3 but not that of CG628 (a pathogen of pea). This suggests that pathogens may attach equally well to wounded and unwounded roots of hosts, whereas nonpathogens attach more efficiently to wounds. It will be necessary to test other nonpathogens to verify this hypothesis. Other factors may have also affected the difference observed in the attachment of biovar 3 to pea roots. The presence of a Ti plasmid was not associated with attachment in these experiments, since the nontumorigenic strain CG484 contains no detectable plasmids.

The production of PG appears to affect the attachment of bacteria to pea and grape roots and may be functioning by interacting with specific bacterial receptor sites on root cells, thereby increasing the efficiency of attachment. Different levels of attachment to grape roots were observed when they were submersed in a bacterial suspension of 10^7 CFU ml⁻¹ but not in a 10^8 CFU ml⁻¹ suspension. Apparently, after 1 h of incubation in the 10^7 CFU ml⁻¹ suspension, receptor sites on grape root cells were saturated with strains CG49 and CG484 but not with CG50 or CG628. At 10^8 CFU ml⁻¹, attachment levels remained the same for CG49 and CG484 but were significantly increased for CG50 and CG628. These results suggest that PG production may be affecting the efficiency of bacterial attachment. PG may be interacting with various pectin or pectin-related components of the plant cell wall that may affect the attachment process of *A. tumefaciens* (21). For example, it has been shown that a pectin or pectin-associated receptor for *Agrobacterium* attachment exists on the plant cell wall (16). By adding a pectin-enriched soluble cell wall fraction from tomato plants to cell suspensions of several species, attachment is inhibited. The role of PG in attachment seems most logical for grape roots, where the enzyme is produced by biovar 3 and is associated with root decay (15). It is not known whether PG is produced by biovar 3 on pea plants; however, no decay has been observed. The apparent increased level of attachment by pectinolytic compared with nonpectinolytic biovar 3 strains on pea plants may result from enzyme being produced on pea plants or from enzyme being introduced into the system along with the bacteria. PG production has not been detected for other *Agrobacterium* spp. (15), and it will be interesting to determine whether PG can affect

attachment and the subsequent level of transformation by other *Agrobacterium* spp. and on different hosts.

It has been demonstrated that plant wounds release compounds that induce Vir gene activity and are thus essential for tumorigenesis by *A. tumefaciens* (1). In the grape system, crown gall usually occurs on the lower trunks of vines at injury sites and is rarely detected on roots, although the bacterium is detectable in the rhizosphere (5). It is possible, therefore, that wounded grape roots secrete specific compounds that induce or enhance PG production and subsequent root decay. No decay developed on unwounded grape roots. Vir genes, therefore, may not be induced by grape root extracts, or tumorigenesis may be inhibited on roots following root decay and cell death. On grape stems, however, PG may enhance tumorigenicity by affecting attachment and induction of Vir gene activity. It was previously reported that, although tumorigenic biovar 3 strains incite decay of grape roots, they cause tumors on stems (3). Ankenbauer and Nester (1) have recently shown that various monosaccharides, including galacturonic acid, induce Vir gene activity of *A. tumefaciens*. If this hypothesis is confirmed, PG could be an important factor in various aspects of the pathogenesis of biovar 3 including host specificity, plant colonization, and cell infection.

The detection of population increases of biovar 3 over time at wound sites on grape roots is another indication of the specificity of this bacterium for grape roots and the potential role of PG. The smallest populations were always measured for strain CG50, and populations of CG628 were also significantly smaller than the wild-type biovar 3 strains.

Our conclusions on the potential significance of PG are based on the assumption that the mutation in CG50 is only affecting PG production and not other potentially important traits of the bacterium. Recent results which support this idea show that the mutation in CG50 is located in the 8.5-kb fragment that contains the PG structural gene (23). These experiments provide the first direct indication that PG may be involved in host specialization of *A. tumefaciens* bv. 3.

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REFERENCES

1. Ankenbauer, R. G., and E. W. Nester. 1990. Sugar-mediated induction of *Agrobacterium tumefaciens* virulence genes: structural specificity and activities of monosaccharides. *J. Bacteriol.* 172:6442-6446.
2. Binns, A. N. 1988. Cell biology of *Agrobacterium* infection and transformation of plants. *Annu. Rev. Microbiol.* 42:575-606.
3. Burr, T. J., B. H. Bishop, B. H. Katz, L. M. Blanchard, and C. Bazzi. 1988. A root-specific decay of grapevine caused by *Agrobacterium tumefaciens* and *A. radiobacter* biovar 3. *Phytopathology* 77:1424-1427.
4. Burr, T. J., and B. H. Katz. 1983. Isolation of *Agrobacterium tumefaciens* biovar 3 from grapevine galls and sap and from vineyard soil. *Phytopathology* 73:163-165.
5. Burr, T. J., B. H. Katz, and A. L. Bishop. 1987. Populations of *Agrobacterium* in vineyard and nonvineyard soils and grape roots in vineyards and nurseries. *Plant Dis.* 71:617-620.
6. Burr, T. J., J. L. Norelli, B. H. Katz, and A. L. Bishop. 1990. Use of Ti plasmid DNA probes for determining tumorigenicity of *Agrobacterium* strains. *Appl. Environ. Microbiol.* 56:1782-1785.
7. DeCleene, M., and J. DeLey. 1976. The host range of crown gall. *Bot. Rev.* 42:389-466.
8. Hawes, M. C., L. Y. Smith, and A. J. Howarth. 1988. *Agrobacterium tumefaciens* mutants deficient in chemotaxis to root exudates. *Mol. Plant-Microbe Interact.* 1:182-186.

9. Kerr, A., and C. G. Panagopoulos. 1977. Biotypes of *Agrobacterium radiobacter* var. *tumefaciens* and their biological control. *Phytopathol. Z.* **90**:172-179.
10. Knauf, V. C., C. G. Panagopoulos, and E. W. Nester. 1982. Genetic factors controlling the host range of *Agrobacterium tumefaciens*. *Phytopathology* **72**:1545-1549.
11. Lippincott, J. A., and B. B. Lippincott. 1975. The genus *Agrobacterium* and plant tumorigenesis. *Annu. Rev. Microbiol.* **29**:377-405.
12. Loubser, J. T. 1978. Identification of *Agrobacterium tumefaciens* biovar 3 on grapevine in South Africa. *Plant Dis. Rep.* **62**:730-731.
13. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. Matthysse, A. G. 1984. Interaction of *Agrobacterium tumefaciens* with plant cell surface, p. 33-54. In D. P. S. Verma and T. Hohn (ed.), *Plant gene research. Genes involved in microbe-plant interactions*. Springer-Verlag, Inc., New York.
15. McGuire, R. G., P. Rodriguez-Palenzuela, A. Collmer, and T. J. Burr. 1991. Polygalacturonase production by *Agrobacterium tumefaciens* biovar 3. *Appl. Environ. Microbiol.* **57**:660-664.
16. Neff, N. T., A. N. Binns, and C. Brandt. 1987. Inhibitory effects of a pectin-enriched tomato cell wall fraction on *Agrobacterium tumefaciens* binding and tumor formation. *Plant Physiol.* **83**:525-528.
17. Nester, E. W., M. P. Gordon, R. M. Amasino, and M. F. Yanofsky. 1984. Crown gall: a molecular and physiological analysis. *Annu. Rev. Plant Physiol.* **35**:387-413.
18. Ophel, K., and A. Kerr. 1990. *Agrobacterium vitis* sp. nov. for strains of *Agrobacterium* biovar 3 from grapevines. *Int. J. Syst. Bacteriol.* **40**:236-241.
19. Ophel, K. M., D. A. Jones, and A. Kerr. 1990. Molecular cloning of cell wall degrading enzymes from *Agrobacterium tumefaciens* biovar 3. *Phytopathology* **80**:982 (Abstr.).
20. Perry, K. L., and C. I. Kado. 1982. Characteristics of Ti plasmids from broad-host-range and ecologically specific biotype 2 and 3 strains of *Agrobacterium tumefaciens*. *J. Bacteriol.* **151**:343-350.
21. Pueppke, S. G., and U. K. Benny. 1984. Adsorption of tumorigenic *Agrobacterium tumefaciens* cells to susceptible potato tuber tissues. *Can. J. Microbiol.* **30**:1030-1037.
22. Reed, K. C., and D. A. Mann. 1985. Rapid transfer of DNA from agarose gels to nylon membranes. *Nucleic Acids Res.* **13**:7207-7221.
23. Rodriguez-Palenzuela, P., T. J. Burr, and A. Collmer. 1991. Polygalacturonase is a virulence factor in *Agrobacterium tumefaciens* biovar 3. *J. Bacteriol.* **173**:6547-6552.
24. Simon, R., U. Priefer, and A. Puhler. 1983. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram-negative bacteria. *Biotechnology* **1**:784-791.
25. Slota, J. E., and S. K. Farrand. 1982. Genetic isolation and physical characterization of pAgK84, the plasmid responsible for agrocin 84 production. *Plasmid* **8**:175-186.
26. Süle, S. 1978. Biotypes of *Agrobacterium tumefaciens* in Hungary. *J. Appl. Bacteriol.* **44**:207-213.

