Correlative Association between Resident Plasmids and the Host Chromosome in a Diverse Agrobacterium Soil Population

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Soil samples collected from a fallow field which had not been cultivated for 5 years harbored a population of Agrobacterium spp. estimated at 3 × 10^7 CFU/g. Characterization of 72 strains selected from four different isolation media showed the presence of biovar 1 (56%) and bv. 2 (44%) strains. Pathogenicity assays on five different test plants revealed a high proportion (33%) of tumorigenic strains in the resident population. All tumorigenic strains belonged to bv. 1. Differentiation of the strains by restriction fragment length polymorphism analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cellular proteins, and utilization patterns of 95 carbon substrates (Biolog GN microplate) revealed a diversified bv. 1 population, composed of five distinct chromosomal backgrounds (chr A, C, D, E, and F), and a homogeneous bv. 2 population (chr B). chr A, B, C, and D were detected at similar levels throughout the study site. According to opine metabolism, pathogenicity, and agrocin sensitivity, chr A strains carried a nopaline Ti plasmid (pTi), whereas chr C strains had an octopine pTi. In addition, four of six nontumorigenic bv. 1 strains (two chr D, one chr E, and one chr F) had distinct and unusual opine catabolism patterns. chr B (bv. 2) strains were nonpathogenic and catabolized nopaline. Although agrocin sensitivity is a pTi-borne trait, 14 chr B strains were sensitive to agrocin 84, apparently harboring a defective nopaline pTi similar to pAtK84b. The other two chr B strains were agrocin resistant. The present analysis of chromosomal and plasmid phenotypes suggests that in this Agrobacterium soil population, there is a preferential association between the resident plasmids and their bacterial host.

Agrobacterium species are gram-negative soil bacteria belonging to the family Rhizobiaceae (29). This genus is composed of at least three basic chromosomal groups which have been allocated the rank of biovar (29). Each biovar is identified by an array of biochemical and physiological tests (36). Some Agrobacterium strains can induce a neoplastic disease, known as crown gall, on a large number of plant families (15). This tumorigenic ability is borne on large extrachromosomal DNA elements, called Ti plasmids (pTi), which are transferable by conjugation to nontumorigenic Agrobacterium strains (56). During the infection process, a specific DNA fragment (the T-DNA) is transferred from the pTi to wounded plant cells and integrated into the nuclear genome (12). Transfer of T-DNA is directed by a set of virulence (vir) genes located in the nontransferred region of the pTi (54). The T-DNA carries both oncogenes, which perturb plant cell division (1, 50), and genes coding for the synthesis of low-molecular-weight compounds, termed opines, specifically metabolized by the tumor-inducing bacterium for its growth (4, 44). Some opines also induce conjugal transfer of the pTi to nontumorigenic agrobacteria (46). However, different opines are synthesized in tumors incited by different pTi, and induction of conjugation is relatively specific. Octopine induces conjugation by strains carrying an octopine-type pTi, whereas nopaline strains are not induced to conjugate by octopine or even nopaline but by another opine, agrocinopine A, which is also present in tissue transformed by T-DNA of nopaline-type pTi. This selective opine induction contributes to the propagation of pathogenicity genes and, consequently, plays a subtle role in the ecology of this bacterium and the epidemiology of the disease it causes (16).

Crown gall infection may have significant detrimental effects on the host plant, generating major economic losses (25, 37, 51). To evaluate the potential for crown gall epidemics and the possible efficacy of control measures such as the introduction of antagonists (e.g., strain K84 (27)), more information on the behavior of this bacterium and of its pTi outside the tumor environment is needed. At present, information on survival potential and genetic stability of tumorigenic agrobacteria in the soil environment is, at best, limited. This paucity of information is due in part to the rare detection of tumorigenic strains in the soil and on roots, even though nontumorigenic agrobacteria are commonly found (2, 7, 10, 35, 52). These apparently low levels of tumorigenic bacteria in soil are in sharp contrast to the regular occurrence of crown gall epidemics and the efficacious protection against soil infections by the biocontrol agent K84 (5, 35).

The need for a better understanding of the ecology of agrobacteria and resident pathogenic plasmids led us to examine the composition of an Agrobacterium soil population and the type of plasmids associated with the host bacterium. This bacterial population was indigenous to a soil

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which was fallow for 5 years after 2 years of stone-fruit tree nursery production.

(A portion of this work was presented at the Annual Meeting of the American Phytopathological Society, St. Louis, Mo. [8].)

MATERIALS AND METHODS

Isolation and culture of bacteria. Bacteria were isolated from soil samples collected at the experiment station of the Institut Technique d’Arboriculture Fruitière et de Viticulture located in the Mitidja plain (Algeria). Four sampling sites were randomly chosen from a plot (32 by 38 m). Samples for analysis were taken at a soil depth of 5 to 10 cm. Soil analysis revealed a loamy texture and an alkaline reaction (pH 8.5). From each sample, 1.0 g of soil was suspended by shaking in 10 ml of sterile distilled water, and the suspension was allowed to settle for 5 min. Tenfold serial dilutions were made, and a 100-μl aliquot of selected dilutions was spread onto duplicate plates containing the following media: 1A (selective for bv. 1) (9), 2E (selective for bv. 2) (9), RS (selective for bv. 3) (48), and MG (nonselective) (24). Plates were incubated for 5 days at 27°C before colony counts were made. From each soil sample, 10 colonies resembling Agrobacterium spp. (36) were selected from each medium and purified by successive streakings on potato dextrose agar (PDA; Difco Laboratories, Detroit, Mich.) supplemented with 0.05% (wt/vol) calcium carbonate and King’s medium B (30). Gram-negative strains (20), nonfluorescent on King’s medium B, were stored at 10°C on PDA-CaCO₃ slants and in sterile distilled water (36).

Bacterial identification. The biovar affiliation of each putative Agrobacterium strain was determined by classical biochemical and physiological tests (36). To determine the taxonomic structure of this Agrobacterium community below the biovar level, subpopulations were differentiated by morphology of colonies growing on PDA-CaCO₃ at 27°C, profiles of cellular proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and profiles of HindIII- and BamHI-digested genomic DNA from individual strains. In addition, strains representative of each subpopulation were compared by their ability to utilize mannopine (2.5 mM). Cocktail C contained the opines octopine and nopaline (0.8% NaCl and 5 mM). The inoculated cocktails were incubated at 28°C for up to 7 days.

Opine utilization was tested by inoculating individual strains into three degradation cocktails which differed in opine composition. Experiments were performed in a final volume of 100 μl. The cocktails consisted of AT minimal medium supplemented with ammonium sulfate (1.0 g/liter), yeast extract (100 mg/liter), and the appropriate opines (providing for F. Guyon, Laboratoire de Biologie Cellulaire, Institut National de la Recherche Agronomique, Versailles, France). Degradation cocktail A contained the octopine (5 mM), nopaline (5 mM), and cumicopine (4 mM). Cocktail B was supplemented with agropine (2.5 mM) and manopine (2.5 mM). Cocktail C contained the opine manopinic acid (5 mM). The inoculated cocktails were incubated at 28°C for up to 7 days. Opine utilization was assessed by investigating their disappearance from the degradation cocktails by high-voltage paper electrophoresis as described above.

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A feature associated with nopaline pTi is sensitivity of the host bacterium to agrocin 84, a bacteriocin produced by the biological control agent Agrobacterium radiobacter K84 (23). Strains were tested for agrocin sensitivity on agar plates.
TABLE 1. Chromosome and plasmid phenotypes of 24 pathogenic and 22 nonpathogenic Agrobacterium strains isolated from four soil samples

<table>
<thead>
<tr>
<th>Strain type</th>
<th>No. of strains from soil samplea</th>
<th>Chromosome</th>
<th>Plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I     II    III    IV</td>
<td>Biovar</td>
<td>Subgroup</td>
</tr>
<tr>
<td>B17 (16)bd</td>
<td>5     4      3      4</td>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>B14 (8)</td>
<td>3     2      1      2</td>
<td>1</td>
<td>C</td>
</tr>
<tr>
<td>E35 (2)</td>
<td>1     1      1      1</td>
<td>1</td>
<td>D</td>
</tr>
<tr>
<td>B11 (2)</td>
<td>1     1      1      1</td>
<td>1</td>
<td>D</td>
</tr>
<tr>
<td>E34 (1)</td>
<td>1     1      1      1</td>
<td>1</td>
<td>E</td>
</tr>
<tr>
<td>F13 (1)</td>
<td>1     1      1      1</td>
<td>1</td>
<td>F</td>
</tr>
<tr>
<td>B16 (14)</td>
<td>4     4      4      2</td>
<td>2</td>
<td>B</td>
</tr>
<tr>
<td>E44 (2)</td>
<td>2     2      2      2</td>
<td>2</td>
<td>B</td>
</tr>
</tbody>
</table>

* Number of strains sharing the same phenotype recovered from each soil sample.
  * Chromosomal subtyping by DNA and protein profiles (see Materials and Methods).
  * Abbreviations: N, nopaline; O, octopine; M, mannopine; Ma, mannopinic acid; Aa, agropine; Ag, agropinic acid; C, cucumopine.
  * The number of strains with the same phenotype are shown in parentheses.
  * Octopine was used only in the presence of nopaline.

by the method of Stonier (55). The agrocin-sensitive strain C58 and the agrocin-resistant derivative C58C1 (our collection) were used as references.

To determine whether nontumorigenic opine-utilizing strains carried oncogenes or vir genes, genomic DNA digests were hybridized, as described above, with gel-purified segments of the T-DNA region (HindIII fragment 1 which encompasses nts1, tmr, 6a, 6b, and ocs) and the vir region (HindIII fragment 3 which includes virG and most of the virB operon) of the octopine plasmid pTi15955, respectively. Both probes were obtained from HindIII digests of cosmid clones of pTi15955 (17).

To determine whether nopaline degradation in nontumorigenic agrobacteria was plasmid borne, plasmids present in these strains were transferred by conjugation to the recipient strain C58C1RSpA1-, a rifampin- and streptomycin-resistant mutant of a plasmid-free derivative of strain GM19017 (47). Bacterial conjugation was performed by using a drop-mating technique. Donors were grown in AT medium supplemented with nopaline (10 mM), whereas the recipient strain was grown in AT medium supplemented with mannitol (2.0 g/liter), ammonium sulfate (2.0 g/liter), rifampin (0.1 mg/ml), and streptomycin (0.5 mg/ml). Drops of the donor log-phase culture were spotted onto a freshly plated lawn of the recipient strain. The cross was performed directly on the selective medium which was AT supplemented with nopaline (10 mM), rifampin (0.1 mg/ml), and streptomycin (0.5 mg/ml). Transconjugants were picked up after 6 days at 28°C, purified under the same conditions on the same medium, and analyzed for the concomitant presence of a plasmid and the ability to catabolize nopaline. The plasmid content of Agrobacterium strains was visualized in an 0.8% agarose gel by the Eckhardt procedure (22).

RESULTS

Agrobacterium-like colonies were recovered from all four soil samples and at density levels estimated at about 3 × 10⁷ CFU per g of soil. Biochemical and physiological tests performed on gram-negative nonfluorescent isolates obtained from purified Agrobacterium-like colonies identified 40 bv. 1 and 32 bv. 2 isolates. Most bv. 1 strains were recovered on medium 1A (24 of 40) and medium RS (13 of 40), whereas most bv. 2 strains were isolated from medium 2E (19 of 32) and medium MG (9 of 32). Several Agrobacterium-like colonies isolated on RS medium were fluorescent pseudomonads (data not shown).

Testing for pathogenicity and sensitivity to agrocin 84 led to the identification of 24 tumorigenic and 30 agrocin-sensitive strains (Table 1). All 24 strains which induced the development of tumors on test plants belonged to bv. 1, of which 16 were sensitive to agrocin. The 14 remaining agrocin-sensitive strains were nonpathogenic bv. 2. These pathogenic and/or agrocin-sensitive strains were recovered from all soil samples and in about the same proportions (Table 1). An assay for the presence of opines in tumors induced by the 24 pathogenic bv. 1 strains revealed the presence of both nopaline- and octopine-producing tumors. Nopaline was detected in tumors incited by 16 of the strains. Octopine was detected in tumors of the remaining eight strains. In addition to synthesizing octopine, these tumors synthesized the manityl opines mannopine, agropine, and agropinic acid (Table 1).

Characterization below the biovar level by DNA digestion profile analysis of the 24 tumorigenic bv. 1, the 14 nonpathogenic and agrocin-sensitive bv. 2 strains, and 8 randomly selected nonpathogenic and agrocin-resistant strains (6 bv. 1 and 2 bv. 2 strains) revealed six distinct genomic fingerprints (Fig. 1). Five different chromosome backgrounds (chr A, C, D, E, and F) were identified among bv. 1 strains, and one (chr B) was identified among bv. 2 (Table 1; Fig. 1). Southern blot analysis with the bv. 1 chromosome probe PMI confirmed the distinctiveness of each chromosome group within bv. 1 strains. Strains with the same DNA digestion profile shared a unique hybridization signal (data not shown). Similar observations were made when the Put probe was used on a limited number of strains representative of each chromosomal group (Fig. 2).

Interestingly, neither the PMI (data not shown) nor the Put (Fig. 2) probe hybridized to chr B (i.e., bv. 2) strains. This is consistent with a previous study which showed that DNA of bv. 2 strains does not usually react with bv. 1 chromosome probes (33). When whole-cell proteins from these strains were separated by SDS-PAGE, the resulting electrophoretic profiles were identical for strains of the same chromosome background; each chromosome group had a unique profile

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Cluster analysis of utilization patterns of 95 carbon substrates confirmed the segregation of the strains by the aforementioned chromosome grouping (Fig. 4); each cluster consisted of strains that had distinct metabolic patterns. Colony morphology was also correlated to chromosomal background. Although colonies of all strains were cream-white in color, circular (i.e., round with smooth edges), and had entire margins, several chromosome groups were distinguishable after 48 h on PDA-CaCO₃. Colonies of chr A strains were 3 mm in diameter, umbonate, and presented the unusual property of producing a pellicle which held together and could not be easily dispersed. Chr D, E, and F strains had the typical convex *Agrobacterium* colony morphology, and these colonies were 3 to 4 mm in diameter. Chr C strains formed pulvinate (i.e., raised) colonies approximately 1.5 mm in diameter. Chr B strains produced puntiform (i.e., less-than-1-mm) colonies. After 4 days, chr B colonies were pulvinate and cleared the CaCO₃ from the medium. Clearing of the medium is typical of bv. 2 strains and is the result of acid production from glucose (6). Analysis of chromosome phenotypes showed the predominance among the 30 bv. 1 strains of 16 chr A strains, followed by 8 chr C strains and 4 chr D strains; chr E and F types were each identified only once (Table 1). Strains with chr A, B, C, or D were recovered at similar levels from all soil samples. The two unique chromosome types (chr E and F) were recovered from different samples (Table 1).

Opine utilization analysis revealed the presence of different opine catabolic patterns (Table 1). The tumorigenic, agrocin-sensitive chr A strains utilized both nopaline and octopine, but the latter was utilized only in the presence of nopaline, a property common to nopaline-type pTi. The remaining eight tumorigenic bv. 1 strains had patterns typi-
cal of octopine pTi: they catabolized octopine and the mannityl opines (i.e., mannopine, mannopinic acid, agropine, and agropinic acid) and were resistant to agrocin. All eight octopine strains were of the chr C type. Interestingly, opine utilization by the six nonpathogenic bv. 1 agrobacteria revealed the presence of strains diverse in opine metabolism. Two chr D strains degraded octopine but differed from regular octopine-utilizing strains by their inability to utilize any of the mannityl opines. The other two chr D strains were unable to utilize any of the opines used in the present study. The strain with chr E catabolized agropinic acid but none of the other mannityl opines. The other bv. 1 strain with a unique chromosome background (chr F) had the unusual ability to metabolize cucumopine, nopaline, and octopine. Fourteen of the bv. 2 (chr B) strains had utilization patterns typical of nopaline pTi. These strains were agrocin sensitive and utilized nopaline and octopine, the latter only in the presence of nopaline. However, these strains were unable to induce tumors on any of the plants used in pathogenicity assays. The last two chr B strains were undistinguishable from the other chr B strains, except for their resistance to agrocin. Southern blot hybridization of digested DNA from strains representative of the different chr groups with T-DNA and vir probes corroborated the pathogenicity tests. On the basis of the position of the hybridization signal, nopaline pTi present in chr A strains were identical or very closely related and the same held true for octopine pTi of chr C strains (Fig. 5). DNA from the nonpathogenic opine-utilizing strains did not hybridize to either the vir or T-DNA probes (not shown), confirming the nontumorigenic nature of the strains with chr B, D, E, or F. To determine whether the ability of chr B strains to catabolize nopaline was plasmid borne, selected strains were conjugated with the plasmid-free strain C58C1RSpAt* that does not utilize opines. The resulting transconjugants were selected on nopaline and found to harbor one of two plasmids detected in the selected donor strains (data not shown), clearly demonstrating that nopaline utilization by chr B strains was plasmid borne. Strains carrying a nopaline pTi, an octopine pTi, or a nontumorigenic nopaline plasmid were recovered from the different sampling sites at similar levels (Table 1).

**FIG. 4.** Dendrogram of reference *Agrobacterium* strains and representative soil strains based on utilization of 95 carbon sources (Biolog GN microplate). Letters (A to F) and numbers (1 to 3) to the right of the brackets represent chromosome subgroups and biovars, respectively. The following strains were included as references: C58, B6, and FACH (biovar 1); AB2/73, AR5/K, and K84 (biovar 2); and, CG660, 3/2, and CG1005 (biovar 3).

**FIG. 5.** HindIII-digested DNA hybridized with the vir probe. (A) chr A strains (lanes 1 to 16); (B) chr C strains (lanes 1 to 8), λ DNA (lane 9), and strain C58 (lane 10). The symbol O and the arrow indicate the origin and direction of migration, respectively.

**DISCUSSION**

The present study assessed the distribution and diversity of the *Agrobacterium* population of an alkaline soil which was continuously fallow for 5 years and where there was apparently no galled host. The soil in this fallow field
harbored a population of agrobacteria estimated at $3 \times 10^7$ CFU/g. One in three Agrobacterium strains induced tumors on at least one of the test-plant species. To our knowledge, this is the first report of a soil with a tumorigenic population estimated at $10^7$ CFU/g. Previously, populations of agrobacteria found in soil have been estimated to vary between $10^3$ and $10^6$ CFU/g, with pathogenic strains seldom being detected except in the vicinity of infected roots (7, 10, 52).

Although artificially introduced tumorigenic strains survive poorly in soil devoid of susceptible hosts (3, 19, 35, 41), naturally occurring pathogenic agrobacteria have been reported in some instances to persist for years in the soil under natural conditions (14, 52). The apparent maintenance of such a large population of tumorigenic strains in the present fallow field may be due to properties intrinsic to that particular soil and/or to the rhizosphere of some of the weeds growing in that field. One edaphic factor which may have contributed to the survival of this Agrobacterium population is the loamy texture of the soil. Because of its higher capacity for water retention, such a texture has been shown to contribute to the multiplication and propagation of bacteria (32). Another parameter that may have promoted survival of the tumorigenic strains in this soil is pH. Acid pH has been reported to reduce survival of introduced pathogenic agrobacteria in the absence of a host (19), and crown gall has been reported to be more serious in regions where the soil is alkaline (34, 53). For instance, the disease is rarely observed in the Willamette Valley of Oregon where soils are acidic, whereas infections are common in alkaline soils of the Sacramento River valley of California (53) and central Washington (34). It has also been shown that alkaline pH positively influences survival and nodulation ability of Rhizobium spp. (21), soil bacteria closely related to Agrobacterium spp.

On the basis of biochemical tests, the resident Agrobacterium population was composed predominantly of bv. 1 isolates. In addition to bv. 1, bv. 2 appears to be the only other biovar present in this fallow soil. The higher rate of recovery of bv. 1 and bv. 2 strains on medium LA and 2E, respectively, confirmed the selectivity of these two media. Recovery from RS medium of bv. 1 strains and of a large number of fluorescent pseudomonads, which had colonies that appeared identical on that medium to those of Agrobacterium spp., indicates that this medium should be used with caution in studies targeting recovery of bv. 3 strains from soil. Selective isolation of bv. 1 and/or pseudomonads by this medium has been reported elsewhere (5, 10). It should be noted, however, that RS and MG media were useful in complementing the other two media for the isolation of additional strains, some of which had peculiar chromosome and plasmid phenotypes. Surprisingly, all pathogenic strains in this soil were bv. 1, whereas bv. 2 pathogens predominated in tumors of nursery trees cultivated in the same region (5), apparently confirming the fitness of bv. 1 in soil (38, 39). The prevalence of bv. 1 in our soil may have been the result of the differential motility reported among agrobacteria; indeed, bv. 1 strains are more motile in alkaline pH than bv. 2 strains, whereas bv. 3 strains lack motility at high pH (11). It should be noted, however, that when two representative bv. 1 strains from this soil were tested under laboratory conditions, they grew better at pH 7 than at pH 8.5 (11a).

Cellular protein patterns correlated well with digested genomic DNA patterns; such a correlation between DNA and protein fingerprints was not surprising since proteins result from the expression of genetic information. The same groupings were obtained from a comparison of utilization patterns of 95 carbon substrates. By using these techniques, six distinct chromosome backgrounds were identified in this collection of strains. The bv. 1 population was diverse, and its strains were segregated into five distinct chromosomal backgrounds (chr A, C, D, E, and F), whereas bv. 2 strains formed a homogeneous cluster (chr B). In addition, there was a good relationship between colony morphology and chromosomal background. With the exception of the six chr D, E, and F strains which produced identical colonies on PDA-CaCO₃, strains of other chromosome groups had colonies with distinct features. Such differences in colony morphology reflect apparently fundamental differences between strains and may serve as indicators of genetic diversity. Differences in protein profiles, genomic DNA, carbon metabolism, and colony morphology suggest that these subpopulations are distinct and probably differ in a number of physiological characteristics of unknown importance for survival fitness and pathogenic ability. chr A, B, C, and D were the most common chromosome groups and were evenly distributed throughout the field. The rare occurrence of strains with chr E and F did not allow us to determine whether these atypical bv. 1 strains were present in other sites; interestingly, these two strains have rare or unique opine catabolic patterns. More work is needed to determine whether all of these unusual opine degradation properties are plasmid borne or chromosomally encoded.

On the basis of opine metabolism, pathogenicity, reaction to agrocin 84, and hybridization with T-DNA and vir probes, three plasmid types were found in relative abundance in all sampling sites. The most common one was the nopaline pTi, followed by both the octopine pTi and a plasmid similar to pAtK84b (also called pNoc), which is a nonpathogenic, nopaline-agrocinopine plasmid present in strain K84 (13). In addition to nopaline utilization, this nonpathogenic plasmid shares with pAtK84b a similar-size plasmid and plasmid transfer induced by nopaline (15a); transfer of nopaline pTi is generally induced by agrocinopine A or B. The relative abundance of the pAtK84b-type plasmid was surprising and remains unexplained, although it may be correlated with the relatively high abundance of the pathogenic nopaline-type plasmid in the population. All analyzed bv. 2 strains isolated in this study harbored a second plasmid, the function of which is unknown. Preliminary analysis performed on a limited number of strains showed that this plasmid does not encode for synthesis of an agrocin (data not shown).

In this study of indigenous agrobacteria and their opine catabolic plasmids, the type of plasmid maintained in a bacterial cell was correlated with the chromosomal background. Indeed, bv. 1 strains with chr A carried a nopaline-type pTi, while those with chr C had an octopine-type pTi; bv. 2 (chr B) strains harbored a pAtK84b-type plasmid (Table 1). Although this population could reflect the types of strains that were introduced to the field during the years of stone-fruit nursery production, it seems improbable that plasmid exchange would not have occurred in the presence of such highly susceptible hosts unless some type of selection pressure was occurring. The affinity of a pTi for a particular chromosomal background was previously suspected in a survey of pathogenic strains, where it was noticed that bv. 2 strains were three times more likely to carry a nopaline-agrocinopine-type pTi than bv. 1 strains (5). Also, when pTi from a collection of bv. 3 strains were analyzed for the presence of two related insertion elements, it was suggested that vitopine, octopine-cucumopine, and nopaline pTi are not or only rarely transmitted to strains with
a different chromosomal background (42). In apparent contradiction to the findings described above, plasmid exchange was suggested when analysis of strains from a forest nursery revealed the presence of the same plasmids in different chromosomal backgrounds (33). These apparently conflicting results may reflect different behaviors of Agrobacterium pathogenic plasmids in different environmental conditions. In a tumor, plasmids conjugate between bacteria, as seen experimentally in the Kerr cross (26, 28); in such an environment, opines would induce the conjugal transfer of plasmids and would likely provide valuable carbon and energy sources to account for the maintenance of the plasmids in different chromosomal backgrounds. In a gall-free, opine-free environment, there is no apparent selective pressure for transfer and propagation of the pathogenic plasmids; however, under such conditions, some plasmid-chromosome background combinations are probably more stable than others. The biological reasons underlying this apparent better stability or fitness are not yet understood.

Finally, the present phenotypic diversity observed in this soil may reflect the agricultural history of the field. The even distribution among samples of the different chromosome groups and their associated plasmids may have occurred because of the regular annual plowing of the land. Limited analysis of four additional soil samples from this study site confirmed the distribution of both plasmids and chromosome populations reported in the other samples (40). The concomitant presence at the time of isolation of phenotypically distinct Agrobacterium populations suggests successful colonization of this soil and stable cohabitation among members of a relatively diverse community with apparently little or no plasmid exchange. Additional work is required to determine the ecological significance of the phenotypic diversity seen in this field. Identification of the factors influencing composition of Agrobacterium communities and the distribution of their pTi in the soil will help advance our understanding of the epidemiology of crown gall disease.

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