Common Loci for *Agrobacterium tumefaciens* and *Rhizobium meliloti* Exopolysaccharide Synthesis and Their Roles in Plant Interactions

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Mutants of *Rhizobium meliloti* have been isolated which are deficient in exopolysaccharide (EPS) production and effective nodulation of alfalfa (J. A. Leigh, E. R. Signer, and G. C. Walker, Proc. Natl. Acad. Sci. USA 82:6231–6235, 1985). We isolated approximately 100 analogous EPS-deficient (Exo) mutants of the closely related plant pathogen *Agrobacterium tumefaciens*, including strains whose EPS deficiencies were specifically complemented by each of five cloned *R. meliloti* *exo* loci. We also cloned *A. tumefaciens* genes which complemented EPS defects in three of the *R. meliloti* Exo mutants. In two of these cases, symbiotic defects were also complemented. All of the *A. tumefaciens* Exo mutants formed normal crown gall tumors on four different plant hosts, except ExoC mutants, which were nontumorigenic and unable to attach to plant cells in vitro. Like their *R. meliloti* counterparts, *A. tumefaciens* Exo mutants were deficient in production of succinoglycan, the major acidic EPS species produced by both genera. *A. tumefaciens* ExoC mutants also produced extremely low levels of another major EPS, cyclic 1,2- β -D-glucan. This deficiency has been noted previously in a different set of nontumorigenic, attachment-defective *A. tumefaciens* mutants.

The family *Rhizobiaceae* includes the genera *Agrobacterium* and *Rhizobium*, which differ from each other primarily in the types of associations they form with plants (18). *Rhizobium* species form nitrogen-fixing root nodules in association with legumes, whereas *Agrobacterium* species induce crown gall tumor formation on a wide range of dicotyledonous plants. These associative properties are specified primarily by large plasmids. The Ti plasmids of *A. tumefaciens* carry the T-DNA, which is transferred and integrated into the host plant genome during crown gall infection, as well as genetic information required for T-DNA transfer (3, 14, 16, 28–30). *Rhizobium* strains have symbiosis plasmids which carry genetic information for most nodulation functions (12, 21). Some bacterial chromosomal genes are also involved in both infections (4, 9, 12, 13, 21, 26).

Given the close phylogenetic relationship between the two organisms, it is likely that they share some genetic information which functions in both infectious processes. An example of this has been found. The chromosomal virulence (*chv*) genes of *A. tumefaciens* are structurally and functionally analogous to the *R. meliloti* *ndv* genes. The *Agrobacterium* loci *chvA* and *chvB* are required for attachment to plant cells and tumorigenesis (8, 9). *R. meliloti* strains carrying mutations in the *ndvA* and *ndvB* loci form empty, ineffective nodules on alfalfa (10). The *chv* and *ndv* genes share sequence homology and are functionally interchangeable in heterologous complementation experiments (10). Both bacteria synthesize a neutral cellular and (in some cases) extracellular polysaccharide, cyclic 1,2- β -D-glucan (5, 27, 31), and the *chvB* locus is required for this synthesis in *A. tumefaciens* (23). A role for the glucan in either infectious process has not been conclusively established.

The major acidic exopolysaccharide (EPS) produced by both genera is succinoglycan, which consists of repeating units of β -linked glucose and galactose (7:1) with acidic sidegroups (15, 27, 31). Mutants of *R. meliloti* which lack this EPS were isolated as dark colonies on media containing calcofluor, a fluorescent stain for β -linked polysaccharides (11, 19). These Exo[−] mutants induced empty, ineffective nodules on alfalfa. By complementation with an *R. meliloti* cosmid bank, the *exo* mutations were sorted into six different genetic complementation groups (19). Two of these groups (*exoC* and *exoD*) are chromosomal loci, while the other four (*exoA*, *exoB*, *exoE*, and *exoF*) map on pRmeSU47b, one of the two symbiosis plasmids of *R. meliloti* SU47 (12).

Since *A. tumefaciens* also synthesizes succinoglycan, we were prompted to investigate the occurrence in *A. tumefaciens* of succinoglycan-specifying (*exo*) loci analogous to those of *R. meliloti* and to determine their role, if any, in crown gall tumor formation.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *A. tumefaciens* was grown on MG/L medium (13), and *R. meliloti* was grown on Luria broth with 2.5 mM CaCl₂ and 2.5 mM MgCl₂. When appropriate, media were supplemented with the following (in micrograms per milliliter): rifampin, 20; chloramphenicol, 20; kanamycin, 100; tetracycline, 8; spectinomycin, 50; gentamicin, 15; and calcofluor, 200 (all obtained from Sigma Chemical Co., St. Louis, Mo.). Plates containing calcofluor were buffered with 25 mM HEPES (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid), pH 7.2. The bacterial strains used in this study are listed in Table 1.

Mutagenesis. Tn5 mutagenesis of *A. tumefaciens* was carried out as previously described (13). Kanamycin-resistant colonies on calcofluor plates were screened for

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant markers	Source or reference
<i>A. tumefaciens</i>		
A348	Rp ^r Cm ^r pTiA6	Parent strain; 14
A5017	ExoA ⁻ Km ^r	Tn5 mutagenesis of A348
A5512	ExoA ⁻	NTG mutagenesis of A348
A5094	ExoB ⁻ Km ^r	Tn5 mutagenesis of A348
A5126	ExoB ⁻ Km ^r	Tn5 mutagenesis of A348
A5129	ExoC ⁻ Km ^r	Tn5 mutagenesis of A348
A5503	ExoC ⁻	NTG mutagenesis of A348
A5090	ExoD ⁻ Km ^r	Tn5 mutagenesis of A348
A5575	ExoF ⁻	NTG mutagenesis of A348
A5015	ExoG ⁻ Km ^r	Tn5 mutagenesis of A348
<i>R. meliloti</i>		
1021	Sm ^r derivative of <i>R. meliloti</i> SU47	Parent strain; 19
6061	ExoA ⁻ Gm ^r Sp ^r	See Materials and Methods
6024	ExoB ⁻ Gm ^r Sp ^r	See Materials and Methods
6025	ExoC ⁻ Gm ^r Sp ^r	See Materials and Methods
6027	ExoD ⁻ Gm ^r Sp ^r	See Materials and Methods
6087	ExoF ⁻ Gm ^r Sp ^r	See Materials and Methods
Plasmids		
pD34	pLFR1 (Tc ^r) with <i>R. meliloti</i> <i>exoA</i>	19
pD2	pLFR1 (Tc ^r) with <i>R. meliloti</i> <i>exoB</i>	19
pD15	pLFR1 (Tc ^r) with <i>R. meliloti</i> <i>exoC</i>	19
pD5	pLFR1 (Tc ^r) with <i>R. meliloti</i> <i>exoD</i>	19
pD56	pLFR1 (Tc ^r) with <i>R. meliloti</i> <i>exoB</i> <i>exoF</i>	19
pFC6244	pVK102 (Km ^r) with <i>A. tumefaciens</i> <i>exoB</i>	See Materials and Methods
pFC6251	pVK102 (Km ^r) with <i>A. tumefaciens</i> <i>exoC</i>	See Materials and Methods
pFC6273	pVK102 (Km ^r) with <i>A. tumefaciens</i> <i>exoD</i>	See Materials and Methods

fluorescence under long-wave UV light. Mutagenesis with *N*-methyl-*N'*-nitrosoguanidine (NTG) was performed as previously described (25). The identities of *Agrobacterium* mutants were checked by microscopic observation, parental rifampin and chloramphenicol resistance markers, and ketolactose production. *R. meliloti* mutant strains 6061, 6024, 6025, 6027, and 6087 were derived from previously reported Exo mutant strains 7061, 7094, 7025, 7017, and 7055, respectively (19). To construct these mutants, the neomycin resistance marker on the Tn5 insertions in the original mutants were replaced in situ with the Gm^r Sp^r marker on the Tn5 derivative Tn5-233 as previously described (6).

Genetic complementation. Plasmids pD34, pD2, pD15, pD5, and pD56 (19), which code for tetracycline resistance

and carry the *R. meliloti* *exo* loci (Table 1), were mated individually from *Escherichia coli* HB101 into each of the *A. tumefaciens* Tn5 mutants and most of the NTG mutants by triparental mating, with pRK2073 (20) as a helper. Tc^r exconjugants were screened for complementation of the calcofluor-dark phenotype.

Agrobacterium *exo* genes were isolated from a cosmid bank of DNA from the Ti plasmid-lacking strain A136 (14) by standard techniques (17). Genomic DNA was cloned into the *Sall* site of cosmid vector pVK102 (17), packaged, and transfected into *E. coli* HB101, selecting for kanamycin resistance. The cosmid bank was then transferred in triparental matings into each of the *R. meliloti* Exo mutants in Table 1. Km^r Gm^r Sp^r exconjugant colonies were screened for their fluorescence on calcofluor plates. Bright colonies were purified, and plasmids from these colonies were mated back into *E. coli* HB101 by selecting for Km^r at 37°C and then mated back into the *R. meliloti* mutants. All exconjugants in these secondary matings were calcofluor bright, confirming that true genetic complementation had occurred. Representative complementing plasmids were mated into other Exo mutants to ensure that complementation was specific (e.g., pFC6244, which complemented ExoB⁻ mutant strain 6024, did not complement ExoA⁻, ExoC⁻, ExoD⁻, or ExoF⁻ mutants).

Plant associations. The ability of *A. tumefaciens* strains to induce tumors was determined in multiple experiments by (i) inoculation of wounds on *Kalanchoe* leaves, (ii) inoculation of surface-sterilized carrot disks on water agar plates, and (iii) inoculation of wounded pea and tomato seedlings. Attachment to *Zinnia* leaf mesophyll cells in vitro was determined as previously described (8).

The ability of *R. meliloti* strains to form effective nodules on alfalfa was determined by inoculating 4-day-old seedlings as previously described (19). Nodules were aseptically excised after 18 days. Acetylene reduction was determined in triplicate samples by incubating nodules in 2-ml vials with 10% acetylene at room temperature for 90 min and measuring ethylene by gas chromatography. Nodule occupancy was determined by crushing surface-sterilized nodules (19) and plating the contents on selective solid media.

EPS analysis. For size fractionation of EPS, *A. tumefaciens* strains were grown to an optical density at 600 nm of 0.6 to 0.8 on mannitol-glutamate medium (2), and bacteria were removed by centrifugation at 16,000 × g for 15 min. Culture supernatants were concentrated by freeze-drying, redissolved, and loaded onto a BioGel A5-m column (35 by 4 cm). EPS was eluted with 10 mM sodium phosphate buffer, pH 7.0, at a flow rate of 0.4 ml/min. Fractions (5 ml) were collected, and their hexose contents were determined by anthrone analysis (7).

For neutral-sugar analysis, culture supernatants were concentrated by rotary evaporation, filtered (Millipore Corp.; 0.45-μm pore size), and dialyzed extensively against distilled water before freeze-drying. Samples were treated with 2 N trifluoroacetic acid at 120°C for 2 h, reduced with sodium borohydride, and analyzed by gas-liquid chromatography as alditol acetates on a packed column of 3% SP2330 on 100/120-mesh Supelcoport. For charge separation, dialyzed and freeze-dried samples were applied to a DEAE-BioGel column (12 by 5 cm). The column was eluted first with water, until no carbohydrate was detected in the effluent by the phenol-sulfuric acid method (1), and then with aqueous 1 M potassium acetate. Peak fractions from the water washes (determined by the phenol-sulfuric acid method) were combined, and neutral glucans were recovered by freeze-drying.

FIG. 1. Calcofluor-dark mutant strains of *A. tumefaciens* grown on a calcofluor (200 μ g/ml) plate and illuminated with long-wave UV light. A, A348 (wild type); B, A5015 (type 1); C, A5094 (type 2); D, A5129 (type 3).

Acidic EPS was similarly recovered from the potassium acetate washes, except that combined peak fractions were dialyzed before freeze-drying.

RESULTS

Isolation of calcofluor-dark mutants of *A. tumefaciens*. Wild-type *A. tumefaciens* A348 produces a large amount of extracellular slime when grown on rich or minimal medium. Colonies fluoresce with a bright green-blue color on calcofluor plates (Fig. 1A). After mutagenesis with Tn5, over 50,000 Km² colonies were screened for calcofluor staining, and about 40 independently derived clones with altered staining were obtained. Three different types could be distinguished. Type 1 mutants fluoresced with a blue-green color but less brightly than the wild type, type 2 mutants had a dull blue color and were darker than type 1 mutants, and type 3 mutants were completely dark and somewhat slow growing (Fig. 1B to D). All three types produced less slime than did the wild type, forming colonies that appeared smaller and dryer. Upon microscopic observation of bacteria picked from MG/L plates, type 1 and 3 mutants were found to be nonmotile at all stages of growth, whereas type 2 mutants and the wild type were motile (Table 2). NTG mutagenesis yielded approximately 50 mutants, all of the same three types. Approximately 3/4 of the mutants isolated by both procedures were type 2. Cellulose fibril-negative mutants of *A. tumefaciens* have been isolated with minimal

TABLE 2. Summary of phenotypes and complementation of calcofluor-dark mutants of *A. tumefaciens*

Type	Calcofluor staining (growth)	Motility	Complementation group	Virulence
Wild	Bright blue-green	+	—	+
1	Light blue-green	—	G	+
2	Dull blue	+	A B D F	+
3	Dark (slow growing)	—	C	—

TABLE 3. Complementation of EPS mutants of *R. meliloti* by cloned *A. tumefaciens* DNA

Host	Complementation group	Plasmid ^a	Nodule occupancy (CFU/nodule) ^b	Acetylene reduction (% of wild type)
1012	Wild type	None	1,000	100.0
6024	B	None pFC6244	1 239	1.0 3.4
6025	C	None pFC6251	33 551	1.6 31.3
6027	D	None pFC6273	1 1	4.8 5.6

^a Isolation of complementing *A. tumefaciens* cosmid clones is described in the text.

^b Average of at least four nodules each.

media containing calcofluor (22). We did not obtain such mutants with MG/L, a rich medium.

Complementation of *A. tumefaciens* mutants with *R. meliloti* *exo* genes. Recombinant plasmids carrying the *R. meliloti* *exo* genes (Table 1) were mated into each of the *A. tumefaciens* calcofluor-dark mutants to determine whether these genes could complement the calcofluor-dark phenotypes *in trans*. None of the type 1 mutants were complemented. By complementation with cloned *A. tumefaciens* DNA, these were found to make up a single complementation group which has not yet been reported in *Rhizobium* spp. (complementation group G; data will be presented elsewhere). Plasmids pD34 (ExoA) and pD2 (ExoB) each complemented several type 2 mutants. pD5 (ExoD) complemented one type 2 mutant. pD56 (ExoB and ExoF) complemented several type 2 mutants, all but two of which were also complemented by pD2 (ExoB). The two which were complemented only by pD56 were assigned to complementation group F. All of the type 3 mutants were complemented by pD15 (ExoC). Each mutant was complemented by only one *exo* locus. The data summarized in Table 2 indicate that genes complementary to all of the *R. meliloti* *exo* loci are present in *A. tumefaciens*. *exoE* (19) is an uncloned complementation group in *R. meliloti*, and its representatives appear to carry deletions in other *exo* loci (T. Finan, unpublished data); it was not considered in this work.

Complementation of *R. meliloti* symbiotic mutants with *A. tumefaciens* *exo* genes. To test the presence of *Agrobacterium* loci complementary to the *R. meliloti* *exo* genes further, we mobilized a cosmid bank of *A. tumefaciens* A136 DNA (with no Ti plasmid) into representative *R. meliloti* mutants from each of the complementation groups. In this manner, we identified clones which specifically complement the calcofluor-dark phenotypes of the *R. meliloti* mutant strains 6024 (ExoB), 6025 (ExoC), and 6027 (ExoD). These cosmid clones specifically complemented ExoB, ExoC, and ExoD mutants of *A. tumefaciens* as well. No complementation of mutant strains 6061 (ExoA) and 6087 (ExoF) was observed with this cosmid library or a library of pTiA6 DNA. The abilities of recombinant plasmids pFC6244 (ExoB), pFC6251 (ExoC), and pFC6273 (ExoD) to complement the symbiotic deficiencies of their respective Exo[−] *R. meliloti* hosts *in trans* were determined. Invasiveness of alfalfa (as determined by the presence of viable bacteria inside surface-sterilized nodules) was partially restored to the ExoB and ExoC mutants, as was the ability of the ExoC mutant to form nodules that reduce acetylene (Table 3). Only partial resto-

TABLE 4. Attachment of *A. tumefaciens* cells to *Zinnia* leaf mesophyll cells

Strain	Locus	Mean ($\pm$ SEM) % attached ^a
A438	— ^b	22.3 $\pm$ 1.3
A1038	<i>chvB</i>	5.8 $\pm$ 0.3
A5129	<i>exoC</i>	2.4 $\pm$ 0.1

^a The percentages of radiolabeled bacteria attached to *Zinnia* leaf mesophyll cells were determined as previously described (8). The values are from triplicate samples.

^b —, Wild type.

ration of the invasiveness phenotype was normally observed in homologous complementation experiments as well (data not shown). Nodules formed by the ExoB and ExoC mutants carrying the complementing plasmids were larger and pinker than those formed by the mutants alone. Plasmid pFC6273 restored calcofluor staining only to ExoD mutants, but it severely inhibited the growth of any *R. meliloti* strain that carried it; this may account for its inability to restore nodule invasiveness to strain 6027.

Virulence properties of *A. tumefaciens* Exo mutants. With few exceptions, all of the *A. tumefaciens* Exo mutants were fully tumorigenic on *Kalanchoe* leaves and carrot disks, and representative mutants from each complementation group (listed in Table 1) formed normal tumors on pea and tomato seedlings (Table 2). Washing *Kalanchoe* leaves with sterile water 2 h after inoculation, which was reported to dislodge cellulose fibril-negative *A. tumefaciens* mutants from wound sites (22), had no noticeable effect on tumor formation by representatives of each complementation group (data not shown).

The exceptions were the type 3 (ExoC) mutants, which were avirulent on all four plants. Tumor formation by these mutants appears to be blocked in an early step, since the representative ExoC[−] strain A5129 does not bind to plant cells in vitro. Radiolabeled cells attached to *Zinnia* leaf mesophyll cells at an efficiency even lower than that of A1038 (8), an attachment-deficient ChvB mutant which has been described previously (Table 4). In addition to being avirulent, ExoC mutants are nonmotile and grow more slowly than the wild type on solid media. Introduction of the *R. meliloti* ExoC plasmid pD15 restores wild-type growth, motility, and virulence on *Kalanchoe* leaves, in addition to calcofluor staining.

EPS synthesis by Exo mutants. *A. tumefaciens* Exo mutants were analyzed to determine whether, like their *R. meliloti* counterparts, they were deficient in succinoglycan production. When EPS in wild-type *A. tumefaciens* culture supernatants were separated by size on BioGel A-5m, two peaks were observed (31; Fig. 2A; the appearance of the small intermediate peak was variable). The high-molecular-weight peak consisted primarily of acidic succinoglycan, and the low-molecular-weight peak was neutral β -1,2-glucan (31). With the ExoA mutant strain A5017 (type 2), the high-molecular-weight peaks were missing (Fig. 2B). Identical results were obtained with representative *A. tumefaciens* ExoB, ExoD, ExoF (type 2), and ExoG (type 1) mutants. These profiles are consistent with the succinoglycan-negative phenotypes of *R. meliloti* Exo mutants. ExoC (type 3) mutants were deficient in both peaks (Fig. 2C; a high background in the low-molecular-weight peak, which covers the included volume, can be expected with these undialyzed samples).

Dialyzed culture supernatants from A5017 (ExoA) and

FIG. 2. Size fractionation of culture supernatants. Culture supernatants were fractionated on BioGel A-5m, and the hexose contents of the fractions were determined as described in Materials and Methods. The curves were normalized for culture optical density and percentage of total crude culture supernatant loaded. Results from single experiments are shown; duplicate experiments yielded nearly identical results. A, A348 (wild type); B, A5017 (ExoA); C, A5129 (ExoC); D, A1038 (*chvB*). Size fractionation profiles of culture supernatants from strains A5512 (ExoA), A5094 (ExoB), A5126 (ExoB), A5090 (ExoD), A5575 (ExoF), and A5015 (ExoG) were identical to that of A5017 (curve B). ExoC mutant A5503 generated a curve with smaller peaks than those of A5129 (curve C).

A5129 (ExoC) were analyzed further. Galactose is a component of succinoglycan (occurring at a 1:7 ratio with glucose), whereas β -1,2-glucan consists entirely of glucose (15, 27, 31). Galactose was completely missing from total A5017 EPS and reduced relative to the wild type in A5129 EPS (Table 5). No acidic EPS (material which binds to DEAE-BioGel) was detected in the A5017 supernatant. These data are consistent with the size fractionation profile from this strain and with the lack of acidic EPS in *Rhizobium* Exo mutants. No neutral or acidic EPS was detected in fractions of A5129

TABLE 5. Sugar content and charge separation of EPS from *A. tumefaciens* Exo mutants^a

Strain	Total sample concn (mg/liter) ^b	Neutral sugar content (% of total sample)		Charge separation (% of total sample) ^c	
		Glucose	Galactose	Acidic	Neutral
A348 (wild type)	15	55.0	5.0	60	20
A5017 (ExoA)	20	62.5	Trace	Trace	70
A5129 (ExoC)	10	5.0	0.25	ND ^d	ND

^a The data presented are results of single experiments; duplicate experiments yielded nearly identical results.

^b Total sample refers to dialyzed and freeze-dried culture supernatants.

^c Determined by separating sample on DEAE-BioGel columns as described in Materials and Methods. Freeze-dried EPS recovered from neutral and acidic fractions was weighed; values are expressed as percentages of the total weight of the sample applied to the column.

^d ND, None detected. Fractions eluted from DEAE-BioGel contained undetectable (by the phenol-sulfuric acid method) carbohydrate.

culture supernatants separated on DEAE-BioGel (Table 5). This result is consistent with the very low yield obtained from BioGel A-5m columns (Fig. 2C) and suggests that ExoC mutants are deficient in the production of both succinoglycan and β -1,2-glucan. The crude lyophilized culture supernatant from this strain may consist largely of excreted protein or other nonsaccharide components. This deficiency in production of extracellular β -1,2-glucan is shared by *A. tumefaciens* ChvB mutants (23; Fig. 2D). Like ExoC mutants, ChvB mutants are also deficient in attachment and virulence. However, mutations in *chvB* do not reduce calcofluor staining or production of high-molecular-weight, acidic EPS (23; Fig. 2D).

DISCUSSION

The close similarities in cellular and extracellular polysaccharide production by *Rhizobium* and *Agrobacterium* spp. have been previously noted (5, 15, 27, 31). In both cases, the major acidic EPS is succinoglycan, and both genera produce a neutral cyclic β -1,2-glucan. Recent mutational analysis has provided evidence that succinoglycan plays a role in nodulation by *R. meliloti* (11, 19). Succinoglycan-negative (Exo) mutants from several different complementation groups were found to be defective in nodulation. We have isolated nearly 100 analogous mutants of *A. tumefaciens*. By extensive complementation analysis, we have shown that all of the reported *R. meliloti* complementation groups are represented among these mutants, as expected from the close phylogenetic relationship between these bacteria. Since all of our Exo mutants (except ExoC) formed tumors on a wide range of plant hosts, we were not able to demonstrate a role for most of these loci (and therefore for their product) in crown gall tumor formation. These results are consistent with a role for succinoglycan in invasion of plant roots by *Rhizobium* spp. (11, 19), which does not occur in *Agrobacterium* infections.

The two genera differ in the genomic distribution of the *exo* loci. In *R. meliloti*, three of the *exo* loci (*exoA*, *exoB*, and *exoF*) are carried on a plasmid (12), whereas all of the *exo* loci appear to be chromosomal in *A. tumefaciens*. This is inferred from the fact that *A. tumefaciens* GMI9023, which is cured of its Ti plasmid, as well as the naturally occurring cryptic plasmid pAtC58 (24), is completely Exo⁺ as determined by calcofluor staining. Calcofluor-dark derivatives of this strain, including ExoF and ExoG mutants, have been previously isolated (T. Finan and G. Cangelosi, unpublished data). Symbiotic functions in *Rhizobium* and *Agrobacterium* species are largely plasmid coded (26). This tendency is particularly striking in the case of the *exo* genes, which appear to be involved in plant interactions in *Rhizobium* spp. but not in *Agrobacterium* spp.

β -1,2-Glucan synthesis is at least partially determined in *A. tumefaciens* by the chromosomal *chvB* locus (23), which, along with the neighboring *chvA* locus, is required for effective attachment to plant cells and tumor formation (8, 9). *R. meliloti* has closely analogous loci, *ndvA* and *ndvB*, which are required for effective nodulation (10). The involvement of the *ndv* loci in β -1,2-glucan formation is not known at this writing. Since ChvB mutants were originally isolated as nonvirulent clones and subsequently found to be deficient in β -glucan production, the correlation between β -glucan and tumorigenesis is more tenuous than the correlation between succinoglycan and nodulation. It is strengthened by the observation in this study that *Agrobacterium* ExoC mutants, which are unique among our Exo mutants in that

they lack or are extremely deficient in the glucan, are also deficient in attachment and tumorigenesis. Like the *chvB* locus, the *exoC* locus does not map on the Ti plasmid, since the cosmid clone carrying it (pFC6251) came from a library of *A. tumefaciens* A136 (no Ti plasmid) DNA. It is not linked to ChvB, since this cosmid clone complements neither ChvA nor ChvB mutants (data not shown). Therefore, *exoC* provides independent support for a role for β -1,2-glucan in tumorigenesis. However, we interpret these results with caution. The ExoC mutants are more pleiotropic than ChvB mutants, being partially deficient in extracellular succinoglycan and also slow growing. ExoC mutants of *R. meliloti* are similarly pleiotropic (19), suggesting that this locus codes for a general membrane or cell surface structure which affects many surface-associated phenotypes. Further correlation of the β -1,2-glucan with attachment and virulence requires isolation of still more independently derived β -1,2-glucan mutants.

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