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Effect of Plasmid pSa and of Auxin on Attachment of *Agrobacterium tumefaciens* to Carrot Cells

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When the plasmid pSa is introduced into *Agrobacterium tumefaciens*, its presence results in the suppression of bacterial virulence. *A. tumefaciens*(pSa) cells are virulent on *Bryophyllum diagremontiana* only when inoculated with auxin. *A. tumefaciens*(pSa) cells also bind to plant cells only in the presence of auxin. The effect of auxin is on the bacteria rather than on the plant cells, since the bacteria require auxin to bind to heat-killed carrot cells. Bacteria containing pSa and grown in the absence of auxin showed a lag in binding to carrot cells in auxin-containing medium. This lag was not seen during the binding of wild-type strains. Tetracycline inhibited the binding of *A. tumefaciens*(pSa) in auxin-containing medium, suggesting that bacterial protein synthesis is required for the auxin effect. No difference was seen in the size or ability to inhibit bacterial binding of lipopolysaccharides from bacteria containing or lacking pSa and grown with or without auxin. *A. tumefaciens*(pSa) cells grown in the absence of auxin lacked surface polypeptide(s) found in bacteria grown in the presence of auxin and in the wild-type bacteria, which do not contain pSa. Thus, the presence of certain polypeptides appears to be associated with the ability of the bacteria to bind to plant cells.

The incompatibility group W plasmid pSa, which confers resistance to many antibiotics, including streptomycin, gentamicin, kanamycin, and chloramphenicol, was discovered serendipitously to suppress the virulence of *Agrobacterium tumefaciens* when introduced into this host (8). The mechanism of virulence suppression does not involve any irreversible change in the bacteria, since they can be cured of pSa and they then recover virulence (4). Recently, Chernin et al. (2) reported that the plant hormone auxin can reverse the effect of pSa on bacterial virulence. I report here the effect of the plasmid pSa and of auxin on the attachment of *A. tumefaciens* to plant suspension culture cells.

MATERIALS AND METHODS

Bacteria were grown in minimal medium with 0.2% glucose, and viable cell counts were determined as previously described (12). Bacteria containing pSa were maintained on Luria agar containing 100 μg of streptomycin per ml. The sources of virulent *A. tumefaciens* C58 and NT1 were as previously described (12). The isolation and characterization of the virulent cellulose-minus mutant Cel-12 were as described previously (9). Virulent strain 1D1 and avirulent strains 1D1(pSa), which is sometimes referred to as SATC22, C58(pSa), and Cel-12(pSa) were obtained from C. Ireland Valentine, Oral Roberts University. Suspension cultures of *Daucus carota* were obtained from W. Boss, North Carolina State University. They were maintained on Murashige and Skoog (MS; 14) medium with weekly transfers.

To determine the effect of auxin on bacterial attachment, it was necessary to transfer carrot cultures to medium lacking auxin. In addition, carrot cells retain the synthetic auxin used in MS medium and release it slowly into the medium. Therefore, the carrot cells were transferred to medium containing the natural auxin, indoleacetic acid (IAA). This auxin is not retained by the carrot cells. The carrot cell line used in these experiments requires auxin for growth (unpublished observations). Thus, to obtain carrot cell cultures which could be incubated with bacteria in the absence of auxin, carrot cells were grown for 1 week in IAA, and the carrot cells were collected on Miracloth filters (Calbiochem) and washed with 10 volumes of MS medium containing no auxin. The cells were then suspended in MS medium containing no auxin and incubated for 1 or 2 days. The cells were then collected and suspended in MS medium containing 0 or 1 mg of IAA per liter.

Bacterial attachment to carrot suspension culture cells was measured as previously described (12). For all experiments 1×10^5 to 4×10^5 carrot cells per ml were used. For kinetic experiments, bacteria were added to the carrot cell suspension to a final concentration of 1×10^3 to 4×10^3 bacteria per ml. For light microscope observations, bacteria were added to a final concentration of 2×10^6 to 10×10^6 cells per ml. For competition experiments, the numbers of attached bacteria were determined by collecting the carrot cells from 5 ml of medium on Miracloth filters (which do not retain free bacteria), washing the carrot cells with five times their volume of medium, resuspending the carrot cells in 15 ml, and grinding them in a Waring blender at top speed for 45 s to release attached bacteria. The number of bacteria was then determined by viable cell counts on Luria agar followed by replica plating onto Luria agar containing 100 μg of streptomycin per ml when bacteria containing and lacking pSa were to be distinguished (pSa carries streptomycin resistance). For some experiments, 10 μg of tetracycline per ml was added to the carrot cell culture medium. This concentration is sufficient to inhibit bacterial protein synthesis under these conditions (10). The plasmid pSa does not confer resistance to tetracycline (8).

For some attachment studies, carrot cells were killed by heating at 65°C for 20 min. They were then collected on a Miracloth filter, washed three times with 10 ml of MS medium without auxin, and resuspended in fresh medium for use in attachment measurements.

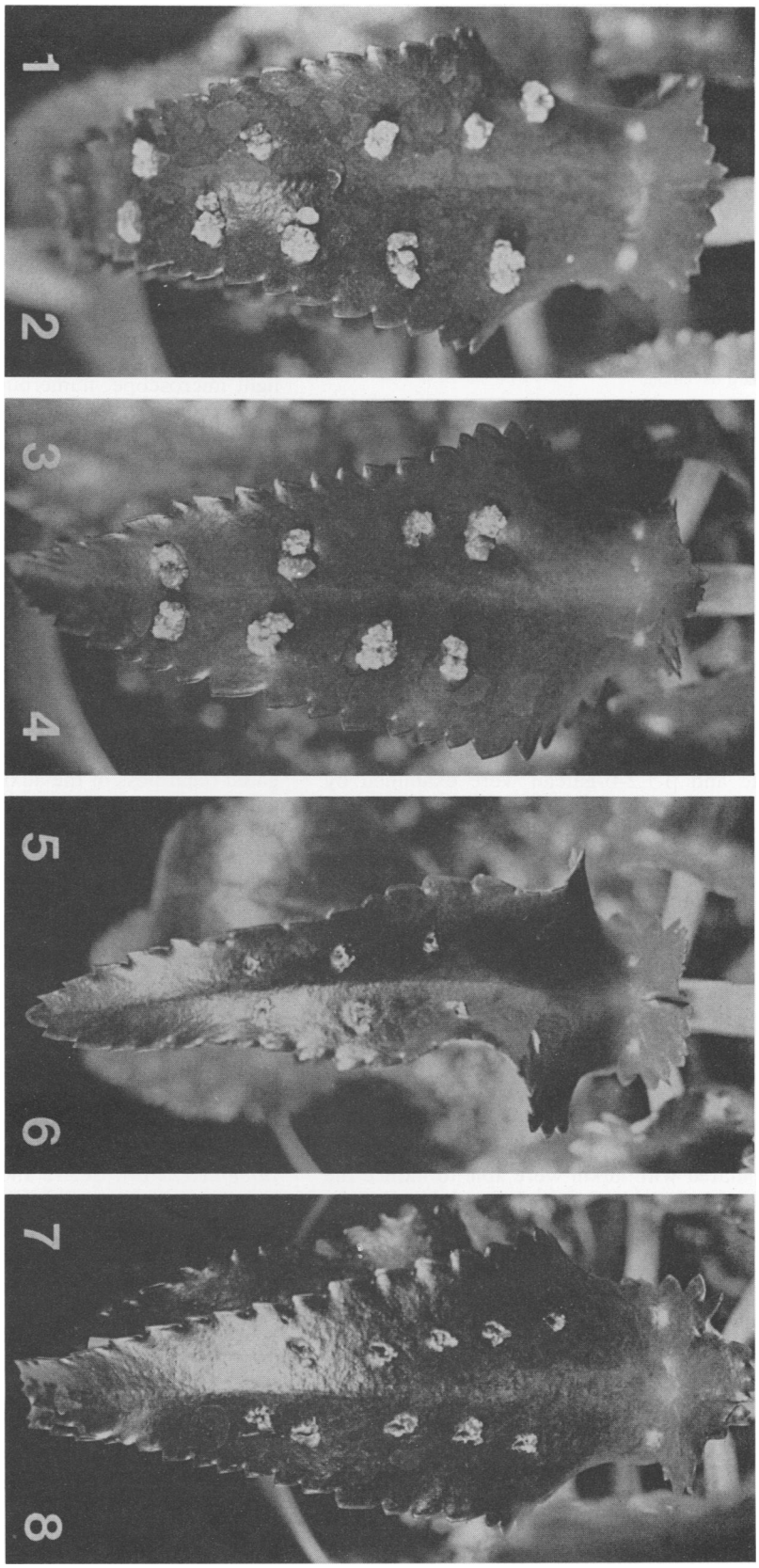

FIG. 1. Virulence of *A. tumefaciens* strains on leaves of *Bryophyllum diagremontiana*. Bacteria were inoculated into toothpick wounds on the leaf surface. Strains: 1, 1D1; 2 and 3, 1D1 with 1 μg of IAA per ml; 4, 1D1(pSa) with 1 μg of IAA per ml; 5 and 6, 1D1(pSa); 7, NT1 with 1 μg of IAA per ml; 8, NT1. Strain 1D1 produced tumors in the presence or absence of IAA. Strain 1D1(pSa) produced tumors only in the presence of IAA. IAA did not cause the production of tumors by avirulent strains such as NT1, which lacks the Ti plasmid. The small bumps visible on the leaves in 5, 6, 7, and 8 at the wound sites are dried droplets of sap which bled from the wound site.

TABLE 1. Effect of auxin on attachment of *A. tumefaciens* strains containing or lacking pSA

Bacterial strain	MS medium with (+) or without (−) IAA	% Bacterial inoculum attached[a]
1D1	+	55 ± 10
	−	50 ± 10
1D1(pSa)	+	50 ± 5
	−	0 ± 3
Cel-12	+	20 ± 5
	−	22 ± 6
Cel-12(pSa)	+	23 ± 7
	−	1 ± 4

[a] Attachment was measured after 120 min of incubation. Initial bacterial concentration varied with the experiment but was between 1×10^3 and 4×10^3 per ml. Each value is the mean of a minimum of three experiments ± the standard deviation.

For light microscopy, live preparations were examined and photographed with a Zeiss photoscope 2 by using Nomarski optics.

For some purposes, bacteria were incubated in MS medium with or without auxin before they were collected by centrifugation. Bacterial lipopolysaccharide (LPS) was extracted, and the amount of ketodeoxyoctanate was estimated as previously described (12). Surface proteins were extracted by using the method of Sonoki and Kado (17), which extracts outer-membrane and periplasmic-space proteins. LPS and protein extracts were analyzed by using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis followed by silver staining as described by Laemmli (6), Merril et al. (13), and Tsai and Frasch (18). Bacterial exopolysaccharide and β-1,2-D-glucan were examined by using the techniques of Carlson and Lee (1) and Puvanesarajah et al. (16).

Bacterial virulence was tested by inoculating toothpick wounds on *Bryophyllum* leaves with the bacteria. The leaves were scored for the presence of tumors for 6 weeks following inoculation.

RESULTS

Virulence and attachment to carrot cells of *A. tumefaciens*(pSa). When the plasmid pSa is introduced into virulent *A. tumefaciens* strains, the bacteria become avirulent (8). This avirulence is not due to any irreversible change in the bacteria, since they can be cured of pSa and they then recover virulence (4). Chernin et al. (2) reported that *A. tumefaciens*(pSa) cells were avirulent if inoculated alone but that bacteria inoculated with auxin were able to induce the formation of tumors. I was able to repeat these observations using virulent *A. tumefaciens* 1D1 and its avirulent derivative 1D1(pSa). Strain 1D1 was virulent on *Bryophyllum* leaves when inoculated in the presence or absence of auxin (Fig. 1). Strain 1D1(pSa) was avirulent when inoculated alone but was virulent when inoculated with 1 μg of IAA per ml. The plant showed no response to avirulent strains such as NT1, which lacks a pTi, whether or not they were inoculated with IAA (Fig. 1). Similar results were obtained with virulent strain C58 and avirulent C58(pSa) and with virulent strain Cel-12 and avirulent Cel-12(pSa). Although the plant produces auxin, this hormone is produced in apical meristems and in expanding leaves. The leaves inoculated in these experiments were mature leaves and thus would be expected to produce little or no auxin.

Wild-type virulent *A. tumefaciens* cells bind to carrot suspension culture cells (11). This site-specific binding of the bacteria to the plant cell surface is believed to be one of the early steps in tumor formation by the bacteria (7). The inhibition of tumor formation on wounded bean leaves by the addition of avirulent bacteria prior to the addition of virulent bacteria has been used as an indirect measure of the ability of bacteria to bind to the plant cell surface (7). *A. tumefaciens*(pSa) cells fail to inhibit tumor formation on wounded bean leaves (15). New et al. suggest that this failure may be due to the failure of the bacteria carrying pSa to attach to plant cells (15). These observations suggested that it would be useful to examine the binding of strains containing pSa to tissue culture cells. Virulent *A. tumefaciens* 1D1 bound to carrot cells in medium containing or lacking auxin (Table 1). Avirulent strain 1D1(pSa) showed significant binding to carrot cells only in medium containing auxin (Table 1). When bacterial attachment to carrot cells was examined under the light microscope, numerous bacteria and bacterial clusters were seen on the surfaces of carrot cells incubated with strain 1D1 in medium with or without auxin (Fig. 2). Bacteria were seen on the surface of carrot cells incubated with strain 1D1(pSa) only if the medium contained auxin; in the absence of auxin no bacteria were observed bound to the plant cells (Fig. 2). Similar results were obtained with bacterial strains C58 and C58(pSa) and with Cel-12 and Cel-12(pSa). In each case, the binding of the bacteria containing pSa, but not of the parent strain, was dependent on the presence of auxin in the medium.

Bacterial attachment to carrot suspension cultures and subsequent cellulose synthesis by the attached bacteria entrapping additional bacteria results in the formation of aggregates of carrot cells which are visible to the naked eye (11). Carrot cells incubated with strain 1D1 became aggregated regardless of the auxin content of the medium. Carrot cells incubated with strain 1D1(pSa) were aggregated only if the medium contained auxin (Fig. 3). In the absence of auxin, small aggregates of the bacteria containing pSa were formed owing to the induction of bacterial cellulose synthesis by the presence of the plant cells (11). These small aggregates are visible in Fig. 3A, dish 3.

To determine whether the binding of bacteria containing pSa to carrot cells in auxin-containing media is to the same site on the plant cell as is the binding of wild-type bacteria lacking pSa, measurements were made of the competition for binding sites between Cel-12 and Cel-12(pSa) in auxin-containing medium. A cellulose-minus mutant was used for this experiment, since these bacteria are able to bind only directly to the plant cell surface and do not show the indirect attachment via cellulose fibrils seen with wild-type *A. tumefaciens* (9). Cel-12 and Cel-12(pSa) were distinguished by their differing antibiotic sensitivities. Carrot cells can

TABLE 2. Effect of LPS on the attachment of *A. tumefaciens* A6 to carrot tissue culture cells

Source of LPS[a]	Concn of LPS (μg of KDO/ml)[b]	% Control attachment[c]
None		
C58	0.04	49 ± 10
1D1	0.04	38 ± 8
1D1(pSa)	0.05	35 ± 10

[a] Bacteria were grown in the absence of auxin. LPS was added to the carrot cells 15 min before the addition of the bacteria.

[b] KDO, Ketodeoxyoctanate.

[c] Control, 100% ± 8. Each value is the mean of three experiments ± the standard deviation. Bacteria (1×10^3 to 4×10^3/ml) were incubated with 10^5 carrot cells per ml. In the control, 55% of the bacteria inoculated bound to the carrot cells in 120 min.

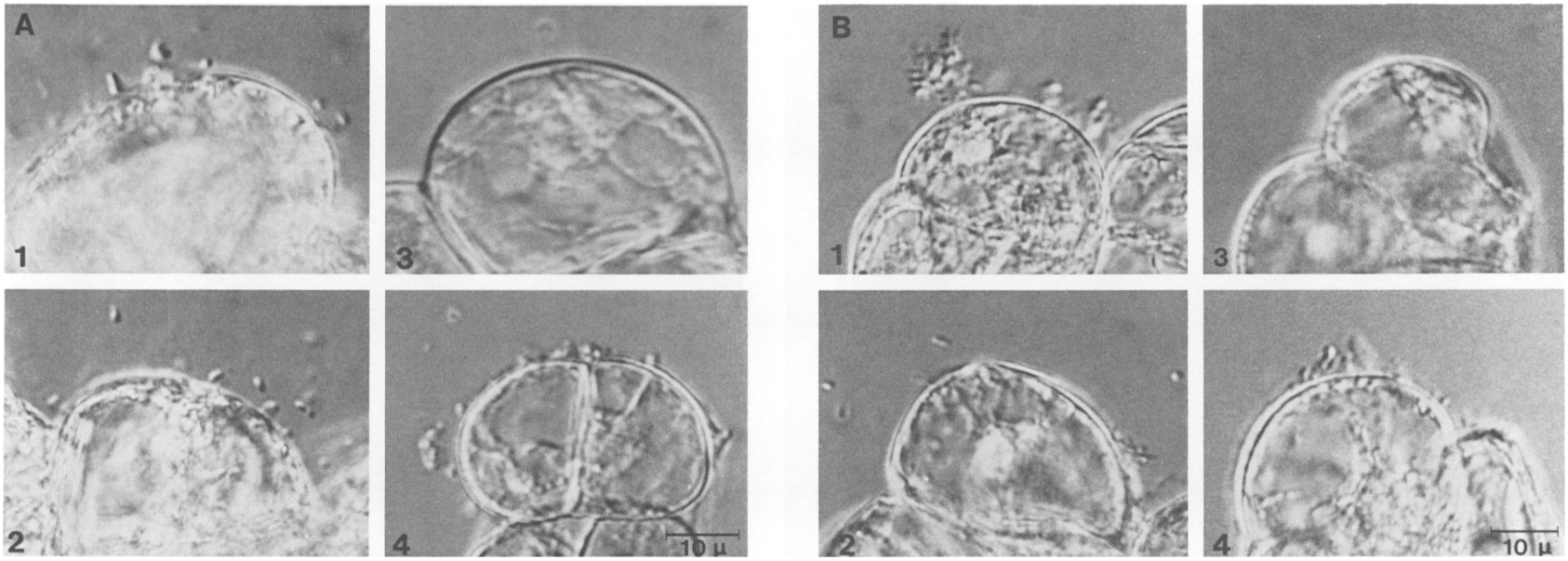

FIG. 2. Photomicrographs of carrot suspension culture cells incubated with *A. tumefaciens* for 2 h in MS medium. Bacteria (10^7/ml) were incubated with 10^5 carrot cells per ml. (A) Live carrot cells. (B) Heat-killed carrot cells. Strains: 1, *A. tumefaciens* 1D1 in the absence of IAA; 2, 1D1 in the presence of 1 μg of IAA per ml; 3, 1D1(pSa) in the absence of IAA; 4, 1D1(pSa) in the presence of 1 μg of IAA per ml. Note that the attachment of 1D1 to carrot cells was independent of IAA, while the attachment of 1D1(pSa) was dependent on the presence of IAA. The effect of IAA on the ability of strain 1D1(pSa) to attach to carrot cells was not dependent on the presence of live carrot cells.

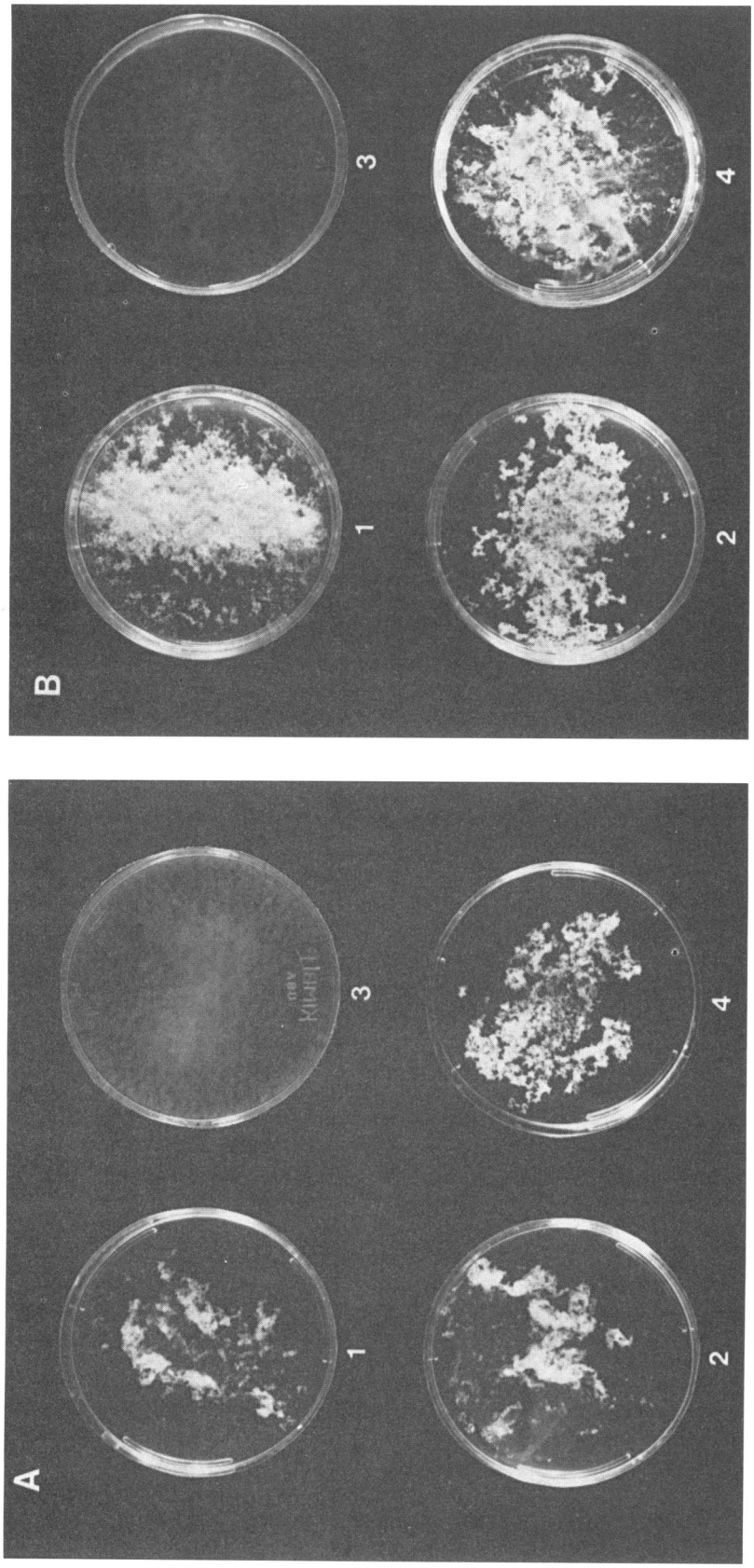

FIG. 3. Aggregation of carrot suspension cells incubated for 24 h with *A. tumefaciens*. (A) Live carrot cells. (B) Heat-killed carrot cells incubated in MS medium with strain 1D1 in the absence of IAA (dishes labeled 1), strain 1D1 in the presence of 1 μg of IAA per ml (dishes 2), strain 1D1(pSa) in the absence of IAA (dishes 3), and 4 strain 1D1(pSa) in the presence of 1 μg of IAA per ml (dishes 4). Note that although strain 1D1 did not require IAA for carrot cell aggregation, strain 1D1(pSa) did require IAA in order to form aggregates with carrot cells. Living carrot cells were not required for this response.

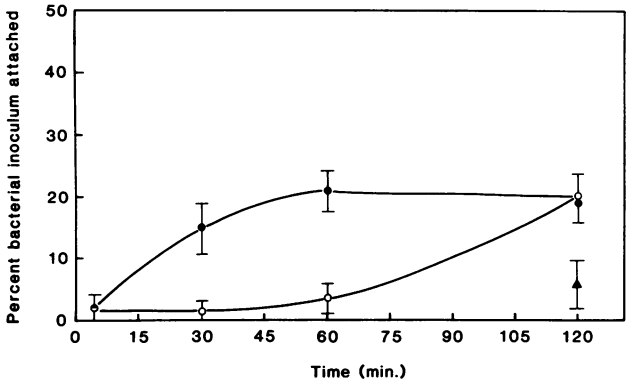

FIG. 4. Time course of attachment of *A. tumefaciens* to carrot suspension cells. Bacteria (2×10^3/ml) were incubated with 10^5 carrot cells per ml in MS medium. ●, Strain 1D1 in the absence of IAA; ○, strain 1D1(pSa) in the presence of 1 μg of IAA per ml; ▲, strain 1D1(pSa) in the absence of IAA. Note the lag in the attachment of 1D1(pSa) in the presence of IAA.

bind on the average about 200 Cel-12 bacteria (5). Cel-12(pSa) also bound to carrot cells at a level of about 200 bacteria per carrot cell (data not shown). When Cel-12 and Cel-12(pSa) were inoculated into carrot suspension cultures at ratios ranging from 1:100 to 100:1 by using a total bacterial inoculum of 10^8 bacteria per ml and 5×10^4 to 10×10^4 carrot cells per ml, the ratio of the attached bacteria reflected the ratio of the bacteria in the inoculum (data not shown). At this ratio of bacteria to plant cells, all of the approximately 200 sites per carrot cell which bind the wild-type bacteria on the plant cell surface should be occupied (5). If the bacteria bind to different sites on the plant surface, the presence of the wild-type bacteria would not affect the binding of the pSa-containing strain and vice versa. For example, when 10^8 Cel-12 cells were inoculated with 10^6 Cel-12(pSa), only 10% of the Cel-12 cells could bind to the 10^7 total sites available on the surfaces of the 5×10^6 plant cells. If Cel-12(pSa) cells bind to the same site, only 10% of these bacteria would be bound, but if Cel-12(pSa) cells bind to a different site which is also present at about 200 sites per carrot cell (or a total of 10^7 sites), then all of the Cel-12(pSa) cells could potentially bind to the carrot cells. The fact that the ratio of the bacteria bound reflected the ratio of the bacteria in the inoculum suggests that Cel-12 and Cel-12(pSa) bind to the same site on the carrot cell surface under these conditions, although other interpretations, such as neighboring sites and stearic hindrance, are possible.

Carrot cells apparently do not play an active role in the binding of *A. tumefaciens*. Bacteria bind to dead carrot cells with little change in strain specificity or in the kinetics of binding (11). To determine whether the auxin effect on the binding of bacteria containing pSa is an effect on the bacteria or on the plant cells, carrot cells were depleted of auxin, heat killed, and incubated with bacteria in medium with or without auxin as described in Materials and Methods. As expected, wild-type bacteria bound to and caused the aggregation of heat-killed carrot cells; the binding and aggregation were not dependent on the presence of auxin. However, bacteria containing pSa bound to and aggregated heat-killed carrot cells only in the presence of auxin. In the absence of added auxin, they failed to bind to or to aggregate the plant cells (Fig. 2 and 3). The aggregates of heat-killed carrot cells formed by strains carrying pSa differed in texture from the aggregates formed by wild-type strains (Fig. 3B, dishes 2 and 4). The reason for this difference is unknown. However, the observation that bacteria carrying pSa bound to and aggregated heat-killed carrot cells only in the presence of added auxin suggests that the requirement for auxin for the binding of these bacteria is due to an effect of the auxin on the bacteria rather than on the plant cells.

Time course of bacterial binding to carrot cells. When the time course of the attachment of the bacteria to carrot cells was examined, it was observed that although strain 1D1 bound to carrot cells without any detectable lag in the binding, strain 1D1(pSa) showed a lag of about 60 min in attaching to carrot cells in the presence of auxin. In the absence of auxin, no binding of 1D1(pSa) was detectable after 120 min (Fig. 4). This lag in the binding of 1D1(pSa) suggested that the bacteria might have to synthesize some substance before they can attach to the carrot cells. To examine this possibility, tetracycline was added to a suspension of strains 1D1 and 1D1(pSa); the bacteria were then added to carrot cells in MS medium containing tetracycline with or without auxin. The attachment of strain 1D1 in medium with or without auxin was not affected by the presence of tetracycline, but the antibiotic inhibited the attachment of 1D1(pSa) in auxin-containing medium, suggesting that bacterial protein synthesis is required for the attachment of 1D1(pSa) in the presence of auxin (Fig. 5 and 6).

Characterization of bacterial surface components. Previous research suggested that bacterial LPS is altered in the pSa-containing strains of *A. tumefaciens* (15). Therefore, LPS was extracted from strains 1D1 and 1D1(pSa) grown in the presence or absence of auxin. No difference in the size of these LPS preparations was detected when they were examined by SDS-polyacrylamide gel electrophoresis (data not shown). LPS from wild-type strains of *A. tumefaciens* inhibits the binding of the bacteria to carrot or tobacco suspension culture cells if it is added to the culture before the addition of the bacteria (12). When the inhibitory ability of LPS from strains C58, 1D1, and 1D1(pSa) (all grown in the absence of auxin) was examined, LPS from strain 1D1(pSa) was found to be effective in inhibiting bacterial attachment at a concentration of LPS comparable to that from strain 1D1 (Table 2). The reason for the discrepancy between this result and the inability of LPS from 1D1(pSa) to inhibit tumor formation on wounded bean leaves (15) is not known. Since no difference in the ability of the LPS to inhibit bacterial binding was observed, the LPS was not characterized further.

No change in bacterial motility was seen in strains containing pSa; thus, presumably their flagella were unaffected by the presence of the plasmid. No change in the amount of extracellular polysaccharide or in β-1,2-D-glucan produced by the bacteria was seen in strains containing pSa (data not shown). Neither extracellular polysaccharide nor β-1,2-D-glucan from wild-type strains or from strains carrying pSa inhibits the binding of *A. tumefaciens* to carrot cells (unpublished observation). Therefore, these components of the bacterial surface and periplasmic space were not examined further.

When the surface polypeptides of the strains containing pSa were extracted by the method of Sonoki and Kado (17; the method extracts outer-membrane and periplasmic-space proteins from *A. tumefaciens*) and compared with those of their parent strains, differences between the parent strain and the pSa-containing strains grown in the presence or in the absence of auxin were observed (Fig. 7 and 8). One or more bands were seen which were present in the parent

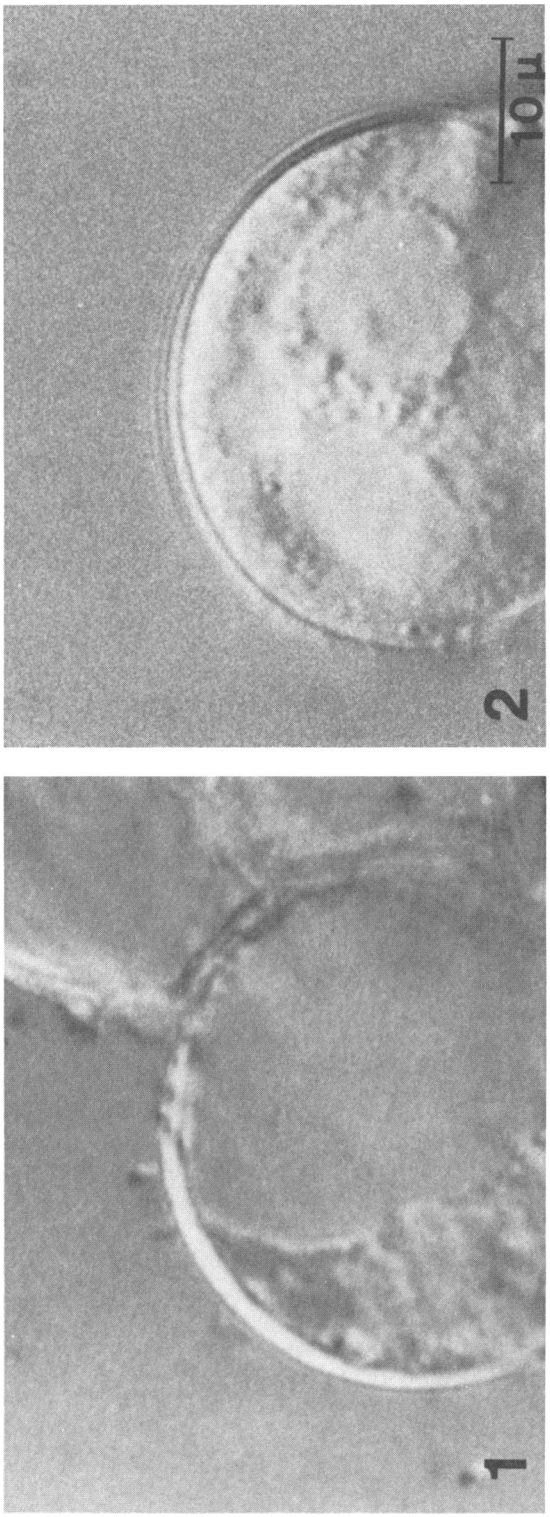

FIG. 5. Photomicrographs of live carrot cells incubated with *A. tumefaciens* for 2 h in MS medium containing 10 μg of tetracycline per ml. Bacteria (10^7/ml) were incubated with 10^5 carrot cells per ml. Panel 1, Strain 1D1 in the presence of 1 μg of IAA per ml. Panel 2, Strain 1D1(pSa) in the presence of 1 μg of IAA per ml. Although tetracycline had no effect on the ability of strain 1D1 to attach to carrot cells, the antibiotic prevented the attachment of strain 1D1(pSa). This result suggests that bacterial protein synthesis is required for the response of 1D1(pSa) to the presence of IAA.

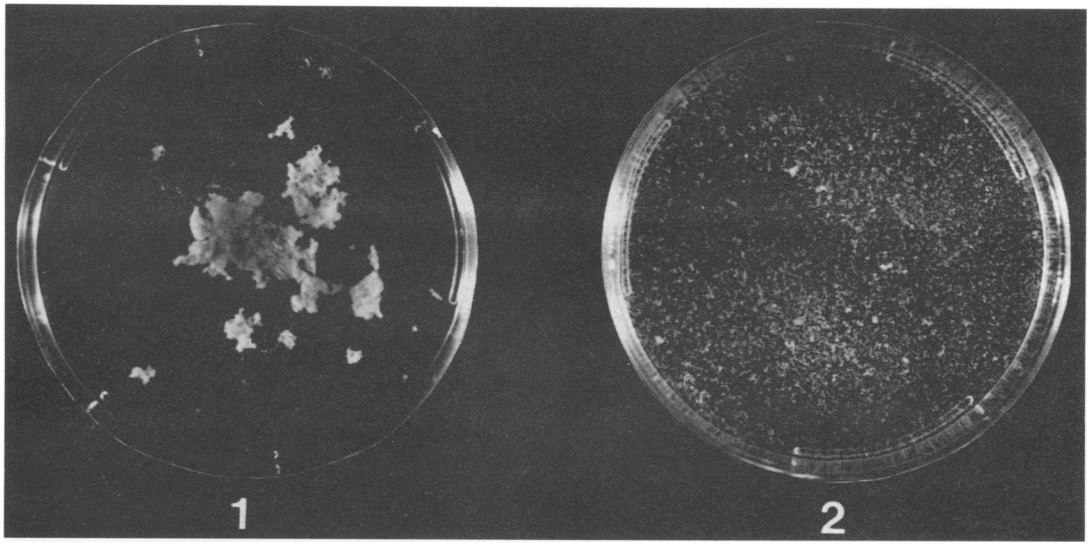

FIG. 6. Effect of tetracycline on the ability of *A. tumefaciens* to form aggregates with carrot suspension culture cells. The bacteria were incubated with live carrot cells in MS medium containing 1 μg of IAA per ml and 10 μg of tetracycline per ml. Dish 1, Strain 1D1. Dish 2, Strain 1D1(pSa). Although tetracycline had no effect on the aggregation of carrot cells by strain 1D1, the antibiotic prevented the aggregation by strain 1D1(pSa).

strain and in the pSa-containing strain grown with auxin but which were absent or much reduced in the pSa-containing strain grown without auxin. The exact changes observed differed for different bacterial strains.

DISCUSSION

The introduction of the plasmid pSa into *A. tumefaciens* suppresses bacterial virulence (8). The lack of virulence is apparently due to the failure of strains carrying pSa to bind to plant host cells; bacterial mutants which fail to bind to host cells are avirulent (3, 10). Virulence (2) and host cell binding can be restored by the addition of auxin. The effect of auxin is on the bacteria rather than on the plant cells, since the auxin promotion of bacterial binding is seen with live bacteria carrying pSa and with dead carrot cells. Auxin promotion of binding of *A. tumefaciens*(pSa) requires bacterial protein synthesis. The examination of the question of the nature of the alteration in strains carrying pSa and grown in the absence of auxin so that they fail to bind to plant cells has resulted in different answers in different systems. No alterations in bacterial flagella, polysaccharides, or LPS were detected under the conditions used in this study. LPS from strain 1D1(pSa) grown in the absence of auxin was as effective as LPS from strain 1D1 in blocking the binding of virulent *A. tumefaciens* cells to carrot suspension culture cells. However, New et al. (15) reported that they detected a difference in the ability of bacterial LPS to inhibit tumor formation by virulent *A. tumefaciens* on wounded bean leaves when they compared LPS extracted from strains carrying pSa with that from strains lacking pSa. These

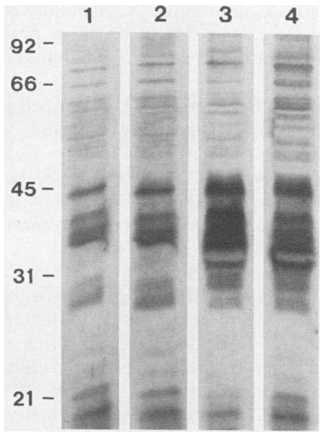

FIG. 7. Surface polypeptides extracted from *A. tumefaciens* separated by SDS-polyacrylamide gel electrophoresis (11% acrylamide) and stained with silver. Bacteria were grown in minimal medium with glucose and incubated in MS medium with or without 1 μg of IAA per ml for 2 h prior to their extraction. Lanes: 1, strain C58(pSa) incubated without IAA; 2, C58(pSa) incubated without IAA (twice as much sample was loaded as in lane 1); 3, C58(pSa) incubated with IAA; 4, C58 incubated without IAA. Each lane (except lane 2) contained the extract from approximately 10^8 bacteria. Note the band of approximately 33,000 daltons missing in strains containing pSa incubated in the absence of auxin. Other more minor differences in the bacteria containing pSa can be seen. The significance of these differences, if any, is unknown. Numbers to the left of the gel show the positions and sizes in kilodaltons of molecular size standards.

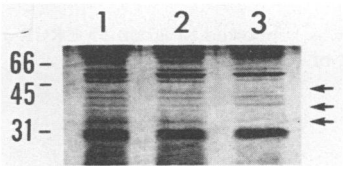

FIG. 8. Surface polypeptides extracted from *A. tumefaciens* separated by SDS-polyacrylamide gel electrophoresis (15% acrylamide) and stained with silver. Bacteria were grown in Luria broth and incubated in MS medium with or without 1 μg of IAA per ml for 2 h prior to their extraction. Lanes: 1, strain 1D1 incubated without auxin; 2, 1D1(pSa) incubated with IAA; 3, 1D1(pSa) incubated without auxin. Arrows indicate bands missing in 1D1(pSa) incubated in the absence of auxin. Numbers to the left of the gel show the positions and sizes in kilodaltons of molecular size standards.

investigators attributed the lack of virulence of strains carrying pSa to this difference in the inhibitory ability of LPS, which they felt reflected a lack of binding of the bacteria carrying pSa to wound sites on the leaves (15). The reason for the difference in the effect of LPS from strains carrying pSa in the bean leaf system and in suspension culture is not known. New et al. (15) did not examine bacterial polypeptides. Surface polypeptides of C58 and C58(pSa) grown in the absence of auxin showed a missing band in C58(pSa) on SDS-polyacrylamide gel electrophoresis. This polypeptide was present in extracts of C58(pSa) grown in the presence of auxin. The polypeptide appears to be of approximately the same size as one of the polypeptides missing in the nonattaching mutant of *A. tumefaciens* Att-C43 (10). Thus, the lack of bacterial binding in C58(pSa) may be due to the lack of this protein.

Two classes of bacterial mutants which fail to bind to suspension culture cells and, presumably as a consequence, are avirulent have been described by Douglas et al. (3) and Matthysse (10). In both cases, the mutants fail to attach to suspension culture cells in the presence of auxin. Thus, the mutants are not directly parallel to strains carrying pSa. The mutants described by Douglas et al. were altered in motility and in β-1,2-D-glucan (3, 16). These mutants show no alterations in surface polypeptides (unpublished observation). Strains carrying pSa do not show any detectable alteration in motility or in β-1,2-D-glucan content in the absence of auxin. The mutants described by Matthysse were altered in surface polypeptides (10). These mutants were isolated from strain C58. Therefore, polypeptides isolated from strains C58 and C58(pSa) grown in MS medium with or without auxin were examined. One of the polypeptides missing in the nonattaching mutant Att-C43 appears to be of a size similar to that of a polypeptide band lacking in C58(pSa) grown in the absence of auxin. This polypeptide was present in C58 grown with or without auxin and in C58(pSa) grown with auxin (Fig. 7). Thus, this may be a protein whose presence is required for bacterial attachment to plant cells and whose synthesis is regulated by auxin in strains carrying pSa.

The questions of why bacteria carrying pSa produce less auxin (2) and of how and why bacterial auxin production regulates the expression of polypeptides required for bacterial attachment to host cells remain to be answered.

ACKNOWLEDGMENTS

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