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# Agglutination of *Erwinia stewartii* Strains with a Corn Agglutinin: Correlation with Extracellular Polysaccharide Production and Pathogenicity

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A bacterial agglutinin was extracted from ground corn (WI hybrid 64A × W117) seed with phosphate-buffered saline (pH 6.0) and precipitated with $(NH_4)_2SO_4$ at 70% saturation. The activities of this agglutinin against 22 strains of *Erwinia stewartii* (agent of bacterial wilt of corn) that varied in virulence were determined. Specific agglutination (agglutination titer per milligram of protein per milliliter) values were correlated negatively with virulence ratings. Strains with high specific agglutination values (15 or higher) were avirulent or weakly virulent; strains with low specific agglutination values (10 or lower) were highly virulent, with two exceptions. Avirulent strains produced butyrous colonies and released only small amounts of extracellular polysaccharide (EPS) into the medium, and the cells lacked capsules; virulent strains produced fluidal colonies and released large amounts of EPS, and the cells were capsulated. There was a strong correlation between the amount of EPS produced by each strain (as determined by increase in viscosity of the medium) and the specific agglutination value; in contrast, lipopolysaccharide compositions were similar in all strains. When cells of six fluidal strains were washed by repeatedly centrifuging and resuspending them in buffer, they were agglutinated more strongly by corn agglutinin than were unwashed cells. When avirulent cells were washed, their specific agglutination values did not increase significantly. Eight EPS-deficient mutants of *E. stewartii*, selected for resistance to the capsule-dependent bacteriophage K9, had lower virulence but higher specific agglutination than did their corresponding wild-type parents. Production of EPS appears to be essential for virulence; EPS may prevent agglutination of bacteria in the host, thus allowing their multiplication.

The initial interaction between bacteria and plants that results in disease may be determined by the natures of the surfaces of these organisms (16, 18). In this interaction, lipopolysaccharides (LPS) or extracellular polysaccharides (EPS) often have been implicated as the essential cell surface components of bacteria, whereas lectins are considered to be the determinants in plant cell walls (8, 16, 18, 21, 22; W. D. Bauer, T. V. Bhuvaneswari, A. J. Mort, and G. Sturgeon, Plant Physiol. **63**(Suppl.):134, 1979). When the crown gall pathogen, *Agrobacterium tumefaciens*, was used in a pinto bean tumor assay, Whatley et al. (21) showed that LPS and cell envelope preparations from virulent and avirulent (site-binding) strains of the bacterium inhibited tumor induction. Conversely, LPS from non-site-binding, avirulent strains were not inhibitory.

Sequeira and Graham (17) showed that lectins extracted from either tobacco or potato agglutinated cells of avirulent ($B_1$), but not virulent ($K_{60}$), strains of *Pseudomonas solanacearum*. Strains of *P. solanacearum* have two distinct kinds of LPS based on size and sugar composition: avirulent ($B_1$) strains have rough LPS, which lacks the O-antigen component, and virulent ($K_{60}$) strains have smooth, complete LPS (22). The question arises of whether differences in agglutination also can be observed in a monocot invaded by a vascular bacterial pathogen.

The causal agent of Stewart's wilt of corn, *Erwinia stewartii*, produces copious amounts of an EPS composed mostly of glucose, galactose, and glucuronic acid (7). The virulence of *E. stewartii* has been associated with EPS production in culture (9, 19) and in planta (E. J. Braun, Phytopathology **71**:205, 1981).

Hemagglutinating activity has been detected in protein fractions from maize coleoptile, me-

socotyl, and root (3). An agglutinin has been isolated from corn seed and has been partially characterized, but no sugar hapten has been determined for it (A. Woods, N. Hunter, L. Sequeira, and A. Kelman, Plant Physiol. **63**(Suppl.):134, 1979). The objective of this study was to determine whether capsulation of *E. stewartii* and agglutination with this corn agglutinin were correlated with virulence.

## MATERIALS AND METHODS

**Bacterial strains and cultural conditions.** Stock cultures of *E. stewartii* (Table 1) were stored at −20°C in a mixture containing 60% glycerol and either 40% of the medium of Ayers et al. (4), but without carbohydrate, or 40% of Luria medium (14). Strains to be tested for agglutination and relative viscosity were streaked initially on a Casamino Acids-glucose-peptone agar medium containing 0.005% triphenyltetrazolium chloride (11) and grown for 48 h at 28°C. Cultures grown longer than 48 h on the triphenyltetrazolium chloride medium lost viability rapidly; thus, in later experiments, the concentration of triphenyltetrazolium chloride was decreased to 0.001%. In experiments involving extraction of LPS, strains were grown in Casamino Acids-glucose-peptone broth for 48 h at 28°C on a rotary shaker.

Colony types on triphenyltetrazolium chloride agar cultures were determined with oblique lighting under a dissecting microscope (11). Strains were categorized into three major colony types: fluidal, intermediate, and butyrous. Fluidal colonies were large and smooth in appearance; butyrous colonies were small and rough.

Several of our stock cultures contained more than one colony type. Butyrous mutants were isolated from cultures of DC2, DC11, DC18, DC19, DC45, DC70, DC168, and DC170. The identities of these mutants with their corresponding fluidal parent strains were confirmed by comparisons of plasmid deoxyribonucleic acid electrophoretic profiles (2). Plasmids with a $1 \times 10^6$-dalton size difference could be detected for plasmids of less than $6 \times 10^7$ daltons.

**Bacteriophage K9.** The capsule-dependent bacteriophage from *E. stewartii* K9 (22) was obtained from A. Karr. High-titer lysates of phage K9 were prepared by infecting DC283 ($7 \times 10^8$ cells per ml) at a multiplicity of infection of 1 to 2 plaque-forming units per cell in Luria broth at 30°C. After lysis, the culture was treated with chloroform, cells and debris were removed by centrifugation at 8,000 × *g* for 15 min, and the phage was precipitated by addition of $(NH_4)_2SO_4$ to 60% saturation. After 6 h at 4°C, the precipitate was collected by centrifugation, suspended in 0.01 M potassium phosphate buffer (pH 7.0), dialyzed overnight against the same buffer at 4°C, and then stored at −20°C. This procedure yielded stocks with titers of $10^{10}$ to $10^{12}$ plaque-forming units per ml. Sensitivity to phage K9 was tested in Casamino Acids-glucose-peptone medium by means of a double-layer agar technique (15); phage suspensions ($10^8$ plaque-forming units per ml) were spotted on soft agar overlays of the test strain, and they were then incubated overnight at 30°C. To isolate K9-resistant mutants, a culture of DC283 in Casamino Acids-glucose-peptone broth ($2 \times 10^8$ cells per ml) was infected at an multiplicity of infection of 2 plaque-forming units per cell and incubated with shaking overnight. The survivors were plated on Casamino Acids-glucose-peptone agar, and butyrous colonies were selected. The K9 resistance of these clones was verified by spot tests.

TABLE 1. *Strains of E. stewartii used in this study*

| Strain no. | | Geographic origin | Source |
|---|---|---|---|
| Current | Original | | |
| DC2 | SW2 | Ohio | D. Coplin |
| DC11 | SW11 | Illinois | D. Coplin |
| DC13 | SW13 | Illinois | D. Coplin |
| DC18 | SW18 | Ohio | D. Coplin |
| DC19 | SW19 | Kentucky | D. Coplin |
| DC20 | SW20 | Kentucky | D. Coplin |
| DC36 | SW36 | Tennessee | D. Coplin |
| DC39 | SW39 | North Carolina | D. Coplin |
| DC45 | SW45 | Missouri | D. Coplin |
| DC51 | SW51 | Virginia | D. Coplin |
| DC63 | SW63 | Ohio | D. Coplin |
| DC65 | SW65 | Ohio | D. Coplin |
| DC70 | SW70 | Ohio | D. Coplin |
| DC100 | SS12 | | ICPPB[a] |
| DC108 | SS10 | | ICPPB |
| DC109 | SS13 | | ICPPB |
| DC110 | SS104 | Illinois | ICPPB |
| DC150 | | Illinois | M. Turner |
| DC158 | ES-1 | New York | T. Woods |
| DC159 | ES-2 | New York | T. Woods |
| DC161 | ES-4 | New York | T. Woods |
| DC168 | GC6 | Missouri | A. Karr |
| DC169 | LC | Missouri | A. Karr |
| DC170 | 22A | Missouri | A. Karr |
| DC283 | DC110 $Nal^r$ | | Nalidixic acid-resistant mutant of DC110 |

[a] ICPPB, International Collection of Plant Pathogenic Bacteria, University of California at Davis, Davis, Calif. (M. P. Starr, Curator).

**Extraction of corn agglutinin.** Corn agglutinin was extracted from corn seed of hybrid 64A × W117. Five hundred grams of freshly harvested seed was ground in a Wiley mill (A. H. Thomas Co., Philadelphia, Pa.) until the meal could pass through an 8-mesh sieve. The meal was added to 2 liters of 0.01 M sodium phosphate-buffered saline (pH 6.0) at 4°C. The mixture was stirred for 2 days at 4°C and then filtered through cheesecloth and again through a Celite pad previously washed with buffer. Ammonium sulfate was added to the clear filtrate liquid in a stepwise manner to obtain 35, 70, and 100% saturation at 4°C. Materials precipitating at each saturation level were collected by centrifugation at 12,000 × *g* for 30 min. The precipitate was suspended in 0.1 M sodium acetate buffer (pH 3.6) and then dialyzed against 0.01 M sodium acetate buffer (pH 4.0) at 4°C. Insoluble material remaining after dialysis was removed by centrifugation. The supernatant initially was tested for agglutinating activity by means of an assay with *Erwinia chrysanthemi* (strain SR261, isolated from corn) (Table 2) (Woods et al., Plant Physiol. **63**(Suppl.):134, 1979). Active fractions were dialyzed against 0.5 M NaCl and stored at 4°C. The amount of protein in

TABLE 2. *Specific activity of corn agglutinin*[a]

| % Saturation with $(NH_4)_2SO_4$ | Titer[b] | μg of protein/ml[c] | Sp act[d] |
|---|---|---|---|
| 35 | 512 | 1,000 | 0.51 |
| 70 | 16,384 | 7,400 | 2.20 |
| 100 | 1,200 | 3,125 | 0.04 |

[a] Obtained by ammonium sulfate precipitation of corn seed extracts.
[b] Tested by agglutination of *E. chrysanthemi* strain SR261.
[c] Determined by the method of Lowry et al. (13).
[d] Specific activity = titer/milligrams of protein per milliliter.

each fraction was determined by the technique of Lowry et al. (13).

**Agglutination assay.** The 48-h growth from a plate culture of *E. stewartii* on triphenyltetrazolium chloride medium was suspended in distilled water, and the optical density at 600 nm was adjusted to 0.4 (approximately $7 \times 10^8$ cells per ml) with a Spectronic 20 colorimeter (Bausch & Lomb, Inc., Rochester, N.Y.). Corn seed agglutinin was dialyzed against 0.01 M sodium acetate buffer, pH 4.6, and adjusted to 740 μg of protein per ml. This solution was diluted serially with buffer to provide seven agglutinin concentrations, ranging from 740 to 12 μg of protein per ml. Drops (25 μl) from each solution of agglutinin were placed on each square of a grid drawn in a polystyrene petri dish. Bacterial suspension (25 μl) was added to each drop of agglutinin as well as to drops of distilled water. This procedure was followed for each of the 30 *E. stewartii* strains and their mutants. Plates were incubated at 24 to 25°C for 2 h on a reciprocal shaker and then examined under a dissecting microscope. Agglutination was rated according to the procedure of Sequeira and Graham (17). When *E. chrysanthemi* was used in the assay, bacteria were suspended in 0.01 M sodium acetate buffer, pH 4.0, and added to agglutinin dialyzed in the same buffer. In some experiments, bacteria were washed by repeatedly centrifuging them at $12,000 \times g$ for 10 min at 4°C and resuspending them in 0.01 M sodium acetate buffer, pH 4.6.

A prior evaluation of the agglutination assay, involving 10 strains of *E. stewartii* and three separate samples per strain in a two-way analysis of variance, showed no significant differences between replicates ($F = 0.85$) but significant differences between strains ($F = 42.7$) at the 95% confidence level, with a pooled standard deviation of 5.7.

**Assay for relative viscosity.** Bacterial suspensions previously used for agglutination assays were stored overnight at 4°C and then centrifuged at 12,000 $\times g$ for 30 min at 4°C to remove bacteria. The relative viscosity of each 6-ml sample was determined at 24 to 25°C by measuring flow time through a 0.4-mm-bore Ostwald viscosimeter (E. H. Sargent & Co., Cleveland, Ohio). Relative viscosity was calculated as the quotient of the flow time in the sample divided by flow time for water. In an evaluation of this relative viscosity assay, involving 10 strains of *E. stewartii* and three separate samples per strain, two-way analysis of variance showed no significant differences between replicates ($F = 0.2$). However, differences between strains ($F = 235$) at the 95% confidence level were highly significant, with a pooled standard deviation of 0.025.

**Measurement of capsule size.** The India ink method of Duguid (5) was followed to determine the presence or absence of capsules. Capsules were measured with an eyepiece micrometer in a Zeiss microscope.

**Extraction and analysis of LPS.** LPS was extracted from each of nine strains of *E. stewartii* by the standard hot phenol-water method (20). Lyophilized LPS samples were hydrolyzed with 2 N trifluoroacetic acid at 121°C for 1 h, and then alditol acetate derivatives were prepared (1). The sugar derivatives were extracted from the acetylation mixture and then analyzed with a Varian model 3740 gas chromatograph (Varian/Instrument Group, Park Ridge, Ill.) equipped with glass columns packed with Sp 2340 Supelcoport.

**Electrophoresis of LPS.** One hundred twenty micrograms of each LPS sample was dissolved in 0.1 ml of sodium dodecyl sulfate buffer [62.5 mM tris-(hydroxymethyl)aminomethane hydrochloride, 10% glycerol, 2.5% dithiothreitol, and 3% sodium dodecyl sulfate (pH 6.8)] and incubated for 5 min at 100°C (10). Disc gel electrophoresis was carried out in 5.8% polyacrylamide gels with 3% polyacrylamide stacking gels at 1 to 2 mA per tube until the dye fronts were near the bottoms of the tubes (12). Gels were fixed overnight in 25% isopropanol and then stained by the periodate-Schiff method (6).

**Pathogenicity tests.** Bacterial strains were grown for 1 to 2 days on Luria (14) agar, and then cell suspensions in 0.15 M NaCl were adjusted to a population of $10^8$ colony-forming units per ml. Plants of the sweet corn cultivar Earliking or of a hybrid field corn line (64A × W117) were grown in sterile soil in 10-cm pots, four plants per pot, in a controlled environment chamber at 30°C with a 16-h photoperiod. Seedlings were inoculated 8 days after planting. Each plant was injected at one site about 4 cm from the soil line (just below the first leaf) by means of a 2-ml plastic syringe fitted with a 22-gauge needle. Each isolate was tested on four plants, and each test was repeated at least once. Disease index ratings were taken on each plant at 8, 21, or 27 days after inoculation. The following continuous disease index was used for evaluation of symptoms in inoculated plants: 1, no symptoms; 1.5, scattered lesions on inoculated leaves; 2, multiple lesions on inoculated leaves; 3, wilting of inoculated leaves, lesions on new leaves; 4, wilting of about 50% of the leaves, lesions on all leaves; 5, plant dead.

## RESULTS

**Comparison of butyrous and fluidal strains.** Strains of *E. stewartii* could be separated on the basis of the butyrous or fluidal appearances of the colonies; cells of the butyrous strains lacked capsules that could be visualized by India ink staining, whereas capsules were present on cells of fluidal strains (Table 3 and Figure 1). Furthermore, butyrous strains did not produce much slime in culture, as indicated by the relative viscosities of cell-free filtrates (Tables 4 and 5), and were resistant to the capsule-

dependent bacteriophage K9. Neither colony type was able to induce a hypersensitive response in *Nicotiana glutinosa* leaves when infiltrated at $2 \times 10^8$ cells per ml.

The virulence of the butyrous mutants was very low in the susceptible sweet corn cultivar or hybrid field corn line used. Only the data for the hybrid line are shown in Table 5. The butyrous strains caused elongated, limited, water-soaked lesions on inoculated leaves, but did not cause systemic wilting or stunting of the plants. Symptoms did not appear on new leaves. The apparent decrease in disease indices for the mutants between 8 and 21 days (Table 5) was due to senescence and loss of the inoculated leaves. All mutant strains used contained between 9 and 13 plasmids, which enabled us to establish the identities of the mutants on the basis of their electrophoretic profiles as compared with those of the parent strains. In each case, the plasmid content of the mutant was identical to that of its parent. This finding confirmed that the mutants were not the result of cross-contamination of our cultures and that they arose by mutation rather than loss of a plasmid.

TABLE 3. *Sizes of the cell capsules of 10 strains of E. stewartii*[a]

| Strain no. | Capsule size[b] (μm) of strain classified as: | |
|---|---|---|
| | Fluidal | Butyrous |
| DC2 | 0.6–1.7 | 0.0 |
| DC18 | 0.8–1.5 | 0.0 |
| DC11 | 0.6–1.0 | 0.0 |
| DC110 | 0.6–1.2 | |
| DC169 | 0.8–1.2 | |
| DC170 | 0.6–1.2 | 0.0 |
| DC100 | | 0.0 |
| DC108 | | 0.0 |
| DC109 | | 0.0 |
| DC161 | | 0.0 |

[a] Determined by the method of Duguid (5), with 6-day-old cultures grown on a tetrazolium chloride agar medium.
[b] Radial thickness of capsular layer.

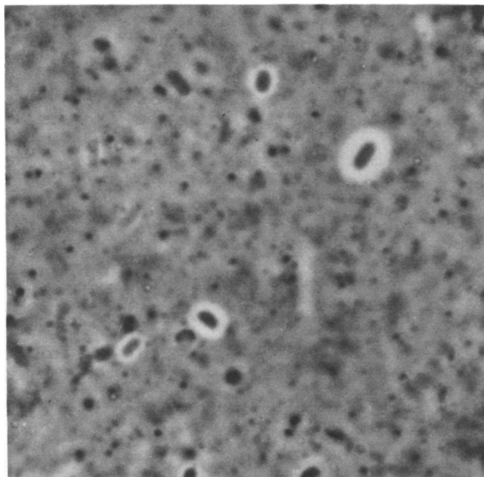

FIG. 1. *Capsule of virulent strain DC110 of E. stewartii, revealed by using India ink stain.* ×830.

TABLE 4. *Relative viscosities, specific agglutinations by corn agglutinin, and virulences on maize (cultivar 64A × W117) of representative strains of E. stewartii*

| Strain no. | Colony type[a] | Relative viscosity (avg ± standard deviation)[b] | Specific agglutination (avg ± standard deviation)[c] | Disease index[d] at: (days) | |
|---|---|---|---|---|---|
| | | | | 8 | 27 |
| DC2 | F | 1.59 ± 0.10 | 5 ± 2 | 3.0 | 3.8 |
| DC11 | F | 1.64 ± 0.16 | 6 ± 4 | 3.2 | 4.6 |
| DC13 | F | 1.35 ± 0.10 | 10 ± 4 | 3.2 | 4.4 |
| DC18 | F | 1.27 ± 0.17 | 6 ± 1 | 3.0 | 4.1 |
| DC19 | F | 1.56 ± 0.00 | 8 | 2.7 | 3.4 |
| DC36 | F | 1.57 ± 0.17 | 9 ± 6 | 3.3 | 4.6 |
| DC39 | F | 1.36 ± 0.17 | 7 ± 3 | 2.9 | 4.2 |
| DC45 | F | 1.30 ± 0.09 | 5 ± 1 | 3.1 | 4.1 |
| DC65 | F | 1.34 | 12 ± 4 | 3.2 | 3.9 |
| DC70 | F | 1.37 ± 0.0 | 5 ± 3 | 3.0 | 4.7 |
| DC110 | F | 1.51 ± 0.00 | 5 ± 2 | 3.7 | 5.0 |
| DC168 | F | 1.38 ± 0.00 | 5 ± 1 | 3.8 | 4.6 |
| DC170 | F | 1.17 ± 0.04 | 5 ± 0 | 3.2 | 4.2 |
| DC20 | F | 1.48 ± 0.05 | 9 ± 3 | 1.8 | 1.0 |
| DC159 | F | 1.59 ± 0.44 | 4 ± 2 | 1.0 | 1.0 |
| DC63 | I | 1.21 ± 0.21 | 42 ± 1 | 1.6 | 1.0 |
| DC100 | B | 1.00 ± 0.02 | 47 ± 14 | 1.0 | 1.0 |
| DC108 | B | 1.00 ± 0.03 | 51 ± 9 | 1.0 | 1.0 |
| DC109 | B | 1.01 ± 0.01 | 34 ± 7 | 1.0 | 1.0 |
| DC150 | I | 1.08 ± 0.00 | 16 ± 6 | 1.0 | 1.0 |
| DC161 | B | 1.01 ± 0.02 | 60 ± 16 | 1.8 | 1.0 |
| DC169 | I | 1.07 ± 0.00 | 63 ± 32 | 2.4 | 1.7 |

[a] F, Fluidal, I, intermediate, B, butyrous.
[b] Relative viscosity = flow time (in seconds) of filtrate/flow time of water.
[c] Specific agglutination = titer/milligrams of protein per milliliter.
[d] Average of at least four values.

The mutants that were resistant to bacteriophage K9 were mostly butyrous. The virulence of 13 K9-resistant, butyrous mutants of DC283 was compared with that of 18 K9-sensitive, fluidal, single-colony isolates from the same strain. Respective disease indices on Earliking sweet corn at 10 and 21 days after inoculation were 1.6 ± 0.5 and 1.2 ± 0.2 for the mutant clones and 3.9 ± 0.6 and 4.8 ± 0.2 for the wild-type clones. Similar results also were obtained with nine K9-resistant clones of DC69.

**Correlation between capsulation, agglutination, and virulence.** Of the 22 strains of *E. stewartii* tested for virulence, 13 were highly virulent, their ratings ranging from 3.9 to 5.0 by 27 days after inoculation (Table 4). The culture filtrates of these strains had relative viscosities of 1.17 to 1.64. The specific agglutination values of these strains were 12 or less. The remaining

TABLE 5. *Relative viscosities, specific agglutinations, and virulences on corn (64A × W117) of wild-type strains and EPS-deficient mutants of these strains of E. stewartii*

| Strain no. | Fluidal colony type | | | | Butyrous colony type | | | |
|---|---|---|---|---|---|---|---|---|
| | Relative viscosity (avg ± SD[a])[b] | Specific agglutination (avg ± SD)[c] | Disease index[d] at: 8 days | Disease index[d] at: 21 days | Relative viscosity (avg ± SD) | Specific agglutination (avg ± SD) | Disease index[d] at: 8 days | Disease index[d] at: 21 days |
| DC2 | 1.59 ± 0.10 | 5 ± 2 | 4.3 | 5.0 | 1.00 ± 0.01 | 80 ± 28 | 2.4 | 1.0 |
| DC11 | 1.64 ± 0.16 | 6 ± 4 | 3.8 | 3.4 | 1.00 ± 0.01 | 65 ± 14 | 2.4 | 1.0 |
| DC18 | 1.27 ± 0.17 | 6 ± 1 | 4.5 | 5.0 | 1.04 ± 0.05 | 47 ± 13 | 2.5 | 1.0 |
| DC19 | 1.56 ± 0.00 | 8 | 4.8 | 5.0 | 1.00 ± 0.02 | 78 ± 34 | 3.0 | 1.0 |
| DC45 | 1.30 ± 0.09 | 5 ± 1 | 3.8 | 4.6 | 1.00 ± 0.02 | 54 ± 8 | 2.9 | 1.0 |
| DC70 | 1.37 ± 0.00 | 5 ± 3 | 4.3 | 4.6 | 1.01 ± 0.02 | 117 ± 0 | 2.0 | 1.0 |
| DC168 | 1.36 ± 0.00 | 5 ± 1 | 4.8 | 5.0 | 1.01 ± 0.01 | 15 ± 0 | 3.8 | 1.7 |
| DC170 | 1.17 ± 0.04 | 5 ± 0 | 3.8 | 5.0 | 1.00 ± 0.04 | 58 ± 0 | 2.3 | 1.0 |
| Mean ± SD | 1.41 ± 0.17 | 6 ± 1 | 4.3 ± 0.4 | 4.7 ± 0.6 | 1.01 ± 0.01 | 64 ± 29 | 2.7 ± 0.6 | 1.1 ± 0.2 |

[a] SD, Standard deviation.
[b] Relative viscosity = flow time (in seconds) of filtrate/flow time of water.
[c] Specific agglutination = titer/milligrams of protein per milliliter.
[d] Average of at least four values.

nine strains were avirulent or weakly avirulent and had disease indices of 1.7 or less after 27 days (Table 4). Seven of these strains were butyrous or intermediate colony types and had specific agglutination values of 16 or greater. Culture filtrates had low viscosity indices (1.00 to 1.21). Only two avirulent strains, DC159 and DC20, had fluidal colony types (viscosity indices, 1.48 to 1.59) and had specific agglutination values of 9 or less. When fluidal strains were compared with their corresponding butyrous mutants (Table 5), the mean viscosity, agglutination, and disease indices showed highly significant ($P = 0.0001$) differences.

Relative viscosity indices were correlated with colony type; that is, butyrous strains had low viscosity indices (1.00 to 1.04), whereas fluidal strains had high indices (1.17 to 1.64). Viscosity indices also were correlated directly with virulence ($r^2$ = 29% after 8 days and 32% after 27 days), with the exception of the two avirulent fluidal strains (DC159 and DC20) (Table 4).

Specific agglutination values were correlated negatively with disease indices ($r^2$ = 34% after 8 days and 48% after 27 days [Table 4]; $r^2$ = 78% after 8 days and 71% after 21 days (Table 5]). Strains with high specific agglutination values (15 or higher) were avirulent or weakly virulent, whereas those with low specific agglutination values (10 or less) were highly virulent, with the exception of DC159 and DC20, which were avirulent.

Specific agglutination values and relative viscosity indices were negatively correlated ($r^2$ = 58% [Table 4], 53% [Table 5]). In general, strains with high specific agglutination values had low relative viscosity indices; strains with low specific agglutinations had high relative viscosity indices.

Bacterial cells from four butyrous strains were tested for specific agglutination before and after washing in buffer; no significant differences were found ($P = 0.0865$). In contrast, washed cells of six fluidal strains of *E. stewartii* gave significantly higher specific agglutination values than did unwashed cells ($P = 0.0286$) (Table 6).

**Analysis of the LPS of virulent and avirulent strains.** The major sugar components of the LPS of all strains of *E. stewartii* were galactose, glucose, and heptose. No differences in sugar composition, either qualitative or quantitative, could be correlated with either virulence or agglutination (Table 7). When LPS samples were electrophoresed and stained by the Schiff-periodate method, all showed one major, fast-

TABLE 6. *Differences in specific agglutination between unwashed and buffer-washed cells from 10 strains of Erwinia stewartii*

| Strain no. | Colony type[a] | Specific agglutination[b]: Unwashed cells[c] | Specific agglutination[b]: Washed cells[d] |
|---|---|---|---|
| DC100 | B | 48 | 117 |
| DC108 | B | 58 | 58 |
| DC161 | B | 58 | 117 |
| DC109 | B | 29 | 58 |
| DC13 | F | 10 | 29 |
| DC18 | F | 7 | 29 |
| DC36 | F | 12 | 29 |
| DC39 | F | 7 | 7 |
| DC65 | F | 15 | 29 |
| DC170 | F | 7 | 7 |

[a] F, Fluidal; B, butyrous.
[b] Specific agglutination = titer/milligrams of protein per milliliter.
[c] Average of three replicates.
[d] Average of two replicates.

TABLE 7. *Sugars in the LPS of virulent and avirulent strains of E. stewartii*

| Strain no. | Colony type[a] | Virulence | Agglutination with corn agglutinin[b] | Identified sugars (%) | | | | Appearance of LPS on gels[c] |
|---|---|---|---|---|---|---|---|---|
| | | | | Arabinose | Galactose | Glucose | Heptose | |
| DC18 | F | + | − | 7 | 20 | 30 | 18 | R |
| DC19 | F | + | − | 5 | 20 | 31 | 22 | R |
| DC39 | F | + | − | 1 | 23 | 54 | 6 | S & R |
| DC51 | F | + | − | | 19 | 33 | 14 | R |
| DC110 | F | + | − | 7 | 27 | 23 | 12 | S & R |
| DC100 | B | − | + | 13 | 13 | 28 | 16 | R |
| DC161 | B | − | + | 5 | 16 | 18 | 26 | R |
| DC108 | B | − | + | 2 | 18 | 27 | 21 | R |
| DC109 | B | − | + | 9 | 11 | 27 | 16 | R |

[a] F, Fluidal; B, butyrous.
[b] +, Specific agglutination of ≥30; −, specific agglutination of ≤10.
[c] R, Rough, or a fast-moving band; S, smooth, or a slow-moving band.

moving band that corresponded to rough LPS in other bacteria (10). Two strains (DC39 and DC110) had an additional slow-moving band that may have represented smooth LPS. However, the presence of this extra band also was not correlated with either agglutination or virulence.

## DISCUSSION

Colony type has been correlated with virulence in *E. stewartii* previously (9, 19). Our studies have extended these early observations. In addition, we have shown that the ability of this pathogen to cause systemic wilting and stunting is associated with slime production in culture, the presence of an organized capsule, and the inability to interact with corn agglutinin. EPS-deficient mutants were able to cause local lesions, but did not spread from inoculated leaves. Significantly, no virulent strains were found that either lacked EPS or were agglutinated by corn agglutinin, although two strains were avirulent and fluidal. These strains, however, may lack factors other than EPS that are required for virulence. Similarly, the complete avirulence of strains DC100, DC108, DC109, and DC150, which have been maintained in culture for many years, may be due to mutations that affect virulence in other ways.

Differences between the agglutinations of avirulent and virulent strains of *P. solanacearum* with potato lectin have been related to differences in LPS structure (22). However, the virulent and avirulent strains of *E. stewartii* tested in this study were similar in LPS composition. Avirulent strains of *E. stewartii* that were agglutinated most strongly by the corn agglutinin also lacked an organized capsule or slime (EPS). Therefore, the agglutinin must be interacting with other cell surface components. The presence of an organized capsule in the virulent strains of *E. stewartii* appeared to interfere with agglutination.

We have presented evidence that EPS production is necessary for full virulence of *E. stewartii* and that a corn agglutinin can be used to differentiate between encapsulated and nonencapsulated strains. Although the primary role of EPS in pathogenesis is assumed to be the plugging of xylem vessels, EPS may also enhance the ability of the bacteria to spread throughout the plant without becoming agglutinated. It is not known whether the reduced virulence of nonencapsulated strains is a direct result of their binding to corn agglutinin. However, it is possible that avirulent bacteria may be immobilized by an LPS-agglutinin interaction in the plant and that this binding can be prevented by EPS. Further studies are needed to determine whether the binding of the corn agglutinin to LPS is sugar specific or ionic in nature, whether this interaction is inhibited by EPS, and whether other agglutinins or lectins exist in resistant cultivars of corn.

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