Comparison of Three Serodiagnostic Assays for Detection of Corynebacterium sepedonicum

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ABSTRACT

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The sensitivity and specificity of an antiserum prepared against glutaraldehyde-fixed cells of *Corynebacterium sepedonicum* were evaluated in agglutination (AG), Ouchterlony double diffusion (DD), and indirect fluorescent antibody stain (IFAS) tests. Test sensitivity was about 2 $\times 10^7$ cells/ml for AG and DD, and 10^1–10^2 cells/ml for IFAS. A cell concentration of 10^2 cells/ml also could be detected by the Gram stain procedure. The ring rot bacterium was detected in stem smears from infected eggplant, potato, and tomato plants by DD and IFAS, and in infected potato tubers by AG, DD, and IFAS. Eighteen cultures of *C. sepedonicum* from diverse geographic origins in North America gave positive reactions with the antiserum. Cross-reactivity was observed by AG but not by DD or IFAS in comparisons with five other species of phytopathogenic corynebacteria that do not attack potato. The AG, DD, and IFAS tests with strains of *Erwinia carotovora* var. *atroseptica*, *E. carotovora* var. *carotovora*, *Pseudomonas solanacearum*, *Bacillus* spp., and *Clostridium* spp. were negative. Sensitivity and specificity data indicate that serodiagnostic procedures, particularly IFAS, are valuable for ring rot diagnosis.

Additional key words: bacterial ring rot, agglutination, immunodiffusion, immunofluorescence, serology.

Approximately 60% of the total seed potato acreage that is rejected annually for certification in North America involves bacterial ring rot of potato (*Solanum tuberosum* L.) incited by *Corynebacterium sepedonicum* (Spieck. & Kotth.) Skapt. & Burkh. (19). Bacterial ring rot is the only disease for which all potato seed certification agencies in North America will reject a seed lot because a single infected plant is in a seed field (12). This rule, which partially accounts for the large percentage of rejections, became necessary because widespread infection frequently resulted from planting seed obtained from a field in which only a trace of disease was evident the previous year (2,5,6). In North America, production of seed potatoes under the "zero tolerance" regulation and close attention to sanitation practices have minimized losses from bacterial ring rot but have not eliminated the disease.

When bacterial ring rot is detected in a field, the economic impact on a seed potato producer is considerable; thus, accurate diagnosis is essential. Currently, diagnosis is based on foliar or tuber symptoms or both and generally is confirmed by a Gram stain test. Although certain species in the genus *Corynebacterium* are the primary Gram-positive bacteria that are considered pathogens of growing plants, other Gram-positive bacteria have been observed as endophytic flora in apparently healthy potato plants (4), and some clostridia have been implicated in the decay of potato tubers or as secondary organisms (13,14). The validity of the diagnosis therefore depends on the investigator's familiarity with the disease and the pathogen.

Rapid and specific serologic procedures for certain organisms have been evaluated and used effectively for studies on bacterial plant pathogens (1,15,17,23). Serologic studies on *C. sepedonicum* based on agglutination and immunodiffusion procedures have been reported previously (3,8,9,11,16,21,22). This investigation was initiated to compare the sensitivity and specificity of agglutination, immunodiffusion, and immunofluorescence tests with an antiserum produced against glutaraldehyde-fixed cells of *C. sepedonicum* and to determine the reliability of these tests for detecting the bacterium in infected plant parts. A preliminary report was presented (20).

MATERIALS AND METHODS

Sources and maintenance of bacterial isolates. Strain SC-3 of *C. sepedonicum* originally was isolated from potato (*S. tuberosum* L.) in Wisconsin. The following individuals or institutions provided one or more cultures of *C. sepedonicum* from the indicated geographic areas: American Type Culture Collection (ATCC) 9850 (Burkholder CS-5); R. J. Copeman—Washington and Alberta and British Columbia, Canada; D. Gross—California and Montana; D. Hammond—Maine; R. Lachance—Quebec, Canada; M. Lai—California; F. Manzer—Maine (infected tubers); and R. McKenzie—New Brunswick, Canada. D. Gross also provided *C. flaccumfaciens*, *C. flaccumfaciens* var. *aurantiacum*, *C. flaccumfaciens* var. *violaceum*, *C. fascians*, *C. insidiosum*, *C. michiganense*, and *C. poinsettiae*. R. S. Hanson provided *Bacillus brevis*, *B. cereus*, *B. circulans*, *B. megaterium*, and *B. polymyxa*. *Clostridium* spp. were obtained from rotting potatoes and *Erwinia carotovora* var. *carotovora* (SR-53), *E. carotovora* var. *atroseptica* (SR-8), and *Pseudomonas solanacearum* (K60) were selected from the culture collections of A. Kelman and L. Sequeira.

All bacteria were grown on nutrient dextrose agar (NDA: 0.3% beef extract, 1.0% peptone, 1.0% dextrose, and 1.8% agar) at 24 or 28 C unless otherwise specified. *Corynebacterium* spp. also were grown on Difco tryptic soy agar (TSA) or yeast-glucose-calcium carbonate agar (YGC: 1.0% yeast extract, 2.0% dextrose, 2.0% calcium carbonate, and 2.0% agar) in some experiments. Cultures were stored on NDA or NA (NDA without dextrose) at 4 C.

Antiserum production. *C. sepedonicum* (SC-3) was grown for 5 days on TSA plates. Cells were washed from the plates with 0.01 M phosphate (pH 7.2) plus 0.85% sodium chloride (PBS). Cells were washed three times by centrifuging at 12,000 $\times g$ for 15 min and resuspending in PBS. Washed cells were dialyzed overnight against 2% glutaraldehyde in PBS and then extensively dialyzed against PBS alone at 4 C. After an initial bleeding to obtain normal serum,

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two rabbits were given intramuscular injections in the hind leg at weekly intervals for 5 wk. The first four injections consisted of 0.75 ml of bacterial suspension ($1–2 \times 10^9$ cells/ml) emulsified 1:1 (v/v) with Freund's incomplete adjuvant, and the fifth injection consisted of 1 ml of bacterial suspension (5×10^9 cells/ml) emulsified 1:1 (v/v) with adjuvant. Three weeks after the fifth injection, a booster injection of 1 ml of bacterial suspension (2×10^9 cells/ml) emulsified 1:1 (v/v) with adjuvant was given. Cell concentrations were adjusted spectrophotometrically at A_{600nm} = 0.1, equivalent to 2×10^8 cells/ml. Animals were bled at weekly intervals starting 14 days after the initial injection. Antiserum titers were determined by making twofold serum dilutions and adding an equal volume of the bacterial suspension (2×10^8 cells/ml) to each dilution. The titer was the highest dilution at which agglutination could be detected after incubating at 4 C for 24 hr. Sera were stored at −20 C.

Serologic tests. Agglutination tests (AG) were performed as described by Claflin and Shepard (3) or by suspending washed or unwashed antigen cells in PBS, incubating for 2 hr at room temperature and 24 hr at 4 C, and determining the amount of agglutination with a stereomicroscope. Reactants were mixed with a gentle airstream or a fine glass rod.

The Ouchterlony double diffusion test (DD) was as described by Allan and Kelman (1). Gels consisted of 0.7% agarose (Oxoid; Consolidated Laboratories, Inc., Chicago Heights, IL 60411) in 0.05 M Tris (pH 7.2) plus 0.85% NaCl (TBS), or PBS plus 0.1% NaN_3. Bacterial cells from cultures were suspended in TBS or PBS ($>10^8$ cells/ml); only TBS was used when plant materials were tested. After squeezing sap from severed eggplant, potato, or tomato stems or excising vascular tissue from potato tubers, samples were collected in small vials containing 0.1–0.2 ml TBS plus 5% NaN_3. Prior to charging antigen wells, the samples were mixed on a Vortex Genie Mixer (Scientific Products, McGraw Park, IL 60085) for 2 min at high speed with one-half volume of glass beads (0.17–0.18 mm diameter).

The direct fluorescent antibody stain (DFAS) procedure as described by Allan and Kelman (1) and an indirect fluorescent antibody stain (IFAS) procedure described below were followed. A droplet from bacterial suspension ($>10^8$ cells/ml) or a smear from a plant stem or a potato tuber was air-dried and heat-fixed on a slide. A droplet of ammonium sulfate-fractionated antiserum (ASF) was spread over the sample with a sterile toothpick, and the slide was incubated in a darkened moist chamber for 30 min. The slide was rinsed in PBS for 10 min, rinsed briefly in glass-distilled water and then blotted dry. A droplet of fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit serum (Baltimore Biological Laboratories, Baltimore, MD 21030) was then spread over the sample, and the slide was incubated for another 30 min in a darkened moist chamber. Slides were rinsed as before and mounted with a droplet of 0.02 M phosphate (pH 7.6) in 90% glycerol and a No. 1 coverslip. Slides were examined by epi-illumination as previously described (1), except that an oil-immersion objective was used.

Pathogenicity and morphology. Pathogenicity of *C. sepedonicum* cultures was tested on eggplant (*Solanum melongena* L. 'Black Beauty'), potato 'Russet Burbank', or tomato (*Lycopersicon esculentum* Mill. 'Bonny Best') (10). Young plants were inoculated immediately below the first true leaf by introducing bacteria with a sterile presharpened toothpick into a 1-cm longitudinal slit made with a razor blade. Desiccation was prevented by covering the inoculation point with petrolatum. Potato tubers were inoculated by rubbing freshly cut surfaces with those of infected tubers before planting. Plants were grown in a greenhouse at about 24 C. Supplemental fluorescent lighting maintained a 16-hr day length. Cell morphology was observed after staining by the Gram stain protocol recommended by Glick et al (7).

TABLE 1. Titers of antisera from rabbits injected with glutaraldehyde-fixed *Corynebacterium sepedonicum* cells[a]

Rabbit	Titer at weeks after initial injection[b]							
	2	3	4	5	6	7	8–9	10–11
A	2	4	8	128	64	512	4,064	16,256
B	4	16	128	2,048	2,048	2,048	16,256	8,128

[a] Cells were dialyzed against 2% glutaraldehyde overnight at 4 C before extensive dialysis against phosphate sodium chloride (PBS) to remove excess glutaraldehyde. Antigen concentration for agglutination tests = 2×10^8 cells/ml or A_{600} = 0.1 in PBS.

[b] Bleedings were started 14 days after initial injection and continued at 7-day intervals. Weekly injections for 5 wk were followed by a booster injection the eighth week.

RESULTS

Specificity of antiserum. Antiserum titers as high as 16,256 were produced in rabbits injected with glutaraldehyde-fixed whole cells of *C. sepedonicum*, strain SC-3 (Table 1). Strong agglutination was not observed beyond a titer of 128, however. In addition to AG, the antisera were suitable for DD and IFAS. Repeated attempts were made to label the antisera directly with FITC. Although a specific fluorescent product was obtained, the intensity of the fluorescence in DFAS tests was weak compared with that in IFAS tests. Primary emphasis was therefore placed on the IFAS procedure.

Positive reactions were obtained in AG, DD, and IFAS tests with 18 strains of *C. sepedonicum* obtained from diverse geographic regions of Canada and the United States. No differences were noted among strains in these tests (Fig. 1B). In AG and DD tests, either crude or ASF antisera were satisfactory.

No reaction was noted in DD and IFAS tests when 10 *Corynebacterium* strains representing seven additional species or varieties were tested (Fig. 1A); however, in AG tests, cross-reactions were obtained. Several factors that may affect AG specificity were evaluated as follows: (i) bacteria were grown on

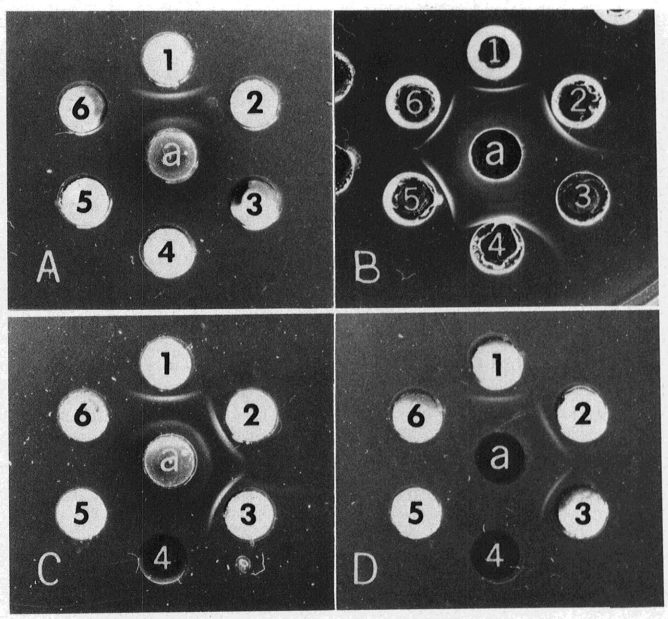

Fig. 1. Double diffusion test demonstrating precipitin bands after 48 hr (A, C, and D) and about 1 wk (B). Central wells (a) were charged with undiluted crude antiserum (A–C) or the γG fraction of the antiserum (D). Antigen wells in A–D contained (1) *Corynebacterium sepedonicum* strain SC-3. In A, wells contained (2) *C. flaccumfaciens*, (3) *C. flaccumfaciens* var. *aurantiacum*, (4) *C. fascians*, (5) *C. michiganense*, and (6) *C. poinsettiae*; in B, wells contained (2) *C. sepedonicum* strain ATCC 9850 (Burkholder CS-5), (3) *Shigella flexneri* serotype 6 Newcastle, (4) *C. sepedonicum* strain ATCC 9850 (Burkholder CS-5), (5) *C. sepedonicum* strain from California, and (6) *C. sepedonicum* strain from Montana; and in C and D, wells contained (2) *C. sepedonicum* strain from Montana, (3) *C. sepedonicum* strain ATCC 9850 (Burkholder CS-5), (4) *E. carotovora* var. *atroseptica* strain SR-8, (5) *E. carotovora* var. *carotovora* strain SR-53, and (6) *P. solanacearum* strain K60. Gels were 0.7% agarose buffered with 0.05 M Tris (pH 7.2) containing 0.85% NaCl and 0.1% NaN_3. Before charging wells, antigens were mixed for 2 min at top speed in the presence of one-half volume of glass beads (0.17–0.18 mm diam).

YGC rather than NDA or TSA, (ii) bacteria were washed with PBS three times before testing, (iii) bacteria were suspended in 0.85% NaCl rather than PBS, (iv) observations were made during a span of 30 min to 2 hr at room temperature rather than after 24 hr at 4 C, (v) a cell suspension with $A_{640} = 0.15$ rather than $A_{600} = 0.10$ (2×10^8 cells/ml was used), and (vi) ASF antiserum rather than crude antiserum was used. Although varying these factors eliminated some heterologous reactions, other heterologous reactions remained. For species other than *C. sepedonicum*, clumping of cells in normal serum also was observed commonly.

No positive reactions were detected by IFAS or DD with *Bacillus* spp., *Clostridium* spp., *E. carotovora* var. *atroseptica* (SR-8), *E. carotovora* var. *carotovora* (SR-53), or *P. solanacearum* (K60) (Fig. 1C and D). Slight agglutination was noted only in some tests with *P. solanacearum*, but the reactions were quite variable and this bacterium is known to clump nonspecifically in salt solutions (L. Sequeira, *personal communication*). In DFAS tests with antiserum for *E. carotovora* var. *atroseptica* (1), *C. sepedonicum* (SC-3) did not fluoresce.

Sensitivity of tests. Of the tests used for detecting *C. sepedonicum*, the Gram stain and IFAS tests were more sensitive than the AG or DD tests (Table 2). This higher level of sensitivity may not be essential to confirm diagnostic symptoms because the AG and DD tests were sensitive enough to detect the bacterium in visibly infected plant parts. Because *Bacillus* spp. can comprise a significant portion of the endophytic flora in potato stems and tubers (4), assays also were performed in the presence of *B. polymyxa*. Assay sensitivity was not appreciably affected by the addition of approximately 10^5 cells/ml of *B. polymyxa* or by making dilutions in a potato tuber extract rather than saline.

We attempted to increase DD sensitivity by disrupting cell integrity. Treatment with 30% pyridine or 2.5% pyrrolidine (18) did not enhance sensitivity, but mixing cells with glass beads for 2 min (1) was effective. Cell breakage was determined by estimating the number of cells in a solution before and after each treatment with the Gram stain procedure. Cell numbers did not visibly decrease within 1–2 min of chemical treatment or after 1 min of mixing with glass beads, but only 25–50% of the cells remained intact after 2 min of the latter treatment. Precipitin bands developed in the same relative positions for untreated cells ($\geq 1 \times 10^9$ cells/ml) as treated cells. A second diffuse precipitin band adjacent to the antiserum well was not visible in tests with ASF antiserum (Fig. 1C and D).

Fixation of *C. sepedonicum* cells with 2% glutaraldehyde prior to use in AG tests did not alter the antiserum titers. At lower antiserum dilutions, however, the amount of agglutination observed was slightly greater after fixation.

Detection in potato stems. To determine efficacy of procedures to detect *C. sepedonicum* in potato stems, plants were inoculated with four strains of the bacterium (ATCC 9850, Montana, New Brunswick, and Wisconsin). Also, tubers were inoculated by contact with infected tubers received from Maine. Samples were collected before symptom development or as initial symptoms were observed (Table 3). Each stem was cut with a new razor blade approximately 10 cm above the soil line and about 5 cm above stem inoculation sites. The ring rot bacterium was detected in the greatest number of samples with the Gram and IFAS tests. Bacterial cells could be detected readily in the lower stems of many inoculated plants before symptoms developed. Gram stain and IFAS tests also were made near the stem apex for the six plants showing symptoms, and cells of *C. sepedonicum* were observed in all cases. Because the DD test is less sensitive than the Gram stain and IFAS tests, the fewer positive tests obtained by the former procedure might be expected. A positive DD test was recorded, however, for each of the six plants showing symptoms. The sensitivity of the serologic tests was not affected by the source of *C. sepedonicum* strain. Four uninoculated plants maintained as controls were negative in all tests.

Detection in potato tubers. The bacterium was detected readily by all procedures when the milky ooze from freshly cut infected tubers was sampled. Nonspecific reactions were not observed in these tests. In one test, two ring rot-infected tubers were each tested at six sites by the Gram stain, DD, and IFAS. All tests were positive. Tests were negative when applied to randomly selected "healthy" tubers of the cultivars Monona, Russet Burbank, Ontario, Superior, Red LaSoda, and Norgold Russet. No reactions were observed in duplicate samples with normal serum as a control. In another test, two tubers each that were either healthy, exhibiting soft rot breakdown, or infected with *C. sepedonicum* were collected. In AG and DD tests, positive reactions were obtained only for the two tubers infected by *C. sepidonicum*.. A positive DD test was obtained with and without mixing the bacteria-tissue suspension with glass beads. No reactions were observed in duplicate samples with normal serum as a control.

Detection in field samples. During the summer of 1977, duplicate slides were smeared and sap was collected in a vial containing TBS-NaN_3 for each of three plants from separate fields after an experienced potato inspector diagnosed ring rot by the symptoms. One slide was used for a Gram stain and the other for IFAS, and the sap solution was used for DD tests. In each case, the field diagnosis was confirmed. The IFAS procedure could be used after a Gram stain, but the amount of autofluorescence due to accumulation of salts and dyes considerably reduced the IFAS contrast. Therefore, the use of one slide for both the Gram stain and IFAS probably would be impractical.

Detection in eggplant and tomato plants. Cells of *C. sepedonicum* could be detected also in eggplant and tomato plants by the Gram stain, DD, and IFAS (Table 4). The four bacterial ring rot strains used to inoculate plants were ATCC 9850 and those from California, Montana, and Wisconsin. After symptoms developed, plants were sampled by cutting the stem about 5 cm above the inoculation site. All four strains of *C. sepedonicum* were

TABLE 2. Minimum number of *Cornebacterium sepedonicum* cells detected by the Gram stain, agglutination (AG), Ouchterlony double diffusion (DD), and indirect fluorescent antibody stain (IFAS) procedures alone or in mixtures with *Bacillus polymyxa*

Bacterium	Test sensitivity (cells/ml)[a]			
	Gram	AG	DD	IFAS
C. sepedonicum	1×10^2	2×10^7	2×10^7	$1 \times 10-10^2$
C. sepedonicum + *B. polymyxa*[b]	1×10^3	NT[c]	2×10^7	1×10^3

[a] All cells initially suspended in 0.85% sodium chloride. Cell number indicates last cell concentration of *C. sepedonicum* detectable in a tenfold dilution series.

[b] Cell concentration of *B. polymyxa* held constant at about 10^5 cells/ml. Dilutions of bacterial suspensions made in potato tuber extract (3:1 v/v). Extract made by blending tubers in 0.85% sodium chloride (1:1 w/v) and filtering through Whatman No. 1 filter paper.

[c] NT = not tested.

TABLE 3. Number of potato plants in which *Corynebacterium sepedonicum* could be detected by the Gram stain, Ouchterlony double diffusion (DD), and indirect fluorescent antibody stain (IFAS) procedures

Plant Part	Number of plants assayed by:			
	Symptoms[a]	Gram	DD	IFAS
Stem[b]	0/16[c]	8/16	5/16	9/16
Tuber[d]				
Infected	2/3	3/3	2/3	3/3
Inoculated	4/18	18/18	18/18	18/18
Totals	6/37	29/37	25/37[e]	30/37

[a] Symptoms were determined and samples collected 55 days after planting.

[b] The lower stem of each plant was inoculated with the indicated *C. sepedonicum* isolate 23 days after planting. Four *C. sepedonicum* strains were used to inoculate plants (four plants/strain).

[c] Numerator is number of plants positive and denominator is number of plants tested.

[d] Before planting, freshly cut surfaces of healthy and previously injected tubers were rubbed together.

[e] A positive DD test was obtained for each plant showing symptoms; for every positive obtained by DD, the Gram and IFAS tests also were positive.

detected in infected eggplants and tomatoes by the Gram stain, IFAS, and DD tests. In the DD test with tomato sap as the antigen, a nonspecific precipitin band appeared midway between the antiserum and antigen wells in several tests. The nonspecific band did not interfere with observations of specific precipitates and could be identified easily because it also developed in duplicate normal serum controls. This band was not observed in DD tests with eggplant or potato sap.

DISCUSSION

A highly specific antiserum was produced against glutaraldehyde-fixed cells of *C. sepedonicum*. Positive reactions were obtained by all three test procedures with the 18 strains of *C. sepedonicum* tested, and the ring rot bacterium could be detected reliably in infected plant parts. Cross-reactions were not observed by the serologic tests with selected noncoryneform bacteria or by DD or IFAS tests with five other species of phytopathogenic corynebacteria. An interesting feature of the DD tests was that reactions of identity always were observed between *C. sepedonicum* strains placed in adjacent wells. However, we observed cross-reactions by AG with the other species of corynebacteria.

The DFAS procedure was not used because the fluorescence lacked the desired intensity. The low intensity with DFAS in this study, in contrast with results with *E. carotovora* var. *atroseptica* (1) may be attributable to the small size of the ring rot bacterium cell (0.5–1.0 × 1.0–3.0 μm vs. 0.4–0.6 × 0.8–1.2 μm), which resulted in reduced contrast.

Before serodiagnosis of bacterial ring rot can be used confidently as a routine assay, resolution of certain problems may be needed. For example, nonpathogenic, endophytic coryneform bacteria that are morphologically indistinguishable from *C. sepedonicum* have been found in apparently healthy potato plants (4). If these coryneform bacteria and *C. sepedonicum* have common antigenic determinants, cross-reactions could lead to incorrect conclusions. Cross-reactions were not observed, however, by Claflin and Shepard (3) in AG tests with several *Corynebacterium* spp. with an antiserum prepared against a Montana strain of *C. sepedonicum*. Earlier studies reported variable results in cross-reactivity tests with *Corynebacterium* spp., but *C. sepedonicum* generally was considered to be distinct (9,11,16,21). These studies indicate that the bacterial strain, method of antigen preparation, and serologic procedure may affect test specificity.

Another problem may be the detection of *C. sepedonicum* in badly decayed potato tuber samples. Mixed populations of bacteria are invariably encountered with these specimens and diagnosis is generally difficult. A sensitive test such as the IFAS procedure probably would be desirable in such cases. Although we did not encounter severe problems with autofluorescence, counterstaining (eg, with gelatin conjugated with rhodamine β-isothiocyanate) such samples may be necessary in order to use the IFAS procedure.

At present, ring rot diagnoses rely mainly on symptom development and the Gram stain reaction, which lacks specificity. Our data indicate that the confidence level in diagnoses can be increased significantly by serologic assays. Serologic testing for *C. sepedonicum*, particularly IFAS, can be used to complement current diagnostic procedures and to aid in ecological studies.

TABLE 4. Number of infected eggplant and tomato plants in which *Corynebacterium sepedonicum* could be detected by the Gram stain, indirect fluorescent antibody stain (IFAS), and Ouchterlony double diffusion (DD) procedures

	Number of plants assayed at:				
	29 days[a]		41 days[a]		
Host	Symptoms	DD	Gram	IFAS	DD
Eggplant	15/15[b]	5/5	10/10	10/10	8/10
Tomato	15/16	4/4	12/12	12/12	10/12
Totals	30/31	9/9	22/22	22/22	18/22

[a]Number of days after inoculation. Four *C. sepedonicum* strains were used to inoculate eggplants and tomatoes (three to four plants of each host/strain).

[b]Numerator is number of plants positive and denominator is number of plants tested.

LITERATURE CITED

1. ALLAN, E., and A. KELMAN. 1977. Immunofluorescent stain procedures for detection and identification of Erwinia carotovora var. atroseptica. Phytopathology 67:1305-1312.
2. BONDE, R. 1939. Bacterial wilt and soft-rot of the potato. Am. Potato J. 16:109-114.
3. CLAFLIN, L. E., and J. F. SHEPARD. 1977. An agglutination test for the serodiagnosis of Corynebacterium sepedonicum. Am. Potato J. 54:331-338.
4. DE BOER, S. H., and R. J. COPEMAN. 1974. Endophytic bacterial flora in Solanum tuberosum and its significance in bacterial ring rot diagnosis. Can. J. Plant Sci. 54:115-122.
5. DYKSTRA, T. P. 1941. Results of experiments in control of bacterial ring rot of potatoes in 1940. Am. Potato J. 18:27-55.
6. EDDINS, A. H. 1939. Some characteristics of bacterial ring rot of potatoes. Am. Potato J. 16:309-322.
7. GLICK, D. P., P. A. ARK, and H. N. RACICOT. 1944. Outline of procedure for the diagnosis of bacterial ring rot of potatoes—Report of the committee of the Potato Association of America. Am. Potato J. 21:311-314.
8. HARRISON, D. E., and H. FREEMAN. 1961. Bacterial wilt of potatoes. II. Serological relationship of two strains of Pseudomonas solanacearum and a culture of Corynebacterium sepedonicum. Aust. J. Agric. Res. 12:872-877.
9. KATZNELSON, H., and M. D. SUTTON. 1956. Laboratory detection of Corynebacterium sepedonicum, causal agent of bacterial ring rot of potatoes. Can. J. Bot. 34:48-53.
10. LARSON, R. H. 1944. The ring rot bacterium in relation to tomato and eggplant. J. Agric. Res. 69:309-325.
11. LAZAR, I. 1968. Serological relationships of corynebacteria. J. Gen. Microbiol. 52:77-88.
12. LEACH, J. G., T. P. DYKSTRA, and R. W. GOSS. 1940. Report of the committee to coordinate research on new and unusual potato diseases. Am. Potato J. 17:81-88.
13. LUND, B. M. 1972. Isolation of pectolytic clostridia from potatoes. J. Appl. Bacteriol. 35:609-614.
14. LUND, B. M., and A. KELMAN. 1977. Determination of the potential for development of bacterial soft rot of potatoes. Am. Potato J. 54:211-225.
15. LUCAS, L. T., and R. G. GROGAN. 1969. Serological variation and identification of Pseudomonas lachrymans and other phytopathogenic Pseudomonas nomenspecies. Phytopathology 59:1908-1912.
16. ROSENTHAL, S. A., and C. D. COX. 1954. An antigenic analysis of some plant and soil corynebacteria. Phytopathology 44:603-604.
17. SCHAAD, N. W. 1978. Use of direct and indirect immunofluorescence tests for identification of Xanthomonas campestris. Phytopathology 68:249-252.
18. SHEPARD, J. F. 1972. Gel-diffusion methods for the serological detection of potato viruses X, S and M. Mont. Agric. Exp. Stn. Bull. 662. 72 pp.
19. SHEPARD, J. F., and L. E. CLAFLIN. 1975. Critical analyses of the principles of seed potato certification. Annu. Rev. Phytopathol. 13:271-293.
20. SLACK, S. A., A. KELMAN, and J. B. PERRY. 1978. Evaluation of agglutination, Ouchterlony double diffusion, and indirect fluorescent antibody stain techniques for serodiagnosis of Corynebacterium sepedonicum. (Abstr.) Am. Potato J. 55:395.
21. SNIESZKO, S. F., and R. BONDE. 1943. Studies on the morphology, physiology, serology, longevity and pathogenicity of Corynebacterium sepedonicum. Phytopathology 33:1032-1044.
22. STROBEL, G. A., and P. V. RAI. 1968. A rapid serodiagnostic test for potato ring rot. Plant Dis. Rep. 52:502-504.
23. VRUGGINK, H., and H. P. MAAS GEESTERANUS. 1975. Serological recognition of Erwinia carotovora var. atroseptica, the causal organism of potato blackleg. Potato Res. 18:546-555.