An ELISA Test for Bacterial Ring Rot of Potato with a New Monoclonal Antibody

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ABSTRACT

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A monoclonal antibody was produced to an extracellular soluble fraction of a culture of *Corynebacterium sepedonicum* and used in several serological testing procedures. The antibody reacted with *C. sepedonicum* antigen in enzyme-linked immunosorbent assay (ELISA), immunodiffusion, and latex agglutination, but not in immunofluorescence tests. In ELISA, it reacted with all strains of *C. sepedonicum* tested, but not with any other plant pathogenic corynebacteria. However, it did cross-react with one of 12 unidentified bacteria that had been previously selected from among many strains isolated from potato on the basis of serological cross-reaction with *C. sepedonicum* polyclonal antiserum. The ELISA test applied to potato tubers was highly sensitive, giving positive reactions at greater than 10^{-6} dilution of tissue extracts from ring rot-infected tubers. Furthermore, apparent contamination of healthy tubers from an external source was detected.

Detection and diagnosis of bacterial ring rot of potato (*Corynebacterium sepedonicum* (Spieck. & Kotth.) Skapt. & Burkh. [syn. *Clavibacter michiganense* subsp. *sepedonicum* (Spieck. & Kotth.) Davis et al]) has improved considerably with the advent of monoclonal antibodies for the immunofluorescence test (9,10). This test permits detection of even low populations of the bacterium with a high degree of accuracy. Unfortunately, however, the immunofluorescence procedure is tedious to perform, requires a considerable amount of skill and patience to read carefully, and requires a costly fluorescence microscope. Consequently, a simpler test such as an enzyme-linked immunosorbent assay (ELISA) or a latex agglutination test that can be performed easily on a large number of samples would be useful for diagnostic and testing laboratories.

A latex agglutination test has been described for detection of *C. sepedonicum* (17) and is used routinely in some laboratories, although difficulties with nonspecific reactions have been reported by others. The use of ELISA for ring rot detection and diagnosis has only recently been described (3). The advantages of using ELISA for detecting plant pathogenic bacteria, however, have been recognized for some time (1).

Our monoclonal 9A1 (McAb 5 in De Boer and Wieczorek [10]) against a *C. sepedonicum* cell wall antigen reacted well in immunofluorescence (10), but reacted poorly in ELISA and latex agglutination tests (*unpublished*). However, previous work with polyclonal antisera indicated that *C. sepedonicum* did possess a soluble antigen of diagnostic value that may be better than cell wall antigens in some serological tests (6).

In this study, we produced a monoclonal antibody to a soluble antigen of *C. sepedonicum* and evaluated its usefulness in several serological procedures.

MATERIALS AND METHODS

Bacterial strains. *C. sepedonicum* strain R8, isolated from a potato tuber (cv. Russet Burbank) in 1984, was used as

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the source of antigen preparation for production of monoclonal antibodies. Additional *C. sepedonicum* strains used to test antibody specificity included the same 18 strains used to test previous monoclonals (10), four strains obtained from Europe, and six strains isolated from potato in British Columbia. Antibody specificity was also tested against the same plant pathogenic corynebacteria used previously (10), five additional *C. michiganense* subsp. *insidiosum* strains, four additional *C. michiganense* subsp. *michiganense* strains, and 12 unidentified bacteria isolated from potato stems and tubers, and selected on the basis of cross-reactivity with polyclonal rabbit antiserum prepared to *C. sepedonicum* (4). Bacteria were stored at −80 C with 10% glycerol and were grown on yeast extract/glucose/mineral salts medium (YGM) at 23 C (8).

Purification of immunogen. Cultures of strain R8 were grown on YGM for 7 days and harvested in 0.01 M phosphate-buffered saline (PBS) (pH 7.2), vortexed vigorously for 1 min, and centrifuged at 10,000 *g* for 20 min. The supernatant was collected, mixed with an equal volume of cold acetone, and incubated overnight at −20 C. The resulting precipitate was collected by centrifugation and dissolved in PBS. The concentration of carbohydrate was estimated at 1 mg/ml by the phenol-sulfuric acid assay using glucose as a standard (2). Protein was not detected with the Coomassie Blue microassay procedure. The extract was designated as the antigen preparation.

Monoclonal antibody production. BALB/c mice were immunized subcutaneously with 60 μg of antigen emulsified with Freund's incomplete adjuvant. Four weeks later the mice were given an intraperitoneal booster injection with 100 μg of antigen or whole cells without adjuvant. Two days after the booster, the antibody titer of each mouse was determined by ELISA and the one with the highest titer was used for the fusion experiment the following day. The splenocytes were fused with FOX-NY myeloma cells using 50% polyethylene glycol/10% dimethylsulfoxide as fusogen. Subsequent plating, selection, and cloning of hybridomas were done as described previously (10). Initial screening of monoclonal antibodies was done against antigen preparations from *C. sepedonicum*, *C. michiganense* subsp. *michiganense*, and *C. m.* subsp. *insidiosum*. A stable clone producing monoclonal antibodies that gave a high ELISA value for *C. sepedonicum* antigen in comparison with antigen from the other two bacteria was selected for further study. The isotype of the monoclonal was determined with a mouse hybridoma subisotyping kit. The monoclonal antibody for routine use was produced as ascitic fluid that was clarified by centrifugation and stored at −20 C. Aliquots for regular use were stored in 50% ammonium sulfate at 4 C.

ELISA. For initial screening of monoclonal antibodies, polystyrene 96-well plates were coated with antigen in PBS at 100 μg/ml, or bacterial culture supernatant. Culture supernatants were prepared by suspending bacteria from YGM plates in PBS (3 ml/15 × 100 mm petri dish), vortexing the suspension vigorously for 1 min, and then centrifuging it at 10,000 *g* for 20 min to remove the cells. In one experiment, bacterial cells were bound to 96-well polystyrene plates by centrifugation of plates containing bacterial cells (100 μl/well of cell suspension $OD_{660} = 0.1$) at 2,000 *g* for 15 min, removing the supernatant, and fixing the cells deposited on the bottom of the wells with methanol for 5 min. For tests with plant samples, plates were coated with polyclonal rabbit IgG (antiserum A in De Boer [5]) at 10 μg/ml in 0.1 M carbonate buffer (pH 9.6). Plant samples consisted of about 0.5 g of tissue cut from the stolon ends of tubers and were prepared and diluted in PBS containing 2% BLOTTO (13), 2% polyvinylpyrrolidone, and 0.05% Tween 20 by the same procedures as used previously (9). After incubation with plant samples, plates were blocked for 0.5 hr with 2% BLOTTO in PBS. Undiluted hybridoma culture supernatant was used for initial screening, but in later tests the ascitic fluid/ammonium sulfate solution was used at a 1/1,000 dilution in PBS plus 1% BLOTTO. Goat anti-mouse IgG + IgM/alkaline phosphatase conjugate was used at a 1/3,000 dilution in PBS/BLOTTO. All incubations with antibody were for 1 hr at 37 C. Plates were developed with *p*-nitrophenyl phosphate substrate at 0.5 mg/ml in 1 M diethanolamine buffer (pH 9.8). Substrate conversion was quantified after 0.5 and 1 hr by measuring absorbance at 405 nm with a Titertek Multiskan MC plate reader (Flow Laboratories Inc., Mississauga, Ont, L5T 1A3).

Immunofluorescence and immunodiffusion. For immunofluorescence, the indirect procedure was applied to cells fixed by acetone treatment to multi-well microscope slides as described previously (9). Immunodiffusion was carried out in agar plates (15 ml of 0.8% Difco purified agar, 0.85% of NaCl, and 200 ppm of sodium azide per 15 × 100 mm petri dish) and the presence of precipitin bands was recorded after 24 hr incubation at room temperature (6). Unpurified polyclonal rabbit antiserum (5) was used for reference.

Latex agglutination. To sensitize latex beads, 400 μl polystyrene beads 0.8 μ in diameter were washed twice in 0.05 M glycine/0.03 M NaCl buffer (pH 8.2). The beads were centrifuged at 10,000 *g* for 15 min at 20 C and resuspended in 10 ml glycine/NaCl. A 150 μl aliquot of ascitic fluid was added to the latex bead preparation and stirred gently for 2 hr at room temperature. The beads were again washed twice in glycine/NaCl and finally were suspended in 10 ml 0.27 M glycine/0.15 M NaCl buffer (pH 8.2) with 0.1% (w/v) sodium azide and 0.1% (w/v) bovine serum albumin. Treated beads were stored at 4 C. The latex agglutination test was performed in capillary tubes as described by Khan and Slack (14).

Tuber samples. To test efficacy of ELISA with a monoclonal antibody for detecting ring rot in potato tubers, several tuber lots were analyzed. Ring rot-free tubers were obtained from the Pemberton seed-growing area where the disease has not been detected for more than 15 years. Ring rot-diseased tubers were obtained from a local market gardener where a ring rot outbreak had been confirmed. The ring rot-infected tubers were also used for the latex agglutination tests. Additional tubers

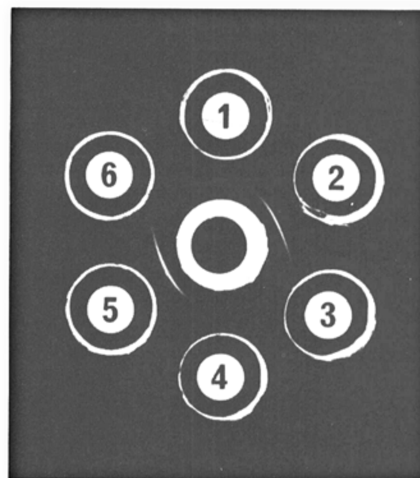

Fig. 1. Reaction of undiluted monoclonal 1H3 ascitic fluid (wells 1 and 4) and undiluted polyclonal antiserum (wells 2 and 5) with *C. sepedonicum* culture supernatant (center well) in immunodiffusion. Wells 3 and 6 are empty.

Table 1. Reaction of various bacteria[a] in ELISA with monoclonal 1H3

Bacteria	No. of strains	Range of ELISA values (A_{405})
Corynebacterium sepedonicum	29	0.428–1.380
Other plant pathogenic corynebacteria	29	0.011–0.082
Cross-reactants[b]	11	0.017–0.077
Strain F[c]	1	0.658
Buffer control	18	0.001–0.008

[a] Supernatant fraction of each bacterium was tested. For coating ELISA plates, supernatants were prepared with phosphate-buffered saline.
[b] Bacteria isolated from potato and selected on the basis of cross-reaction with polyclonal antiserum.
[c] One of the cross-reactants.

with unidentified decay lesions, obtained from a local packaging firm, were also tested. Symptoms on these tubers varied widely, but none resembled ring rot.

RESULTS

After immunization, antisera from all mice had a titer of greater than 1/2,000 in ELISA with antigen from *C. sepedonicum*, *C. m.* subsp. *michiganense*, and *C. m.* subsp. *insidiosum*, but the ELISA absorbance (A_{405}) values were lowest with the *C. sepedonicum* antigen. Initial screening of all hybridomas (>1,000/fusion) in the first three fusion experiments in which purified antigen was used for the booster injection failed to detect any that were producing monoclonal antibodies specific for the *C. sepedonicum* antigen preparation and did not cross-react with those of *C. m.* subsp. *michiganense* and *C. m.* subsp. *insidiosum*. In the fourth fusion experiment in which whole cells had been used for the booster injection, two hybridomas were detected that produced antibodies to the *C. sepedonicum* antigen but did not react with antigen from the other two bacteria. One of these hybridomas was unstable and lost antibody production ability, but the other one appeared to be stable after recloning several times. This hybridoma produced a monoclonal antibody which typed out as an IgM, was designated 1H3, and was used in all further experiments.

In ELISA, monoclonal 1H3 reacted better than monoclonal 9A1. Strain R8 culture supernatant reacted with A_{405} values of 2.460 and 0.209 for 1H3 and 9A1, respectively. Monoclonal 1H3 also reacted better against *C. sepedonicum* culture supernatant (A_{405} = 2.460) than against whole cells fixed onto plates (A_{405} = 1.245). Monoclonal 1H3 did not react with cells in immunofluorescence but did react with a soluble antigen in immunodiffusion, giving a precipitin band that fused completely with the precipitin band produced by the polyclonal antiserum (Fig. 1).

The monoclonal reacted with culture supernatants of all *C. sepedonicum* strains tested, but not with supernatants of other plant pathogenic corynebacteria (Table 1). It did, however, react with one Gram-negative bacterium (strain F) from a group of 12 unidentified bacteria that had been isolated from potato and selected from among a large number of strains on the basis of reaction with polyclonal *C. sepedonicum* antisera (4).

Sensitivity of ELISA for detecting ring rot was determined by testing serial 10-fold dilutions of preparations made from tubers with typical ring rot symptoms. Sample dilutions up to 10^{-6} generally were still positive in ELISA (Fig. 2A). Similarly, the latex agglutination test was positive to 10^{-6} dilution of sample (Fig. 2B).

Tissue samples from ring rot-free tubers obtained from the Pemberton seed-growing area had ELISA readings of <0.05 (Fig. 3A). Similarly, samples from decayed tubers from the local packaging firm had low ELISA values (Fig. 3B). Samples from tubers with ring rot symptoms, in contrast, gave ELISA values generally >0.1 (Fig. 3C). Unexpectedly, when healthy Pemberton tubers had been stored for 1–2 wk in the testing laboratory in sacks separate from, but adjacent to, sacks with ring rot symptoms, some of the previously ring rot-free tubers tested positive in ELISA (Fig. 4A). Also a number of tubers with fungal decay that had been stored near ring rot-infected tubers tested positive (Fig. 4B). Because fruit flies were the most obvious means by which *C. sepedonicum* or the soluble antigen could have been transferred to the previously negative samples, these were also tested by ELISA after grinding them individually or in groups in PBS in a porcelain depression plate. A few of the flies tested positive (Table 2).

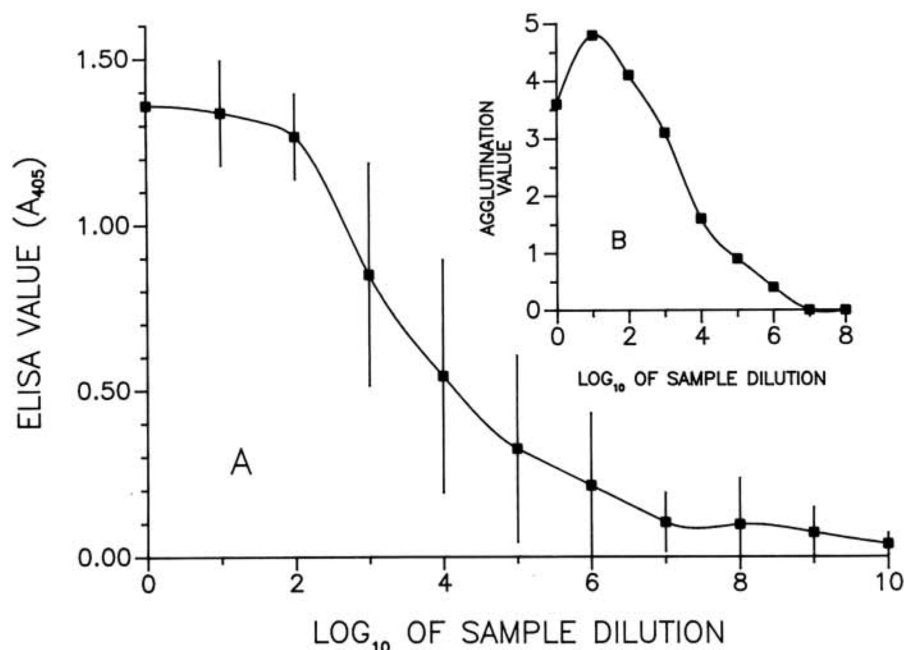

Fig. 2. Reaction of ring rot-infected potato tuber sample dilutions in **(A)** ELISA and **(B)** latex agglutination with monoclonal 1H3. Each ELISA data point represents mean of eight samples tested in duplicate; substrate was developed for 1 hr. Vertical bars give standard deviation. Latex agglutination test was done on the same samples as ELISA. Degree of agglutination was rated on an arbitrary scale of 0–5; 0 = no agglutination, 5 = large floccules and clearing of fluid.

DISCUSSION

The strong reaction of the polyclonal mouse antisera from the test bleedings with *C. m.* subsp. *michiganense* and *C. m.* subsp. *insidiosum* antigen preparations in comparison with the reaction with *C. sepedonicum* was unexpected. These results, however, were analogous to those of Strobel and Rai (18), who produced antiserum against *C. m.* subsp. *michiganense* for a serological test to detect *C. sepedonicum* in bacterial ring rot-infected potatoes. The cross-reactions confirm that there is a strong serological relationship between these two bacteria. Nevertheless, our previous work with polyclonal antisera indicated that *C. sepedonicum* possessed a distinct soluble antigen. The *C. sepedonicum* antiserum formed a precipitin band with *C. sepedonicum* that did not fuse with the precipitin band formed by *C. m.* subsp. *michiganense* (5).

The antigen preparation reacted with the polyclonal antiserum in double diffusion (*data not shown*) and was immunogenic, yielding high antibody titers in mice. However, cell fusions using splenocytes from mice immunized with the antigen preparation alone did not yield hybridomas that produced antibodies that discriminated between *C. sepedonicum*, *C. m.* subsp. *michiganense*, and *C. m.* subsp. *insidiosum* antigen preparations in ELISA tests. From the fusion in which whole cells were used for the booster injection, two hybridomas out of more than a thousand clones produced antibodies that were reactive with *C. sepedonicum* but not reactive with *C. m.* subsp. *michiganense* and *C. m.* subsp. *insidiosum* antigen preparations. Whether development of these two hybridomas was fortuitous or resulted because whole cells rather than antigen preparation was used in the booster injection was not determined.

Monoclonal 1H3 reacted in immunodiffusion with the same soluble antigen as the polyclonal antiserum (Fig. 1). The antigen is probably the extracellular polysaccharide because identical extracts

from another *C. sepedonicum* strain were shown by gas and paper chromatography to contain glucose, galactose, and fucose (S. H. De Boer, R. J. Copeman, and P. Lee Wing, *unpublished*). These monosaccharides are also components of the extracellular polysaccharide of related *Corynebacterium* spp. (11). The failure of 1H3 to react in immunofluorescence, and its strong ELISA reaction with culture supernatant in comparison with the weaker reaction with bacterial cells, confirmed that it did not react with a structural component of the bacterium.

The latex agglutination procedure also worked with monoclonal 1H3 (Fig 2B). This test has proven to be a valuable, rapid test in some laboratories for ring rot testing. More testing, however, will be required to determine whether some of the problems encountered in latex agglutination testing with polyclonal antiserum are eliminated when the monoclonal is used.

Monoclonal 1H3 could be used in ELISA for a highly sensitive test to detect the bacterial ring rot disease (Fig. 2A); ring rot-infected tubers were clearly discriminated from healthy ones (Fig. 3). Moreover, probable contamination of tubers with *C. sepedonicum* cells or extracellular antigen from an external source was also detected (Fig. 4). Unfortunately, the spread of *C. sepedonicum* from infected to healthy tubers could not be confirmed because the bacterium is very difficult to isolate unless it is present at high concentrations, as in potato stems and tubers with ring rot symptoms. Although the apparent contamination of our ring rot-free control samples can only be attributed in a very preliminary way to spread of the antigen by fruit flies, spread of the bacterium by insects has been observed previously by others (15,16). The possible involvement of cross-reacting bacteria such as strain F in the apparent cross-contamination cannot be entirely excluded, but other ring rot-free control tubers did not test positive when sampled later.

The reaction of monoclonal 1H3 with strain F is of concern and may limit the usefulness of the monoclonal. This bacterium is a Gram-negative rod and is not pathogenic on eggplant, an indicator host for *C. sepedonicum* (4). However, the frequency with which this bacterium occurs in association with potato is unknown. The results of our tests with samples from healthy tubers and tubers with decay other than ring rot suggest that strain F occurs infrequently or at too low a population to be detected in the serological tests. Nevertheless, it would be advisable to confirm ELISA-positive samples with a second test such as immunofluorescence with 9A1, which does not react with strain F (10), rather than relying entirely on reaction with 1H3. Cross-reactivity is a problem with all serodiagnostic tests, but specificity of tests with monoclonal antibodies that react with a single antigenic determinant is potentially much better than that with polyclonal antibodies which react with a large number of different determinants. The specificity of monoclonal antibodies for one particular bacterial pathogen depends on the frequency with which the antigenic determinant is present among other bacteria. The difficulty in selecting hybridomas that produce monoclonal antibodies to an antigen unique to the target bacterium is clear from the large number of hybridomas that needed to be screened in this and the previous study (10) to obtain antibodies with a degree of specificity to *C. sepedonicum*. Furthermore, since it is impossible to test all bacteria that might be encountered for possible cross-reactivity, the true extent of possible cross-reactions cannot be determined. The possibility of false-positive serological tests remains a reality, even when highly specific monoclonals are used. However, diagnosis on the basis of reaction with two monoclonals, each reacting with a different antigenic determinant, enhances test validity. It is less likely that a cross-reacting organism has two antigenic determinants in common with the target bacterium than a single determinant.

In addition to the need for diagnostic tests to confirm ring rot diagnosis, there is a need for tests to determine whether consignments of seed potatoes without external ring rot symptoms are free of *C. sepedonicum* (7,12). Composite samples are often used for this purpose (7,12). The ELISA procedure appears to be sensitive enough so that it can be applied to such composite tuber samples. If a sample from an infected tuber can be diluted 10^{-6} and still be detected (Fig. 2A), it is likely that a bulked sample consisting of tissue from one infected tuber in a composite of several hundred tuber samples would also yield a positive test result. In one

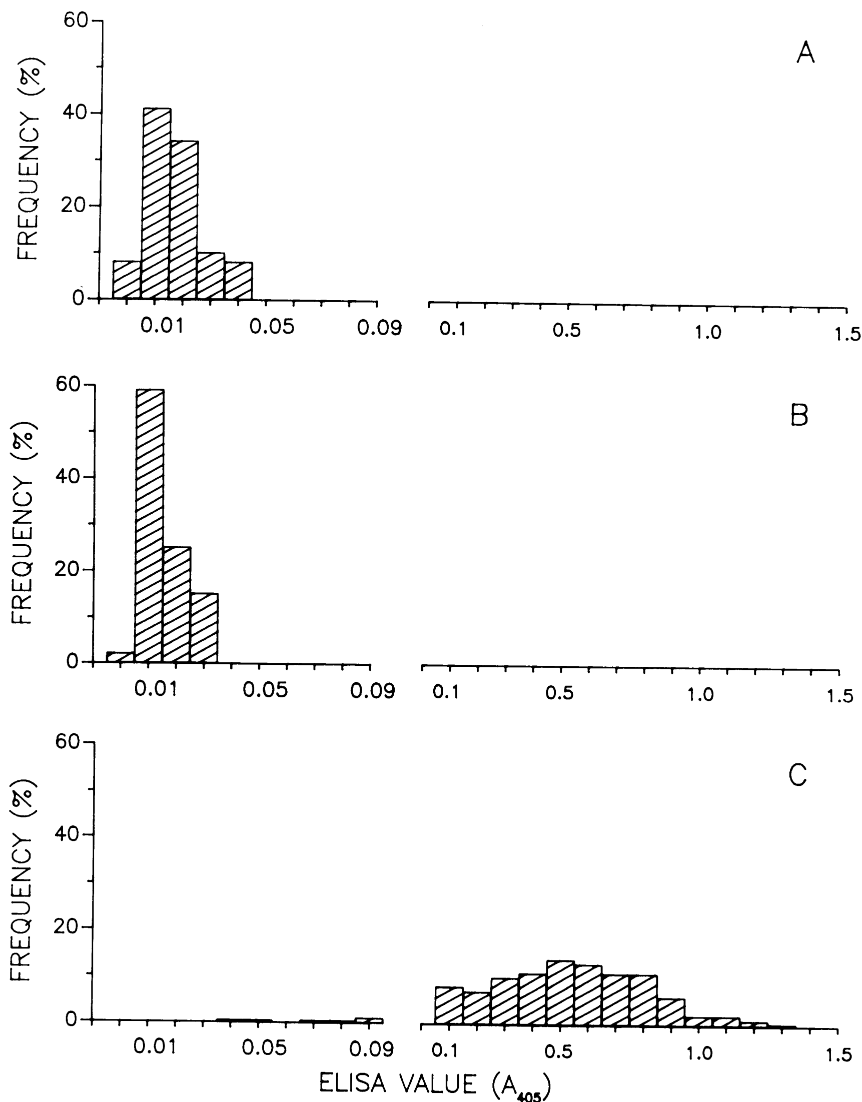

Fig. 3. Frequency distribution of ELISA values (A_{405}) obtained with **(A)** samples of healthy tubers, **(B)** ring rot-free decayed tubers, and **(C)** ring rot-infected tubers. Substrate was developed for 0.5 hr for all samples.

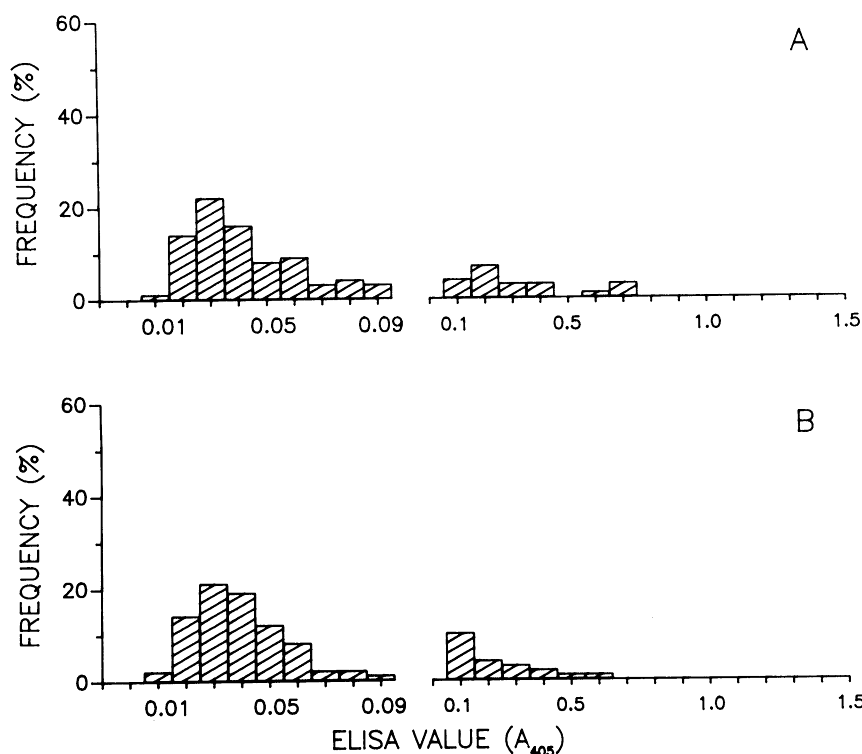

Fig. 4. Frequency distribution of ELISA values (A_{405}) obtained with samples of **(A)** healthy tubers that had been stored adjacent to ring rot-infected tubers and **(B)** ring rot-free decayed tubers stored adjacent to ring rot-infected tubers. Substrate was developed for 0.5 hr for all samples.

Table 2. Reaction of fruit flies (*Drosophila* sp.) in ELISA with monoclonal 1H3

No. flies per sample	No. samples tested	No. samples positive	ELISA values (A_{405})	
			Mean positive samples	Mean negative samples
1	16	2	0.193 ± 0.096	0.028 ± 0.010
2	11	1	0.097	0.028 ± 0.008
3	9	0	···	0.032 ± 0.011
4	3	1	0.409	0.046 ± 0.004

preliminary test, 23 100-tuber samples were tested by ELISA using monoclonal 1H3. A mean value of 0.019 was obtained with ostensibly healthy tubers, whereas a positive control in which one infected sample was added to a healthy 99-tuber sample gave an A_{405} value of 0.815. Sensitivity of the ELISA, however, is difficult to interpret in terms of cells/preparation detected, because 1H3 reacts with a soluble, extracellular antigen. Production and secretion of the antigen may vary with growth conditions of the bacterium and, therefore, its concentration is not necessarily correlated with cell numbers.

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LITERATURE CITED

1. Alvarez, A. M., and Lou, K. 1985. Rapid identification of *Xanthomonas campestris* pv. *campestris* by ELISA. Plant Dis. 69:1082-1086.
2. Ashwell, G. 1966. New colorimetric methods of sugar analysis. Pages 85-86 in: Methods in Enzymology. Vol. VIII. Complex Carbohydrates. E. F. Neufeld and V. Ginsburg, eds. Academic Press, New York. 759 pp.
3. Corbiere, R., Hingand, L., and Jouan, B. 1987. Application des methodes ELISA et immunofluorescence pour la detection de *Corynebacterium sepedonicum*: reponses varietales de la pomme de terre au fletrissement bacterien. Potato Res. 30:539-549.
4. Crowley, C. F., and De Boer, S. H. 1982. Nonpathogenic bacteria associated with potato stems cross-react with *Corynebacterium sepedonicum* antisera in immunofluorescence. Am. Potato J. 59:1-8.
5. De Boer, S. H. 1982. Cross-reaction of *Corynebacterium sepedonicum* antisera with *C. insidiosum*, *C. michiganense*, and an unidentified coryneform bacterium. Phytopathology 72:1474-1478.
6. De Boer, S. H. 1983. Evaluation of an agar immunodiffusion procedure for confirming bacterial ring rot diagnoses. Am. Potato J. 60:661-669.
7. De Boer, S. H. 1987. The relationship between bacterial ring rot and North American seed potato export markets. Am. Potato J. 64:683-694.
8. De Boer, S. H., and Copeman, R. J. 1980. Bacterial ring rot testing with the indirect fluorescent antibody staining procedure. Am. Potato J. 57:457-465.
9. De Boer, S. H., and McNaughton, M. E. 1986. Evaluation of immunofluorescence with monoclonal antibodies for detecting latent bacteria ring rot infections. Am. Potato J. 63:533-543.
10. De Boer, S. H., Wieczorek, A. 1984. Production of monoclonal antibodies to *Corynebacterium sepedonicum*. Phytopathology 74:1431-1434.
11. Gorin, P. A. J., and Spencer, J. F. T. 1961. Extracellular acidic polysaccharides from *C. insidiosum* and other *Corynebacterium* spp. Can. J. Chem. 39:2274-2281.
12. Janse, J. D., and Van Vaerenbergh, J. 1987. Interpretation of the EC method for the detection of latent *Corynebacterium sepedonicum* infections in potato. EPPO Bull. 17:1-10.
13. Johnson, D. A., Gautsch, J. W., Sportsman, J. R., and Elder, J. H. 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene 1:3-8.
14. Khan, M. A., and Slack, S. A. 1978. Studies on the sensitivity of a latex agglutination test for the serological detection of potato virus S and potato virus X in Wisconsin. Am. Potato J. 55:627-638.
15. List, G. M., and Kreutzer, W. A. 1942. Transmission of the causal agent of the ring-rot disease of potatoes by insects. J. Econ. Ent. 35:455-456.
16. Perrault, C. 1948. Etudes sur la pourriture du cerne des pommes de terre causee par *Corynebacterium sepedonicum* (Spieck. & Kott). Skaptason & Burkholder. I. Les agents de dissemination. Sci. Agric. 28:244-260.
17. Slack, S. A., Sanford, H. A., and Manzer, F. E. 1979. The latex agglutination test as a rapid serological assay for *Corynebacterium sepedonicum*. Am. Potato J. 56:441-446.
18. Strobel, G. A., and Rai, P. V. 1968. A rapid serodiagnostic test for potato ring rot. Plant Dis. Rep. 52:502-504.