Use of Monoclonal Antibodies Specific for Pectate Lyase as Serological Probes in the Identification of Soft Rot *Erwinia* spp.

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This research was supported in part by a grant from the Bi-National Agricultural Research and Development Program (BARD), Project 1-581-82.

We wish to thank S. H. DeBoer, R. S. Dickey, and G. Kritzman for supplying cultures; E. A. Maher and D. A. Galuska for helpful suggestions throughout this study; and R. S. Livingston for preparation of the antigen and antibodies.

Accepted for publication 23 June 1988 (submitted for electronic processing).

ABSTRACT

Klopmeyer, M. J., and Kelman, A. 1988. Use of monoclonal antibodies specific for pectate lyase as serological probes in the identification of soft rot *Erwinia* spp. Phytopathology 78:1430-1434.

Polyclonal and monoclonal antibodies (MCAs) were prepared against a purified extracellular endopectate lyase (PL) from *Erwinia carotovora* subsp. *carotovora*, causal agent of bacterial soft rot of potato. The antibodies were tested on crude PL preparations from representative soft rot *Erwinia* spp. including *E. c. carotovora*, *E. c. atroseptica*, and *E. chrysanthemi* in a dot blot immunoassay. The polyclonal anti-PL sera reacted with PL from all soft rot *Erwinia* spp. tested, but cross-reacted weakly with PL from pectolytic *Pseudomonas* and *Xanthomonas* strains. The MCAs reacted with PL from 55 strains of *E. c. carotovora*, 10 of 11 *E. c. atroseptica* strains, and most of 20 potato strains of *E. chrysanthemi* tested. Reaction with PL from 24 non-potato *E. chrysanthemi* strains was variable and was not related to the host plant or geographical origin of the strain. The MCAs did not react with PL from pectolytic pseudomonads, a pectolytic xanthomonad, or commercially prepared pectic enzymes from *Aspergillus* spp.

Additional keywords: blackleg of potato, *Pseudomonas fluorescens*, *Solanum tuberosum* L., *Xanthomonas campestris*.

Identification of the soft rot *Erwinia* spp., including *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, and *E. chrysanthemi* may be accomplished by using biochemical tests (8,14), DNA homology (2,22), immunofluorescent staining (1), double gel diffusion tests (31), and fatty acid composition (7). However, these methods are either time-consuming or require special equipment. An alternate approach would be to use standard serological procedures such as enzyme-linked immunosorbent assays (ELISA) or dot blot immunoassays. Serological relationships among the soft rot *Erwinia* have been extensively studied with polyclonal antisera against fixed or live whole cells (1,5,28,31), membrane protein complexes (33), and flagella (4). However, the presence of undefined antigens on cell surfaces has led to difficulties in the attempts to produce conventional antisera that do not cross-react with other soft rot or non-soft rot bacteria.

Soft rot *Erwinia* spp. are characterized by their ability to produce extracellular pectic enzymes that are involved in plant tissue maceration (26,27,29). The major pectic enzyme produced is pectate lyase (PL). Because large amounts of PL can be released by these erwinias in the presence of a suitable substrate, a rapid and reliable method to detect this enzyme may be of value in diagnosis or detection of these pathogens. The use of a specific protein antigen such as PL would thus facilitate production of antibodies with greater specificity and sensitivity. Mazzucchi et al (20) previously reported the production of polyclonal antisera against a purified pectate lyase from a carnation strain of *E. chrysanthemi*. This antiserum reacted with PL from all *E. chrysanthemi* strains tested; however, it did not react with PL from the 19 *E. c. carotovora* or 14 *E. c. atroseptica* strains tested.

The use of monoclonal antibodies (MCAs) against a specific protein, such as PL, would be a valuable tool in the identification of the soft rot erwinias. MCAs have been produced against the lipopolysaccharide from *E. c. atroseptica* (6) and PL from an *E. chrysanthemi* strain (30). In a preliminary report, Ward and DeBoer (32) reported that MCAs prepared against 41 and 44 kd forms of PL reacted in Western blot assays with three general classes of *Erwinia* supernatant proteins. In addition some MCAs reacted with very large (7,250 kd) and small (18–20 kd) molecular weight proteins. Our objective was to determine whether soft rot *Erwinia* spp. could be identified with polyclonal and monoclonal antibodies prepared against the major extracellular endopectate lyase from *E. c. carotovora*. Preliminary results of this study have been reported previously (12).

MATERIALS AND METHODS

Bacterial strains. Pectolytic *E. c. carotovora*, *E. c. atroseptica*, and *E. chrysanthemi* strains isolated from potato seed and stems in Israel were obtained from G. Kritzman, Department of Plant Pathology, Agriculture Research Organization, The Volcani Center, Bet Dagan, Israel. *E. carotovora* cultures, representing 40 whole cell serogroups of *E. c. carotovora* and *E. c. atroseptica*, were obtained from S. H. DeBoer of Agriculture Canada, Vancouver, British Columbia. Twelve strains of *E. chrysanthemi* isolated from potato and other hosts were obtained from R. S. Dickey, Cornell University, Ithaca, NY. Additional strains of pectolytic *Erwinia* spp., *Pseudomonas* spp., *Xanthomonas campestris*, and nonpectolytic *E. amylovora*, *E. herbicola*, and *Escherichia coli* (HB101) were obtained from a collection maintained at the University of Wisconsin-Madison. Commercially prepared fungal pectic enzymes including pectolyase (endopolygalacturonase-PG and endo-pectin lyase) from *Aspergillus japonicus* (P-3026) and pectinase (PG) from *A. niger* (P-5146) (Sigma Chemical Co., St. Louis, MO) were also tested. All strains were grown on the crystal-violet pectate medium (3) for detection of pectolytic enzyme activity.

Production of antigen and antibodies. Rabbit polyclonal antiserum and MCAs were prepared against a purified endopectate lyase from an *E. c. carotovora* strain (SR 319-Serogroup XXIX) isolated from potato field soil in Wisconsin. PL was purified from supernatants of cultures shaken at 22 C for 15 hr in minimal salts broth (27) plus sodium polypectate. Supernatants

were brought to 90% saturation with ammonium sulfate at 4 C for 15 hr. The resulting precipitate was pelleted and redissolved in a minimum amount of distilled water dialyzed and purified by ion exchange and agarose affinity chromatography (15,16). Purified PL (42 kd, pI approx. 9.2–9.7) from *E. c. carotovora* (SR319) was injected subcutaneously into New Zealand white rabbits at monthly intervals for 5 mo. Rabbits were bled and antiserum titer tested by indirect ELISA using purified PL as the antigen.

Forty-two-day-old female Balb/C mice were immunized with 50 μg of purified PL mixed with an equal volume of Freund's complete adjuvant (first injection only) or Freund's incomplete adjuvant (subsequent intraperitoneal injections). Immunizations were continued monthly for two additional months and at 3, 2, and 1 day before the fusion, 5–50 μg in PBS were injected into a tail vein. Spleen cells were then fused with NS-1 myeloma cells by using polyethylene glycol as described previously (21). Antibody-producing hybridomas were screened by indirect ELISA by using PL as the antigen, cloned, and characterized as to class and subisotype (Calbiochem, Behring Diagnostics, La Jolla, CA). Stable lines were injected into pristane-primed Balb/C mice for the generation of ascites. After 10–20 days, swollen mice were tapped, and the ascites fluid clarified by centrifugation, before storage in 1-ml aliquots at −20 C.

Dot blot immunoassay. Experimental procedures were designed to simplify techniques used for screening PL produced by these soft rot bacteria without extensive enzyme purification or analysis. Bacterial strains were grown in minimal salts pectate broth or LB broth (19) plus sodium polypectate for 48 hr at 28 C and frozen at −20 C. Pectolytic enzymes produced in this growth medium were screened by placing 10 μl of this frozen and thawed cell suspension on a nitrocellulose membrane (NCM) (0.45 μm, Schleicher and Schuell, Keene, NH). The NCM was treated with 5% skim milk in Tris-buffered (2 M, pH 7.4) saline (11) to block nonspecific protein binding sites and incubated with either the rabbit anti-PL serum (1:1,000 dilution) or the monoclonal antibodies (1:100 dilution). The NCM was washed and blocked again before incubating with either goat anti-rabbit (Sigma) or goat anti-mouse immunoglobulin-alkaline phosphatase conjugate (Kirkegaard and Perry, Gaithersburg, MD) at a 1:1,000 dilution. The NCM was again washed thoroughly and placed on a 1% agarose gel containing the alkaline phosphatase substrate, 5-bromo-4-chloro-3-indolyl phosphate (BCIP-Sigma), at 0.05% suspended in 0.75 M Tris-HCl, buffer (pH 9.7) (13) and incubated overnight at room temperature (22 C). Presence of PL in the cell suspensions was indicated by a dark blue spot. Gradation in the color response from dark blue to no color was classified as strong positive, weak positive, or no reaction. Negative controls included cell suspensions of nonpectolytic bacteria, *E. amylovora*, *E. herbicola*, and *Escherichia coli* (HB101).

Detection of pectic enzyme activity in NCM. Presence of PL activity in the cell suspensions spotted on the NCM was verified by placing the NCM face down on a 1% agarose gel containing 0.1% polygalacturonic acid suspended in a 50 mM Tris-HCl (pH 8.5)-1.5 mM $CaCl_2$ buffer (25). Polygalacturonase (PG) activity was determined by using a pectate agarose gel suspended in a 50 mM sodium acetate (pH 5.3) and 5 mM EDTA buffer. After 1 hr of incubation at room temperature (22 C), the gel was stained in a 0.1% ruthenium red solution for 20 min. If pectate lyase or polygalacturonase activity was present, a cleared zone appeared in the ruthenium red-stained pectate agarose background. The intensity of clearing on this gel (relative PL activity) was classified as strong, medium, or weak.

RESULTS

All strains designated as pectolytic that were grown on the crystal-violet pectate medium formed the pits (deep for soft rot erwinias and shallow for pseudomonads) that indicated presence of pectolytic enzyme activity (3). The nonpectolytic strains did not form pits on this medium. Pectate lyase activity (as determined by the pectate agarose gel technique) was also present in all cell suspensions of the pectolytic *Erwinia*, *Pseudomonas*, and *Xanthomonas* strains grown in the sodium polypectate medium. High PG activity was present in the pectolyase (endo PG and endo pectin lyase) and pectinase (PG) from *Aspergillus* spp., but no PL activity was detected in these preparations. Results are presented as positive reactions of the polyclonal and monoclonal antibodies for the cell suspensions, indicating that the antibodies reacted with or failed to react with PL in these suspensions. The MCAs reacted with PL from whole cell preparations of *E. c. carotovora* in a Western blot (17,18).

The rabbit polyclonal anti-PL serum gave positive reactions with all pectolytic *Erwinia* strains tested. These strains included the 40 *E. c. carotovora* and *E. c. atroseptica* serogroups (Table 1), as well as other *E. c. carotovora* and *E. c. atroseptica* strains and all *E. chrysanthemi* strains tested (Tables 2 and 3). The rabbit polyclonal anti-PL serum also reacted weakly with four pectolytic pseudomonads (including *P. marginalis*) and a pectolytic *Xanthomonas* sp. This antiserum, however, did not react with non-soft rotting *Erwinia* spp. including *E. amylovora* and *E. herbicola*, the *Escherichia coli* wild-type strain (HB101), and the fungal PL and PG enzymes.

Of the 30 monoclonal antibodies produced, four (4-7, 4-10, 5-9, 5-12, all of IgG_1 subisotype) that reacted strongly to purified PL in a dot blot immunoassay were used to screen the pectolytic *Erwinia* spp. (Table 1). These four MCAs reacted with all *E. c. carotovora* strains (from both potato and non-potato hosts) including representatives of all 40 *Erwinia carotovora* serogroups identified by DeBoer (5, *personal communication*), and six *E. c. carotovora* strains and one *E. c. atroseptica* strain from Israel. These MCAs also did not react with commercially prepared fungal PL and PG enzymes, a non-pectolytic strain of *Erwinia amylovora*, four non-pectolytic strains of *E. herbicola*, four pectolytic pseudomonads, and one pectolytic xanthomonad.

Reactions of the MCAs with the *E. chrysanthemi* strains isolated from potato and non-potato hosts were variable (Tables 2 and 3). The MCAs reacted with the *E. chrysanthemi* potato strains isolated in Israel and also a Taiwan strain (Table 2). However, with

TABLE 1. Reaction of rabbit polyclonal anti-PL serum and four monoclonal antibodies (4-7, 4-10, 5-9, and 5-12) specific for pectate lyase (PL) from *Erwinia carotovora* subsp. *carotovora* (SR319) with PL from various pectolytic and non-pectolytic bacterial strains using a dot blot immunoassay[a]

	Rabbit	Monoclonal antibodies			
	Strains positive (no.)/Strains tested (no.)				
Strain/host[b]	Anti-PL	4-7	4-10	5-9	5-12
Ecc (potato)	55/55	55/55	55/55	55/55	55/55
Ecc (non-potato)	4/4	4/4	4/4	4/4	4/4
Eca (potato)	11/11	10/11	11/11	11/11	11/11
Echr (potato)	20/20	13/20	16/20	16/20	14/20
Echr (non-potato)	24/24	7/24	8/24	10/24	6/24
E. amylovora	0/1	0/1	0/1	0/1	0/1
E. herbicola	0/4	0/4	0/4	0/4	0/4
Pseudomonas fluorescens[c]	4/4	0/4	0/4	0/4	0/4
P. syringae pv. *tabaci*	0/3	0/3	0/3	0/3	0/3
Xanthomonas campestris[c]	1/1	0/1	0/1	0/1	0/1
Escherichia coli[d] (HB101)	0/1	0/1	0/1	0/1	0/1

[a] Cells grown in minimal salts broth plus sodium polypectate used as antigen and a 1:100 dilution of the monoclonal antibody or a 1:1,000 dilution of rabbit anti-PL with a 1:1,000 dilution of goat anti-mouse or goat anti-rabbit immunoglobulin alkaline phosphatase conjugate and BCIP substrate. Dark blue spot indicated positive reaction with PL in the cell suspensions.

[b] Ecc = *Erwinia carotovora* subsp. *carotovora*; Eca = *E. carotovora* subsp. *atroseptica*; Echr = *E. chrysanthemi*. Non-potato Ecc hosts include strains isolated from carrot, cabbage, celery and broccoli. Non-potato Echr hosts are listed in Table 3.

[c] *Pseudomonas fluorescens* includes pectolytic strains; the strain of *Xanthomonas campestris* was a pectolytic nonpathogenic strain.

the exception of the Taiwan strain, the MCAs either reacted weakly or did not react with the non-Israeli *E. chrysanthemi* potato strains.

The MCAs exhibited less uniformity in reaction with the non-potato *E. chrysanthemi* strains including those that infect corn as compared to the *E. c. carotovora*, *E. c. atroseptica*, and *E. chrysanthemi* potato strains (Table 3). All four MCAs reacted strongly with only one non-potato *E. chrysanthemi* strain SR142, a corn strain from Italy, but they varied in their degree of reaction with the other corn *E. chrysanthemi* strains.

The MCAs also did not react with monocot host strains of *E. chrysanthemi* other than those from corn (SR231, SR233, SR234, and SR235). The MCAs also failed to react with most *E. chrysanthemi* strains isolated from dicots with the exception of one carnation strain (SR229), one chrysanthemum strain (0862), and a Parthenium strain (1015).

DISCUSSION

The polyclonal and monoclonal antibodies produced against the major pectate lyase from *E. c. carotovora* were used as diagnostic probes in studies with representative strains of *E. c. carotovora*, *E. c. atroseptica*, and *E. chrysanthemi*. The polyclonal rabbit anti-PL serum reacted with all pectolytic *Erwinia* spp. tested including *E. c. carotovora*, *E. c. atroseptica*, and *E. chrysanthemi*. The polyclonal antiserum reacted with the pectolytic pseudomonads and one pectolytic species of *Xanthomonas*; it did not react with the three pectolytic strains of *P. syringae tabaci*. In contrast to our observations, polyclonal antisera prepared by Mazzucchi et al (20) against PL from a carnation strain of *E. chrysanthemi* cross-reacted with PL from all other *E. chrysanthemi* strains, but not with PL from *E. c. carotovora* or *E. c. atroseptica* strains. Although our polyclonal antiserum was prepared against a purified PL from *E. c. carotovora*, it still reacted positively with all *E. chrysanthemi* strains tested, indicating *E. c. carotovora* PL may contain antigenic sites that are present in PL of all soft rot *Erwinia* spp. In contrast, *E. chrysanthemi* PL preparations may have dominant antigenic sites not present on *E. c. carotovora* PL that elicit high concentrations of antibodies in the rabbit unique for *E. chrysanthemi* PL.

Serological studies of *E. c. carotovora* and *E. c. atroseptica* (5, S. H. DeBoer, *personal communication*) identified 40 serogroups by using cell extracts as the antigen. Most *E. c. atroseptica* strains were placed in serogroup I, with small numbers in serogroups XVIII, XX, and XXII, whereas *E. c. carotovora* strains were separated into the other 36 serogroups. In our survey of the strains representative of the 40 serogroups using the MCAs against an *E. c. carotovora* PL, the MCAs reacted with PL from all 55 *E. c. carotovora* and 10 of 11 *E. c. atroseptica* strains tested. Differences between PL from *E. c. carotovora* and *E. c. atroseptica* could not be detected by our technique. *E. c. carotovora* and *E. c. atroseptica* pectic enzymes also were not differentiated by Ried and Collmer (25) using isoelectric focusing profiles of culture supernatants.

The results of tests with the MCAs against the *E. c. carotovora* PL revealed certain immunological differences in the different *E. chrysanthemi* strains. Reaction with potato strains of *E. chrysanthemi* appeared to depend on geographical location. The MCAs strongly reacted with the *E. chrysanthemi* potato strains from Israel and Taiwan. However, the MCAs either weakly reacted or did not react with strains from Peru and the Netherlands. It is not known whether the Israeli *E. chrysanthemi* strains produce a PL enzyme or isozyme not present in other potato *E. chrysanthemi* strains.

E. chrysanthemi strains from non-potato hosts were serologically quite diverse in their reactions with the MCAs against an *E. c. carotovora* PL. The MCAs weakly reacted with most corn strains tested irrespective of geographical or climatic origin. The only exception was that all four MCAs strongly reacted with a corn strain from Italy (SR142). However, none of the MCAs reacted with corn strains SR58 and SR120A. In the *E. chrysanthemi*

TABLE 2. Reaction of four monclonal antibodies (4-7, 4-10, 5-9, and 5-12) specific for pectate lyase (PL) from *Erwinia carotovora* subsp. *carotovora* (SR319) with PL from *Erwinia chrysanthemi* strains from potato using a dot blot immunoassay[a]

Strain[b]	Location	PL[c] activity	Monoclonal antibody 4-7	4-10	5-9	5-12
SR297	Peru	+	−	w	w	−
SR298	Peru	s	−	−	w	−
1088	Peru	+	−	−	−	−
1686,1687	Peru	s,+	−	w	w	−
1591	Taiwan	s	+	+	+	+
1669	Netherlands	+	−	−	−	w
1682	Netherlands	w	−	−	−	−
Isr302, 305	Israel	+,w	+	+	+	+
Isr307	Israel	w	w	+	w	+
Isr308	Israel	+	w	w	w	w
Isr309,312,317	Israel	w,w,w	+	+	w	−
Isr321	Israel	+	+	+	w	w
Isr325	Israel	+	+	+	+	w
Isr327,329	Israel	+,w	+	+	w	w
Isr331	Israel	w	+	+	w	w

[a]Cells grown in minimal salts broth plus sodium polypectate used as antigen with a 1:100 dilution of the monoclonal antibody and 1:1,000 dilution of goat anti-mouse immunoglobulin alkaline phosphatase conjugate using BCIP as the substrate. Dark blue spot indicated a positive reaction with PL in the cell suspensions; + = strong reaction, w = weak reaction, − = no reaction.

[b]Strain numbers preceded with SR from University of Wisconsin; Isr from G. Kritzman; all others from R. Dickey.

[c]PL activity of each strain as classified according to relative clearing of pectate agarose gel after 1 hr of incubation on dot blot membrane; s = strong clearing, + = medium clearing, w = weak clearing.

TABLE 3. Reaction of four monoclonal antibodies (4-7, 4-10, 5-9, and 5-12) specific for pectate lyase (PL) from *Erwinia carotovora* subsp. *carotovora* (SR319) with PL from *Erwinia chrysanthemi* strains isolated from various hosts using a dot blot immunoassay[a]

Strain[b]	Host	Location	PL[c] activity	Monoclonal antibody 4-7	4-10	5-9	5-12
SR58	corn	N. Carolina	s	−	−	−	−
SR72	corn	Lincoln Co., WI	+	w	+	+	−
SR74	corn	Lincoln Co., WI	s	−	w	+	−
SR80	corn	Spooner, WI	s	−	w	w	w
SR83A	corn	Spooner, WI	w	−	−	−	−
SR84	corn	India	+	−	−	w	−
SR120A	corn	Hawaii	s	−	−	w	−
SR142	corn	Italy	s	+	+	+	+
SR171	corn	Colombia, S.A.	+	w	w	w	−
1030	corn	France	+	w	−	w	−
SR147	grass	Australia	s	−	−	−	−
SR231	*Philodendron*	Florida	w	−	−	−	−
SR233	*Syngonium*	Florida	+	−	−	−	−
SR234	banana	Honduras	w	−	−	−	−
SR235	*Dieffenbachia*	Honduras	+	−	−	−	w
SR32	chrysanthemum	New York	+	−	−	w	−
0862	chrysanthemum	USA	+	w	w	w	−
SR228	poinsettia	Ohio	+	−	−	−	−
SR229	carnation	Pennsylvania	s	w	w	w	w
1237	carnation	France	+	−	−	−	w
SR243	tomato	France	+	−	−	−	−
SR244	*Pelargonium*	Comores Islands	+	−	−	w	−
0706	plantain	Colombia, S.A.	w	−	−	−	−
1015	*Parthenium*	USA	+	w	+	+	w

[a]Cells grown in minimal salts broth plus sodium polypectate used as antigen with a 1:100 dilution of monoclonal antibody and 1:1,000 dilution of goat anti-mouse immunoglobulin alkaline phosphatase conjugate using BCIP as substrate. Dark blue spot indicated a positive reaction with PL in the cell suspensions; + = strong positive reaction; w = weak positive reaction; − = no reaction.

[b]Strain numbers preceded with SR from University of Wisconsin, all others from R. Dickey.

[c]PL activity of each strain as classified according to relative clearing of pectate agarose gel after 1 hr of incubation on dot blot membrane s = strong clearing, + = medium clearing, 2 = weak clearing.

strains studied, no correlation was observed between strain recognition and the subdivision designations developed by Dickey (8–10).

The MCAs either weakly reacted or did not react with *E. chrysanthemi* strains from dicot hosts. Recognition of the dicot strains of *E. chrysanthemi* appears to be independent of subdivision designation and probably dependent entirely on the particular strain studied. Serological relationships among 27 strains of *E. chrysanthemi* using polyclonal antibodies against membrane protein complexes also revealed no correlation between host of origin and serovar (33). Thus, similarity or lack of similarity in PL recognition from various host strains by the MCAs may be because the plant from which the strain was isolated may not necessarily be the only host to which the strain is adapted under natural conditions (8,20).

Multiple isozymes of PL are present in culture supernatants of all *E. chrysanthemi* strains from many different hosts that have been examined (23,26,29). These isozymes can be divided into basic, neutral, and acidic groups (26). Although multiple isozymes of PL were present in *E. c. carotovora* and *E. c. atroseptica* supernatants, only isozymes with alkaline isoelectric points were detected (24,26). Our polyclonal and monoclonal antibodies were prepared against a purified PL from *E. c. carotovora* with a basic isoelectric point. Because PL isozymes can be differentiated immunologically by using polyclonal antisera (23), the MCAs may only react with isozymes of PL with basic isoelectric points and these isozymes may not be present in sufficient amounts in the nonpositive *E. chrysanthemi* strains to react with the MCAs. However, the MCAs are capable of detecting PL in picogram quantities in a dot blot immunoassay (M. Klopmeyer, *unpublished*); therefore, presence or absence of a particular PL isozyme rather than isozyme concentration may be the reason for variability observed in the tests for presence of PL in the *E. chrysanthemi* strains in our study. Because these MCAs recognize at least six distinct sites on PL (17), it is unlikely that low numbers of available epitopes on the PL molecule may account for the lack of recognition by the MCAs for the PL from the nonreactive *E. chrysanthemi* strains.

Recognition of PL by these MCAs may also depend on cultural conditions for PL production, length of storage of cultures, limitations in sensitivity of technique, affinity of the MCAs for PL, and different epitopes recognized by the different MCAs (17,32).

Because the MCAs obtained in this study appear to be specific for PL from *E. c. carotovora*, *E. c. atroseptica*, and certain strains of *E. chrysanthemi* and do not cross-react with pectic enzymes from a fungus, pectolytic pseudomonads or a pectolytic xanthomonad, they may be valuable aids in the detection and identification of *E. c. carotovora* or *E. c. atroseptica* in decaying plant tissue using PL as the target antigen. They have value in studies on the role of PL in pathogenesis; presence of PL isozymes in host tissue are detected with certain of these MCAs (17,18). However, soft rot bacteria can remain quiescent in bruised plant tissues or in lenticels of stored potato tubers and, thus, may not produce pectic enzymes at levels detectable by the MCAs. Therefore, an added step in the detection of this enzyme, such as enrichment for the bacterium in a sodium polypectate-containing medium, would be required if decay is not present.

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onto the spikes and the water agar to the extent that the inoculum