Selective Medium for Isolation of Pectolytic *Erwinia* sp.

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ABSTRACT

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Miller-Schroth medium modified by replacement of most of the agar with sodium polypectate appeared superior to the crystal violet polypectate medium for the selective isolation of pectolytic *Erwinia* sp. NaOH and MOPS (3-[*N*-morpholino]propanesulfonic acid) were added to raise the pH and buffer the medium, respectively. Pectolytic erwinias appeared as pink to orange colonies located in deep pits. Nonpectolytic erwinias formed pink-orange colonies with no pits. *Pseudomonas* spp. either did not grow on the medium or formed green colonies with no pits or pits that were very shallow. Recovery of pectolytic erwinias was significantly greater ($P = 0.05$) in 19 of 22 pectolytic strains tested with the modified medium than with the crystal violet pectate medium.

Miller-Schroth (MS) medium (4) is effective for isolation of *Erwinia amylovora* (Burrill) Winslow et al strains from plant material diseased by fire blight and for isolation of many other *Erwinia* species. This medium has excellent selectivity for *Erwinia* spp., but it is difficult to distinguish among strains of *E. amylovora*, pectolytic *Erwinia* spp., *E. herbicola* (Löhnis) Dye, and miscellaneous enterobacteria. A medium in which pectate rather than agar is the solidifying agent should enable the differentiation of pectolytic *Erwinia* spp. from nonpectolytic enterobacteria because of pits that formed by pectate degradation.

Our objective was to retain the selectivity of MS while substituting pectate for agar as a solidifying agent. The first problem was finding a suitable pectate source. Pectate is a general term for a wide group of polysaccharide substances found in plant cell walls. The biochemical and physical properties of the pectate depend on the source and method of preparation. Solidification of high methoxyl pectates requires the presence of 58–75% sugar in the medium and a low pH (2.8–3.5) (5). These types are used by the food industry for jams and jellies and are not suitable for bacterial media because of the low pH required for gel formation. Low-methoxyl pectate is preferred for use in bacteriological media. Calcium is needed for gelation at pHs between 7 and 8 (5). Most commercial sources (chemical supply houses) of pectate are not satisfactory. Some will not gel at neutral pH, and others gel but pectolytic *Erwinia* spp. do not degrade them (1). We know of only one commercial source of pectate suitable for our purpose in the United States (M. Burger Enterprises, 2225 Eton Ridge, Madison, WI 53705). Alternately, pectate can be extracted from orange peels or apples (1). In this paper, we compare a new pectate medium (Miller-Schroth-pectate [MSP]) with crystal violet pectate (CVP) for recovery of soft rot erwinias from diseased tissue.

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MATERIALS AND METHODS

To modify the Miller-Schroth medium, first, heat 1 L of distilled water to 80–90 C. Add 10 g of mannitol, 0.5 g of nicotinic acid, 3 g of L-asparagine, 2 g of K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, and 2.5 g of sodium taurocholate to the water while magnetic stirring. Next, mix 0.1 ml of Tergitol 7 (sodium heptadecyl sulfate), 10 ml of 2% nitrilotriacetic acid (20 g + 14.5 g of KOH per liter), 9 ml of 0.5% bromthymol blue (Na salt), 2.5 ml of 0.5% neutral red, 5 ml of 1 N NaOH, 1.75 ml of 1% thallium nitrate, and 50 ml of 0.33% $CoCl_2$ in a container and add the mixture to the hot medium. Next, add 15–30 ml of 1 N NaOH, 4 g of MOPS (3-[*N*-morpholino]propanesulfonic acid), 12 ml of 10% $CaCl_2 \cdot 2H_2O$, and 4 g of agar separately to the heated stirring media. Finally, add 18 g of sodium polypectate (M. Burger Enterprises) a little at a time to the vortex until it dissolves.

Maintain the temperature of the medium at 80–90 C at all times before autoclaving and pouring. Pectate media will solidify at 70 C and will not form an acceptable gel if remelted and poured. The plates must not have any surface water present when used. Allow them to dry for several days. The NaOH must be added before the pectate is incorporated or precipitation will result. The optimal amount of 1 N NaOH to be added varies with the batch of pectate used. Final pH should be 7.5 to 7.7. It may be necessary to vary the amount of NaOH to find the optimum amount to provide a firm gel for a specific batch of pectate. The color of the medium should be blue-green. Five milliliters of 1% cycloheximide may be added after autoclaving if fungal contam-

ination is a problem.

The efficiency of the recovery of pectolytic erwinias on MSP and CVP (2) was compared by dilution plating soft-rotted potato tissue from tissue slices that were inoculated with various pectolytic erwinias. These bacteria originally were isolated from a variety of plants with soft rot symptoms. Inoculated potato slices were used to determine the performance of the selective medium with typical soft-rotted plant material containing numerous nonpectolytic bacteria. A potato was peeled and cut into slices 5–8 mm thick and placed in a petri dish with 10 ml of sterile water. A test strain grown on King's medium B for 24 hr at 24 C was inoculated with a loopful of bacteria in a groove along the center of the potato slice, and the slices were incubated for 2 days at 28 C. Approximately 1 cm^2 of rotted tissue was thoroughly mixed with 10 ml of sterile water, and dilution series were made using 10-ml tubes of sterile water. Three dilutions each were plated on CVP and MSP media. King's medium B was included as a control to determine total populations (3). There were five plates for each dilution. Plates were incubated at 28 C for 2 days, and the dilutions with 30–200 colonies per plate were chosen for counting. Only pink to orange colonies in pits were counted. An analysis of variance comparing populations of pectolytic erwinias on MSP and CVP was calculated for each *Erwinia* sp. tested. With a selective media, it is useful to know which nontarget organism will grow and if they are distinguishable from the target organism. Therefore, 18 nonsoft-rotting (determined by the potato soft-rot test) erwinias and pseudomonads isolated from miscellaneous plant disease specimens were streaked onto MSP and incubated for 2–4 days at 28 C.

RESULTS AND DISCUSSION

Growth of nontarget bacteria was not a problem on MSP. Dilution plates on MSP prepared from soft-rotted potato tissue usually yielded only colonies of pectolytic erwinias. The populations of nontarget bacteria in the rotted potato tissue probably was low. Dilution plate counts on King's medium B agar yielded counts in the same range as on CVP or MS (*data not shown*), indicating that nontarget bacteria were not present in high numbers.

The populations recovered from soft-rotted potato tissue were extremely high. Populations ranged from 10^{10} to 10^{12} cfu/ml when a very heavy suspension of rotted potato tissue in water was prepared. The variation could be attributable to differing growth rates among various pathovars and species of pectolytic erwinias.

Pectolytic erwinias formed pink to orange colonies and deep pits within 1–2 days of incubation at 28 C. After 2 days, the colonies began to turn green and the medium became soft and disintegrated if large numbers of soft rotting erwinias were present. Thus, the plates should be examined within 2 days of incubation. In 19 of 22 strains tested (Table 1), significantly more soft-rotting erwinias were recovered from soft-rotted potatoes with MSP than with CVP.

None of the 18 nonpectolytic bacteria streaked on MSP formed pits (Table 2). Nonsoft-rotting erwinias formed pink colonies changing to green but did not form pits. Pseudomonads either did not grow or formed green colonies without pits. One unidentified *Pseudomonas* sp. formed green colonies with slight shallow depressions in the medium. MSP is an excellent medium for isolation of pectolytic erwinias because of the easily detectable pits. Plates may be stored for several months in sealed containers under refrigeration (L. Pierce and A. H. McCain, *unpublished*).

Table 1. Comparison of MSP[w] and CVP[x] selective media for recovery of pectolytic erwinias from inoculated potato slices

Strain	Species	Source	Number of cfu ($\times 10^{10}$) per milliliter[y]	
			MSP	CVP
144	*Erwinia carotovora* subsp. *atroseptica*	Potato	67.8	29.0*[z]
152	*E. c. atroseptica*	Potato	99.0	56.0*
147	*E. c. atroseptica*	Potato	62.2	55.5
765-1	*E. c. carotovora*	Calla	60.6	16.6*
849-1	*E. c. carotovora*	Calla	37.5	20.0*
730-1	*E. c. carotovora*	Calla	3.0	2.2*
665-2B	*E. c. carotovora*	Carnation	35.1	23.6*
325	*E. c. carotovora*	Christmas cactus	30.7	16.8*
691-2	*E. c. carotovora*	Cyclamen	110.0	72.6*
277-3	*E. c. carotovora*	Dieffenbachia	2.3	1.5*
773	*E. c. carotovora*	Impatiens	97.8	53.6*
576-1	*E. c. carotovora*	Iris	5.0	3.1*
82-4	*E. c. carotovora*	Iris	67.2	43.8*
976-2	*E. c. carotovora*	Ivy	51.0	13.0*
412-2	*E. c. carotovora*	Poinsettia	116.8	55.2*
538-3	*E. c. carotovora*	Primrose	117.8	73.8*
425-2	*E. c. carotovora*	Nephthytis	48.6	38.8
745-4	*E. c. carotovora*	Aborvitae	40.0	7.6*
691-1	*E. c.* subsp. unknown	Cyclamen	36.4	24.8*
830-1A	*E. c.* subsp. unknown	Cyclamen	325.8	228.2*
109	*E. chrysanthemi*	Alfalfa	1.0	0.2*
128	*E. chrysanthemi*	Chrysanthemum	1.1	0.7

[w] MSP = Miller-Schroth-pectate medium.
[x] CVP = crystal violet pectate medium.
[y] Estimated (by dilution plant count) colony-forming units recovered on MSP or CVP from the same heavy suspension of rotted potato tissue for each *Erwinia* strain. Analysis of variance calculated for each strain comparing MSP and CVP media.
[z] * = Significantly different at $P = 0.05$.

Table 2. Growth of nonsoft-rotting[x] bacteria on MSP[y] after 2–4 days of growth

Strain	Species[z]	Source	Growth
718-3	*Erwinia* sp.	Cyclamen	Pink-orange, no pits
626-2	*Erwinia* sp	Iris	Pink-orange, no pits
635-2	*Erwinia* sp	Pear	Pink-orange, no pits
658-1	*Erwinia* sp.	Ivy	Pink-orange, no pits
681-2	*Pseudomonas syringae*	Liquidambar	No growth
364-1	*P. cattalayae*	Orchid	No growth
440-1	*E. amylovora*	Pear	Pink-orange, no pits
495-4	*E. amylovora*	Raphiolepis	Pink-orange, no pits
161	*E. herbicola*	Cyclamen	Green, no pits
327	*P. fluorescens*	Pea	Green, no pits
765-1	*E. herbicola*	Anigozanthos	Green, no pits
764-3	*E. herbicola*	Cactus	Green, no pits
115	*P. tolaasii*	Agaricus mushrooms	Green, no pits
765-3	*Pseudomonas* sp.	Anigozanthos	Green, no pits
140	*Pseudomonas* sp.	Potato	Green, no pits
141	*P. putida*	Soil	Green, no pits
107	*P. syringae* pv. *savastonoi*	Olive	Green, no pits
856	*Pseudomonas* sp.	Potato	Green, slight shallow pits

[x] Determined by potato soft rot test.
[y] MSP = Miller-Schroth-pectate medium.
[z] Strains isolated from plant specimens received in our laboratory 1985–1990. Identifications by the authors.

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