Pectate and Pectin Gels for Differentiation of Pseudomonas sp. and Other Bacterial Plant Pathogens

D. C. Hildebrand

Associate Research Plant Pathologist, Department of Plant Pathology, University of California, Berkeley 94720.

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ABSTRACT

One hundred and sixty-five strains of 54 *Pseudomonas* sp., 32 strains of 12 *Erwinia* sp., three *Agrobacterium* sp., one *Corynebacterium* sp., and seven *Xanthomonas* sp. were tested for pit formation on polypectate gels of three different pH ranges (4.9-5.1, 6.9-7.1, and 8.3-8.5). Of the *Pseudomonas* sp., 92 strains produced pitting at the low pH (medium A), and 29 formed pits at the highest pH (medium C). Only *P. solanacearum* strains, the angular leaf-spotting pathogens, and one of two strains of *P. caryophylli* formed pits at all pH levels. Pitting at a given pH was consistent among strains of the same species. The addition of metabolizable substrates to the gels affected pit formation, presumably through a shift in pH. Pitting on pectin gels by most organisms was similar to that on polypectate gels, except that pits were smaller. All *Erwinia* sp., except *E. amylovora*, and all *Xanthomonas* sp. tested also formed pits on the polypectate gels, and, as was found for *Pseudomonas* sp., the pH levels at which pitting occurred depended upon the species of pathogen. That strains of a species usually were consistent in their abilities to cause pitting on polypectate gels makes this property a useful taxonomic character in the differentiation of various plant pathogens.

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Additional key words: physiology, taxonomy.

Pectate gel media have been used extensively for detecting the presence of pectolytic enzymes in *Erwinia* sp. Pseudomonads also have been tested on these gels, but with variable results. For example, Oxford (3) and Smith (6) reported that two of eleven and four of five strains, respectively, of *Pseudomonas syringae* produced pectolytic enzymes on pectate gels; whereas Burkholder & Starr (2), Prunier & Kaiser (4), and Sands et al. (5) were unable to detect these enzymes for a number of strains. Since pectolytic enzymes vary considerably in pH optima, response to ions, and mode of degradation (1), these erratic results, although possibly due to normal variation within a population, more likely were caused by differences in techniques and conditions used. Consequently, the effect of different pH levels and substrates on the abilities of *Pseudomonas* sp. to produce pectolytic enzymes on gels was examined in terms of the potential usefulness of this property in the differentiation of the species. Several species of other genera were included for comparative purposes.

MATERIALS AND METHODS.–*Sodium polypectate gels.*–The following basal medium, adjusted to different pH levels, was used for detection of pectolytic enzymes. It consisted of ingredients added in the following order: 1,000 ml distilled water (heated to near boiling); 1 ml bromothymol blue (1.5% alcoholic solution); 6 ml 10% $CaCl_2 \cdot 2H_2O$ (freshly prepared); and 22 g sodium polypectate (Sunkist 6024 or Sigma P-1879). The basal medium was autoclaved (15 lb. for 15 min), and was adjusted either to pH 4.9-5.1 (medium A) by the addition of 3.4 to 4.2 ml 1 *N* HCl (depending upon polypectate source) or 6.9-7.1 (medium B) by addition of 2.3 to 3.0 ml 1 *N* NaOH before autoclaving (15 lb. for 15 min). Medium C (pH 8.3-8.5) was prepared by the addition of 6.4 to 8.5 ml sterile 1 *N* NaOH after the basal medium had been autoclaved. One hundred ml sterile 4% agar solution was added to all media after they were adjusted and autoclaved. The medium, while above 70 C, was added to petri dishes which were then stored at room temperature for several days until the surface of the gel was dry. Masses of bacteria from 24- to 48-hr cultures grown on King's B medium were spotted, four/plate, on the gel surface. The dishes were kept incubated for 1-6 days at 28 C.

Pectin gels.–The pectin basal medium consisted of the following ingredients added in order: 1,000 ml distilled water (heated to near boiling); 1 ml bromothymol blue (1.5% alcoholic solution); and 60 g pectin (Matheson, Coleman, and Bell). The medium was autoclaved (15 lb. for 15 min), then adjusted to pH 4.8-5.0 (medium D) by the addition of 8.5 ml sterile 1 *N* NaOH or pH 6.8-7.0 (medium E) by the addition of 11.3 ml sterile 1 *N* NaOH. One hundred ml sterile 4% agar solution was added to both media after they were autoclaved and adjusted. Immediately after the addition of the pectin to the dishes, the gel surface was dried in a 40-C chamber for 20 min, and bacteria were spotted on the surface. Results were read after 48-hr incubation at 28 C.

Bacterial cultures.–The strains used were designated by an abbreviation of the name of the institution from which they were received: CNBUS (Comité National de Biologistes de l'Union Sovietique); ICPB (International Collection of Phytopathogenic Bacteria); NCPPB (National Collection of Plant Pathogenic Bacteria); NEAP (Estacao Agronomica Nacional, Portugal). Cultures not designated as to source are from our laboratory. The *Pseudomonas* sp. tested were as follows: *P. alboprecipitans*, ICPB-PA-117; *P. alliicola*, ICPB-PA-8, ICPB-PM-7, ICPB-PM-8, ICPB-PM-9, ICPB-PM-15, ICPB-PM-16, Dickey 61-3; *P. andropagonis*, NCPPB-933, NCPPB-934; *P. angulata*, NCPPB-79,

NCPPB-1238; *P. antirrhinii*, NCPPB-1398; *P. aptata*, NCPPB-873, ICPB-PA-122; *P. atrofaciens*, NCPPB-117; *P. barkeri*, NCPPB-244; *P. betle*, NCPPB-323; *P. cannabina*, NCPPB-1410, NCPPB-1437; *P. caryophylli*, B2, NCPPB-349; *P. cattleyae*, PC-1; *P. cepacia*, Stanier 249, Stanier 382, Dickey-63-53, Dickey 63-75, Dickey 64-20; *P. cerasi* var. *pyri*, CNBUS-103; *P. cichorii*, Stall 140, Stall 62-4, Stall 62-22, NCPPB-907, NCPPB-1512; *P. citriputeale*, CNBUS-500; *P. coronafaciens*, NCPPB-600, NCPPB-1348, NCPPB-1351, NCPPB-1355; *P. coronafaciens* var. *atropurpurea*, NCPPB-1328; *P. delphinii*, NCPPB-650; *P. dysoxyli*, NCPPB-225; *P. eriobotryae*, NCPPB-2321, 408, 424, 425; *P. flectans*, NCPPB-539; *P. fluorescens*, Stanier 2, Stanier 31, Stanier 108, Stanier 188, Stanier 192, Stanier 411; *P. garcae*, NCPPB-412, NCPPB-1399; *P. glycinea*, NCPPB-1271, Kennedy R-1, Kennedy R-2, Kennedy R-3, Kennedy R-4, Leben 564; *P. hibisciola*, NCPPB-1683; *P. lachrymans*, NCPPB-277, NCPPB-467, NCPPB-1436, PL-5, PL-15, PL-17, PL-18, PL-19; *P. mangiferaeindicae*, NCPPB-212; *P. marginalis*, NCPPB-247, NCPPB-667, NCPPB-1689, Stanier 400, Stanier 401; *P. marginata*, ICPB-PM-2, ICPB-PC-144, ICPB-PM-144, NCPPB-1888, NCPPB-1889, NCPPB-1891, NCPPB-1051; *P. mellea*, NCPPB-280; *P. mori*, NCPPB-1415, NCPPB-1445, Grogan 18-G526, Grogan 126-547-2, ICPB-PM-135; *P. mori* var. *huzi*, NCPPB-1037, NCPPB-1413; *P. morsprunorum*, NCPPB-1095; *P. oryzicola*, NCPPB-1417; *P. panacis*, NCPPB-1498; *P. papaveris*, NCPPB-285; *P. passiflorae*, ICPB-PP-111, ICPB-PP-120; *P. phaseolicola*, Grogan G-2, Grogan G-16, Grogan G-55, Grogan G-60, Grogan G-69, Grogan G-71, Grogan G-72, Grogan G-73, Grogan G-75, Grogan G-77, Grogan G-101, Grogan G-114, Grogan G-115, Grogan G-123, Grogan G-124, Natti-Burkholder Race 1, HB-35, HB-36; *P. pisi*, NCPPB-300, NCPPB-1359, Lawyer 7362, Lawyer 73662, Lawyer 7444, Lawyer 74702; *P. polycolor*, NCPPB-1224, PP-3; *P. pomi*, NCPPB-463; *P. primulae*, NCPPB-1618, NCPPB-1619; *P. putida*, Stanier 90; *P. rubrilineans*, NCPPB-359, NCPPB-920, NCPPB-1118, *P. rubrisubalbicans*, NCPPB-520, NCPPB-1027; *P. savastanoi*, NCPPB-639, NCPPB-1464, NCPPB-1481, OK-5, Wilson 1006, NEAP E-4; *P. sesami*, NCPPB-1017, NCPPB-1033, NCPPB-1330; *P. setariae*, NCPPB-1392; *P. solanacearum*, Buddenhagen B-139, Buddenhagen D-100, Buddenhagen P-28-T; Buddenhagen blue mutant; *P. syringae*, S-3, S-9, S-10, S-35, S-36, S-40, DeVay B-3, Grogan 28, Grogan 61, CNBUS-421, Leben 620, Leben 622; *P. tabaci*, Klement *P. tabaci; P. tomato*, P. tom. 1, P. tom. 4, P. tom. 8, P. tom. c.; *P. viridiflava*, Billing 1249; *P. woodsii*, ICPB PW-2; *Pseudomonas* sp. B62-1.

Strains of other genera and species tested were: *Agrobacterium radiobacter*, ICPB-TT-103; *A. rhizogenes*, ICPB TR-108; *A. tumefaciens*, ICPB TT-103, CG-68, *Corynebacterium michiganense*, Grogan G-120, CM-Davis; *Erwinia amylovora*, FB-1, FB-9, FB-12, FB-15, FB-16, FB-17; *E. aroideae*, ICPB EA-14; *E. atroseptica*, EA-201; *E. carotovora*, ICPB EC-109; *E. carotovora* var. *partenii*, ICPB EC-124; *E. chrysanthemi*, ICPB EC-174, ICPB EC-201; *E. dieffenbachiae*, ICPB ED-103; *E. herbicola*, ICPB 2556, ICPB 3162; *E. mangiferae*, ICPB EM-101; *E. nigrifluens*, EN-1, EN-2, EN-3, EN-4, EN-5, EN-6, EN-7; *E. quercina*, ACC, AC-1, AC-3, AC-4; *E. rubrifaciens*, ER-1, ER-4, ER-6; *E. uredovora*, Kado 20D3, Kado 20D31; *Xanthomonas campestris*, XC-2; *X. dieffenbachiae*, XD-1; *X. fragariae*, XF-32, ICPB XF-102; *X. incanae*, XI-3; *X. juglandis*, B62-69, B62-69(-4), B62-69(-6); *X. phaseoli*, ICPB XP-2; *X. vitians*, XV-201.

RESULTS.–*Action on polypectate gels.*–Many *Pseudomonas* sp. and other bacteria produced enzymes which degraded polypectate, as evidenced by the formation of pits in the polypectate gels (Table 1). Three types of activity patterns were observed for the various strains: some formed pits at low pH (medium A); some formed pits at high pH (medium C); and others caused pitting at all pH levels. Generally, all strains of a given species exhibited the same type of activity; all 17 strains of *P. phaseolicola* produced moderate- to large-sized pits on medium A, smaller pits on medium B, and none on medium C, whereas all five strains of *P. marginalis* were moderately to highly active on media B and C, but inactive on medium A. Other bacteria such as *P. coronafaciens* did not cause pitting.

The source of the polypectate also slightly influenced the results. Strains of *P. syringae*, *P. fluorescens*, and *E. amylovora* on the Sigma polypectate gels often formed pits in 24 to 48 hr which usually disappeared after 6 days' incubation. Whether these pits were due to degradation of the polypectate or to another factor, such as loss of water around the colony, was not established; these reactions, therefore, are questionable.

Effect of additional substrates in polypectate gels.–Preliminary tests indicated that the addition of 0.5% yeast extract (a common additive to polypectate gels) to the gels prevented formation of pits by certain *Pseudomonas* sp. Several substrates, therefore, were tested as additives to the gels to determine whether the effect possibly was caused by catabolite repression or whether pH changes associated with utilization of the substrate influenced activity of the enzyme. The additional substrates used were glucose, sodium succinate, sodium asparaginate, and β-alanine. All were added at a level of 0.5% to the autoclaved gels, and an adjustment of the pH was made.

The addition of various substrates to the basal gels considerably altered the patterns of pectolytic activity of the various organisms (Table 2). Most of the changes could be ascribed to changes in pH (as detected by the indicator in the gels) which occurred during metabolism of the added substrate by the organisms. Thus, the growth of *P. marginata* strains on succinate or asparaginate raised the pH of the gels, and pit formation was not observed. In contrast, growth of these and several other strains of *Pseudomonas* sp. on glucose often resulted in lowering the pH, and pitting usually occurred. Unlike *E. amylovora* strains, *Pseudomonas* sp. which produced questionable pitting on the Sigma polypectate also

TABLE 1. Activity of *Pseudomonas* sp. and other bacteria on polypectate[a] gels at different pH levels

		No. strains showing activity								
		4.9-5.1 (medium A)			6.9-7.1 (medium B)			8.3-8.5 (medium C)		
Species	No. strains tested	Moderate to high[b]	Slight	None	Moderate to high	Slight	None	Moderate to high	Slight	None
Pseudomonas sp.										
P. alboprecipitans	1	1				1				1
P. alliicola	7	7			7					7
P. andropagonis	2	2				2				2
P. angulata	2	1	1		1	1		1	1	
P. antirrhinii	1	1					1			1
P. aptata	2			2			2			2
P. atrofaciens	1			1			1			1
P. barkeri	1			1			1			1
P. betle	1			1			1			1
P. cannabina	2	2				2				2
P. caryophylli	2	2			2				1	1
P. cattleyae	1			1			1			1
P. cepacia	5	5			5					5
P. cerasi	1			1			1			1
P. cichorii	5			5			5			5
P. citriputeale	1			1			1			1
P. coronafaciens	4			4			4			4
P. delphinii	1	1					1			1
P. dysoxyli	1			1			1			1
P. eriobotryae	4	4					4			4
P. flectans	1	1					1			1
P. fluorescens	6			6	1[c]		5	1		5
P. garcae	2			2			2			2
P. glycinea	6	3	3				6			6
P. hibisciola	1			1			1			1
P. lachrymans	8	4	4		6	2		6	2	
P. mangiferaeindicae	1			1	1			1		
P. marginalis	5			5	5			5		
P. marginata	7	7			6	1				7
P. mellea	1			1			1			1
P. mori	7	7			4	1	2			7
P. morsprunorum	1	1					1			1
P. oryzicola	1			1			1			1
P. panacis	1			1			1			1
P. papaveris	1			1			1			1
P. passiflorae	2			2			2			2
P. phaseolicola	18	18			12	5	1			18
P. pisi	6			6			6			6
P. polycolor	2			2			2			2
P. pomi	1			1			1			1
P. primulae	2			2			2			2
P. putida	1			1			1			1
P. rubrilineans	3			3	3			3		
P. rubrisubalbicans	2		1	1		1	1			2
P. savastanoi	6	2	2	2	1	3	2			6
P. sesami	3	3			2	1		2	1	
P. setariae	1			1			1			1
P. solanacearum	4	4			4			4		
P. syringae	12			12			12			12
P. tabaci	1	1				1			1	
P. tomato	4	4				1	3			4
P. viridiflava	1			1	1			1		
P. woodsii	1	1				1				1
Pseudomonas sp. B62-1	1			1			1			1
Agrobacterium sp.										
A. radiobacter	1			1			1			1
A. rhizogenes	1			1			1			1
A. tumefaciens	2			2			2			2
Corynebacterium sp.										
C. michiganense	2	2					2			2

TABLE 1. (continued)

		No. strains showing activity								
		4.9-5.1 (medium A)			6.9-7.1 (medium B)			8.3-8.5 (medium C)		
Species	No. strains tested	Moderate to high[b]	Slight	None	Moderate to high	Slight	None	Moderate to high	Slight	None
Erwinia sp.										
E. amylovora	6			6			6			6
E. aroideae	1	1			1			1		
E. atroseptica	1	1			1			1		
E. carotovora	2	2			2			2		
E. chrysanthemi	2	2			2			2		
E. dieffenbachiae	1	1			1			1		
E. herbicola	2			2		2			2	
E. mangiferae	1			1		1			1	
E. nigrifluens	7			7	6	1		6	1	
E. quercina	4		1	3	4			4		
E. rubrifaciens	3			3	3			3		
E. uredovora	2	1	1				2			2
Xanthomonas sp.										
X. campestris	1	1			1			1		
X. dieffenbachiae	1		1			1				1
X. fragariae	2		2				2		2	
X. incanae	1	1			1			1		
X. juglandis	3	3			3				2	1
X. phaseoli	1	1					1			1
X. vitians	1	1					1			1

[a] Sunkist sodium polypectate 6024.

[b] Activity rated according to degree of pitting produced. "Moderate to high" indicates a pit with a radius of > 1 mm from colony margin, "slight" indicates a pit < 1 mm in diam from colony margin, and "none" indicates no pit formed.

[c] Strain rots lettuce; may be *P. marginalis.*

produced questionable pitting on Sunkist sodium polypectate 6024 when glucose was added.

Action on pectin gels.—Media D (pH 5) and E (pH 7) were used either alone or supplemented with 0.06% $CaCl_2 \cdot 2H_2O$ or 0.5% glucose, or both, and the pH was adjusted.

The results for *Pseudomonas* sp. (Table 3) in general were similar to those recorded for media A and B (Table 2). Those which produced pits on either medium A or B usually did likewise on medium D or E. Pits were smaller on media D and E, but this could be accounted for by the shortened incubation time necessary because of rapid disintegration of these gels. This shorter incubation time could also account for the lack of pitting by some strains which were slightly active on the pectate gels after the longer incubation. The addition of $CaCl_2$ with or without glucose appeared to enhance the activity of the two fluorescent species (*P. mori* and *P. marginalis*), whereas none of the supplements appeared to affect the nonfluorescent strains, with the possible exception of the *P. caryophylli* strain on medium D. Usually the margins of the pits were somewhat better defined on media with $CaCl_2$, which made results easier to read.

Erwinia sp. behaved differently from *Pseudomonas* sp. on the pectin gels. Only the *E. chrysanthemi* strain formed pits on the pectin gels, and these were much smaller than could be accounted for on the basis of a shorter incubation time. The other soft-rotting strain, *E. carotovora*, did not form pits on either medium D or E, although it rapidly formed large pits on media B and C.

DISCUSSION.—Based on formation of pits below colonies, it appears that many of the *Pseudomonas* sp. produce pectolytic enzymes on pectin and pectate gels. Although the method does not indicate the type of pectolytic enzyme responsible for the pitting, it appears useful as a diagnostic test to aid in the identification of species of *Pseudomonas* and *Erwinia*. Members of the same species in most cases showed similar patterns of activity on the different gels. Usually the pattern of pitting could not be correlated to the type of disease produced. The soft-rotting organisms, *P. marginalis* and *P. cepacia*, produced pitting at the high and low pH levels, respectively (Table 1); and, in diseases caused by these organisms, the pH of rotted tissue increases and decreases, respectively.

Strains of the angular leaf-spotting organisms (*P. angulata* [*P. tabaci* without toxin?], *P. lachrymans*, and *P. sesami*) caused pitting of sodium polypectate at all pH levels. The only other organisms which exhibited similar patterns were *P. tabaci* and *P. solanacearum*; the rapidity of formation, as well as configuration of the pits formed by the latter organism, was quite different from those of the angular leaf-spotting organisms.

TABLE 2. Effect of additional substrates on activity of *Pseudomonas* sp. and *Erwinia* sp. on polypectate gels[a] of different pH levels

Strains	Activity														
	4.9-5.1 (medium A)					6.9-7.1 (medium B)					8.3-8.5 (medium C)				
	Without supplements	+0.5% Glucose	+0.5% Succinate	+0.5% Sodium asparaginate	+0.5% β-alanine	Without supplements	+0.5% Glucose	+0.5% Succinate	+0.5% Sodium asparaginate	+0.5% β-alanine	Without supplements	+0.5% Glucose	+0.5% Succinate	+0.5% Sodium asparaginate	+0.5% β-alanine
Pseudomonas sp.															
P. alliicola PA-8	M[b]	H	S	0	0	H	H	0	0	0	0	H	S	0	0
P. caryophylli 349	H	M	M	0	M	H	M	0	0	S	S	M	0	0	0
P. cepacia 63-53	H	H	S	0	0	H	H	0	0	0	0	H	0	0	0
P. coronafaciens 1348	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. eriobotryae 424	M	0	0	0	S	0	M	0	0	0	0	0	0	0	0
P. fluorescens 192	0	0	0	0	0	0	S	0	0	0	0	S	0	0	0
P. fluorescens 108	0	0	0	0	0	0	S	0	0	0	0	0	S	0	0
P. lachrymans 277	M	H	0	0	M	M	H	S	S	M	H	M	0	S	S
P. lachrymans PL-5	M	M	0	0	S	S	M	M	S	M	H	M	S	0	S
P. marginalis 667	0	0	H	H	H	H	S	M	M	H	H	H	M	M	H
P. marginalis 1689	0	0	H	H	H	H	M	H	H	H	H	H	H	M	H
P. marginata 1888	H	H	0	0	H	H	H	0	0	0	0	H	0	0	0
P. marginata 1889	H	H	0	0	S	H	H	0	0	0	0	H	0	0	0
P. mori 1415	H	M	S	0	H	H	H	0	0	H	0	H	0	0	0
P. morsprunorum 1095	H	M	0	0	M	0	M	0	0	0	0	M	0	0	0
P. polycolor 3	0	0	0	0	0	0	M	0	0	0	0	M	S	0	0
P. sesami 1017	H	M	S	0	M	M	H	0	0	0	S	M	0	0	0
P. sesami 1033	H	H	M	0	H	M	H	0	0	S	M	H	0	S	M
P. solanacearum B-139	H	H	M	M	H	H	H	M	M	H	H	H	M	M	M
P. solanacearum P-28-T	H	H	H	H	H	H	H	H	M	H	M	M	H	M	M
P. syringae 3	0	0	0	0	0	0	S	0	0	0	0	S	0	0	0
P. syringae 9	0	0	0	0	0	0	S	0	0	0	0	S	0	0	0
P. tabaci	M	M	0	0	S	0	M	0	0	0	0	M	0	0	0
P. tomato 1	M	M	S	S	M	M	M	0	0	M	0	M	0	0	0
P. sp. B62-1	0	0	0	0	0	0	0	S	0	0	0	0	0	0	0
Erwinia sp.															
E. amylovora 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. carotovora 109	M	0	M	H	H	H	S	H	H	H	H	H	H	H	H
E. chrysanthemi 201	H	S	0	H	H	H	H	H	H	H	H	H	H	H	H
E. herbicola 3162	0	S	0	0	0	S	S	S	0	0	S	S	0	0	0
E. quercina 1	0	0	0	M	0	H	M	S	S	M	M	M	S	S	M
E. rubrifaciens 4	0	0	0	0	0	H	S	S	S	M	H	M	M	M	M

[a] Sunkist sodium polypectate 6024.

[b] Activity rated according to degree of pitting produced. H = high activity, or a pit with a radius of > 5 mm from colony margin; M = moderate activity or a pit of 1-5 mm radius; S = slight activity or a pit < 1 mm radius; and 0 = no activity.

The inconsistencies noted for previous reports, which seemed to indicate that pectate gels were unsuitable for use in *Pseudomonas* identification, appear to be due primarily to differences in techniques. Not only is pH critical, but the presence of different substrates affects the formation of pits. Thus, the addition of yeast extract or other metabolizable organic nitrogen source to the gels, a common practice when testing pectolytic enzyme production by *Erwinia* sp., completely inhibited pit formation by many *Pseudomonas* sp. Presumably, this inhibition of pit formation was caused by unfavorable changes in pH, as pitting was unaffected unless the pH of the gel changed to a point where pitting normally did not occur. Such changes in the pH probably account for the report by Zucker & Hankin (8) that the pectate lyase produced by a *P. fluorescens* strain was unstable and readily inactivated by 0.1% glucose. We observed that glucose per se had no effect on pitting produced by *P. marginalis* [included in *P. fluorescens* biotype

TABLE 3. Activity of *Pseudomonas* sp. and *Erwinia* sp. on pectin gels of different pH levels

	Activity							
	4.8-5.0 (medium D)				6.8-7.0 (medium E)			
Strains	Without supplement	Plus 0.5% glucose	Plus 0.06% $CaCl_2$	Plus 0.06% $CaCl_2$ and 0.5% glucose	Without supplement	Plus 0.5% glucose	Plus 0.06% $CaCl_2$	Plus 0.06% $CaCl_2$ and 0.5% glucose
Pseudomonas sp.								
P. alliicola PA-8	H[a]	M	M	H	S	S	M	M
P. caryophylli 349	0	0	S	S	S	S	S	S
P. cepacia 63-53	M	M	M	M	S	S	M	M
P. eriobotryae 424	0	0	0	0	0	0	0	0
P. fluorescens 192	0	0	0	0	0	0	0	0
P. fluorescens 108	0	0	0	0	0	0	0	0
P. lachrymans 277	0	0	0	0	0	0	0	0
P. lachrymans PL-5	0	0	0	0	0	0	0	0
P. marginalis 667	0	0	0	0	0	S	S	S
P. marginalis 1689	0	0	M	0	0	S	0	S
P. marginata 1888	H	M	M	H	S	S	M	M
P. marginata 1889	H	M	M	H	S	S	M	M
P. mori 1415	0	0	M	S	0	0	0	0
P. morsprunorum 1095	0	0	0	0	0	0	0	0
P. polycolor 3	0	0	0	0	0	S	0	S
P. sesami 1017	0	0	0	0	0	0	0	0
P. sesami 1033	0	0	0	0	0	0	0	0
P. solanacearum B-139	M	M	H	S	H	S	H	M
P. solanacearum P-28-T	M	M	H	M	H	M	H	M
P. syringae 3	0	0	0	0	0	0	0	0
P. syringae 9	0	0	0	0	0	0	0	0
P. tabaci	0	0	0	0	0	0	0	0
P. tomato 1	0	0	0	0	0	0	0	0
P. sp. B62-1	0	0	0	0	0	0	0	0
Erwinia sp.								
E. amylovora 9	0	0	0	0	0	0	0	0
E. carotovora 109	0	0	0	0	0	0	0	0
E. chrysanthemi 201	S	0	S	0	S	0	S	S
E. herbicola 3162	0	0	0	0	0	0	0	0
E. quercina 1	0	0	0	0	0	0	0	0
E. rubrifaciens 4	0	0	0	0	0	0	0	0

[a] Activity rated according to degree of pitting produced. H = high activity or a pit with a radius of $>$5 mm from colony margin; M = moderate activity or a pit of 1-5 mm radius; S = slight activity or a pit $<$1 mm radius; and 0 = no activity.

B, Stanier et al. (7)] unless unfavorable pH changes occurred.

There are a number of different pectolytic enzymes, and these, depending on the source of the enzyme, vary widely in pH optima and conditions under which they are active. This diversity indicates that our tests, although useful, cannot be considered as detecting all enzymes which might be produced, or all organisms which might form pectolytic enzymes. Thus, the questionable results we obtained with *P. syringae* and *E. amylovora* with the polypectate from Sigma might well be an indication that pectolytic enzymes can be produced by these organisms under the proper circumstances. Likewise, the diversity in enzymes means that although organisms such as *P. mori* and *P. cepacia* may show similar patterns of activity on the different gels, the enzymes responsible for this pitting may be different. Therefore, the enzymes responsible for pitting must be characterized before the true significance of pit formation can be ascertained with regard to both the relationships of organisms to each other and to the symptoms they cause.

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