Isolation of Pectolytic Fluorescent Pseudomonads from Soil and Potatoes

Diane A. Cuppels and Arthur Kelman

Former graduate research assistant and professor, respectively, Department of Plant Pathology, University of Wisconsin-Madison, Madison 53706. Present address of senior author: Agriculture Canada Res. Inst., Univ. Sub Post Office, London, Ont., Canada. N6A 5B7.
Research supported by the College of Agricultural and Life Sciences.
Appreciation is expressed to H. M. Darling for helpful advice, to J. Gullings-Handley for assistance with the biochemical tests, to G. Gaard for preparation of electron photomicrographs, and to S. A. Vicen for preparation of illustrations.
Accepted for publication 17 May 1980.

ABSTRACT

CUPPELS, D. A., and A. KELMAN. 1980. Isolation of pectolytic fluorescent pseudomonads from soil and potatoes. Phytopathology 70:1110-1115.

Fluorescent pectolytic pseudomonads capable of causing soft rot of fleshy vegtables were isolated frequently from the surfaces and lenticels of stored potato tubers. On crystal violet pectate medium (CVP), colonies examined with oblique light appeared pink and slightly translucent and were surrounded by wide, shallow depressions. Pseudomonads of this colony morphology also were detected in the root zones of potato plants, washwater from a potato chip processing plant, field soils, a Wisconsin river and lake, and from decaying carrot and cabbage heads. Strains were found in Wisconsin soils just after the spring thaw and thus probably overwintered there. When inoculated into potato tuber and carrot slices, these strains rapidly produced a soft rot which fluoresced under ultraviolet light. When inoculated into stems of potato plants or lettuce heads, they caused a localized browning and softening of tissue. On the basis of the biochemical and physiological properties of 62 strains (including 45 from potato tuber surfaces), these bacteria were identified as strains of *Pseudomonas marginalis*.

Additional key words: bacterial soft rot of carrot and cabbage.

Pectolytic fluorescent pseudomonads have been isolated from soil, the rhizospheres of several plants, and decaying plant material (6,8,15,28,30). Several soft rot diseases of vegetables have been attributed to strains of these bacteria (11,14,33,34), which frequently were classified as *Pseudomonas marginalis* (10,24,26,34) or *P. fluorescens* (2,11). *P. marginalis* has not been thoroughly studied and its ability to macerate potato tissue, at present, serves as the principal character separating it from other groups in *P. fluorescens* and *P. putida* (30). Bacteria related or belonging to the species *P. fluorescens* also have been implicated in a number of other plant diseases, such as pinkeye, browneye, and red-xylem disease of potatoes (12,13,28) and marginal leaf spot (23) and pink rib (16) of lettuce.

Soft-rot-causing fluorescent pseudomonads with a distinctive colony morphology on crystal violet pectate (CVP) medium have been isolated from Wisconsin cabbage and carrot field soils (6). Under unusually wet conditions in the muck soil of a carrot field, as many as 10^9 pectolytic pseudomonads per gram of soil were recorded. A preliminary study indicated that these bacteria resembled *P. marginalis* (6). Subsequently, these bacteria have been detected in potato field soils and on potato tubers. The objectives of the present study were to extend the biochemical and physiological characterization of these bacteria and to determine their prevalence in soil, in potato plant root zones, and on potato tubers.

MATERIALS AND METHODS

Bacterial strains and media. The 62 pectolytic pseudomonads that were physiologically and pathologically characterized in this study were isolated independently from Wisconsin cabbage and carrot soils, from the surfaces of both healthy and diseased potato tubers, from decaying carrots and cabbage heads, and from potato chip plant washwater. *Pseudomonas marginalis* (ATCC 10844 - from endive) and *Erwinia carotovora* subsp. *atroseptica* (SR8-from potato) were included in the characterization tests as controls. The pseudomonads were maintained on nutrient agar (Difco) slants stored at 4 C; SR8 was stored in sterile double-distilled water at 21 C.

The two isolation media employed in this study were CPG (casamino acids-peptone-glucose) agar and CVP (crystal violet pectate) medium (6). Inoculated plates were incubated 3 days at 21 C.

Isolation methods. *Soil.* Soil samples (25 g) were suspended in sterile, distilled water (1:10 dilution) and shaken for 50 min on a rotary shaker (120 rpm) (20). The suspensions were diluted serially and plated on CVP and CPG media. Samples were collected from the top 10-cm of a Wisconsin cabbage seedbed soil (July 1970) and from muck soil in a Wisconsin carrot field (April 1970) in which there had been severe soft rot incidence the previous season. Samples also were taken from three Connecticut potato field soils (in 1975) (kindly supplied by David Sands) and from two Wisconsin corn field soils (in 1972).

Rhizosphere soil. Soil samples from potato plant (cultivar Russet Sebago) root zones were collected at designated times throughout the 1972 growing season (30 plants per sampling) and assayed for the presence of pectolytic pseudomonads according to the techniques described by DeBoer et al (8).

Lake mud samples. In November 1972, 25 × 6.4-cm core samples of mud were removed from the bottom of Lake Wingra, a eutrophic lake in Madison, Wisconsin. The cores were divided into six sampling levels. One gram (wet weight) from each level was placed in 0.5 ml of sterile tap water and mixed well with a Vortex mixer. The suspensions were streaked on CVP medium.

Lake water. Water samples were collected approximately 6–7 m from the shore of Lake Wingra. Water depth in the sampling area was 1 m. Samples were taken 10 cm below the surface or 10 cm above the mud bottom before and after the bottom had been disturbed. The samples were diluted and plated on CVP and CPG media.

Potato tuber surfaces. Northern Wisconsin foundation seed potatoes (Early Gem, Russet Burbank, LaChipper, Norchip, Norland, Sebago, Norgold Russet, and Red LaSoda), which had been stored at 4 C and 90% relative humidity (RH) for 6 mo, were assayed for total surface bacteria and pectolytic pseudomonads. No soft rot was evident in the tubers selected for these tests. For each cultivar, 10 samples of 10 tubers each were taken. Each 10-tuber sample was weighed, placed in a 20 × 30-cm covered container with 500 ml of sterile tap water, and shaken for 60 min at 21 C. The washwater samples were diluted and plated on CVP medium. The results were expressed as number of viable cells per square centimeter of potato surface. Approximate surface area for

0031-949X/80/11111006/$03.00/0

the 100 weighed and washed tubers of each cultivar was calculated by the formula, $S = 58.3 (V_T/4.2)^{2/3}$, in which V_T = the total volume of the tubers. This formula was based on the assumption that the tubers were spherical and the fact that potatoes occupy 0.9 $m^3/10^3$ kg fresh weight (5). If the tubers were cylindrical with an approximate length of 10 cm, the number of bacteria per square centimeter of tuber surface would be nearly the same as that for the spherical shape.

Potato tuber lenticels. The lenticels of 50 tubers each of cultivars Sebago and Russet Burbank and 20 tubers each of cultivars Superior, Red LaSoda, and Norchip (obtained from certified seed potato growers) were sampled for pectolytic pseudomonads. A 10-lenticel sample from each tuber was ground in 0.1 ml of sterile distilled water with a sterilized glass rod and the suspension was streaked on CVP medium.

Potato chip plant washwater. Samples were taken from a washing tank in a potato chip plant after approximately 9,072 kg (10 tons) of potato tubers originating from one shipment from a given grower had passed through the tank. Shipments came from Florida, Alabama, and North Dakota. The procedure used to detect pectolytic bacteria in this washwater was described previously (6).

Cabbage and carrot tissue. Samples of tissue from decaying cabbage heads and carrots were ground in 0.1 ml sterile water and streaked on CVP medium. The cabbage was collected from a university field plot and the carrots from a grower's field, in which a large number of carrots were showing soft rot symptoms (6).

Test methods for characterization of pectolytic pseudomonads. Procedures for testing the ability to rot vegetable tissue (potato tuber, carrot slices, or cabbage leaves), to produce arginine dihydrolase and cytochrome oxidase, to metabolize glucose oxidatively, and to liquefy gelatin were described previously (6). Tests for production of levan, utilization of 2-ketogluconate, formation of lipase, and nitrate reduction were those described by Lelliott et al (26).

The minimal medium (for fluorescent pseudomonads) and assay procedures for β-alanine, ethanol, and *n*-propanol utilization were described by Misaghi and Grogan (27).

The ability to form phenazine and fluorescent pigments was determined with the A and B media of King et al (22).

The hypersensitive reaction was tested in 30 day-old tobacco plants (*Nicotiana tabacum* L. 'Bottom Special') by the method of Klement et al (23).

Ability to grow at 37 C was determined by inoculating prewarmed tubes of CPG broth (10 ml) with exponentially-growing cells of the test strains and incubating the cultures in a water bath shaker set at 37 ± 0.5 C for 24 hr.

Acid production from xylose, mannitol, trehalose, sorbitol, or inositol was tested in a medium containing 2 g of Bacto casamino acids, 3 g of Bacto-purified agar, 0.03 g of phenol red, and 10 g of the test compound in 1 L distilled water. Readings were taken after 1, 2, and 7 days of incubation at 22 C.

Acid production from sucrose was detected by using the minimal medium of Misaghi and Grogan (27) supplemented with 1.5% Difco-agar, 0.0016% bromcresol purple, and 1% filter-sterilized sucrose.

Each strain, grown for 18 hr in 100 ml of 1% peptone plus 1% glycerol broth on a water bath shaker at 25 C, was tested for poly-β-hydroxybutyrate (PHB) accumulation by two methods: Burdon's fat stain with Sudan Black B (3) and chloroform extraction (17).

The number and location of flagella on the cells of strains grown 12 hr at 25 C in CPG broth under aerobic conditions were determined by using Leifson's flagella stain (25). One hundred cells per strain were examined.

Electron microscopy. Electron photomicrographs of strain PM7 were prepared as described previously (21). Strain PM7 was a typical tissue-macerating pseudomonad isolated from a potato tuber surface.

Pathogenicity tests. Procedures for pathogenicity tests on potato plants were described previously (6). Lettuce plants (*Lactuca sativa* 'Oak Leaf') (6 wk-old) were inoculated with cells from 24-hr-old pseudomonad cultures grown on CPG agar. Bacteria were smeared in cuts made near the center of each head with a sterile scalpel. Inoculated plants (two per strain) were incubated in a greenhouse moist chamber (approximately 100% RH) for 7 days at 25 C. The reaction of each plant to inoculation was rated on a 0 to 5 scale with 5 representing total collapse and death. The mean reaction rating for all plants inoculated with the same strain was the disease index of that strain.

RESULTS

Pectolytic bacteria that formed pinkish, translucent, mosaic-like (PTM) colonies (as seen with oblique lighting) with wide shallow pits on CVP medium (Fig. 1) were identified as fluorescent pseudomonads because of their ability to metabolize glucose aerobically but not anaerobically and to produce a fluorescent pigment on King's B medium (22). When potato and carrot slices were inoculated with these bacteria, extensive soft rot occurred within 18 hr. After 48 hr, the decayed slices were covered with fluorescent, light yellow, fluidal bacterial growth. The appearance of the decay differed markedly from that caused by *Erwinia carotovora* subsp. *atroseptica*. In the case of the latter, decaying tissue usually was rimmed by a dark brown or black zone (Fig. 2).

Pseudomonads with the PTM colony morphology were common on northern Wisconsin seed potato tubers, where their numbers ranged from 2×10^3 to 2×10^6 bacteria per square centimeter of tuber surface (Table 1). For the eight cultivars tested, PTM pseudomonads averaged 4.7% of the total bacterial population and 30% of the pectolytic population able to grow on CVP. No pectolytic strains of *Erwinia* were detected on these foundation seed tuber samples.

PTM pseudomonads were found in the lenticels of a large number of the potato tubers that were assayed (Table 2). The incidence for three of five cultivars from certified seed potato farms (Farms B and C) was ≤ 90% whereas that for two cultivars from a foundation seed farm (A) was approximately 35%. Although the incidence of *Erwinia carotovora* on tubers from the certified seed potato farms (Farms B and C) also was higher than that on tubers from

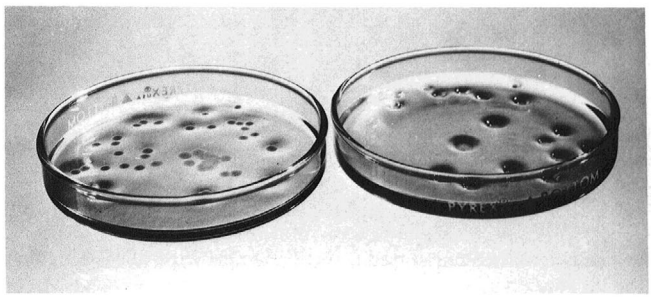

Fig. 1. Colonies of (left) a typical PTM pseudomonad (PM7) and (right) *Erwinia carotovora* subsp. *atroseptica* SR8 on crystal violet pectate (CVP) medium after incubation at 21 C for 3 days.

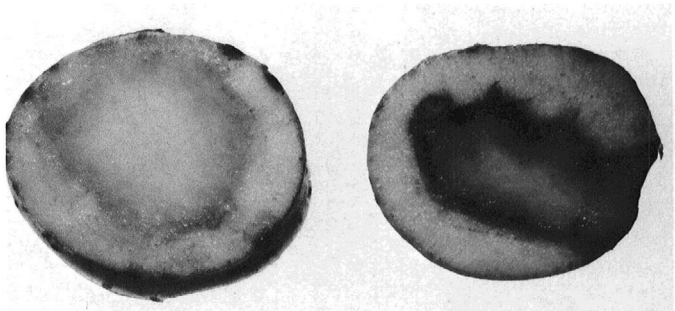

Fig. 2. Appearance of decay caused by (left) PTM pseudomonad (PM7) and (right) *Erwinia carotovora* subsp. *atroseptica* (SR8) on tuber slices of potato (cultivar Sebago). Note the distinct dark margin around the decayed tissue in the slice inoculated with SR8.

TABLE 1. Recovery of pectolytic bacteria from the surfaces of potato tubers[a] after 6 mo of storage

Potato cultivar	Bacterial populations (cells/cm^2)[b]			PTM[c] pseudomonads in Gram-negative population (%)
	Total bacteria	Pectolytic bacteria	PTM pseudomonads	
Early Gem	2.0×10^5	2.5×10^4	2.3×10^4	12
Norland	1.2×10^5	5.9×10^4	2.2×10^3	2
Norchip	8.7×10^5	1.1×10^5	4.0×10^4	5
Red LaSoda	2.5×10^7	2.6×10^6	3.4×10^3	0.014
Norgold Russet	6.6×10^7	1.8×10^7	5.7×10^5	0.87
Russet Burbank	5.4×10^5	2.5×10^5	5.1×10^4	9.5
LaChipper	3.2×10^7	3.9×10^6	2.1×10^6	6.3
Sebago	1.3×10^6	6.5×10^4	2.0×10^4	1.6

[a] Tubers were harvested in October, 1971, and stored at 4 C and 90% relative humidity until they were sampled in April, 1972.
[b] Ten tubers per sample were weighed and then washed in 500 ml sterile tap water. Serial dilutions of the washwater were plated on CVP medium. Ten ten-tuber samples of each variety were assayed. The figures presented in these columns represent the mean number of bacterial cells per square centimeter of potato tuber surface.
[c] PTM = pectolytic pseudomonads that form pink, translucent, mosaiclike colonies on crystal violet pectate (CVP) medium.

the foundation farm (Farm A), the degree of infestation for the two soft-rotting bacteria was not directly correlated.

PTM pseudomonads were isolated from the root zones (root and adhering soil) of Sebago potato plants throughout the 1972 Wisconsin growing season. Thirty different plants were assayed at 10,24,38,56,105, and 145 days after planting; the percentage of plants infested was 33, 13, 33, 53, 0, and 13, respectively. When PTM pseudomonads were present in a root zone sample, their numbers ranged from 10^3 to 10^7/g dry wt. The highest number of PTM pseudomonads occurred in samples taken at the time of seed piece decay.

TABLE 2. Recovery of PTM[a] pseudomonads and soft rot *Erwinia* from the lenticels of stored potato tubers[b]

Tuber source[c] and cultivar names	Tubers with:	
	PTM pseudomonads (%)	*Erwinia carotovora* (%)
Farm A[d]		
Sebago	36	2
Russet Burbank	34	0
Farm B[d]		
Sebago	90	92
Russet Burbank	22	76
Farm C[e]		
Superior	52	40
Red LaSoda	92	16
Norchip	100	42

[a] PTM = pectolytic pseudomonads that form pink, translucent, mosaic-like colonies on crystal violet pectate medium (CVP).
[b] Ten-lenticel samples from each tuber were ground in 0.1 ml sterile distilled water. Two plates of CVP medium were streaked with each suspension.
[c] A was a potato foundation seed farm, B and C were certified seed farms.
[d] Fifty tubers of each variety were sampled after 60 days of storage.
[e] Twenty-five tubers of each variety were sampled after 90-days storage.

In April, 1972, just after the ground thawed, soils from two Wisconsin corn fields, one sandy and the other a heavy clay, were assayed for PTM pseudomonads. Three samples of each soil were taken from 20–25 cm below the field surface (Table 3). As measured with CPG medium, the total number of bacteria averaged 1.2×10^7 cells per gram dry weight in sandy soil and 1.6×10^8 cells per gram dry weight in clay soil. The PTM pseudomonads, therefore, represented 0.07% and 0.004% of the detectable bacterial populations in sandy and clay soil, respectively. In May, 1975, samples of field soil from three potato fields in Connecticut also were examined for pectolytic pseudomonads (Table 3). In contrast to the Wisconsin corn field samples, two of these soils contained relatively high numbers of PTM pseudomonads.

In late November, 1972, mud and water samples from Lake Wingra, taken before and after the bottom had been disturbed, were assayed for PTM pseudomonads. These bacteria were found in mud samples, but not in the undisturbed water. They also were present in one of four Wisconsin river water samples, where they numbered 1.7×10^3 cells per milliliter.

PTM pseudomonads were isolated from decaying carrots and cabbage heads collected in a commercial carrot field and a cabbage seedbed, respectively. They were the only soft-rotting bacteria that were detected in numerous samples of rotting tissue from either field. When inoculated into carrots or cabbage tissue, the isolated strains produced extensive soft rot.

When 62 PTM pseudomonad strains were characterized by the determinative scheme of Lelliott et al (26) (Table 4), 61 gave LOPAT reactions typical of group IVa (*Pseudomonas marginalis*).

TABLE 3. Occurrence of PTM pseudomonads[a] in potato and cornfield soil samples collected in the early spring

Source of soil sample	Bacterial populations on CVP medium[b] (cells/g dry wt soil)		PTM pseudomonads in the Gram-negative population (%)[c]
	Total	PTM pseudomonads	
Wisconsin corn fields			
A (sandy)	6.4×10^5	8.5×10^3	1.3
B (clay)	8.1×10^5	6.8×10^3	0.84
Connecticut potato fields			
A	6.0×10^5	2.6×10^4	4.0
B	6.8×10^5	3.3×10^5	50.0
C	7.8×10^5	$< 10^3$	<0.1

[a] PTM pseudomonads = pectolytic bacteria that form pink, translucent, mosaic-like colonies on CVP medium.
[b] The Wisconsin cornfield samples were taken in April, 1972, just after the ground had thawed. The number given for each soil type is the average calculated from three separate samples. The Connecticut potato field samples were taken in May, 1975, and platings were made within 2 wk.
[c] %PTM pseudomonads = ([No. of PTM bacteria per gram of soil as determined on CVP]/[total no. of bacteria per gram of soil as determined on CVP]) × 100.

The subsidiary characteristics of the scheme confirmed placement of these bacteria in Group IVa, even though three strains were lipase-negative and one strain did not produce acid from sucrose. Since Stanier et al (32) suggested that *P. marginalis* belonged to *Pseudomonas fluorescens* biotype II, several additional characterization tests were performed (Table 5). These tests (gelatin hydrolysis, lack of growth at 37 C, flagella number (>1), lack of pyocyanine pigment on King's A, use of trehalose, β-alanine, and mesoinositol, and lack of polyhydroxybutyrate accumulation), in addition to several of the characteristics listed in Table 4 (levan, 2-ketogluconate, oxidase, and arginine dihydrolase production), indicated that the PTM pseudomonads were members of the species *P. fluorescens* as described by Doudoroff and Palleroni (9). Nitrate reduction, levan formation, and ethanol and propanol utilization, which are among the distinguishing characteristics for *P. fluorescens* biotype II, were positive for 100, 98, and 87%, respectively, of the 62 PTM strains tested. A diffusible pink pigment was produced by 53 of the 62 PTM strains after 36–72 hr of incubation on King's A medium (22). Seven of the nine strains unable to form the pigment also could not utilize ethanol or propanol. The nonpigmented strains came from potato surfaces, cabbage field soil, or cabbage tissue. The authentic strain of *P. marginalis* (ATCC 10844 from endive), although positive for ethanol and propanol utilization, did not form the pink pigment. Only one strain of those forming the pink pigment could not use ethanol and propanol.

PTM pseudomonads possessed 1–12 polar flagella per cell; the mean for the 45 strains examined was five. A cell of potato strain PM 7, which had 11 flagella, is shown in Fig. 3.

Under the test conditions followed, these pseudomonads were weakly pathogenic for both potato and lettuce plants in greenhouse inoculations (Table 6). Disease indices, based on the extent of browning and softening of tissue around the inoculation site, did not exceed 3.0 for any strain. As a control, potato plants (cultivar Russet Burbank) were stem-inoculated with *E. carotovora* subsp. *atroseptica* (SR8). The stems of these plants blackened and collapsed within 1 wk (disease index = 5.0). The disease index (2.8) on lettuce of strains isolated from cabbage tissue approximated that of the endive strain (3.0). These cabbage strains had the highest disease rating of all the PTM strains tested.

DISCUSSION

All samples of Connecticut and Wisconsin field soil collected in the early spring contained approximately the same number of bacteria able to grow on CVP medium ($6–8 \times 10^5$ cells per gram dry wt). However, the percentage of PTM pseudomonads in these bacterial populations varied considerably (<0.1 to 50%). The

TABLE 4. Characteristics of Wisconsin PTM pseudomonads[a]

			Source and Number of Strains Tested:				
	Typical reactions[b] of group IVa (*P. marginalis*)	*P. marginalis* (ATCC 10844)	Potato Surface 45	Potato Washwater 5	Carrot Field Soil 3	Cabbage Field Soil 5	Cabbage Tissue 4
Main characteristics			(number of positive reactions)				
Levan	+	+	44	5	3	5	4
Oxidase	+	+	45	5	3	5	4
Potato Rot	+	+	45	5	3	5	4
Arginine Dihydrolase	+	+	45	5	3	5	4
Tobacco Hypersensitivity	−	−	0	0	0	0	0
Subsidiary characteristics							
2-Ketogluconate	+	+	45	5	3	5	4
Lipase (margarine)	+	sl+	43	5	3	5	3
Nitrate reduction	+	+(G)[c]	45(8G)	5(3G)	3(1G)	5(4G)	4(1G)
Acid from sucrose	+	−	45	4	3	5	4

[a] These tests were used in a determinative scheme for fluorescent plant pathogenic pseudomonads developed by Lelliott, et al (26).
[b] LOPAT reactions of six strains designated as Group IVa (*P. marginalis*) by Lelliott et al (26).
[c] G = gas production.

TABLE 5. Supplementary biochemical tests for characterization of PTM pseudomonads

		Number of positive strains from:				
Test	*P. marginalis* (ATCC 10844) (1)	Potato surface (45)[a]	Potato washwater (5)	Carrot field soil (3)	Cabbage field soil (5)	Cabbage tissue (4)
Gelatin hydrolysis	1	45	5	3	5	4
Polyhydroxybutyrate accumulation	0	0	0	0	0	0
Growth at 37 C	0	0	0	0	0	0
Utilization of:						
β-alanine	1	45	5	3	5	4
Ethanol	1	40	5	3	3	3
n-propanol	1	40	5	3	3	3
Acid production:						
Mannitol	s1[b]	s45	s5	s3	s5	s4
Trehalose	1	45	5	3	5	4
Sorbitol	1	44	5	3	5	3
Meso-Inositol	1	s45	5	s3	5	3
D-Xylose	1	42	5	3	5	4
Pigment formation:						
King's A (pink)	0	38	5	3	5	2
King's B (fluorescent)	1	45	5	3	5	4

[a] Number of strains tested.
[b] s = slight acid production after 7 days.

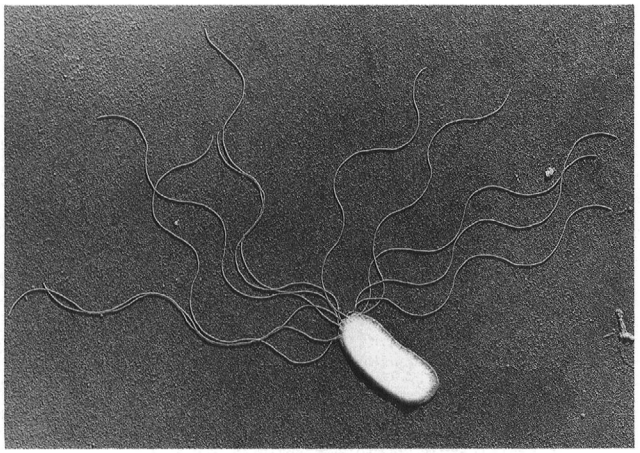

Fig. 3. Flagellated cell of PTM pseudomonad PM7 from a culture grown for 12 hr at 25 C in CPG broth (see methods) (× 15,000).

percentage of nonpectolytic fluorescent pseudomonads in the aerobic bacterial populations of Australian soils likewise showed considerable variation (31); this variation was attributed to fluctuations in the state of decomposition of soil organic matter. Sands and Hankin (30) reported a relatively low incidence of pectolytic pseudomonads in Connecticut field soils (one in 28 samples). Their low numbers may have been due to the fact that fewer PTM pseudomonads (and perhaps other pectolytic pseudomonads as well) can be recovered from soil with their FPA medium than with CVP medium (A. Kelman, *unpublished*).

Since PTM pseudomonads were detected in relatively high numbers by direct plating of samples of Wisconsin soil taken shortly after the spring thaw, they appear to withstand winter conditions in a northern climate. In contrast, strains of *E. carotovora*, although isolated from Wisconsin soils in the spring by baiting and enrichment techniques (7), have not been isolated by direct plating (6). A similar observation was made in Scotland where soft rot-causing pseudomonads were the only soft rot organisms that could be recovered in the spring, even from soils that had been heavily inoculated with *E. carotovora* subsp. *atroseptica* the previous fall (15). PTM pseudomonads can also withstand winter conditions in aqueous environments such as a lake bottom or river water. Thus, these bacteria may survive from one potato growing season to the next not only on seeds, as indicated by the survey of stored seed tubers (Table 1), but also in soil and water.

PTM pseudomonads, like *E. carotovora* (8), were present in higher numbers around potato roots and tubers than in plant-free soil. These results are in accord with previous observations on the selective increase of short Gram-negative rods, particularly pseudomonads, in plant root zones (1,18). Holding (18) found that 50% of the Gram-negative population present in plant rhizospheres were pseudomonads, one-third of which were pectolytic. In contrast to our results, he found no pectolytic Gram-negative bacteria in "plant-free" soil; ie, soil beyond the root zone of growing plants.

All the PTM pseudomonads tested were identified as *P. fluorescens* following the criteria of Doudoroff and Palleroni (9) or as *P. marginalis* following the description of this species given by Lelliott et al (26). Although not all the differentiating characters for the *P. fluorescens* biotype were determined for the PTM pseudomonads, on the basis of the tests completed, 87% of these strains belonged to biotype II. The remaining 13% more closely resembled biotype II than any of the other three biotypes or the 'miscellaneous strains' group (Biotype G of Stanier et al [32]), even though they were unable to utilize ethanol or *n*-propanol.

Ninety-five percent of the PTM pseudomonad strains differed from biotype II in being lipolytic; 85% differed in being able to produce a pink pigment on King's medium A (22). However, biotype II strains, as originally described (32), were tested for the ability to hydrolyze Tween 80, but not margarine (the latter

TABLE 6. Pathogenicity of pectolytic pseudomonad pathogens of potato and lettuce plants compared with that of the potato blackleg pathogen, *Erwinia carotovora* subsp. *carotovora*

Source of strains	Number of strains	Disease index[a]	
		Potato	Lettuce
Potato	28	1.1	1.1
Cabbage	4	1.1	1.1
Carrot field soil	3	2.3	1.3
Cabbage field soil	5	1.2	1.8
Potato tuber washwater[b]	5	0.8	1.4
P. marginalis ATCC 10844 (endive)	1	1.0	3.0
E. carotovora subsp. *atroseptica* SR8	1	5.0	2.0

[a] Pectolytic strains were tested for pathogenicity by inoculation of 4-wk-old potato plants (cultivar Russet Sebago) and 6-wk-old lettuce plants (cultivar Oak Leaf). Plant reaction was rated on a 0 to 5 scale (5 = total collapse and death). Each strain was inoculated into four potato plants and two lettuce plants. The number is an average of the reactions obtained with all strains from a particular source.

[b] Washwater came from a potato chip processing plant.

procedure was used in our study). The distinctive pink pigment of the PTM pseudomonads (produced on King's A) varied in color from a light pink to a pinkish brown, depending upon the strain; it differed distinctly from the orange-yellow pigment described for *Pseudomonas aureofaciens* (9). Furthermore, PTM pseudomonads differed from *P. aureofaciens* not only in pigment color, but also in utilization of D-xylose, sorbitol, ethanol, and *n*-propanol. Other fluroescent pseudomonads, including strains unable to cause soft rot of plants, should be tested for this pink pigment to determine whether its production might be unique to *P. marginalis*. Burkholder (4) also noticed this pigment (but not on King's A) in certain lettuce strains of *P. marginalis*.

Bacteria identified as *P. fluorescens* have been associated with pinkeye or browneye disease of potatoes (11,19). They have many characteristics in common with the PTM pseudomonads, which frequently were isolated both from potato tubers with pinkeye lesions (Kelman, *unpublished* data) and from healthy and rotting tubers with no pinkeye symptoms (Tables 1 and 2). We wish to emphasize that neither our studies nor previously published reports conclusively demonstrate that pectolytic pseudomonads identified as *P. fluorescens* or *P. marginalis* cause pinkeye. Other investigators have suggested that pathogens such as *Verticillium* or *Rhizoctonia* may predispose potato plants to this disease (12,13,29). The exact cause of pinkeye will require further investigation.

As clearly indicated by Sands and Hankin (30), pectolytic fluorescent pseudomonads that macerate potato tissue are a biochemically diverse group of bacteria. Because of this diversity, the status of *P. marginalis* (10) is unclear. The PTM pseudomonads, which are prevalent on potato tubers and in Wisconsin potato field soil, strongly resemble the lettuce (4) and chicory (14) strains of *P. marginalis*, even though they are weakly pathogenic for lettuce in greenhouse tests. Until taxonomic questions concerning *P. marginalis* can be resolved, we recommend that it be retained as a means of identifying all soft-rot causing, oxidase-positive, arginine dihydrolase-positive, fluorescent pseudomonads, including the PTM pseudomonads (35).

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