

## Diversity of Pectolytic, Fluorescent Pseudomonads Causing Soft Rots of Fresh Vegetables at Produce Markets

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### ABSTRACT

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Pectolytic bacteria (128 strains) isolated from rotted specimens of 10 vegetables were characterized. Sixty-four strains (50%) were identified as *Erwinia carotovora*, 55 strains (43%) as fluorescent *Pseudomonas* spp., and the remaining nine strains (7%) as *Bacillus* sp., *Cytophaga johnsonae*, or *Xanthomonas campestris*. Fluorescent pseudomonads accounting for almost half of vegetable rots found at markets were divided into four groups based on six physiological tests. Each group was nutritionally heterogeneous and could be divided into several (four to 11) subgroups

according to their ability to use 10 out of 30 organic compounds tested as energy or carbon sources. Oxidase-positive strains (Groups 1-3) were similar to but did not exactly fit into the ideal phenotype of *P. fluorescens* biovar II, biovar IV, or biovar V. Oxidase-negative strains (Group 4) constituting 29% (16 strains) of the total pseudomonads isolated were closely related to *P. viridiflava*, but 11 strains were unable to induce hypersensitive response on tobacco and four strains unable to use sorbitol.

*Additional key words:* *Erwinia carotovora*, postharvest decays, *Pseudomonas fluorescens*, *P. marginalis*, soft-rot bacteria.

Bacterial soft rot is the leading cause of decays of perishable vegetables at retail and wholesale markets (23). It has been shown to affect up to 6% of head-lettuce shipments inspected at New York and Chicago metropolitan areas (1,5). Although pectolytic *Erwinia* species are commonly assumed to be the principal cause of the problem (1,5,23), extensive efforts have not yet been made to identify and to compare the relative importance of pectolytic bacteria of other genera (*Pseudomonas*, *Bacillus*, *Clostridium*, and *Flavobacterium*) (14). This deficiency was probably in part due to the unavailability of a selective medium that could be used to isolate pectolytic bacteria of different genera with high recovery frequencies. The crystal-violet polypectate (CVP) medium, initially formulated by Cuppels and Kelman (6) to investigate the ecology of soft-rotting erwinias, was found to be useful for detection of pectolytic fluorescent (PF) pseudomonads present in various environments (7). Because of its low selectivity and high recovery efficiency (7), the CVP medium provides a valuable aid for studying pectolytic bacteria, especially PF pseudomonads associated with rots of fleshy vegetables in the marketplace.

The PF pseudomonads have been previously reported as a cause of postharvest rots of celery (25), chicory (8), lettuce (1,5), Chinese chard (4), cabbage (3), and potato (9). The organisms, although frequently named *P. marginalis* (7,10,15,16,21), probably consist of a nutritionally and physiologically heterogeneous group. Sands and Hankin (19) examined 140 strains of PF pseudomonads from plant tissues, soil, and sewage and found that strains causing maceration of potato disks fall within the phenotype from *P. fluorescens* to *P. putida*. Wang and Kelman (27) detected PF pseudomonads with phenotypes representative of various biovars of *P. fluorescens* in soil at different depths. It is presently unknown if PF pseudomonads associated with rots of fresh vegetables at markets are as diverse as those detected from other sources.

The present study was undertaken to examine the spectrum and relative importance of pectolytic bacteria associated with rots of

fresh vegetables; to investigate the degree of physiological and nutritional variability of PF pseudomonads isolated from naturally rotted vegetable specimens; and to determine whether they can be classified and characterized according to the scheme described for various biovars of *P. fluorescens* (17,24).

### MATERIALS AND METHODS

**Bacterial reference strains.** The following reference strains were used: *P. fluorescens* biovar I strain 13525, *P. fluorescens* biovar II strain 17816, *P. marginalis* strain 10844, *P. viridiflava* strain 12633 (all obtained from The American Type Culture Collection, Rockville, MD). *E. carotovora* subsp. *carotovora* strain SR 319, *E. carotovora* subsp. *atroseptica* strain SR-8, and *E. chrysanthemi* strain 120A (all kindly provided by A. Kelman, Dept. of Plant Pathology, University of Wisconsin).

**Rotted specimens.** A total of 154 rotted vegetable specimens, mostly bell pepper and zucchini squash, were randomly collected from local supermarkets during March–August 1984. Soft-rot of bacterial origin was usually indicated by the formation of wet and totally disintegrated lesions, which could be visually differentiated from firm and coherent lesions characteristic of rots induced by rotting fungi.

**Isolation.** Isolations were made from individual specimens on the same day they were brought into the laboratory. A loopful of rotted tissue was streaked onto an agar plate containing CVP medium (6), and the plate was incubated at 26 C for 2 days. The CVP medium was sometimes enriched with yeast extract (0.1%) to enhance the growth of oxidase-negative PF pseudomonads. Single colonies exhibiting pectolytic activity were isolated and used for further characterization. *Pseudomonas* agar F (Difco) was used for general culturing and for diagnosis of fluorescence.

**Identification of unusual strains of soft-rot bacteria.** Nine unusual strains of soft-rot bacteria identified, respectively, as *Xanthomonas campestris*, *Cytophaga johnsonae*, and *Bacillus* sp. were detected initially on CVP medium based on their colony-morphology and pigmentation. Physiological and pathological properties of these strains have been characterized separately and have been reported elsewhere (12,13).

**Characterization of bacterial isolates.** Isolates showing colonies typical of erwinias (7) were subjected to nine diagnostic tests: erythromycin sensitivity, sucrose reduction, phosphatase reaction,

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TABLE 1. Pectolytic, bacterial flora associated with soft rots of fresh vegetables at food markets

Rotted specimens	Total* strains isolated	<i>Erwinia</i> spp.		<i>Pseudomonas</i> spp.		Miscellaneous <sup>b</sup>	
		Strains (no.)	Incidence (%)	Strains (no.)	Incidence (%)	Strains (no.)	Incidence (%)
Zucchini squash	39	22	56	17	44	0	0
Bell pepper	42	21	50	18	43	3	7
Tomato	8	5	63	2	25	1	12
Cucumber	7	5	71	0	0	2	29
Lettuce	7	1	14	6	86	0	0
Broccoli	5	1	20	4	80	0	0
Spinach	9	4	44	5	56	0	0
Onion	3	2	67	0	0	1	33
Cubanell pepper	2	1	50	1	50	0	0
Carrot	2	1	50	1	50	0	0
Papaya	1	0	0	0	0	1	100
Asparagus	3	1	33	1	33	1	33
Total	128	64		55		9	
Average % incidence			50		43		7

\* A total of 154 specimens was examined; data indicate the total number of pectolytic bacteria isolated from each crop.

<sup>b</sup> Respectively identified as *Xanthomonas campestris* (13), *Cytophaga johnsonae* (12), or *Bacillus* sp.

growth at 37 C, anaerobic growth, potato rot, use of lactose, palatinose, and  $\alpha$ -methylglucoside. The tests were performed and isolates classified according to the protocols previously described (22). Initial grouping of PF pseudomonads was based on six physiological tests (LOPAT tests and nitrate reduction) as described by Lelliott et al (11). Six week-old plants (*Nicotiana tabacum* var. *xanthi*) were used to determine ability to induce hypersensitive response. Denitrification was tested by the method of Stanier et al (24).

Ability to use various carbon sources was tested using a minimal salt solution containing  $K_2HPO_4$  (0.7%),  $KH_2PO_4$  (0.2%),  $MgSO_4 \cdot 7H_2O$  (0.02%) and  $(NH_4)_2SO_4$  (0.1%) at pH 7.0. The 30 hydrocarbon sources tested were: glucose, betaine, D-tartrate, L-tartrate,  $\alpha$ -ketogluconate, adonitol, propylene glycol, erythritol, propionate, benzoate, DL-arginine, ethanol,  $\beta$ -alanine, myoinositol, L-rhamnose,  $\beta$ -cellobiose, D(+)-trehalose, sorbitol, DL-homoserine, dulcitol, D-mannitol, quinate, butyrate, trigonelline, raffinose, sucrose, maltose, xylose, melibiose, and L-arabinose. Each carbon source was sterilized by filtration and was added to the minimal salt agar medium at a final concentration of 0.2% except for ethanol and propylene glycol, which were added to a final concentration of 4%. Inoculation was made with a toothpick and inoculated agar plates were incubated at 26 C for 1 wk.

In addition to their pectolytic activity on CVP medium, PF pseudomonads were tested for the ability to degrade carboxymethyl cellulose, starch, Tween 80, and gelatin (22). Secretion of cellulases was assayed as previously described (13).

PF pseudomonads were tested for their ability to grow at 4 C on *Pseudomonas* agar F (Difco). Growth was visually determined after 10 days' incubation. Ability to macerate potato disks was tested according to the methods described by Sands and Hankin (19).

## RESULTS

**Spectrum of pectolytic bacteria isolated.** By using CVP medium, 128 strains of pectolytic bacteria representing five different genera were isolated from 154 decayed specimens showing bacterial soft-rot symptoms. The majority of strains were identified as erwinias or pseudomonads, which represented 50% (64 strains) and 43% (55 strains), respectively, of total isolates obtained. Nine unusual strains identified as members of *Bacillus*, *Xanthomonas*, or *Cytophaga* were also isolated from various rotted vegetables. The physiological and pathological properties of these strains were reported elsewhere (12,13). Table 1 summarizes the diversity of pectolytic bacteria involved in soft-rot of vegetables after harvest. Results indicate that PF pseudomonads are responsible for a much greater proportion of vegetable rots than previously assumed (1,5,23) or reported (26).

**Properties of erwinias.** All of 64 strains were identified as *E.*

TABLE 2. Grouping of pectolytic, fluorescent pseudomonads isolated from naturally rotted vegetable specimens

Tests	Group 1 (19 strains)	Group 2 (9 strains)	Group 3 (11 strains)	Group 4 (16 strains)
Levan formation	+	+	-	$\pm^a$
Oxidase reaction	+	+	+	-
Potato rot	+	+	+	+
Arginine dihydrolase	+	+	+	-
Tobacco hypersensitivity	-	-	-	$\pm^b$
Nitrate reduction	+	w <sup>c</sup>	-	-
% Incidence	35	16	20	29

<sup>a</sup> Seven strains formed mucoid slime similar to levan on sucrose medium.

<sup>b</sup> Eleven strains failed to induce the hypersensitive reaction on tobacco.

<sup>c</sup> Delayed or very weak reaction.

*carotovora* based on the following observed properties: formation of typical colonies on CVP medium (7), peritrichously flagellated, facultatively anaerobic, gram-negative, nonspore forming, phosphatase-negative, and insensitive to erythromycin and unable to grow at 37 C. Four strains (6%) were able to reduce sucrose and form acid from palatinose, and  $\alpha$ -methylglucoside, and these were classified as *E. c.* subsp. *atroseptica*. The other 60 strains (94%) possessed properties typical of *E. c.* subsp. *carotovora* (22). These results confirm the earlier report of Vincelli and Cappellini (26) who showed that *E. c.* subsp. *atroseptica* is occasionally involved in soft-rot of bell pepper originated from Florida.

**General properties of PF pseudomonads.** The 55 strains of pseudomonads isolated exhibited pectolytic activity on CVP medium and produced fluorescent pigment on *Pseudomonas* agar F. All strains grew at 4 C but not at 37 C, and all but two strains were able to liquefy gelatin. Production of amylase and cellulase was not detected for any strain. Ability to hydrolyze Tween 80 varied among the strains. Strong hydrolysis was generally detected for oxidase-positive strains, whereas weak or no hydrolysis was found for oxidase-negative strains. Constitutive or inducible pectate lyase activity was found for all strains.

**Physiological grouping of PF pseudomonads.** The PF pseudomonads obtained in the study were divided into four groups according to six physiological tests (Table 2). Groups 1-3 were positive for oxidase and arginine dihydrolase and could be named collectively as *P. marginalis* as previously suggested (7). However, each group was distinct in its ability to form levan or to reduce nitrate or both. Group 4, consisting of 29% (or 16 out of 55 strains) of PF pseudomonads obtained, could be readily differentiated from Groups 1-3 based on its members' negative reaction in the tests for oxidase, arginine dihydrolase, and nitrate reductase. Furthermore, members of Group 4 grew poorly on CVP medium, usually giving small and rigid colonies. Rots of vegetables at retail

markets were caused by four different physiological groups of PF pseudomonads, and rots of a given vegetable could be associated with more than one physiological group of organisms (Table 3).

**Nutritional variability of PF pseudomonads.** To determine if each physiological group had characteristics comparable to either biovars of *P. fluorescens* or to *P. viridiflava*, all strains were tested for their ability to use 30 compounds as carbon and energy sources. Six compounds used by all strains were glucose, D(+)-xylose, L-arabinose, D-mannose, D-mannitol, betaine, and  $\alpha$ -ketoglucuronate. Nine compounds not used by any strains were benzoate, maltose, dulcitol, D(+)-raffinose, DL-arginine, trigonelline, quinate, and DL-homoserine. The four physiological groups and strains within each group varied in their ability to utilize 10 selective compounds (sorbitol, propionate, trehalose, L-rhamnose, sucrose, erythritol, propylene glycol, ethanol, D-tartrate, and adonitol). Based on their selective use of these 10 compounds, each physiological group could be further divided into several nutritional subgroups (Tables 4-7). The relationship of each physiological group to a known biovar of *P. fluorescens* or to *P. viridiflava* was compared. Apart from pectolytic activity, some properties that were unique for each group were as follows:

**Group 1.** This group positive for all but one (tobacco-hypersensitivity) physiological tests was closely related to biovar II of *P. fluorescens* proposed by Stanier et al (17,24) or to Group IV of *P. marginalis* reported by Lelliott et al (11). On the basis of their ability to use 10 selective compounds, seven nutritional subgroups were found (Table 4). This group did not fit exactly into the ideal phenotype of *P. fluorescens* biovar II with regard to use of four organic compounds (adonitol, sucrose, propylene glycol, and ethanol). Sucrose was used by only one strain, whereas nine out of 19 strains were able to catabolize adonitol, a characteristic of biovar I of *P. fluorescens*.

**Group 2.** This group was also positive for most of physiological tests (Table 2) and could be readily differentiated from Group 1 by its weak activity to denitrify and by its unique pattern of use of 10

selective carbon sources. Members of this group were closely related to *P. fluorescens* biovar IV based on their nutritional properties, but some differences in their ability to use sucrose, propylene glycol, and ethanol were observed (Table 5).

**Group 3.** A total of 11 strains was placed in this group. Like *P. fluorescens* biovar V (17), members of this group were positive for reactions of oxidase and arginine dihydrolase but negative for levan formations and nitrate reduction. Nutritional variability among PF pseudomonads was greatly amplified in Group 3. Table 6 illustrates 11 different nutritional patterns conferred by 11 strains within the group.

**Group 4.** This group accounted for 29% (16 strains) of the total pseudomonads (55 strains) isolated. Members of this group were characterized by negative test reactions for oxidase, arginine-dihydrolase, and nitrate-reductase, and by less nutritional versatility than other groups. Several carbon sources (such as propionate, trehalose, propylene glycol, ethanol, rhamnose, sucrose, and adonitol) used by most if not all of oxidase-positive strains were not used by any strains within the group. They were closely related to, but by no means identical to, the phenotype of *P. viridiflava* described by Billing (2) or to the Group II plant-pathogenic pseudomonads described by Lelliott et al (11). Only five out of 16 strains placed in the group were able to induce a hypersensitive reaction in tobacco. Seven strains were able to form mucoid slime similar to levan on sucrose-containing medium. Like oxidase-positive strains (Groups 1-3) described earlier, oxidase-negative strains also constitute a physiologically and nutritionally heterogeneous group. Five subgroups within Group 4 are listed in Table 7.

## DISCUSSION

Pectolytic bacteria belonging to the genera of *Erwinia*, *Pseudomonas*, *Clostridium*, *Bacillus*, and *Flavobacterium* have been previously reported (14) to be associated with postharvest rots of various food crops. This study shows that two other bacteria in the genera of *Xanthomonas* and *Cytophaga* are also involved. Furthermore, we found that pectolytic, fluorescent (PF) pseudomonads are much more important as soft-rot pathogens than previously thought as 43% (55 out of 128 strains) of the pectolytic bacteria isolated are PF pseudomonads (Table 1). The PF pseudomonads may not be as destructive as soft-rotting erwinias in the field, but they undoubtedly represent a threat to fresh produce stored at low temperatures. An earlier study of Brokheurst and Lund (3) and the one described here have shown that PF pseudomonads are able to grow and subsequently macerate plant tissues at 6 C or below. Refrigeration, frequently employed to prolong the shelf-life and to reduce decay of fresh produce caused by erwinias (23) is apparently ineffective against PF pseudomonads. A new control strategy, therefore, needs to be designed for this group of organisms.

TABLE 3. Soft-rots of vegetables found at food markets caused by various physiological groups of pectolytic fluorescent pseudomonads

Rotted specimens	Total no. strains isolated	Strains assigned to each physiological group (no.)			
		Group 1	Group 2	Group 3	Group 4
Bell pepper	18	7	7	0	4
Zucchini squash	17	7	1	0	9
Tomato	2	0	0	0	2
Lettuce	6	1	1	3	1
Broccoli	5	1	0	4	0
Spinach	5	2	0	3	0
Cubanell pepper	1	1	0	0	0
Asparagus	1	0	0	1	0

TABLE 4. Nutritional variation among 19 strains of Group 1 and their relation to *Pseudomonas fluorescens* biovar II

Utilization of:	Subgroups (No. strains) <sup>a</sup>							No. <sup>b</sup> Strains (+)	Percent <sup>c</sup> (+)	Percent (+) <sup>d</sup> <i>P.f.</i> biovar II
	a (1)	b (8)	c (1)	d (2)	e (2)	f (3)	g (2)			
Sorbitol	+	+	+	+	+	+	+	19	100	90-100
Trehalose	+	+	-	+	+	+	+	18	95	90-100
Erythritol	+	+	+	+	+	+	-	17	89	11-89
Propionate	+	+	+	+	+	+	+	19	100	90-100
Propylene glycol	+	+	+	+	-	-	-	12	63	90-100
Ethanol	+	+	+	+	-	-	-	12	63	90-100
Tartrate	+	+	+	-	+	-	-	12	63	11-89
Rhamnose	+	+	+	+	-	-	-	12	63	11-89
Sucrose	+	-	-	-	-	-	-	1	5	90-100
Adonitol	+	-	+	-	+	+	+	9	47	0-10

<sup>a</sup> Nineteen strains in the group were tested for their ability to use 10 selective C-sources. Seven subgroups (a-g) were identified; each subgroup exhibited a distinct pattern of utilization. The number of strains belonging to each subgroup was listed in parenthesis. +: ability to use; -: inability to use.

<sup>b</sup> The total number of strains showing positive growth in the minimum medium containing a given C-source.

<sup>c</sup> Calculated with the formula: No. strains (+) / 19 × 100.

<sup>d</sup> Data from Palleroni (17). % (+) indicates the percentage of strains in biovar II of *P. fluorescens* that is positive for growth in the minimum medium containing a given C-source.

The usefulness of CVP medium for isolation of pectolytic erwinias and pseudomonads has been previously discussed (6,7). The low selectivity of this medium offers a great advantage over other selective media such as the FPA medium of Sands et al (20). During the study, we found that strains of *Bacillus*, *Xanthomonas*, *Cytophaga*, and oxidase-negative pseudomonads (Group 4) in our collection failed to grow on FPA medium. This may partially explain the discrepancy existing between our results and those of Vincelli and Cappellini (26), who employed FPA medium and found that only 3% of bacterial rots of bell pepper were associated with PF pseudomonads.

Oxidase-negative *P. viridiflava* is usually considered a field pathogen (2,28) and has not been suspected of being an important cause of postharvest decays (14). In this study, 29% (16 out of 55 strains) of PF pseudomonads were oxidase-negative and were placed together as Group 4. Although all of them possess properties resembling *P. viridiflava*, some strains did not use sorbitol or cause a hypersensitive response on tobacco in contrast to *P. viridiflava*. It awaits to be determined if *P. viridiflava* represents a continuum group that falls between *P. syringae* and *P. fluorescens* (or *P. marginalis*).

Oxidase-positive PF pseudomonads associated with rots of fresh produce are nutritionally and physiologically as diverse as those detected in other environments (15,16,18,19,26). We tried to classify them according to the determinative scheme described for biovars of *P. fluorescens* (17,24). We found that they were similar

TABLE 5. Nutritional variation among nine strains of Group 2 and their relation to *Pseudomonas fluorescens* biovar IV

Utilization	Subgroups (no. strains) <sup>a</sup>				Strains <sup>b</sup> (+)	% <sup>c</sup> (+)	% (+) <sup>d</sup> P.f. bio. IV
	a (1)	b (1)	c (3)	d (4)			
Sorbitol	+	+	+	+	9	100	90-100
Propionate	+	+	+	+	9	100	90-100
Trehalose	+	-	+	+	9	100	90-100
Erythritol	+	-	-	-	1	11	11-89
Propylene glycol	-	-	+	-	3	33	0-10
Ethanol	-	-	+	-	3	33	0-10
Tartrate	+	-	-	-	1	11	0-10
Rhamnose	-	+	-	-	1	11	90-100
Sucrose	-	-	-	-	0	0	0-10
Adonitol	-	-	-	-	0	0	0-10

<sup>a</sup>Nine strains in the group were tested for their ability to use 10 selective C-sources. Four subgroups (a-d) were identified; each subgroup exhibited a distinct pattern of utilization. The number of strains belonging to each subgroup was listed in parentheses. +: ability to use; -: inability to use.

<sup>b</sup>The total number of strains showing positive growth in the minimum medium containing a given C-source.

<sup>c</sup>Calculated with the formula: No. strains (+)/9 × 100.

<sup>d</sup>Data from Palleroni (17). % (+) indicates the percentage of strains in biovar IV of *P. fluorescens* that is positive for growth in the minimum medium containing a given C-source.

TABLE 6. Nutritional variation among 11 strains of Group 3 and their relation to *P. fluorescens* biovar (one subgroup contains one strain)

Utilization	Subgroups <sup>a</sup>											Strains <sup>b</sup> (+)	Percent <sup>c</sup> (+)	% (+) <sup>d</sup> P.f. biovar V
	a	b	c	d	e	f	g	h	i	j	k			
Propionate	+	+	-	+	+	-	+	+	-	+	-	9	69	90-100
Rhamnose	+	+	+	+	+	+	-	-	-	-	-	8	62	11-89
Trehalose	+	-	-	+	+	+	+	+	+	-	-	9	69	11-89
Sorbitol	+	-	-	+	+	+	-	-	-	-	-	6	46	11-89
Tartrate	-	+	+	+	-	-	+	+	+	-	-	6	46	11-89
Sucrose	-	+	+	-	-	+	+	-	+	-	-	5	38	11-89
Ethanol	+	-	+	-	-	-	-	-	-	-	-	3	23	11-89
Propylene glycol	+	-	-	-	-	-	-	-	-	+	-	3	23	11-89
Adonitol	+	+	-	-	-	-	-	-	-	-	-	2	15	11-89
Erythritol	-	+	+	-	-	-	-	-	-	-	-	2	15	11-89

<sup>a</sup>Eleven strains in the group were tested for their ability to use 10 selective C-sources. Seven subgroups (a-k) were identified; each subgroup exhibited a distinct pattern of utilization. One subgroup represents one strain. +: ability to use; -: inability to use.

<sup>b</sup>The total number of strains showing positive growth in the minimum medium containing a given C-source.

<sup>c</sup>Calculated with the formula: No. strains (+)/11 × 100.

<sup>d</sup>Data from Palleroni (17). % (+) indicates the percentage of strains in biovar V of *P. fluorescens* that is positive for growth in the minimum medium containing a given C-source.

TABLE 7. Physiological and nutritional variation among 16 strains of Group 4 and their relation to *Pseudomonas viridiflava*

Tests	Subgroups (No. strains) <sup>a</sup>					No. strains <sup>b</sup> (+)	Percent <sup>c</sup> (+)	<i>P. viridiflava</i> <sup>d</sup> % (5)
	a (1)	b (6)	c (3)	d (5)	e (1)			
Mucoid growth	+	+	-	-	-	7	44	0-10
Tobacco hypersensitivity	-	-	-	+	-	5	31	90-100
Gelatin liquefaction	+	+	+	+	-	15	94	90-100
Utilization of:								
Sorbitol	+	+	-	+	-	12	75	90-100
Propionate	+	-	-	-	-	1	6	11-89
Trehalose	-	-	-	-	-	0	0	0-10
Erythritol	+	+	+	+	-	15	94	90-100
Propylene glycol	-	-	-	-	-	0	0	0-10
Ethanol	-	-	-	-	-	0	0	0-10
D-Tartrate	+	+	+	+	+	16	100	90-100
Rhamnose	-	-	-	-	-	0	0	0-10
Sucrose	-	-	-	-	-	0	0	0-10
Adonitol	-	-	-	-	-	0	0	0-10

<sup>a</sup>Sixteen strains in the group were tested for their ability to use 10 selective C-sources. Seven subgroups (a-e) were identified; each subgroup exhibited a distinct pattern of utilization. The number of strains belonging to each subgroup was listed in parentheses. +: ability to use; -: inability to use.

<sup>b</sup>The total number of strains showing positive growth in the minimum medium containing a given C-source.

<sup>c</sup>Calculated with the formula: No. strains (+)/16 × 100.

<sup>d</sup>Data from Palleroni (17). % (+) indicates the percentage of strains in *P. viridiflava* that is positive for growth in the minimum medium containing a given C-source.

but not identical to biovar II, biovar IV, and biovar V of *P. fluorescens*. Whether oxidase-positive PF pseudomonads should be classified as *P. fluorescens* (3,9,18,19,26) can be determined only after a broader collection of strains are examined. From results as presented earlier (3,18,19,26) and in this study, it is clear that a scheme for classification of soft-rotting fluorescent pseudomonads that are positive for reactions of oxidase and arginine dihydrolase has to be devised. The name *P. marginalis* apparently is not sufficient to reflect the physiological and nutritional diversity of the group. The six physiological tests (Table 2) and the differential ability to use 10 selective compounds (Tables 4-7) have been used temporarily in our laboratory to characterize soft-rotting, fluorescent pseudomonads associated with market problems.

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