CULTURAL, PHYSIOLOGICAL, AND BIOCHEMICAL PROPERTIES OF ISOLATES OF PHILIPPINE PSEUDOMONAS SOLANACEARUM

ELDON I. ZEHR

Reprinted from

PHILIPPINE PHYTOPATHOLOGY

Volume 6, Number 1 and 2, Pages 29 to 43

CULTURAL, PHYSIOLOGICAL, AND BIOCHEMICAL PROPERTIES OF ISOLATES OF PHILIPPINE PSEUDOMONAS SOLANACEARUM

ELDON I. ZEHR

Former Graduate Assistant, Department of Plant Pathology, Cornell University, Ithaca, New York 14850. Present address: Department of Plant Pathology and Physiology, Clemson University, Clemson, South Carolina 29631.

Portion of Ph. D. thesis.

The interest and advice of R. S. Dickey, K. G. Parker, and H. D. Thurston is gratefully acknowledged.

This research was supported in part by Ford Foundation funds under the University of the Philippines-Cornell Graduate Education Program.

ABSTRACT

The cultural, physiological, and biochemical properties of 35 isolates of *Pseudomonas solanacearum* from the Philippines and one isolate each from North Carolina and Colombia were comparatively investigated. Among isolates from the Philippines, differences were observed in colony characteristics, production of brown pigment in several media, hydrogen sulfide production, reaction in litmus milk with cream, and utilization of maltose, lactose, and cellobiose. Philippine isolates differed from North Carolina isolate K-60 in colony characteristics and utilization of dulcitol, mannitol, arabinose, and tartrate; and from Colombia isolate S-208 in colony characteristics, pellicle formation in nutrient broth, optimum temperature for growth, diffusible pigment production in certain media, sensitivity to sodium chloride, growth characteristics in nutrient broth, and utilization of sorbitol, dulcitol, mannitol, i-inositol, ethanol, and arabinose. Among Philippine isolates, there was no apparent correlation between cultural, physiological, or biochemical properties, and host range.

Many investigations on the cultural, physiological, and biochemical properties of *Pseudomonas solanacearum* E. F. Smith have been made, but widespread disagreement concerning these properties remains unsettled (Kelman, 1953). No doubt, some differences resulted from the use of different techniques and/or methods by various investigators, but several investigations that utilized uniform techniques and environmental conditions indicate that there are true differences among strains. Hayward (1964) observed that isolates differ in utilization of arabinose, lactose, maltose, cellobiose, sorbitol, dulcitor, mannitol, malonate, and i-inositol as carbon sources in a semisynthetic medium. Harrison (1961) observed that isolates collected in Australia differ in certain properties, including utilizaton of lactose, maltose, and xylose; starch hydrolysis; and production of a diffusible brown pigment in agar media. In other comparative studies, Vasudeva (1955)

observed that isolates from India differ in utilization of xylose, raffinose, and salicin. In Japan (Okabe and Goto, 1952) some isolates produce gas from mannitol and dextrose, while others do not; and they differ qualitatively in utilization of mannitol and lactose.

In the Philippines, isolates of *P. solanacearum* differ considerably in virulence to tobacco and ginger, and to a lesser extent to peanut, pepper, and tomato (Zehr, 1971). An investigation was undertaken to determine whether these isolates also differ in cultural, physiological, and biochemical properties, and to determine whether such differences might be related to differences in virulence.

Materials and Methods

Thirty-five isolates of *P. solanacearum* from the Philippines, isolate K-60 from North Carolina, and isolates S-208 from Colombia, which were used in host range studies (Zehr, 1971), were compared in cultural, physiological, and biochemical tests. Philippine isolates were identified as *P. solanacearum* by pathogenicity tests on tomato (*Lycopersicon esculentum* Mill.) or other known suscepts of this pathogen, gram reaction, and colony appearance on tetrazolium medium (TTC) (Kelman, 1954). Stock cultures maintained in distilled water at room temperature (23-32 C) were tested periodically for virulence and for purity by streaking on TTC medium. Before each test, a loopful of bacterial suspension was transferred from stock cultures to nutrient agar slants and incubated at 32 C for 48 hr. The resulting bacterial growth was used for inoculum.

Most of the tests were conducted using procedures described in the Manual of Microbiological Methods (Society of American Bacteriologists, 1957). Additional tests or deviations from these procedures will be described in detail. Tests were replicated 4 to 6 times and made at 32-33 C, unless otherwise, indicated. Experimental results were analyzed numerically by computer and isolates were grouped according to similarities as described by Lessel and Holt (1970).

Gram reaction. — The Hucker modification of the gram stain was used.

Colony characteristics. — Colonies were observed on nutrient agar, potato-dextrose agar plus 1% peptone (PDPA), and TTC medium using a minimum of three plates per isolate. Difco or Baltimore Biological Laboratory (BBL) nutrient broth plus 1.5% agar was used for the nutrient agar formula. PDPA was prepared from fresh potatoes, including 1.5% agar and 2.0% dextrose, and pH was adjusted to 6.8 to 7.0.

Nutrient broth. — Difco nutrient broth was used.

Litmus milk. — All isolates were tested both in cream-free litmus milk and in litmus milk with cream. Difco litmus milk was used for the cream-free test, but for the test with cream a saturated aqueous solution of

litmus was added to fresh milk containing 2% butterfat, until a light lavender color was observed (Society of American Bacteriologists, 1957).

Gelatin utilization. — Nine isolates were tested at 20 C for liquefaction of gelatin at a 12% aqueous concentration, however, incorporation of gelatin (0.4%) in nutrient agar proved to be a superior method for this test. After growth appeared (48-72 hr), the medium was flooded with a mercuric chloride-HCl solution (Society of American Bacteriologists, 1957) to precipitate the gelatin in the medium. A clear zone surrounding the bacterial growth was interpreted as a positive test for gelatin utilization.

Steamed potato slants. — Fresh potatoes were cut into slants 5-7 mm^2 at the base, placed in test tubes with 1 ml of 1.5% water agar to hold the slants in position, and sterilized for 15 min at 121 C. Tests were made after incubation at room temperature (23-30 C) and at 32 C.

Motility. — Motility was tested in the Difco motility medium (Anonymous, 1953), to which 0.005% 2, 3, 5-triphenyl tetrazolium chloride was added to aid detection of motility (Gershman, O'Meara, and Chute, 1959).

Sodium chloride tolerance. — Growth was determined in Difco nutrient broth containing 0.5, 1.0, and 2.0% sodium chloride.

Hydrogen sulfide production. — Hydrogen sulfide (H_2S) production from 1% tryptone broth was tested by the lead acetate-impregnated filter paper method. Sixteen isolate were tested for H_2S production from cystine, using the Ayers, Rupp, and Johnson basal salts medium with dextrose as a carbon source (Rhodes, 1959). Nine isolates were tested further, using peptone-iron agar as an indicator of H_2S production.

Pectolytic activity. — Starr's method (Starr, 1947) for testing the presence of pectolytic enzymes was used. Sodium polypectate (Nutritional Biochemicals Corp.) was used as the substrate for this test.

Indole production. — After growing the bacteria in 1% tryptone broth for 24 hr, Kovacs' reagent was added to test for the presence of indole.

Catalase production. — Isolates were transferred from stock cultures to nutrient agar slants and grown at room temperature (23-30 C) for 48 hr. Two ml 10-vol (3%) hydrogen peroxide was added to each tube and observed for evolution of gas, indicating catalase activity.

Kovacs' oxidase test. — One loopful of bacteria from 4-day-old nutrient agar cultures was transferred to filter paper moistened with a drop of 1% aqueous N,N,N',N'-tetramethyl-p-phenylene-diamine dihydrochloride. Appearance of a violet color was considered a positive oxidase reaction (Kovacs, 1956).

Production of green fluorescent pigment. — Clara's medium (Clara, 1934) was used for this test.

Nitrate reduction. — Reduction of nitrates was tested in nutrient broth plus 0.1% sodium nitrate. Sulfanilic acid and a-naphthylamine reagents were added after 12 hr, 24 hr, and one week to test for nitrites.

Starch hydrolysis. — Nutrient agar containing 0.2% starch was poured in plates. A single streak of bacterial suspension was made on the medium, and incubated at 32 C for 3 days. Plates then were flooded with Gram's iodine solution to test for starch hydrolysis.

Methyl Red-Voges Proskauer test. — The medium and reagents for this test were prepared as suggested in the Manual of Microbiological Methods (Society of American Bacteriologists, 1957). Reagents were added two days after setting the test.

Optimum temperature. — This test was made in nutrient broth at temperatures of 15, 21, 27, 33, and 39 C. Optimum temperature for growth was determined by observing turbidity after 16 hr, 24 hr, 2 days, and 8 days.

Utilization of amino acids as sources of carbon and nitrogen. — Utilization of asparagine was tested according to the method of Starr and Weiss (1943) in a nitrogen and carbon-free basal medium. Three consecutive transfers were made to tubes of fresh medium to ensure that growth did not result from nutrient carry-over in the initial transfer. Glassware used in the experiment was washed in acid, rinsed thoroughly first in tap water and then in distilled water. Asparagine was sterilized by filtration and added aseptically to the autoclaved basal medium for a final concentration of 0.5%.

Tyrosine was tested similarly as a substrate for carbon and nitrogen, but repeated transfers from the tyrosine medium were not made. The concentration of tyrosine used was 0.025%.

Organic sources of carbon. — The synthetic basal salts medium of Ayers, Rupp, and Johnson (Society of American Bacteriologists, 1957) was used to test the utilization of 23 different materials as sources of carbon. These were: dextrose, sucrose, mannose, galactose, raffinose, levulose, maltose, rhamnose, lactose, cellobiose, salicin, sorbitol, dulcitol, mannitol, i-inositol, arabinose, ethanol, glycerol, tartrate, acetate, citrate, malonate, and formate. Brom thymol blue was added to the medium initially as a 1.6% alcoholic solution but investigation showed that the alcohol could support bacterial growth. Therefore, the indicator was added as an aqueous suspension for most of the tests. Glassware was washed thoroughly in an acid cleaning solution and rinsed in distilled water before use. The basal medium was dispensed in test tubes and autoclaved, while the carbon sources were sterilized by Millipore filtration and added aseptically to the sterilized basal medium. Organic acids were tested at 0.5% concentration; other materials were tested at 1.0%. All tube cultures were incubated at 32 C, except

for brief intervals during hot weather when the incubation temperature rose to 36 C. A maximum of 3 weeks was allowed for completion of these tests.

Results

Gram stain. — All isolates were short, gram-negative rods.

Colony characteristics. — Differences in colony size, pigmentation, viscosity, and production of a diffusible brown pigment were apparent among many of the 37 isolates when they were grown on TT or PDPA media. On TTC medium, most Philippine isolates grew rapidly and in 48 hr produced convex, irregularly round colonies (4-6 mm diam) that tended to flow readily. A few isolates, notably LB-6 and LC-1, always produced smaller colonies (2-3 mm diam) that remained discrete. All Philippine isolates produced colonies that were white with glistening, reddish-pink centers. Colonies from isolates K-60 (North Carolina) and S-208 (Colombia) were similar, but had dull, red centers and were smaller than Philippine isolates (except LB-6 and LC-1). Colonies of K-60 were more fluid than were those of S-208. All isolates, except S-208, produced a diffusible brown pigment in the medium, but isolates DC-3. DC-4, V-1, and K-60 were especially proficient in production of this pigment (Table 1).

On PDPA, greater variation among Philippine isolates was noted, especially in pigmentation of the colonies. Colonies after 48 hr were 3-5 mm diam and very fluid. Some were pinkish-buff, others were white and opaque, and still others grayish-white and translucent. Colonies derived from isolate LC-1 were smaller (1-3 mm) on this medium, as was observed on TTC, but the diameter of those from LB-6 did not differ from other isolates. Isolates S-208 and K-60 were brownish-white, relatively small (1-3 mm diam for S-208; 2-4 mm diam for K-60), and less fluid than Philippine isolates. As was observed in TTC medium, isolates DC-3, DC-4 V-1, and K-60 were notable in production of a diffusible brown pigment in PDPA.

On nutrient agar, colonies were circular, convex with irregular margins, fluid, grayish-white or brownish-white, 2-3 mm diam, and there were few apparent differences among isolates. Little diffusible brown pigment was produced when Difco nutrient agar was used, but on BBL nutrient agar pigment production was similar to that noted with TTC and PDPA media.

Growth in nutrient broth. — All isolates grew well in nutrient broth and produced abundant, diffused growth with white sediment. All isolates except S-208 produced a thin pellicle (S-208 produced none), and cultures of S-208 and K-60 were less turbid than those of other isolates. Isolates DC-3, DC-4, V-1, and K-60 produced brown pigment in the medium after 6-10 days.

Reaction in litmus milk. — Smith (1914) described as "var. *asiaticum*" a strain of *P. solanacearum* that produced acid from litmus milk with

TABLE 1. Summary of differences observed in cultural, physiological, and biochemical properties of 37 isolates of *Pseudomonas solanacearum*

Isolates	Optimum temp (°C)	Pellicle (nutrient broth)	Diffusible brown pigment				Growth 1% NaCl	Black pigment on steamed potato	Acid in litmus milk with cream	H_2S produced			Acid from carbon sources									
			TTC	PDPA	Nutrient broth	Tyrosine				Peptone-iron agar	Tryptone	Cystine	Lactose	Maltose	Cellobiose	Sorbitol	Dulcitol	Mannitol	i-inositol	Ethanol	Arabinose	Tartrate
A-2	33	+[a]	±	+	-	+	+	±	-	-	-		+[b]	++	+	++	++	++	++	+	+	++
A-3	33	+	+	+	-	+	+	±	+		-	+	+	++	+	++	++	++	++	+	+	++
Ba-1	33	+	+	+	-	+	+	-	-		-		++	++	+	++	++	++	++	+	+	++
BC-1	33	+	+	+	-	+	+	-	-		-		++	++	++	++	++	++	++	++	+	++
Ca-1	33	+	+	+	-	+	+	±	-		-		+	+	+	++	++	++	++	+	+	++
Ce-1	33	+	+	+	-	+	+	±	-		-	-	++	++	+	++	++	++	++	+	+	++
CO-7	33	+	+	+	-	+	+	±	+	-	-		+	+	+	++	++	++	++	+	+	++
Da-1	33	+	+	+	-	+	+	±	+		-		+	++	+	++	++	++	++	+	+	++
DC-1	33	+	+	+	-	+	+	-	-		-		+	++	+	++	++	++	++	+	+	++
DC-7	33	+	+	+	-	+	+	±	-		-	+	+	++	+	++	++	++	++	+	+	++

TABLE 1.—contd — 2

Isolates	Optimum temp (°C)	Pellicle (nutrient broth)	Diffusible brown pigment			Tyrosine	Growth 1% NaCl	Black pigment on steamed potato	Acid in litmus milk with cream	H_2S produced			Acid from carbon sources									
			TTC	PDPA	Nutrient broth					Peptone-iron agar	Tryptone	Cystine	Lactose	Maltose	Cellobiose	Sorbitol	Dulcitol	Mannitol	i-inositol	Ethanol	Arabinose	Tartrate
GS-2	33	+	+	+	-	+	+	-	+		-		+	+	++	++	++	++	++	+	+	++
GS-4	33	+	+	+	-	+	+	-	±	-	-	+	+	++	++	++	++	++	++	+	+	++
I-2	33	+	+	+	-	+	+	±	-		-	±	+	++	+	++	++	++	++	+	+	++
I-3	33	+	+	+	-	+	+	±	±	-	-		++	++	++	++	++	++	++	+	+	++
I-4	33	+	+	+	-	+	+	±	±		-	+	++	++	+	++	++	++	++	+	+	++
Ki-1	33	+	+	+	-	+	+	-	±		-	+	+	++	++	++	++	++	++	+	+	++
La-1	33	+	+	+	-	+	+	±	-		-		++	++	+	++	++	++	++	++	+	++
La-2	33	+	+	+	-	+	+	±	±		-		++	++	+	++	++	++	++	+	+	++
LB-4	33	+	+	+	-	+	+	±	-	-	-		++	++	+	++	++	++	++	+	+	++
LB-5	33	+	+	+	-	+	+	±	±	-	-	±	++	++	+	++	++	++	++	+	+	++
LB-6	33	+	+	+	-	+	+	±	-	-	-	+	++	++	+	++	++	++	++	+	+	++
LC-1	33	+	+	+	-	+	+	±	±		-	-	++	++	++	++	++	++	++	+	+	++

TABLE 1.—contd — 3

Isolates	Optimum temp (°C)	Pellicle (nutrient broth)	Diffusible brown pigment				Growth 1% NaCl	Black pigment on steamed potato	Acid in litmus milk with cream	H_2S produced			Acid from carbon sources									
			TTC	PDPA	Nutrient broth	Tyrosine				Peptone-iron agar	Tryptone	Cystine	Lactose	Maltose	Cellobiose	Sorbitol	Dulcitol	Mannitol	i-inositol	Ethanol	Arabinose	Tartrate
Li-1	33	+	+	+	-	+	+	-	-		-		+	++	+	++	++	++	++	+	+	++
S-2	33	+	+	+	-	+	+	±	+	-	-		+	+	+	++	++	++	++	+	+	++
S-3	33	+	+	+	-	+	+	±	-		-		++	++	+	++	++	++	++	+	+	++
Ab-1	33	+	+	+	-	+	+	-	-		-		-	-	-	++	++	++	++	+	+	++
Ab-2	33	+	+	+	-	+	+	-	-		-		-	-	-	++	++	++	++	+	+	++
CO-4	33	+	+	+	-	+	+	±	-		-		-	-	-	++	++	++	++	+	+	++
CO-8	33	+	+	+	-	+	+	±	-		-	-	-	-	-	++	++	++	++	+	+	++
D-2	33	+	+	+	-	+	+	-	-		-	-	-	-	-	++	++	++	++	+	+	++
Ta-1	33	+	+	+	-	+	+	±	-		-	±	-	-	-	++	++	++	++	+	+	++
SI-1	33	+	+	+	-	+	+	±	-		-	+	-	-	-	++	++	++	++	+	+	++
DC-3	33	+	++	++	+	++	+	±	-	-	+	+	-	-	+ c/	++	++	++	++	+	+	++
DC-4	33	+	++	++	+	++	+	±	-		+		-	-	+	++	++	++	++	+	+	++

TABLE 1.--contd — 4

Isolates	Optimum temp (°C)	Pellicle (nutrient broth)	Diffusible brown pigment				Growth 1% NaCl	Black pigment on steamed potato	Acid in litmus milk with cream	H_2S produced			Acid from carbon sources									
			TTC	PDA	Nutrient broth	Tyrosine				Peptone-iron agar	Tryptone	Cystine	Lactose	Maltose	Cellobiose	Sorbitol	Dulcitol	Mannitol	i-inositol	Ethanol	Arabinose	Tartrate
V-1	33	+	++	++	+	++	+	±	-		+	+	-	-	+	++	++	++	++	+	+	++
K-60	33	+	++	++	+	+	+	-	-		+		-	-	-	++	-	-	++	+	-	-
S-208	27	-	-	-	-	+	-	-	-		-		-	-	-	-	-	-	-	-	-	+

a/ + = positive test; ++ = strongly positive test; ± = results differ among replicates; - = negative test.

b/ For carbon sources: ++ = acid produced in synthetic basal medium within one week at 32 C. + = acid produced in 1-3 weeks; - = no acid produced in three weeks.

c/ Isolates DC-3, DC-4, and V-1 produced acid from cellobiose in Hayward's medium (Hayward, 1964), but not in Ayers, Rupp, and Johnson medium (Society of American Bacteriologists, 1957).

cream. More recently, cream-free litmus milk has become widely used among bacteriologists. This experiment was made to determine whether Philippine isolates produce acid from cream-free litmus milk, litmus milk with cream, or both.

In cream-free litmus milk, all isolates produced an alkaline reaction in 3-4 days which gradually increased in intensity for 2-3 weeks without clearing or litmus reduction. In litmus milk with cream, all isolates produced an alkaline reaction initially, but some changed to acid after one week. The acid reaction began in the cream layer of the surface, gradually spreading throughout the medium. Sometimes, however, the acid reaction was weak and restricted to the surface layer. Isolates with an alkaline reaction did not reduce litmus, but change to acid was accompanied by litmus reduction and sometimes by acid curd formation. Twelve isolates produced an acid reaction. However, the acid reaction of isolates GS-4 and LB-5 reverted to alkalne within 3 weeks, and some others were variable among replicates.

Gelatin utilization. — All isolates utilized gelatin when it was incorporated in nutrient agar. All 9 isolates that were tested for gelatin liquefaction produced crateriform liquefaction after 4 weeks.

Growth on steamed potato slants. — Production of a tar-black pigment on steamed potato slants has been reported as a useful diagnostic tool for *P. solanacearum* (Harrison, 1961; Hutchinson, 1913; and Smith, 1896). This experiment examined the usefulness of this technique for Philippine isolates.

Glistening white bacterial growth appeared on the slants in 2 days, and a golden-brown pigment was present after 1 week. Sometimes a tar-black pigment also appeared within 1 week. Twenty-five of the 37 test isolates produced the black pigment within 3 weeks, but production was variable among replicates. Isolates K-60, S-208, and 10 Philippine isolates produced no black pigments on steamed potato slants.

Motility. — All isolates were motile in the semi-solid medium. However, growth of S-208 was poor in the medium, perhaps due to sodium chloride sensitivity.

Sodium chloride tolerance. — The rate of growth of all isolates was reduced when NaCl added to the medium. Most isolates grew fairly well at concentrations of 0.5% and 1.0% NaCl, but none grew at 2.0% NaCl. However, with S-208, growth was much reduced at 0.5% NaCl, and no growth was visible at the 1.0 or 2.0% concentrations. Sodium chloride was bacteriostatic at the 2.0% concentration. The bacteria settled to the bottom of the medium in a stringy mass, but when streaked on TTC medium after one week, they produced normal virulent (fluidal) colonies.

Hydrogen sulfide production. — Only 4 of the 37 isolates tested produced hydrogen sulfide (H_2S) from tryptone, and these were only weakly positive. The 4 positive isolates also produced considerable brown pig-

ment in the medium (none was produced by all other isolates). A stronger positive H_2S reaction was obtained when cystine replaced tryptone as the substrate, and some isolates that had given a negative test for H_2S with tryptone were positive in this test. However, other isolates were negative for H_2S production in both media. In peptone-iron agar all 9 isolates tested were negative for H_2S production.

Pectolytic activity.—Most isolates grew well in the pectin medium and produced acid with crateriform liquefaction within 1 week. Isolates GS-1 and S-208, however, grew very slowly in this medium and produced little or no liquefaction or change in pH.

Indole production.—All isolates were negative for indole production.

Catalase and oxidase reactions.—All isolates were positive for both the catalase and oxidase tests.

Production of green fluorescent pigment.—All isolates grew well in Clara's medium, but none produced a green fluorescent pigment in the medium.

Nitrate reduction.—All isolates reduced nitrates to nitrites.

Starch hydrolysis.—None of the isolates hydrolyzed starch.

Methyl red-Voges Proskauer test.—All isolates gave negative reactions in the methyl red and Voges Proskauer test.

Optimum temperature for growth.—The most rapid growth occurred at 33 C for all isolates except S-208, which grew best at 27 C. However, the range of temperatures in which isolates grew differed considerably. Most isolates grew slowly at 15 C, and grew well from 21-33 C, but isolates GS-2 and Li-1 produced no visible growth in eight days at 15 C and grew slowly at 21 C. Isolates K-60, La 2, Ki-1, 1-3, 1-4, and Da-1 grew well at 39 C, but the other isolates did not grow at this temperature.

Utilization of amino acids as sources carbon and nitrogen.—When asparagine or tyrosine was the sole source of carbon and nitrogen, all isolates grew rapidly. No pigment formation was apparent from asparagine, but in the tyrosine medium isolates V-1, DC-3, and DC-4 produced a pink pigment within 3 days. After 5 days, other isolates also produced a pink pigment in the medium and when the cultures were 12 days old, all were positive for pigment production. At 12 days, when the last observation was made, the pigment had changed to brownish-orange.

Organic sources of carbon.—Of the 23 materials investigated as possible sources of carbon, all isolates utilized glycerol, acetate, citrate, dextrose, sucrose, mannose, galactose, and levulose. None of the isolates utilized rhamnose, raffinose, malonate, formate, or salicin. Isolates differed in utilization of the remaining 10 materials, however. None of the isolates produced gas from the materials tested.

Hayward's (1964) medium and Difco OF Basal Medium were compared with Ayers, Rupp, and Johnson's basal medium of determine whether the basal medium might influence results of tests with lactose, maltose, and cellobiose. Twelve isolates that had given negative results for acid production with these materials previously were tested in the 3 media and compared with isolate LC-1, which readily utilized lactose, maltose, and cellobiose in previous experiments. Hayward's medium was slightly more sensitive than the other two, giving the most rapid indication of acid production. In Hayward's medium isolates V-1, DC-3 and DC-1 produced slight acid from cellobiose which was not detected in the other 2 media. When no additional carbon source was added to the OF medium, growth and a strong alkaline reaction occurred; but when the medium was covered with melted petrolatum, no growth or change in pH occurred, even when an additional carbon source was added. This indicated that all 13 isolates tested had strictly oxidative metabolism.

Examination of similarities of isolates. — Results of 56 tests were analyzed by computer for similarities among the 37 isolates. This analysis showed a high percentage similarity (over 90%) among most isolates from the Philippines (Fig. 1). Philippine isolates were distributed in two relatively distinct, homogeneous groups that are similar to Biotypes 3 and 4 (Hayward, 1964), respectively. However, isolates DC-3, DC-4, and V-1 were not consistent with any of the 4 biotypes that have been proposed. Isolates K-60 and S-208 were distinct from all Philippine isolates and also were of relatively low similarity (76.8%) to one another.

Discussion

The 37 isolates of *P. solanacearum* used in this study differed in many tests, including colony characteristics, production of brown diffusible pigment, optimum temperature and temperature range for growth in nutrient broth, sodium chloride tolerance, pigment production on steamed potato slants, acid production in litmus milk with cream, hydrogen sulfide production, and carbon sources utilized. Production of black pigment on steamed potato slants, acid production in litmus milk with cream, and hydrogen sulfide production were not stable characters that might be useful for defining strains of *P. solanacearum*. The steamed potato and litmus milk reactions differed among replicates, and the hydrogen sulfide reaction depended on the substrate and method of testing.

Latin American workers (Buddenhagen, 1960; French and Sequeira, 1970) have found colony characteristics on TTC medium useful for identifying certain strains of *P. solanacearum*. Among the Philippine isolates tested, colony characteristics were useful for identifying isolates LB-6 and LC-1. Although certain differences among other isolates were apparent, they were distinct and tended to disappear as the colonies became older.

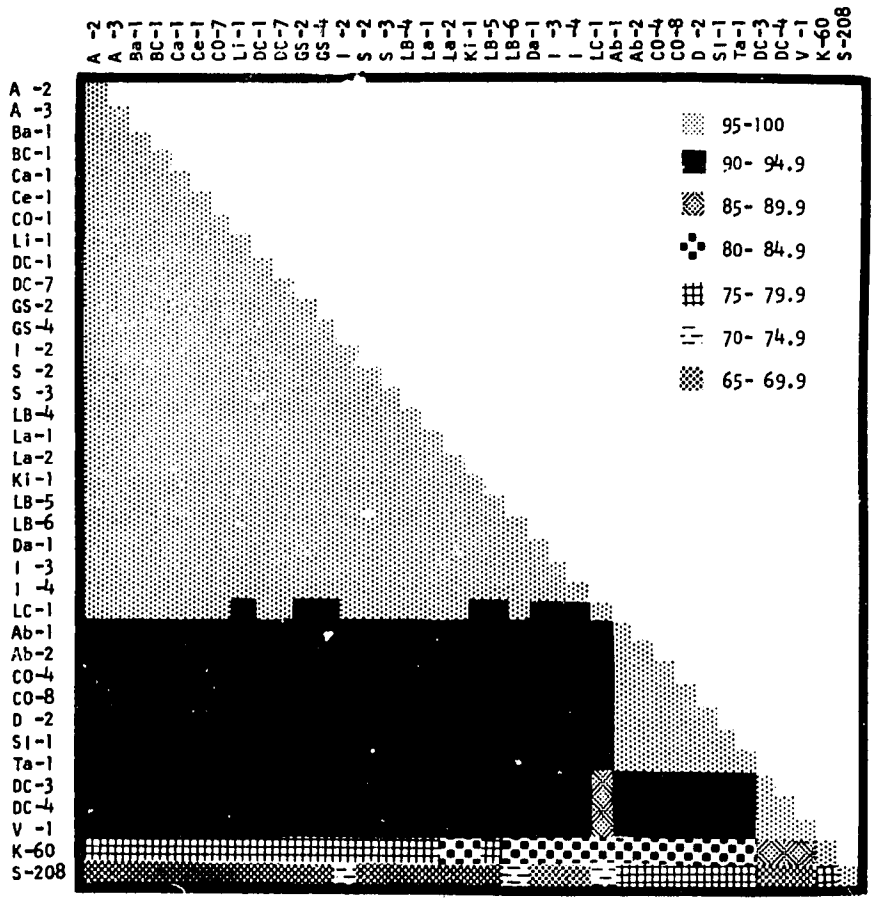

Fig. 1. Percentage similarity of 56 cultural, physiological, and biochemical properties of 37 isolates (A-2, A-3, etc.) of *Pseudomonas solanacearum*.

Isolates K-60 and S-208 were easily distinguished from Philippine isolates by colony form and pigmentation on TTC medium.

The designation "var. *asiaticum*" solely according to acid production in litmus milk with cream (Smith, 1914) is of dubious value. All isolates produced alkaline reaction in this medium within 1 week and only after 1 week did some of the isolates show an acid reaction. Designation, therefore, depends on the time that the observations are made. Perhaps, a more serious objection is that the reaction sometimes differed among replicates of the same isolate.

Reactions of Philippine isolates in other tests were fairly uniform, except in utilization of lactose, maltose, and cellobiose. Apparently, Biotypes 3 and 4, which differ in utilization of these 3 compounds (Hayward, 1964), are common in the Philippines, but Biotypes 1 and 2 were not found. Isolates DC-3, DC-4, and V-1, which were intermediate between Biotypes 3

and 4, were more active in the production of a brown pigment in various types of media than were other Philippine isolates.

Isolates K-60, from North Carolina, and S-208, from Colombia, were distinct from Philippine isolates in a number of characters, especially in the range of carbon sources that were utilized. These 2 isolates also were distinct from one another in these tests. Isolate S-208 differed so markedly from other isolates in its cultural and physiological properties that one might be tempted to define it as a distinct species. However, in pathogenicity tests (Zehr, 1971), S-208 produced typical symptoms of bacterial wilt in tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.), and diploid banana (*Musa balbisiana* Colla). Therefore, the writer is not inclined to consider this isolate as another species.

Little correlation was found between physiological properties and host range (Zehr, 1971). Okave and Goto (1961) reported that Japanese isolates that attacked tobacco oxidized lactose as a carbon source. All Philippine isolates of high virulence to tobacco also utilized lactose, but North Carolina isolate K-60, which is virulent to tobacco, did not utilize lactose. As was observed by Okabe and Goto (1961), lactose utilization was not a good index of virulence to tobacco because many isolates of low virulence to tobacco also utilized lactose. Colony form was not related to pathogenicity, and also, was not correlated with difference in physiological or biochemical characteristics.

Literature Cited

Anonymous. 1953. Difco Manual (Ninth Edition). Difco Laboratories, Detroit, 350 p.

Buddenhagen, I. W. 1960. Strains of *Pseudomonas solanacearum* in indigenous hosts in banana plantations of Costa Rica, and their relationship to bacterial wilt of bananas. Phytopathology 50: 660-664.

Clara, F. M. 1934. A comparative study of the green-fluorescent bacterial plant pathogens. New York (Cornell) Agr. Exp. Sta. Mem. 159. 34 p.

French, E. R. & L. Sequeira. 1970. Strains of *Pseudomonas solanacearum* from Central and South America: a comparative study. Phytopathology 60: 506-512.

Gershman, M., D. C. O'Meara, & H. L. Chute. 1959. Use of a tetrazolium salt for an easily discernible sulfide-motility reaction. J. Bacteriol. 78: 739-740.

Harrisson, D. E. 1961. Bacterial wilt of potatoes. I. Field symptoms of the disease and studies on the causal organism, *Pseudomonas solanacearum* variety *asiaticum*. Australian J. Agr. Res. 12: 854-871.

Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. J .Appl. Bacteriol. 27: 265-277.

Hutchinson, C. M. 1913. Rangpur tobacco wilt. Mem. Dept. Agr. India, Bact. Ser. 1: 67-84.

Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. North Carolina Agr. Exp. Sta. Tech. Bull. 99. 194 p.

Kelman, A. 1954. The relationship of pathogenicity of *Pseudomonas solanacearum* to colony appearance in a tetrazolium medium. Phytopathology 44: 693-695.

KOVACS, N. 1956. Identification of *Pseudomonas pyocyanae* by the oxidase reaction. Nature 178: 703.

LESSEL, E. G. & J. G. HOLT. 1970. Presenting and interpreting the results. p. 50-58. *In* W. R. Lockhart and J. Liston (ed.) Methods for Numerical Taxonomy. American Soc. for Microbiol., Bethesda, Md.

OKABE, N. & M. GOTO. 1952. Studies on *Bact. solanacearum* with special reference to the kinds of strains and their classification. Shizuoka Univ. Fac. Agr. Rept. 2: 64-93.

OKABE, N. & M. GOTO. 1961. Studies on *Pseudomonas solanacearum*. XI. Pathotypes in Japan. Shizuoka Univ. Fac. Agr. Rept. 11: 25-42.

RHODES, MURIEL J. 1959. The characterization of *Pseudomonas fluorescens*. J. Gen. Microbiol. 21: 211-263.

SMITH, E. F. 1896. A bacterial disease of tomato, eggplant, and Irish potato, U.S. Dept. Agr. Div. Veg. Physiol. and Pathol. Bull. 12. 28 p.

SMITH, E. F. 1914. Bacteria in relation to plant diseases. Carnegie Institute, Washington, D. C. Vol. 3. 398 p.

SOCIETY OF AMERICAN BACTERIOLOGISTS. 1957. Manual of microbiological methods. McGraw-Hill, New York. 315 p.

STARR, M. P. 1947. The causal agent of bacterial root and stem disease of guayule. Phytopathology 37: 391-300.

STARR, M. P. & J. E. WEISS. 1943. Growth of phytopathogenic bacteria in a synthetic asparagine medium. Phytopathology 33: 314-318.

VASUDEVA, R. S. 1955. Report of the Division of Mycology and Plant Pathology. Sci. Rept. Agr. Res. Inst. New Delhi, 1953-1954. p. 93.

ZEHR, E. I. 1970. Strains of *Pseudomonas solanacearum* in the Philippines as determined by cross-inoculation of hosts at different temperatures. Philippine Phytopathol. 6: 44-51.