TARC Notes

Strain differentiation of *Pseudomonas solanacearum* affecting solanaceous crops in the Philippines

The bacterial wilt disease caused by *Pseudomonas solanacearum* E. F. Smith is at present one of important plant diseases of bacterial origin in the world. In the Philippines, the disease affects abaca, banana, eggplant, pepper, potato, tomato, tobacco, peanut, ginger, castor bean, cowpea and bush lima bean. Its destructiveness, however, seems to be limited to solanaceous crops, banana, castor bean and ginger (Quimio, 1976) at present. The objective of this research was to classify *P. solanacearum* strains infecting solanaceous crops in the Philippines on the basis of their ability to utilize three hexose alcohols and three disaccharides (Hayward, 1964), relative sensitivities to bacteriophages, and cardinal temperatures for growth.

Eighty eight isolates, representing 20 Provinces of the Philippines, were selected from the National Culture Collection of *P. solanacearum* in the Department of Plant Pathology, University of the Philippines at Los Baños for this study. Kelman's (1954) differential medium (peptone 10 g, dextrose 5 g, casein hydrolysate 1 g, 1,2,3,5-trephenyltetrazolium chloride (0.5% solution) 10 ml, agar 18 g and distilled water 1,000 ml) was used for isolations and fresh inoculum was prepared using the same medium without tetrazolium salt.

Biovar: Hugh and Leifson's medium (1935) was used as basal medium (peptone 1 g, $NH_4H_2PO_4$ 1 g, KCl 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, agar 3 g, bromothymol blue 0.08 g, distilled water 1,000 ml, pH 7). All the test carbohydrates were sterilized through millipore filtration except dulcitol which was sterilized together with the basal medium because of its poor solubility. The carbohydrate solution was added to give a final concentration of 1% in the basal medium (Hayward, 1964).

All the four Biovar, Biovar I, II, III and IV, reported for *P. solanacearum* (Hayward, 1964, 1976) were found in the isolates used. Sixty seven isolates (80.7%), however, showed characteristics of Biovar III. The distribution of the isolates by Biovar on different host plants and by areas indicates that the dominant and widely distributed strain of *P. solanacearum* affecting solanaceous crops in the Philippines belongs to Biovar III (Table 1).

Bacteriophage and bacteriophage typing: Bacteriophages were isolated as follow; about 100 g rhizosphere soil of infested plant was mixed and shaken for a few minutes with sterile water in the Erlenmeyer flask. One ml of the soil suspension and one ml of inoculum (1×10^9 cells/ml) were plated with 10 ml of melted Kelman's medium without tetrazolium salt. Bacteriophage plaque which appeared within 12—15 hrs after incubation of the plate at 30°C was picked up to Vitamin-free casein hydrolysate medium (vitamin-free casein hydrolysate (10% solution) 2 ml, $CaCl_2$ 0.5 g, distilled water 1,000 ml, pH 6.5; Wakimoto, 1955) and stored in the refrigerator at about 10°C until further used. PSP_1 was isolated from ginger (Kam, 1977), and PSP_2 and PSP_3 from tomato. Bacteriophage typing was

Table 1. Hayward's classification of *P. solanacearum* biovars on the basis of utilization of carbohydrates

Biovar	Acid production from						No. of isolates
	lactose	maltose	cellobiose	mannitol	sorbitol	dulcitol	
I	−	−	−	−	−	−	6 (7.2%)
II	+	+	+	−	−	−	5 (6.0%)
III	+	+	+	+	+	+	67 (80.7%)
IV	−	−	−	+	+	+	5 (6.0%)

done as follows; 2 ml of bacterial suspension taken from 36—48 hrs slant cultures on Kelman's medium without tetrazolium salt was plated with 5 ml of the same medium previously melted and allowed to cougeal. A loopful of the bacteriophage suspension was then streaked on to the agar surface and the plate incubated at 30°C. Appearance of clear areas on the path where the phage suspension was streaked was recorded as positive for a particular phage and isolate combination used.

The Philippines isolates of *P. solanacearum* were classified into six phage types, designated here as A, B, C, D, E and F based on their relative sensitivities to the three isolates of bacteriophage used. Tentative numbering given to the Philippine *P. solanacearum* bacteriophage isolates, PSP_1, PSP_2 and PSP_3 was used for convenience, because proper naming of the phage should be made based on their morphological and serological characteristics and also on the comparative studies using bacteriophage of *P. solanacearum* from other countries. Phage type F was most common since it was observed to be well distributed all over the country. The results of the bacteriophage typing studies offer a simple pattern for the Philippine isolates. A problem, however, is that 67.4% of the isolates (type F) are resistant to the three bacteriophage used (Table 2). No correlation was observed between biovar of the isolates and bacteriophage type.

Temperature relations: Cardinal temperatures for growth of isolates were determined in the Kelman's liquid medium without tetrazolium salt. The minimum, optimum and maximum temperatures were determined using a Temperature Gradient Incubator (Model TN-3, Toyo Kagaku Sangyo), which was set at 30 different temperature gradient from 3.5°C to 45.0°C. Optimum temperature for growth was recorded 5—6 hrs after inoculation using a spectrophotometer (Spectronic 20, Baush & Lomb) at 425 mm, while minimum and maximum temperatures were checked two days after inoculation.

The optimum temperatures ranged from 31.0 to 36.5°C, minimum temperatures 3.5 to 15.5°C and maximum temperatures 36.5 to 45.0°C. For the purpose of classification, the isolates may be grouped on optimum temperatures for growth as follows; a) 31.0 —31.5°C, b) 33.0—33.5°C, c) 34.0—34.5°C, d) 35.0—35.5°C and e) 36.0—36.5°C. Majority of the isolates (about 68%), however, showed optimum temperatures of 33.0 to 34.5°C (Table 3). There was no correlation between optimum temperature ranges and minimum or maximum temperatures. However, isolates with the highest optimum temperature (36.0—36.5°C) had also highest maximum temperature (45.0°C). The data also showed that with the exception of tomato isolates the range of optimum temperatures was quite narrow for isolates taken from the same host plant; Potato isolates 33.0—33.5°C, pepper isolates 33.0—34.0°C, tobacco isolates 33.0—34.0°C, egglant isolates 33.0—35.0°C, bean isolates 34.0—35.0°C and tomato isolates 31.0—36.5°C. Cardinal temperatures for growth of isolates from high elevations

Table 2. Bacteriophage and bacteriophage types of *P. solanacearum* in the Philippines

Phage type	Bacteriophage PSP_1	PSP_2	PSP_3	No. of isolates
A	+	+	+	1 (1.2%)
B	+	+	−	18 (21.0%)
C	+	−	−	2 (2.3%)
D	−	+	−	3 (3.5%)
E	−	−	+	4 (4.7%)
F	−	−	−	58 (67.4%)

Table 3. Classification of *P. solanacearum* isolates based on optimum temperature for growth

Temperature (℃)	No. of isolates
a) 31.0−31.5	4 (6.1%)
b) 33.0−33.5	17 (25.8%)
c) 34.0−34.5	28 (42.4%)
d) 35.0−35.5	13 (19.7%)
e) 36.0−36.5	4 (6.1%)

(4,999 ft, 3,020 ft and 2,215 ft) showed no difference from those of low elevations (below 100 ft above sea level). Thus, no correlation between *in vitro* temperature requirements and the isolate's origin based on elevations.

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Bacterial brown spot of *Phalaenopsis* orchids in the Philippines

A causal bacterium was isolated from diseased *Phalaenopsis* leaves collected from the campus of the University of the Philippines at Los Baños. Studies of the bacteriological characters were made according to the methods outlines by the Society of American Bacteriologists (1957), unless otherwise indicated. The characters of the bacterium causing brown spot of *Phalaenopsis* orchids under consideration are similar to those of *Pseudomonas cattleyae* (Pavarino) Savulescu.

The disease appears first as small, water-soaked lesion on the leaves. These lesion increase in size rapidly, changing from light brown to dark brown in color with age. The tissues surrounding the older spots exhibit light green to light yellow irregular halo. Spots coalesce and form irregular patches of dark brown to black areas of dead tissues. When the conditions are favorable for disease development, the disease attack the crown and kill the plant.

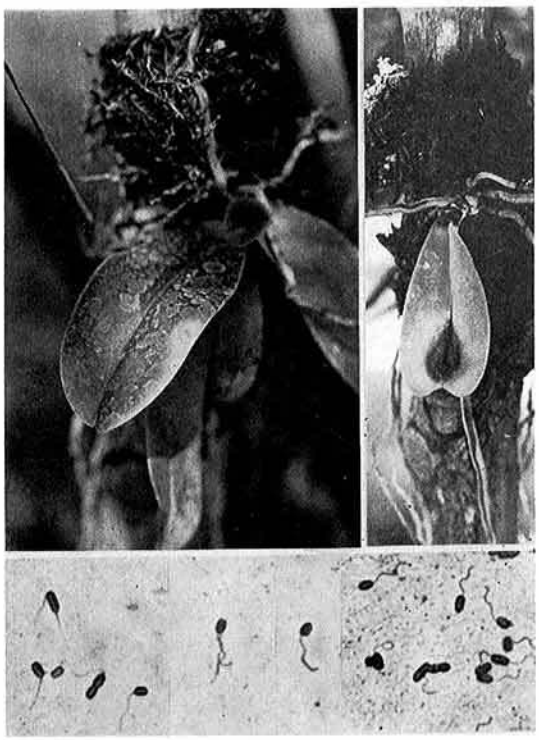

Plate 1. Bacterial brown spot of *Phalaenopsis*
Left : Early symptom
Right : Advanced symptom
Lower : Causal bacterium

Bacteriological characters:

Rods: 0.5×1.0—2.0 microns, with round ends, occurring singly or occassionally in pairs, motile by means of a single polar flagellum or rarely bipolar flagella.

Gram negative.

Poly-B-hydroxybutyrate: positive.

Aerobic.

Nutrient agar colonies: grayish white, circular, convex, entire, smooth, butyrous.

Nutrient agar slant: growth grayish white, filiform, non fluorescent, odorless, brittle to viscid, medium unchanged.

Nutrient broth: growth odorless, moderate clouding, viscid sediment, thin pellicle.

Fermi's solution: good growth.

Chromogenesis: no pigment in Clara's medium or on medium B of King, Ward and Raney.

Gelatin not liquified.

Fresh milk and litmus milk unchanged.

Ammonia produced.

Nitrates reduced to nitrites.

Indol production: negative.

Hydrogen sulfide production: negative.

Pectolytic activity: positive.

Starch hydrolyzed.

Glucose metabolize fermentatively in Hugh and Leifson's medium.

Levan formation: negative.

Oxidase reaction: positive.

Arginin dihydrolase: negative.

Tween 80 hydrolysis: positive.

Acid but no gas from fructose, galactose, glucose, glycerol, mannitol, sorbitol and xylose.

No acid or gas from arabinose, dextrin, escurin, inositol, inulin, lactose, maltose, mannose, raffinose, rhamnose, saricin, soluble starch, sucrose and trehalose.

Optimum temperature 29.5°C, minimum temperature 7.0°C and maximum temperature 39.5°C.

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