# Characteristics of Strains of Pseudomonas solanacearum from China

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### **ABSTRACT**

He, L. Y., Sequeira, L., and Kelman, A. 1983. Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Disease 67:1357-1361.

Twenty-nine strains of *Pseudomonas solanacearum* isolated from 14 cultivated and wild host plants in different locations in China were compared in terms of physiological characteristics in culture and pathogenicity to six differential host plants. Based on Hayward's classification scheme, two strains were placed in biotype II, 14 in biotype III, and 10 in biotype IV. Three strains from mulberry, however, differed from established biotypes because of their ability to produce acid from lactose, maltose, cellobiose, and mannitol but not from dulcitol and sorbitol. Also, they were only slightly virulent on potato (cultivar Russet Burbank) and eggplant (cultivar Black Beauty). Most other strains were highly virulent on these hosts. The strains from mulberry were tentatively classified as race 4, biotype V.

Additional key words: bacterial wilt

Bacterial wilt caused by Pseudomonas solanacearum E. F. Sm. is one of the most important and widespread bacterial diseases of crops in China, especially in the southern provinces. Early surveys for the disease on economically important crops were made by Yu and Fang (29), Ma and Gao (20), and Li (17). Until the 1970s, there were only a few reports concerning strains of P. solanacearum that attack these crops (5,22,30). In recent years, the disease was observed on mulberry, olive, and casuarina and substantial losses have occurred on these hosts (8,23,26). Strains that attack these species have not been reported from other regions of the world and may be indigenous to China.

P. solanacearum has been divided into different races on the basis of host range (2) and into different biotypes on the basis of physiological characteristics in

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The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact. culture (10). Within each of these races or biotypes, there are numerous subtypes that may be associated with particular geographical locations (1,9). In a previous study involving 22 strains of P. solanacearum from different locations in China, strains from tomato, eggplant, ginger, peanut, sweet potato, olive, mulberry, and casuarina were grouped in race 1 and those from potato in race 3 (23). Strains from mulberry were grouped in biotype I, those from potato in biotype III, and the rest in biotype IV. Similar studies in Taiwan with strains mainly from solanaceous hosts revealed numerous "pathotypes" within race 1 with characteristics typical of biotypes II-IV (3,12,28).

Because of the variation in reports on classification of these strains of *P. solanacearum*, investigations were initiated to fully characterize the strains collected in different locations in China from a wide range of hosts.

# MATERIALS AND METHODS

Cultures. Strains of *P. solanacearum* were collected from 14 host plants at different locations in southern China (Table 1). These were compared with several strains (K60, B1, and J8418F)

obtained from the collection at the Department of Plant Pathology, University of Wisconsin, Madison. Inoculum was prepared from cultures of the virulent, fluidal colony type grown on tetrazolium chloride medium (TZC) at 28 C for 48 hr (14). For long-term storage, cultures were lyophilized or maintained as suspensions in distilled water in capped test tubes (15).

**Plants.** Plants used in pathogenicity tests were tobacco (*Nicotiana tabacum* L. 'Bottom Special'), potato (*Solanum* 

**Table 1.** Strains of *Pseudomonas solanacearum* from China used in physiological and pathogenicity tests

Strain Host plant  P1 Peanut P6 Peanut P7 Peanut P9 Peanut P9 Peanut P11 Peanut P12 Peanut P13 Peanut P14 Peanut P14 Peanut P15 Tomato P16 Olive P17 Olive P18 Olive P19 Olive P2 Olive P3 Olive P4 Olive P5 Olive P6 Olive P6 Olive P7 Olive P7 Olive P8 Olive P8 Olive P9 Olive P1 Olive P1 Olive P1 Olive P1 Olive P2 Olive P3 Olive P4 Olive P5 Olive P6 Olive P6 Olive P6 Olive P6 Olive P6 Olive P7 Olive P8 Olive P8 Olive P9 Olive P1 Olive P10 Olive
P6 Peanut Guangdong P7 Peanut Nanning, Guangxi P9 Peanut Luzhai, Guangxi P11 Peanut Nathing, Guangxi P13 Peanut Huanjiang, Guangxi P14 Peanut Quanzhou, Guangxi TM1 Tomato Guangdong TM2 Tomato Liuzhou, Guangxi O1 Olive Hupeh O2 Olive Nanning, Guangxi C1 Ginger Shandong P01 Potato Shandong P02 Potato Fujian P03 Potato Fujian P03 Potato Yunnan PE1 Pepper Liuzhou, Guangxi S1 Sesame Luzhai, Guangxi C1 Casuarina sp. B2 Sweet potato B3 Sweet potato M2 Mulberry Guangzhou, Guangdong
P7 Peanut Nanning, Guangxi P9 Peanut Luzhai, Guangxi P11 Peanut Nanning, Guangxi P13 Peanut Huanjiang, Guangxi P14 Peanut Quanzhou, Guangxi TM1 Tomato Guangdong TM2 Tomato Liuzhou, Guangxi O1 Olive Hupeh O2 Olive Nanning, Guangxi C1 Ginger Shandong P01 Potato Shandong P02 Potato Fujian P03 Potato Fujian P03 Potato Fujian P03 Potato Yunnan PE1 Pepper Liuzhou, Guangxi C1 Casuarina sp. C1 Casuarina sp. C1 Casuarina sp. C1 Casuarina Sp. C2 Sweet potato C3 Sweet potato C4 Casuarina Sp. C5 Sweet potato C6 Mulberry Guangdong C6 Guangdong C7 Casuarina Sp. C8 Sweet potato C9 Mulberry Guangzhou, Guangdong
P9 Peanut Luzhai, Guangxi P11 Peanut Nanning, Guangxi P13 Peanut Huanjiang, Guangxi P14 Peanut Quanzhou, Guangt TM1 Tomato Guangdong TM2 Tomato Liuzhou, Guangxi O1 Olive Hupeh O2 Olive Nanning, Guangxi Z1 Ginger Shandong Z2 Ginger Shandong P01 Potato Shandong P02 Potato Fujian P03 Potato Fujian P03 Potato Yunnan PE1 Pepper Liuzhou, Guangxi S1 Sesame Luzhai, Guangxi C1 Casuarina sp. B2 Sweet potato B3 Sweet potato B3 Sweet potato M2 Mulberry Guangzhou, Guangdong
P11 Peanut Nanning, Guangxi P13 Peanut Huanjiang, Guangxi P14 Peanut Quanzhou, Guangxi TM1 Tomato Guangdong TM2 Tomato Liuzhou, Guangxi O1 Olive Hupeh O2 Olive Nanning, Guangxi Z1 Ginger Shandong Z2 Ginger Shandong P01 Potato Shandong P02 Potato Fujian P03 Potato Fujian P03 Potato Fujian P04 Sesame Luzhai, Guangxi C1 Casuarina sp. B2 Sweet potato B3 Sweet potato B3 Sweet potato B4 Mulberry Guangzhou, Guangdong
P13 Peanut Huanjiang, Guang. P14 Peanut Quanzhou, Guangs. TM1 Tomato Guangdong TM2 Tomato Liuzhou, Guangxi O1 Olive Hupeh O2 Olive Nanning, Guangxi Z1 Ginger Shandong Z2 Ginger Shandong P01 Potato Shandong P02 Potato Fujian P03 Potato Yunnan PE1 Pepper Liuzhou, Guangxi S1 Sesame Luzhai, Guangxi C1 Casuarina sp. B2 Sweet potato B3 Sweet potato B3 Sweet potato M2 Mulberry Guangzhou, Guangdong
P14 Peanut Quanzhou, Guang: TM1 Tomato Guangdong TM2 Tomato Liuzhou, Guangxi O1 Olive Hupeh O2 Olive Nanning, Guangxi O3 Olive Liuzhou, Guangxi Z1 Ginger Shandong PO1 Potato Shandong PO2 Potato Fujian PO3 Potato Fujian PO3 Potato Yunnan PE1 Pepper Liuzhou, Guangxi S1 Sesame C1 Casuarina sp. B2 Sweet potato B3 Sweet potato B3 Sweet potato Mulberry Guangzhou, Guangdong
TM1 Tomato Guangdong TM2 Tomato Liuzhou, Guangxi O1 Olive Hupeh O2 Olive Nanning, Guangxi O3 Olive Liuzhou, Guangxi Z1 Ginger Shandong Z2 Ginger Shandong PO1 Potato Shandong PO2 Potato Fujian PO3 Potato Yunnan PE1 Pepper Liuzhou, Guangxi S1 Sesame Luzhai, Guangxi C1 Casuarina sp. B2 Sweet potato B3 Sweet potato B3 Sweet potato Mulberry Guangdong Mulberry Guangdong Guangdong
TM2 Tomato Olive Olive Olive Olive Nanning, Guangxi Hupeh Nanning, Guangxi Liuzhou, Guangxi Liuzhou, Guangxi Liuzhou, Guangxi Shandong Clainger Polato Potato Potato Potato Potato Potato Posame Liuzhou, Guangxi Luzhai, Guangxi Clasuarina sp. B2 Sweet potato B3 Sweet potato Mulberry Liuzhou, Fujian Clangdong Vianyou, Fujian Clangdong Clangdong Clangdong Clangdong
O1 Olive Hupeh O2 Olive Nanning, Guangxi O3 Olive Liuzhou, Guangxi Z1 Ginger Shandong Z2 Ginger Shandong PO1 Potato Fujian PO3 Potato Fujian PO3 Potato Yunnan PE1 Pepper Liuzhou, Guangxi S1 Sesame Luzhai, Guangxi C1 Casuarina sp. B2 Sweet potato B3 Sweet potato M2 Mulberry Guangdong Guangdong Guangdong
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Z1 Ginger Shandong Z2 Ginger Shandong PO1 Potato Shandong PO2 Potato Fujian PO3 Potato Yunnan PE1 Pepper Liuzhou, Guangxi S1 Sesame Luzhai, Guangxi C1 Casuarina sp. B2 Sweet potato Xianyou, Fujian B3 Sweet potato Mulberry Guangzhou, Guangdong
Z2 Ginger Shandong PO1 Potato Shandong PO2 Potato Fujian PO3 Potato Yunnan PE1 Pepper Liuzhou, Guangxi S1 Sesame Luzhai, Guangxi C1 Casuarina sp. B2 Sweet potato Sweet potato B3 Sweet potato Zhaoan, Fujian M2 Mulberry Guangdong Guangdong
Z2 Ginger Shandong PO1 Potato Shandong PO2 Potato Fujian PO3 Potato Yunnan PE1 Pepper Liuzhou, Guangxi S1 Sesame Luzhai, Guangxi C1 Casuarina sp. B2 Sweet potato Sweet potato B3 Sweet potato Zhaoan, Fujian M2 Mulberry Guangdong Guangdong
PO2 Potato Fujian PO3 Potato Yunnan PE1 Pepper Liuzhou, Guangxi S1 Sesame Luzhai, Guangxi C1 Casuarina sp. Guangdong B2 Sweet potato Xianyou, Fujian B3 Sweet potato Zhaoan, Fujian M2 Mulberry Guangdong
PO3 Potato Yunnan PE1 Pepper Liuzhou, Guangxi S1 Sesame Luzhai, Guangxi C1 Casuarina sp. Guangdong B2 Sweet potato Xianyou, Fujian B3 Sweet potato Zhaoan, Fujian M2 Mulberry Guangzhou, Guangdong
PEI Pepper Liuzhou, Guangxi S1 Sesame Luzhai, Guangxi C1 Casuarina sp. Guangdong B2 Sweet potato Xianyou, Fujian B3 Sweet potato Zhaoan, Fujian M2 Mulberry Guangzhou, Guangdong
S1 Sesame Luzhai, Guangxi C1 Casuarina sp. B2 Sweet potato Xianyou, Fujian B3 Sweet potato Zhaoan, Fujian M2 Mulberry Guangzhou, Guangdong
C1 Casuarina sp. Guangdong B2 Sweet potato Xianyou, Fujian B3 Sweet potato Zhaoan, Fujian M2 Mulberry Guangzhou, Guangdong
B2 Sweet potato Xianyou, Fujian B3 Sweet potato Zhaoan, Fujian M2 Mulberry Guangzhou, Guangdong
B3 Sweet potato Zhaoan, Fujian M2 Mulberry Guangzhou, Guangdong
M2 Mulberry Guangzhou, Guangdong
Guangdong
M4 Mulberry Guangzhou,
Guangdong
M5 Mulberry Shunde, Guangdo
TB2 Tobacco Guizhou
E1 Eggplant Fujian
SN1 Black nightshade Fujian
U1 Urtica nivea Zhejiang

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tuberosum L. 'Russet Burbank'), tomato (Lycopersicon esculentum Mill. 'Bonny Best'), eggplant (Solanum melongena L. 'Black Beauty'), pepper (Capsicum annum L. 'Sweet Yellow'), and peanut (Arachis hypogaea L. 'NC 6' and 'NC 4'). Seeds were planted in vermiculite or Jiffy Mix (Jiffy Mix Products, Chicago, IL 60185) and seedlings were transplanted one each in a 10-cm pot containing a soilpeat-sand (2:2:1.5) mixture that had been steamed for 1 hr. Plants (except tobacco) were grown in the greenhouse at 22-23 C under supplemental lighting  $(9 \times 10^3 \text{ lux})$ provided by a combination of Sylvania Gro-Lux and cool-white fluorescent tubes. Tobacco plants were grown in a greenhouse at  $28 \pm 9 \, \text{C}$ .

Inoculation. Plants were steminoculated when 15-20 cm high by procedures similar to those described by Winstead and Kelman (27). Five to 10 plants of each host were inoculated with each strain of the bacterium. Plants in one series were inoculated by inserting a micropipette containing 50 µl of inoculum in the stem at the axil of the third fully expanded leaf from the top (19). The micropipette was left in position until the inoculum was absorbed. Root inoculations in one experiment were completed by pouring inoculum (10 ml/plant) around the base of the stem, then inserting a knife 4 cm into the soil and cutting the roots along one side.

Inoculated plants were placed in a greenhouse at 28±9 C with supplemental lighting as indicated previously. Plants were watered alternately with Hoagland's

nutrient solution and tap water.

Severity of wilting was ranked at weekly intervals after inoculation on the following scale: 1 = no symptoms, 2 = inoculated leaf wilted, 3 = two or three leaves wilted, 4 = four or more leaves wilted, and 5 = plant dead.

**Hypersensitive reaction.** To test the ability of different strains to induce a hypersensitive reaction (HR) on tobacco leaves, the method of Lozano and Sequeira (18) was used. Completely expanded tobacco leaves were infiltrated with a bacterial suspension adjusted to  $A_{600} = 0.1$  (about  $10^8$  cells per milliliter). Reactions were recorded at 24 and 48 hr.

**Physiological tests.** Tests for formation of fluorescent and melanin pigments were completed on King's B medium (24) and casamino acids-peptone-glucose (CPG) medium (4) containing 0.1% tyrosine, respectively. For gram stain, the procedure of Gregersen (7) was followed. Poly- $\beta$ -hydroxybutyrate was detected by the staining procedure of Sands et al (24).

To test for oxidation of carbohydrates, the medium described by Hayward (10) was used. Lactose, maltose, cellobiose, arabinose, trehalose, fructose, and sucrose solutions were filter-sterilized. p-Glucose, mannitol, and sorbitol were autoclaved for 20 min as 10% (w/v) solutions. Dulcitol was added directly to the basal medium, which was then autoclaved for 20 min. Oxidative vs. fermentative metabolism was tested in Hayward's medium containing glucose in plugged test tubes sealed with a 2-cm layer of sterile melted Vaseline or left

unsealed. Cultures were incubated at 28 C and checked for acid production at various intervals up to 28 days.

Susceptibility to antibiotics was tested with Bacto antibiotic sensitivity disks as recommended by the manufacturer (Difco Laboratories, Detroit, MI 48232). Pectinase production was detected by the characteristic pitting of a crystal violet-pectate (CVP) medium (4). Reducing sugars were determined with the 2,2′-bicinchoninate reagent (21). Other physiological tests (see Results) were carried out as recommended in standard laboratory manuals (11,13,16,24,25).

#### RESULTS

Cultural characteristics. All strains of P. solanacearum from China had cultural characteristics similar to those of strains from other regions of the world. On CPG medium, all virulent isolates produced smooth, opaque, highly fluidal colonies. A brown or tan pigment appeared on CPG medium after certain strains had grown for several days. On TZC medium, all virulent strains produced fluidal colonies with pink or light red centers after 48 hr. Most strains produced rough, butyrous colonies spontaneously in culture as reported previously for other strains (14). All strains were rod-shaped, gram-negative, non-spore-forming, and positive for poly- $\beta$ -hydroxybutyrate. None of the strains produced fluorescent pigments on King's B medium. On

**Table 2.** Pathogenicity of strains of *Pseudomonas solanacearum* from China to six major hosts and classification of these strains as to pathogenicity group and race

Strain									
	Original host	Eggplant	Potato	Tomato	Pepper	Peanut	Tobacco	Group Rac	
TB2	Tobacco	Н	L	M	M	М	Н	1	1
O3	Olive	Н	Н	M	L	L	Н	1	1
B3	Sweet potato	M	M	Н	Н	M	0	2	1
B2	Sweet potato	Н	Н	Н	Н	L	0	2	1
P7	Peanut	Н	Н	M	M	L	0	2	1
Z2	Ginger	Н	Н	Н	M	L	0	2	1
O2	Olive	Н	Н	M	M	L	0	2	1
01	Olive	Н	L	M	L	L	0	2	1
SI	Sesame	Н	Н	M	L	M	0	2	1
PO1	Potato	Н	Н	Н	Н	0	$0_{p}$	3	1
TM2	Tomato	Н	Н	M	H	0	0	3	1
UI	Urtica	Н	Н	M	M	0	$0_{\mathrm{p}}$	3	1
PE1	Pepper	Н	Н	L	Н	0	0	3	1
Z1	Ginger	Н	L	M	L	0	0	3	1
C1	Casuarina sp.	Н	Н	Н	0	Н	0	4	1
E1	Eggplant	Н	Н	Н	0	Н	0	4	1
P14	Peanut	Н	Н	Н	0	Н	0	4	1
P6	Peanut	Н	Н	Н	0	M	0	4	1
TMI	Tomato	Н	Н	M	0	Н	0	4	1
PH	Peanut	Н	Н	M	0	M	0	4	1
P9	Peanut	Н	Н	L	0	Н	0	4	1
P13	Peanut	M	Н	L	0	Н	0	4	1
P1	Peanut	Н	L	M	0	Н	0	4	1
PO3	Potato	M	Н	Н	M	0	0	5	3
M4	Mulberry	L	L	0	0	0	0	6	4
M5	Mulberry	L	L	0	0	0	0	6	4

<sup>&</sup>lt;sup>a</sup> Results based on average disease indices of 5-10 plants 21 days after inoculation. H = high (4.1-5.0), M = medium (2.6-4.0); L = low (1.1-2.5), and 0 = none (1.0).

**Table 3.** Reaction of tobacco leaves to infiltration with strains of *Pseudomonas solanacearum* 

	Hours after	infiltration
Strain	24	48
TB2	a	N
O3	_	N
P1	HR	HR
P6	C	HR
P7	C	HR
P9	C	HR
P11	C	HR
P13	HR	HR
P14	HR	HR
TM1	HR	HR
TM2	_	C
O1	С	HR
O2	HR	HR
Z1	HR	HR
PO1	C	HR
PE1	С	C
S1	HR	HR
C1	C	HR
B2	Ċ	C
В3	C	HR
E1	C	HR
PO3	_	C
M4	C	HR
M5	HR	HR
K60		N
B1	C	HR

<sup>&</sup>lt;sup>a</sup>-= No reaction, N = infiltrated area became necrotic with chlorotic margins, HR = hypersensitive reaction, and C = infiltrated area became chlorotic.

<sup>&</sup>lt;sup>b</sup>Plant showed limited yellowing of the foliage but no wilting.

tyrosine medium, most isolates produced a brown diffusable pigment but three (TBI, SI, and Z2) did not. All strains except P14 failed to grow at temperatures higher than 37 C; strain P14 showed slight growth at 41 C.

Pathogenicity. In susceptible hosts, symptoms began to appear 3-4 days after stem inoculation. Initial symptoms usually consisted of wilting of the inoculated leaf and stunting of growth. In some hosts, inoculation was followed by considerable decay of the pith surrounding the point of inoculation, but no systemic symptoms appeared. Because wilting was not observed, these plants were given a

value of 1 in the disease severity index even though the symptoms were clearly different from those on inoculated, immune hosts that received the same ranking.

All strains caused rapid wilting of eggplant (Table 2). Potato was susceptible to most strains, but plants varied as to rapidity of wilting. Tomato was susceptible to all strains except those from mulberry. Pepper, peanut, and tobacco showed marked differences in their reactions to specific strains but were resistant to the vast majority (92.3%) of strains.

On the basis of host reaction, the strains could be grouped into five

pathogenicity groups: strains of group 1 were virulent on the six hosts; group 2 included strains virulent on all hosts except tobacco; strains in group 3 were not virulent on tobacco and peanut; strains in group 4 were not virulent on tobacco and pepper; group 5 included a single strain from potato that was highly virulent only on potato and tomato; and group 6 included only strains from mulberry and these were weakly virulent on eggplant and potato and not virulent on the other hosts. Because of their wide host range, strains in groups 1-4 were considered members of race 1; those from potato were included in race 3, but those

Table 4. Physiological characteristics of strains of Pseudomonas solanacearum from China

Tests	Reactions to strains														
		P1	P6	P7	P9	P11	P13	P14	TM1	TM2	01	02	03	<b>Z1</b>	Z2
Starch hydrolysis		_a	_		_	_	_	_		_	_	_	_	-	-
H <sub>2</sub> S production		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole production		_	_	_	_	_	_	_	_	_	_	_		-	_
MR test		_	_	_		_	_	_		_	_	_		_	_
VP test		_	_		_	_	_	_	-	_	_		_	-	_
Gelatin liquification		_	_	_	_	_	_	_	_	_	_	-	_	-	-
Nitrite from nitrate		+	+	+	+	+	+	+	+	+	+	+	+	+	NE
Gas from nitrate		+	+	+	+	+	+	_	+	+	+	+	+	+	NE
Arginine dihydrolase		_	_	_			_	_	_	_	_	_		_	_
Hydrolysis of aesculin		_	_	_	_	_	_		_	_	_	_		_	_
Hydrolysis of Tween 80		+	+	_	+	+	+	+	+	+	+		+	+	+
Levan production		+	+	_		+	+	+	+	+	+	+	+	+	+
Litmus milk		alk	alk	alk	alk	alk	alk	alk	alk	alk	alk	alk	alk	alk	alk
Oxidase	č	41K +	+	+	+	+	+	+	+	+	+	+	+	+	+
		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase		+	+	+	+	± -⊥	+	+	+	+	+	+	+	+	+
Urease					+	+	+	+	+	+	+	+	+	+	+
Pectinase		+	+	+ +	+	+	+	+	+	+	+	+	+	+	+
Oxidation of acetate						+	+	+	+	+	+	+	+	+	+
Oxidation of citrate		+	+	+	+	_	_	_	T	_	_	- -	_	_	
Oxidation of malonate				_	_		_	_		_	_	_	_	_	_
Oxidation of gluconate		_	_	_	_	_	_	_		_	_	_	_	_	
NaCl tolerance												,			NIE
1%		+	+	+	+	+	+	+	+	+	+	+	+	+	NE
1.4%		+	+	+	+	+	+	+	+	+	+	+	+	+	NE
1.7%		+	+	_	+	+	+	+	+	_	_	±	+	+	NE
2.0%		-	_	_	_	_	_	_		_	_	_	_	-	NE
	PO1	PO2	PO3	PE1	SI	<b>C1</b>	<b>B2</b>	В3	M2	M4	M5	TB2	EP1	SN1	U1
Starch hydrolysis	_	_	_	_	_	_	_	_	-	_	_	_	_	_	
H <sub>2</sub> S production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole production	_	_	_	_	_	_		_	-	_	_	_	_	_	_
MR test	_	_	_	_	_	_	_	_	_	-	_	-		_	_
VP test		_	_	_		-	_	_		_		_	_	_	_
Gelatin liquification	_	_			_	_	_	_	_	_	_	_	_	_	-
Nitrite from nitrate	+	+	+	+	+	+	+	ND	+	+	+	ND	ND	ND	ΝĽ
Gas from nitrate	+	_	±	+	+	+	+	ND	+	+	+	ND	ND	ND	NI
Arginine dihydrolase	_	_	_	_	_	_	_	_	_		-	_	_		
Hydrolysis of aesculin		_	· —	_	_	_	_	-	_	-	-	_		-	_
Hydrolysis of Tween 80	_	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Levan production	+	+	+	_	+	+	+	+	+	+	_	+	+	+	+
Litmus milk	alk	alk	alk	alk	alk	alk	alk	alk	alk	alk	alk	alk	alk	alk	all
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pectinase	+	±	+	+	+	+	+	+	+	+	+	+	+	+	NI
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidation of acetate		T 1	T .	1.	1		<u> </u>	<u> </u>	<u> </u>	+	÷	+	<u>.</u>	<u>.</u>	+
Oxidation of citrate	+		T	T	—	_	_	_	_	_	_	_		_	_
Oxidation of malonate	_	_			_	_	_	=	_	_	_	_	_	_	_
Oxidation of gluconate	_	_	waters	_	_	_	_		_	_	_	_	_	_	
NaCl tolerance								NE				NID	NID	NIP	N.I.
1%	+	+	+	+	+	+	+	ND	+	+	+	ND	ND	ND	NI
1.4%	+	+	+	+	+	+	+	ND	+	+	+	ND	ND	ND	NI
					<b>±</b>	+		ND	_		_	ND	ND	ND	NI
1.7% 2.0%	+	± _	_	± -	<u> </u>	_	_	ND	_	_	_	ND	ND	ND	NI

 $<sup>^</sup>a+$  = Positive reaction or growth, - = negative reaction or no growth,  $\pm$  = reaction not definite, and alk = alkaline reaction. MR = methyl red, VP = Voges-Proskauer reaction, and ND = not determined.

Table 5. Oxidation of carbohydrates by strains of Pseudomonas solanacearum from China

	Carbohydrate												
Biotype	Glucose												
Strain	Sealed	Unsealed	Sucrose	Fructose	Arabinose	Trehalose	Lactose	Maltose	Cellobiose	Dulcitol	Mannitol	Sorbitol	
Biotype II													
PO2	_a	+	+	+	+	-	+	+	+	_	_		
PO3	_	+	+	+	+	_	+	+	+	_	_	_	
Biotype III													
P7	-	+	+	+	+	+	+	+	+	+	+	+	
P11	_	+	+	+	+	+	+	+	+	+	+	+	
P13	_	+	+	+	+	+	+	+	+	+	+	+	
TM2		+	+	+	+	+	+	+	+	+	+	+	
O2	-	+	+	+	+	+	+	+	+	+	+	+	
O3	_	+	+	+	+	+	+	+	+	+	+	+	
Z2	_	+	+	+	+	+	+	+	+	+	+	+	
PO1	_	+	+	+	+	+	+	+	+	+	+	+	
PE1	_	+	+	+	+	+	+	+	+	+	+	+	
S1	_	+	+	+	+	+	+	+	+	+	+	+	
C1	_	+	+	+	+	+	+	+	+	+	+	+	
TB2	_	+	+	+	+	+	+	+	+	+	+	+	
SN1	-	+	+	+	+	+	+	+	+	+	+	+	
U1	_	+	+	+	+	_	+	+	+	+	+	+	
Biotype IV													
ΡĬ		+	+	+		+		_	_	+	+	+	
P6	_	+	+	+	_	+		_	_	+	+	+	
P9		+	+	+		+	_	_	_	+	+	+	
P14	_	+	+	+	_	+		_	_	+	+	+	
TM1	_	+	+	+	_	+		-		+	+	+	
01	_	+	+	+	_	+		_	******	+	+	+	
ZI	_	+	+	+	+	+	_	_	_	+	+	+	
B2	_	+	+	+	+	+	_	_	_	+	+	+	
B3	_	+	+	+	+	+	_	_		+	+	+	
EI	_	+	+	+	_	+	_	_	_	+	+	+	
Biotype V		•	•	•		•				•	•	•	
M2	_	+ .	+	+	+	+	+	+	+	_	+		
M4	_	+	+	+	+	+	+	+	+	_	+	_	
M5	_	+	+	+	+	+	+	+	+	_	+	_	

 $<sup>^{</sup>a}-=$  Negative and += positive reaction.

Table 6. Susceptibility of some strains of Pseudomonas solanacearum to antibiotics

		Strain							
Antibiotic	Concentration $(\mu \mathbf{g}/\mathbf{ml})$	82, PO2, PO3, 276	P11, O3, C1, PO1, TB1 P6, O1, Z1, B2	M4, M5					
Viomycin	2	R <sup>a</sup>	R	R					
•	10	WS	R-WS	WS					
Chloramphenicol	5	R	R	R					
•	30	R	R-WS	S					
Oleandomycin	15	R	S	R-WS					
Penicillin	10	R	R	R					
Streptomycin	10	S	S	S					

<sup>&</sup>lt;sup>a</sup> R = resistant, zone of inhibition absent; S = susceptible, wide zone of inhibition (>12 mm); and WS = weakly susceptible, zone of inhibition indefinite or small (<11 mm).

from mulberry did not fit into any of the known races.

Hypersensitivity. Most strains of *P. solanacearum* from China caused a typical HR in tobacco leaves (Table 3). Exceptions were strains pathogenic on tobacco (pathogenicity group 1) that caused a slow, spreading necrosis similar to that caused by strain K60, a virulent race 1 strain from the United States. Other strains, such as TM2, PE1, B2, and PO3, caused only a slow collapse of the infiltrated area after 48 hr, but symptoms were indistinguishable from the HR after 5 days.

Physiological characteristics. All strains produced H<sub>2</sub>S from cysteine and reduced nitrate; most hydrolyzed Tween 20 and produced levan. All strains were

arginine dihydrolase negative and oxidase, catalase, and urease positive. All strains oxidized acetate and citrate but not malonate or gluconate. The methyl red (MR) test and Voges-Proskauer (VP) reaction were negative with all strains. No strains hydrolyzed starch or aesculin, produced indole, or liquified gelatin. As is characteristic of other strains of this species, all strains from China showed a very high sensitivity to NaCl (none grew at 2% NaCl). Most strains formed shallow pits on the CVP medium, indicating formation of pectic enzymes (Table 4).

Marked differences were observed in the ability of strains from China to oxidize sugar alcohols and disaccharides (Table 5). In accordance with Hayward's classification (10), 14 of 29 strains were classified as biotype III and 10 as biotype IV. Two strains from potato were classified as biotype II. The remaining three strains, all from mulberry, could not be placed in any of the known biotypes. These strains produced acid from lactose, maltose, cellobiose, and mannitol but failed to oxidize dulcitol and sorbitol, even after 28 days of incubation.

All isolates from China were resistant to penicillin  $(10 \,\mu\text{g})$ , viomycin  $(2 \,\mu\text{g})$ , and chloramphenicol  $(5 \,\mu\text{g})$  and were susceptible to streptomycin  $(10 \,\mu\text{g})$ . They differed from one another in susceptibility to oleandomycin  $(15 \,\mu\text{g})$ ; strains belonging to biotypes III and IV were susceptible but other strains were resistant or only weakly susceptible. All mulberry isolates were susceptible to chloramphenicol  $(30 \,\mu\text{g})$  (Table 6).

## DISCUSSION

Evolution of *P. solanacearum* in China seems to have occurred along lines somewhat divergent from patterns in other parts of the world. Evidence for this is the existence of strains that attack sweet potato, olive, casuarina, mulberry, etc., plants that have not been described previously as hosts in other geographical locations where the pathogen occurs and these crops are grown. On the basis of

their cultural, biochemical, and physiological characteristics, most strains of *P. solanacearum* from China, except those that attack mulberry, resemble races or biotypes known from other regions of the world. It is surprising, however, that no representatives of biotype I were present in the 29 isolates from China that were examined. This biotype is present in most parts of the world. It was not surprising that race 2 representatives were not among those collected in China because the center of origin of this race appears to be in the Caribbean (1).

Strains from mulberry that we tested were unusual because they were only weakly virulent on eggplant and potato and not virulent on tomato, pepper, peanut, or tobacco. Physiologically, these strains also were unusual in their ability to oxidize lactose, maltose, cellobiose, and mannitol, a combination of traits not found in biotypes described previously by Hayward (10). For this reason, it is proposed that this group be designated race 4, biotype V. This will require confirmation by further research with additional strains from mulberry. Other strains may exist on mulberry because Ren et al (23) classified as biotype I the strains they isolated from mulberry in the same province of China (Guangdong).

The lack of virulence to tobacco of many strains from China was unexpected, but similar results were obtained by Hsu et al (12) with strains from Taiwan. The high susceptibility of eggplant (cultivar Black Beauty) to most strains from China supports the previous report that eggplant is a universal suscept for race 1 (6).

On the basis of pathogenicity tests with a relatively small sample (29 strains), most strains of *P. solanacearum* from China appear to belong to race I. Within this broad group, however, there are at least four pathogenicity groups. According to recommendations made at the International Planning Conference and Workshop on Bacterial Wilt (25), we have not designated these groups as pathotypes. Identification of these groups and establishment of their geographic distribution, however, are important for design of crop rotation schemes and other control measures.

Unfortunately, as Hayward (10) and others have pointed out, there is no direct correlation between physiological characteristics and pathogenicity in most instances. Therefore, biotype designation in the laboratory is not particularly useful in determining the potential host range of a strain. Host range must be determined by inoculating differential hosts in the greenhouse, which in turn is subject to the variability inherent in the inoculation procedures. In addition, certain host plants wilt when inoculated in the greenhouse but do not do so in nature. This study, therefore, can be considered only preliminary and must be confirmed by additional research with a much larger number of strains from additional locations in China.

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