Strains of *Pseudomonas solanacearum* Affecting Solanaceae in the Americas

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ABSTRACT

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Of 85 strains of *Pseudomonas solanacearum* from 10 countries in the Americas and from four solanaceous hosts, 28 strains were biotype I, 52 were biotype II, and 5 were biotype III. Biotype I was identified among strains from Brazil, Colombia, Costa Rica, Peru, and the USA. Biotype III was found among strains from Costa Rica, Peru, and Brazil only; but none of the strains were from potatoes. Biotype IV was not found. Thirty representative strains were selected for pathogenicity tests on tomato (*Lycopersicon esculentum* 'Huando'), potato (*Solanum tuberosum* 'Ticahuasi'), and *Nicotiana glutinosa*. Twenty-three strains were pathogenic on all three hosts, by a soil infestation method. Biotype II had more pathogenic strains than biotype I under glasshouse conditions. Only one strain of biotype III, from tomato, affected potatoes.

Bacterial wilt, caused by *Pseudomonas solanacearum*, is the most important bacterial disease of potato, tobacco, tomato, and banana in the tropical and subtropical regions of the world (1,3,12,16). This pathogen has many pathovars and a wide range of host plants. On the basis of host range, three races have been proposed (2). Two races can infect potatoes: race 1 affects most solanaceous crops including potatoes, and race 3 affects potatoes primarily and other solanaceous species to a lesser extent. Race 1 is believed to have originated in the lowland tropics and race 3 in the highland tropics (2,5,18,19).

Bacterial wilt affects potatoes and other solanaceous crops in North, Central, and South America (3,6–8, 11,12,17). In most instances, identification of the pathogen has not included race determination, and the biotype concept developed by Hayward in 1964 (9,10) has been used more in other continents than in the Americas. Both the race and biotype systems of classification are useful in characterizing the pathovars in each region (4).

For this study, we assembled a representative group of strains from the Americas to determine their principal characteristics, and we selected strains isolated from different host species and obtained in different geographic and climatic conditions. The geographic distribution of *P. solanacearum* affecting potatoes and other solanaceous hosts will be of value to pathologists and breeders who are trying to find controls for this

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disease problem. A preliminary report has been published (15).

MATERIALS AND METHODS

Bacterial strains. Eighty-five strains of *P. solanacearum* from 10 countries in North, Central, and South America and originating from four solanaceous hosts were assembled.

After purification, strains were stored in sterile tap water in screw-cap tubes at room temperature. Strains were streaked on tetrazolium chloride (TZC) medium to prepare inoculum; fluidal, wild-type colonies were selected (13) and transferred to tubes containing 5 ml of sterile tap water. Inoculum was obtained from 48-hr growth at 30 C of each strain on TZC medium without tetrazolium salts.

Biotype determination. The biotype of the strains was determined according to the method described by Hayward (9,10) in which four biotypes are established on the basis of each strain's ability to utilize three hexose alcohols and to produce acid from three disaccharides. Media were prepared, placed in test tubes, inoculated with a loopful of each strain, and maintained at 30 C for 14 days. The color change of the media was recorded daily throughout the experiment. Thirty representative strains were selected for further studies (Table 1).

Pathogenicity tests. Pathogenicity of the 30 strains was tested on potato (*Solanum tuberosum* 'Ticahuasi'), tomato (*Lycopersicon esculentum* 'Huando'), and *Nicotiana glutinosa*. These were grown in 13-cm-diameter clay pots containing a sterilized mixture of soil, peat moss, and Jiffy Mix (2:1:1) with a pH of about 6.5. Initial growth was in a screenhouse at 14–23 C. Five days before inoculation, plants were transferred to a glasshouse at 26–32 C. Potato plants were inoculated 20 days after emergence (15–20 cm tall) and the other two hosts 14 days after transplanting.

Inoculation. Plants were inoculated by pouring 50 ml of a bacterial suspension (approximately 2×10^7 cells per milliliter in sterilized tap water) onto the soil in each pot. Four to five hours before inoculation, pots were watered to field capacity in order to distribute the inoculum uniformly throughout the soil.

Host reactions were recorded three times per week until 45 days after inoculation. Five plants per host and per strain were inoculated in the first test and eight in the second.

When a plant became wilted, several stem sections were taken to isolate the bacteria on TZC medium to confirm the presence of *P. solanacearum* (12). Plants remaining after 45 days were similarly assayed.

RESULTS

Biotype determination. Three of the four biotypes (9) were determined among the 85 strains. Their geographic distribution is listed in Table 2. There were 28 strains of biotype I, 52 of biotype II, and 5 of biotype III. Of the 30 selected strains, 15 were biotype I, 12 were biotype II, and 3 were biotype III (Table 1). No strains of biotype IV were found in this collection of strains. All three biotypes were found only in Brazil, Costa Rica, and Peru, but only biotype II was found in Argentina, Mexico, Panama, Uruguay, and Venezuela (Table 2). Biotype I was identified among strains from Brazil, Colombia, Costa Rica, Peru, and the USA. Biotype III was found among strains from Costa Rica, Peru, and Brazil only, but none of the strains were from potatoes.

Pathogenicity tests. Only strain 051 did not wilt any of the three hosts species (Table 1). Root isolations were not made, but although this strain retained its fluidal wild-type characteristic, it probably was an avirulent mutant. The other 29 strains were pathogenic on at least one host.

For differential purposes, strains that wilted up to 50% of the tested plants were considered pathogenic and those that wilted 50–100% of the plants were considered highly pathogenic. Twenty-three were pathogenic on all three hosts

Table 1. Pathogenicity of *Pseudomonas solanacearum* strains from potato and other solanaceous crops from the Americas

Strain[a]	Collection site	Isolated by	Year of isolation	Biotype	Pathogenicity[b] on *Nicotiana glutinosa*	Tomato	Potato
010-Te Potato	Balcarce, Argentina	A. Calderoni, O. Malamud	1979	II	++	++	++
069-ST Tomato	Sao Paulo, Brazil	J. Neto	1976	I	+	+	++
075-ST Potato	Sao Paulo, Brazil	J. Neto	1976	II	++	+	++
098-LT Tomato	Manaus, Brazil	A. Takatsu	1976	III	0	++	0
045-LJ Potato	Rio de Janeiro, Brazil	S. Drummond	1975	I	+	+	++
100-LJ Tobacco	Santander, Colombia	G. Granada	1972	I	++	0	0
104-HT Potato	Antioquia, Colombia	G. Granada	1976	II	+	+	++
127-HT Tomato	Antioquia, Colombia	G. Granada	1977	I	+	0	++
048-LT Potato	Costa Rica	L. Sequeira	1965	II	++	++	++
065-LT Chili	La Garita, Costa Rica	L. Gonzalez	1972	III	++	+	0
066-HT Potato	Pacayas, Costa Rica	L. Gonzalez	1972	I	0	+	++
092-LT Tomato	Paraiso, Costa Rica	E. French	1976	I	++	++	0
105-LT Tobacco	Puriscal, Costa Rica	L. Gonzalez	1977	I	+	+	++
124-LT Potato	Turrialba, Costa Rica	M. Jackson	1977	I	++	+	++
068-HT Potato	Tlaxcala, Mexico	L. Fucikovsky	1976	II	++	+	++
071-HT Potato	Sinaloa, Mexico	L. Fucikovsky	1976	II	++	++	++
067-HT Potato	Chiriqui, Panama	C. Martin	1976	II	++	++	++
003-HT Potato	Piura, Peru	I. Herrera	1974	I	++	++	++
013-HT Potato	Piura, Peru	I. Herrera	1973	II	++	++	++
015-HT Potato	Tingo Maria, Peru	I. Herrera	1972	I	+	+	+
018-LT Potato	Yurimaguas, Peru	C. Martin	1975	I	++	+	+
061-LT Potato	Yurimaguas, Peru	C. Martin	1976	II	+	++	+
072-LT Tomato	Iquitos, Peru	E. French	1966	III	++	+	+
096-HT Potato	Huanuco, Peru	C. Martin	1976	II	++	++	++
130-LT Potato	San Ramon, Peru	C. Martin	1977	I	++	+	++
058-Te Potato	Rocha, Uruguay	S. Garcia	1975	II	++	++	++
051-Te Potato	North Carolina, USA	A. Kelman	1953	I	0	0	0
060-Te Tomato	North Carolina, USA	A. Kelman	1953	I	+	+	+
109-Te Potato	Florida, USA	R. Stall	1978	I	++	++	++
146-HT Potato	Anzoategui, Venezuela	C. Martin	1978	II	++	++	++

[a] Numbers are those of the International Potato Center collection. The letter codes after number designate the climatic origin as follows: Te = temperate region at latitudes greater than 35° south or north of the equator and below 1,500-m elevation; ST = subtropical, between the tropics of Cancer and Capricorn and 35° north or south; LT = lowland tropics, below 1,500-m elevation; and HT = highland tropics, above 1,500 m.

[b] Host susceptibility: + = 1–50% wilted plants; ++ = 51–100% wilted plants; 0 = no wilted plants, 45 days after inoculation.

Table 2. Distribution of biotypes and races of *Pseudomonas solanacearum* affecting potatoes and other solanaceous crops in the Americas

Country	Climatic region[a]	Biotype	Race
Argentina	Te	II	3
Brazil	LT	I,III	1
	ST	I,II	1,3
Colombia	LT	I	1
	HT	II	3
Costa Rica	LT	I,II,III	1,3
	HT	I,II	1,3
Mexico	LT	II	3
	HT	II	3
Panama	HT	II	3
Peru	LT	I,II,III	1,3
	HT	I,II	1,3
Uruguay	Te	II	3
USA	Te	I	1
	ST	I	1
Venezuela	HT	II	3

[a]Te = temperate region at latitudes greater than 35° south or north of the equator and below 1,500-m elevation; ST = subtropical, between the tropics of Cancer and Capricorn and 35° north or south; LT = lowland tropics, below 1,500-m elevation; HT = highland tropics, above 1,500-m.

and strains 003, 109, 010, 013, 048, 058, 067, 071, 096, and 146 were highly pathogenic. Highly pathogenic strains were more frequent among biotype II than among biotype I samples. Only one strain of biotype III, from tomato near Iquitos on the banks of the Amazon River in Peru, affected potato. It was previously shown to be highly pathogenic to potato by stem inoculation (5).

DISCUSSION

We used relatively few strains in this research, for such a large geographic region as the Americas, and these strains represent primarily the tropical belt with only a few strains from the subtropics and temperate zone. No strains were available from tropical countries such as Bolivia, Paraguay, or Ecuador in South America or Nicaragua, El Salvador, Guatemala, or Honduras in Central America. Bacterial wilt has never been reported on any solanaceous crop in Chile, a temperate country.

The inoculation experiments by soil infestation, which involves no wounding and thus simulates nature better than the stem puncture inoculation technique most often used (5), show that some biotype I strains are as pathogenic as some biotype II strains. This seems to contradict the concept of a specialized, highland potato strain of *P. solanacearum* that is always biotype II and is homologous with race 3 (10,18,19). This concept is further weakened by the pathogenicity of strains from potato in the Amazon basin at a 170-m elevation at Yurimaguas, Peru (14). Isolate 061, the only biotype II strain from that site used in the inoculations was, however, less pathogenic to potatoes than other biotype II strains but was similar in pathogenicity to the biotype I strain 018 from the same location.

The greatest genetic diversity of *P. solanacearum*, as reflected by the incidence of different biotypes and pathogenicity to several hosts, appears to be in the tropical lowlands of Brazil, Costa Rica, and Peru. Conversely, genetic diversity of the bacteria apparently is less at greater distances from the equator.

In the United States, only biotype I has been reported from Florida to North Carolina (one report of race 3 did not include enough information to validate its identification as race 3), and there it is reported to be indigenous (12). Our results also confirm the report (4) that biotypes I and II are the most widespread in the Americas, that biotype III is rare, and that biotype IV is not found at all.

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