Numerical Analysis of Phenotypic Features of *Pseudomonas solanacearum* Strains Isolated from Tobacco and Other Hosts in South Africa

MARTHIE C. ENGELBRECHT and M. J. HATTINGH, Department of Plant Pathology, University of Stellenbosch, Stellenbosch 7600, South Africa

ABSTRACT

Engelbrecht, M. C., and Hattingh, M. J. 1989. Numerical analysis of phenotypic features of *Pseudomonas solanacearum* strains isolated from tobacco and other hosts in South Africa. Plant Disease 73:893-898.

Forty-six strains of *Pseudomonas solanacearum* from South Africa and 13 reference strains were subjected to numerical phenetic analysis using 55 unit characters. Data were analyzed by applying unweighted pair-grouping clustering on the simple matching (S_{sm}) coefficient. At 85% S_{sm}, three phenons and two solitary strains were distinguished. Phenons 1, 2, and 3 corresponded, respectively, with biovars III, I, and II. All strains in phenons 1 and 2 were assigned to race 1 and those of phenon 3 to race 3. At 95% S_{sm}, three groups were recognized within each phenon. None of the reference strains corresponded with South African strains in all of the features examined. Apart from five potato strains of group 3A, all South African strains of *P. solanacearum* were assigned to groups 1A and 1B of phenon 1. Local race 1 tobacco strains were highly virulent on other host species tested.

Additional keywords: bacterial wilt

Bacterial wilt caused by *Pseudomonas solanacearum* (Smith) Smith is one of the most widespread and destructive diseases of many crops of economic importance (22,26). Before 1980, the disease was known to occur in South Africa on castor oil, dahlia, eggplant, groundnut, nasturtium, potato, pepper, and tomato (12). Significantly, tobacco was not listed as a host. McClean (32) found that a groundnut strain of *P. solanacearum* caused some symptoms on inoculated tobacco but the plants recovered. Wager (42) speculated that tobacco grown in South Africa is immune to the disease. Early in the present decade, however, outbreaks of bacterial wilt appeared locally in tobacco fields in the Lowveld area of Transvaal province, and the pathogen was subsequently isolated from diseased plants (8).

Strains of *P. solanacearum* have traditionally been differentiated into three races according to host range (5). Numerous known and unknown pathogenic strains, some with host specialization, occur within each race (4,10,23,31). These strains probably evolved independently in different regions of the world (3). More recently, the unique ginger and mulberry strains have been added as races 4 and 5, respectively (3). Apart from races, four biovars have been defined within *P. solanacearum* on the basis of their ability to oxidize certain carbohydrates (20). The mulberry strains were later designated as biovar V (24). Races and biovars usually do not correspond. Until now, however, strains of race 3 always belonged to biovar II, although the reverse is not necessarily true (3).

Swanepoel and Young (40) found that six South African tobacco strains of *P. solanacearum* were representative of biovar III. Little else is known of the characteristics of strains causing bacterial wilt of tobacco in this country. The spread of the disease to other tobacco-growing regions of Transvaal and the origin of the inoculum are of particular concern to the industry. The present study examines the phenotypic grouping of local and reference strains from tobacco and other hosts. The race and biovar of each test strain were also determined.

Present address of first author: Tobacco and Cotton Research Institute, Private Bag X82075, Rustenburg 0300, South Africa.

Part of an M.Sc. thesis submitted by the first author to the University of Stellenbosch.

Accepted for publication 5 June 1989 (submitted for electronic processing).

© 1989 The American Phytopathological Society

MATERIALS AND METHODS

Origin of cultures. The source and original hosts of the 59 strains of *P. solanacearum* studied are given in Table 1. Thirteen reference strains included were revived on tetrazolium chloride (TZC) agar (27) from lyophilized cultures. Transfers from single fluidal colonies of each strain were kept as stock cultures in sterile tap water at 20 C (28). These suspensions were regularly streaked on TZC agar plates to select virulent type colonies used throughout the investigation.

Physiological and biochemical features. Unless stated otherwise, solid test media were inoculated by transferring growth from 48-hr-old virulent-type colonies on TZC agar with a sterile loop. Tubes of liquid or semisolid test media were inoculated from slightly turbid suspensions prepared by suspending growth from 24-hr-old colonies on nutrient agar (NA) in sterile distilled water. Tests were performed at 30 C (unless otherwise required) and were then repeated, on two separate days. Uninoculated control media were included.

Tyrosinase activity was tested on the medium described by Hayward (20) and on a modified TZC medium (11). Results were read after 48 hr. Ammonia production was tested according to Harrison (17). Hydrolysis of Tween 40, 60, and 80 was observed after 6 days, following the method of Sierra (36). Phosphatase activity was detected on NA containing a 0.1% filter-sterilized solution of phenolphthalein diphosphate sodium salt (25). Nitrate reduction was determined by the method of Hayward (21) after culturing strains (three serial transfers) on his medium. Growth of strains at 12 and 39 C in yeast salts broth was determined after 7 and 14 days (9).

Oxidation of individual carbohydrates (10 g/L) was tested in the basal medium of Hayward (20) containing 0.08 g/L of bromothymol blue indicator (21). For this, a 10% solution of each carbohydrate was filter-sterilized and 1-ml volumes were dispensed in tubes each containing 9 ml of autoclaved basal medium; dulcitol, which is poorly soluble, was autoclaved in the medium. Strains were also checked in carbohydrate-free control medium. Final readings were taken after 21 days' incubation.

Utilization of individual organic acids at a concentration of 0.1% (based on the active carbon radical) was tested in standard mineral base (SMB) medium (9); agar was omitted, however, and pH was adjusted to 7. Soluble organic acid salts were neutralized and filter-sterilized before being added to autoclaved SMB. Insoluble L-tyrosine, DL-tryptophan, hippurate, azelate, sebacate, or *p*-hydroxybenzoate was added directly to SMB before autoclaving. Turbidity due to bacterial growth was recorded until 21 days after inoculation. Each strain was

also tested in nonsupplemented SMB.

Catalase activity was tested in a sucrose-peptone medium (19) by using 20 vol of hydrogen peroxide (20). Tests for starch hydrolysis (37) and gelatin liquefaction (41) were performed on NA plates supplemented with 0.2% soluble starch or 0.4% gelatin. Levan production was observed on NA plates containing 50 g/L of sucrose (20). Oxidation of gluconate was determined after 7 and 14 days following the method of Haynes (18) but with 40 g/L of sodium gluconate instead of potassium gluconate. Sensitivity of strains to streptomycin (10 and 25 μg), chloramphenicol (10, 30, and 50 μg), oleandomycin (15 μg), and bacitracin (10 μg) incorporated in Oxoid sensitivity disks was recorded after 48 hr on seeded TZC agar plates. Tolerance of strains to five concentrations of NaCl (1, 1.4, 1.6, 1.7, and 2%) was tested in the basal medium of Hayward (20) after 48 hr.

Biovar determination. The ability of each strain to oxidize the disaccharides maltose, lactose, and cellobiose and the hexose alcohols mannitol, dulcitol, and sorbitol was tested on media the same as that used to test oxidation of carbohydrates. Acid production and the pattern of growth in the tubes were recorded weekly for 21 days. The test was repeated four times on separate days.

Differentiation of races. The reactions of infiltrated leaves were used to determine the race of each strain according to Lozano and Sequeira (29). Races were also separated on the basis of host response as proposed by Buddenhagen et al (5).

Fully developed intact leaves on 10-wk-old tobacco plants (*Nicotiana tabacum* L. 'Bottom Special') grown in a greenhouse (26–34 C) were used for infiltration. Bacterial suspensions were prepared from cultures in the exponential growth phase and standardized turbidometrically to approximately 10^9 cfu according to Sequeira (35). Hypodermic syringes without needles were used to infiltrate each strain into the abaxial sides of two leaves on two different plants on three separate occasions.

Pathogenicity of 15 selected strains of *P. solanacearum* from South Africa (local quarantine regulations precluded the use of imported strains) was determined on the following host species: tobacco cv. Bottom Special, tomato (*Lycopersicon esculentum* Mill.) cvs. Rooi Kaki (susceptible) and Rodade (resistant), potato (*Solanum tuberosum* L.) cv. Vanderplank, eggplant (*S. melongena* L.) cv. Black Beauty, green pepper (*Capsicum annuum* L.) cv. California Wonder, chili pepper (*C. frutescens* L.) cv. Kroondal Cayenne, groundnut (*Arachis hypogaea* L.) cvs. White Pearl Spanish and Sellie, sunflower (*Helianthus annuus* L.), cotton (*Gossypium hirsutum* L.) cv. Acala 1517-70, dahlia (*Dahlia rosea* Cav.) cv. Extra Dwarf Early Bird, triploid banana (*Musa acuminata* Colla) cv. Dwarf Cavendish, and diploid banana (*Ensete ventricosum* (Welw.) Cheesman).

Test plants were grown from seed planted singly in 10-cm-diameter pots kept in a greenhouse (26–34 C). However, tobacco was sown in seed trays before being transplanted to pots after 3 wk and triploid banana plants were grown from callus cultures. Plants in pots were grown to a height of 20–35 cm. This took 4–8 wk except for triploid banana (4 mo) and diploid banana (6 mo). Inoculum was prepared by suspending 24-hr-old NA agar cultures in deionized water and adjusting the concentration turbidometrically to approximately 10^8 cfu, as described by Kelman and Person (28). Stem and roots of separate plants were inoculated according to Winstead and Kelman (43). Four plants were used per host for each of the two methods of inoculation. Control plants were treated with sterile water. Disease readings were taken at 5-day intervals until 35 days after inoculation.

Numerical analysis of phenotypic features. Results obtained from the standard physiological and biochemical tests as well as data obtained to identify biovars and the reaction of strains in infiltrated tobacco leaves were used for the computer-assisted numerical analysis. From the 73 tests applied, 18 were redundant and thus not included. All tests were scored as positive (coded 2) or negative (coded 1). Multistate features (response in tobacco leaves and reaction in nitrate, mannitol, dulcitol, and sorbitol media) were divided into mutually exclusive states. Missing or doubtful results (at an average of 0.12% per strain) were coded as 0 (no comparison).

The similarity coefficient S_{sm} (39), which includes positive and negative matches, was determined with the Bonham-Carter (1) program modified by K. Kersters (Laboratorium voor Microbiologie en Microbiële Genetica, Rijksuniversiteit, Ghent, Belgium). A Sperry Univac 1100 computer was used for the calculations. Strains were clustered by the unweighted average pair-group method (38), and the levels of association were used to construct a dendrogram.

Table 1. Origin of strains of *Pseudomonas solanacearum* used in numerical analysis of phenotypic features and assignment of strains to biovars

Original host	Strain number	Biovar[a]	Source[b]
Tobacco	G11(246)	I	Santander, Colombia (G. A. Granada)
	K105(27)	I	Florida, USA (A. Kelman)
	S240(147)	IV	Ingham, Australia (A. C. Hayward)
	Z1, Z2, Z3	I	Zimbabwe (C. R. Fischer)
	L1,[c] L5,[c] L6,[c] L7,[c] L9[c]	III	Barberton (M. C. Engelbrecht)
	L2, L12, L13, L18, L19, L22	III	Hazyview (M. C. Engelbrecht)
	L3	III	Carino (M. C. Engelbrecht)
	L4, L11	III	Schagen (M. C. Engelbrecht)
	L8, L14, L15, L23	III	Plaston (M. C. Engelbrecht)
	L10[c]	III	White Hills (M. C. Engelbrecht)
	L16, L17	III	Friedenheim Estate (M. C. Engelbrecht)
	M1,[c] M2,[c] M3,[c] M5,[c] M6,[c] M11,[c] M12,[c] M13[c]	III	Groblersdal (M. C. Engelbrecht)
	M7,[c] M8[c]	III	Brits (M. C. Engelbrecht)
Potato	K235(37)	II	Bogotá, Colombia (H. D. Thurston)
	S213(119)	II	Paraiso, Costa Rica (L. Sequeira)
	S221(134)	I	Nairobi, Kenya (R. Robinson)
	S243(150)	II	Nambour, Australia (A. C. Hayward)
	257(257)	II	Chicua, Costa Rica (L. Gonzalez)
	H2, H4	II	Soekmekaar (A. E. Swanepoel)
	H5	II	Makoppa (A. E. Swanepoel)
	H6	II	Clanwilliam (A. E. Swanepoel)
	H7	III	New Hanover (A. E. Swanepoel)
Tomato	K60(25)	I	North Carolina, USA (A. Kelman)
	S236(143)	III	Nambour, Australia (A. C. Hayward)
	H1, H3	III	Nelspruit (A. E. Swanepoel)
	H8[c]	III	Boons (M. C. Engelbrecht)
	H11	III	Empangeni (M. C. Engelbrecht)
	H14[c]	III	Groblersdal (M. C. Engelbrecht)
	H15	III	Hazyview (M. C. Engelbrecht)
Pepper	H13	III	Port Shepstone (M. C. Engelbrecht)
Eggplant	H17	III	Brits (M. C. Engelbrecht)
Datura ferox	L20	III	Heidelberg, Transvaal (M. C. Engelbrecht)
	M4[c]	III	Groblersdal (M. C. Engelbrecht)

[a] Based on oxidation of disaccharides (lactose, maltose, cellobiose) and hexose alcohols (mannitol, sorbitol, dulcitol) according to Hayward (20). Biovar I oxidizes none of the carbohydrates; biovar II, disaccharides only; biovar III, both groups; biovar IV, alcohols only.

[b] Unless otherwise stated, locations are in South Africa. Strains were isolated by or obtained from investigators mentioned in parentheses.

[c] Strains grew in a ring below the surface of media containing hexose alcohols, although acid was produced. Other biovar III strains and the biovar IV strain formed the typical pellicle on these media.

RESULTS

Clustering of strains. The phenotypic similarities among the 59 strains of *P. solanacearum* are illustrated in the dendrogram (Fig. 1). All strains were associated at 40.28% S_{sm}. At 85% S_{sm}, three phenons and two solitary strains were distinguished. Strains isolated from tobacco occurred in two of the three phenons. Forty-three (72.9%) of the strains were assigned to phenon 1. At 95% S_{sm}, three groups were recognized within each of the three phenons. However, groups 1C, 2C, 3B, and 3C each comprised only a single strain.

Characterization of clusters. All strains tested showed catalase activity and utilized acetate, fumarate, and succinate as sole carbon sources. None hydrolyzed starch, liquefied gelatin, produced levan, or formed 2-ketogluconate. Acid was not produced from D(−)arabinose or D(−)ribose, and DL-phenylalanine was not utilized as sole carbon source. All strains were to some extent sensitive to each of the four antibiotics tested, irrespective of concentration. The results obtained on media containing different concentrations of NaCl were not reproducible. These data were therefore not used to distinguish clusters.

Characterization of phenons and groups was based on a combination of 55 valid test responses listed in Table 2. Several tests (production of ammonia; oxidation of lactose, maltose, cellobiose, mannitol, dulcitol, and sorbitol; utilization of sarcosine, glycollate, and Ca lactate; and response in tobacco leaves) are highly specific and can be applied to distinguish among the three phenons.

A single characteristic (formation of a pellicle on liquid media containing mannitol, dulcitol, or sorbitol) discriminates group 1A from groups 1B and 1C. Unlike strains of group 1A and 1B, the single strain of group 1C did not produce tyrosinase and failed to grow at 39 C but produced gas in nitrate medium.

Within phenon 2, groups 2A and 2B were separated by tests for hydrolysis of Tween 80, growth at 12 and 39 C, and utilization of hippurate, L-(+)-tartrate, DL-tartrate, and *p*-hydroxybenzoate. In contrast, only the single strain of group 2C failed to oxidize *m*-inositol but utilized malonate and quinate.

The three groups of phenon 3 were separated by results obtained in six tests: reduction of nitrate; oxidation of trehalose and D(+)xylose; and utilization of β-alanine, pentanoate, and *p*-hydroxybenzoate.

Phenon 1 contained 42 of the 46 strains isolated in South Africa from tobacco, potato, tomato, pepper, eggplant, and *Datura ferox* L. Phenon 2 held reference strains only. Three strains (Z1, Z2, and Z3) isolated from tobacco in Zimbabwe

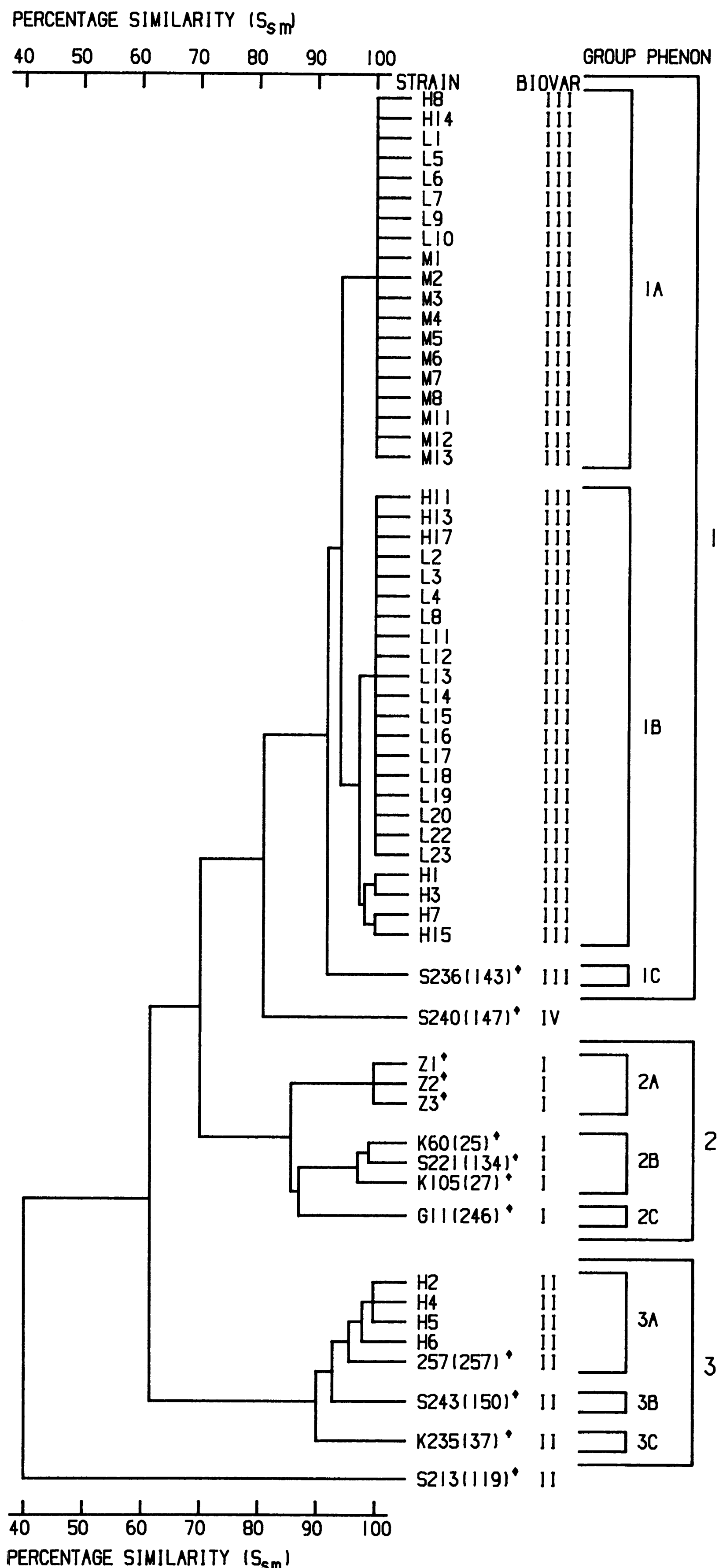

Fig. 1. Dendrogram of S_{sm} similarity coefficients, clustered by the average unweighted pair-grouping method, showing the phenotypic similarities among 46 South African and 13 reference strains (*) of *Pseudomonas solanacearum*.

clustered in group 2A. Strain K60(25) (derivative of the type strain ATCC 11696) was assigned to group 2B. All seven strains in phenon 3 were isolated from potato. Four local strains formed a tight cluster within group 3A.

Biovar determination. Allocation of the 59 strains to the four biovars proposed by Hayward (20) is shown in Table 1. All strains isolated from tobacco, tomato, pepper, eggplant, and *D. ferox* in South Africa were assigned to biovar III. Apart from the single biovar IV representative, all reference strains isolated from tobacco in other

Table 2. Physiological and biochemical features of phenons,[a] groups,[b] and two solitary strains of *Pseudomonas solanacearum* discerned by unweighted pair-group analysis on the simple matching coefficient

	Strains positive (%)												Solitary strains[c]	
Features	**1**	**1A**	**1B**	**1C**	**2**	**2A**	**2B**	**2C**	**3**	**3A**	**3B**	**3C**	**S213**	**S240**
Tyrosinase[d]	98	100	100	0	100	100	100	100	0	0	0	0	+	−
Ammonia	100	100	100	100	100	100	100	100	0	0	0	0	+	+
Tween 80 hydrolysis	100	100	100	100	57	0	100	100	100	100	100	100	+	+
Phosphatase	0	0	0	0	29	0	33	100	100	100	100	100	−	−
Reaction in nitrate medium														
Reduction to nitrite	100	100	100	100	100	100	100	100	86	100	100	0	+	+
Gas production	2	0	0	100	0	0	0	0	0	0	0	0	+	+
Growth at														
12 C	0	0	0	0	43	100	0	0	0	0	0	0	−	−
39 C	98	100	100	0	57	0	100	100	100	100	100	100	+	−
Pellicle on broth containing														
Mannitol	56	0	100	100	0	0	0	0	0	0	0	0	−	+
Dulcitol	56	0	100	100	0	0	0	0	0	0	0	0	−	+
Sorbitol	56	0	100	100	0	0	0	0	0	0	0	0	−	+
Oxidation of carbohydrates														
Lactose	100	100	100	100	0	0	0	0	100	100	100	100	+	−
Maltose	100	100	100	100	0	0	0	0	100	100	100	100	+	−
Cellobiose	100	100	100	100	0	0	0	0	100	100	100	100	+	−
Mannitol	100	100	100	100	0	0	0	0	0	0	0	0	−	+
Dulcitol	100	100	100	100	0	0	0	0	0	0	0	0	−	+
Sorbitol	100	100	100	100	0	0	0	0	0	0	0	0	−	+
Glycerol	100	100	100	100	100	100	100	100	100	100	100	100	−	+
Sucrose	100	100	100	100	100	100	100	100	100	100	100	100	−	+
D(+)Galactose	100	100	100	100	0	0	0	0	43	20	100	100	−	+
D(−)Fructose	100	100	100	100	100	100	100	100	100	100	100	100	−	+
Trehalose	100	100	100	100	100	100	100	100	14	0	0	100	+	−
m-Inositol	100	100	100	100	86	100	100	0	0	0	0	0	−	+
D(+)Xylose	0	0	0	0	0	0	0	0	14	0	100	0	−	−
D(+)Mannose	100	100	100	100	14	0	33	0	100	100	100	100	+	−
L(+)Arabinose	5	0	9	0	0	0	0	0	0	0	0	0	−	−
Utilization of organic acids														
Betaine	9	0	17	0	0	0	0	0	0	0	0	0	−	−
Sarcosine	100	100	100	100	0	0	0	0	0	0	0	0	−	+
DL-Asparagine	100	100	100	100	100	100	100	100	100	100	100	100	−	+
L-Threonine	100	100	100	100	100	100	100	100	100	100	100	100	−	+
β-Alanine	100	100	100	100	100	100	100	100	86	100	100	0	−	+
L-Ornithine	100	100	100	100	100	100	100	100	100	100	100	100	−	+
DL-Tryptophan	100	100	100	100	100	100	100	100	0	0	0	0	−	−
Hippurate	0	0	0	0	57	0	100	100	100	100	100	100	−	−
Gluconate	100	100	100	100	100	100	100	100	100	100	100	100	−	+
DL-Glutamate	100	100	100	100	100	100	100	100	100	100	100	100	−	+
Propionate	100	100	100	100	100	100	100	100	100	100	100	100	−	+
Sebacate	100	100	100	100	100	100	100	100	100	100	100	100	−	+
Isobutyrate	100	100	100	100	100	100	100	100	100	100	100	100	−	+
Pentanoate	100	100	100	100	100	100	100	100	86	100	100	0	−	+
Malonate	0	0	0	0	14	0	0	100	0	0	0	0	−	−
Azelate	100	100	100	100	100	100	100	100	100	100	100	100	−	+
Quinate	100	100	100	100	14	0	0	100	100	100	100	100	−	+
Glycollate	100	100	100	100	0	0	0	0	0	0	0	0	−	+
L-(+)-Tartrate	100	100	100	100	57	100	0	100	0	0	0	0	+	+
DL-Tartrate	100	100	100	100	57	100	0	100	0	0	0	0	+	+
p-Hydroxybenzoate	100	100	100	100	57	100	0	100	14	0	100	0	−	+
Lactate (Ca)	100	100	100	100	100	100	100	100	0	0	0	0	−	+
Lactate (Na)	100	100	100	100	100	100	100	100	100	100	100	100	−	+
Hexanoate	100	100	100	100	100	100	100	100	86	80	100	100	−	+
Laevulinate	100	100	100	100	100	100	100	100	29	20	100	0	−	+
L-Citrulline	100	100	100	100	100	100	100	100	100	100	100	100	−	+
L-Tyrosine	100	100	100	100	100	100	100	100	100	100	100	100	−	+
Reaction in tobacco leaves														
Necrosis	100	100	100	100	100	100	100	100	0	0	0	0	−	+
Yellowing	0	0	0	0	0	0	0	0	100	100	100	100	−	−

[a] Phenons 1 (43 strains), 2 (7 strains), and 3 (7 strains).
[b] Groups 1A (19 strains), 1B (23 strains), and 1C (1 strain) of phenon 1; groups 2A (3 strains), 2B (3 strains), and 2C (1 strain) of phenon 2; and groups 3A (5 strains), 3B (1 strain), and 3C (1 strain) of phenon 3.
[c] Full codes S213(119) and S240(147).
[d] Two methods used for testing tyrosinase activity gave identical results.

countries belong to biovar I. Most strains from potato were assigned to biovar II. The earlier report (20) that strain K60(25) belongs to biovar I is confirmed.

Two distinct types of responses, which were particularly striking during the first 15 days after inoculation, occurred in the mannitol, dulcitol, and sorbitol media of Hayward (20). Actively growing strains either produced a dense surface pellicle accompanied by a positive acidic reaction or formed a ring of growth about 3 cm below the surface, without an acid reaction. However, 19 local biovar III strains, which also formed the typical ring, consistently produced some acid near the end of the incubation period.

Differentiation of races. After 48 hr, all 49 strains of *P. solanacearum* originally isolated from tobacco, tomato, pepper, eggplant, and *D. ferox* (Table 1) caused a dark-brown necrotic lesion surrounded by a yellow halo in the infiltrated area of tobacco leaves. This reaction is typical for race 1 of *P. solanacearum* (29). Strains H7 and S221(134) isolated from potato were also assigned to race 1. With the exception of S213(119), which gave no visible reaction, all other strains from potato caused only a yellow discoloration within 48 hr after infiltration and were classified as race 3.

None of the 15 South African strains of *P. solanacearum* was pathogenic to the two groundnut cultivars, cotton, or triploid banana. Since root and stem inoculations gave similar results, these data for each host-pathogen combination were combined. None of the control plants became diseased.

Eleven strains—H1, H3, H7, H8, H11, H13, H14, H15, H17, L1, and M1—were assigned to race 1 according to the criteria of Buddenhagen et al (5). With the exception of strains H8 (avirulent on pepper and diploid banana) and H14 (avirulent on chili pepper), all these strains were virulent on the solanaceous hosts and diploid banana. Among the Compositae, dahlia was more susceptible than sunflower to race 1 strains. Apart from H5 (highly virulent on eggplant), the other strains (H2, H4, and H6) placed in race 3 all had high levels of virulence on tomato and potato only. However, these strains also had lesser effects on pepper, eggplant, and dahlia. All strains except H7 were equally virulent on tomato cultivars Rooi Kaki (susceptible) and Rodade, which has been reported to be resistant to race 1 (2).

DISCUSSION

The phenotypic dendrogram (Fig. 1) of a collection of strains of *P. solanacearum* was structured mainly on biochemical, physiological, and nutritional properties that have been used in other studies to discriminate groups within the species (14,16,17,20,24, 33,34,41). Three distinct phenons were recognized. Significantly, phenons 1, 2, and 3 corresponded respectively with biovars III, I, and II as defined by Hayward (20). Furthermore, based on their response in infiltrated tobacco leaves (29), all strains in phenons 1 and 2 were assigned to race 1 and those of phenon 3 to race 3. The race of each of 15 selected South African strains was confirmed by their differential reaction on a range of host plants (5).

Apart from five potato strains of group 3A, all South African strains of *P. solanacearum* were assigned to groups 1A and 1B of phenon 1. The reference strain S236(143) separated from these two clusters to form group 1C. The distinctive ability of group 1A strains to produce acid without a marked increase of surface growth on media containing mannitol, sorbitol, and dulcitol appears to be unusual. However, sparse growth is typical for strains producing acid in maltose, lactose, or cellobiose media (20; present investigation). Apparently these strains oxidize rather than hydrolyze the disaccharides to form bionic acids (20). Acid produced from the six carbohydrates is less readily detected unless the concentration of bromothymol blue in the basal medium is increased from 0.003 g/L (20) to 0.08 g/L (21). Engelbrecht and Prinsloo (8) previously used the lower concentration and thus inadvertently placed two isolates of *P. solanacearum* from tobacco in South Africa in biovar I instead of biovar III.

None of the 13 reference strains corresponded with the South African strains in all of the features examined. However, most of the reference strains conformed with results obtained previously by other investigators (6,15,29). Furthermore, except for potato strain 257(257) from Costa Rica, which clustered with four local potato strains, all formed separate groups within phenons or diverged as solitary strains (Fig. 1). The status of the two solitary strains is uncertain. First, strain S240(147) was assigned to biovar IV and not to III as determined by Granada and Sequeira (13). Second, S213(119) was the only strain in the entire collection that failed to elicit a reaction in tobacco leaves. In contrast, Lozano and Sequeira (29) used the reaction to place the strain in race 1. Granada and Sequeira (14) initially agreed with this but later listed the strain under race 3 (15).

Local strains attacking tobacco belong to two homogeneous groups within biovar III. Unlike reference strains from neighboring Zimbabwe and other countries, biovar I tobacco strains are not known to occur in South Africa. To our knowledge, the presence of biovar III tobacco strains in Zimbabwe has not yet been recorded. The existence of biovar III strains with wide host range, biovar II strains confined to potato, and the absence of biovar I and IV strains from South Africa agree with recent findings of Swanepoel and Young (40). Biovar II is believed to have originated in Latin America (3) and was probably introduced locally on infected potato seed tubers.

The reason why bacterial wilt of tobacco caused by *P. solanacearum* had not been recorded in South Africa before 1984 (8) remains obscure. Nevertheless, the occurrence of race 1 on tobacco in this country has obvious epidemiological implications. Race 1 has an extensive host range, and local tobacco strains were highly virulent on several other host species tested. Similarly, tomato strain H8 was highly virulent on tobacco plants. In addition, preliminary investigations (M. C. Engelbrecht, *unpublished*) indicate that tobacco introduction line 448A, used in the United States as the main source for breeding for resistance (7,30), is highly susceptible to local strains of *P. solanacearum*. Cultivars such as NC 95, derived from line 448A, are also highly susceptible.

The reference strains used in this investigation were selected on the basis of races and biovars suspected to occur in South Africa. Within this limitation, numerical analysis of the phenotypic features of 59 strains supports the present biovar and race schemes, in spite of the well-documented overlapping of biochemical characteristics between groups (16,21,34).

ACKNOWLEDGMENTS

We thank Isabel M. M. Roos for expert advice, P. S. Knox-Davies for comments on the manuscript, and C. R. Fischer, L. Sequeira, and Anita E. Swanepoel for supplying reference strains.

LITERATURE CITED

1. Bonham-Carter, C. F. 1967. Fortran IV program for Q-mode cluster analysis of non-quantitative data using IBM 7090/7094 computers. Kans. Geol. Surv. Prog.
2. Bosch, S. E., Louw, A. J., and Aucamp, E. 1985. 'Rodade' bacterial wilt resistant tomato. HortScience 20:458-459.
3. Buddenhagen, I. W. 1986. Bacterial wilt revisited. Pages 126-143 in: Proc. Int. Workshop Bact. Wilt Dis. Asia South Pac. G. J. Persley, ed. ACIAR Proc. 13.
4. Buddenhagen, I. W., and Kelman, A. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 2:203-230.
5. Buddenhagen, I. W., Sequeira, L., and Kelman, A. 1962. Designation of races in *Pseudomonas solanacearum*. (Abstr.) Phytopathology 52:726.
6. Ciampi, L., and Sequeira, L. 1980. Influence of temperature on virulence of race 3 strains of *Pseudomonas solanacearum*. Am. Potato J. 57:307-317.
7. Clayton, E. E., and Smith, T. E. 1942. Resistance of tobacco to bacterial wilt (*Bacterium solanacearum*). J. Agric. Res. 65:547-554.
8. Engelbrecht, M. C., and Prinsloo, G. C. 1985. *Pseudomonas solanacearum* on tobacco in South Africa. Phytophylactica 17:171-172.
9. Fahy, P. C., and Hayward, A. C. 1983. Media and methods for isolation and diagnostic tests. Pages 337-378 in: Plant Bacterial Diseases: A Diagnostic Guide. P. C. Fahy and G. J. Persley, eds. Academic Press, Sydney.
10. French, E. R. 1986. Interaction between strains of *Pseudomonas solanacearum*, its hosts and the environment. Pages 99-104 in: Proc. Int. Workshop Bact. Wilt Dis. Asia South Pac. G. J. Persley, ed. ACIAR Proc. 13.

11. French, E. R., and Sequeira, L. 1970. Strains of *Pseudomonas solanacearum* from Central and South America: A comparative study. Phytopathology 60:506-512.
12. Gorter, G. J. M. A. 1977. Index of plant pathogens and the diseases they cause in cultivated plants in South Africa. S. Afr. Dep. Agric. Tech. Serv. Sci. Bull. 392. 177 pp.
13. Granada, G. A., and Sequeira, L. 1975. A hypersensitive reaction induced in tobacco leaves by a compatible (race 1) isolate of *Pseudomonas solanacearum*. Phytopathology 65:731-733.
14. Granada, G. A., and Sequeira, L. 1975. Characteristics of Colombian isolates of *Pseudomonas solanacearum* from tobacco. Phytopathology 65:1004-1009.
15. Granada, G. A., and Sequeira, L. 1983. A new selective medium for *Pseudomonas solanacearum*. Plant Dis. 67:1084-1088.
16. Harris, D. C. 1972. Intra-specific variation in *Pseudomonas solanacearum*. Pages 289-292 in: Proc. Int. Conf. Plant Pathog. Bact. 3rd. H. P. Maas Geesteranus, ed.
17. Harrison, D. E. 1961. Bacterial wilt of potatoes. I. Field symptoms of the disease and studies on the causal organism, *Pseudomonas solanacearum* variety *asiaticum*. Aust. J. Agric. Res. 12:854-871.
18. Haynes, W. C. 1951. *Pseudomonas aeruginosa*—its characterization and identification. J. Gen. Microbiol. 5:939-950.
19. Hayward, A. C. 1960. A method for characterizing *Pseudomonas solanacearum*. Nature (Lond.) 186:405-406.
20. Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. J. Appl. Bacteriol. 27:265-277.
21. Hayward, A. C. 1976. Some techniques of importance in the identification of *Pseudomonas solanacearum*. Pages 137-142 in: Proc. Int. Plann. Conf. Workshop Ecol. Control Bact. Wilt 1st. L. Sequeira and A. Kelman, eds. North Carolina State University, Raleigh.
22. Hayward, A. C. 1986. Bacterial wilt caused by *Pseudomonas solanacearum* in Asia and Australia: An overview. Pages 15-24 in: Proc. Int. Workshop Bact. Wilt Dis. Asia South Pac. G. J. Persley, ed. ACIAR Proc. 13.
23. He, L. Y. 1986. Bacterial wilt in the People's Republic of China. Pages 40-48 in: Proc. Int. Workshop Bact. Wilt Dis. Asia South Pac. G. J. Persley, ed. ACIAR Proc. 13.
24. He, L. Y., Sequeira, L., and Kelman, A. 1983. Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Dis. 67:1357-1361.
25. Holding, A. J., and Collee, J. G. 1971. Routine biochemical tests. Pages 2-32 in: Methods in Microbiology. Vol. 6A. J. R. Norris and D. W. Ribbons, eds. Academic Press, New York.
26. Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. N.C. Agric. Exp. Stn. Tech. Bull. 99. 194 pp.
27. Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.
28. Kelman, A., and Person, L. H. 1961. Strains of *Pseudomonas solanacearum* differing in pathogenicity to tobacco and peanut. Phytopathology 51:158-161.
29. Lozano, J. C., and Sequeira, L. 1970. Differentiation of races of *Pseudomonas solanacearum* by a leaf infiltration technique. Phytopathology 60:833-838.
30. Lucas, G. B. 1975. Diseases of Tobacco. 3rd ed. Biological Consulting Associates, Raleigh, NC. 621 pp.
31. Martin, C., French, E. R., and Nydegger, U. 1982. Strains of *Pseudomonas solanacearum* affecting Solanaceae in the Americas. Plant Dis. 66:458-460.
32. McClean, A. P. D. 1930. The bacterial wilt disease of peanuts. S. Afr. Dep. Agric. Sci. Bull. 87. 14 pp.
33. Palleroni, N. J. 1984. Genus I. *Pseudomonas migula* 1894. Pages 141-199 in: Bergey's Manual of Systematic Bacteriology. Vol. 1. N. R. Krieg and J. G. Holt, eds. Williams & Wilkins, Baltimore.
34. Palleroni, N. J., and Doudoroff, M. 1971. Phenotypic characterization and deoxyribonucleic acid homologies of *Pseudomonas solanacearum*. J. Bacteriol. 107:690-696.
35. Sequeira, L. 1979. Bacterial hypersensitivity. Pages 111-120 in: *Nicotiana*: Procedures for Experimental Use. R. D. Durbin, ed. U.S. Dep. Agric. Tech. Bull. 1586.
36. Sierra, G. 1957. A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek 23:15-22.
37. Smibert, R. M., and Krieg, N. R. 1981. General characterization. Pages 409-443 in: Manual of Methods for General Bacteriology. P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips, eds. American Society for Microbiology, Washington, DC.
38. Sneath, P. H. A., and Sokal, R. R. 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification. W. H. Freeman and Co., San Francisco. 573 pp.
39. Sokal, R. R., and Michener, C. D. 1958. A statistical method for evaluating systematic relationships. Univ. Kans. Sci. Bull. 38:1409-1438.
40. Swanepoel, A. E., and Young, B. W. 1988. Characteristics of South African strains of *Pseudomonas solanacearum*. Plant Dis. 72:403-405.
41. Thurston, H. D. 1963. Bacterial wilt of potatoes in Colombia. Am. Potato J. 40:381-390.
42. Wager, V. A. 1944. Bacterial wilt of the eggplant. Farming S. Afr. 19:661-664.
43. Winstead, N. N., and Kelman, A. 1952. Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. Phytopathology 42:628-634.