Characteristics of South African Strains of *Pseudomonas solanacearum*

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ABSTRACT

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Forty-five strains of *Pseudomonas solanacearum* were isolated from host plants grown in different locations in South Africa. With one exception, the strains isolated from potatoes were identified as biotype II, and all but one of the strains from tomatoes and tobacco as biotype III. Distinct differences were found between the two biotypes with respect to their ability to oxidize eight carbohydrates dissolved in a synthetic medium. One strain from potatoes, identified as biotype II, was distinctly different from the other potato strains in its ability to oxidize the carbohydrates. Five strains were selected for virulence tests on eight host plants. All these strains were virulent to potatoes, tomatoes, eggplant, and pepper but there were clear differences in virulence towards tobacco, sunflower, peanut, and the weed large thorn apple (*Datura ferox*).

Additional keywords: bacterial wilt

Bacterial wilt caused by *Pseudomonas solanacearum* E. F. Sm. was first reported in South Africa in 1914 (5) on potato. Subsequently, it was reported as attacking tomato and eggplant (6), pepper (19), peanut (16), and recently tobacco (7). After the initial report, there were occasional outbreaks of the disease on potatoes, and in 1978 the presence of bacterial wilt was established in a number of seed potato crops.

South Africa is a country with climatic conditions varying from temperate to subtropical. The disease occurs from time to time in the temperate regions, but is endemic in the low-altitude (subtropical) areas such as the Transvaal Lowveld, the Natal Coastal Regions, and the Flats of the Cape Province, where the climate is characterized by hot, dry summers and cool, wet winters. Farmers in these endemic areas can produce susceptible crops, other than seed potatoes, during the cool winter months, but avoid susceptible crops in other seasons.

Buddenhagen, Sequeira, and Kelman (4) differentiated strains of *P. solanacearum* into three races. Race 1 affects tobacco, tomato, many solanaceous and other weeds, and certain diploid bananas. Race 2 causes wilt of triploid bananas (Moko disease), *Heliconia* spp., or both. Race 3 affects potatoes and tomatoes, but is not highly virulent on other solanaceous crops.

Hayward (9) differentiated strains of the pathogen into four biotypes according to their ability to oxidize three disaccharides (lactose, maltose, and cellobiose) and three hexose alcohols (mannitol, sorbitol, and dulcitol). Biotype I oxidizes none of the carbohydrates, biotype II only the disaccharides, biotype III oxidizes both the disaccharides and the hexose alcohols, and biotype IV the hexose alcohols only. Biotype II can, according to Buddenhagen and Kelman, be designated as race 3, the potato race. It is the only grouping which can be correlated with race or pathotype, as defined by other workers (3).

A survey was conducted in South Africa to establish the identity and distribution of the different biotypes defined by Hayward (9). In addition to the standard tests for the identification of the biotypes, other physiological tests were carried out to ascertain the possible existence of variation among different strains. Isolates were also compared for virulence in a series of host plants, including *Helianthus annuus* L. and *Datura ferox* L., on which the disease has not yet been reported in South Africa. Sunflower is sometimes used in this country in a rotational crop system that also includes potatoes, and *D. ferox* is a solanaceous weed generally found in cultivated lands.

MATERIALS AND METHODS

Identification tests. From 1984 to 1986, 45 strains of *P. solanacearum* were isolated from potatoes, tomatoes, and tobacco from different locations in South Africa. These strains were isolated on tetrazolium chloride medium (TZC) (12) and identified by using standard laboratory techniques (8,10,17). These included microscopic examination, the Gram stain, and the following physiological tests: the formation of fluorescent pigments on medium B of King et al (14), nitrate reduction, indole production, starch hydrolysis, gelatin liquefaction, hydrolysis of Tween 80, and catalase activity. Tests for oxidation/fermentation of glucose, Kovac's oxidase, production of a brown, diffusible pigment on a tyrosine medium, and determination of biotypes were done according to Hayward (9,10). All these tests were repeated five times. Cultures were stored in McCartney bottles in sterile, distilled water at ambient temperatures (13).

To determine if differences existed among strains assigned to the same biotype, tests were conducted to determine the ability of the strains to oxidize eight carbohydrates using the synthetic medium of Ayers, Rupp, and Johnson (1). The following carbohydrates were included: sucrose, *meso*-inositol, D+galactose, rhamnose, D+trehalose, D-ribose, D-mannose, and D+xylose. The carbohydrates were filter-sterilized and added to the presterilized medium in capped test tubes to obtain a final concentration of 0.2%. The indicator bromothymol blue (0.008%) was included in the medium to detect acid production by change in color from green to yellow, compared with an inoculated control without any carbohydrate. Strains were cultured on slants of TZC medium at 30 C for 48 hr. Bacteria were suspended in sterile, distilled water. This suspension was diluted with sterile, distilled water until the absorbance equalled 0.2 (at 620 nm) on the scale of a Bausch & Lomb Spectronic 21. Test tubes were inoculated with 0.05 ml of the suspension and incubated up to 30 days at 30 C.

Virulence tests. Plants used in virulence tests were potato (*Solanum tuberosum* L. 'BP-1'), tomato (*Lycopersicon esculentum* Mill. 'Roma'), tobacco (*Nicotiana tabacum* L. 'Hicks'), eggplant (*Solanum melongena* L. 'Black Beauty'), pepper (*Capsicum annuum* L. 'California Wonder'), peanut (*Arachis hypogaea* L. 'Sellie'), sunflower (*Helianthus annuus* L. 'SO 323'), and large thorn apple (*D. ferox*).

Seed potato tubers, sunflower seed, and peanuts were planted directly in 15-cm plastic pots. Seeds of the other plants were sown in seedling trays and were transplanted to plastic pots 4–5 wk after sowing. The root inoculation technique (15,20) was used on 10- to 15-cm high plants; 30-ml inoculum (with an absorbance value of 0.001) per plant was applied. Five strains were selected for

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virulence tests: strains 1 and 5 were both isolated from potato, but from different parts of the country; strain 3 was isolated from potato, but with distinct physiological differences from other potato isolates; strain 4 was isolated from tomato and physiologically representative of all tomato isolates; and strain 10 was isolated from tobacco. Each isolate was inoculated into a total of at least 15 plants of each host. Inoculated plants were grown in a glasshouse at 28 C (day) and 25 C (night). Disease indices were recorded 40 days after inoculation on a scale of 1–5, where 1 = no symptoms, 2 = wilt of one leaf, 3 = wilt of up to half of the leaves, 4 = wilt of nearly all the leaves, and 5 = complete wilt or death (11). Virulence ratings were based on average disease indices according to the scale of He, Sequeira, and Kelman (11).

RESULTS

Identification tests. All strains were rod-shaped and gram-negative. On TZC medium all strains produced fluidal colonies with pink centers after 48 hr, but colonies of strain 3 were red-pink and less fluidal.

All strains gave a negative reaction for indole production, formation of fluorescent pigments on King's B medium, starch hydrolysis, and gelatin liquefaction. All strains were oxidative and oxidase-positive, produced a brown pigment on a tyrosine medium, and produced catalase. Nitrate was reduced by all strains, but gas was produced by strain 3 only. All strains hydrolyzed Tween 80 except strain 3. Based on these results, the strains were identified as *Pseudomonas solanacearum*. Assignment of the strains to biotype groups is presented in Table 1. Sucrose, *meso*-inositol, and D+galactose were oxidized by all strains. Results of the oxidation of the other carbohydrates are given in Table 1.

Virulence tests. Results of the virulence tests are given in Table 2. The strains differentiated as biotype II showed no virulence to sunflower, tobacco, and peanut; virulence to the other hosts varied. Strain 3 was the only biotype II strain that showed virulence to *D. ferox*. Biotype III strains were virulent to all eight hosts and differed only in the high virulence rating of strain 10 to tobacco.

Table 1. Oxidation[a] of carbohydrates by South African strains of *Pseudomonas solanacearum*

Strain	Host plant	Rhamnose	Trehalose	Ribose	Mannose	Xylose
			Biotype II			
1	Potato	–[b]	–	–	V^+	V^-
2	Potato	–	–	–	V^+	V^-
3	Potato	+	W^+	+	+	+
5	Potato	–	–	–	V^+	–
6	Potato	–	–	–	V^+	–
9	Potato	–	–	–	V^+	V^-
19	Potato	–	–	–	V^+	V^-
20	Potato	–	–	–	V^+	–
21	Potato	–	–	–	V^+	–
22	Potato	–	–	–	V^+	V^-
23	Potato	–	–	–	V^+	V^-
24	Potato	–	–	–	V^+	–
25	Potato	–	–	–	V^+	V^-
26	Potato	–	–	–	+	V^+
27	Potato	–	–	–	+	W^+
28	Potato	–	–	–	+	V^+
30	Potato	–	–	–	+	V^+
39	Tomato	–	–	–	+	V^-
45	Potato	–	–	–	W^+	–
			Biotype III			
4	Tomato	–	+	+	+	V^-
7	Tomato	–	+	+	+	V^-
8	Potato	–	+	+	V^+	–
10	Tobacco	–	+	+	V^+	V^-
11	Tomato	–	+	+	+	–
12	Tobacco	–	+	+	V^+	–
13	Tomato	–	+	+	V^+	–
14	Tomato	–	+	+	+	V^-
15	Tomato	–	+	+	+	–
16	Tomato	–	+	+	V^+	–
17	Tomato	–	+	+	V^+	–
18	Tomato	–	+	+	+	–
29	Tobacco	–	+	+	+	V^-
31	Tobacco	–	+	+	+	–
32	Tobacco	–	+	+	+	–
33	Tomato	–	+	+	+	–
34	Tobacco	–	+	+	+	V^+
35	Tomato	–	+	+	+	–
36	Tomato	–	+	+	+	W^+
37	Tomato	–	+	+	V^+	V^-
38	Tomato	–	+	+	+	–
40	Tomato	–	+	+	W^+	W^+
41	Tomato	–	+	+	+	W^+
42	Tomato	–	+	+	+	W^+
43	Tomato	–	+	+	+	V^-
44	Tomato	–	+	+	+	W^+

[a] Results based on the method of Stanier et al (18).
[b] – = Negative, + = positive, W^+ = weakly positive, V^+ = variable (usually positive), V^- = variable (usually negative).

DISCUSSION

Although bacterial wilt has been known in South Africa for more than 70 years, the characteristics of the strains of the pathogen occurring in this country have not previously been determined. With one exception, the strains isolated from diseased potatoes were representative of biotype II and, also with one exception, the strains isolated from diseased tomato and tobacco plants were representative of biotype III. Biotype III was mainly found in the hot, subtropical areas of the country and biotype II in the temperate regions. The tendency to isolate biotype III from tomatoes and biotype II from potatoes may reflect the practice of cultivating tomatoes in the warm regions and potatoes either in the cooler regions or during the cool seasons in the warm regions. There are few production areas where the two crops are grown together. The isolation of biotype III (strain 8) from potatoes was an exceptional case: the potatoes were grown on an old tomato field. Similarly, biotype II (strain 39) was isolated from diseased tomatoes grown in a field with a history of early summer potato production.

Strains of biotype III were isolated from diseased tobacco plants found in the Transvaal Lowveld. Tomatoes are also widely grown in this area where the pathogen is considered to be endemic. Although tobacco has been produced for a number of years in this area, the disease was only recently observed (1985) (7) and the identity of the pathogen on this crop confirmed. Biotypes I and IV were not isolated from either potato, tomato, or tobacco in this survey.

According to the race identification system of Buddenhagen, Sequeira, and Kelman (4), the isolated strains probably belong either to race 1 or to race 3, as the disease has not yet been reported on banana in this country (race 2 attacks banana and *Heliconia* spp.). Isolates of biotype II can be designated as race 3 (3) and the biotype III strains as race 1, based on the results of the virulence tests of the two representative strains.

No physiological differences were

Table 2. Virulence rating[a] of South African strains of *Pseudomonas solanacearum* on eight hosts

Strain	Original host	Biotype	Potato	Tomato	Eggplant	Pepper	Sunflower	Tobacco	Peanut	*Datura ferox*
1	Potato	II	H[b]	H	H	M	0	0	0	0
3	Potato	II	H	H	M	L	0	0	0	L
5	Potato	II	H	M	M	M	0	0	0	0
4	Tomato	III	M	H	H	H	L	L	L	L
10	Tobacco	III	M	H	H	H	L	H	L	L

[a] Results based on average disease indices on a scale of 1–5 of 15–20 plants 40 days after inoculation.
[b] H = High (4.1–5.0), M = medium (2.6–4.0), L = low (1.1–2.5), 0 = none (1.0).

observed between strains isolated from diseased tobacco and tomato plants. With the exception of strain 3, all biotype II strains failed to oxidize trehalose and ribose. These sugars were oxidized by all biotype III strains. No consistent results were obtained with mannose and xylose. Strain 3 was consistently identified as biotype II, as defined by Hayward, but it was similar to the biotype III strains in its ability to oxidize trehalose and ribose. It was the only strain capable of oxidizing rhamnose, and differed from all the other strains in its ability to produce gas from nitrate and its inability to hydrolize Tween 80. Since strain 3 was the only virulent strain found with these particular characteristics, it may be a mutant.

At the Vegetable and Ornamental Plant Research Institute, potatoes and tomatoes are bred for resistance to bacterial wilt. The tomato cultivar Rodade (2) was released by this Institute in 1982 and has been widely accepted by tomato growers, particularly in the warm areas. Bosch, Louw, and Aucamp (2) reported its resistance to race 1 of the pathogen. In preliminary (*unpublished*) tests done by the authors, Rodade was found to have no resistance to an isolate of biotype II at a 28 C (day) and 25 C (night) temperature regime compared with a resistance of 94% to an isolate of biotype III at the same temperature regime. This cultivar cannot, therefore, be planted in biotype II-infested soil, but as discussed earlier, biotype II is more or less restricted to the potato production areas. Resistance of a potato cultivar to biotype II is obviously essential, but whereas the potato breeding program is also geared for tolerance to hotter growing conditions, it would be short-sighted not to examine resistance of the selections to all biotypes of *P. solanacearum* present in this country.

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LITERATURE CITED

1. Ayers, S. H., Rupp, P., and Johnson, W. T. 1919. A study of the alkaliforming bacteria in milk. U.S. Dep. Agric. Bull. 782.
2. Bosch, S. E., Louw, A. J., and Aucamp, E. 1985. Rodade: Bacterial wilt resistant tomato. HortScience 20:458-459.
3. Buddenhagen, I., and Kelman, A. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 2:203-230.
4. Buddenhagen, I., Sequeira, L., and Kelman, A. 1962. Designation of races in *Pseudomonas solanacearum*. (Abstr.) Phytopathology 52:726.
5. Doidge, E. M. 1914. Some diseases of the potato. Union S. Afr. Agric. J. 7:698-703.
6. Doidge, E. M. 1924. A preliminary checklist of plant diseases occurring in South Africa. Bot. Surv. S. Afr. 11.
7. Engelbrecht, M. C., and Prinsloo, G. C. 1985. *Pseudomonas solanacearum* on tobacco in South Africa. Phytophylactica 17:171-172.
8. Harrigan, W. F., and McCance, M. E. 1966. Laboratory Methods in Microbiology. Academic Press, London and New York.
9. Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. J. Appl. Bacteriol. 27:265-277.
10. Hayward, A. C. 1976. Some techniques of importance in the identification of *Pseudomonas solanacearum*. Pages 137-142 in: Proc. Int. Plan. Conf. Workshop Ecol. Control Bact. Wilt Caused by *Pseudomonas solanacearum* 1st. L. Sequiera and A. Kelman, eds. North Carolina State University, Raleigh. 166 pp.
11. He, L. Y., Sequeira, L., and Kelman, A. 1983. Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Dis. 67:1357-1361.
12. Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.
13. Kelman, A., and Person, L. H. 1961. Strains of *Pseudomonas solanacearum* differing in pathogenicity to tobacco and peanut. Phytopathology 51:158-161.
14. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301.
15. Lallmahomed, G. M., and Ricaud, C. 1978. Root versus stem inoculation in pathogenicity tests and in the assessment of resistance to bacterial wilt in tobacco and tomato. Proc. Int. Conf. Plant Pathog. Bact. 4th.
16. McClean, A. P. D. 1930. The bacterial wilt disease of peanuts. Sci. Bull. Dep. Agric. S. Afr. 87:1-14.
17. Sands, D. C., Schroth, M. N., and Hildebrand, D. C. 1980. *Pseudomonas*. Pages 36-44 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN. 68 pp.
18. Stanier, R. Y., Palleroni, N. J., and Doudoroff, M. 1966. The aerobic pseudomonads: A taxonomic study. J. Gen. Microbiol. 43:179.
19. Verwoerd, L. 1929. A preliminary checklist of diseases of cultivated plants in the winter rainfall area of the Cape Province. Sci. Bull. Dep. Agric. S. Afr. 88:1-128.
20. Winstead, N. N., and Kelman, A. 1952. Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. Phytopathology 42:628-634.