Fluorescent Pseudomonads Associated with Bacterial Canker of Stone Fruit in South Africa

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ABSTRACT

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A total of 403 oxidase-negative, green fluorescent pseudomonads were isolated from cankers, symptomless branches, and symptomless buds on plum, apricot, peach, and nectarine trees and from healthy leaves of the first two hosts. Isolates were characterized by GATTa tests for gelatin liquefaction, aesculin hydrolysis, tyrosinase activity, and tartrate utilization. Most isolates were assigned to *Pseudomonas syringae* pv. *syringae* (*Pss*) but *P. syringae* pv. *morsprunorum* and intermediate forms were also identified. The hypersensitive reaction on tobacco leaves was a reliable criterion for establishing pathogenicity to plum and apricot host plants. A resident phase of *Pss* was found on symptomless leaves and buds. *Pss* appeared to be the major pathogen causing bacterial canker of stone fruit in Cape Province, South Africa.

Additional key words: epidemiology, ice nucleation, *Prunus*, syringomycin

Dieback of stone fruit trees is common in most of the fruit-growing areas in southwestern Cape Province, South Africa. In recent years, the condition has become serious on plums (*Prunus salicina* Lindl.) and losses are a cause for concern. Apricot (*P. armeniaca* L.), peach (*P. persica* (L.) Batsch), and nectarine (*P. persica* var. *nectarina* (Ait.) Maxim.) orchards have also exhibited tree dieback. Apart from a single report (7) implicating *Pseudomonas syringae* pv. *syringae* (*Pss*) van Hall (5) on apricots, there is no experimental evidence for the role of this pathogen and *P. syringae* pv. *morsprunorum* (*Psm*) (Wormald) Young et al (5) in bacterial canker of stone fruit trees in South Africa.

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This paper reports the isolation, characterization, and pathogenicity of bacteria isolated from diseased and symptomless *Prunus* trees in South Africa.

MATERIALS AND METHODS

Host material. Samples of plant material were taken at 2-wk intervals between September 1981 and August 1982 from a heavily infected Harry Pickstone plum orchard near Franschhoek and a Malan Royal apricot orchard at Bien Donné. The apricot orchard was on the experimental farm of the Fruit and Fruit Technology Research Institute in Stellenbosch. Samples were also obtained from plum, apricot, peach, and nectarine orchards in most of the other fruit-growing areas of southwestern Cape Province, and on a single occasion, from plum and nectarine orchards in the Knysna district of southern Cape Province.

Isolation. Bacteria were isolated from surface-disinfested (swabbed with 70% ethanol and flamed) stem cankers, symptomless buds, and symptomless branches. Individual buds, tissue from margins of lesions, and symptomless branches were removed aseptically, cut into small pieces, and transferred to test tubes (buds) or 20-ml screw-cap bottles (tissue) containing 1 or 10 ml sterile buffered saline (NaCl, 8.5 g/L; 0.01 M NaH_2PO_4-Na_2HPO_4 buffer, pH 7), respectively, then shaken vigorously. After 1–2 hr, loopfuls of suspensions were streaked in duplicate onto medium B (MB) of King et al (8) and nutrient agar (Difco) supplemented with 5% sucrose (NSA) (11).

Leaves were collected from trees in apricot and plum orchards. All leaves appeared healthy; we were unable to find leaf spotting, which is characteristic of the summer phase of bacterial canker in other countries (1,3). Each sample included 100 leaves selected at random from five separate trees. Leaves were washed by a method modified from Crosse (2). Each sample was placed in a 2-L Erlenmeyer flask containing 1 L sterile deionized water, 1 g peptone (Difco), and 1 ml Tween 80. The flask was agitated on a NBS Gyrotory shaker for 4 min at 30-min intervals. After 4 hr, dilution series of the wash fluids were plated in duplicate onto MB and NSA.

Agar plates were incubated at 26 C for 3 days. Randomly selected representative colonies of bacteria, which fluoresced on MB or produced levan (11) on NSA, were isolated and purified by repeated streaking on these media.

Reference strains. Cultures of *Pss* (PDDCC 3023, PDDCC 3676, and PDDCC 3687) and *Psm* (PDDCC 3715 and PDDCC 5795) obtained from the Plant Disease Division Culture Collection, Auckland, New Zealand, were included in the investigation.

Characterization of bacterial isolates. Isolates were tested for cytochrome oxidase activity with Difco oxidase-

differentiation disks. Isolates that fluoresced on MB, resembled the reference strains on NSA, and showed no cytochrome oxidase activity were subjected to the GATTa determinative tests (10) for gelatin liquefaction (G), aesculin hydrolysis (A), tyrosinase activity (T), and tartrate utilization (Ta). Presence of syringomycin was established by the bioassay developed by Gross and De Vay (6). Representative GATTa$^+$, GATTa$^-$, and GATTa$^\pm$ isolates were tested for ice-nucleation activity as described by Lindow et al (12).

Uninoculated control media were included in all tests, which were repeated at least twice on separate days. An incubation temperature of 26 C was used.

Hypersensitivity and pathogenicity. The pathogenic potential of each isolate (24- to 48-hr cultures) was estimated by its ability to induce a hypersensitive reaction (HR) within 24 hr on White Burley tobacco by the method of Klement et al (9).

Certain isolates were also tested for pathogenicity to plum shoots and leaves. Each isolate, including the five reference strains, was cultured on MB for 24–72 hr, suspended in water, and adjusted to 10^7 cells per milliliter as described by Latorre and Jones (10). Green shoots were inoculated by injecting inoculum with a hypodermic syringe fitted with a 26-gauge needle. The four youngest leaves on a shoot were inoculated by infiltrating inoculum on the abaxial side with a hypodermic syringe without a needle. In addition, young leaves were spray-inoculated with a series of suspensions containing 10^3, 10^4, 10^5, 10^6, and 10^7 cells per milliliter until water-soaked lesions were visible on the abaxial sides. Inoculated trees, kept in a greenhouse at 25 C, were covered with moist plastic bags for 2 days. Results were recorded 8 days after inoculation. Woody stems of plum and apricot trees were inoculated separately with several isolates, as described by Latorre and Jones (10). Control trees were injected or sprayed with sterile distilled water.

Table 1. Physiological characterization of 403 oxidase-negative, fluorescent bacteria recovered from symptomless and diseased plum, apricot, peach, and nectarine trees in Cape Province, South Africa in 1981 and 1982

Source of isolates	Number of isolates	Group		
		GATTa^{+a}	GATTa^{-b}	GATTa$^{\pm c}$
Plum				
Branches	62	35	12	15
Leaves	33	29	1	3
Buds	12	9	2	1
Cankers	197	147	28	22
Subtotals	304	220 (72.4%)[d]	43 (14.1%)	41 (13.5%)
Apricot				
Buds	12	10	0	2
Leaves	12	11	0	1
Cankers	30	29	0	1
Subtotals	55	51 (92.7%)	0 (0%)	4 (7.3%)
Peach				
Buds	4	1	0	3
Cankers	19	17	0	2
Subtotals	23	18 (78.3%)	0 (0%)	5 (21.7%)
Nectarine				
Buds	10	4	1	5
Cankers	11	2	0	9
Subtotals	21	6 (28.6%)	1 (4.7%)	14 (66.7%)
Totals	403	295 (73.2%)	44 (10.9%)	64 (15.9%)

[a] Positive for gelatin liquefaction and aesculin hydrolysis but negative for tyrosinase activity and tartrate utilization.
[b] Negative for gelatin liquefaction and aesculin hydrolysis but positive for tyrosinase activity and tartrate utilization.
[c] Heterogeneous for one or more of the four GATTa tests.
[d] Percentage of isolates per column relative to the total tested.

RESULTS

Characterization of bacterial isolates. A total of 403 oxidase-negative, green fluorescent pseudomonads were characterized and separated into three groups based on the GATTa tests (Table 1). Of these, 73.2% of the isolates were GATTa$^+$,

Table 2. Additional characteristics of three groups of *Pseudomonas syringae* isolated from symptomless and diseased plum, apricot, peach, and nectarine trees[a]

Group[b]	Source of isolates	Levan production	Syringomycin activity	Ice-nucleation activity	Hypersensitivity reaction on tobacco
Pss	Plum	164/216	136/166	17/24	169/216
	Apricot	39/35	17/21	6/13	29/32
	Peach	12/17	13/14	2/3	13/16
	Nectarine	6/6	2/4	4/6	0/3
	Totals	211/274 (76.7%)	168/205 (81.9%)	29/46 (63%)	211/267 (79%)
Psm	Plum	44/44	3/44	0/8	42/45
	Apricot	1/1	0/1	0/1	0/1
	Nectarine	1/1	0/1	0/1	0/1
	Totals	46/46 (100%)	3/46 (6.5%)	0/10 (0%)	42/47 (89.3%)
Intermediate forms	Plum	40/45	13/38	1/4	19/41
	Apricot	3/4	0/4	1/4	1/4
	Peach	6/7	1/7	2/7	2/7
	Nectarine	14/14	5/14	1/14	8/14
Totals		63/70 (90%)	19/63 (30.2%)	5/29 (17.2%)	30/66 (45.5%)

[a] Ratios indicate number of positive isolates relative to total number tested.
[b] *Pss* = *P. syringae* pv. *syringae* and *Psm* = *P. syringae* pv. *morsprunorum*.

10.9% were GATTa$^-$, and 15.9% were GATTa$^{\pm}$. The South African GATTa$^+$, GATTa$^-$, and GATTa$^{\pm}$ isolates were regarded as *Pss*, *Psm*, and intermediate forms, respectively. This agreed with the reactions produced by the reference strains of the two pathovars.

All *Psm* isolates, most *Pss* isolates, and most isolates of intermediate form produced levan (Table 2); however, some *Pss* isolates, mostly from samples collected in the Franschhoek area, developed flat, non-levan-forming colonies and tended to produce fluorescent pigment on NSA. Apart from the isolates listed in Table 1, five oxidase-negative, levan producing, nonfluorescent isolates were also assigned to *Pss*.

Most *Pss* isolates produced syringomycin and showed ice-nucleation activity (Table 2). Only three *Psm* isolates produced syringomycin, whereas none of the *Psm* isolates tested showed ice-nucleation activity. On the other hand, several of the intermediate forms gave positive results in both of these tests.

Hypersensitivity and pathogenicity. Two hundred eighty-three (74.5%) of the 380 original isolates tested induced the HR (Table 2). Of those tested, all isolates of *Pss, Psm*, and the intermediate form, which induced the HR were also pathogenic to plum leaves and shoots and to woody stems of plum and apricot.

Pathogenic isolates caused dark brown necrotic lesions on succulent plum shoots. Dark brown sunken lesions developed on woody stems and gummosis resulted within 20–30 days. Pathogenic bacteria were reisolated from several of the typical lesions at monthly intervals throughout the investigation. Disease symptoms or gummosis failed to develop on control trees and on trees inoculated with HR-negative isolates.

Water-soaked lesions developed 2–3 days after inoculation on plum leaves. Necrotic lesions resulted within 5–7 days. Eventually, the necrotic tissue fell out and shot holes resulted. No spots developed on leaves inoculated with HR-negative isolates or sprayed with sterile distilled water. Typical spots appeared on leaves that had been sprayed with inoculum containing 10^5 cells per milliliter. At higher concentrations, the spots coalesced to form necrotic areas. No spots were visible on leaves sprayed with suspensions containing the two lowest cell concentrations.

DISCUSSION

Most isolates recovered from stone fruit trees in southwestern Cape Province were identified as pathogenic *Pss*. Although intermediate forms and a few *Psm* isolates were also detected, *Pss* is probably the causal organism of bacterial canker in the region. Nevertheless, *Psm* and the intermediate forms should not be disregarded because pathogenic isolates of both groups were isolated throughout the growing season.

Detection of nonfluorescent strains and at least two colony types of *Pss* from stone fruit orchards in southwestern Cape Province indicates the existence of a heterogeneous population. The variants might be ecotypes (4) or physiotypes of *Pss* (3). Furthermore, the host is known to affect the composition of the pathogen population (3). In our investigation, *Pss* was the dominant population on plum, apricot, and peach. Intermediate forms predominated on nectarines, whereas nonfluorescent strains were detected only on this host. Development of effective control procedures will be complicated if there are important differences in pathological and epidemiological relations between local physiotypes.

The HR on tobacco seemed to be a reliable criterion for testing pathogenicity of South African isolates. Syringomycin activity was fairly specific for *Pss* but 30.2% of the intermediate forms also produced the toxin. Latorre and Jones (10) warned that a potential error could result if these tests alone are used to detect pathogenic strains of *Pss*. The diagnostic value of the ice-nucleation test appeared to be limited. Although none of the *Psm* isolates showed ice-nucleation activity, many *Pss* isolates and intermediate forms also gave negative results in this test.

Dieback of stone fruit trees in South Africa is typical of bacterial canker (1,3). Canker formation on branches, usually accompanied by gum exudation, was the most characteristic and pronounced symptom (I. M. M. Roos and M. J. Hattingh, *unpublished*). There were no symptoms on leaves or fruit of any of the stone fruit trees examined in the field but spots developed on inoculated young plum leaves under humid conditions in the greenhouse. Detection of *Pss* on symptomless leaves and buds of stone fruit trees in orchards indicated the existence of a resident phase of the pathogen under local conditions. This agrees with reports from other countries (1,3).

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