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BACTERIAL CANKER OF AVOCADO

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PROGRESS REPORT

OPSOMMING:

*'n Virulente bakteriese isolaat geassosieer met kanker op avokado, geidentifiseer as **Pseudomonas syringae** van Hail is vergelyk t.o.v. morfologies, fisiologías en biochemiese karaktereienskappe met vyfander isolate. Die avokado isolaat het nou ooreengestem met die ander **P. syringae** isolate.*

SUMMARY:

*A Virulent bacterial isolate associated with canker of avocado, identified as **Pseudomonas syringae** van Hall, was compared to five related isolates. After morphological, physiological and biochemical characterization studies the avocado isolate was found to be closely related to the other **P. syringae** isolates tested.*

INTRODUCTION

Canker of avocado was described by Myburgh & Kotzé (1982) who isolated various bacteria from disease lesions. A virulent fluorescent strain of *Pseudomonas syringaevan* Hall was found tobe associated with this disease (Korsten & Kotzé, 1984). In this report the virulent isolate W-1b will be characterized and compared to five bacterial strains.

MATERIALS AND METHODS

Reference Strains.

Cultures of *P. syringae* pav, *savastoni* (P.s) (ATCC 13527) was obtained from the American Collection, *P. syringae* pav. *Syringae* (R7) causing canker on cherry trees, and P. *syringae* pav. *syringae* (138) causing canker on apricot trees, were both obtained from the Research Institute for Fruit and Fruit Technology at Stellen-bosch, *P. aeruginosa* (P.a) isolate UP 65, was obtained from the culture collection of the Department of Microbiology and Plant Pathology, University of Pretoria. The test isolate W-16 was obtained from a canker on avocado tree whereas a fourth strain Ko-16 was isolated from cankers on Hass avocado inoculated with strain W-16.

CHARACTERIZATION OF BACTERIAL ISOLATES

Test isolates from avocado and isolates for comparison were characterized by the following tests: Gram stain, production of fluorescent pigment, Gelatinize proteolytic activity, lipolytic activity, amylase activity, case in hydrolysis, cellulose and catalase activities (Harrigan & McCance, 1966); LOPAT test (Viljoen, 1972); GATT a test (Latorre & Jones, 1979), Flagella arrangement as well as size and shape of bacteria were determined by transmission electron microscopy (TEM) using the

negative staining technique (Home, 1967). Nutritional screening using Ayres, Rubb & Johnson's mineral salt solution (Sands, Schroth & Hildebrand, 1970) and the compounds listed in Table 2. Uninoculated control media were included in all tests, which were repeated at least twice on separate days. Incubation temperature of 25ºC was used.

RESULTS

Results of morphological-, biochemical land physiological tests given in Table 1, indicated that the isolates from avocado were similar to *P. syringae.* Morphologically there was no difference between the isolates although cell size and number of flagella differed (Fig. 1). All tested isolates had three flagella except *P. aeruginosa* which had one. Biochemically and physiologically the isolates differed as follows:

1. Gelatinase activity, only *P. aeruginosa* positive;
2. Lipolytic activity, all isolates positive except W-1 B and Ko-1 B;
3. Amylase activity, all isolates positive except R7, 138 and *P. aeruginosa.*

Isolates W-1B, Ko-1B and *P. syringae* reacted similarly to the various components of the LOPAT test being positive only in tobacco hypersensitivity. Isolates R7 and 138 differed from these three isolates by giving a positive levan reaction, while *P. aeruginosa* reacted completely differently. Although all six isolates were grouped as being GETTa ±, W-1B and Ko-IB were aesculin and tart rate positive, while P.S., R7 and 138 were only positive for tart rate utilization. *P. aeruginosa* reacted differently, being either + or — in all the tests. Results of nutritional screening are given in Table 2.

The biochemical and physiological tests were statistically analyzed using cluster analyses (Fig. 2). This showed a very close affinity between isolate W-1 B and Ko-1 B. Closely related also are isolates R7 and 138. Both these pairs of isolates in turn show closer affinity with isolate P.s. than with isolate *P. aeruginosa.*

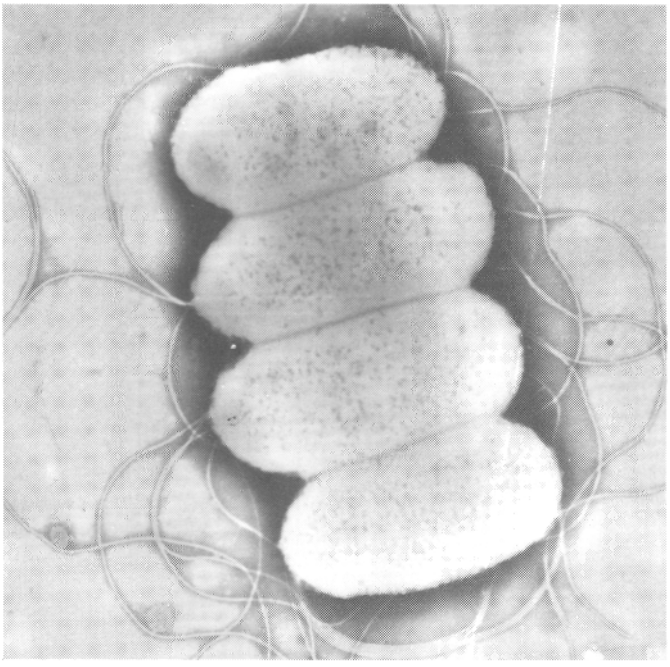

Fig. 1. Isolate W-1b viewed under the TEM using the negative staining technique.

1 mm = 0,05 μm

DISCUSSION

The isolates from avocado canker showed a high degree of similarity to *Pseudomonas syringae* in respect of morphology, their biochemical reactions, and their ability to utilize certain compounds and the LOPAT and GATTa tests. Statistical analyses of these results show that the avocado canker strain W-1 B and Ko-1 B are closely similar and maybe grouped together whereas isolates R7, 138 from cherry and apricot respectively, and *P. syringae* have a slightly lower but still significantly high degree of similarity. The isolates from avocado must therefore be regarded as identical to *P. syringae* but possibly belonging to a different pathovar. The identity of this pathovar is still unknown at present.

TABLE 1: Biochemical and physiological characteristics of the various test isolates.

Characteristic	W-1B	Ko-1B	P.s	R7	138	*P. aeruginosa*
1. Morphological characteristics						
Flagella present	+	+	+	+	+	+
Cell shape	Rods	Rods	Rods	Rods	Rods	Rods
Cell size (μm)	4-1,3	4,1-1,2	4,5-1,5	4,5-1,2	4,5-1,6	5 - 1
2. Biochemical and physiological						
Gram stain	-	-	-	-	-	-
Fluorescence on KB	+	+	+	+	+	+
Gelatinase activity	-	-	-	-	-	+
Lipolytic activity	-	-	+	+	+	+ +
Amylase activity	+	+	+	-	-	-
Casein hydrolysis	-	-	+	-	-	+
Cellulase activity	-	-	-	-	-	-
Catalase activity	+	+	+	+	+	+
3. LOPAT test						
Levan formation (L)	-	-	-	+	+	-
Oxidase activity (O)	-	-	-	-	-	+
Potato soft rot (P)	-	-	-	-	-	+
Arginine hydrolysis (A)	-	-	-	-	-	+
Tobacco hypersensitivity (T)	+	+	+	+	+	-
4. GATTa test						
Gelatin liquefaction (G)	-	-	-	-	-	+
Aesculin hydrolysis (A)	+	+	-	-	-	±
Tyrosinase activity (T)	-	-	-	-	-	+
Tartratra utilization (TA)	+	+	+	+	+	±

Fig. 2: Cluster analysis of the biochemical, physiological and nutritional screening tests.

TABLE 2: Utilisation of carbon and other compounds by the various bacterial isolates.

Compound	W-1B	Ko-1B	P.s	R 7	138	P.a
Adonitol	G-	G-	G-	-	-	+
β-Alanine	G-	G-	-	-	-	B-
Anthranilic Acid	+	+	-	-	-	+
L-Arabinose	G-	G-	-	-	-	+
L+Arabinose	+	+	G-	+	+	+
D-Arabinose	G-	-	-	-	-	+
DL-Arabitol	G-	G-	+	G-	-	+
P-Arbitin	G-	G-	-	-	-	±
Benzoic Acid	+	+	-	-	-	+
Betaine HCℓ	-	-	-	-	-	B-
L+Calcium Tartrate	-	-	B-	-	-	+
Cellobiose	G-	-	G-	-	-	G-
I-Erythritol	+	+	-	G-	+	+
D-Fructose	G-	+	G-	G-	+	+
D+Galactose	+	+	±	+	+	+
D+Glucose	+	+	+	+	+	+
Glycogen	G-	G-	-	-	-	G-
Inositol	±	G-	G-	G-	-	+
Inulin	±	+	-	G-	-	G-
L-Lactic acid	-	-	-	-	-	-
Lactose	G-	G-	-	G-	±	G-
D+Maltose	G-	G-	G-	G-	-	±
D-Mannitol	-	-	-	-	-	+
D-Mannose	±	±	+	+	+	+
D+Mannose	±	+	-	-	-	+
Melibiose	G-	G-	G-	-	+	±
L-D-Melezitose	G-	G-	-	-	±	±
Quinaldic acid	+	+	-	+	-	+
Raffinose	G-	G-	-	G-	+	G-
L+Rhamnose	±	±	-	+	+	+
Salicin	G-	G-	-	-	-	G-
D-Sorbitol	+	-	+	+	+	+
D-Ribose	+	+	-	+	+	+
Starch	+	+	-	-	-	+
Sucrose	G-	G-	+	+	+	+
D-Tartaric acid	B-	B-	B-	B-	B-	+
M-Tartaric acid	-	-	-	B-	-	-
Meso-Tartaric acid	-	-	-	B-	-	±
Trehalose	G-	G-	-	-	-	±
Trigonelline hydrochloride	-	-	-	-	-	+
Xylit	G-	G-	G-	-	-	±
D+Xylose	G-	G-	G-	+	+	+

±= Late positive reaction or a pos. & neg. reaction
G = Growth occurred, but no acid production
B = Indicates a colour change to blue.

REFERENCES

HARRIGAIM, WF & McCance, ME (Eds.), 1966. Laboratory methods in microbiology. London. Academic Press, 362 pp.

HORNE, RW, 1967. Negative staining methods, pp. 328-355 in: D. Kay, Ed., *Techniques for electron microscopy,* 560 pp.

HUGH, R. & LEIFSON, E, 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. *J. Bacterial.* 66, 24 - 26.

KORSTEN, L. & KOTZE, JM, 1984. Bacterial canker of avocado. *S. Afr. Avocado Growers' Ass. Yearbook* 7, 88 - 89.

LATORRE, BA & JONES, AL, 1979. *Pseudomonas morsprunorum,* The cause of bacterial canker of

sour cherry in Michigan and its epiphytic association with *P. syringae. Phytopathology* 69, 335 - 339.

MYBURGH, L. & KOTZE, J.M, 1982. Bacterial disease of avocado. *S. Afr. Avocado Growers' Ass. Yrbk* 5, 105 - 106.

SANDS, DC, SCHROTH, MN & HILDEBRAND, DC, 1970. Taxonomy of phytopathogenlc *pseudomonad's. J. Bacteriol.* 101, 9 - 23

VILJOEN, NM, 1972. Oorsaak, epiderniologie en bestryding van 'n bakteriese siekte op Manigifere indica L. M.Sc. (Agric) University Pretoria, 89 pp.