Further Characterization of *Xanthomonas campestris* pv. *mangiferaeindicae*

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The classification of the causal organism of bacterial black spot of mango has long been an issue. A total of 15 strains, including the type strain, were subjected to 156 tests used for species identification of members of *Xanthomonas* and *Pseudomonas*. The current classification of this organism as *Xanthomonas campestris* pv. *mangiferaeindicae* (ISPP list, 1980) is confirmed, and the description is extended.

Bacterial black spot is a serious necrotic disease of mangoes which has been reported from most mango-growing areas of the world, including the Indian Subcontinent (10, 18), Africa (4), Australia (15), South America (10), Taiwan (1), and Reunion (2). Patel et al. (18) first described the causal organism of this disease under the name *Pseudomonas mangiferae-indicae*, a species pathogenic on *Mangifera indica* L. and *Anacardium occidentale* L. Liao later stated that the latter plant was not a host (12), whereas *Bignonia chamberlaynii* Sims (13) was. Robbs et al. found sporadic occurrence on *A. occidentale*. In 1974 Robbs et al. (19) reclassified the bacterium as *Xanthomonas campestris* subsp. *mangiferae-indicae*, whereas in the same year Steyn et al. (22), although noting deviations from the description of *P. mangiferae-indicae*, retained this name. In 1975 Daniel et al. (3) also identified the organism as *P. mangiferae-indicae* and suggested that its taxonomic position be redefined. In 1980 the current designation as *Xanthomonas campestris* pv. *mangiferaeindicae* (Patel, Moniz and Kulkarni 1948) Robbs, Ribiero and Kimura 1974 was proposed (6). The neopathotype strains were strains PDDCC 5740, NCPPB 490, and ATCC 11637.

We sought clarification of the characteristics, which varied in different reports, and the taxonomic position of this organism. Accordingly, we compared local isolates with the type strain, strains from Taiwan and Reunion, and two isolates of *Xanthomonas campestris* pv. *campestris*. Tests suitable for the identification and species identification of both *Xanthomonas* and *Pseudomonas* species were used.

The following strains were used: ATCC 11637^T (T = type strain), from the American Type Culture Collection, Rockville, Md.; NCPPB 490^T, from the National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom; T1 and T2, from P.-J. Ann, Taiwan Agricultural Research Institute, Taiwan; R, B. Aubert, IRFA, Réunion; TZ, isolated from *M. indica* L. cv. Kent, Tzaneen, Republic of South Africa; ZM, from *M. indica* cv. Zill, Malelane, Republic of South Africa; V, from *M. indica* cv. Peach; B, from *M. indica* cv. Brooks; P, from *M. indica* cv. Palmer; K, from *M. indica* cv. Kent; Z, from *M. indica* cv. Zill; and F1, F2, and F3, from *M. indica* cv. Fascell, Nelspruit, Republic of South Africa. Two isolates (strain X1, isolated from cabbage at Nelspruit; strain X2, from F. M. Wallis, Natal University, Republic of South Africa) of *X. campestris* pv. *campestris* were included. The strains were maintained on nutrient agar and boost agar (23 g of nutrient agar, 20 g of glucose, 10 g of yeast extract, 1,000 ml of distilled water) at 4°C.

The Jensen modification of Gram stain (7) was used to examine cell morphology; the flagella were observed with an electron microscope in negatively stained preparations. Colony morphology and pigment production were determined on nutrient agar and King medium B (11). Unless otherwise stated, the tests were performed by the methods of Dye (5). The methods described by Misaghi and Grogan (14) were used to determine the presence of arginine dihydrolase and the production of 2-ketogluconate, levan, lipase, and cytochrome oxidase and to establish the potato soft rotting and tobacco hypersensitivity potentials of the strains. Cellulase production was determined by the method of Oshira et al. (16); gelatinase, indole production, and starch hydrolysis were determined as described by Schaffer (20). The method of Stanier et al. (21) was used to assay poly- β -hydroxybutyrate. Triphenyl tetrazolium chloride tolerance was tested on boost agar plates. Pectinase activity was determined on polypectate gels (8). The basal medium of Palleroni and Doudoroff (17) and the replica plating technique and methods described by Stanier et al. (21) were used to determine the use of compounds as sole carbon sources. All tests were performed at least twice. The pathogenicities of the strains were tested by pressure infiltrating suspensions containing 10⁶ colony-forming units per ml into the leaves of *M. indica* (cv. Haden) plants. These plants were observed daily for the development of typical, angular, raised necrotic lesions with water-soaked edges.

All strains were gram negative, aerobic, nonsporulating, rod shaped, and motile by means of a single polar flagellum. On nutrient agar the cell size was 0.4 to 0.5 by 1.0 to 1.5 μ m. On nutrient agar or King medium B plates, round, shallowly convex colonies with entire margins were formed. The color was initially smoke grey but soon became white to cream. With age, the colonies became pale yellowish brown. The pigment was not soluble in ethanol or petroleum ether. One strain, strain T1, produced a brown diffusible pigment. No fluorescent pigments were present in any of the strains. The bacteria were strictly oxidative. All isolates grew at 37°C; none grew at 41°C. At 39°C strains NCPPB 490^T, B, P, R, and T1 did not grow.

All strains were positive for esculin hydrolysis, caseinase, catalase, cellulase, gelatinase, hydrogen sulfide, levan, and lipase production, and starch hydrolysis and negative for acetoin production, arginine dihydrolase activity, growth with asparagine as the sole C and N source, indole production, 2-ketogluconate production, nitrate reduction, cytochrome oxidase, poly- β -hydroxybutyrate synthesis, soft rotting of potatoes, tobacco hypersensitivity, and urease production.

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All strains were able to utilize acetate, DL-alanine, cellobiose, citrate, L-cysteine, dextrin, erythritol, fumarate, galactitol, D-galactose, D-glucose, glycerol, glycogen, *myo*-inositol, inulin, DL-lactate, malate, malonate, maltose, D-mannitol, D-mannose, melibiose, pyruvate, raffinose, L-rhamnose, D-ribose, salicin, starch, succinate, sucrose, trehalose, and D-xylose as sole carbon sources. The strains were not able to use esculin, β -alanine, L-arabinose, L-arginine, ascorbate, L-asparagine, DL-asparagine, benzoate, 2,5-dihydroxybenzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, betaine, DL-3-hydroxybutyrate, cholesterol, citrulline, cystine, ethanol, formate, D-galacturonate, D-glucitol, D-gluconate, 2-keto-D-gluconate, L-glutamine, glycine, L-histidine, DL-histidine, L-leucine, isoleucine, L-leucosine, L-lysine, melezitose, L-methionine, methanol, orcinol, DL-ornithine, oxalate, phenylalanine, hydroxyproline, propylene glycol, resorcinol, ribitol, sarcosine, L-serine, DL-serine, D-tartrate, L-tartrate, L-threonine, tryptamine, L-tryptophan, uracil, urate, L-valine, or DL-valine. The method of Dye (5) verified the utilization of the organic acids acetate, citrate, fumarate, lactate, malate, malonate, and succinate and the nonutilization of benzoate, formate, galacturonate, gluconate, oxalate, and tartrate. The Dye method (5) of testing for acid production from sugars as an indication of utilization produced anomalous results. Thus, the results obtained for esculin, cellobiose, dextrin, galactose, glucose, glycerol, mannose, melibiose, melezitose, ribitol, D-ribose, sucrose, sorbitol, trehalose, and xylose agreed with those obtained previously in that when growth occurred, acid was produced. However, there was no growth or acid production on galactitol, *myo*-inositol, inulin, mannitol, or rhamnose, whereas there was growth without the production of acid on raffinose and starch and very slight growth without acid production on salicin. L-arabinose permitted growth and acid production on Dye medium (5) but did not serve as a sole carbon source on the medium of Palleroni and Doudoroff (17).

Strains R, T1, and T2 did not produce ammonia. No strain pitted pectate gels at pH 5, but at pH 8 strains B, F1, F2, T2, V, P, Z, and ZM were positive. All strains except strain T2 tolerated 2% NaCl; none tolerated 4% NaCl. Only strains Z and T2 grew in the presence of 0.02% triphenyl tetrazolium chloride; none grew in the presence of 0.05% triphenyl tetrazolium chloride. Strains T1 and T2 did not produce acid from lactose, fructose, or maltose, although both tests indicated that these strains could use the latter two sugars as sole carbon sources. Strain T1 was able to use L-proline, and strains P, F2, F3, T1, and V grew slightly on arbutin. All strains of the mango pathogen were pathogenic, as shown by the appearance of typical lesions on inoculated mango leaves after 6 days.

We found a number of discrepancies with previously published descriptions which relate to the taxonomy of the mango pathogen. Steyn et al. (22) reported anaerobic production of acids from lactose, raffinose, and glycerol when the method of Hugh and Liefson was used (9).

The organism has been reported as being negative for H_2S production (1, 3, 22), positive for urease (22), negative for acid production from arabinose (12), and positive for acid production from glucitol (12), as causing tobacco hypersensitivity (22), and as using asparagine as a sole carbon source (22). None of these results could be confirmed by repeated testing.

Of the approximately 30 tests described by Robbs et al. (19), identical results were obtained for all but acid production from fructose, which was found to vary among strains.

Allowing for those characteristics which were variable, our mango pathogen strains were identical to the two isolates of *X. campestris* pv. *campestris* except for the lack of a carotenoid pigment, and these results in turn agreed with previously published results for *X. campestris* pv. *campestris*. There were several other differences with previously published data, but these can perhaps be ascribed to the different basal media used, a compelling argument for the standardization of basal media used in taxonomic studies. A comparison of the data with previously published descriptions of *Pseudomonas* indicated the nonidentity of the mango pathogen with any *Pseudomonas* species. We conclude that the current classification of the mango pathogen as *X. campestris* pv. *mangiferaeindicae* (6) is correct.

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