Transfer of *Pseudomonas cissicola* (Takimoto 1939) Burkholder 1948 to the Genus *Xanthomonas*

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The type strain of *Pseudomonas cissicola* (Takimoto 1939) Burkholder 1948, strain ICMP 8561 (= NCPPB 2982), was examined. The strain has the determinative characteristics and the fatty acid profile of the genus *Xanthomonas*. The nutritional characteristics of the strain as determined by Biolog microplate screening were also consistent with identification as a *Xanthomonas* species. Some characteristics of the fatty acid profile and reactions to tests on the Biolog microplate were not consistent with characteristics of known *Xanthomonas* species, suggesting that the strain represents a previously unreported species.

Pseudomonas cissicola (Takimoto 1939 [10]) Burkholder 1948 (1) was included on the Approved Lists of Bacterial Names (7) on the basis of an emended description (4). The proposed neotype strain, strain PC1, was deposited in the International Collection of Microorganisms from Plants (ICMP). The mislabelling of the type strain in the ICMP and the subsequent mislabelling of strains in other collections later identified as strain PC1^T (T = type strain) have been fully discussed (3). Authentic cultures of strain PC1^T were redistributed and are designated ATCC 33616^T, CFBP 2432^T, ICMP 8561^T, LMG 2167^T, and NCPPB 2982^T. However, investigations of this strain (8) indicated that it was a xanthomonad. Further investigation of the type strain of *P. cissicola* based on the redistributed cultures was performed to clarify the position of this species.

The following characteristics of strain PC1^T (as ICMP 8561^T) were examined: (i) cell morphology and flagellar insertion were ascertained by staining suspensions from a 16-h culture grown in nutrient broth on Formvar-curdon-coated 400-mesh grids by using 0.7% phosphotungstic acid (pH 7.2) and examining the prepared grids with a JEOL model Jem 1200EXII transmission electron microscope; (ii) inhibition in the presence of increasing concentrations of 2,3,5-triphenyltetrazolium chloride was investigated (6); (iii) development of poly- β -hydroxybutyrate inclusions was examined (5); (iv) colony characteristics in glucose media were determined following growth for 72 h at 27°C on glucose-yeast extract-carbonate agar (2); (v) utilization of various carbon sources was investigated by inoculating a Biolog GN microplate as recommended by the manufacturer; and (vi) the fatty acid profiles of strains NCPPB 2982^T and ICMP 8561^T were determined (8).

Strain ICMP 8561^T is a rod-shaped bacterium that is motile by means of a single polar flagellum (Fig. 1), grows in the presence of 2,3,5-triphenyltetrazolium chloride only at concentrations less than 0.1%, lacks observable poly- β -hydroxybutyrate inclusions, and produces white, mucoid colonies on glucose-containing media. The 24 fatty acid methyl esters (FAMES) which were detected included large amounts of iso and anteiso acids. In particular, 15:0 iso and 15:0 anteiso acids, as well as 12:0 3-OH, 11:0 iso 3-OH, and 13:0 iso 3-OH, were

found together with a high proportion of branched FAMES. Large amounts of 10:0 3-OH and small amounts of 12:0 3-OH, 11:0 iso 3-OH, and 11:0 iso were present. ICMP 8561^T gave positive reactions in the following Biolog tests: Tween 40, Tween 80, *N*-acetyl-D-glucosamine, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, α -D-glucose, D-psicose, sucrose, D-trehalose, methylpyruvate, monomethylsuccinate, α -ketoglutarate, succinate, bromosuccinate, succinamate, L-alanylglycine, and glycyl-L-glutamate. Weak or variable reactions were observed with dextrin, glycogen, lactulose, D-mannose, turanose, *cis*-aconitate, alaninamide, L-alanine, L-aspartate, L-glutamate, L-proline, L-serine, glycerol, DL- α -glycerolphosphate, glucose 1-phosphate, and glucose 6-phosphate. Negative reactions were observed with α -cyclodextrin, *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, *meso*-erythritol, *meso*-inositol, α -D-lactose, maltose, D-mannitol, D-melibiose, β -methyl-D-glucoside, D-raffinose, L-rhamnose, D-sorbitol, xylitol, acetate, citrate, formate, D-galactonic acid lactone, D-galacturonate, D-gluconate, D-glucosaminat, D-glucuronate, α -hydroxybutyrate, β -hydroxybutyrate, γ -hydroxybutyrate, ρ -hydroxyphenylacetate, itaconate, α -ketobutyrate, α -ketovalerate, DL-lactate, malonate, propionate, quinate, D-saccharate, sebacate, glucuronamide, D-alanine, L-asparagine, glycyl-L-aspartate, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylamine, L-pyroglytamate, D-serine, L-threonine, DL-carnitine, γ -aminobutyrate, urocanate, inosine, uridine, thymidine, phenyl ethylamine, putrescine, 2-aminoethanol, and 2,3-butanediol.

The generic determinative characteristics of ICMP 8561^T (= NCPPB 2982^T), which is a rod-shaped bacterium that is motile by means of a single polar flagellum, grows in the presence of 2,3,5-triphenyltetrazolium chloride only at concentrations less than 0.1%, lacks observable poly- β -hydroxybutyrate inclusions, and produces xanthan-like mucoid extracellular material on glucose-containing media, are diagnostic characteristics for *Xanthomonas* spp. The strain also has the fatty acid profile of a xanthomonad with three characteristic acids, 13:0 iso 3-OH, 12:0 3-OH, and 11:0 iso 3-OH. We conclude, therefore, that the type strain of *P. cissicola*, strain PC1, which was received independently at the ICMP and the National Collection of Plant-Pathogenic Bacteria, is a member of the genus *Xanthomonas*. When they placed the species in the genus *Pseudomonas*, Goto and Makino (4) were perhaps misled by the lack of production of the yellow xanthomonadin pigments which are common to, but not universally produced by, members of this genus (9). These authors also appear to

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FIG. 1. Electron micrographs of *P. cissicola* ICMP 8561^T, showing cell morphology and flagellar insertion. Bars = 200 nm.

have reported a false-positive reaction in the test for poly- β -hydroxybutyrate production. Nevertheless, the body of data reported here clearly supports reclassification of the strain in the genus *Xanthomonas*. The metabolic reactions of *Xanthomonas* spp. as determined by Biolog tests have been described previously (12). An analysis of the data showed that if nonvariable reactions were scored as reactions in which more than 90% of strains gave positive or negative reactions, then species were not discriminated. We arbitrarily set test variability at 20 to 79% for the data of Vauterin et al. (12) in order to permit a comparison of ICMP 8561^T data with their data. Even on this basis, *P. cissicola* exhibited more than 95% similarity with *Xanthomonas oryzae* and *Xanthomonas translucens* and

more than 90% similarity with 15 genomic species, but not with *Xanthomonas albilineans*, *Xanthomonas codiae*, *Xanthomonas melonis*, *Xanthomonas pisi*, or *Xanthomonas sacchari* as characterized (12). Inspection of the results of Biolog tests (12) indicated that discrimination depended on a very high level of reproducibility. Future application of the determinative characteristics of Vauterin et al. (12), who also expressed reservations, should show whether their determinative scheme is efficacious. The strain which we studied differed from all recognized species in two or more tests. A comparison of the strain ICMP 8561^T FAME profile with the profiles of 80 *Xanthomonas* pathovars belonging to 17 of the proposed *Xanthomonas* species (13) was performed by using unweighted pair

grouping of libraries. The results showed that strain ICMP 8561^T was most similar to strains of *X. translucens* and an atypical strain of *Xanthomonas vesicatoria*. Nevertheless, strain ICMP 8561^T could not be placed in *X. translucens* because it contained 10:0 3-OH and an unknown fatty acid with an equivalent chain length of 11.8 that is not present in this species (13). The large amounts of 10:0 3-OH and small amounts of 12:0 3-OH, 11:0 iso 3-OH, and 11:0 iso are unusual for the genus *Xanthomonas*. These fatty acid profile and biochemical test data support placement of strain ICMP 8561^T in a previously unreported *Xanthomonas* species.

It is not appropriate to propose the new combination *Xanthomonas cissicola* without investigating more extensively the genomic and phenotypic characteristics of this species compared with previously described species. To do this, a larger number of strains than are available at present is essential (11).

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