Nomenclatural Status of *Pseudomonas barkeri* (Berridge 1924) Dowson 1943 and *Pseudomonas washingtoniae* (Pine 1943) Elliott 1951

Request for an opinion

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Strains NCPPB 244 and NCPPB 967, the only known isolates reported to belong to *Pseudomonas barkeri* (Berridge 1924) Dowson 1943 and *Pseudomonas washingtoniae* (Pine 1943) Elliott 1951, respectively, were received from the National Collection of Plant Pathogenic Bacteria, Harpenden, England. Cultures of these strains were examined by the standard cultural and biochemical tests currently in use for characterizing pseudomonads and were identified as belonging to *Pseudomonas putida* (Trevisan 1889) Migula 1895 and *Pseudomonas fluorescens* Migula 1895, respectively. In the absence of authentic cultures and adequate descriptions, it is requested that the Judicial Commission of the International Committee on Systematic Bacteriology place the names *P. barkeri* and *P. washingtoniae* and their objective synonyms on the list of rejected names as "doubtful names" (*nomina dubia*).

Berridge (3) referred to *Bacterium barkeri* as a pathogen causing blight of pear (*Pyrus communis* L.) blossom in England, and Dowson (7) reclassified the organism within the genus *Pseudomonas* Migula (20). Pine (23) isolated and identified a pathogen from Washington palm (*Washingtonia filifera* Wendl.) as *Phytomonas washingtoniae*, and Elliott (11) placed this organism in *Pseudomonas*. Doudoroff and Palleroni (6) included *Pseudomonas washingtoniae* in an addendum to the genus of species which have been incompletely described but which conform to the generic definition. They made no reference to *Pseudomonas barkeri*. Dye et al. (9) proposed that these *nomenspecies* should be retained pending further research. Only one isolate of each of these organisms is known, of which representatives are held in the Plant Diseases Division Culture Collection (PDDCC), Department of Scientific and Industrial Research, Auckland, New Zealand.

This study is part of a larger examination of plant pathogenic pseudomonads in which cultures of both pathogenic and nonpathogenic species are included for testing. The only species reported here are those which could usefully be compared with *P. barkeri* and *P. washingtoniae*.

Bacterial strains. *P. barkeri* PDDCC strain 3880 was received from the National Collection of Plant Pathogenic Bacteria (NCPPB) Harpenden, England, as NCPPB 244 (= NCTC 393 ex B.T.P. Barker = ATCC 6876). *P. washington-*

iae PDDCC 3966 was received as NCPPB 967 (= ICPB PW2 ex Brown = NRRL B-899). No strains have been designated as the type or neotype of these organisms. *Pseudomonas fluorescens* Migula (21) PDDCC strains 3512 and 3520 and *Pseudomonas putida* Trevisan (28) Migula (21) PDDCC strains 3510 and 3511, received from the National Collection of Industrial Bacteria, Aberdeen, Scotland, as NCIB strains 9046, 9886, 9494, and 9887, respectively, were included for comparison. Strain NCIB 9046 is the neotype of *P. fluorescens* and strain NCIB 9494 is the suggested neotype of *P. putida*.

Maintenance of cultures and preparation of inoculum. Plate cultures were prepared from freeze-dried cultures, and 10 colonies were transferred collectively to slopes of a medium called YCA (NH₄H₂PO₄, 1.0 g; K₂HPO₄, 2.8 g; KCl, 0.2 g; MgSO₄·7H₂O, 0.2 g; yeast extract [Difco], 5 g; agar [Davis], 12 g; distilled water, 1 liter; pH 7.0) incubated at 25°C for 2 days and stored at 12°C. Fresh cultures (incubated for 24 to 48 h at 25°C) on YCA slopes were suspended in sterile distilled water to give faintly turbid suspensions of approximately 10⁸ cells per ml. Solid media were streaked with these suspensions, and broth media were inoculated with one or two drops of the suspensions dispensed with sterile Pasteur pipettes.

Oxygen requirements and respiratory nitrate reductase activity. Freshly prepared slopes of a medium containing the following were inoculated in pairs: NH₄H₂PO₄, 1 g; KCl,

0.2 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g; KNO_3 , 10 g; glucose, 3 g; yeast extract (Difco), 3 g; agar (Davis), 12 g; distilled water, 1 liter; pH 7.0. One of each pair of tubes was placed in a Torbal anaerobic jar containing a GasPak (Becton, Dickinson & Co.) and was incubated for 14 days at 25°C, and the other tube was incubated aerobically at 25°C. Cultures which grew under aerobic conditions but which failed to grow in the anaerobic jar were presumed to require oxygen or nitrate as ultimate electron acceptors and were recorded as obligate aerobes. Cultures which grew in the anaerobic jar either were anaerobes or were anaerobes capable of utilizing nitrate as an electron acceptor. They were inoculated on the medium described above from which nitrate had been excluded, were incubated anaerobically, and were examined for growth after 14 days.

The methods of other tests used are summarized with their results in Table 1.

The results of the characterization of the strains included in this study are given in Table 1.

P. barkeri PDDCC 3880 and *P. washingtoniae* PDDCC 3966 were found to be gram-negative rods which were motile by means of a single polar flagellum; therefore they are members of the family *Pseudomonadaceae* as de-

finied by Doudoroff and Palleroni (6). The non-dependence on growth factor requirements and the ability to grow in a medium containing 0.1% TTC indicated that the organisms were not species of *Xanthomonas*, a genus composed entirely of plant pathogens with close affinities to *Pseudomonas* (10). Failure to accumulate poly- β -hydroxybutyrate showed that these organisms cannot be included in the groups of species represented by the pathogen *Pseudomonas solanacearum* (25, 26).

Although *P. barkeri* PDDCC 3880 did not produce fluorescent pigment, the fact that it, like *P. washingtoniae* PDDCC 3966, exhibited the determinative characters of the fluorescent pseudomonads indicates that both of these bacteria are pseudomonads.

The results of the LOPAT tests (see Table 1) showed that both *P. barkeri* 3880 and *P. washingtoniae* 3966 belong in group V of the LOPAT determinative scheme. This group of organisms contains a single plant-pathogenic species, *P. tolaasii* Paine (22), and nonpathogenic fluorescent pseudomonads, including *P. fluorescens*. Doudoroff and Palleroni (6) described the results of tests which differentiated *P. fluorescens* from related organisms. Results from the present study show that *P. washingtoniae*

TABLE 1. Reactions of strains of *P. barkeri*, *P. fluorescens*, *P. putida*, and *P. washingtoniae* to comparative tests

Test	Method reference	<i>P. barkeri</i> PDDCC 3880	<i>P. washingtoniae</i> PDDCC 3966	<i>P. fluorescens</i> PDDCC 3512, 3520	<i>P. putida</i> PDDCC 3511, 3512
Gram reaction	2, 27	—	—	—	—
Morphology	18	Rod	Rod	Rod	Rod
Flagellation	18	1 Polar	1 Polar	1-3 Polar	1-3 Polar
Oxygen requirement		Obligate aerobe	Obligate aerobe	Aerobe	Aerobe
Nitrate reductase		—	—	3512— 3520+	W ^a
Growth factors required	8	—	—	—	—
Fluorescent pigment	14	—	+	+	+
Growth in 0.1% TTC	17	+	+	+	+
Poly- β -hydroxybutyrate accumulated	4, 12, 13	—	—	—	—
Gelatin liquefaction	8	—	+	+	—
Growth at 41°C		—	—	—	—
Carbon-source utilization	29				
β -Alanine		+	+	+	+
Glycine		+	+	3512— 3520+	+
Valine		+	+	+	+
meso-inositol		+	+	+	+
2-Keto-D-gluconate		+	+	+	+
Trehalose		+	+	+	+
LOPAT ^b tests	16				
Levan production		—	—	—	—
Oxidase activity		+	+	+	+
Potato rot		—	—	—	—
Arginine dihydrolase activity		+	+	+	+
Tobacco hypersensitivity reaction		—	—	—	—

^a Weakly positive reaction.

^b LOPAT is the acronym for the five tests listed.

PDDCC 3966 is indistinguishable from the strains of *P. fluorescens* tested.

Doudoroff and Palleroni (6) reported that reactions on meso-inositol, trehalose, and gelatin distinguish *P. putida* from *P. fluorescens*. In the tests reported here, these two species were distinguished only by their gelatin reaction, a major determinative test separating these species. *P. barkeri*, like *P. putida*, did not liquefy gelatin. Rhodes (24) and Lysenko (19) suggested that *P. fluorescens* and *P. putida* are elements of a cluster of organisms which are not taxonomically distinct. However, the identity of *P. fluorescens* and *P. putida* are not questioned in this paper, and therefore *P. barkeri* is regarded as having more affinities to *P. putida* than to *P. fluorescens*.

In reporting "*Bacterium barkeri*," Berridge made incidental reference to a subculture of one of Barker's isolates from blossom blight of pears (1). The description published by Barker and Grove (1) and by Doidge (5) are inadequate, in terms of present-day knowledge, to enable identification of the organism. Furthermore, the name "*Bacterium barkeri*" was not formally proposed by Berridge, and therefore it is not valid according to Rule 28b of the International Code of Nomenclature of Bacteria (15). From their description of the disease syndrome, it is possible that Barker and Grove were observing the effects of *Pseudomonas syringae* van Hall (C. J. J. Van Hall, Inaugural Dissertation, Amsterdam, 1902) but that their isolates were contaminated with *P. putida*, a saprophyte, which has become the dominant organism in storage. Similarly, Pine's (23) description of *P. washingtoniae* is also not adequate to allow identification of this pathogen, and hence both of these names should be considered nomina dubia according to Rule 56a (2) of the International Code of Nomenclature of Bacteria (15), i.e., they are names whose application is uncertain.

The results presented here show that the only available strain purported to be a member of *P. washingtoniae*, PDDCC 3966, is indistinguishable from *P. fluorescens*. *P. barkeri* PDDCC 3880 is identified as a member of *P. putida*.

Therefore, it is requested that the Judicial Commission of the International Committee on Systematic Bacteriology place the names *Pseudomonas barkeri* (Berridge) Dowson and *Pseudomonas washingtoniae* (Pine) Elliott together with their objective synonyms on the list of rejected names as doubtful names (nomina dubia).

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REPRINT REQUESTS

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