Pseudomonas pickettii, a New Species of Clinical Origin Related to *Pseudomonas* *solanacearum*

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Twenty strains of *Pseudomonas* isolated from clinical specimens were found to constitute a homogeneous group which we recognize as a species and name *Pseudomonas pickettii*. On the basis of deoxyribonucleic acid hybridization data, this species appears to be most closely related to the phytopathogenic species *P. solanacearum*. The type strain of *P. pickettii* is K-288 (ATCC 27511).

Thirty-five bacterial strains isolated from hospital specimens were received by us from M. J. Pickett, with the tentative identification of *Pseudomonas pseudoalcaligenes* type 2. When tested by our methods (4, 7) 19 of these strains were found to constitute a phenotypically homogeneous group which differs in over 40 characters from both *P. pseudoalcaligenes* and *P. alcaligenes* as described by us (7). Another strain, also received from M. J. Pickett as an "unclassified denitrifier," could also be assigned to this homogeneous group. We further compared the phenotypic properties of this group of strains with those of other well-characterized *Pseudomonas* species and performed a limited number of deoxyribonucleic acid (DNA)-DNA hybridization experiments to determine whether it may represent a new species.

MATERIALS AND METHODS

Bacterial strains. All the bacterial strains used by us are designated by the numbers under which they were received from M. J. Pickett, Department of Bacteriology, University of California, Los Angeles. All, except strain K-300, which was sent as an "unclassified denitrifier," were tentatively identified by Pickett as *P. pseudoalcaligenes* type 2. The original source of strain K-214 was a "Sephadex eluate" from Olive View Hospital; the other 19 strains were isolated in the Clinical Laboratories of the University of California, Los Angeles. Ten strains (K-288, K-298, K-472, K-507, K-529, K-550, K-560, K-572, K-615, and K-633) were isolated from patients who had undergone tracheotomies. Two strains (K-618 and K-619) were isolated from the blood of a patient. Strain K-599 was isolated from hospital equipment. The original sources of six strains (K-48, K-300, K-421, K-444, K-487, and K-551) are not recorded. Strains of all other *Pseudomonas* species reported here are

designated as in our previous publications (1, 2, 3, 5, 7).

Methods. The methods used for the phenotypic characterization of the strains have been described by Stanier et al. (7). The phenotypic similarity coefficients (6) for positive characters only (S_p) and for positive and negative matches (S_{SM}) were calculated by using 169 morphological, nutritional, and biochemical characters that we had found useful for differentiating among *Pseudomonas* species. DNA-DNA hybridizations were performed by the competition and direct binding techniques as described by Ballard et al. (1) and by Palleroni and Doudoroff (3).

RESULTS AND DISCUSSION

The twenty strains studied by us formed a phenotypically homogeneous group which could be distinguished from all other *Pseudomonas* species that we had studied or that we could recognize from the literature. Because strains of this group appear to be of somewhat common occurrence in clinical specimens, and can apparently be misidentified as members of other species (e.g., *P. pseudoalcaligenes*), we propose to create a new species which we name after M. J. Pickett, *P. pickettii*. The phenotypic properties of the type strain and of other members of the group are presented in the formal description and in Table 1.

DNA-DNA hybridization experiments showed a high degree of homology among three representative strains of *P. pickettii* and a relatively low degree of homology with strains of other selected species (see Table 2). On the basis of DNA homology, it appears that the organisms of this group are most closely related to *P. solanacearum* and somewhat less closely related to members of the *pseudomallei-cepacia* group (4).

TABLE 1. *Nutritional properties of the strains of P. pickettii^a*

Substrate	Growth	% positive	Substrate	Growth	% positive
Hydrocarbons			Aliphatic acids (cont.)		
<i>n</i> -Dodecane ^b	—	0	Azelate ^b	+	100
<i>n</i> -Hexadecane ^b	—	0	Sebacate ^b	+	100
Alcohols and polyols			Glycollate	+	100
Ethanol ^b	—	0	Lactate	+	100
<i>n</i> -Propanol	—	20	Pyruvate	+	100
Isopropanol	—	0	Glycerate ^b	+	100
Butanol	—	30	D-Malate	+	85
Isobutanol	—	50	L-Malate	+	100
Geraniol	—	5	D(—)Tartrate	—	0
Ethylene glycol	—	0	L(+)Tartrate	+	90
Propylene glycol	—	5	<i>m</i> -Tartrate	+	90
2,3-Butylene glycol	—	15	β -Hydroxybutyrate	+	100
Glycerol	+	100	Citrate	+	100
Erythritol	—	0	α -Ketoglutarate	+	100
Mannitol	—	0	Aconitate	+	100
Sorbitol	—	0	Levulinate	+	100
<i>m</i> -Inositol	—	0	Citraconate ^b	+	100
Adonitol	—	0	Itaconate	—	0
Carbohydrates and sugar acids			Mesaconate	—	0
D-Ribose	—	0	Hydroxymethylglutarate ^b	+	100
D-Xylose	+	100	Nonnitrogenous aromatic and other cyclic compounds		
D-Arabinose	—	0	D-Mandelate	—	0
L-Arabinose	+	100	L-Mandelate	+	95
D-Fucose	—	0	Benzoyl formate	+	90
L-Rhamnose	—	0	<i>o</i> -Hydroxybenzoate	—	0
Glucose	+	100	<i>m</i> -Hydroxybenzoate	—	0
Mannose	—	0	<i>p</i> -Hydroxybenzoate	+	95
Galactose ^b	+	100	Phthalate	—	0
Fructose	+	100	Phenylacetate	—	0
Sucrose	—	0	Naphthalene	—	0
Trehalose	—	0	Phenol	—	0
Maltose	—	0	Quinate ^b	+	100
Cellobiose	—	0	Testosterone ^b	—	0
Lactose	—	0	Amino acids and related compounds		
Starch	—	0	Glycine	+	100
Salicin	—	0	L-Alanine	+	100
Gluconate	+	100	D-Alanine	+	100
2-Ketogluconate ^b	+	100	β -Alanine ^b	+	100
Saccharate	+	100	L-Serine	+	100
Mucate	+	100	L-Threonine ^b	+	100
Aliphatic acids			L-Leucine	—	0
Acetate	+	100	L-Isoleucine	—	0
Propionate	+	100	L-Norleucine	—	0
Butyrate	+	100	L-Valine	+	100
Isobutyrate	+	100	L-Aspartate ^b	+	100
Valerate ^b	+	80	L-Glutamate	+	100
Isovalerate	+	100	L-Lysine	—	0
Caproate ^b	+	80	L-Arginine	—	0
Heptanoate ^b	+	100	L-Ornithine	—	0
Caprylate ^b	+	100	L-Citrulline	—	0
Pelargonate	—	0	α -Aminobutyrate	—	0
Caprate	+	100	γ -Aminobutyrate	+	100
Malonate	+	100	α -Aminovalerate	—	0
Succinate ^b	+	100	δ -Aminovalerate	—	0
Maleate	+	90	L-Histidine ^b	+	80
Fumarate	+	100	L-Proline ^b	+	100
Glutarate	+	85	L-Tyrosine ^b	+	100
Adipate	+	100	L-Phenylalanine	—	5
Pimelate ^b	+	100			
Suberate ^b	+	100			

TABLE 1—Continued

Substrate	Growth	% positive	Substrate	Growth	% positive
Amino acids and related compounds (cont.)			Miscellaneous compounds		
L-Tryptophan	+	100	Spermine	—	0
D-Tryptophan	—	55	Butylamine	—	0
Kynurenine ^b	+	80	α -Amylamine	—	0
Kynurenate	+	100	Betaine	—	0
Anthranilate	—	0	Sarcosine	—	0
Histamine ^b	—	20	Creatine	—	0
Tryptamine	—	0	Hippurate	+	100
Miscellaneous compounds			Pantothenate	—	0
Ethanolamine	—	0	Acetamide	—	0
Benzylamine	—	0	Nicotinate	—	0
Putrescine	—	0	Trigonelline	—	5

^a For each substrate tested as a sole carbon source, growth or no growth of the type strain is indicated by + or —. Numbers refer to percent of all strains tested that are positive for the character. Strain numbers of positive strains for each substrate used by a fraction of the strains are the following: *n*-propanol, K-214, K-472, K-615, K-618; butanol, K-48, K-214, K-615, K-618, K-619, K-633; isobutanol, K-214, K-298, K-421, K-472, K-487, K-507, K-529, K-550, K-615, K-618; geraniol, K-214; 2,3-butylene glycol, K-550, K-615, K-618; valerate, all except K-300; caproate, all except K-300; maleate, all except K-615, K-618; glutarate, all except K-300, K-615, K-618; D-malate, all except K-300, K-615, K-618; L-(+)-tartrate, all except K-615, K-618, *m*-tartrate, all except K-615, K-618; L-mandelate, all except K-214; benzoyl formate, all except K-214, K-300; *p*-hydroxybenzoate, all except K-214; L-histidine, all except K-300; L-phenylalanine, K-300; D-tryptophan, K-48, K-214, K-472, K-507, K-529, K-551, K-560, K-615, K-618, K-619, K-633; kynurenine, all except K-633; histamine, K-300; trigonelline, K-550.

^b Only 5 of the 20 strains were tested: K-288, K-300, K-421, K-550, K-633.

TABLE 2. DNA competition experiments using *P. pickettii* strain K-288 as reference, compared with similarity (S_J) and matching (S_{SM}) coefficients

DNA from	Mol % guanine plus cytosine	Competition at 71 C (%)	Direct binding (%)	S_J	S_{SM}
<i>Pseudomonas pickettii</i> K-288	64	"100"	"100"	"100"	"100"
<i>P. pickettii</i> K-550	64	100	ND ^c	97	99
<i>P. pickettii</i> K-300	64	90	ND	88	94
<i>P. solanacearum</i> 769	66	56	39	46	74
<i>P. solanacearum</i> 773	66	37	ND	46	73
<i>P. solanacearum</i> 777A	66	29	ND	54	75
<i>P. solanacearum</i> 780	69	29	ND	56	77
<i>P. solanacearum</i> 776	66	ND	17 ^a	55	76
<i>P. cepacia</i> 382	67	26	12	57	69
<i>P. mallei</i> 3873	68	21	ND	46	63
<i>P. pseudomallei</i> 165	69 ^b	11	ND	ND	ND
<i>P. acidovorans</i> 105	66	0	ND	56	74
<i>P. testosteroni</i> 78	61	10	ND	58	78
<i>P. facilis</i> 332	64	15	ND	39	67
<i>P. delafieldii</i> 134	65	0	8	52	75
<i>P. aeruginosa</i> 131	68	4	5	51	68
<i>P. stutzeri</i> 222	65	8	ND	48	71
<i>P. pseudoalcaligenes</i> 63	62	2	3 ^a	34	64
<i>P. alcaligenes</i> 142	66	2 ^a	ND	28	64

^a Values obtained in reciprocal hybridization experiments using *P. solanacearum* 776, *P. pseudoalcaligenes* 63, or *P. alcaligenes* 142 as reference.

^b DNA composition not determined for this strain but is very uniform for all other strains of the species tested.

^c Not determined.

In the following description, the characters of the species apply to the type strain except where otherwise indicated. The nutritional properties of the type strain and of other strains of *P. pickettii* are indicated in Table 1. The constellations of phenotypic characters that distinguish this species from all other *Pseudomonas* species that have been thoroughly characterized by our methods are indicated in the footnote to the formal description.

Pseudomonas pickettii sp. nov. pick. et' ti.i. Pickett patronymic; M. L. gen. noun *pickettii* of Pickett; named for M. J. Pickett.

Rods, approximately 0.5 to 0.6 by 1.5 to 3.0 μm , occurring singly, in pairs, and more rarely in short chains. Accumulate poly- β -hydroxybutyrate granules as carbon reserve^a. Motile by means of a single polar flagellum^b (cells with two flagella are also observed, although very infrequently). The wavelength of the flagella is approximately 1.7 μm (Fig. 1). Gram negative.

Colonies are not pigmented^c, and soluble pigments are not produced^a.

Nitrate reduced to nitrogen gas^e. Arginine dihydrolase reaction, negative^f. Oxidase reaction, positive^g. No hydrolysis of gelatin^h, starchⁱ, or extracellular poly- β -hydroxybutyrate^j. Polyethylene sorbitan monooleate (Tween 80) is hydrolyzed. Egg yolk reaction, negative.

Organic growth factors not required^k. At least 66 different organic compounds can be used as sole carbon sources by the type strain and at least nine other compounds by other strains (Table 1). These include several monosaccharides and sugar acids and a large number of mono-, di-, and tricarboxylic acids and amino acids. Not used as carbon sources are disaccharides, most polyols, and amines. Substrates of diagnostic importance in distinguishing this species from all other species that have been well characterized include glucose^l, galactose^m,

FIG. 1. *Pseudomonas pickettii*, strain K-288. Left, phase-contrast photograph of exponentially grown cells. Right, Leifson flagella stain. Magnification, $\times 3,000$.

L-arabinoseⁿ, malonate^o, suberate^p, and L-tryptophan^q, which are utilized by all strains, as well as D-arabinose^r, maltose^s, sucrose^t, ethanol^u, mannitol^v, and ethylene glycol^w, which are not utilized by any strain. Almost all strains, including the type strain, use mandelate and *p*-hydroxybenzoate. The latter compound is metabolized via the *ortho* cleavage of protocatechuate. All strains use ammonium salts or nitrate as nitrogen sources.

Does not grow autotrophically with the oxidation of H₂ as a source of energy^x.

Obligately aerobic except in media with nitrate as an electron acceptor. Optimal temperature, approximately 35 C. Growth at 41 C^y but not at 4 C.

The guanine plus cytosine content of the DNA is approximately 64 mol % (by buoyant density). DNA-DNA hybridization experiments indicate a relatively close relationship of this species to *P. solanacearum*, a more distant relationship to *P. cepacia* and *P. mallei*, and virtually none with *P. pseudoalcaligenes* and other *Pseudomonas* species tested (Table 2).

Type strain: K-288. Deposited in the American Type Culture Collection (ATCC), Rockville, Md., under the number 27511.

^{a-y}Positive and negative characters useful for differentiating *P. pickettii* from some other *Pseudomonas* species as follows: ^a,^d,^f,^h,^u,^v*P. aeruginosa*; ^a,^b,^d,^f,^h,^y*P. fluorescens*; ^a,^b,^d,^e,^f,^y*P. putida*; ^a,^b,^d,^g,ⁿ,^y*P. syringae* group; ^a,^b,^d,^e,^q,^t,^y*P. cichorii*; ^e,^f,^l,^m,ⁿ,^o,^p*P. alcaligenes*; ^e,^l,^m,ⁿ,^o,^p*P. pseudoalcaligenes*; ^a,^c,^f,^m,^u*P. mendocina*; ^a,ⁱ,ⁿ,^s,^w*P. stutzeri*; ^b,^e,^l,^m,ⁿ,^y*P. acidovorans*; ^b,^e,^l,^m,ⁿ,^o,^y*P. testosteroni*; ^b,^e,^r,^t,^v*P. cepacia*; ^b,^e,^h,^r,^t,^u,^v*P. marginata*; ^b,^f,^r,^t,^v*P. caryophylli*; ^o,^q,^t,^x,^y*P. solanacearum*; ^b,^f,^h,ⁱ,^j,^r,^s,^t,^v*P. pseudomallei*; ^b,^f,^h,^j,^t,^v*P. mallei*; ^e,^k,^l,^m,ⁿ,^q*P. diminuta*; ^c,^e,^k,ⁿ,^q,^y*P. vesicularis*; ^a,^b,^e,^g,^h,^k,^s,^t,^y*P. maltophilia*; ^e,^j,^l,^m,ⁿ,

^o,^p,^y*P. lemoignei*; ^e,^j,^q,^v,^y*P. delafieldii*; ^e,^h,^j,^q,^x,^y*P. facilis*; ^e,ⁱ,^o,^q,^t,^s,^x,^y*P. saccharophila*; ^e,^m,ⁿ,^o,^u,^x,^y*P. ruhlandii*; ^c,^e,^o,^s,^t,^x,^y*P. flava*; ^c,^e,^m,ⁿ,^o,^u,^x,^y*P. palleroni*.

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