Transfer of *Pseudomonas plantarii* and *Pseudomonas glumae* to *Burkholderia* as *Burkholderia* spp. and Description of *Burkholderia vandii* sp. nov.

TEIZI URAKAMI,^{1*} CHIEKO ITO-YOSHIDA,² HISAYA ARAKI,² TOSHIO KIJIMA,³
KEN-ICHIRO SUZUKI,⁴ AND KAZUO KOMAGATA^{5†}

Biochemicals Division, Mitsubishi Gas Chemical Co., Shibaura, Minato-ku, Tokyo 105,¹ Niigata Research Laboratory, Mitsubishi Gas Chemical Co., Tayuhama, Niigata 950-31,² Plant Pathological Division of Biotechnology, Tochigi Agricultural Experiment Station, Utsunomiya 320,³ Japan Collection of Microorganisms, The Institute of Physical and Chemical Research, Wako-shi, Saitama 351-01,⁴ and Institute of Molecular Cell and Biology, The University of Tokyo, Bunkyo-ku, Tokyo 113,⁵ Japan

Plant-associated bacteria were characterized and are discussed in relation to authentic members of the genus *Pseudomonas* sensu stricto. Bacteria belonging to *Pseudomonas* rRNA group II are separated clearly from members of the genus *Pseudomonas* sensu stricto (*Pseudomonas fluorescens* rRNA group) on the basis of plant association characteristics, chemotaxonomic characteristics, DNA-DNA hybridization data, rRNA-DNA hybridization data, and the sequences of 5S and 16S rRNAs. The transfer of *Pseudomonas cepacia*, *Pseudomonas mallei*, *Pseudomonas pseudomallei*, *Pseudomonas caryophylli*, *Pseudomonas gladioli*, *Pseudomonas pickettii*, and *Pseudomonas solanacearum* to the new genus *Burkholderia* is supported; we also propose that *Pseudomonas plantarii* and *Pseudomonas glumae* should be transferred to the genus *Burkholderia*. Isolate VA-1316^T (T = type strain) was distinguished from *Burkholderia* species on the basis of physiological characteristics and DNA-DNA hybridization data. A new species, *Burkholderia vandii* sp. nov. is proposed for this organism; the type strain of *B. vandii* is VA-1316 (= JCM 7957).

Palleroni et al. (32) reported in 1973 that *Pseudomonas* species could be divided into at least five distinct groups on the basis of levels of rRNA-DNA homology. This finding was supported by the variable enzymological patterns observed for tyrosine biosynthesis (6), cellular fatty acid composition data (27), hydroxy fatty acid composition data (27, 43), quinone system data (27, 61), DNA-DNA homology data (13, 20, 29), rRNA-DNA homology data (8, 9, 11, 52–54), 16S rRNA sequence data (24, 40, 56–59), and 5S rRNA sequence data (7, 12, 41), and it is clear that the five groups of Palleroni et al. differ at least at the genus level.

Many plant-associated or plant-pathogenic strains of *Pseudomonas* species (36, 42, 46), particularly strains of *Pseudomonas cepacia* (1, 4, 51), *Pseudomonas gladioli* (1, 4, 16, 51), *Pseudomonas glumae* (51), and *Pseudomonas avenae* (17, 35, 51), have been isolated from various kinds of plants and have been identified as bacteria that exhibit growth-inhibiting activity against plant-pathogenic microorganisms, such as *Clavibacter michiganensis* subsp. *michiganensis*, *Agrobacterium tumefaciens*, *Fusarium oxysporum* f. sp. *cepae*, and other organisms. *P. cepacia* strains appear to be widely distributed in nature and have been isolated from rotten onions, soils, and clinical specimens (e.g., urinary tract infection specimens) (28). Furthermore, *P. avenae* ("Pseudomonas alboprecipitans" [38], *Pseudomonas rubrilineans* [35]) and *Pseudomonas cattleyae* ("Bacterium cattleyae") (2) were isolated as plant-associated or plant-pathogenic bacteria.

P. cepacia, *P. gladioli* ("Pseudomonas marginata"), *Pseudomonas caryophylli*, *Pseudomonas solanacearum* (5, 15), *Pseudomonas pickettii* (23, 33), *Pseudomonas mallei*, and *Pseudomonas pseudomallei* were included in rRNA group II of the genus *Pseudomonas* by Palleroni et al. (32) and are identified as section II bacteria in Bergey's *Manual of Systematic Bacteriology* (28). *P. glumae* was included in rRNA group II on the basis of the rRNA-DNA hybridization data of De Vos et al. (9). *Pseudomonas plantarii* was isolated as the causal agent of rice seedling blight by Azegami et al. and was shown to be related to *P. gladioli* (3). Recently, the rRNA sequences of several *Pseudomonas* species were studied (7, 12, 24, 40, 41, 56–59), and *P. cepacia*, a typical species of rRNA group II, was assigned to the β-purple bacteria (40). This phylogenetic characterization supported the differentiation of *Pseudomonas* rRNA group II bacteria (28) (*P. solanacearum* rRNA complex in rRNA superfamily III [11]) from *Pseudomonas* rRNA group I bacteria (28) (*Pseudomonas fluorescens* rRNA branch in rRNA superfamily II [11]) at least at the genus level. In 1992, Yabuuchi et al. (60) proposed that seven species belonging to *Pseudomonas* rRNA group II (*P. cepacia*, *P. mallei*, *P. pseudomallei*, *P. caryophylli*, *P. gladioli*, *P. pickettii*, and *P. solanacearum*) should be transferred to a new genus, *Burkholderia*, on the basis of polyphasic taxonomy data. *P. avenae* and *P. cattleyae* were included in section V of the genus *Pseudomonas* in Bergey's *Manual of Systematic Bacteriology* (28). The results of rRNA-DNA hybridization experiments performed with *P. avenae* and *P. cattleyae* (9, 11, 55) demonstrated that these bacteria belong in the *Pseudomonas acidovorans* rRNA complex in rRNA superfamily III (11, 28). In 1992, Willems et al. (54) proposed the transfer of *P. avenae* and *P. cattleyae* to the new genus *Acidovorax* (53) as *Acidovorax avenae* subsp. *avenae* and *Acidovorax avenae* subsp. *cattleyae*, respectively.

In this paper, we describe a detailed recharacterization of plant-associated bacteria, clarify the differentiation of these

* Corresponding author. Mailing address: Biochemicals Division, Mitsubishi Gas Chemical Co., Seavans-N Building, Shibaura, Minato-ku, Tokyo 105, Japan. Phone: (03) 5440-3218. Fax: (03) 5440-3229.

† Present address: Department of Agricultural Chemistry, Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagaya-ku, Tokyo 156, Japan.

TABLE 1. Bacterial strains studied

Strain ^a	Other designations ^a	Reference(s)
<i>Burkholderia cepacia</i> ATCC 25416 ^T	ICP 25 ^T , JCM 5964 ^T , NCTC 10743 ^T	4, 28, 31, 60
<i>Burkholderia cepacia</i> ALQ 8281 ^b		60
<i>Burkholderia gladioli</i> ATCC 10248 ^T	IFO 13700 ^T , NCPPB 1891 ^T , PDDCC 2804 ^T	4, 16, 28, 60, 62
<i>Burkholderia gladioli</i> ATCC 19302	NCPPB 947, ICPB PA7, PDDCC 2804	4, 16, 28, 60, 62
<i>Pseudomonas plantarii</i> JCM 5492 ^T	NIAES 1723 ^T	3
<i>Burkholderia caryophylli</i> ATCC 25418 ^T	ICPB PC113 ^T , NCPPB 2151 ^T , PDDCC 512 ^T	4, 28, 60
<i>Pseudomonas glumae</i> NIAES 1169 ^T	ATCC 33617 ^T , NCPPB 2981 ^T	28, 46
<i>Burkholderia pickettii</i> JCM 5969 ^T	ATCC 27511 ^T , ICPB 3981 ^T	23, 28, 34, 36, 60
<i>Burkholderia solanacearum</i> ATCC 11696 ^T	NCPPB 325 ^T , PDDCC 5721 ^T	5, 15, 23, 28, 30, 60
<i>Acidovorax avenae</i> subsp. <i>avenae</i> ATCC 19860 ^T	NCPPB 1011 ^T	28, 35, 38, 54
<i>Acidovorax avenae</i> subsp. <i>cattleyae</i> ATCC 33619 ^T	NCPPB 961 ^T	2, 28, 54
<i>Pseudomonas aeruginosa</i> JCM 5962 ^T	ATCC 10145 ^T , NCIB 8295 ^T , NCTC 10332 ^T	28
<i>Pseudomonas fluorescens</i> NCIB 9046 ^T	ATCC 13525 ^T , IAM 12022 ^T , ICPB 3200 ^T , IFO 14160 ^T , JCM 5963 ^T , NCPPB 1964 ^T	28
Isolate VA-1316 ^T		This study
Isolate D-2251		This study
Isolate CY-0619		This study
Isolate CY-0627		This study
Isolate VU-0563		This study

^a Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; ICPB, International Collection of Phytopathogenic Bacteria, University of California, Davis; IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, The Institute of Physical and Chemical Research, Wako-shi, Saitama, Japan; NCIB, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, United Kingdom; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom; NCTC, National Collection of Type Cultures, London, United Kingdom; NIAES, National Institute of Agro-Environmental Science, Ibaragi, Japan; PDDCC, Plant Disease Division Culture Collection, Auckland, New Zealand.

^b This bacterium was supplied by M. Goto, Faculty of Agriculture, Shizuoka University, Shizuoka, Japan.

bacteria from authentic members of the genus *Pseudomonas* sensu stricto, and propose the transfer of *P. plantarii* and *P. glumae* to the genus *Burkholderia* and the establishment of a new species, *Burkholderia vandii*.

MATERIALS AND METHODS

Bacterial strains. The strains which we studied are shown in Table 1. Names which do not appear on the Approved Lists of Bacterial Names (39) are enclosed in quotation marks. Strains VA-1316^T (T = type strain), D-2251, CY-0619, CY-0627, and VU-0563 were isolated by us as antibiotic-producing bacteria that are active against *C. michiganensis* subsp. *michiganensis*. Strain VA-1316^T was isolated from roots of *Vanda* species, strain D-2251 was isolated from roots of *Dendrobium* species, strains CY-0619 and CY-0627 were isolated from roots of *Cymbidium* species, and strain VU-0563 was isolated from roots of *Vuytstekeara* species. These bacteria were maintained on PYG agar (47).

Morphological, biochemical, and physiological characterization. PYG agar was used as the preculture and basal medium for bacterial strains. Unless indicated otherwise, the strains were cultivated at 30°C. Morphological, biochemical, and physiological characteristics were investigated by using methods described previously (47). Production of indole was determined with Kovács reagent in 1% tryptone broth (catalog no. 0123; Difco Laboratories, Detroit, Mich.). Production of hydrogen sulfide was tested on triple sugar iron agar for 2 weeks. The requirement for vitamins was tested in liquid basal medium B (49), and methanol was replaced with 1% (wt/vol) D-glucose or 1% (wt/vol) D-galactose. Utilization of carbon compounds was determined in liquid basal medium B, and methanol was replaced with other carbon compounds as reported previously (47). In addition, we used the same method to determine the utilization of L-rhamnose, adonitol, glycine, leucine, L-isoleucine, L-valine, L-threonine, ornithine, DL-norleucine, DL-citrulline, sarcosine, *n*-butanol, isobutanol, *n*-propanol, propylene glycol, 2,3-butanediol, monoethanol-

amine, *n*-amylamine, tryptamine, benzylamine, acetamide, suberic acid, levulinic acid, mesaconic acid, L-tartaric acid, D-tartaric acid, itaconic acid, pimelic acid, citraconic acid, caprylic acid, nicotinic acid, sebacic acid, benzoylformic acid, L-mandelic acid, 4-hydroxy-2-quinoline carboxylic acid, *n*-capric acid, glutaric acid, DL-2-aminobutyric acid, *n*-caproic acid, *n*-heptanoic acid, benzoic acid, *p*-aminobenzoic acid, *m*-hydroxybenzoic acid, adipic acid, salicylic acid, *n*-nonanoic acid, azelaic acid, and glycolic acid, except that each of these compounds was tested at a concentration of 0.15% (wt/vol).

Cellular fatty acid and hydroxy fatty acid compositions. Cells cultivated in PYG medium (pH 7.0) at 30°C for 1 day with shaking were used to determine cellular fatty acid and hydroxy fatty acid compositions by methods described previously (48, 50).

Quinone systems and quinone homologs. Cells cultivated in PYG medium (pH 7.0) at 30°C for 2 days with shaking were used to determine the quinone systems as described previously (48, 49).

DNA base composition. DNA was extracted by the method of Saito and Miura (37), and the guanine-plus-cytosine (G+C) content was determined by reversed-phase high-performance liquid chromatography (45).

DNA-DNA hybridization. DNA-DNA hybridization was carried out at 68°C by using the method of Kaneko et al. (21). DNAs of *Burkholderia cepacia* ATCC 25416^T, *Burkholderia gladioli* ATCC 10248^T, *P. glumae* NIAES 1169^T, and new isolate VA-1316^T were labelled with (1',2',5'-³H)dCTP by the nick translation method, using an Amersham kit (catalog no. TRK 700; Amersham International plc, Amersham, United Kingdom). DNA-DNA hybridization experiments were performed with the *Pseudomonas* rRNA group II bacteria, our new isolates, *Acidovorax avenae* subsp. *avenae* ATCC 19860^T, *Acidovorax avenae* subsp. *cattleyae* ATCC 33619^T, and *Pseudomonas aeruginosa* JCM 5962^T.

Resistance to antibiotic compounds. Resistance to antibiotic compounds by *Pseudomonas* strains was tested by using Showa

TABLE 2. Differentiating physiological characteristics of plant-associated bacteria

Strain	Denitrification	Hydrolysis of gelatin	Urease activity	Growth in the presence of 3% (wt/vol) NaCl	Utilization of the following nitrogen sources:		Growth at:		
					Nitrate	Urea	pH 4	pH 8	pH 9
<i>B. cepacia</i> ATCC 25416 ^T	— ^a	—	+	w	w	+	+	+	—
<i>B. cepacia</i> ALQ 8281	—	—	+	+	w	+	+	+	—
<i>B. gladioli</i> ATCC 10248 ^T	—	w	+	—	+	+	+	+	—
<i>B. gladioli</i> ATCC 19302	—	+	+	—	w	w	+	+	—
Isolate D-2251	—	+	+	w	+	+	+	+	—
Isolate CY-0619	w	w	+	—	w	w	+	+	—
Isolate CY-0627	—	+	—	w	w	w	+	+	—
Isolate VU-0563	—	w	—	—	+	+	+	+	—
Isolate VA-1316 ^T	+	+	+	—	—	+	+	w	—
<i>P. plantarii</i> JCM 5492 ^T	+	+	+	—	w	w	+	—	—
<i>B. caryophylli</i> ATCC 25418 ^T	+	—	—	—	—	+	—	—	—
<i>P. glumae</i> NIAES 1169 ^T	+	+	+	+	w	w	—	w	—
<i>B. solanacearum</i> ATCC 11696 ^T	—	—	+	—	—	w	—	w	—
<i>B. pickettii</i> JCM 5969 ^T	+	—	+	—	—	—	—	+	—
<i>Acidovorax avenae</i> subsp. <i>avenae</i> ATCC 19860 ^T	+	—	+	—	—	—	—	+	+
<i>Acidovorax avenae</i> subsp. <i>cattleyae</i> ATCC 33619 ^T	—	—	+	—	—	—	—	+	w

^a +, positive; —, negative; w, weakly positive.

disks (diameter, 9 mm; Showa Yakuhin Kakou, Tokyo, Japan). Each disk contained 30 µg of cefotiam hydrochloride, 30 µg of cefmetazole (sodium salt), 30 µg of cefotaxime (sodium salt), 30 µg of cefmetanoxin sulfate, 30 µg of lotamoxysufu (sodium salt), 30 µg of ceftizoxime (sodium salt), 30 µg of aminobenzylpenicillin, 30 µg of gentamicin sulfate, 30 µg of amikacin sulfate, or 200 µg of minocycline hydroxychloride.

Production of antibiotic substances. To detect the production of antibiotic substances, *C. michiganensis* subsp. *michiganensis* and *F. oxysporum* f. sp. *lycopersici* J-3, which are distantly related to each other, were used as indicator organisms. These indicator organisms are known to be distributed

widely and to cause serious plant diseases. The inhibitory activities of the test strains against the growth of *C. michiganensis* were studied by the following method. Each strain tested was streaked onto nutrient agar overlaid with *C. michiganensis*, the preparation was incubated at 30°C, and the size of the growth inhibition zone formed on the agar plate was measured. To test the inhibitory activities of the test strains against the growth of *F. oxysporum*, each strain tested and *F. oxysporum* were streaked separately on PDA agar (Nissui, Tokyo, Japan), the preparation was incubated for 7 days at 25°C, and inhibition of *F. oxysporum* growth by the test strain was measured.

TABLE 3. Oxidation of sugars by plant-associated bacteria

Strain	Oxidation of:													
	L-Arabinose	D-Xylose	D-Glucose	D-Mannose	D-Fructose	Galactose	Maltose	Sucrose	Lactose	Trehalose	D-Sorbitol	D-Mannitol	Inositol	Glycerol
<i>B. cepacia</i> ATCC 25416 ^T	— ^a	—	+	—	—	+	—	+	—	+	—	—	—	—
<i>B. cepacia</i> ALQ 8281	—	—	+	—	—	+	—	+	—	—	—	—	—	—
<i>B. gladioli</i> ATCC 10248 ^T	+	+	+	+	+	+	—	—	—	+	—	—	—	—
<i>B. gladioli</i> ATCC 19302	+	+	+	+	+	+	—	—	—	—	—	—	—	—
Isolate D-2251	+	+	+	+	+	+	—	—	—	—	—	—	—	—
Isolate CY-0619	+	+	+	+	+	+	—	—	—	—	—	—	—	w
Isolate CY-0627	+	+	+	+	+	+	—	—	—	+	—	—	—	—
Isolate VU-0563	+	+	+	+	+	+	—	—	—	—	—	—	—	—
Isolate VA-1316 ^T	—	—	—	—	—	—	—	—	—	—	—	—	w	w
<i>P. plantarii</i> JCM 5492 ^T	+	+	+	+	+	+	—	—	—	—	—	—	—	w
<i>B. caryophylli</i> ATCC 25418 ^T	—	—	—	—	—	—	—	—	—	—	—	—	—	w
<i>P. glumae</i> NIAES 1169 ^T	—	—	—	—	—	—	—	—	—	—	—	—	—	w
<i>B. solanacearum</i> ATCC 11696 ^T	—	—	—	—	—	—	—	—	—	—	—	—	—	w
<i>B. pickettii</i> JCM 5969 ^T	—	—	+	w	—	w	+	—	w	—	—	—	—	—
<i>Acidovorax avenae</i> subsp. <i>avenae</i> ATCC 19860 ^T	—	—	—	—	—	+	—	—	—	—	—	—	—	w
<i>Acidovorax avenae</i> subsp. <i>cattleyae</i> ATCC 33619 ^T	—	w	—	—	—	+	—	—	—	—	—	—	—	w

^a +, positive; —, negative; w, weakly positive.

TABLE 4. Utilization of carbon compounds by plant-associated bacteria

Strain	Utilization of:															
	L-Arabinose	D-Xylose	D-Glucose	D-Mannose	D-Galactose	Sucrose	Lactose	Trehalose	D-Sorbitol	D-Mannitol	Inositol	Glycerol	L-Rhamnose	Adonitol	Glycine	L-Isoleucine
<i>B. cepacia</i> ATCC 25416 ^T	+	+	+	+	+	+	w	+	+	+	+	+	—	+	w	+
<i>B. cepacia</i> ALQ 8281	+	+	+	+	+	+	—	+	+	+	+	+	—	+	—	+
<i>B. gladioli</i> ATCC 10248 ^T	+	+	+	+	+	—	w	+	+	+	+	+	—	+	—	+
<i>B. gladioli</i> ATCC 19302	+	+	+	+	+	—	w	+	+	+	+	+	—	+	+	+
Isolate D-2251	+	+	+	+	+	—	w	+	+	+	+	+	—	+	w	+
Isolate CY-0619	+	+	+	+	+	—	—	+	+	+	+	+	—	+	w	+
Isolate CY-0627	+	+	+	+	+	—	—	+	+	+	+	+	—	+	—	+
Isolate VU-0563	+	+	+	+	+	—	w	+	+	+	+	+	—	+	—	+
Isolate VA-1316 ^T	+	+	+	+	+	—	w	+	+	+	+	+	—	—	—	+
<i>P. plantarii</i> JCM 5492 ^T	+	+	+	+	+	w	—	—	+	+	+	+	w	—	—	w
<i>B. caryophylli</i> ATCC 25418 ^T	+	+	+	+	+	+	—	+	+	+	+	+	—	—	—	w
<i>P. glumae</i> NIAES 1169 ^T	+	+	+	+	+	—	—	+	+	+	+	w	—	+	—	w
<i>B. solanacearum</i> ATCC 11696 ^T	—	—	+	—	—	+	—	—	—	—	+	+	—	—	—	+
<i>B. pickettii</i> JCM 5969 ^T	+	+	+	—	+	—	—	—	—	—	—	+	—	—	w	+
<i>Acidovorax avenae</i> subsp. <i>avenae</i> ATCC 19860 ^T	+	—	—	—	+	—	—	—	+	+	—	+	—	—	—	—
<i>Acidovorax avenae</i> subsp. <i>cattleyae</i> ATCC 33619 ^T	+	—	—	—	+	—	—	—	+	+	—	+	—	—	—	+

^a +, positive; —, negative; w, weakly positive.

Tolerance to fusaric acid. Most strains tested inhibited the growth of *F. oxysporum* f. sp. *lycopersici* J-3. Meanwhile, *F. oxysporum* produced fusaric acid, which inhibits the growth of microorganisms (22). Therefore, the tolerance of the test strains to fusaric acid was investigated. Fusaric acid was added to PYG broth at a concentration of 0.01 to 1.0% (wt/vol).

RESULTS

Phenotypic characteristics of the test strains. All strains were gram negative, non-spore-forming, rod-shaped organisms whose cells were 0.5 to 1.0 µm wide by 1.5 to 3.0 µm long and had rounded ends. The cells occurred singly, rarely in pairs, and were motile by means of one or several polar flagella. Colonies were white to light yellow. Abundant growth was observed in nutrient broth, PYG broth, and peptone water. No water-soluble fluorescent pigment was produced on King B medium. Only *P. glumae* produced a fluorescent pigment on potato agar. Granules of poly-β-hydroxybutyrate accumulated in the cells. Nitrate was reduced to nitrite by all strains. The methyl red test and the Voges-Proskauer reaction were negative. Production of indole in 1% tryptone broth (Difco) and production of hydrogen sulfide in triple sugar iron medium were not observed. Hydrolysis of starch was not observed. Ammonia was produced in peptone water. Litmus milk was not changed. Catalase and oxidase were produced. All strains produced acid from sugars oxidatively. However, the kinds of sugars that produced acid were not identical to the kinds of sugars that produced acid in previous studies (15, 28, 60) for reasons which are not clear. The acid formation patterns of *B. gladioli*, *P. plantarii*, and four new isolates (but not isolate VA-1316^T) resembled each other. All strains utilized D-fruc-

tose, L-leucine, succinic acid, acetic acid, sebacic acid, and adipic acid, but did not utilize maltose, soluble starch, methanol, monomethylamine, dimethylamine, trimethylamine, *N,N*-dimethylformamide, methane, hydrogen, and formic acid. Growth factors were not required as essential supplements. All strains utilized ammonia and peptone as sole nitrogen sources. All strains grew at pH 5.0 to 7.5, but did not grow at pH values below 3.0 or above 9.5. All strains grew at 30 and 37°C but not at 42°C. Biochemical and physiological characteristics that differentiate strains are shown in Tables 2 to 4. The rRNA group II organisms *B. cepacia*, *B. gladioli*, *P. plantarii*, *Burkholderia caryophylli*, *P. glumae*, *Burkholderia solanacearum*, and *Burkholderia pickettii* and *Acidovorax avenae* strains were distinguished from each other on the basis of biochemical and physiological characteristics (Tables 2 to 4). New isolates D-2251, CY-0619, CY-0627, and CY-0563 were identical to *B. gladioli* on the basis of phenotypic characteristics, but new isolate VA-1316^T differed from the other strains.

Cellular fatty acid compositions. The cellular fatty acids of all of the strains tested consisted of large amounts of *n*-C_{16:0}, *n*-C_{16:1}, and *n*-C_{18:1} acids (Table 5).

Hydroxy fatty acid compositions. All of the *Pseudomonas* rRNA group II bacteria except *B. solanacearum* and *B. pickettii* and the new isolates contained large amounts of 3-hydroxy C_{14:0}, 3-hydroxy C_{16:0}, and 2-hydroxy C_{16:0} acids as their hydroxy fatty acid components. *B. solanacearum* contained large amounts of 3-hydroxy C_{14:0} and 2-hydroxy C_{16:0} acids. *B. pickettii* contained large amounts of 3-hydroxy C_{14:0}, 2-hydroxy C_{14:0}, and 2-hydroxy C_{16:0} acids. In contrast, *Acidovorax avenae* strains contained a large amount of 3-hydroxy C_{10:0} acid, and the *Pseudomonas* rRNA group I bacteria (*P. aeruginosa* and *P.*

TABLE 4—Continued

Utilization of:																							
L-Threonine	Ornithine	D,L-Norleucine	D,L-Citrulline	Sarcosine	Ethanol	n-Butanol	Isobutanol	n-Propanol	Propylene glycol	2,3-Butanediol	Mono-ethanolamine	n-Amylamine	Tryptamine	Benzylamine	Acetoamide	Citric acid	Suberic acid	Levulinic acid	Mesaconic acid	L-Tartaric acid	D-Tartaric acid	Itaconic acid	Pimelic acid
+	+	+	w	w	+	+	w	+	w	+	+	+	+	+	+	w	+	+	-	w	w	-	+
+	+	w	w	w	w	w	w	-	w	+	+	+	+	w	+	w	w	w	-	w	w	-	w
+	+	+	+	+	w	+	-	-	+	-	w	-	-	-	-	w	w	w	+	w	w	-	w
+	+	w	+	w	w	+	+	+	+	+	w	-	+	+	+	w	w	w	+	w	w	+	w
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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fluorescens) contained 3-hydroxy C_{10:0}, 3-hydroxy C_{12:0}, and 2-hydroxy C_{12:0} acids (Table 6).

Quinone systems and quinone homologs. *Pseudomonas* rRNA group II and III bacteria and all of the new isolates contained a large amount of ubiquinone Q-8. In contrast, the *Pseudomonas* rRNA group I organisms *P. aeruginosa* and *P. fluorescens* contained large amounts of ubiquinone Q-9 (Table 6). These results are consistent with the data of Yamada et al. (61) and Oyaizu and Komagata (27).

DNA base compositions. The DNA base compositions of all of the *Pseudomonas* rRNA group II bacteria, our new isolates, and *Acidovorax avenae* strains ranged from 64 to 70 mol% G+C (Table 6). The G+C contents of *P. aeruginosa* and *P. fluorescens* were 65.9 and 60.2 mol%, respectively.

DNA-DNA hybridization. The levels of DNA-DNA similarity among *Pseudomonas* rRNA group II bacteria, our new isolates, *Acidovorax avenae* strains, and *P. aeruginosa* are shown in Table 7. The *Pseudomonas* rRNA group II bacteria and our new isolates were clearly distinguished from *Acidovorax avenae* strains and *P. aeruginosa*. Our new isolates were clearly divided into two groups. Four of our new isolates (D-2251, CY-0619, CY-0627, and VU-0563) exhibited high similarity values (68 to 82%) with *B. gladioli*, but new isolate VA-1316^T exhibited low similarity values (17 to 57%) with *Pseudomonas* rRNA group II bacteria. *B. solanacearum* and *B. pickettii* exhibited less than 20% similarity with other *Pseudomonas* rRNA group II bacteria and our new isolates.

Resistance to antibiotic compounds. All of the strains except *P. aeruginosa* and *P. fluorescens* strains and our new isolates were susceptible to cefotaxime (sodium salt), cefmetanoxin sulfate, ceftizoxime (sodium salt), gentamicin sulfate, amikacin sulfate, and minocycline hydrochloride. *B. gladioli*, *P. plantarii*, *P. glumae*, *B. solanacearum*, *Acidovorax avenae* subsp.

cattleyae, and all of our new isolates were susceptible to all of the antibiotic compounds tested. *B. cepacia* strains were resistant to cefotiam hydrochloride and aminobenzylpenicillin, *B. caryophylli* was resistant to cefotiam hydrochloride, cefmetazole (sodium salt), lotamoxysulfu (sodium salt), *B. pickettii* was resistant to cefotiam hydrochloride, and *Acidovorax avenae* subsp. *avenae* was resistant to aminobenzylpenicillin.

Antibiotic activity against plant-pathogenic microorganisms. Some saprophytic *Pseudomonas* strains are known to produce antibiotics, pyrrolnitrin (produced by *B. cepacia*) and/or pyoluteorin (18, 25, 26, 51). Most *Pseudomonas* rRNA group II strains and all of our new isolates inhibited the growth of the plant-pathogenic microorganisms *C. michiganensis* subsp. *michiganensis* and *F. oxysporum* f. sp. *lycopersici* J-3 (Table 8). Figure 1 shows the growth inhibition zone formed by new isolate VA-1316^T on a lawn of *C. michiganensis* subsp. *michiganensis*. However, *Acidovorax avenae* strains did not inhibit the growth of plant-pathogenic microorganisms.

Tolerance to fusaric acid. All *Pseudomonas* rRNA group II strains and our new isolates grew in medium containing 0.01% (wt/vol) fusaric acid, but *Acidovorax avenae* strains did not grow in this medium (Table 8). All of the *Pseudomonas* rRNA group II bacteria except *B. solanacearum* and *B. pickettii* and all of our new isolates grew in medium containing 0.05% (wt/vol) fusaric acid.

DISCUSSION

Pseudomonas rRNA group II strains, *B. cepacia*, *B. gladioli*, *P. plantarii*, *B. caryophylli*, *P. glumae*, *B. solanacearum*, *B. pickettii*, and our new isolates are plant-associated bacteria that have the same morphological characteristics. However, the results of our study of utilization of carbon compounds (D-

TABLE 4—Continued

Strain	Utilization of:																	
	Citraconic acid	Caprylic acid	Nicotinic acid	Benzoylformic acid	L-Mandelic acid	4-Hydroxy-2-quinoline carboxylic acid	n-Capric acid	Glutaric acid	DL-2-Aminobutyric acid	n-Caproic acid	n-Heptanoic acid	Benzoic acid	p-Aminobenzoic acid	m-Hydroxybenzoic acid	Salicylic acid	n-Nonanoic acid	Azelatic acid	Glycolic acid
<i>B. cepacia</i> ATCC 25416 ^T	+	+	—	+	+	+	+	+	+	+	+	+	+	w	+	+	+	+
<i>B. cepacia</i> ALQ 8281	+	+	—	+	+	+	+	+	w	+	+	+	+	w	+	+	+	+
<i>B. gladioli</i> ATCC 10248 ^T	—	+	+	—	—	+	+	—	—	+	+	+	+	+	—	+	+	—
<i>B. gladioli</i> ATCC 19302	+	+	+	—	—	+	+	—	w	+	+	+	+	+	—	+	+	—
Isolate D-2251	—	+	+	—	—	+	+	—	+	+	+	+	+	—	—	+	+	—
Isolate CY-0619	+	+	+	—	—	+	+	—	+	+	+	+	+	—	—	+	+	—
Isolate CY-0627	+	+	+	—	—	+	+	—	+	+	—	+	+	—	—	+	+	—
Isolate VU-0563	+	+	+	—	—	+	+	—	+	+	+	+	+	—	—	+	+	—
Isolate VA-1316 ^T	+	+	+	—	—	+	+	—	—	—	+	—	—	—	—	+	—	—
<i>P. plantarii</i> JCM 5492 ^T	w	w	+	—	—	w	w	—	—	w	+	—	—	—	—	+	w	—
<i>B. caryophylli</i> ATCC 25418 ^T	—	—	—	—	—	—	—	w	—	—	—	w	—	—	—	—	w	+
<i>P. glumae</i> NIAES 1169 ^T	—	+	w	—	—	—	w	—	—	w	+	—	w	—	+	—	+	—
<i>B. solanacearum</i> ATCC 11696 ^T	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—	—	+	—
<i>B. pickettii</i> JCM 5969 ^T	—	—	—	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Acidovorax avenae</i> subsp. <i>avenae</i> ATCC 19860 ^T	—	—	—	—	—	—	—	+	w	w	—	—	—	—	—	—	+	—
<i>Acidovorax avenae</i> subsp. <i>cattleiae</i> ATCC 33619 ^T	w	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	+	—

xylose, L-rhamnose, and L-valine for *B. cepacia*; sucrose and glycine for *B. gladioli*; L-rhamnose, trehalose, L-valine, and sebacic acid for *B. caryophylli*; L-leucine for *B. solanacearum*; and L-leucine for *B. pickettii*) were different from those described in *Bergey's Manual of Systematic Bacteriology* (28).

Furthermore, the data for acid formation from sugars obtained in this study were different from the data obtained by Yabuuchi et al. (60). The reasons for these unexpected results are not apparent. These bacteria have the same major cellular fatty acid and ubiquinone system components. Galbraith and

TABLE 5. Cellular fatty acid compositions of plant-associated bacteria

Strain	% of total acids																	
	Straight-chain acids									Cyclopropane acids		3-Hydroxy acids				2-Hydroxy acids		
	C _{12:0}	C _{14:0}	C _{15:0}	C _{16:0}	C _{16:1}	C _{17:0}	C _{18:0}	C _{18:1}	C _{19:0}	C _{17:0}	C _{19:0}	3-OH C _{10:0}	3-OH C _{12:0}	3-OH C _{14:0}	3-OH C _{16:0}	2-OH C _{12:0}	2-OH C _{14:0}	2-OH C _{16:0}
<i>B. cepacia</i> ATCC 25416 ^T		3.6	0.1	25.4	22.9	0.1	0.5	41.6		3.1	1.0			0.4	0.3			1.0
<i>B. cepacia</i> ALQ 8281	0.1	3.5	0.1	23.4	25.2	0.1	1.0	41.0		2.3	0.6			1.2	0.5			1.0
<i>B. gladioli</i> ATCC 10248 ^T		3.6		26.6	22.4	0.1	1.2	39.1		3.0	0.9			1.6	0.5			1.0
<i>B. gladioli</i> ATCC 19302		3.8		28.0	24.9		0.7	37.8		2.9	0.7			0.4	0.1			0.7
Isolate D-2251		3.9	0.1	27.8	22.4	0.2	1.2	33.4	1.6	2.3	0.6			3.8	1.3			1.4
Isolate CY-0619		3.7	0.2	26.4	22.6	0.3	1.1	39.3		1.7	0.4			2.7	0.8			0.8
Isolate CY-0627		3.8	0.2	27.7	22.9	0.3	1.2	36.3		2.4	0.5			2.8	0.9			1.0
Isolate VU-0563		4.0	0.1	26.7	21.7	0.2	1.3	38.6		2.1	0.6			2.5	0.7			1.5
Isolate VA-1316 ^T		4.1		27.8	19.7	0.1	1.3	34.7	0.6	4.7	0.7			3.4	2.1			0.8
<i>P. plantarii</i> JCM 5492 ^T		4.2	0.1	25.9	21.2	0.6	1.0	29.0	0.8	3.8	3.6			2.5	0.1			2.0
<i>B. caryophylli</i> ATCC 25418 ^T		3.1	0.4	22.0	15.0	0.6	1.2	48.1		1.7	0.5			2.5	0.9			4.0
<i>P. glumae</i> NIAES 1169 ^T		4.6	0.2	28.1	22.6	0.4	0.9	33.4	0.3	2.3	1.3			4.5	0.9			0.6
<i>B. solanacearum</i> ATCC 11696 ^T		4.2	1.0	25.5	28.4	1.9	0.9	30.4	0.3	5.8	0.2			0.4				1.0
<i>B. pickettii</i> JCM 5969 ^T		3.3	0.6	27.2	31.0	2.0	0.9	33.0		1.3				0.3		0.2		0.2
<i>Acidovorax avenae</i> subsp. <i>avenae</i> ATCC 19860 ^T	2.4	2.1	0.5	33.7	45.4	0.2	0.3	13.8		0.7		0.9						
<i>Acidovorax avenae</i> subsp. <i>cattleiae</i> ATCC 33619 ^T	2.0	1.3	0.9	33.4	42.4	0.6	0.3	14.4		4.0		0.7						
<i>P. aeruginosa</i> JCM 5962 ^T		1.1		23.2	19.6		0.3	46.8		0.5	0.3	2.3	0.6			5.3		
<i>P. fluorescens</i> NCIB 9046 ^T		1.6		30.8	35.2		1.5	22.0		0.1	0.2	3.0	1.1			4.5		

TABLE 6. DNA base compositions, ubiquinone homologs, and hydroxy fatty acid compositions of plant-associated bacteria

Strain	G+C content (mol%)	% of total ubiquinones				% of total 3-hydroxy fatty acids				% of total 2-hydroxy fatty acids		
		Q-7	Q-8	Q-9	Q-10	3-OH C _{10:0}	3-OH C _{12:0}	3-OH C _{14:0}	3-OH C _{16:0}	2-OH C _{12:0}	2-OH C _{14:0}	2-OH C _{16:0}
<i>B. cepacia</i> ATCC 25416 ^T	67.1	0.9	98.2	0.9				72.8	27.3			100
<i>B. cepacia</i> ALQ 8281		2.7	96.6	0.7				72.7	27.3			100
<i>B. gladioli</i> ATCC 10248 ^T	67.9	0.9	98.2	0.9				77.1	22.9			100
<i>B. gladioli</i> ATCC 19302	68.3	0.9	98.2	0.9				73.4	26.6			100
Isolate D-2251	69.4	0.6	98.9	0.5				74.8	25.2			100
Isolate CY-0619	68.8	0.5	98.8	0.7				77.5	22.5			100
Isolate CY-0627	68.5	0.3	98.9	0.8				76.4	23.6			100
Isolate VU-0563	69.5	0.4	98.6	1.0				77.5	22.5			100
Isolate VA-1316 ^T	68.5	1.6	97.9	0.7				61.6	38.4			100
<i>P. plantarii</i> JCM 5492 ^T	68.7	3.9	95.7	0.4				64.9	35.1			100
<i>B. caryophylli</i> ATCC 25418 ^T	65.2	2.3	97.6	0.1				74.1	25.9			100
<i>P. glumae</i> NIAES 1169 ^T	68.2	2.0	97.4	0.6			1.3	81.9	16.8			100
<i>B. solanacearum</i> ATCC 11696 ^T	66.6	3.3	96.4	0.3				100				100
<i>B. pickettii</i> JCM 5969 ^T	64.0	1.5	98.1	0.4				97.3	2.7		47.2	52.8
<i>Acidovorax avenae</i> subsp. <i>avenae</i> ATCC 19860 ^T	69.5	1.2	96.0	2.8		100						
<i>Acidovorax avenae</i> subsp. <i>cattleyae</i> ATCC 33619 ^T	69.0	1.1	94.1	4.8		100						
<i>P. aeruginosa</i> JCM 5962 ^T	65.9	0.3	6.2	92.8	0.7	69.9	30.1			100		
<i>P. fluorescens</i> NCIB 9046 ^T	60.2		4.0	92.9	3.1	66.3	33.4			100		

Wilkinson (14) reported that the nonhydroxy fatty acids of *B. caryophylli* and *B. gladioli* consisted of large amounts of *n*-C_{16:0}, *n*-C_{18:1}, and cyclopropane C_{17:0} acids and that *B. pickettii* contained large amounts of *n*-C_{16:0}, *n*-C_{16:1}, *n*-C_{18:1}, and cyclopropane C_{17:0} acids. The differences in our results may have been due to the culture age of the cells used, because a decrease in the amount of *n*-C_{16:1} acid and an increase in the amount of cyclopropane C_{17:0} acid with age have been reported previously (48). On the other hand, Stead (43) and Janse (19) described the cellular fatty acid compositions of *Pseudomonas* species, and their results for nonhydroxy fatty acid composition and 3-hydroxy fatty acid composition were identical to our results. However, their results for 2-hydroxy fatty acid composition were different from our results. The failure to detect 2-OH C_{16:1} and 2-OH C_{18:1} acids in this study may have been due to a difference in the detection limits of these 2-hydroxy fatty acids. These bacteria (*B. cepacia*, *B. gladioli*, *B. plantarii*, *B. caryophylli*, *P. glumae*, and our new

isolates) were identical to each other in physiological characteristics, ubiquinone systems, cellular fatty acid compositions, and hydroxy fatty acid compositions. On the basis of phenotypic and chemotaxonomic characteristics, *P. plantarii* and *P. glumae* should be included in the genus *Burkholderia*. New isolates D-2251, CY-0619, CY-0627, and VU-0563 were identified as *B. gladioli* strains on the basis of physiological characteristics (Tables 2 to 4) and DNA-DNA similarity values (Table 7). On the other hand, the DNA-DNA similarity values obtained for new isolate VA-1316^T, *P. glumae* NIAES 1169^T, and *P. plantarii* JCM 5492^T were approximately 51 to 57%, and these values are at the borderline for the separating species. However, these three strains could be clearly distinguished from each other on the basis of carbon compound utilization patterns. Therefore, we believe that new isolate VA-1316^T should be separated from previously described *Burkholderia* species at the species level, and we propose a new species for this strain. Recently, Li et al. (24) studied the 16S rRNA

TABLE 7. Levels of DNA-DNA homology among plant-associated bacteria

Strain	% DNA-DNA homology with:			
	Strain ATCC 25416 ^T	Strain ATCC 10248 ^T	Strain VA-1316 ^T	Strain NIAES 1169 ^T
<i>B. cepacia</i> ATCC 25416 ^T	100	23	24	23
<i>B. gladioli</i> ATCC 10248 ^T	30	100	45	41
<i>B. gladioli</i> ATCC 19302	26	68	40	37
Isolate D-2251	31	81	46	44
Isolate CY-0619	26	68	40	38
Isolate CY-0627	29	80	45	41
Isolate VU-0563	29	82	44	37
Isolate VA-1316 ^T	30	42	100	55
<i>P. glumae</i> NIAES 1169 ^T	27	37	51	100
<i>P. plantarii</i> JCM 5492 ^T	25	33	57	48
<i>B. caryophylli</i> ATCC 25418 ^T	21	18	23	19
<i>B. solanacearum</i> ATCC 11696 ^T	14	17	17	16
<i>B. pickettii</i> JCM 5969 ^T	15	15	17	14
<i>Acidovorax avenae</i> subsp. <i>avenae</i> ATCC 19860 ^T	8	7	8	6
<i>Acidovorax avenae</i> subsp. <i>cattleyae</i> ATCC 33619 ^T	7	8	8	8
<i>P. aeruginosa</i> JCM 5962 ^T	6	9	6	9

TABLE 8. Production of an antibiotic substance and tolerance to fusaric acid

Strain	Activity against the following indicators:		Tolerance to a fusaric acid concn of:			
	<i>C. michiganensis</i> ^a	<i>F. oxysporum</i> ^b	0.01% (wt/vol)	0.05% (wt/vol)	0.1% (wt/vol)	0.5% (wt/vol)
<i>B. cepacia</i> ATCC 25416 ^T	++	+	+	+	+	—
<i>B. cepacia</i> ALQ 8281	++	+	+	+	+	—
<i>B. gladioli</i> ATCC 10248 ^T	+	+	+	+	+	+
<i>B. gladioli</i> ATCC 19302	—	+	+	+	+	+
Isolate D-2251	+	+	+	+	+	+
Isolate CY-0619	+	+	+	+	+	+
Isolate CY-0627	+	+	+	+	+	+
Isolate VU-0563	++	+	+	+	+	—
Isolate VA-1316 ^T	++	+	+	+	+	—
<i>P. plantarii</i> JCM 5492 ^T	—	+	+	+	+	+
<i>B. caryophylli</i> ATCC 25418 ^T	—	—	+	+	—	—
<i>P. glumae</i> NIAES 1169 ^T	+	+	+	+	+	—
<i>B. solanacearum</i> ATCC 11696 ^T	—	—	+	—	—	—
<i>B. pickettii</i> JCM 5969 ^T	—	—	+	—	—	—
<i>Acidovorax avenae</i> subsp. <i>avenae</i> ATCC 19860 ^T	—	—	—	—	—	—
<i>Acidovorax avenae</i> subsp. <i>cattleyae</i> ATCC 33619 ^T	—	—	—	—	—	—

^a —, activity not detected; +, width of inhibition zone less than 5 mm; ++, width inhibition zone more than 5 mm.

^b —, activity not detected; +, growth inhibition observed.

sequences of the rRNA group II pseudomonads and found evidence which suggested a dichotomy in this group. One subgroup contained *Pseudomonas andropogonis*, *B. caryophylli*, and *B. gladioli*, and the second subgroup contained *B. solanacearum* and *B. pickettii*. Furthermore, Stead (43) reported that *P. solanacearum* could be distinguished from other *Pseudomonas* rRNA group II bacteria on the basis of hydroxy fatty acid composition. These results were supported by the DNA-DNA similarity and hydroxy fatty acid composition data obtained in this study. Therefore, we believe that the genus

Burkholderia should be characterized more precisely in the future.

Acidovorax avenae subsp. *avenae* and *Acidovorax avenae* subsp. *cattleyae* had the same chemotaxonomic characteristics and could be distinguished clearly from the genus *Pseudomonas* sensu stricto (*P. fluorescens* rRNA branch [11]), *Burkholderia* species, *Comamonas* species, and *Hydrogenophaga* species on the basis of phenotypic characteristics (10, 17, 28, 35, 38, 44), ubiquinone systems, DNA base compositions, DNA-DNA homology data, and rRNA-DNA hybridization data (9, 11, 52, 53) (Table 9). Minimal characteristics for differentiating the genera *Burkholderia*, *Acidovorax*, *Comamonas*, *Hydrogenophaga*, and *Pseudomonas* are shown in Table 9. Characteristics which differentiate among *Burkholderia* species are shown in Tables 3 to 7 and are described in *Bergey's Manual of Systematic Bacteriology* (28).

Transfer of *Pseudomonas plantarii* and *Pseudomonas glumae* to the genus *Burkholderia* as *Burkholderia* species. *Pseudomonas plantarii* was transferred previously to the genus *Burkholderia* as *Burkholderia plantarii* (Azegami, Nishiyama, Watanabe, Kadota, Ohuchi, and Fukazawa 1987) comb. nov. This species is described in this study and in reference 3, and the type strain is strain JCM 5492 (= NIAES 1723). *Pseudomonas glumae* has also been transferred to the genus *Burkholderia* as *Burkholderia glumae* (Uematsu, Yoshimura, Nishiyama, Ibaraki, and Fujii 1976) comb. nov. This species is described in this study and in references 28 and 46, and the type strain is NIAES 1169 (= ATCC 33617 = NCPPB 2981).

Description of *Burkholderia vandii* Urakami, Ito-Yoshida, Araki, Kijima, Suzuki, and Komagata sp. nov. *Burkholderia vandii* (vand' i. i. M.L. adj. *vandii*, coming from Hindi *Vanda*.) Non-spore-forming, gram-negative, rod-shaped organisms. The cells are 0.5 to 1.0 by 1.5 to 3.0 µm and have rounded ends. The cells occur singly, rarely in pairs, and are motile by means of one or several polar flagella. Granules of poly-β-hydroxybutyrate accumulate in the cells. Colonies are white to light yellow. No water-soluble fluorescent pigment is produced. Abundant growth occurs in nutrient broth, PYG broth, and peptone water. Nitrate is reduced to nitrite. The methyl red

FIG. 1. Growth inhibition zone formed by isolate VA-1316^T on a lawn of *C. michiganensis* subsp. *michiganensis*.

TABLE 9. Characteristics that differentiate the genera of plant-associated bacteria from *Pseudomonas* strains

Genus	Typical species	Quinone system	Major cellular fatty acids	Major 3-hydroxy acid(s)	Major 2-hydroxy acid	G+C content (mol%)	rRNA group ^a	16S rRNA sequence ^b
<i>Burkholderia</i>	<i>B. cepacia</i>	Q-8	C _{16:0} , C _{16:1} , C _{18:1}	3-OH C _{14:0} (3-OH C _{16:0})	2-OH C _{16:0}	67-68	III-2	β-2
<i>Acidovorax</i>	<i>A. avenae</i>	Q-8	C _{16:0} , C _{16:1} (C _{18:1})	3-OH C _{10:0}	ND ^c	70-71	III-1	
<i>Comamonas</i>	<i>C. acidovorans</i> ^d	Q-8	C _{16:0} , C _{16:1} , C _{18:1}	3-OH C _{10:0}	2-OH C _{16:0}	66-67	III-1	β-1
<i>Hydrogenophaga</i>	<i>H. flava</i> ^e	Q-8	C _{16:0} , C _{16:1} , C _{18:1}	3-OH C _{10:0}	ND	66-67	III-1	β-1
<i>Pseudomonas</i>	<i>P. aeruginosa</i>	Q-9	C _{16:0} , C _{16:1} , C _{18:1}	3-OH C _{10:0} (3-OH C _{12:0})	2-OH C _{12:0}	65-66	II-1	γ-3

^a Data from studies of De Vos et al. (9, 11) and Willems et al. (52-55). Group II-1, *P. fluorescens* rRNA branch in rRNA superfamily II; group III-1, *P. acidovorans* rRNA complex in rRNA superfamily III; group III-2, *P. solanacearum* rRNA complex in rRNA superfamily III.

^b Data from references 56 through 59.

^c ND, not detected.

^d Data from reference 44.

^e Data from references 27 and 52.

test and the Voges-Proskauer reaction are negative. Indole and hydrogen sulfide are not produced. Starch is not hydrolyzed. Ammonia is produced. Litmus milk is not changed. Positive for denitrification and hydrolysis of gelatin. Acids are weakly produced from inositol and glycerol oxidatively, but are not produced from L-arabinose, D-xylose, D-glucose, D-mannose, D-fructose, D-galactose, maltose, sucrose, lactose, trehalose, D-sorbitol, D-mannitol, and soluble starch. Acid is not produced from sugars fermentatively. L-Arabinose, D-xylose, D-glucose, D-fructose, D-mannose, D-galactose, lactose, trehalose, D-sorbitol, D-mannitol, inositol, glycerol, L-leucine, L-isoleucine, L-threonine, ornithine, sarcosine, ethanol, *n*-propanol, propylene glycol, succinic acid, acetic acid, sebacic acid, adipic acid, mesaconic acid, D-tartaric acid, citraconic acid, caprylic acid, nicotinic acid, 4-hydroxy-2-quinoline carboxylic acid, *n*-capric acid, *n*-heptanoic acid, and *n*-nonanoic acid are utilized, but sucrose, L-rhamnose, maltose, soluble starch, adonitol, glycine, DL-norleucine, DL-citrulline, methanol, *n*-butanol, isobutanol, 2,3-butanediol, monoethanolamine, *n*-amylamine, tryptamine, benzylamine, acetamide, formic acid, citric acid, suberic acid, levulinic acid, L-tartaric acid, itaconic acid, pimelic acid, benzoylformic acid, L-mandelic acid, glutaric acid, DL-2-aminobutyric acid, *n*-caproic acid, benzoic acid, *p*-amino-benzoic acid, *m*-hydroxybenzoic acid, salicylic acid, azelaic acid, glycolic acid, monomethylamine, dimethylamine, trimethylamine, *N,N*-dimethylformamide, methane, and hydrogen are not utilized. Growth factors are not required. Ammonia, urea, and peptone are utilized as nitrogen sources, but nitrate is not utilized. Oxidase, catalase, and urease are produced. Aerobic. Metabolism is strictly respiratory and not fermentative. Good growth occurs between pH 5.0 and 7.5, and weak growth occurs at pH 4.0 and 8.0. Growth does not occur at pH values above 9.0 and below 3.0. Good growth occurs at 30 and 37°C, but does not occur at 42°C. Growth does not occur in the presence of 3% sodium chloride. Susceptible to cefotaxime, cefmetaxim, ceftizoxime, gentamicin, amikacin, minocycline, cefotiam, cefmetazole, lotamoxysu, and aminobenzylpenicillin. Growth occurs in the presence of 0.1% (wt/vol) fusaric acid. Growing cells inhibit the growth of the phytopathogenic microorganisms *C. michiganensis* and *F. oxysporum*. The cellular fatty acids include large amounts of straight-chain saturated *n*-C_{16:0} acid and unsaturated *n*-C_{16:1} and C_{18:1} acids. The hydroxy acids include large amounts of 3-OH C_{14:0} and 2-OH C_{16:0} acids. The ubiquinone system is Q-8.

The type strain is strain VA-1316. Strain VA-1316^T has all of the characteristics described above for the species. The DNA base composition is 68.5 mol% G+C. This strain was isolated from *Vanda* species by Urakami et al. as a antibiotic-producing bacterium active against *C. michiganensis* subsp. *michiganensis*.

The type strain has been deposited in the Japan Collection of Microorganisms, Institute of Physical and Chemical Research, under accession number JCM 7957.

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REFERENCES

- Arie, T., S. Namba, S. Yamashita, Y. Doi, and T. Kijima. 1987. Biological control of *Fusarium* wilt of bottle gourd by mix-cropping with welsh onion or Chinese chive inoculated with *Pseudomonas gladioli*. Ann. Phytopathol. Soc. Jpn. 53:531-539.
- Ark, P. A., and H. E. Thomas. 1946. Bacterial leaf spot and bud rot of orchids caused by *Phytophthora cactleyae*. Phytopathology 36: 695-698.
- Azegami, K., K. Nishiyama, Y. Watanabe, I. Kadota, A. Ohuchi, and C. Fukazawa. 1987. *Pseudomonas plantarii* sp. nov., the causal agent of rice seedling blight. Int. J. Syst. Bacteriol. 37:144-152.
- Ballard, R. W., N. J. Palleroni, M. Doudoroff, R. Y. Stanier, and M. Mandel. 1970. Taxonomy of the aerobic pseudomonads: *Pseudomonas cepacia*, *P. marginata*, *P. allicola*, and *P. caryophylli*. J. Gen. Microbiol. 60:199-214.
- Buddenhagen, I. W., and A. Kelman. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 2:203-230.
- Byng, G. S., R. J. Whitaker, R. L. Gherna, and R. A. Jensen. 1980. Variable enzymological patterning in tyrosine biosynthesis as a means of determining natural relatedness among the *Pseudomonadaceae*. J. Bacteriol. 144:247-257.
- Dams, E., A. Vandenberghe, and R. D. Wachter. 1983. Sequences of the 5S rRNAs of *Azotobacter vinelandii*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* with some notes on 5S RNA secondary structure. Nucleic Acids Res. 11:1245-1252.
- De Vos, P., and J. De Ley. 1983. Intra- and intergeneric similarities of *Pseudomonas* and *Xanthomonas* ribosomal ribonucleic acid cistrons. Int. J. Syst. Bacteriol. 33:487-509.
- De Vos, P., M. Goor, M. Gillis, and J. De Ley. 1985. Ribosomal ribonucleic acid cistron similarities of phytopathogenic *Pseudomonas* species. Int. J. Syst. Bacteriol. 35:169-184.
- De Vos, P., K. Kersters, E. Falsen, B. Pot, M. Gillis, P. Segers, and J. De Ley. 1985. *Comamonas* Davis and Park 1962 gen. nov., nom. rev. emend., and *Comamonas terrigena* Hugh 1962 sp. nov., nom. rev. Int. J. Syst. Bacteriol. 35:443-453.
- De Vos, P., A. Van Landschoot, P. Segers, R. Tytgat, M. Gillis, M. Bauwens, R. Rossau, M. Goor, B. Pot, K. Kersters, P. Lizzaraga, and J. De Ley. 1989. Genotypic relationships and taxonomic localization of unclassified *Pseudomonas* and *Pseudomonas*-like strains by deoxyribonucleic acid-ribosomal ribonucleic acid hybridizations. Int. J. Syst. Bacteriol. 39:35-49.
- Erdmann, V. A., and J. Wolters. 1986. Collection of published 5S, 5.8S and 4.5S ribosomal RNA sequences. Nucleic Acids Res. 14:r1-r59.

13. Ezaki, T., Y. Hashimoto, and E. Yabuuchi. 1989. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.* **39**:224–229.
14. Galbraith, L., and S. G. Wilkinson. 1991. Polar lipids and fatty acids of *Pseudomonas caryophylli*, *Pseudomonas gladioli* and *Pseudomonas pickettii*. *J. Gen. Microbiol.* **137**:197–202.
15. Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. *J. Appl. Bacteriol.* **27**:265–277.
16. Hildebrand, D. C., N. J. Palleroni, and M. Doudoroff. 1973. Synonymy of *Pseudomonas gladioli* Severini 1913 and *Pseudomonas marginata* (McCulloch 1921) Stapp 1928. *Int. J. Syst. Bacteriol.* **23**:433–437.
17. Hu, F.-P., J. M. Young, and C. M. Triggs. 1991. Numerical analysis and determinative tests for nonfluorescent plant-pathogenic *Pseudomonas* spp. and genomic analysis and reclassification of species related to *Pseudomonas avenae* Manns 1909. *Int. J. Syst. Bacteriol.* **41**:516–525.
18. Imanaka, H., M. Kousaka, G. Tamura, and K. Arima. 1965. Studies on pyrrolnitrin, a new antibiotic. II. *J. Antibiot. Ser. A* **18**:205–206.
19. Janse, J. D. 1991. Infra- and intraspecific classification of *Pseudomonas solanacearum* strains, using whole cell fatty acid analysis. *Syst. Appl. Microbiol.* **14**:335–345.
20. Johnson, J. L., and N. J. Palleroni. 1989. Deoxyribonucleic acid similarities among *Pseudomonas* species. *Int. J. Syst. Bacteriol.* **39**:230–235.
21. Kaneko, T., R. Nozaki, and K. Aizawa. 1978. Deoxyribonucleic acid relatedness between *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*. *Microbiol. Immunol.* **22**:639–641.
22. Kern, H. 1972. Phytotoxins produced by fusaria, p. 35–48. In *Phytotoxins in plant diseases*. Academic Press, New York.
23. King, A., B. Holmes, I. Phillips, and S. P. Lapage. 1979. A taxonomic study of clinical isolates of *Pseudomonas pickettii*, '*P. thomasi*' and 'group IVd' bacteria. *J. Gen. Microbiol.* **114**:137–147.
24. Li, X., M. Dorsch, D. T. Del, L. I. Sly, E. Stackebrandt, and A. C. Hayward. 1993. Phylogenetic studies of the rRNA group II pseudomonads based on 16S rRNA gene sequences. *J. Appl. Bacteriol.* **74**:324–329.
25. Lindberg, G. D. 1981. An antibiotic lethal to fungi. *Plant Dis.* **65**:680–683.
26. Lindberg, G. D., and J. M. Larkin. 1980. Production of tropolone by *Pseudomonas*. *J. Nat. Prod. (Lloydia)* **43**:592–594.
27. Oyaizu, H., and K. Komagata. 1983. Grouping of *Pseudomonas* species on the basis of cellular fatty acid composition and the quinone system with special reference to the existence of 3-hydroxy fatty acids. *J. Gen. Appl. Microbiol.* **29**:17–40.
28. Palleroni, N. J. 1984. Genus 1. *Pseudomonas* Migula 1894, 237^{AL}. (Nom. cons. Opin. 5, Jud. Comm. 1952, 237), p. 141–199. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
29. Palleroni, N. J., R. W. Ballard, E. Ralston, and M. Doudoroff. 1972. Deoxyribonucleic acid homologies among some *Pseudomonas* species. *J. Bacteriol.* **110**:1–11.
30. Palleroni, N. J., and M. Doudoroff. 1971. Phenotypic characterization and deoxyribonucleic acid homologies of *Pseudomonas solanacearum*. *J. Bacteriol.* **107**:690–696.
31. Palleroni, N. J., and B. Holmes. 1981. *Pseudomonas cepacia* sp. nov., nom. rev. *Int. J. Syst. Bacteriol.* **31**:479–481.
32. Palleroni, N. J., R. Kunisawa, R. Contopoulou, and M. Doudoroff. 1973. Nucleic acid homologies in the genus *Pseudomonas*. *Int. J. Syst. Bacteriol.* **23**:333–339.
33. Pickett, M. J., and J. R. Greenwood. 1980. A study of the Va-1 group of pseudomonads and its relationship to *Pseudomonas pickettii*. *J. Gen. Microbiol.* **120**:439–446.
34. Ralston, E., N. J. Palleroni, and M. Doudoroff. 1973. *Pseudomonas pickettii*, a new species of clinical origin related to *Pseudomonas solanacearum*. *Int. J. Syst. Bacteriol.* **23**:15–19.
35. Ramundo, B. A., and L. E. Claffin. 1990. Demonstration of synonymy between the plant pathogens *Pseudomonas avenae* and *Pseudomonas rubrilineans*. *J. Gen. Microbiol.* **136**:2029–2033.
36. Rosen, H. R. 1922. A bacterial disease of foxtail (*Chaetochloa lutescens*). *Ann. Mo. Bot. Gard.* **9**:333–402.
37. Saito, H., and K. Miura. 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. Biophys. Acta* **72**:619–629.
38. Schaad, N. W., C. I. Kado, and D. R. Sumner. 1975. Synonymy of *Pseudomonas avenae* Manns 1905 and *Pseudomonas alboprecipitans* Rosen 1922. *Int. J. Syst. Bacteriol.* **28**:133–137.
39. Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.). 1980. Approved lists of bacterial names. *Int. J. Syst. Bacteriol.* **30**:225–420.
40. Stackebrandt, E., R. G. E. Murray, and H. G. Truper. 1988. *Proteobacteria* classis nov., a name for the phylogenetic taxon that includes the "purple bacteria and their relatives." *Int. J. Syst. Bacteriol.* **38**:321–325.
41. Stahl, D. A., D. J. Lane, G. J. Olsen, D. J. Heller, T. M. Schmidt, and N. R. Pace. 1987. Phylogenetic analysis of certain sulfide-oxidizing and related morphologically conspicuous bacteria by 5S ribosomal ribonucleic acid sequences. *Int. J. Syst. Bacteriol.* **37**:116–122.
42. Stanier, R. Y., N. J. Palleroni, and M. Doudoroff. 1966. The aerobic pseudomonads: a taxonomic study. *J. Gen. Microbiol.* **43**:159–271.
43. Stead, D. E. 1992. Grouping of plant-pathogenic and some other *Pseudomonas* spp. by using cellular fatty acid profiles. *Int. J. Syst. Bacteriol.* **42**:281–295.
44. Tamaoka, J., D. Ha, and K. Komagata. 1987. Reclassification of *Pseudomonas acidovorans* den Dooren de Jong 1926 and *Pseudomonas testosteroni* Marcus and Talalay 1956 as *Comamonas acidovorans* comb. nov. and *Comamonas testosteroni* comb. nov., with an emended description of the genus *Comamonas*. *Int. J. Syst. Bacteriol.* **37**:52–59.
45. Tamaoka, J., and K. Komagata. 1984. Determination of DNA base composition by reversed high-performance liquid chromatography. *FEMS Microbiol. Lett.* **25**:125–128.
46. Uematsu, T., D. Yoshimura, K. Nishiyama, T. Ibaraki, and H. Fujii. 1976. Pathogenic bacterium causing seedling rot of rice. *Ann. Phytopathol. Soc. Jpn.* **42**:464–471.
47. Urakami, T., H. Araki, H. Oyanagi, K. Suzuki, and K. Komagata. 1990. *Paracoccus aminophilus* sp. nov., and *Paracoccus aminovorans* sp. nov., which utilize *N,N*-dimethylformamide. *Int. J. Syst. Bacteriol.* **40**:287–291.
48. Urakami, T., and K. Komagata. 1979. Cellular fatty acid composition and coenzyme Q system in gram-negative methanol-utilizing bacteria. *J. Gen. Appl. Microbiol.* **25**:343–360.
49. Urakami, T., and K. Komagata. 1986. Occurrence of isoprenoid compounds in gram-negative methanol-, methane-, and methylamine-utilizing bacteria. *J. Gen. Appl. Microbiol.* **32**:317–341.
50. Urakami, T., and K. Komagata. 1987. Cellular fatty acid composition with special reference to the existence of hydroxy fatty acids in gram negative methanol-, methane-, and methylamine-utilizing bacteria. *J. Gen. Appl. Microbiol.* **33**:135–165.
51. Wakimoto, S., K. Hirayae, K. Tsuchiya, Y. Kushima, N. Furuya, and N. Matsuyama. 1986. Production of antibiotics by plant pathogenic *Pseudomonas*. *Ann. Phytopathol. Soc. Jpn.* **52**:835–842.
52. Willems, A., J. Busse, M. Goor, B. Pot, E. Falsen, E. Jantzen, B. Hoste, M. Gillis, K. Kersters, G. Auling, and J. De Ley. 1989. *Hydrogenophaga*, a new genus of hydrogen-oxidizing bacteria that includes *Hydrogenophaga flava* comb. nov. (formerly *Pseudomonas flava*), *Hydrogenophaga palleronii* (formerly *Pseudomonas palleronii*), *Hydrogenophaga pseudoflava* (formerly *Pseudomonas pseudoflava* and "*Pseudomonas carboxydoflava*"), and *Hydrogenophaga taeniospiralis* (formerly *Pseudomonas taeniospiralis*). *Int. J. Syst. Bacteriol.* **39**:319–333.
53. Willems, A., E. Falsen, B. Pot, E. Jantzen, B. Hoste, P. Vandamme, M. Gillis, K. Kersters, and J. De Ley. 1990. *Acidovorax*, a new genus for *Pseudomonas facilis*, *Pseudomonas delafieldii*, EF group 13, EF group 16, and several clinical isolates, with the species *Acidovorax facilis* comb. nov., *Acidovorax delafieldii* comb. nov., and *Acidovorax temperans* sp. nov. *Int. J. Syst. Bacteriol.* **40**:384–398.
54. Willems, A., M. Goor, S. Thielemans, M. Gillis, K. Kersters, and J. De Ley. 1992. Transfer of several phytopathogenic *Pseudomonas* species to *Acidovorax* as *Acidovorax avenae* subsp. *avenae* subsp.

- nov., comb. nov., *Acidovorax avenae* subsp. *citrulli*, *Acidovorax avenae* subsp. *cattleyae*, and *Acidovorax konjaci*. Int. J. Syst. Bacteriol. **42**:107–119.
55. Willems, A., B. Pot, E. Falsen, P. Vandamme, M. Gillis, K. Kersters, and J. De Ley. 1991. Polyphasic taxonomic study of the emended genus *Comamonas*: relationship to *Aquaspirillum aquaticum*, E. Falsen group 10, and other clinical isolates. Int. J. Syst. Bacteriol. **41**:427–444.
56. Woese, C. R. 1987. Bacterial evolution. Microbiol. Rev. **51**:221–271.
57. Woese, C. R., P. Blanz, and C. M. Hahn. 1984. What isn't a pseudomonad: the importance of nomenclature in bacterial classification. Syst. Appl. Microbiol. **5**:179–195.
58. Woese, C. R., W. G. Weisburg, C. M. Hahn, B. J. Paster, L. B. Zablen, B. J. Lewis, T. J. Macke, W. Ludwig, and E. Stackebrandt. 1985. The phylogeny of purple bacteria: the gamma subdivision. Syst. Appl. Microbiol. **6**:25–33.
59. Woese, C. R., W. G. Weisburg, B. J. Paster, C. M. Hahn, R. S. Tanner, N. R. Krieg, H. P. Kooops, H. Harms, and E. Stackebrandt. 1984. The phylogeny of purple bacteria: the beta subdivision. Syst. Appl. Microbiol. **5**:327–336.
60. Yabuuchi, E., Y. Kosako, H. Oyaizu, I. Yano, H. Hotta, Y. Hashimoto, T. Ezaki, and M. Arakawa. 1992. Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. Microbiol. Immunol. **36**:1251–1275.
61. Yamada, Y., H. Takinami-Nakamura, Y. Tahara, H. Oyaizu, and K. Komagata. 1982. The ubiquinone systems in the strains of *Pseudomonas* species. J. Gen. Appl. Microbiol. **28**:7–12.
62. Young, J. M., D. W. Dye, J. F. Bradbury, C. G. Panagopoulos, and C. F. Robbs. 1978. A proposed nomenclature and classification for plant pathogenic bacteria. N. Z. J. Agric. Res. **21**:153–177.