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Polyphasic Taxonomy in the Genus *Burkholderia* Leading to an Emended Description of the Genus and Proposition of *Burkholderia vietnamiensis* sp. nov. for N_2-Fixing Isolates from Rice in Vietnam

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The taxonomic position of nitrogen-fixing strains that were isolated from rhizosphere macerates of rice cultivated in the Bình Thanh region of Vietnam was determined by using polyphasic taxonomy. We determined the phylogenetic relationships of these organisms by performing DNA-rRNA hybridization experiments with a labeled rRNA probe from the type strain of *Burkholderia cepacia*, and we found that they belong to a single rRNA complex. Other members of this rRNA complex were also studied, and the N_2-fixing strains were found to be closely related to *B. cepacia*. In addition, all members of the rRNA complex containing *B. cepacia* were studied by performing auxanographic and DNA-DNA hybridization experiments. Phenotypically and genotypically, the N_2-fixing isolates constitute a single cluster together with two strains of clinical origin. These organisms constitute a new *Burkholderia* species, for which the name *Burkholderia vietnamiensis* is proposed; the type strain of this species is TVV75 (= LMG 10929). All members of this species can fix nitrogen. On the basis of our polyphasic taxonomy results and previously published data we concluded that the genus *Burkholderia* should be restricted to the following species: *B. cepacia* (the type species), *Burkholderia mallei*, *Burkholderia pseudomallei*, *B. vietnamiensis*, *Burkholderia gladioli*, *Burkholderia caryophylli*, *Burkholderia plantarii*, *Burkholderia glumae*, *Burkholderia vandii*, *Burkholderia cocovenenans* comb. nov., and *Burkholderia andropogonis* comb. nov. On the basis of genotypic and phenotypic results [*Alcaligenes*] *eutrophus*, [*Burkholderia*] *solanacearum*, and [*Burkholderia*] *pickettii* belong to two other clusters whose internal structures must be studied further.

On the basis of the results of extensive phenotypic studies and DNA-rRNA and DNA-DNA hybridization experiments performed by Palleroni, Stanier, and their collaborators, the genus *Pseudomonas* was divided into five groups (24, 43, 46, 56). Additional phylogenetic data have shown that these groups are only very remotely related and that each of them contains species belonging to other genera (21–23, 70, 71). In addition to the former pseudomonads that have been shown to belong to the genus *Xanthomonas* and to a smaller group related to *Pseudomonas diminuta* and *Pseudomonas vesicularis* (21–23, 43) classified in the recently described genus *Brevundimonas* (52), three large groups of pseudomonads can be considered. *Pseudomonas* rRNA group I (43) is part of rRNA superfamily II (18) or the gamma subclass of the *Proteobacteria* (55), where it constitutes a separate rRNA complex (18, 21, 22, 68); this group represents the authentic pseudomonads which are grouped with *Pseudomonas aeruginosa*, the type species (43). Although the internal relationships within this group have not been determined yet, it is evident that the genus *Pseudomonas* must be limited to this group and that all other *Pseudomonas* species have been generically misnamed as determined by phylogenetic data. Below the genus names of misnamed taxa are enclosed in brackets. [*Pseudomonas*] rRNA group III (43) is part of the acidovorans rRNA complex in rRNA superfamily III or the beta subclass of the *Proteobacteria*. A detailed polyphasic approach has revealed the finer relationships within the acidovorans rRNA complex, and workers have created a new bacterial family, the *Comamonadaceae* (67), which contains in addition to the genus *Comamonas* several newly described genera, including the genera *Hydrogenophaga*, *Acidovorax*, *Variovorax*, and *Xylophilus*.

[*Pseudomonas*] rRNA group II (43) is also part of rRNA superfamily III, where it constitutes a separate cluster that is only remotely related to the *Comamonadaceae* (18); [*Pseudomonas*] rRNA group II and the *Comamonadaceae* belong to separate large rRNA branches that split off at $T_{m(e)}$ values of about 70°C [$T_{m(e)}$ is the temperature at which 50% of a DNA-rRNA duplex is denatured]. This rRNA cluster contains at least 10 [*Pseudomonas*] species, including several phytopathogenic species, as well as nonphytopathogenic species. These species are [*Pseudomonas*] *cepacia*, [*Pseudomonas*] *solanacearum*, [*Pseudomonas*] *gladioli*, [*Pseudomonas*] *caryophylli*, [*Pseudomonas*] *glumae*, [*Pseudomonas*] *andropogonis*, [*Pseudomonas*] *woodsii*, [*Pseudomonas*] *pickettii*, [*Pseudomonas*] *phenazinium*, and [*Pseudomonas*] *oxalaticus* (18). When rRNA from [*P.*] *solanacearum* NCPPB 325^T (T = type strain) is used to form DNA-rRNA duplexes, these organisms have $T_{m(e)}$ values ranging from 74 to 81.5°C. [*Pseudomonas*] *mallei* and [*Pseudomonas*] *pseudomallei* also belong to this group (43). Four other species ([*Pseudomonas*] *plantarii* [2], [*Pseudomonas*] *mixta* [8], [*Pseudomonas*] *syzygii* [50], and [*Pseudomonas*] *cocovenenans* [74]) have also been described as members of the so-called solanacearum rRNA group (68). Recently, Bowman et al. (9) provided evidence that [*Pseudomonas*] *mixta* belongs to another lineage in the beta subclass, and consequently these

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authors transferred this species to the new genus *Telluria* as *Telluria mixta*. Two generically misnamed *Alcaligenes* species, [*Alcaligenes*] *eutrophus* and "[*Alcaligenes*] *hydrogenophilus*," definitely belong to this rRNA complex (18). It has also been shown that [*P.*] *oxalaticus* is phenotypically and genotypically closely related to [*A.*] *eutrophus* (32). [*Pseudomonas*] *rubrisubalbicans* has also been described as a member of this rRNA group (23), but recently this placement has been refuted since this species constitutes a separate rRNA cluster that splits off at a $T_{m(e)}$ value of 73°C from the solanacearum rRNA branch (18, 25). The broad range of guanine-plus-cytosine (G+C) contents, the relatively broad range of $T_{m(e)}$ values, and the variations in phenotypic characteristics (3) among the species suggest that there is a certain amount of heterogeneity within the solanacearum rRNA complex. An analysis of the sequences of the 16S rRNA genes of representative strains of seven species by Li et al. (38) revealed that there are two lineages that correspond to one genus or possibly two related genera. Yabuuchi et al. (73) transferred seven species that belong to this rRNA complex to the new genus *Burkholderia* on the basis of the results of a polyphasic taxonomic study of the type or reference strains of the following species: *Burkholderia mallei*, *Burkholderia pseudomallei*, *Burkholderia cepacia*, *Burkholderia solanacearum*, *Burkholderia caryophylli*, *Burkholderia gladioli*, and *Burkholderia pickettii*. Later, Urakami et al. (63) also transferred [*P.*] *plantarii* and [*P.*] *glumae* to the genus *Burkholderia* as *Burkholderia plantarii* and *Burkholderia glumae*, respectively, and created a new species, *Burkholderia vandii*, for a strain isolated from the roots of Vanda species.

Recently, Tran Van (62) isolated several N_2-fixing bacterial strains that are associated with roots of rice growing in rice fields in Vietnam. These strains have been further characterized, and it has been shown that they are phenotypically very similar to *B. cepacia*. The aims of this study were to further determine the internal structure of the solanacearum rRNA complex and to determine the relationship between the N_2-fixing isolates and this group (particularly *B. cepacia*). In this study we used a polyphasic approach which included DNA-rRNA hybridization, DNA-DNA hybridization, and auxanographic analysis of as many strains of each species as possible. Our results clearly demonstrated that *B. cepacia*, *B. gladioli*, *B. plantarii*, *B. glumae*, [*P.*] *cocovenenans*, and the N_2-fixing isolates constitute a separate rRNA branch within the rRNA complex. Genotypically, the N_2-fixing isolates are closely related to *B. cepacia*. We propose that the genus *Burkholderia* should be restricted to the members of this rRNA branch plus *B. caryophylli*, *B. mallei*, *B. pseudomallei*, *B. vandii*, and *Burkholderia andropogonis* comb. nov. and that a new species, *Burkholderia vietnamiensis*, should be created for the N_2-fixing taxon. The former [*P.*] *cocovenenans* also belongs in the genus *Burkholderia* as *Burkholderia cocovenenans* comb. nov. [*Alcaligenes*] *eutrophus* and [*P.*] *oxalaticus* constitute another rRNA branch, as do [*Burkholderia*] *solanacearum* and [*Burkholderia*] *pickettii*, and the finer relationships of these two branches have to be determined. These organisms represent at least one separate genus. Below we use the new nomenclature described above.

MATERIALS AND METHODS

Bacterial strains. The strains that we used are listed in Table 1. Most of these strains are culture collection strains; the exceptions are the TVV strains (TVV69, TVV70, TVV71, TVV72, TVV74, TVV75^T, TVV115, TVV116, TVV127, TVV128, TVV131, and TVV135), which were isolated from rhizosphere macerates (roots plus adhering soil) of rice (*Oryza sativa* cv. Huyêt Rồng) that was cultivated on a soil in the Bình Thanh region of Vietnam, which is 10 km northeast of Hô Chi Minh City (62). This soil is an acid sulfate soil (Thionic Fluvisol according to the Food and Agriculture Organization classification) with a silty clay loam texture. N_2-fixing bacteria were enumerated and isolated by using the procedure described previously by Omar et al. (42). *Azospirillum lipoferum* 4B (5) was used as a positive control for nitrogen fixation.

All strains were maintained on nutrient agar (0.1% [wt/vol] beef extract, 0.2% [wt/vol] yeast extract, 0.5% [wt/vol] NaCl, 0.5% [wt/vol] peptone, 2.0% [wt/vol] agar; pH 7.4). The bacteriological purity of each strain was verified by plating and by microscopic examination of living and Gram-stained cells. When two or more colony types (colony types t1, t2, and t3) were found, they were compared by protein sodium dodecyl sulfate-polyacrylamide gel electrophoresis (47) in order to determine whether they were colony morphology variants. If two colony types produced almost identical protein patterns, only one type was included in this study. All strains were grown at 28°C.

Biochemical tests. The following conventional tests were performed with the TVV strains: tests for pigment production on nutrient agar and King B medium (35), growth at temperatures between 20 and 41°C on nutrient agar, growth on PCAT medium (12), oxidase activity (49), and onion pathogenicity (26). The API 2ONE microtube system method (bioMérieux, La Balme-les-Grottes, France) was used as a standardized method to determine nitrate reduction, hydrolysis of gelatin and esculin, glucose fermentation, arginine dihydrolase activity, production of indole, β-galactosidase activity, and urease activity. Transmission electron microscopy studies were performed with strains TVV75^T, TVV69, TVV70, and TVV71 to observe flagella by the methods described by Berge et al. (7).

Carbon substrate assimilation tests. API galleries (API 50CH, API 50AO, and API 50AA; bioMérieux) were used to determine the assimilation of 147 organic compounds as sole carbon sources. The experimental procedure which we used has been described previously (33). Nine strains were tested in duplicate on separate occasions to verify the reproducibility of the method.

Numerical analysis of phenotypic characteristics. The results of auxanographic tests were scored as described previously (33). A total of 147 characteristics were examined, and the results of tests for growth on inulin, L-sorbose, methyl-xyloside, esculin, isophthalate, gluconate, fumarate, L-malate, L-aspartate, and L-glutamate were not included in the numerical analysis because they were negative for all strains. The levels of interstrain similarity (S) were calculated by using a similarity coefficient derived from the Canberra metric coefficient (d_{CANB}) (54) and the following equation: $S = 100 \times (1 - d_{CANB})$. A cluster analysis was performed by using the unweighted average pair group method (54), the Clustan version 2.1 program of Wishart (69), and the Siemens model 7570-C computer of the Akademisch Rekencentrum, Universiteit Gent, Ghent, Belgium.

Nitrogenase assay. The ability of each strain to fix N_2 was estimated by using the acetylene reduction assay and an agar slant under an air atmosphere. Pure cultures of strains were streaked onto solid WAT4C medium slants (7) containing four carbon sources (0.50% [wt/vol] starch, 0.50% [wt/vol] glucose, 0.50% [wt/vol] mannitol, 0.35% [wt/vol] malic acid). Uninoculated tubes were used as negative controls. The tubes were immediately closed with rubber stoppers (Suba-Seal), and acetylene (1%, vol/vol) was injected. Ethylene production was measured after 4 days of incubation at 28°C with a flame ionization gas chromatograph (Varian series 1400 aerograph).

Isolation of high-molecular-weight DNA. For DNA-rRNA hybridization experiments we used high-molecular-weight DNA prepared by the method of Marmur (39). Cells were grown for 2 to 3 days in Roux flasks on a solid medium as described above.

For DNA-DNA hybridization experiments high-molecular-weight DNA was extracted and purified by using the procedure of Brenner et al. (10).

DNA base composition. Mean G+C contents were determined by using the thermal denaturation method (20) and were calculated by using the equation of Marmur and Doty (40), as modified by De Ley (17).

DNA-rRNA hybridization. DNA was purified by CsCl gradient centrifugation and was fixed on cellulose nitrate filters (type SM 11358; Sartorius, Göttingen, Germany) as described previously (19). The amount of filter-fixed DNA was determined chemically (66). ^{3}H-labeled rRNAs from *B. cepacia* LMG 1222^T and [*A.*] *eutrophus* LMG 1199^T were prepared and purified by using the method described by De Ley and De Smedt (19). Cells were labeled in vivo by adding 0.8 mCi of [2,8-^{3}H]adenine and 2.8 mCi of [5,6-^{3}H]uracil (New England Nuclear Research Products, Boston, Mass.) in 150 ml of medium containing 1% (wt/vol) glucose, 1% (wt/vol) yeast extract, 0.1% $(NH_4)_2SO_4$, and 0.025% (wt/vol) KH_2PO_4 (pH 7). The specific activities of the 23S rRNA fractions were 7,000 cpm for *B. cepacia* LMG 1222^T and 6,000 cpm for [*A.*] *eutrophus* LMG 1199^T. Labeled rRNA from [*B.*] *solanacearum* NCPPB 325^T was available from members of our research group (22). Each hybrid was characterized by its $T_{m(e)}$. $\Delta T_{m(e)}$ is the difference between $T_{m(e)}$s of the homologous and the heterologous duplexes.

DNA-DNA hybridization and thermal stability of duplexes. Native DNA was labeled in vitro by nick translation with tritium-labeled nucleotides (Amersham International, Amersham, England). The procedure used for the hybridization experiments (S1 nuclease-trichloroacetic acid method) has been described previously (28). The reassociation temperature was 70°C. DNA-DNA hybridization experiments were performed by using labeled DNAs from the following strains as probes: *B. cepacia* LMG 1222^T and LMG 6964 and *B. vietnamiensis* LMG 6998 and TVV70.

TABLE 1. Strains used

Taxon	Strain[a]	Other designation[a]	Source of isolation	Phenon
New isolates				
Burkholderia vietnamiensis	TVV69	LMG 10926	Rice rhizosphere soil, Bình Thanh, Vietnam	II
Burkholderia vietnamiensis	TVV70	LMG 10927	Rice rhizosphere soil, Bình Thanh, Vietnam	II
Burkholderia vietnamiensis	TVV71	LMG 10928	Rice rhizosphere soil, Bình Thanh, Vietnam	II
Burkholderia vietnamiensis	TVV75^T	LMG 10929^T	Rice rhizosphere soil, Bình Thanh, Vietnam	II
Burkholderia vietnamiensis	TVV116	LMG 10930	Rice rhizosphere soil, Bình Thanh, Vietnam	II
Burkholderia vietnamiensis	TVV128	LMG 10931	Rice rhizosphere soil, Bình Thanh, Vietnam	II
Burkholderia vietnamiensis	TVV135	LMG 11346	Rice rhizosphere soil, Bình Thanh, Vietnam	II
Burkholderia vietnamiensis	TVV72	LMG 11347	Rice rhizosphere soil, Bình Thanh, Vietnam	II
Burkholderia vietnamiensis	TVV115	LMG 11348	Rice rhizosphere soil, Bình Thanh, Vietnam	II
Burkholderia vietnamiensis	TVV74	LMG 11349	Rice rhizosphere soil, Bình Thanh, Vietnam	II
Burkholderia vietnamiensis	TVV127	LMG 11350	Rice rhizosphere soil, Bình Thanh, Vietnam	II
Burkholderia vietnamiensis	TVV131	LMG 10932	Rice rhizosphere soil, Bình Thanh, Vietnam	
Collection strains				
Burkholderia vietnamiensis	LMG 6998	CCUG 7246	Blood	II
Burkholderia vietnamiensis	LMG 6999	CCUG 9631	Neck abscess, child	II
Burkholderia cepacia	LMG 11351	PHP7	Maize rhizosphere	I
Burkholderia cepacia	LMG 1222^T	ATCC 25416^T	*Allium cepa* L.	I
Burkholderia cepacia	LMG 1275	Stanier 382	Forest soil	I
Burkholderia cepacia	LMG 6032	CCUG 787		I
Burkholderia cepacia	LMG 6859	NCIB 9087	Rotting tree trunk	I
Burkholderia cepacia	LMG 6860t1[b]	NCIB 9091t1	Soil	I
Burkholderia cepacia	LMG 6861t1	NCIB 9092t1	Soil	I
Burkholderia cepacia	LMG 6862t1	NCIB 9093t1	Forest soil	I
Burkholderia cepacia	LMG 6863t1	NCIB 9690t1	River Maraval	I
Burkholderia cepacia	LMG 6864	NCIB 9691	Soil enriched with D-mandelate	I
Burkholderia cepacia	LMG 6865	NCIB 10649		I
Burkholderia cepacia	LMG 6887	NCPPB 945		I
Burkholderia cepacia	LMG 6888	NCPPB 946		I
Burkholderia cepacia	LMG 6889t1	NCPPB 1962t1		I
Burkholderia cepacia	LMG 6959	PDDCC 3177	*Allium cepa* L.	I
Burkholderia cepacia	LMG 6960t1	PDDCC 5837t1		I
Burkholderia cepacia	LMG 6961t1	PDDCC 5841t1		I
Burkholderia cepacia	LMG 6962	PDDCC 5952	*Allium cepa* L.	I
Burkholderia cepacia	LMG 6963t1	ATCC 17460t1	Soil	I
Burkholderia cepacia	LMG 6964	PDDCC 5982	*Lycopersicon lycopersicum* (L.) Karst. ex Farw.	I
Burkholderia cepacia	LMG 6980	CNBP 1434	*Allium cepa* L.	I
Burkholderia cepacia	LMG 6981t1	ATCC 25609t1	Bronchial washings	I
Burkholderia cepacia	LMG 6981t2	ATCC 25609t2	Bronchial washings	I
Burkholderia cepacia	LMG 6981t3	ATCC 25609t3	Bronchial washings	I
Burkholderia cepacia	LMG 6986	CCUG 788	Urine	I
Burkholderia cepacia	LMG 6987t1	CCUG 853t1		I
Burkholderia cepacia	LMG 6988	CCUG 1603	Leg wound	I
Burkholderia cepacia	LMG 6989t1	CCUG 2393t1	Blood	I
Burkholderia cepacia	LMG 6990t1	CCUG 2851t1		I
Burkholderia cepacia	LMG 6991t1	CCUG 2856t1	Forest soil	I
Burkholderia cepacia	LMG 6992	CCUG 2857	Soil	I
Burkholderia cepacia	LMG 6993	DSM 50180	Soil	I
Burkholderia cepacia	LMG 6994	CCUG 2859	Forest soil	I
Burkholderia cepacia	LMG 6996	CCUG 3000	Urine	I
Burkholderia cepacia	LMG 6997	CCUG 3461B	Ear	I
Burkholderia cepacia	LMG 7000	CCUG 13348	Blood	I
Burkholderia cepacia	LMG 6995	CCUG 1440	Urine	sep[c]
Burkholderia gladioli pv. alliicola	LMG 2121[d]	NCPPB 947[d]		III
Burkholderia gladioli pv. alliicola	LMG 6877	NCPPB 948		III
Burkholderia gladioli pv. alliicola	LMG 6878	NCPPB 2478	*Allium cepa* L.	III
Burkholderia gladioli pv. alliicola	LMG 6915	NRRL B-828		III
Burkholderia gladioli pv. alliicola	LMG 6954	PDDCC 3410	*Allium cepa* L.	III
Burkholderia gladioli pv. alliicola	LMG 6955	PDDCC 3411	*Allium cepa* L.	III
Burkholderia gladioli pv. alliicola	LMG 6956	PDDCC 3412	*Allium cepa* L.	III
Burkholderia gladioli pv. alliicola	LMG 6957	PDDCC 5838		III
Burkholderia gladioli pv. alliicola	LMG 6958	PDDCC 5840		III
Burkholderia gladioli pv. alliicola	LMG 6979	CNBP 1435	*Allium cepa* L.	III
Burkholderia gladioli pv. gladioli	LMG 2216^T	ATCC 10248^T	*Gladiolus* sp.	III
Burkholderia gladioli pv. gladioli	LMG 6880	NCPPB 644	*Gladiolus* sp.	III
Burkholderia gladioli pv. gladioli	LMG 6881	NCPPB 1051	*Gladiolus* sp.	III
Burkholderia gladioli pv. gladioli	LMG 6882	NCPPB 1887	*Gladiolus* sp.	III
Burkholderia gladioli pv. gladioli	LMG 6884	NCPPB 1890	*Gladiolus* sp.	III

Continued on following page

TABLE 1—*Continued*

Taxon	Strain[a]	Other designation[a]	Source of isolation	Phenon
Burkholderia caryophylli	LMG 2155^T	ATCC 25418^T	*Dianthus caryophyllus* L.	IV
Burkholderia caryophylli	LMG 2156	NCPPB 609	*Dianthus caryophyllus* L.	IV
Burkholderia caryophylli	LMG 2157	NCPPB 349	*Dianthus caryophyllus* L.	IV
Burkholderia caryophylli	LMG 6876	NCPPB 353	*Dianthus* sp.	IV
Burkholderia caryophylli	LMG 6978	CNBP 1370	*Dianthus caryophyllus* L.	IV
Burkholderia glumae	LMG 2196^T	NCPPB 2981^T	*Oryza sativa* L.	V
Burkholderia glumae	LMG 1277	NCPPB 2391	*Oryza sativa* L.	V
Burkholderia glumae	LMG 6952	PDDCC 3728	*Oryza sativa* L.	V
Burkholderia plantarii	LMG 9035^T	ICMP 9424^T	*Oryza sativa* L.	VI
Burkholderia plantarii	LMG 10908	ICMP 9426	*Oryza sativa* L.	VI
[*Pseudomonas*] *phenazinium*	LMG 2247^T	NCIB 11027^T	Soil enriched with threonine	VII
[*Pseudomonas*] *phenazinium*	LMG 6868t1	NCIB 11431t1		VII
[*Pseudomonas*] *phenazinium*	LMG 6868t2	NCIB 11431t2		VII
Burkholderia andropogonis	LMG 2126	NCPPB 450	*Trifolium repens*	VIII
Burkholderia andropogonis	LMG 2127	NCPPB 451	*Trifolium pratense* L.	VIII
Burkholderia andropogonis	LMG 2128	NCPPB 933	*Zea mays* L.	VIII
Burkholderia andropogonis	LMG 2129^T	NCPPB 934^T	*Sorghum bicolor* (L.) Moench	VIII
Burkholderia andropogonis	LMG 2327	Hayward 0364A	*Trifolium repens* L.	VIII
Burkholderia andropogonis	LMG 2329	NCPPB 1024	*Mucuna deeringianum* (Bort) Merrill	VIII
Burkholderia andropogonis	LMG 6872	NCPPB 1127	*Bougainvillea* sp.	VIII
Burkholderia andropogonis	LMG 6873	NCPPB 1693	*Rheum rhaponticum* L.	VIII
Burkholderia andropogonis	LMG 6874	NCPPB 2179	*Sorghum bicolor* (L.) Moench	VIII
Burkholderia andropogonis	LMG 6875	NCPPB 2868	*Trifolium repens* L.	VIII
Burkholderia andropogonis	LMG 6942	PDDCC 3994	*Mucuna deeringianum* (Bort) Merrill	VIII
Burkholderia andropogonis	LMG 6943	PDDCC 6779	*Coffea arabica* L.	VIII
Burkholderia andropogonis	LMG 6944	PDDCC 6780	*Coffea arabica* L.	VIII
Burkholderia andropogonis	LMG 6945	PDDCC 5980	*Sorghum bicolor* (L.) Moench	VIII
Burkholderia andropogonis	LMG 6946	PDDCC 7076	*Cicer arietinum* L.	VIII
Burkholderia andropogonis[e]	LMG 1279	NCPPB 2441	*Setcreasea pallida* Rose	VIII
Burkholderia andropogonis[e]	LMG 2362[f]	NCPPB 968	*Dianthus caryophyllus* L.	VIII
Burkholderia andropogonis[e]	LMG 2363	NCPPB 2157	*Dianthus barbatus* L.	VIII
Burkholderia andropogonis[e]	LMG 6947	PDDCC 4001	*Dianthus* sp.	VIII
Burkholderia andropogonis[e]	LMG 6948	PDDCC 4003	*Dianthus* sp.	VIII
Burkholderia andropogonis[e]	LMG 6949	PDDCC 6647	*Dianthus caryophyllus* L.	VIII
Burkholderia andropogonis[e]	LMG 6950	PDDCC 6649	*Dianthus caryophyllus* L.	VIII
[*Burkholderia*] *solanacearum*	LMG 2295	NCPPB 173	*Solanum tuberosum* L.	sep
[*Burkholderia*] *solanacearum*	LMG 2296	NCPPB 215	*Lycopersicon lycopersicum* (L.) Karst. ex Farw.	IX
[*Burkholderia*] *solanacearum*	LMG 2297	NCPPB 253	*Casuarina equisetifolia* J. R. Forst. and G. Forst	IX
[*Burkholderia*] *solanacearum*	LMG 2298	NCPPB 282	*Solanum tuberosum* L.	IX
[*Burkholderia*] *solanacearum*	LMG 2299^T	NCPPB 325^T	*Lycopersicon lycopersicum* (L.) Karst. ex. Farw.	IX
[*Burkholderia*] *solanacearum*	LMG 2300	NCPPB 339	*Solanum tuberosum* L.	IX
[*Burkholderia*] *solanacearum*	LMG 2301	NCPPB 446	*Musa* sp.	IX
[*Burkholderia*] *solanacearum*	LMG 2303	NCPPB 789	*Musa* sp.	IX
[*Burkholderia*] *solanacearum*	LMG 2304	NCPPB 792	*Tectona grandis*	IX
[*Burkholderia*] *solanacearum*	LMG 2305	NCPPB 909	*Solanum tuberosum* L.	IX
[*Burkholderia*] *solanacearum*	LMG 2306	NCPPB 1019	*Lycopersicon lycopersicum* (L.) Karst. ex Farw.	IX
[*Burkholderia*] *solanacearum*	LMG 5839	NCPPB 1579	*Zingiber officinale*	IX
[*Alcaligenes*] *eutrophus*	LMG 1199^T	ATCC 17697^T		X
[*Alcaligenes*] *eutrophus*	LMG 1200	ATCC 17698		X
[*Alcaligenes*] *eutrophus*	LMG 1190	ATCC 33178		X
[*Alcaligenes*] *eutrophus*	LMG 1210	ATCC 17710		X
[*Alcaligenes*] *eutrophus*	LMG 1211	ATCC 25207		X
[*Alcaligenes*] *eutrophus*	LMG 1206	ATCC 17706		X
[*Alcaligenes*] *eutrophus*	LMG 1207	ATCC 17707		X
[*Alcaligenes*] *eutrophus*	LMG 1201	ATCC 17699		
[*Alcaligenes*] *eutrophus*	LMG 1202	ATCC 17700		
[*Alcaligenes*] *eutrophus*	LMG 1203	ATCC 17702		
[*Alcaligenes*] *eutrophus*	LMG 1204	ATCC 17704		
[*Alcaligenes*] *eutrophus*	LMG 1205	ATCC 17705		
[*Alcaligenes*] *eutrophus*	LMG 1209	ATCC 17709		
"*Pseudomonas oxalaticus*"	LMG 2235[g]	NCIB 8642[g]	Indian earthworm	X
"*Pseudomonas oxalaticus*"	LMG 2233	NCIB 8543	Indian earthworm	X
"*Pseudomonas oxalaticus*"	LMG 2234t1	NCIB 8544t1	Indian earthworm	X
"*Pseudomonas oxalaticus*"	LMG 2236	NCIB 8643	Indian earthworm	X
[*Burkholderia*] *pickettii*	LMG 5942^T	ATCC 27511^T	Tracheotomy patient	XI
[*Burkholderia*] *pickettii*	LMG 6866	NCIB 10805		XI
[*Burkholderia*] *pickettii*	LMG 6871	CCM 2846	Soil of a rice field	XI
[*Burkholderia*] *pickettii*	LMG 7001	CCUG 3314		XI

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TABLE 1—*Continued*

Taxon	Strain[a]	Other designation[a]	Source of isolation	Phenon
[*Burkholderia*] *pickettii*	LMG 7002	CCUG 3316		XI
[*Burkholderia*] *pickettii*	LMG 7003	CCUG 3317		XI
[*Burkholderia*] *pickettii*	LMG 7004	CCUG 3319		XI
[*Burkholderia*] *pickettii*	LMG 7005	CCUG 1467		XI
[*Burkholderia*] *pickettii*	LMG 7006	CCUG 1742	Blood	XI
[*Burkholderia*] *pickettii*	LMG 7007	CCUG 3324		XI
[*Burkholderia*] *pickettii*	LMG 7008	CCUG 2165	Urine	XI
[*Burkholderia*] *pickettii*	LMG 7009	CCUG 8527	Trachea	XI
[*Burkholderia*] *pickettii*	LMG 7010	CCUG 9984	Urethra	XI
[*Burkholderia*] *pickettii*	LMG 7011	CCUG 11629	Trachea	XI
[*Burkholderia*] *pickettii*	LMG 7012	CCUG 12413		XI
[*Burkholderia*] *pickettii*	LMG 7013	CCUG 12490	Blood	XI
[*Burkholderia*] *pickettii*	LMG 7014	CCUG 12491	Blood	XI
[*Burkholderia*] *pickettii*	LMG 7015	CCUG 12492	Blood	XI
[*Burkholderia*] *pickettii*	LMG 7016	CCUG 12493	Blood	XI
[*Burkholderia*] *pickettii*	LMG 7017	CCUG 12494	Blood	XI
[*Burkholderia*] *pickettii*	LMG 7018	CCUG 3323		XI
[*Burkholderia*] *pickettii*	LMG 7056	CCUG 3321		XI
[*Burkholderia*] *pickettii*	LMG 7057	CCUG 691	Drain fluid	XI
[*Burkholderia*] *pickettii*	LMG 7058	CCUG 3325		XI
[*Burkholderia*] *pickettii*	LMG 7140	Pickett K-638		XI
[*Burkholderia*] *pickettii*	LMG 7141	Pickett K-1303		XI
[*Burkholderia*] *pickettii*	LMG 7142	Pickett K-1466		XI
[*Burkholderia*] *pickettii*	LMG 7143	Pickett K-1503		XI
[*Burkholderia*] *pickettii*	LMG 7144	Pickett K-1510		XI
[*Burkholderia*] *pickettii*	LMG 7145	Pickett K-232		XI
[*Burkholderia*] *pickettii*	LMG 7147t1	Pickett K-1193t1		XI
[*Burkholderia*] *pickettii*	LMG 7159	CIP 73.24		XI
[*Burkholderia*] *pickettii*	LMG 7160	CIP 74.22	Blood	XI
Burkholderia cocovenenans	LMG 11626^T	ATCC 33664^T		
Brevundimonas diminuta	LMG 2089^T	ATCC 11568^T		
Pseudomonas aeruginosa	LMG 6395	DSM 1117		
Pseudomonas stutzeri	LMG 2333^T	ATCC 17588^T		
Pseudomonas putida	LMG 2257^T	ATCC 12633^T		
Sphingomonas paucimobilis	LMG 1227^T	ATCC 29837^T		
Telluria mixta	LMG 11547^T	UQM 1762^T	Black earth soil	
Azospirillum lipoferum	LMG 4348	4B	Rice rhizosphere (Camargue, France)	

[a] ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; CIP, Collection de l'Institut Pasteur, Paris, France; CNBP, Collection Nationale de Bactéries Phytopathogènes, Institut National de la Recherche Agronomique, Station de Pathologie Végétale et Phytobactériologie, Angers, France; DSM, Deutsche Sammlung von Microorganismen, Braunschweig, Germany; LMG, Culture Collection, Laboratorium voor Microbiologie, State University of Ghent, Ghent, Belgium; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; NCPPB, National Collection of Plant Pathogenic Bacteria, Hatching Green, England; NRRL, Agricultural Research Service Culture Collection, Peoria, Ill.; PDDCC, Culture Collection of the Plant Diseases Division, New Zealand Department of Scientific and Industrial Research, Auckland, New Zealand; TVV, Tran Van Van; UQM, Department of Microbiology, University of Queensland, St. Lucia, Queensland, Australia.

[b] The suffixes t1, t2, and t3 indicate colony morphology variants.

[c] sep, separate position.

[d] Pathovar reference strain.

[e] Originally named [*Pseudomonas*] *woodsii*.

[f] Type strain of the former species [*Pseudomonas*] *woodsii*.

[g] Co-type strain.

The thermal stability of reassociated DNAs was estimated by determining the denaturation temperature (T_m) (the temperature at which 50% of the double-stranded DNA was denatured and lysed by S1 nuclease). The T_m was determined by using the method of Crosa et al. (16). The divergence between DNAs was estimated by determining the ΔT_m value (the difference between the T_m of the heteroduplex [in the heterologous reaction] and the T_m of the homoduplex [in the homologous reaction]).

RESULTS

Phenotypic characteristics of the TVV strains. The number of natural nitrogen-fixing bacteria associated with the rhizosphere of rice plants grown in an acid sulfate soil in Bình Thanh, Vietnam, was 2.8×10^8 cells per g (dry weight) of rhizosphere soil. Among these nitrogen-fixing bacteria, the 12 TVV strains were dominant. These strains were motile at 28°C, and polar flagella were observed when transmission electron microscopy was used. They grew well at 28°C, and maximum growth was observed at 41°C. Colonies on nutrient agar were smooth, translucent, and convex with entire margins, and colonies were not pigmented either on nutrient agar or on King B medium. Colonies on PCAT medium were white and smaller than *B. cepacia* LMG 1222^T colonies. Nitrate was reduced to nitrite, β-galactosidase and oxidase were produced, and gelatin was hydrolyzed, but esculin was not hydrolyzed. Glucose fermentation, production of indole, arginine dihydrolase activity, and urease activity were negative. The TVV strains were not pathogenic for onions.

The TVV strains produced three types of ornibactins (41), which are new types of siderophores (60), but did not produce pyochelin or cepabactin, two compounds which are typically produced by *B. cepacia* strains.

TABLE 2. Results of auxanographic studies

No.	Substrate[a] Compound	*B. vandii*[b]	*B. cocovenenans* ($n = 19$)[c]	*B. mallei*[d]	*B. pseudomallei*[d]	*B. cepacia* (phenon I; $n = 36$)	*B. vietnamiensis* (phenon II; $n = 13$)	*B. gladioli* (phenon III; $n = 15$)	*B. caryophylli* (phenon IV; $n = 25$)	*B. glumae* (phenon V; $n = 3$)	*B. plantarii* (phenon VI; $n = 2$)	[*P.*] *phenazinium* (phenon VII; $n = 3$)	*B. andropogonis* (phenon VIII; $n = 22$)	[*B.*] *solanacearum* (phenon IX; $n = 11$)	[*A.*] *eutrophus* (phenon X; $n = 11$)	[*B.*] *pickettii* (phenon XI; $n = 33$)
1	Pyruvate		+[e]	+	+	+	+	+	+	+	+	+	+	+	d^-	+
2	D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
3	Succinate		+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	L-α-Alanine		+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	Inositol	+	+	+	+	+	+	+	+	+	+	+	+	d^+	−	−
7	Acetate		+	+	+	+	+	+	+	+	+	+	+	d^+	+	+
8	D-α-Alanine		+	+	+	+	+	+	+	+	+	+	+	d^+	+	+
9	D-Galactose	+	+	+	+	+	+	+	+	+	+	+	+	d^-	−	+
10	Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	d^-	−	−
11	Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	d^-	−	d^-
12	DL-Lactate		+	+	+	+	+	+	+	+	+	+	+	d^+	+	+
13	L-Threonine[f]	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
14	L-Histidine[f]		+	+	+	+	+	+	+	+	+	+	−	+	+	+
15	L-Tyrosine[f]		+	+	+	+	+	+	+	+	+	+	−	+	+	+
16	Trehalose	+	+	+	+	+	+	+	+	+	+	−	d^-	d^-	−	d^-
17	D-Mannose	+		+	+	+	+	+	+	+	+	+	+	−	−	−
18	DL-Glycerate			+	+	+	+	+	+	+	+	+	+	+	+	+
19	*p*-Hydroxybenzoate			+	+	+	+	+	+	+	+	+	+	d^-	d^-	+
20	DL-4-Aminobutyrate			+	+	+	+	+	+	+	+	+	d^+	+	d^+	+
21	Betaine			+	+	+	+	+	+	+	+	+	−	−	−	−
22	Propionate[f]			+	+	+	+	+	+	+	+	d^+	−	d^+	+	+
23	L-Valine[f]	+	−	d	+	+	+	+	+	+	+	+	−	+	d^+	+
24	L-Proline		+	d	+	+	+	+	+	+	+	+	+	+	+	+
25	Citrate[f]	−	+	d	+	+	+	+	+	+	+	+	+	+	+	+
26	L-Serine		+	d	+	+	+	+	+	+	+	+	+	+	+	+
27	2-Ketogluconate			d	+	+	+	+	+	+	+	+	d^-	−	d^+	+
28	L-Phenylalanine[f]		d^-	d	+	+	+	+	+	+	+	+	−	d^-	+	+
29	D-Fructose		+	d	+	+	+	+	+	+	+	+	+	+	d^+	+
30	D-Arabitol					+	+	+	+	+	+	+	+	d^-	−	d^-
31	DL-3-Hydroxybutyrate		+			+	+	+	+	+	+	+	d^+	+	+	+
32	L-Arabinose		+	d	−	+	+	+	+	+	+	+	+	−	−	+
33	D-Malate			d	d	+	+	+	+	+	+	−	+	−	+	+
34	Isobutyrate[f]		−	−	+	+	+	+	+	+	+	−	−	d^+	d^+	+
35	D-Lyxose[f]		−			+	+	+	+	+	+	d^+	+	−	−	−
36	Butyrate[f]		+	d	+	+	+	+	+	+	+	d^+	−	d^+	+	+
37	Glucosamine[f]					+	+	+	+	+	+	d^+	−	−	−	+
38	Aconitate[f]		+	−	+	+	+	+	+	+	d^+	+	−	−	d^-	+
39	L-Isoleucine[f]		−	−	+	+	+	+	+	+	d^-	+	−	+	+	+
40	L-Arginine[f]		+	+	+	+	+	+	+	+	d^-	+	−	−	−	−
41	*N*-Acetylglucosamine[f]			+		+	+	+	+	+	+	+	−	−	d^+	d^+
42	L-Leucine[f]		−	−	−	+	+	+	+	d^+	+	d^+	−	d^+	+	+
43	L-Tryptophan[f]		−	+	+	+	+	+	+	+	−	+	−	d^+	+	+
44	Sarcosine[f]		+	d	d	+	+	+	+	d^+	−	d^+	−	d^-	−	−
45	β-Alanine[f]		+	+	+	+	+	+	+	d^+	−	+	−	d^+	+	+
46	*n*-Valerate[f]			−	+	+	+	+	d^-	−	−	−	−	d^+	+	+
47	L-Ornithine[f]	+	−	−	+	+	+	+	+	−	−	−	−	d^-	d^-	d^+
48	L-Norleucine[f]		−	−	−	+	+	+	d^-	−	−	d^+	−	−	+	d^+
49	L-Lysine[f]		+	−	+	+	+	+	d^-	−	−	d^+	−	−	−	−
50	DL-Kynurenine[f]			−	+	+	+	+	d^-	−	−	d^+	d^-	d^+	d	d^+
51	*n*-Caproate[f]	−		−	+	+	+	+	−	−	d^+	−	−	−	+	+
52	Heptanoate[f]	+		−	+	+	+	+	−	+	+	+	−	−	+	+
53	Caprylate[f]	+	+	−		+	+	+	−	d^-	+	+	−	−	+	d^+
54	Levulinate[f]	−	−	−	+	+	+	+	−	−	−	+	−	d^+	+	+
55	Pelargonate[f]		+	−	+	+	+	+	−	d^+	+	+	−	−	+	d^-
56	Caprate[f]	+		−	+	+	+	+	−	+	+	d^+	−	−	d^-	d^-
57	DL-5-Aminovalerate[f]					+	+	+	−	−	−	−	−	−	d^-	−
58	Ethanolamine[f]	−	+	−	+	+	+	+	−	+	+	+	d^+	−	−	−
59	2-Aminobenzoate[f]			−	−	+	+	+	−	+	−	+	d^-	−	d^-	d^-
60	Adipate[f]		+	+	+	+	+	+	−	−	−	d^+	−	−	d^+	d^+
61	Azelate[f]	−	+	d	d	+	+	+	+	+	+	+	−	+	+	+

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TABLE 2—*Continued*

No.	Substrate[a] Compound	*B. vandii*[b]	*B. cocovenenans* ($n = 19$)[c]	*B. mallei*[d]	*B. pseudomallei*[d]	*B. cepacia* (phenon I; $n = 36$)	*B. vietnamiensis* (phenon II; $n = 13$)	*B. gladioli* (phenon III; $n = 15$)	*B. caryophylli* (phenon IV; $n = 25$)	*B. glumae* (phenon V; $n = 3$)	*B. plantarii* (phenon VI; $n = 2$)	*[P.] phenazinium* (phenon VII; $n = 3$)	*B. andropogonis* (phenon VIII; $n = 22$)	*[B.] solanacearum* (phenon IX; $n = 11$)	*[A.] eutrophus* (phenon X; $n = 11$)	*[B.] pickettii* (phenon XI; $n = 33$)
62	Sebacate[e]		+	−	+	+	+	+	+	+	+	+	−	d^+	+	+
63	L-Cysteine[e]					+	+	+	+	+	+	+	+	d^+	d^-	+
64	Phenylacetate[e]			+	+	+	+	+	−	−	−	+	−	−	+	d^-
65	Dulcitol[e]					+	+	+	−	+	+	−	−	d^-	−	−
66	D-Arabinose[e]			+	+	+	+	+	+	+	+	+	+	−	−	−
67	D-Tagatose[e]					+	+	+	−	d^+	−	−	−	−	−	−
68	D-Fucose[e]		−	+	+	+	+	+	+	d^+	+	d^+	d^-	−	−	+
69	L-Fucose[e]					+	+	+	+	+	+	+	−	−	−	−
70	Amygdalin[e]					+	d^+	−	−	d^-	+	−	−	−	−	−
71	Glutarate[e]	−	−	d	d	+	+	d^+	−	−	−	−	−	−	+	d^+
72	Ribose[e]		+	−	+	+	d^-	+	+	+	+	+	+	−	−	d^-
73	Malonate[e]		+	d	−	+	d^-	+	−	d^-	d^+	−	−	d^-	d^-	+
74	2-Ketoglutarate[e]			+	+	+	d^+	+	+	+	−	+	+	d^+	d^-	+
75	DL-Norvaline					+	d^-	+	−	−	d^+	−	−	−	d^-	−
76	DL-2-Aminobutyrate[e]	−		d	−	+	d^+	+	−	−	d^+	d^+	−	−	d^-	−
77	DL-3-Aminobutyrate[e]					+	d^+	+	+	−	−	d^+	−	−	d^-	+
78	Suberate[e]	−		d	+	+	d^-	d^+	d^+	d^+	−	d^+	−	d^+	+	+
79	L-Citrulline[e]			−	−	+	d^+	d^+	−	−	−	−	−	d^-	d	d^-
80	Amylamine[e]	−		−	+	+	d^+	−	+	−	−	−	−	−	−	−
81	Arbutin[e]					+	d^+	−	−	d^-	+	−	−	−	−	−
82	Salicin[e]		+	d	+	+	d^+	−	−	d^-	+	−	−	−	−	−
83	Pimelate[e]	−	−	d	d	+	−	d^+	−	−	−	d^+	−	d^+	+	+
84	Diaminobutane[e]					+	+	−	−	−	−	−	−	−	−	−
85	L-Rhamnose[e]	−	−	−	−	−	−	−	+	−	+	d^+	d^+	−	−	−
86	5-Ketogluconate[e]					+	−	+	+	d^-	+	d^+	−	−	d^-	−
87	Xylitol[e]		+			+	−	d^+	+	−	−	+	−	−	−	−
88	L-Arabitol[e]					+	−	−	+	−	−	+	−	−	−	−
89	Glycolate[e]	−	−	−	−	+	−	d^-	d^-	−	−	−	−	−	+	+
90	Citraconate[e]	+	+	−	−	+	−	d^-	−	−	+	−	−	−	+	+
91	*m*-Hydroxybenzoate[e]	−	d^-	−	−	+	−	d^+	−	−	−	−	−	−	d^-	−
92	Butylamine[e]					+	−	−	−	−	−	−	−	−	−	−
93	Tryptamine[e]	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−
94	Adonitol[e]	−	d^-		d	+	−	+	+	+	−	+	+	−	−	−
95	Ethylamine			−		−	−	−	−	−	−	−	−	−	−	−
96	Erythritol[e]		−	−	+	−	−	−	−	−	−	−	−	−	−	−
97	Oxalate[e]		−	−		−	−	−	+	d^-	−	−	−	−	d^-	+
98	L-Methionine		−		−	−	−	−	−	−	−	−	−	−	d^-	d^-
99	Maleate		−	−	−	d^-	−	d^-	−	−	−	−	−	−	d^-	+
100	D-Tartrate[e]	+	+	−	−	−	−	+	−	−	+	−	−	−	d^-	d^+
101	Mesaconate[e]	+	d^+	−	−	−	−	+	−	−	d^-	−	−	−	+	−
102	Terephthalate			−	−	−	−	d^+	−	−	−	−	−	−	−	−
103	Itaconate	−	−	−	−	−	+	−	−	−	−	+	−	−	+	−
104	Lactose	+		−	−	−	d^-	−	−	−	−	−	+	−	−	−
105	D-Xylose[e]	+	+	+	−	d^+	+	+	+	+	+	d^+	−	−	−	+
106	L-Xylose					d^-	−	−	−	−	−	−	−	−	−	−
107	D-Tryptophan			−	−	d^-	−	−	−	−	−	−	−	−	−	−
108	L-Mandelate	−		−	d	d^+	−	d^-	−	−	−	−	−	−	d^-	d^+
109	Methyl-D-glucoside		−			d^-	−	−	−	−	−	−	−	−	−	−
110	D-Turanose		−			d^-	−	−	−	−	−	−	−	−	−	−
111	*o*-Hydroxybenzoate[e]			−	−	d^+	−	−	−	+	−	−	−	−	d^-	−
112	Glycine[e]	−	−	+	−	d^-	−	−	−	−	d^+	d^+	−	−	−	d^+
113	Urea					d^-	−	−	−	−	−	−	−	−	−	−
114	Acetamide					d^-	−	−	−	−	−	−	−	−	−	−
115	D-Melibiose[e]		+			d^-	−	−	−	+	−	−	−	−	−	−
116	L-Tartrate[e]	−	+	−	−	d^+	−	+	−	−	+	−	−	d^-	−	+
117	Benzoate[e]	−	d^-	+	+	d^+	+	+	−	−	−	−	−	d^+	+	d^+
118	D-Cellobiose[e]		+	+	+	d^+	+	d^+	d^-	d^+	+	−	−	−	−	−
119	Benzylamine[e]		−	−	−	d^+	+	d^-	d^+	−	−	d^-	−	−	−	−
120	Histamine[e]		−	−	−	d^+	+	−	−	−	−	−	−	−	−	−
121	Isovalerate[e]					d^+	+	−	d^-	−	−	−	−	d^+	+	+
122	Sucrose[e]	−	−	+	+	d^+	+	−	+	−	−	+	−	+	−	−

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TABLE 2—*Continued*

No.	Substrate[a] Compound	*B. vandii*[b]	*B. cocovenenans* ($n = 19$)[c]	*B. mallei*[d]	*B. pseudomallei*[d]	*B. cepacia* (phenon I; $n = 36$)	*B. vietnamiensis* (phenon II; $n = 13$)	*B. gladioli* (phenon III; $n = 15$)	*B. caryophylli* (phenon IV; $n = 25$)	*B. glumae* (phenon V; $n = 3$)	*B. plantarii* (phenon VI; $n = 2$)	[*P.*] *phenazinium* (phenon VII; $n = 3$)	*B. andropogonis* (phenon VIII; $n = 22$)	[*B.*] *solanacearum* (phenon IX; $n = 11$)	[*A.*] *eutrophus* (phenon X; $n = 11$)	[*B.*] *pickettii* (phenon XI; $n = 33$)
123	β-Gentiobiose[f]					d^+	+	−	d^-	d^-	+	−	−	−	−	−
124	*meso*-Tartrate[f]			−	−	d^+	d^-	+	+	−	−	+	−	−	d^-	d^+
125	D-Raffinose[f]		−			d^+	d^+	−	+	d^+	−	−	−	−	−	−
126	Spermine[f]					d^-	d^-	−	−	−	−	−	−	−	−	−
127	Maltose[f]		−	d	+	d^-	−	−	−	−	−	−	−	−	−	−
128	Methyl-D-mannoside	−				d^-	−	−	−	−	−	−	−	−	−	−
129	D-Melezitose					−	−	−	−	−	−	−	−	−	−	−
130	Starch		−			−	−	−	−	−	−	−	−	−	−	−
131	D-Mandelate					−	−	−	−	−	−	−	−	−	−	−
132	Trigonelline			d	d	−	−	−	−	−	−	−	−	−	−	−
133	Glycogen					−	−	−	−	−	−	−	−	−	−	−
134	Creatine		−	−	−	−	−	−	−	−	−	−	−	−	−	−
135	3-Aminobenzoate			−	−	−	−	−	−	−	−	−	−	−	−	−
136	4-Aminobenzoate	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
137	Phthalate			−	−	−	−	−	−	−	−	−	−	−	−	−

[a] All of the strains which we tested used the following substrates: gluconate, fumarate, L-malate, L-aspartate, and L-glutamate. None of the strains used inulin, methyl-xyloside, L-sorbose, esculin, and isophthalate.

[b] Data from reference 63.

[c] Data from reference 74. *n* is the number of strains studied.

[d] Data for the type strains of *B. mallei* and *B. pseudomallei* from reference 73.

[e] +, more than 90% of the strains gave a positive reaction; −, fewer than 10% of the strains gave a positive reaction, d^+, between 10 and 90% of the strains gave a positive reaction and the type strain was positive; d^-, between 10 and 90% of the strains gave a positive reaction and the type strain was negative.

[f] Characteristics that differentiate members of the genus *Burkholderia*.

Numerical analysis of auxanographic results. The auxanographic characteristics of the members of the solanacearum rRNA complex are presented in Table 2. Figure 1 shows a dendrogram which was obtained by using unweighted average pair group clustering of *S* values. All strains clustered above an *S* value of 64%. The reproducibility of the tests was good; the average *S* values for 10 strains tested in duplicate (Fig. 1) were between 90 and 95%. Eleven phena (phena I to XI) containing two or more strains were delineated; two strains (*B. cepacia* LMG 6995 and [*B.*] *solanacearum* LMG 2295) did not belong to any of these phena. Of the 11 phena which we identified, 10 contain only members of previously described species. Phena I, III, IV, V, VI, VII, IX, and XI contain only *B. cepacia*, *B. gladioli*, *B. caryophylli*, *B. glumae*, *B. plantarii*, [*P.*] *phenazinium*, [*B.*] *solanacearum*, and [*B.*] *pickettii* strains, respectively. "*Pseudomonas oxalaticus*" and [*A.*] *eutrophus* strains constitute phenon X, while phenon VIII contains only [*P.*] *andropogonis* and [*P.*] *woodsii* strains. The isolates from Vietnam are members of phenon II, which also contains two strains received as *B. cepacia* LMG 6998 and LMG 6999.

B. cepacia contains plant-pathogenic organisms, saprophytic bacteria, and some clinical isolates. Despite the internal heterogeneity of this species, all *B. cepacia* strains except the two phenon II strains and strain LMG 6995 are phenotypically identical. One of our isolates, strain PHP7, which was isolated from a maize rhizosphere, also belongs to this cluster (phenon I). The description of *B. cepacia* deduced from the auxanographic phenon I data corresponds well to the description given by Palleroni and Holmes (45). Our auxanographic results for phenon I differ in at least 17 carbon sources from the results that Yabuuchi et al. (73) obtained for the type strain of *B. cepacia* but correspond better to the results of Palleroni (43) and to the results that Urakami et al. obtained for 45 substrates (63). Our results and those of Palleroni (43) differ for only seven carbon sources (ethylamine [substrate 95], L-norleucine [substrate 48], L-rhamnose [substrate 85], L-valine [substrate 23], maltose [substrate 127], DL-2-aminobutyrate [substrate 76], and glycine [substrate 112]), and our results and the results of Urakami et al. (63) differ only for DL-4-aminobutyrate (substrate 20). Although a clear-cut distinction between possible ecological groups could not be made, it is remarkable that all of the strains isolated from *Allium cepa* grew on L-arabinose (substrate 32), D-xylose (substrate 105), sucrose (substrate 122), L-tartrate (substrate 116), and *o*-hydroxybenzoate (substrate 111) but not on maltose (substrate 127) and D-turanose (substrate 110), while this combination of characteristics was never found for a clinical isolate.

The two aberrant *B. cepacia* strains belonging to phenon II were of clinical origin; these strains constitute a phenon together with the N_2-fixing isolates from Vietnam. Within this phenon the TVV strains are more similar to each other than they are to the two aberrant *B. cepacia* strains.

The phenon III *B. gladioli* strains, including the type strain, form a very homogeneous group (mean *S* value, 91.0%) and correspond phenotypically to the species *B. gladioli* as described by Ballard et al. (4), although there is an important difference in two characteristics, growth on sucrose (substrate 122) and growth on trigonelline (substrate 132); these two characteristics were found to be negative in this study and also were found to be negative for the reference strain by Yabuuchi et al. (73) and for the type strain by Urakami et al. (63). We observed that the type strain grew slightly on D-cellobiose (substrate 118), while Yabuuchi et al. (73) reported negative results for this test. Growth on D-cellobiose (substrate 118) is impor-

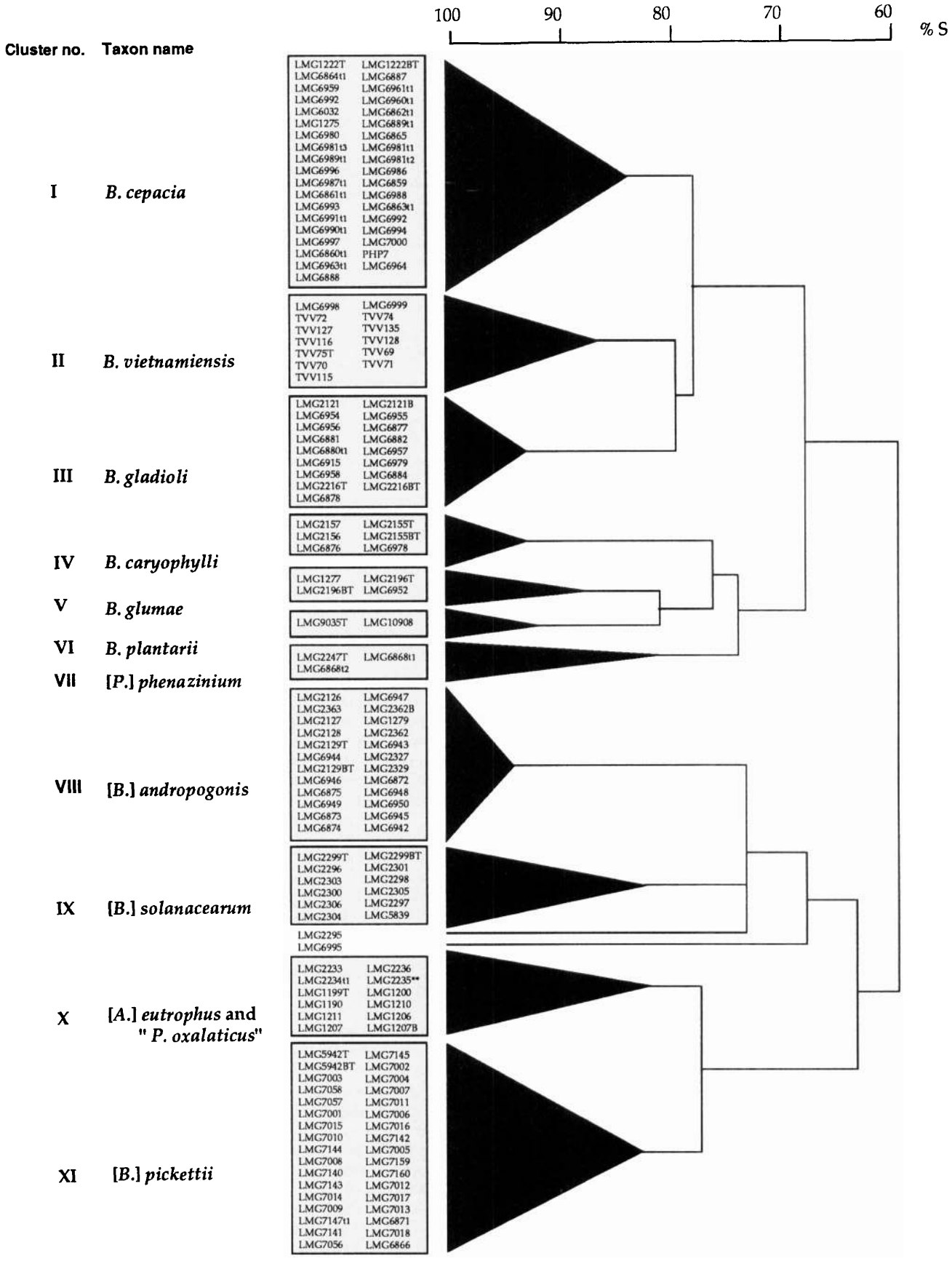

FIG. 1. Dendrogram obtained from an unweighted average pair group cluster analysis of similarity coefficients of strains belonging to the solanacearum rRNA complex. The results of 137 auxanographic tests were compared in this analysis. The suffix B indicates that a strain was tested twice. *B.*, *Burkholderia*; *A.*, *Alcaligenes*; *P.*, *Pseudomonas*.

tant since this characteristic has been used as a diagnostic characteristic for the nonfluorescent phytopathogenic [*Pseudomonas*] species. Most of our results correspond to the results of Urakami et al. (63); the exceptions are the results for 4-aminobenzoate (substrate 136) and DL-2-aminobutyrate (substrate 76). We could not detect auxanographic differences between the two pathovars of *B. gladioli*; the only difference which we could confirm was darkening of the culture medium by some strains of *B. gladioli* pv. alliicola but not by any member of *B. gladioli* pv. gladioli.

Phenon IV corresponds to *B. caryophylli*, although the phenotypic description of Ballard et al. (4) and the results obtained for the type strain by Yabuuchi et al. (73) differ from our data, especially the amino acid data (we found more positive reactions). In addition, differences in growth on salicin (substrate 82), adonitol (substrate 94), malonate (substrate 73), azelate (substrate 61), sebacate (substrate 62), sarcosine (substrate 44), benzylamine (substrate 119), D-cellobiose (substrate 118), *n*-valerate (substrate 46), phenylacetate (substrate 64), oxalate (substrate 97), and amylamine (substrate 80) were found. The five strains included in this phenon exhibit a high level of phenotypic similarity (92.3%), although they have different geographic origins. Most of results are consistent with those of Urakami et al. (63); the exceptions are the data for growth on L-threonine (substrate 13), azelate (substrate 61), L-rhamnose (substrate 85), and adonitol (substrate 94).

All three strains of *B. glumae* that we investigated fell into phenon V, although strain LMG 1277 was somewhat different from the two other strains. The description of this species given by Kurita and Tabei (36) was based mainly on acidification of sugars; consequently, we could not compare this description with most of our auxanographic results, which correspond to the data of Azegami et al. (2) and Urakami et al. (63); the only exceptions were our data for growth on L-threonine (substrate 13), caprylate (substrate 53), levulinate (substrate 54), and adonitol (substrate 94). Some characteristic features of *B. glumae* deduced from our auxanographic data were growth on adonitol (substrate 94), D-xylose (substrate 105), L-fucose (substrate 69), dulcitol (substrate 65), *p*-hydroxybenzoate (substrate 19), and tryptamine (substrate 93) but no growth on citraconate (substrate 90), DL-3-aminobutyrate (substrate 77), *meso*-tartrate (substrate 124), and benzoate (substrate 117).

Azegami et al. (2) described [*P.*] *plantarii*, which was later transferred to the genus *Burkholderia* as *B. plantarii* (63). We investigated two representative strains of *B. plantarii*, including the type strain; these organisms constitute a separate phenon (phenon VI) that is most similar to *B. glumae*. These taxa differ from each other in the following characteristics: growth on adonitol (substrate 94), citraconate (substrate 90), D-tartrate (substrate 100), L-rhamnose (substrate 85), L-tartrate (substrate 116), tryptamine (substrate 93), D-melibiose (substrate 115), *o*-hydroxybenzoate (substrate 111), L-tryptophan (substrate 43), 2-aminobenzoate (substrate 59), and 2-ketoglutarate (substrate 74). Again, most of the results obtained with 45 substrates by Urakami et al. (63) were identical to our results; the only exceptions were the results for growth on D-tartrate (substrate 100), glycine (substrate 112), and sucrose (substrate 122).

Three strains of [*P.*] *phenazinium* constitute phenon VII; although these strains have a similarity value of only approximately 80%, our results confirmed the description given by Bell and Turner (6).

We could not detect any phenotypic differences between the members of phenon VIII ([*P.*] *woodsii* and [*P.*] *andropogonis*); these organisms are almost identical phenotypically and form a very tight cluster (*S* value, 94.3%). Our results are consistent with the description of Goto and Starr (27) for [*P.*] *andropogonis* and with the description of Burkholder and Guterman (14) for [*P.*] *woodsii*. The latter species was originally described as pathogenic for *Dianthus caryophyllus*, while [*P.*] *andropogonis* causes symptoms in at least 12 plant species belonging to 10 genera (1, 13, 15, 27, 30, 51). Although the symptoms can vary between different plants, the names [*P.*] *woodsii* and [*P.*] *andropogonis* can be considered synonyms because of the nearly identical phenotypic profiles of these organisms.

Our results confirmed the description of [*B.*] *solanacearum* (phenon IX), a taxon that has been studied intensively previously (11, 29, 44). However, we observed large variations in growth for the following substrates: acetate (substrate 7), benzoate (substrate 117), isobutyrate (substrate 34), suberate (substrate 78), 2-ketoglutarate (substrate 74), D-α-alanine (substrate 8), L-leucine (substrate 42), and L-tryptophan (substrate 43). Also, the subdivision of this species into four biovars (29) was confirmed, and our results represent a completion of Hayward's study (29). Our results differ from the results that Urakami et al. (63) obtained for the type strain of [*B.*] *solanacearum* in only a few characteristics (growth on D-galactose [substrate 9], *n*-caproate [substrate 51], heptanoate [substrate 52], and benzoate [substrate 117]).

We found that four strains of "*P. oxalaticus*" (34) and seven strains of [*A.*] *eutrophus* are members of phenon X, which confirmed the auxanographic results of Jenni et al. (32). Within this phenon the co-type strain of "*P. oxalaticus*" differs most from the three other "*P. oxalaticus*" strains and from all of the [*A.*] *eutrophus* strains.

Phenon XI contains only [*B.*] *pickettii* strains; in general, our results are consistent with the descriptions given by Ralston et al. (48) and Palleroni (43) but differ from some of the results obtained by Yabuuchi et al. (73) and Urakami et al. (63) for the type strain. The core of this cluster (28 strains) is very homogeneous (mean *S* value, 89.8%). All of the auxanographic features that differentiate the phena are shown in Table 2; for comparison, the results obtained with the type strains of *B. mallei* (73), *Burkholderia pseudomallei* (73), [*P.*] *cocovenenans* (74), and *B. vandii* (63) are included in Table 2.

Nitrogenase activity. All of the *B. vietnamiensis* TVV strains isolated from the rhizosphere of rice plants were positive for nitrogenase activity (Table 3). Only two of the *Burkholderia* reference strains tested in this study (LMG 6998 and LMG 6999) exhibited nitrogenase activity. These strains, together with the TVV strains, constitute phenon II (Fig. 1) and produce ornibactin siderophores (41).

DNA-rRNA hybridization. Previous hybridization results allowed us to define the solanacearum rRNA complex (22, 23) and to show that [*A.*] *eutrophus* is the only group belonging to this complex that does not belong to one of the sections of the genus *Pseudomonas* as defined by Palleroni (43). Hybridization experiments performed with rRNA from [*A.*] *eutrophus* LMG 1199^T clearly revealed that this species constitutes a separate genotypic cluster [$T_{m(e)}$, 78.2 to 80.0°C] on a separate rRNA branch (Fig. 2 and Table 4) that splits off at a $\Delta T_{m(e)}$ value of 7°C. The results of preliminary hybridizations between DNAs from two representative TVV rice isolates and [*B.*] *solanacearum* LMG 2299^T rRNA demonstrated that these rice isolates also belong to the solanacearum rRNA complex. To determine the genotypic relationships among the members of this rRNA complex, we concentrated on the phena that were most closely related to *B. cepacia*. We prepared a 23S rRNA probe from *B. cepacia* LMG 1222^T and hybridized it with DNAs from representative strains of the different phena. The results of the DNA-rRNA hybridization experiments are shown in Table 4. Members of phena I and II had very high $T_{m(e)}$ values (79.6 to

TABLE 3. Nitrogenase activities of reference strains of *Burkholderia* species

Strain	Nitrogenase activity (nmol of C_2H_4 per tube)[a]
Phenon I[b]	
B. cepacia LMG 1222^T	0
B. cepacia LMG 6863	0
B. cepacia LMG 6888	0
B. cepacia LMG 6964	0
B. cepacia LMG 6981	0
B. cepacia LMG 6988	0
B. cepacia LMG 6997	0
B. cepacia PHP7	0
Phenon II	
B. vietnamiensis LMG 6998	958 ± 209
B. vietnamiensis LMG 6999	1,326 ± 255
B. vietnamiensis TVV69	2,089 ± 348
B. vietnamiensis TVV70	988 ± 164
B. vietnamiensis TVV71	686 ± 203
B. vietnamiensis TVV72	1,316 ± 191
B. vietnamiensis TVV74	825 ± 244
B. vietnamiensis TVV75^T	593 ± 206
B. vietnamiensis TVV115	1,352 ± 244
B. vietnamiensis TVV116	507 ± 239
B. vietnamiensis TVV127	1,249 ± 507
B. vietnamiensis TVV128	1,108 ± 610
B. vietnamiensis TVV131	947 ± 250
B. vietnamiensis TVV135	1,060 ± 239
Phenon III	
B. gladioli LMG 2216^T	ND[c]
B. gladioli LMG 6882	0
B. gladioli LMG 6886	0
Phenon IV	
B. caryophylli LMG 2155^T	0
Phenon V	
B. glumae LMG 2196^T	0
B. glumae LMG 1277	0
B. glumae LMG 6952	0
Phenon VI	
B. plantarii LMG 9035	0
B. plantarii LMG 10908	0
Phenon VII	
[*P.*] *phenazinium* LMG 2247^T	0
Phenon IX	
[*B.*] *solanacearum* LMG 2299^T	0
Phenon XI	
[*B.*] *pickettii* LMG 5942^T	0
Reference strain	
Azospirillum lipoferum 4B	732 ± 100

[a] Averages ± standard deviations of the values from three experiments.
[b] See Fig. 1.
[c] ND, not determined.

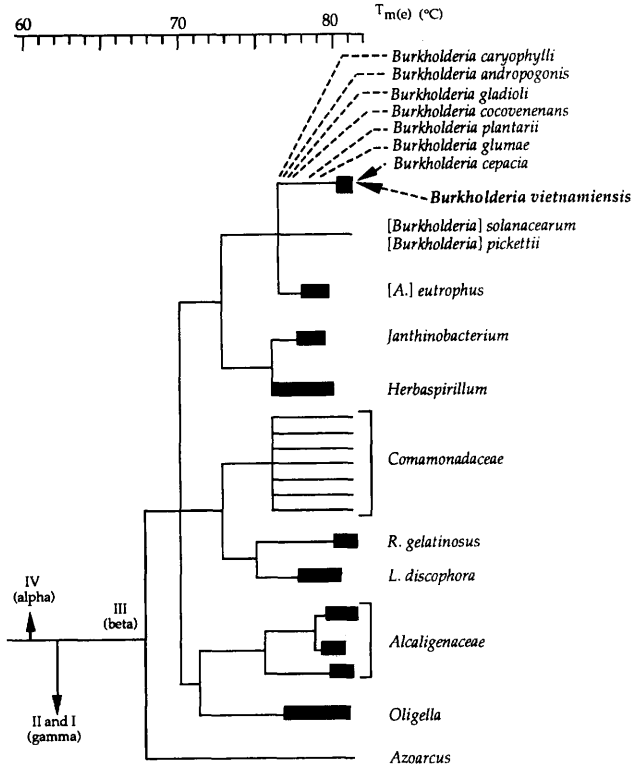

FIG. 2. $T_{m(e)}$ dendrogram showing the position of the *Burkholderia* rRNA branch within rRNA superfamily III (beta subclass). *A.*, *Alcaligenes*; *R.*, *Rubrivivax*; *L.*, *Leptothrix*.

81.1°C), indicating that these two clusters are genotypically very similar. Of all of the other representative strains of the different phena examined, only *B. gladioli* LMG 6882 (phenon III) and the type strains of *B. plantarii* (phenon VI), [*P.*] *cocovenenans*, and *B. glumae* (phenon V) had $T_{m(e)}$ values that were significantly higher (78.4 to 79.1°C) than the $T_{m(e)}$ values of the representatives of the other phena (75.2 to 76.7°C). *T. mixta* LMG 11547^T does indeed not belong in this rRNA complex since its DNA-rRNA hybrid with rRNA from *B. cepacia* LMG 1222^T had a $T_{m(e)}$ of about 70°C.

DNA-DNA hybridization. The unstandardized reassociation values for homoduplexes ranged from 69 to 100%. The levels of reassociation in control tubes containing only labeled DNA ranged from 5 to 13%. The T_m values for homoduplexes (in 0.42 M NaCl) ranged from 95 to 97.5°C. The levels of relative DNA homology at 70°C are shown in Table 5.

The two reference *B. cepacia* strains, LMG 1222^T and LMG 6964, which belong to different subclusters of phenon I, gave comparable results with all of the strains studied. Of the nine collection strains previously identified as *B. cepacia*, seven strains belonging to phenon I exhibited high levels of homology (up to 65%) with *B. cepacia* LMG 6964 and thus belong to this species. The two remaining strains (LMG 6998 and LMG 6999) and the 12 strains isolated from the rice rhizosphere constitute another DNA homology group. Within this group, which corresponds to phenon II (Fig. 1), the strains exhibited levels of homology of more than 85%, and they exhibited levels of homology of less than 50% and ΔT_m values ranging from 5 to 6°C with *B. cepacia* strains (members of phenon I). The members of both of these DNA homology groups exhibited 5 to 26% DNA relatedness with members of the other clusters belonging to this RNA complex and less than 5% DNA relatedness with other pseudomonad reference strains.

DISCUSSION

On the basis of the results of our DNA-rRNA hybridization experiments we concluded that the rRNA complex which we studied contains at least three separate rRNA branches (Fig. 2), namely, the [*B.*] *solanacearum* rRNA branch, the *B. cepacia* rRNA branch, and the [*A.*] *eutrophus* rRNA branch. Recent analyses of about 80 and 89 to 97% of relevant 16S ribosomal DNA sequences provided more details concerning the phylo-

TABLE 4. Results of the DNA-rRNA hybridization experiments performed with rRNAs from *B. cepacia* LMG 1222^T and [*A.*] *eutrophus* LMG 1199^T and DNAs from representatives of the different phena

Source of DNA	$T_{m(e)}$ (°C) of hybrid with rRNA from:	
	B. cepacia LMG 1222^T	[*A.*] *eutrophus* LMG 1199^T
Phenon I[a]		
B. cepacia LMG 1222^T	81.1	75.0
B. cepacia LMG 6998	80.2	
B. cepacia LMG 6883	80.4	
B. cepacia LMG 6993	81.1	
B. cepacia LMG 6888	81.1	
B. cepacia LMG 6981	80.6	
B. cepacia LMG 6988	79.6	
Phenon II		
B. vietnamiensis TVV116	80.5	
B. vietnamiensis TVV69	80.9	
B. vietnamiensis TVV75^T	81.0	
B. vietnamiensis TVV70	80.4	
B. vietnamiensis TVV131	80.1	
Phenon III		
B. gladioli LMG 6882	78.6	
B. gladioli LMG 2216^T	75.0	
Phenon IV		
B. caryophylli LMG 2299^T	75.5	75.0
Phenon V		
B. glumae LMG 2196^T	79.1	
Phenon VI		
B. plantarii LMG 9035^T	78.4	
Phenon VII		
[*P.*] *phenazinium* LMG 2247^T		74.5
Phenon VIII		
B. andropogonis LMG 2729^T	76.7	73.5
B. woodsii LMG 2362^T		74.0
Phenon IX		
[*B.*] *solanacearum* LMG 2299^T	75.2	75.5
[*B.*] *solanacearum* LMG 2297		75.5
Other [*B.*] *solanacearum* strains		
[*B.*] *solanacearum* LMG 2291		76.5
[*B.*] *solanacearum* LMG 2293		76.0
Phenon X		
"*P. oxalaticus*" LMG 2235		76.0
[*A.*] *eutrophus* LMG 1199^T		79.5
[*A.*] *eutrophus* LMG 1200		79.0
[*A.*] *eutrophus* LMG 1201		79.5
[*A.*] *eutrophus* LMG 1206		79.5
[*A.*] *eutrophus* LMG 1207		78.2
[*A.*] *eutrophus* LMG 1209		79.5
Other [*A.*] *eutrophus* strains		
[*A.*] *eutrophus* LMG 1202		78.5
[*A.*] *eutrophus* LMG 1203		79.5
[*A.*] *eutrophus* LMG 1204		80.0
[*A.*] *eutrophus* LMG 1205		80.0
Phenon XI		
[*B.*] *pickettii* ATCC 27511	75.3	
Other strains		
B. cocovenenans LMG 11626^T	77.7	
T. mixta LMG 11547^T	69.6	

[a] See Fig. 1.

genetic relationships among the members of the solanacearum rRNA complex (38, 73). It was shown that *B. cepacia* is closely related to *B. gladioli* and that [*B.*] *solanacearum* is closely related to [*B.*] *pickettii* (38, 73). Both groups constitute separate lineages. *B. caryophylli* can be considered a regular member of the *B. cepacia-B. gladioli* lineage (38), while [*P.*] *andropogonis* represents a somewhat more removed branch of this lineage. On the other hand, [*A.*] *eutrophus* is a further removed branch of the [*B.*] *solanacearum*-[*B.*] *pickettii* lineage (38). It is our experience that members of an rRNA branch that have $\Delta T_{m(e)}$ values of 4 to 5°C (18) can be considered members of a separate genus. Consequently, the *B. cepacia* rRNA branch, which contains, in addition to *B. cepacia* strains, strains representing auxanographic phena II (the N_2-fixing strains), III (*B. gladioli*), V (*B. glumae*), VI (*B. plantarii*) (Table 4), and [*P.*] *cocovenenans*, constitutes at least a separate genus. The ribosomal DNA sequencing results of Li et al. (38) clearly revealed that *B. caryophylli* (phenon IV) and [*P.*] *andropogonis* (phenon VIII) can be considered members of the same genus, as mentioned above. It is our strategy to propose generic status only when there are phenotypic and/or chemotaxonomic parameters available to describe and differentiate the genus. From the assimilation results it is evident that phena I to VI, phenon VIII, [*P.*] *cocovenenans*, *B. mallei*, *B. pseudomallei*, and *B. vandii* do share characteristics that can differentiate them as a taxonomic entity from the other phena representing the other lineage. Fatty acid profiles also differentiate the proposed new genus from the [*B.*] *solanacearum*-[*B.*] *pickettii* lineage since it has been shown that *B. cepacia*, *B. caryophylli*, *B. gladioli*, *B. glumae*, *B. plantarii*, [*P.*] *andropogonis*, *B. mallei*, and *B. pseudomallei* constitute a separate fatty acid cluster (31, 59, 63, 73). This fatty acid cluster is characterized by a relatively high percentage of the 3-hydroxy fatty acid 3-OH $C_{16:0}$ (17 to 38% of the total 3-hydroxy fatty acids), while this fatty acid is absent or present in small amounts in members of the [*B.*] *solanacearum*-[*B.*] *pickettii* lineage. 3-Hydroxy fatty acids are also present in the members of auxanographic phenon II and in [*P.*] *cocovenenans* (64). Because the recently proposed species *B. vandii* (63) has the same physiological and chemotaxonomic characteristics, it is also considered a member of this genus.

In summary, we conclude that (i) the members of phena I to VI and phenon VIII, [*P.*] *cocovenenans*, *B. vandii*, *B. mallei*, and *B. pseudomallei* constitute a separate genus, for which the name *Burkholderia* has to be retained because this taxon contains the type species, *B. cepacia*, and (ii) members of the other phenotypic groups and/or other rRNA lineages have to be removed from this genus. These organisms are [*B.*] *solanacearum*, [*B.*] *pickettii*, and [*A.*] *eutrophus*. As no data on the rRNA of [*P.*] *phenazinium* are available, we cannot decide whether this species also belongs in the emended genus *Burkholderia*. Within the genus *Burkholderia* as defined here, the intrageneric relationships were determined by performing DNA-DNA hybridization studies. No significant levels of DNA-DNA binding were observed between members of the clusters belonging to the *B. cepacia* rRNA branch (Fig. 2) except between members of phenon I (*B. cepacia*) and members of phenon II (the N_2-fixing organisms from Vietnam and two *B. cepacia* strains of clinical origin) (Tables 1 and 2). In addition, the $\Delta T_{m(e)}$ values revealed that the members of the phenotypic clusters designated phena I and II were closely related. However, the levels of DNA relatedness (less than 50% homology as determined by the S1 nuclease-trichloroacetic acid method) were not high enough to place these organisms in one species. Within phenon II high levels of DNA-DNA hybridization were found, leading to the conclusion that strains belonging to phenon II constitute a new species. We propose that this new species should be named *Burkholderia vietnamiensis* because the majority of the strains were isolated in Vietnam. On the basis of other DNA-DNA hybridization results (Table 5) and the DNA-rRNA hybridization results (Table 4), we concluded that the following species should be retained as separate taxa: *B. gladioli* (phenon III), *B. caryophylli* (phenon IV), *B. glumae*

TABLE 5. Results of DNA-DNA hybridization experiments

Source of unlabeled DNA	% Reassociation at 70°C with labeled DNA from:			
	B. cepacia LMG 1222^T	*B. cepacia* LMG 6964	*B. vietnamiensis* LMG 6998	*B. vietnamiensis* TVV70
Phenon I[a]				
Burkholderia cepacia LMG 1222^T	100	80	40 (5.0)[b]	41
Burkholderia cepacia LMG 6964			50	
Burkholderia cepacia LMG 6963		71		42
Burkholderia cepacia LMG 6888		77	40	
Burkholderia cepacia LMG 6981		71	47 (5.5)	
Burkholderia cepacia LMG 6988		68	40 (5.0)	
Burkholderia cepacia LMG 6997		65	35 (6.0)	
Phenon II				
Burkholderia vietnamiensis LMG 6998		48 (5.0)	100	
Burkholderia vietnamiensis LMG 6999		46 (5.0)	100	
Burkholderia vietnamiensis TVV70	35	46 (6.0)	88	100
Burkholderia vietnamiensis TVV69	38	47	92	95
Burkholderia vietnamiensis TVV71	35	47		93
Burkholderia vietnamiensis TVV74	33		85	94
Burkholderia vietnamiensis TVV72	32			98
Burkholderia vietnamiensis TVV75^T	34			102
Burkholderia vietnamiensis TVV115	31			103
Burkholderia vietnamiensis TVV116	34			99
Burkholderia vietnamiensis TVV127	32			95
Burkholderia vietnamiensis TVV128	28			108
Burkholderia vietnamiensis TVV131	36			99
Burkholderia vietnamiensis TVV135	36			101
Phenon III				
Burkholderia gladioli LMG 2216^T				21
Burkholderia gladioli LMG 6882		26	22.2	
Phenon IV				
Burkholderia caryophylli LMG 2155^T		10	8	12
Phenon V				
Burkholderia glumae LMG 2196^T			20	
Phenon VII				
[*Pseudomonas*] *phenazinium* LMG 2247^T		8	5	
Phenon IX				
[*Burkholderia*] *solanacearum* LMG 2299^T	24	5		
Phenon XI				
[*Burkholderia*] *pickettii* LMG 5942^T	9	19	10	16
Reference strains				
Brevundimonas diminuta LMG 1793^T	0			0
Pseudomonas aeruginosa LMG 6395	0			1
Pseudomonas stutzeri LMG 2333^T				1
Pseudomonas putida LMG 2257^T				1
Sphingomonas paucimobilis LMG 1227^T	0			5

[a] See Fig. 1.
[b] The values in parentheses are $\Delta T_{m(e)}$ values (in degrees Celsius).

(phenon V), *B. plantarii* (phenon VI), and *B. andropogonis* (phenon VIII). The names *B. woodsii* and *B. andropogonis* are probably synonyms. Although genotypic evidence concerning this possible synonymy is not yet available, we propose that these two species should be placed in one species, for which the name *Burkholderia andropogonis* is proposed on the basis of Rule 42 of the *International Code of Nomenclature of Bacteria* (37).

From the rRNA and DNA-DNA results of Yabuuchi et al. (73) it is evident that *B. mallei* and *B. pseudomallei* belong to a single genospecies, but it has been proposed that these two species should be kept separate for epidemiological and zoonotic reasons. *B. cocovenenans* has been described as exhibiting 60% DNA-DNA binding with *B. cepacia* ATCC 13945; our DNA-rRNA hybridization results showed that *B. cocovenenans* is genotypically less similar to *B. cepacia* than *B. vietnamiensis*. DNA-DNA hybridization data (64) confirmed the separate status of *B. cocovenenans*. For *B. vandii* (63) the DNA-DNA homology values obtained with *B. glumae* and *B. plantarii* were at the borderline of species separation, and the final conclusion was based on differences in utilization of carbon compounds. The differentiating auxanographic characteristics of all of the species belonging to the redefined genus *Burkholderia* are shown in Table 2. Our results corresponded rather well to those obtained for a few strains of each species (including the type strain) by Urakami et al. (63), who used 45 common substrates in their analysis. In the proposed emended description of the genus *Burkholderia* below we omit characteristics for which different results were obtained by us and Urakami et al. (63).

Emended description of the genus *Burkholderia*. *Burkholderia* (Yabuuchi, Kosako, Oyaio, Yano, and Ezaki 1992) (Burk. hold.er'i.a. M.L. dim. ending *-ia*; M.L. fem. n. *Burkholderia*, named after W. H. Burkholder, the American bacteriologist

TABLE 6. Characteristics that differentiate *B. cepacia*, *B. gladioli*, and *B. vietnamiensis*

Characteristic	*B. cepacia*	*B. vietnamiensis*	*B. gladioli*
Nitrogenase activity	−	+	−
Denitrification	−	+	−
Carbon sources used for growth			
L-Arabitol	+	−	−
Butylamine	+	−	−
Tryptamine	+	−	−
Adonitol	+	−	+
Diaminobutane	+	+	−
5-Ketogluconate	+	−	+
D-Tartrate	−	−	+
Mesaconate	−	−	+
Itaconate	−	+	−
Siderophore production			
Cepabactin	+	−	ND[a]
Pyochelin	+	−	ND
Ornibactin	+	+	ND

[a] ND, not determined.

who first discovered the etiological agent of sour skin of onions). *Burkholderia* cells are gram-negative, nonfermentative, straight rods that have a single polar flagellum or a tuft of polar flagella. A single species, *B. mallei*, is atrichous and nonmotile. Catalase is produced, and oxidase activity varies between species. The cellular fatty acids are characterized by the presence of 3-hydroxy $C_{16:0}$. The type strains of several species are characterized by the presence of two types of ornithine lipids. Most species grow at 40°C. All species can grow with the following substrates as sole carbon sources: glucose, glycerol, inositol, galactose, sorbitol, and mannitol. Some species are pathogenic for humans, animals, or plants. Isolated from plant material, soil, or clinical samples. Can be recognized on the basis of 16S rRNA characteristics (sequence and/or DNA-rRNA hybridization data). Most strains accumulate polyhydroxybutyrate as carbon reserve material and are capable of *ortho* cleavage of protocatechuate.

The G+C content is 59.0 to 69.5 mol%.

The type species is *B. cepacia* (73).

The emended genus *Burkholderia* contains the following species: *B. andropogonis* comb. nov., *B. caryophylli*, *B. cepacia*, *B. cocovenenans* comb. nov., *B. gladioli*, *B. glumae*, *B. mallei*, *B. plantarii*, *B. pseudomallei*, *B. vandii*, and *B. vietnamiensis* sp. nov. Descriptions of *B. caryophylli*, *B. cepacia*, *B. gladioli*, *B. glumae*, *B. mallei*, *B. plantarii*, *B. pseudomallei*, and *B. vandii* can be found elsewhere (2, 43, 63, 73). These taxa can be further differentiated on the basis of phenotypic characteristics (Table 2) and genotypic characteristics (Tables 4 and 5).

Description of *Burkholderia vietnamiensis* sp. nov. *Burkholderia vietnamiensis* (vi.et'na.mi.en.sis. M.L. adj. *vietnamiensis*, referring to Vietnam, the country where the rice strains were isolated). Motile cells that are 0.8 to 2 μm long and 0.3 to 0.8 μm wide. The colonies on nutrient agar are not pigmented; on King B medium colonies do not produce a fluorescent pigment. Growth occurs on nutrient agar at temperatures between 20 and 41°C. Not pathogenic for onions. Produces oxidase, catalase, β-galactosidase, and gelatinase; reduces nitrate to nitrite; and does not produce urease, indole, and arginine dihydrolase. All strains fix atmospheric nitrogen and produce ornibactin siderophores but not pyochelin or cepabactin. Auxanographic characteristics are shown in Table 2. The characteristics that differentiate this species from *B. cepacia* and *B. gladioli* are shown in Table 6. The G+C contents vary from to 66.9 to 68.1 mol%. Isolated from rice field soils and from clinical samples. When rRNA from *B. cepacia* LMG 1222^T is used, DNA-rRNA hybrids have $T_{m(e)}$ values similar to the $T_{m(e)}$ values found for members of *B. cepacia*. The species can be recognized on the basis of DNA-DNA homology data.

Type strain TVV75 has been deposited in the collection of the Laboratory of Microbiology in Ghent, Belgium, as strain LMG 10929. The characteristics of the type strain are the same as those described above for the species. The G+C content of the type strain is 67.9 mol%.

Description of *Burkholderia cocovenenans* (van Damme, Johannes, Cox, and Berends 1960) comb. nov. The description of *B. cocovenenans* is the description given by van Damme et al. (65) and Zhao et al. (74). The type strain is LMG 11626 (= ATCC 33664); the characteristics of the type strain are the same as those of the species. When rRNA from *B. cepacia* LMG 1222^T is used, the DNA-rRNA hybrid has a $T_{m(e)}$ of 77.7°C.

Description of *Burkholderia andropogonis* (Stapp 1928) comb. nov. *Pseudomonas andropogonis* (Smith 1911) stapp 1928; *Pseudomonas stizolobii* (Wolf 1920) Stapp 1935; *Aplanobacter stizolobii* (Wolf 1920); *Pseudomonas Woodsii* (Smith 1911) Stevens 1925. In addition to the description given by Palleroni (43), *B. andropogonis* strains do not grow on L-threonine, L-histidine, and L-tyrosine. More auxanographic characteristics are shown in Table 2. Can be differentiated from other *Burkholderia* species by its 16S rRNA sequence (38). Pathogenic for sorghum, corn, clover, carnation, and velvet bean. The G+C content varies from 59 to 61.3 mol%. The type strain is LMG 2199 (= NCPPB 934). The description of the type strain is the same as that of the species. The G+C content of the type strain is 59 mol%.

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