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*

A. WILLEMS,[†] M. GOOR, S. THIELEMANS, M. GILLIS,* K. KERSTERS, AND J. DE LEY

Laboratorium voor Microbiologie en microbiële Genetica, Rijksuniversiteit Gent,
K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium

DNA-rRNA hybridizations, DNA-DNA hybridizations, polyacrylamide gel electrophoresis of whole-cell proteins, and a numerical analysis of carbon assimilation tests were carried out to determine the relationships among the phylogenetically misnamed phytopathogenic taxa *Pseudomonas avenae*, *Pseudomonas rubrilineans*, "*Pseudomonas setariae*," *Pseudomonas cattleyae*, *Pseudomonas pseudoalcaligenes* subsp. *citrulli*, and *Pseudomonas pseudoalcaligenes* subsp. *konjaci*. These organisms are all members of the family *Comamonadaceae*, within which they constitute a separate rRNA branch. Only *P. pseudoalcaligenes* subsp. *konjaci* is situated on the lower part of this rRNA branch; all of the other taxa cluster very closely around the type strain of *P. avenae*. When they are compared phenotypically, all of the members of this rRNA branch can be differentiated from each other, and they are, as a group, most closely related to the genus *Acidovorax*. DNA-DNA hybridization experiments showed that these organisms constitute two genotypic groups. We propose that the generically misnamed phytopathogenic *Pseudomonas* species should be transferred to the genus *Acidovorax* as *Acidovorax avenae* and *Acidovorax konjaci*. Within *Acidovorax avenae* we distinguished the following three subspecies: *Acidovorax avenae* subsp. *avenae*, *Acidovorax avenae* subsp. *cattleyae*, and *Acidovorax avenae* subsp. *citrulli*. Emended descriptions of the new taxa are presented.

On the basis of the results of extensive phenotypic studies and DNA-rRNA and DNA-DNA hybridizations performed by Palleroni, Stanier, and their collaborators, the genus *Pseudomonas* was divided into five groups (15, 35, 37, 51). Additional phylogenetic data have shown that these groups are only very remotely related and that each of them in fact contains species belonging to several other genera (11, 12, 64). Most authors agree that the taxon *Pseudomonas* includes representatives of multiple genera (3, 11, 36, 50) along with the authentic pseudomonads, which are grouped around *Pseudomonas aeruginosa*. Although several studies have been performed, the taxonomy and nomenclature of the different groups and their relatives are far from resolved.

Pseudomonas rRNA group III (section III [35]) is part of the acidovorans rRNA complex in rRNA superfamily III (7) or the beta subclass of the *Proteobacteria* (49). In addition to the species belonging to *Pseudomonas* section III, the acidovorans rRNA complex comprises a wide variety of bacterial species that belong to several genera that are phylogenetically only remotely related to this rRNA complex (26, 39, 61). This confusing situation is gradually being corrected. In 1985 the genus *Comamonas* was revived (13), and later *Pseudomonas acidovorans* and *Pseudomonas testosteronei* were transferred to this genus as separate species (54). In a polyphasic study of the acidovorans rRNA complex, we revised the classification of several taxa in this group; *Xanthomonas ampelina* was transferred to the new genus *Xylophilus* (61), the genera *Hydrogenophaga* and *Acidovorax* were created for several *Pseudomonas* species (58, 60), and the genus *Variovorax* was created for *Alcaligenes par-*

adoxus (62). We have proposed that the acidovorans rRNA complex should be recognized as a new bacterial family, the *Comamonadaceae* (59).

In this paper we describe the relationships of the following phytopathogenic, generically misnamed *Pseudomonas* species and subspecies which belong to the acidovorans rRNA complex: *Pseudomonas avenae*, *Pseudomonas rubrilineans*, "*Pseudomonas setariae*," *Pseudomonas cattleyae*, *Pseudomonas pseudoalcaligenes* subsp. *citrulli*, and *Pseudomonas pseudoalcaligenes* subsp. *konjaci*.

Pseudomonas avenae was first described by Manns (30) as the cause of leaf blight on oats in Ohio. In 1922 Rosen (42) described a similar disease on foxtail in Arkansas but gave the pathogen a new name, "*Pseudomonas alboprecipitans*," because of physiological differences. Schaad et al. (45) compared isolates from diseased corn in Georgia and Florida with the previous descriptions of *Pseudomonas avenae* and "*Pseudomonas alboprecipitans*" and concluded that the latter name is a later synonym of *Pseudomonas avenae*. The name *Pseudomonas avenae* was retained on the list of Young et al. (66) and on the Approved Lists of Bacterial Names (47).

Red stripe disease on sugarcane is caused by *Phytoplasma rubrilineans* (29). In 1942 this species was transferred to the genus *Xanthomonas* (52), and in 1962 Hayward (22) extended its description and placed it in the genus *Pseudomonas*.

In 1934 Okabe (33) described brown stripe disease on *Setaria italica* and named the pathogen "*Bacterium setariae*." This species was later transferred to the genus *Pseudomonas* (44). Young et al. (66) considered "*Pseudomonas setariae*" to be a separate species, but it was not included on the Approved Lists (47) because the only extant strain was considered to be a member of *Pseudomonas avenae* (65).

"*Pseudomonas panici*" (19) was designated (66) a patho-

* Corresponding author.

[†] Present address: Department of Microbiology, Agricultural and Food Research Council Institute of Food Research, Reading Laboratory, Shinfield, Reading RG2 9AT, United Kingdom.

var of *Pseudomonas syringae* van Hall 1902 on the basis of the single named extant strain. This pathogen has been confused with *Pseudomonas panicis* (Nakata and Takimo 1922) Dowson 1943 (16), an unrelated pathogen isolated from *Panax quinquefolium* (35). None of these species was included on the Approved Lists (47).

"*Bacterium cattleyae*" (later transferred to the genus *Pseudomonas* [44]) was first isolated from orchids by Pavarino (38) together with three other pathogens, "*Bacillus farnetianus*," "*Bacillus pollacii*," and "*Bacterium krameriani*." According to Elliott (17) these four taxa were incompletely described and could not be sufficiently differentiated. Later, pathogenic isolates obtained from *Cattleya* and *Phalaenopsis* species in California, Italy, and The Philippines (1, 40) were identified as *Pseudomonas cattleyae*, which is also the only one of the names mentioned above that was included on the Approved Lists (47).

Two different plant diseases are ascribed to members of the species *Pseudomonas pseudoalcaligenes*. Leaf blight on watermelon, *Citrullus lanatus* (57), is caused by *Pseudomonas pseudoalcaligenes* subsp. *citrulli* (46), while *Pseudomonas pseudoalcaligenes* subsp. *konjaci* (18) is a pathogenic organism that has been isolated from the leaves of the konjac plant (*Amorphophallus konjac*).

MATERIALS AND METHODS

Bacterial strains. The strains which we studied are listed in Table 1. These organisms were grown as described previously (39, 62). For the sake of clarity, names of taxa which are generically misnamed according to our phylogenetic data are enclosed in brackets below.

Polyacrylamide gel electrophoresis of proteins. The strains were grown in Roux flasks for 40 h 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% [wt/vol] agar; pH 7.4) at 28°C. The method used to prepare whole-cell protein extracts and the procedure used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis have been described previously (28).

Isolation of high-molecular-weight DNA. Cells were grown for 2 to 3 days in Roux flasks on the solid medium described above. DNA was prepared by using the method of Marmur (31).

DNA base composition. The mean guanine-plus-cytosine (G+C) contents were determined by using the thermal denaturation method (10) and were calculated by using the equation of Marmur and Doty (32), as modified by De Ley (6).

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 (9). The amount of filter-fixed DNA was determined chemically (9, 55). ³H-labeled rRNA from [*Pseudomonas*] *avenae* NCPPB 1011^T (T = type strain) was prepared and purified by using the method described by De Ley and De Smedt (9). The specific activity of the 23S fraction was 95×10^3 dpm · μg⁻¹. 23S [³H]rRNAs from *Acidovorax facilis* ATCC 11228^T (specific activity, 63×10^3 dpm · μg⁻¹) and *Comamonas acidovorans* Stanier 14^T (60×10^3 dpm · μg⁻¹) and 23S [¹⁴C]rRNA from *Xylophilus ampelinus* NCPPB 2217^T (3×10^3 dpm · μg⁻¹) were available from members of our research group (11, 60, 61). Each hybrid was characterized by its $T_{m(e)}$ [$T_{m(e)}$ is the temperature, in degrees Celsius, at which 50% of a DNA-rRNA duplex is denatured].

DNA-DNA hybridization. The degrees of binding were determined by using the initial renaturation rate method (8) as described previously. Renaturations were performed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0) at the optimal renaturation temperature of 82.0°C, with a total DNA concentration of 0.106 mM base pairs. Degrees of binding of 25% or less indicated that there was no significant DNA homology.

Auxanographic features. We used API galleries (types API 50CH, API 50AO, and API 50AA; API System S. A., Montalieu-Vercieu, France) to verify the assimilation of 147 organic compounds as sole carbon sources. A total of 34 phytopathogenic *Pseudomonas* strains and 29 reference strains were tested by using the procedure described previously (27). Nine strains were tested in duplicate on separate occasions to verify the reproducibility of the test.

Numerical analysis of auxanographic features. The tests were scored as described previously (43). Of the 147 features tested, 25 were not included in the numerical analysis because they had the same quantitative value. The levels of interstrain similarity (*S*) were calculated by using a similarity coefficient derived from the Canberra metric coefficient (d_{CANB}) (48) and the following equation: $S = 100 \times (1 - d_{CANB})$. A cluster analysis was performed by using the unweighted average pair group method (48), the CLUSTAN 2.1 program of Wishart (63), and the Siemens model 7570-C computer of the Centraal Digitaal Rekencentrum, Rijksuniversiteit, Ghent, Belgium.

RESULTS AND DISCUSSION

Generic and suprageneric relationships of the phytopathogenic [*Pseudomonas*] species belonging to the acidovorans rRNA complex. Previously published DNA-rRNA hybridization data (12, 58) have shown that [*Pseudomonas*] *avenae*, [*Pseudomonas*] *cattleyae*, [*Pseudomonas*] *rubrilineans*, "[*Pseudomonas*] *setariae*," and [*Pseudomonas pseudoalcaligenes*] subsp. *citrulli* belong to the acidovorans rRNA complex in rRNA superfamily III. Hybridizations with labeled rRNA from [*Pseudomonas*] *avenae* NCPPB 1011^T (Tables 2 and 3) showed that these phytopathogenic species form a single separate rRNA branch within this complex. These five species formed a tight phylogenetic cluster ($T_{m(e)}$ values versus [*Pseudomonas*] *avenae* NCPPB 1011^T, 81.5 to 80.6°C (Table 2)). Strains of [*Pseudomonas pseudoalcaligenes*] subsp. *konjaci* had $T_{m(e)}$ values of 77.2 to 77.9°C versus strain NCPPB 1011^T and 74.5 to 76.9°C versus rRNAs from the type strains of *Acidovorax facilis*, *C. acidovorans*, and *Xylophilus ampelinus* (Table 2). Therefore, [*Pseudomonas pseudoalcaligenes*] subsp. *konjaci* is located low on the [*Pseudomonas*] *avenae* rRNA branch and is clearly separated from the top cluster containing the other five species. Except for several *Aquaspirillum* strains and some *Acidovorax* strains, no other strain yielded $T_{m(e)}$ values higher than 76.5°C in hybridizations with rRNA from [*Pseudomonas*] *avenae*. Since the values for reciprocal hybridizations (e.g., between DNAs from phytopathogenic strains and rRNAs from the *Aquaspirillum* strains or from *Acidovorax facilis* [39] [Table 2]) were not significantly higher than 77°C, these aquaspirillae and *Acidovorax* strains are not members of the [*Pseudomonas*] *avenae* rRNA branch. The link between the new rRNA branch and the other rRNA branches in the acidovorans rRNA complex was $76.0 \pm 1.1^\circ\text{C}$; this was the mean $T_{m(e)}$ obtained in hybridizations between DNAs from representatives of the acidovorans rRNA complex and rRNA from [*Pseudomonas*] *avenae* NCPPB 1011^T (Table 3).

TABLE 1. Strains used

Name as received	Designation as received ^a	Other strain designation(s) ^a	Source and place and year of isolation
Organisms assigned to <i>Acidovorax</i>			
<i>avenae</i> subsp. <i>avenae</i> [<i>Pseudomonas</i>] <i>avenae</i>	NCPPB 1011 ^T	LMG 2117 ^T , ATCC 19860 ^T , CCUG 15838 ^T	<i>Zea mays</i> , United States, 1958
[<i>P.</i>] <i>avenae</i>	NCPPB 2399	LMG 2118	<i>Oryza sativa</i> , The Philippines
[<i>P.</i>] <i>avenae</i>	NCPPB 2401	LMG 2119	<i>Zea mays</i> , Japan, 1966
[<i>P.</i>] <i>avenae</i>	CCM 2761	LMG 5703, CCUG 18836	<i>Zea mays</i>
[<i>P.</i>] <i>avenae</i>	CCM 2762	LMG 5704, CCUG 18837	<i>Zea mays</i>
[<i>P.</i>] <i>avenae</i>	CCUG 18836	LMG 7378	<i>Zea mays</i>
[<i>P.</i>] <i>avenae</i>	ICMP 3178	LMG 6422	Leaf, <i>Zea mays</i> , Iwata, Japan, 1970
[<i>P.</i>] <i>avenae</i>	ICMP 3180	LMG 6423	Leaf, <i>Zea mays</i> , Iwata, Japan, 1970
[<i>P.</i>] <i>avenae</i>	ICMP 3182	LMG 6424	Leaf, <i>Zea mexicana</i> , Japan, 1970
[<i>P.</i>] <i>avenae</i>	ICMP 3254	LMG 6425	Leaf, <i>Zea mexicana</i> , Japan, 1968
[<i>P.</i>] <i>avenae</i>	ICMP 7083	LMG 6426	<i>Eleusine coracana</i> , brown stripe on sheath and leaf blade, Japan, 1975
[<i>P.</i>] <i>avenae</i>	ICMP 7084	LMG 6427	<i>Eleusine coracana</i> , brown stripe on sheath and leaf blade, Japan, 1975
[<i>Pseudomonas</i>] <i>rubrilineans</i>	NCPPB 359	LMG 2280	<i>Saccharum officinarum</i> , Mauritius, 1955
[<i>P.</i>] <i>rubrilineans</i>	NCPPB 920 ^T	LMG 2281 ^T , ATCC 19307 ^T , CCUG 15837 ^T	<i>Saccharum officinarum</i> cv. R445, Réunion, 1960
[<i>P.</i>] <i>rubrilineans</i>	NCPPB 1118	LMG 2282	<i>Saccharum officinarum</i> , India, 1961
[<i>P.</i>] <i>rubrilineans</i>	ICMP 1656	LMG 6428	<i>Zea mays</i> var. <i>rugosa</i> , Florida, 1963
[<i>P.</i>] <i>rubrilineans</i>	ICMP 3107	LMG 6429	<i>Zea mays</i> , Ludhiana, India, 1969
[<i>P.</i>] <i>rubrilineans</i>	ICMP 3166	LMG 6430	<i>Zea mays</i> cv. CM300, Delhi, India
[<i>P.</i>] <i>rubrilineans</i>	ICMP 3168	LMG 6431	<i>Zea mexicana</i> , Delhi, India
[<i>P.</i>] <i>rubrilineans</i>	ICMP 5811	LMG 6432	<i>Zea mays</i> , Brazil, 1976
[<i>P.</i>] <i>rubrilineans</i>	ICMP 7460	LMG 6433	<i>Canna indica</i> , Brazil, 1978
"[<i>Pseudomonas</i>] <i>setariae</i> "	IRRI ST3	LMG 6516	<i>Oryza sativa</i> , The Philippines, 1980
"[<i>P.</i>] <i>setariae</i> "	IRRI ST10	LMG 6517	<i>Oryza sativa</i> , The Philippines, 1980
"[<i>P.</i>] <i>setariae</i> "	NCPPB 1392	LMG 1806, ATCC 19882, CCUG 15836	<i>Oryza sativa</i> , Japan, 1956
Organisms assigned to <i>Acidovorax</i>			
<i>avenae</i> subsp. <i>cattleyae</i> [<i>Pseudomonas</i>] <i>cattleyae</i>	NCPPB 961 ^T	LMG 2364 ^T , LMG 5286 ^T , ATCC 33619 ^T , CCUG 21975 ^T	1950
[<i>P.</i>] <i>cattleyae</i>	NRRL B-732	LMG 5142, ATCC 10200	Infected leaf of <i>Cattleya</i> sp.
Organisms assigned to <i>Acidovorax</i>			
<i>avenae</i> subsp. <i>citrulli</i> [<i>Pseudomonas pseudoalcaligenes</i>] subsp. <i>citrulli</i>	NCPPB 3055	LMG 5483	<i>Citrullus lanatus</i>
[<i>P. pseudoalcaligenes</i>] subsp. <i>citrulli</i>	ICMP 6521	LMG 2254	<i>Citrullus lanatus</i> , water-soaked lesion on seedling, United States, 1978
[<i>P. pseudoalcaligenes</i>] subsp. <i>citrulli</i>	ICMP 6522	LMG 2255	<i>Citrullus lanatus</i> , water-soaked lesion on seedling, United States, 1977
[<i>P. pseudoalcaligenes</i>] subsp. <i>citrulli</i>	ICMP 7500 ^T	LMG 5376 ^T , ATCC 29625 ^T , CCUG 1739 ^T	<i>Citrullus lanatus</i> , water-soaked lesion on cotyledon, United States, 1977
Organisms assigned to <i>Acidovorax konjaci</i>			
[<i>Pseudomonas pseudoalcaligenes</i>] subsp. <i>konjaci</i>	ICMP 7733 ^T	LMG 5691 ^T , ATCC 33996 ^T , CCUG 17394 ^T	<i>Amorphophallus rivieri</i> cv. Konjac, Shizuoka Prefecture, Japan, 1977
[<i>P. pseudoalcaligenes</i>] subsp. <i>konjaci</i>	ICMP 7734	LMG 5692	<i>Amorphophallus rivieri</i> cv. Konjac, Shizuoka Prefecture, Japan, 1977
[<i>P. pseudoalcaligenes</i>] subsp. <i>konjaci</i>	ICMP 7851	LMG 5693	<i>Amorphophallus rivieri</i> cv. Konjac, Shizuoka Prefecture, Japan, 1977
Reference strains			
<i>Acidovorax delafieldii</i>	ATCC 17505 ^T	LMG 5943 ^T , CCUG 1779 ^T , ATCC 17505 ^T	Soil enriched with PHB as a sole carbon source ^c
<i>A. delafieldii</i>	ATCC 17506t1 ^b	LMG 1792t1, CCUG 14277	Soil enriched with PHB as a sole carbon source
<i>A. delafieldii</i>	CCUG 23830B	LMG 8909	Central venous catheter, 54-yr-old male, Göteborg, Sweden, 1989
<i>Acidovorax facilis</i>	ATCC 11228 ^T	LMG 2193 ^T , CCUG 2113 ^T	Lawn soil, United States
<i>A. facilis</i>	ATCC 15376	LMG 2194, CCUG 14278	Unknown
<i>A. facilis</i>	DSM 550	LMG 6598, CCUG 15919, ATCC 17695	Lawn soil

Continued on following page

TABLE 1—Continued

Name as received	Designation as received ^a	Other strain designation(s) ^a	Source and place and year of isolation
<i>Acidovorax temperans</i>	CIP 239.74	LMG 3332, CCUG 21717	Blood culture, France
<i>A. temperans</i>	CIP 471.74	LMG 3334, CCUG 21716	Unknown
<i>A. temperans</i>	CCUG 11779 ^T	LMG 7169 ^T	Urine, 68-yr-old male, Göteborg, Sweden, 1973
<i>A. temperans</i>	CCUG 16573	LMG 6437	Cervix, Göteborg, Sweden, 1984.
[<i>Alcaligenes</i>] <i>latus</i>	Palleroni-H4 ^T	LMG 3321 ^T , ATCC 29712 ^T	Soil, California
[<i>Aquaspirillum</i>] <i>anulus</i>	NCIB 9012 ^T	LMG 5404 ^T	Pond water
[<i>Aquaspirillum</i>] <i>delicatum</i>	NCIB 9419 ^T	LMG 4327 ^T , ATCC 14667 ^T	Distilled water
[<i>Aquaspirillum</i>] <i>giesbergeri</i>	ATCC 11334 ^T	LMG 4332 ^T	Pond water
[<i>Aquaspirillum</i>] <i>gracile</i>	ATCC 19625	LMG 4334	Pond water
[<i>Aquaspirillum</i>] <i>metamorphum</i>	NCIB 9509 ^T	LMG 4338 ^T , ATCC 15280 ^T	Putrid infusion of freshwater shellfish
[<i>Aquaspirillum</i>] <i>psychrophilum</i>	IFO 13611 ^T	LMG 5408 ^T	<i>Ceratodon purpureus</i> , Antarctica
[<i>Aquaspirillum</i>] <i>sinuosum</i>	ATCC 9786 ^T	LMG 4347 ^T	Freshwater
<i>Comamonas acidovorans</i>	Stanier 14 ^T	LMG 1226 ^T , ATCC 15668 ^T , CCUG 14481 ^T	Soil enriched with acetamide, Delft, The Netherlands, 1926
<i>C. acidovorans</i>	ATCC 17406	LMG 1790, CCUG 15340	Soil enriched with <i>p</i> -hydroxybenzoate
<i>C. acidovorans</i>	ATCC 17476	LMG 1791, CCUG 15337	Great Britain
<i>Comamonas terrigena</i>	NCIB 8193 ^T	LMG 1253 ^T , CCUG 2185 ^T , ATCC 8461 ^T	Hay infusion filtrate, United States
<i>C. terrigena</i>	ATCC 11330	LMG 2370, CCUG 17395	Freshwater
<i>C. terrigena</i>	CCUG 2601	LMG 5323	Feces, Göteborg, Sweden, 1973
<i>Comamonas testosteroni</i>	NCTC 10698 ^T	LMG 1786 ^T , CCUG 1426 ^T , ATCC 1196 ^T	Soil, Berkeley, Calif.
<i>C. testosteroni</i>	ATCC 17407	LMG 1787, CCUG 15341	Soil enriched with anthranilate
<i>C. testosteroni</i>	ATCC 17409	LMG 1788, CCUG 15339	Soil enriched with kynurenate, Berkeley, Calif., 1963
<i>Hydrogenophaga flava</i>	DSM 619 ^T	LMG 2185 ^T , CCUG 1658 ^T , ATCC 33667 ^T	Mud from ditch, 1942
<i>Hydrogenophaga palleronii</i>	Stanier 362t1 ^{Tb}	LMG 2366t1 ^T (= CCUG 20334 ^T), ATCC 17724 ^T (= CCUG 1780 ^T)	Water enriched for hydrogen bacteria in an atmosphere containing 6% O ₂
<i>H. palleronii</i>	RH2	LMG 6348, CCUG 20338	Göttingen, Germany
<i>H. palleronii</i>	Z32	LMG 6347, CCUG 17388, CCUG 20337	USSR
<i>Hydrogenophaga pseudoflava</i>	GA3 ^T	LMG 5945 ^T , CCUG 13799 ^T , ATCC 33668 ^T	Water, River Weende, Germany
<i>H. pseudoflava</i>	GA2	LMG 6351, CCUG 17389, CCUG 20339	Unknown
<i>H. pseudoflava</i>	Z-1107	LMG 8355, CCUG 22764	Mud and soil, River Moskwa, USSR
<i>Hydrogenophaga taeniospiralis</i>	DSM 2082 ^T	LMG 7170 ^T , CCUG 15921 ^T	Soil, Spain
<i>Pseudomonas pseudoalcaligenes</i>	Stanier 63 ^T	LMG 1225 ^T , ATCC 17440 ^T	Sinus drainage
<i>P. pseudoalcaligenes</i> subsp. <i>pseudoalcaligenes</i>	CIP 60.76	LMG 5516, ATCC 17443	Pharyngeal washings
<i>P. pseudoalcaligenes</i> subsp. <i>pseudoalcaligenes</i>	CIP 61.21	LMG 5517, ATCC 17442	Contaminated culture medium
<i>P. pseudoalcaligenes</i> subsp. <i>pseudoalcaligenes</i>	CCUG 15237	LMG 6036	Metalworking fluids, Göteborg, Sweden, 1984
<i>P. pseudoalcaligenes</i> subsp. <i>pseudoalcaligenes</i>	CCUG 15284	LMG 6037	Metalworking fluids, Göteborg, Sweden, 1984
[<i>Pseudomonas</i>] <i>saccharophila</i>	ATCC 15946 ^T	LMG 7831 ^T , LMG 2256 ^T	Mud, San Francisco Bay, Calif., 1940
<i>Rubrivivax gelatinosus</i>	NCIB 8290 ^T	LMG 4311 ^T , ATCC 17011 ^T	Acetate enrichment, pH 6.6, 1944
<i>Sphaerotilus natans</i>	NCIB 11197t1 ^T	LMG 7172t1 ^T	Hay infusion
<i>Variovorax paradoxus</i>	ATCC 17549t1 ^b	LMG 1796t1, CCUG 1778	Soil enriched with pantothenate
<i>V. paradoxus</i>	ATCC 17713t1 ^{Tb}	LMG 1797t1 ^T , CCUG 1777 ^T	Soil in mineral medium under an atmosphere containing 91% H ₂ , 4% O ₂ , and 5% CO ₂
<i>V. paradoxus</i>	ATCC 17722	LMG 3575, DSM 647	Water with an atmosphere containing 89% H ₂ , 6% O ₂ , and 5% CO ₂
<i>Xylophilus ampelinus</i>	NCPPB 2217 ^T	LMG 5856 ^T , ATCC 33914 ^T , CCUG 21976 ^T	<i>Vitis vinifera</i> var. <i>sultana</i> , Crete

^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; CCM, Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia; CIP, Collection de l'Institut Pasteur, Paris, France; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; IRRI, International Rice Research Institute, Los Baños, The Philippines; 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; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; NRRL, Agricultural Research Service Culture Collection, Peoria, Ill.; ICMP, Culture Collection of the Plant Disease Division, New Zealand Department of Scientific and Industrial Research, Auckland, New Zealand.

^b We isolated two stable colony types from the original culture and labeled them t1 and t2. Since both types produced almost identical protein electrophoretic patterns, we only used colony type t1.

^c PHB, poly-β-hydroxybutyrate.

TABLE 2. DNA base compositions and $T_{m(e)}$ values of hybrids between DNAs from phytopathogenic [*Pseudomonas*] strains and labeled rRNAs from *Acidovorax avenae* NCPPB 1011^T, *Acidovorax facilis* ATCC 11228^T, *Comamonas acidovorans* Stanier 14^T, and *Xylophilus ampelinus* NCPPB 2217^T

DNA from strain:	Mean G+C content (mol%)	$T_{m(e)}$ (°C) vs [³ H]rRNA from:			$T_{m(e)}$ (°C) vs [¹⁴ C]rRNA from <i>Xylophilus ampelinus</i> NCPPB 2217 ^T
		<i>Acidovorax avenae</i> NCPPB 1011 ^T	<i>Acidovorax facilis</i> ATCC 11228 ^T	<i>Comamonas acidovorans</i> Stanier 14 ^T	
<i>Acidovorax avenae</i> subsp. <i>avenae</i> NCPPB 1011 ^T	69.8 ^a	80.6	76.6 ^b	76.6 ^c	76.4 ^c
<i>A. avenae</i> subsp. <i>avenae</i> NCPPB 2399	67.8 ^d			77.0 ^d	
<i>A. avenae</i> subsp. <i>avenae</i> NCPPB 2401	66.8 ^d			75.5 ^d	
<i>A. avenae</i> subsp. <i>avenae</i> CCM 2761	68.6	81.0	76.8	76.4	
<i>A. avenae</i> subsp. <i>avenae</i> CCM 2762	68.9	80.7	76.0	76.9	
<i>A. avenae</i> subsp. <i>avenae</i> NCPPB 359	68.1 ^d			76.0 ^d	
<i>A. avenae</i> subsp. <i>avenae</i> NCPPB 920	69.0 ^d	81.3	76.8	77.5 ^d	77.7 ^c
<i>A. avenae</i> subsp. <i>avenae</i> NCPPB 1118	69.6 ^d			76.5 ^d	
<i>A. avenae</i> subsp. <i>avenae</i> NCPPB 1392	69.1 ^d	81.5	76.7	78.0 ^d	77.4 ^c
<i>Acidovorax avenae</i> subsp. <i>cattleyae</i> NCPPB 961 ^T	68.6 ^d	76.4	75.2	77.2 ^c	
<i>A. avenae</i> subsp. <i>cattleyae</i> NRRL B-732	69.1			76.1	
<i>Acidovorax avenae</i> subsp. <i>citrulli</i> ICMP 7500 ^T	68.4	80.6	76.1	76.5 ^c	76.9 ^c
<i>A. avenae</i> subsp. <i>citrulli</i> ICMP 6521	68.1 ^d			77.0 ^d	
<i>A. avenae</i> subsp. <i>citrulli</i> ICMP 6522	67.2 ^d			77.0 ^d	
<i>Acidovorax konjaci</i> ICMP 7733 ^T	68.4	77.2	75.5	76.3	76.9
<i>A. konjaci</i> ICMP 7734	67.7	77.4	75.7	75.7	
<i>A. konjaci</i> ICMP 7851	68.1	77.9	75.2	74.5	

^a Data from reference 58.

^b Data from reference 60.

^c Data from reference 61.

^d Data from reference 12.

TABLE 3. $T_{m(e)}$ values of the hybrids between DNAs from several reference strains belonging to the acidovorans rRNA complex and rRNA from *Acidovorax avenae* NCPPB 1011^T

DNA from strain:	$T_{m(e)}$ (°C) vs [³ H]rRNA from <i>Acidovorax avenae</i> NCPPB 1011 ^T
<i>Acidovorax delafeldii</i> ATCC 17505 ^T	77.3
<i>A. delafeldii</i> ATCC 17506 ^T	77.2
<i>Acidovorax facilis</i> ATCC 11228 ^T	78.0
<i>A. facilis</i> ATCC 15376	76.3
<i>Acidovorax temperans</i> CIP 239.74	77.3
<i>A. temperans</i> CIP 471.74	78.0
[<i>Alcaligenes</i>] <i>latus</i> Palleroni-H4 ^T	72.8
[<i>Aquaspirillum</i>] <i>anulus</i> NCIB 9012 ^T	77.4
[<i>Aquaspirillum</i>] <i>delicatum</i> NCIB 9419 ^T	77.5
[<i>Aquaspirillum</i>] <i>giesbergeri</i> ATCC 11334 ^T	77.0
[<i>Aquaspirillum</i>] <i>gracile</i> ATCC 19625	77.4
[<i>Aquaspirillum</i>] <i>metamorphum</i> NCIB 9509 ^T	77.3
[<i>Aquaspirillum</i>] <i>psychrophilum</i> IFO 13611 ^T	74.2
[<i>Aquaspirillum</i>] <i>sinuosum</i> ATCC 9786 ^T	77.6
<i>Comamonas acidovorans</i> Stanier 14 ^T	75.9
<i>Comamonas terrigena</i> NCIB 8193 ^T	75.7
<i>C. terrigena</i> ATCC 11330	76.3
<i>Comamonas testosteroni</i> NCTC 10698 ^T	75.7
<i>Hydrogenophaga palleronii</i> Stanier 362t1 ^T	76.2
<i>Hydrogenophaga pseudoflava</i> GA3 ^T	76.5
[<i>Pseudomonas</i>] <i>saccharophila</i> ATCC 15946 ^T	73.1
<i>Rubrivivax gelatinosus</i> NCIB 8290 ^T	72.4
<i>Sphaerotilus natans</i> NCIB 11197t1 ^T	72.5
<i>Variovorax paradoxus</i> ATCC 17713t1 ^T	76.4
<i>Xylophilus ampelinus</i> NCPPB 2217 ^T	76.4

and in hybridizations between DNAs from strains belonging to the [*Pseudomonas*] *avenae* branch and rRNAs from members of the other branches in the acidovorans rRNA complex (Table 2) (39, 58, 60, 62).

The rRNA branch containing the type species, *Pseudomonas aeruginosa*, is part of rRNA superfamily II (11) in the gamma subclass of the *Proteobacteria* and represents the authentic pseudomonads. *Pseudomonas pseudoalcaligenes* subsp. *pseudoalcaligenes* is part of this group (11). Phylogenetically, it is evident that all *Pseudomonas* species belonging to the acidovorans rRNA complex (rRNA superfamily III or the beta subclass of the *Proteobacteria*) should be transferred from the genus *Pseudomonas* to a more suitable genus, either existing or newly created. To accommodate the group of phytopathogenic species belonging to the [*Pseudomonas*] *avenae* rRNA branch, a new genus, or the following previously described genera might be candidates: *Acidovorax* (60), *Comamonas* (54), *Hydrogenophaga* (58), *Variovorax* (59), and *Xylophilus* (61). The genus *Xylophilus* is ruled out because it contains only yellow-pigmented strains, is isolated exclusively from infected vines, and grows extremely slowly even at its optimal temperature of 24°C. The genus *Hydrogenophaga* contains only yellow-pigmented hydrogen-oxidizing strains, and the genus *Variovorax* contains yellow-pigmented strains with much greater metabolic versatility than the strains belonging to the [*Pseudomonas*] *avenae* rRNA branch. The genus *Comamonas* contains strains that have bipolar tufts of flagella, are not phytopathogenic, and have a carbon substrate assimilation pattern different from that of the phytopathogenic pseudomonads (Fig. 1). The genus *Acidovorax* is more suitable phenotypically. In the dendrogram obtained by performing a numerical analysis of auxanographic features (Fig. 1), the phytopathogenic species formed a separate cluster that was linked most closely to the *Acidovorax* species. Clear-cut phenotypic

FIG. 1. Dendrogram obtained from unweighted average pair group cluster analysis of similarity coefficients of *Acidovorax* strains and representative reference strains belonging to the *Comamonadaceae*. A total of 122 auxanographic tests were used to produce the similarity coefficients derived from the Canberra metric distance coefficient as follows: $S = 100 \times (1 - d_{CANB})$. A., *Acidovorax*; C., *Comamonas*; H., *Hydrogenophaga*; V., *Variovorax*.

differentiation from this genus was not possible (Table 4). Because of the high level of phenotypic similarity and the high level of rRNA cistron similarity, we propose that the phytopathogenic species should be transferred to an emended genus *Acidovorax*. The fact that the various species of this genus occur in different ecosystems (soil, water, and clinical and plant material) is remarkable but not unique. A similar situation is found in the genus *Xanthomonas* (53). Characteristics that differentiate the emended genus *Acidovorax*

from the other genera in the acidovorans rRNA complex are shown in Table 5.

Relationships of the phytopathogenic strains within the genus *Acidovorax*. We studied the species and infraspecific relationships by using protein gel electrophoresis, DNA-DNA hybridization, DNA-rRNA hybridization, and numerical analysis of auxanographic features. Since the phytopathogenic strains formed a separate rRNA branch (Fig. 2), they were not considered members of the three previously recognized *Acidovorax* species. This was confirmed by the absence of any significant levels of DNA binding between representative strains (Fig. 3) (25). Our results indicated that two main DNA groups of phytopathogenic strains were present.

The first group contained the strains of [*Pseudomonas*] *avenae*, [*Pseudomonas*] *cattleyae*, [*Pseudomonas pseudoalcaligenes*] subsp. *citrulli*, [*Pseudomonas*] *rubriligneans*, and "[*Pseudomonas*] *setariae*." Strains of these taxa had levels of DNA binding of 54 to 100%. Within this group three subgroups were delineated at levels of DNA binding of 54 to 68% (Fig. 3). The first subgroup comprised strains of [*Pseudomonas*] *avenae*, [*Pseudomonas*] *rubriligneans*, and "[*Pseudomonas*] *setariae*," with levels of DNA binding of 75 to 100%. These organisms had very similar protein patterns (Fig. 4), and in the auxanographic analysis all of these strains except strain NCPPB 2399 clustered at an *S* value of more than 91% (Fig. 1); strain NCPPB 2399 was aberrant because it failed to grow on L-arabinose, D-galactose, and D-fucose (Table 6). The names of these three species can be considered synonymous, as was shown previously for [*Pseudomonas*] *rubriligneans* and [*Pseudomonas*] *avenae* by Ramundo and Claflin (41). Although the previous descriptions of [*Pseudomonas*] *avenae*, [*Pseudomonas*] *rubriligneans*, and "[*Pseudomonas*] *setariae*" (20, 22, 24, 45) were rather limited, according to current standards these organisms exhibit high levels of similarity. Phytopathological data and host range studies also support the synonymy of [*Pseudomonas*] *avenae*, [*Pseudomonas*] *rubriligneans* and "[*Pseudomonas*] *setariae*." All of these species cause analogous symptoms on different members of the Poaceae. Usually water-soaked, longitudinal stripes are formed (2, 20, 34), and bacterial exudates appear (2, 5, 34). In a later stage the lesions turn reddish or dark brown (4, 5, 34). Recently, several [*Pseudomonas*] *avenae* strains have been reported to be very different from "[*Pseudomonas*] *setariae*" and [*Pseudomonas*] *rubriligneans* on the basis of the results of electrophoresis of proteins (56). In our analysis, the opposite was found. We included the "[*Pseudomonas*] *setariae*" and [*Pseudomonas*] *rubriligneans* strains that were used by Van Zyl and Steyn (56), but not their [*Pseudomonas*] *avenae* strains. The low correlation coefficients which Van Zyl and Steyn reported for their [*Pseudomonas*] *avenae* strains contrasts with the very homogeneous protein patterns obtained by us for other [*Pseudomonas*] *avenae* strains.

The second subgroup contained two [*Pseudomonas*] *cattleyae* strains with a level of DNA binding of 95% (Fig. 3). The protein patterns of these organisms (Fig. 4) were very similar to those of members of the first subgroup. These strains formed a separate phenon, which was linked to the [*Pseudomonas*] *avenae* phenon at an *S* value of 87% (Fig. 1). One of the strains in this subgroup was the type strain of [*Pseudomonas*] *cattleyae*. Previously, it has been shown that two other strains of this taxon (strains NCPPB 1874 and ICMP 3992) belong to the *Pseudomonas fluorescens* rRNA branch and are not related to the [*Pseudomonas*] *cattleyae* type strain (12). The phenotypic description of [*Pseudomo-*

TABLE 4. Differentiation between the *Acidovorax* species and subspecies

Characteristic	<i>Acidovorax</i> <i>avenae</i> subsp. <i>avenae</i> (n = 24) ^a	<i>Acidovorax</i> <i>avenae</i> subsp. <i>cattleyae</i> (n = 2)	<i>Acidovorax</i> <i>avenae</i> subsp. <i>citrulli</i> (n = 4)	<i>Acidovorax</i> <i>konjac</i> (n = 3)	<i>Acidovorax</i> <i>facilis</i> (n = 4)	<i>Acidovorax</i> <i>delafieldii</i> (n = 14)	<i>Acidovorax</i> <i>temperans</i> (n = 18)
Growth on ^b :							
L-Arabinose or D-galactose	+	+	+	—	+	+	—
Ribose	d	+	+	+	+	+	—
D-Xylose	+	—	d	—	—	—	—
D-Glucose	+	+	+	—	+	+	+
D-Mannose	—	—	—	d	+	+	—
D-Fucose	+	+	d	—	—	+ ^d	—
Mannitol or D-arabitol	+	+	—	+	+	+	d
Sorbitol	+	+	—	—	+	+	d
Isobutyrate	+	+	d	+	—	d	d
Isovalerate	+	+	—	+	+ ^d	+	+
n-Caproate	d	+	—	—	—	d	d
Malonate	—	—	—	+	—	—	—
Aconitate	d	—	—	+	—	d	—
Adipate	+	+	+	+	—	+	+
Pimelate	+	+	+	+	d	+	+
2-Ketogluconate	—	—	d	—	—	+	d
D-Tartrate	+	+	+	+	—	d	—
m-Tartrate	d	—	+ ^d	+	—	d	d
Citraconate	+	+	+	+	—	+	d
2-Ketoglutarate	+	+	+	+	—	+ ^d	d
Citrate	d	+	d	d	—	d	—
p-Hydroxybenzoate	—	—	—	—	d	+	d
L-Threonine or L-histidine	+	+	—	+	+	+	d
L-Tryptophan	+	+	d	+	+ ^d	d	—
DL-3-Aminobutyrate	d	+	d	+	—	—	—
DL-2-Aminobutyrate	d	+	—	—	—	—	d
Acetamide	d	+	—	—	—	—	—
Ethanolamine	+	+	+	—	—	—	—
Nitrate reduction	+ ^{e,f}	+ ^f	— ^g	+ ^h	+	+	+
Gelatinase	Strain dependent ^{e,f}	—	+ ^d	—	+	d	—
Pathogenicity on:							
Watermelon	— ^g		+ ^g	Hypersensitive reaction ^h			
Cantaloupe	— ^g		+ ^g				
Squash	— ^g		+ ^g				
Cucumber	— ^g		+ ^g				
Tomato	— ^g		— ^g				
Cowpea	— ^g		— ^g				
Corn	+ ^g		— ^g				
Konjac			— ^h	+ ^h			
<i>Phalaenopsis</i> sp.		+ ^f					
<i>Cattleya</i> sp.		+ ^f					
G+C content (mol%)	67.8–69.8	68.6–68.9	67.2–68.4	67.7–68.4	64–65	65–66	62–66
T _{m(e)} (°C) vs rRNA from:							
<i>Acidovorax avenae</i> NCPPB 1011 ^T	80.6–81.5	80.8	80.6	77.2–77.9	76.3–78.0	77.2–77.3	77.3–78.0
<i>Acidovorax facilis</i> ATCC 11228 ^T	76.0–76.8	76.4	76.1	75.2–75.7	79.3–80.6	77.2–80.1	77.2–80.3

^a n is the number of strains used in auxanographic tests. The type strain was included for all taxa.^b Tested by using auxanographic API galleries.^c +, present in more than 91% of the strains; —, present in less than 9% of the strains; d, present in 9 to 91% of the strains.^d Late reaction.^e Data from reference 35.^f Data from reference 21.^g Data from reference 46.^h Data from reference 18.

nas] *cattleyae* given by Quimio and Tabei (40) differs from the phenotypic description of [*Pseudomonas*] *avenae* given by Schaad et al. (45) in the following three characters: hydrolysis of gelatin, reaction on litmus milk, and oxidase reaction. The main difference between the two taxa is their sources of isolation. [*Pseudomonas*] *cattleyae* has never been isolated from members of the Poaceae. The symptoms which this organism causes on orchids (round spots that turn

brown to black with yellow to green halos [1, 40]) are clearly different from those caused by *Pseudomonas avenae* on members of the Poaceae.

The third subgroup contained strains of [*Pseudomonas pseudoalcaligenes*] subsp. *citrulli* with levels of DNA binding of 93 to 100% (Fig. 3). These organisms had similar protein patterns that were different from the patterns of the members of the two subgroups described above (Fig. 4). In

TABLE 5. Differentiation of the emended genus *Acidovorax* and other genera in the *Comamonadaceae*

Characteristic	<i>Acidovorax</i> ^a	<i>Comamonas</i> ^b	<i>Hydrogenophaga</i> ^c	<i>Xylophilus</i> ^d	<i>Variovorax</i> ^e
Cell morphology	Rods	Rods to spirilla	Rods	Rods	Rods
Flagella	1 polar	Bipolar tufts of 1 to 5 flagella	1 polar	1 polar	Peritrichous
Yellow insoluble pigment on nutrient agar	—	—	+	+	+
Isolated from:					
Soil	+	+	+	—	+
Water	+	+	+	—	+
Infected plant	+	—	—	+	—
Clinical samples	+	+	—	—	—
Growth at 30°C	+	+	+	—	+
Oxidase	+	+	+	—	+
Carbon sources used for growth ^f :					
Adonitol or L-arabitol	—	—	—	—	+
D-Glucose	+	—	+	+	+
Glycolate	—	d	+	—	+
L-Mandelate	—	d	+	—	d
L-Tyrosine	+	d	d	—	—
G+C content (mol)	62–70	60–69	65–69	68–69	67–69.5

^a Data from this study and reference 60.^b Data from reference 62.^c Data from reference 58.^d Data from reference 61.^e Data from reference 26.^f +, present in at least 90% of the strains; d, present in 11 to 89% of the strains; —, absent in at least 90% of the strains.

the auxanographic analysis these strains formed a separate phenon that was linked to the other phytopathogenic "pseudomonads" at an *S* value of 82% (Fig. 1). Phenotypic differences between [*Pseudomonas pseudoalcaligenes*] subsp. *citrulli* and [*Pseudomonas*] *avenae* have been described by Schaad et al. (45). These authors classified the melon pathogen as a separate subspecies in *Pseudomonas pseudoalcaligenes* because of phenotypic similarities. According to our auxanographic data, all of the strains belonging to the emended genus *Acidovorax* used suberate, azelate, and sebacate as sole carbon sources and did not use pelargonate, diaminobutane, spermine, itaconate, and mesaconate. The reverse is true for *Pseudomonas pseudoalcaligenes* subsp. *pseudoalcaligenes*. These two taxa are in fact only very remotely related according to their low rRNA cistron similarity values (14), their different protein patterns (Fig. 4), and the absence of significant DNA binding between the type strains (Fig. 3).

We propose that the taxa belonging to the first group described above should be placed in a single species, for which, because of precedence, the name *Acidovorax avenae* should be used. Three subspecies in this species are described below. [*Pseudomonas*] *avenae* Manns 1909, [*Pseudomonas*] *rubrilineans* (Lee, Purdy, Barnum, and Martin 1925) Stapp 1928, and "[*Pseudomonas*] *setariae*" Savulescu 1947 are united in *Acidovorax avenae* subsp. *avenae*. *Pseudomonas cattleyae* (Pavarino 1911) Savulescu 1947 and *Pseudomonas pseudoalcaligenes* subsp. *citrulli* Schaad, Sowell, Goth, Colwell, and Webb 1978 are transferred to *Acidovorax avenae* subsp. *cattleyae* and *Acidovorax avenae* subsp. *citrulli*, respectively.

Finally, a second group comprised strains of [*Pseudomonas pseudoalcaligenes*] subsp. *konjaci*. The levels of DNA binding of these strains were 98 to 100%, and they exhibited no significant DNA binding with strains belonging to the first group (Fig. 3). The protein patterns of these organisms were very similar; they clearly differed from those of the strains

belonging to the first DNA group (Fig. 4). These strains formed a separate phenon that was linked to the [*Pseudomonas*] *avenae* and [*Pseudomonas*] *cattleyae* phenon at an *S* value of 85% (Fig. 1). The protein patterns of these strains were similar to those of [*Pseudomonas pseudoalcaligenes*] subsp. *citrulli* and were clearly different from those of *Pseudomonas pseudoalcaligenes* subsp. *pseudoalcaligenes* (Fig. 4); the type strains of the two taxa did not exhibit any significant level of DNA binding (Fig. 3). [*Pseudomonas pseudoalcaligenes*] subsp. *konjaci* Goto 1983 is proposed as a separate species, *Acidovorax konjaci*.

Very recently, strains belonging to the *Acidovorax avenae* rRNA branch and strains of other plant-pathogenic [*Pseudomonas*] species belonging to the solanacearum rRNA branch were studied by Hu et al. (23), who performed a numerical analysis of the results of 50 phenotypic tests. For the members of *Acidovorax avenae*, the four groups which we identified by auxanography corresponded rather well to the four phenotypic groups of Hu et al. (23), although the results on some substrates did differ (e.g., D-tartrate). However, on the basis of DNA-DNA hybridization results, Hu et al. (23) proposed that [*Pseudomonas*] *avenae* and [*Pseudomonas*] *cattleyae* should be considered synonyms and created two subspecies of [*Pseudomonas*] *avenae* for [*Pseudomonas pseudoalcaligenes*] subsp. *citrulli* and [*Pseudomonas pseudoalcaligenes*] subsp. *konjaci*. As discussed above, our DNA-rRNA results clearly showed that [*Pseudomonas pseudoalcaligenes*] subsp. *konjaci* is genotypically further removed from the other members of the [*Pseudomonas*] *avenae* rRNA branch, and our DNA-DNA hybridization results confirmed our DNA-rRNA results. Indeed, different polyphasic studies performed by members of our research group (39, 43, 58–60) have shown that strains with $\Delta T_{m(e)}$ values [$\Delta T_{m(e)}$ is the difference between the $T_{m(e)}$ of a homologous DNA-rRNA hybrid and the $T_{m(e)}$ of a heterologous DNA-rRNA hybrid] of 4°C or above never have levels of DNA-DNA homology as high as the values of 70 to 80%

FIG. 2. Simplified $T_{m(e)}$ dendrogram showing the *Comamonadaceae* and its nearest neighbors in rRNA superfamily III. Each branch represents a labeled rRNA. The solid bars represent $T_{m(e)}$ ranges within the rRNA branches. A., *Acidovorax*; L., *Leptothrix*; P., *Pseudomonas*; R., *Rubrivivax*.

found by Hu et al. (23). Therefore, we cannot agree with the taxonomic conclusions of these authors concerning the konjac plant pathogens. The results of our polyphasic study described above also did not allow placement of [*Pseudomonas*] *cattleyae* in *Acidovorax avenae* sensu stricto; as shown in Fig. 1, 3, and 4, the [*Pseudomonas*] *cattleyae* strains do not belong in *Acidovorax avenae* subsp. *avenae*.

Emended description of *Acidovorax* Willems et al. 1990. The original description of the genus *Acidovorax* is extended with the characteristics given below. Cells are straight to slightly curved rods that are 0.2 to 0.8 by 1.0 to 5.0 μm ; they occur singly, in pairs, or in short chains. Motile by means of one polar flagellum or, rarely, by means of two or three polar flagella. On nutrient agar colonies are round with smooth to slightly scalloped or spreading margins. Sometimes different margin types which cannot be isolated as stable forms occur in one culture. Colonies are convex, smooth to slightly granular, and beige to faintly yellow. A translucent marginal zone may be present. At 30°C diameters of 0.5 to 3 mm in 3 days and 4 mm in 7 days can be attained. *Acidovorax* species occur in soil, water, clinical samples, and infected plant material. Some of the phytopathogenic strains produce a yellow to slightly brown diffusible pigment. The optimal growth temperature is 30 to 35°C. The DNA base composition as determined by thermal denaturation ranges from 62 to 70 mol% G+C. In DNA-rRNA hybridizations *Acidovorax* strains have $T_{m(e)}$ values of at least 77°C versus rRNA from either *Acidovorax avenae* NCPPB 1011^T or *Acidovorax facilis* ATCC 11228^T. The genus *Acidovorax* belongs to the family *Comamonadaceae* in the beta subclass of the *Proteobacteria* (rRNA superfamily III) together with the genera *Comamonas*, *Hydrogenophaga*, *Xylophilus*, and *Variovorax*. Characteristics that differentiate the seven *Acidovorax* taxa are shown in Table 4.

Description of *Acidovorax avenae* (Manns 1909) comb. nov. The description of *Acidovorax avenae* (a' ve. nae. M. L. fem.

FIG. 3. Matrix of DNA binding values among representative *Acidovorax* strains and the type strain of *Pseudomonas pseudoalcaligenes*.

FIG. 4. Patterns obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole-cell protein extracts of phytopathogenic *Acidovorax* strains and *Pseudomonas pseudoalcaligenes* subsp. *pseudoalcaligenes* strains.

TABLE 6. Auxanographic features of the phytopathogenic *Acidovorax* species and subspecies^a

Substrate ^b	<i>Acidovorax avenae</i> subsp. <i>avenae</i>		<i>Acidovorax avenae</i> subsp. <i>cattleyae</i>		<i>Acidovorax avenae</i> subsp. <i>citrulli</i>		<i>Acidovorax konjaci</i>	
	No. of strains positive/no. of strains tested	Reaction of strain NCPPB 1011 ^T	No. of strains positive/no. of strains tested	Reaction of strain NCPPB 961 ^T	No. of strains positive/no. of strains tested	Reaction of strain PDDCC 7500 ^T	No. of strains positive/no. of strains tested	Reaction of strain PDDCC 7733 ^T
L-Arabinose or D-galactose	23/24	+	2/2	+	4/4	+	0/3	—
Ribose	17/24	+	2/2	+	4/4	+	3/3	+
D-Xylose	22/24	+	0/2	—	2/4 ^c	—	0/3	—
D-Glucose	24/24	+	2/2	+	4/4	+	0/3	—
Mannitol or D-arabitol	24/24	+	2/2	+	0/4	—	3/3	+
Sorbitol	24/24	+	2/2	+	0/4	—	0/3	—
D-Fucose	23/24	—	2/2	+	2/4	+	0/3	—
L-Fucose	13/24	+	0/2	—	0/4	—	0/3	—
Gluconate	24/24	+	1/2	—	4/4	+	3/3	+
2-Ketogluconate	0/24	—	0/2	—	1/4 ^c	—	0/3	—
Acetate	24/24	+	2/2	+	4/4	—	1/3 ^c	—
Propionate	24/24	+	2/2	+	1/4 ^c	—	3/3	+
Isobutyrate or <i>n</i> -valerate	24/24	+	2/2	+	3/4	+	3/3	+
Isovalerate	24/24	+	2/2	+	0/4	—	3/3	+
<i>n</i> -Caproate	6/24	+	2/2	+	0/4	—	0/3	—
Caprate	5/24	—	0/2	—	0/4	—	0/3	—
Oxalate	0/24	—	0/2	—	1/4 ^c	—	1/3 ^c	—
Malonate	0/24	—	0/2	—	0/4	—	3/3	+
Maleate	2/24	—	0/2	—	1/4 ^c	—	0/3	—
Suberate, pyruvate, or DL-glycerate	23/24	+	2/2	+	4/4	+	3/3	+
L-Tartrate	0/24	—	0/2	—	0/4	—	1/3	—
<i>m</i> -Tartrate	4/24	—	0/2	—	4/4 ^d	+ ^d	3/3	+
Aconitate	21/24	+	0/2	—	0/4	—	3/3	+
Mesaconate	0/24	—	0/2	—	0/3	—	1/3 ^c	+
Citrate	5/24 ^d	+ ^d	2/2	+	1/4 ^d	—	2/3 ^c	+ ^c
L-Mandelate	1/24 ^c	—	0/2	—	0/4	—	0/3	—
L-Valine	24/24	+	2/2	+	2/4	+	3/3	+
DL-Norvaline	15/24	+	1/2	—	0/4	—	0/3	—
DL-2-Aminobutyrate	3/24	—	2/2	+	0/4	—	0/3	—
L-Threonine	22/24	+	2/2	+	0/4	—	3/3	+
L-Cysteine	0/24	—	0/2	—	2/4	+	0/3	—
L-Histidine	24/24	+	2/2	+	0/4	—	3/3	+
L-Tryptophan	24/24	+	2/2	+	2/4	—	3/3	+
L-Ornithine	15/24	—	2/2	+	3/4	—	2/3	—
L-Arginine	0/24	—	0/2	—	0/4	—	1/3	—
DL-Kynurenine	14/24	—	0/2	—	0/4	—	0/3	—
DL-3-Aminobutyrate	6/24	—	2/2	+	2/4	—	3/3	+
DL-5-Aminovalerate	5/24	+	0/2	—	0/4	—	0/3	—
2-Aminobenzoate	3/24	—	0/2	—	0/4	—	0/3	—
Acetamide	13/24	—	2/2	+	0/4	—	0/3	—
Ethanolamine	21/24	—	2/2	+	4/4	+	0/3	—

^a Data for the other three *Acidovorax* species are given in reference 60.^b All of the phytopathogenic *Acidovorax* strains tested used the following substrates: glycerol, D-fructose, butyrate, succinate, fumarate, glutarate, adipate, pimelate, azelate, sebacate, DL-lactate, DL-3-hydroxybutyrate, D-malonate, L-malonate, D-tartrate, levulinate, 2-ketoglutarate, citraconate, D-α-alanine (weak growth by *Acidovorax avenae* subsp. *citrulli*), L-alanine, L-leucine, L-isoleucine, L-norleucine (weak growth by *Acidovorax avenae* subsp. *citrulli*), L-serine, L-phenylalanine, L-tyrosine, L-aspartate, L-glutamate, L-proline, β-alanine, and DL-4-aminobutyrate. None of the phytopathogenic *Acidovorax* strains tested used the following substrates: erythritol, D-arabinose, L-xylose, adonitol, D-lyxose, L-arabitol, methyl-xyloside, D-mannose, L-sorbose, D-tagatose, L-rhamnose, dulcitol, inositol, methyl-D-mannoside, methyl-D-glucoside, N-acetylglucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, maltose, lactose, D-melibiose, sucrose, β-gentiobiose, D-turanose, trehalose, D-melezitose, D-raffinose, starch, glycogen, inulin, xylitol, 5-ketogluconate, heptanoate, caprylate, pelargonate, glycolate, itaconate, phenylacetate, benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, D-mandelate, phthalate, isophthalate, terephthalate, glycine, L-methionine, D-tryptophan, trigonelline, L-lysine, L-citrulline, betaine, creatine, 3-aminobenzoate, 4-aminobenzoate, urea, sarcosine, ethylamine, butylamine, amylamine, benzylamine, diaminobutane, spermine, histamine, tryptamine, and glucosamine.^c Late reaction (growth after 5 to 7 days).^d Weak growth.

n. *Avena*, a genus of grasses; M. L. gen. n. *avenae*, of *Avena*) is the same as that given above for the genus. Additional characteristics are shown in Tables 4 and 6. The G+C contents of the DNAs range from 66.8 to 69.8 mol%. Three subspecies are described below; each of these subspe-

cies contains a group of strains which can be differentiated phenotypically and genotypically.

Description of *Acidovorax avenae* subsp. *avenae* (Manns 1909) comb. nov. *Acidovorax avenae* subsp. *avenae* contains strains that are pathogenic for various species of the

Poaceae, including oats, corn, wheat, barley, rye, sorghum, sugarcane (red stripe disease), rice seedlings (bacterial stripe disease), Italian millet, and Proso millet. The type strain is strain NCPPB 1011 (= LMG 2117); it was isolated from *Zea mays* in the United States in 1958, and the G+C content of its DNA is 69.8 mol%.

Description of *Acidovorax avenae* subsp. *cattleyae* (Savulescu 1947) comb. nov. *Acidovorax avenae* subsp. *cattleyae* (catt'-ley. ae. M.L. fem. n. *Cattleya*, a genus of orchids; M.L. gen. n. *cattleyae*, of *Cattleya*) contains strains that cause leaf spot and bud rot on *Cattleya* and *Phalaenopsis* orchids. The type strain is strain NCPPB 961 (= LMG 2364); the G+C content of its DNA is 68.6 mol%.

Description of *Acidovorax avenae* subsp. *citrulli* (Schaad, Sowell, Goth, Colwell, and Webb 1978) comb. nov. *Acidovorax avenae* subsp. *citrulli* (ci'trul.li. M.L. mas. n. *Citrullus*, a genus in the cucumber family; M.L. gen. n. *citrulli*, of *Citrullus*) contains strains that are pathogenic for various species of the Cucurbitaceae, including watermelon, cantaloupe, cucumber, and squash. The type strain is ICMP 7500 (= LMG 5376); it was isolated from a water-soaked lesion on a cotyledon of *Citrullus lanatus* in the United States in 1977, and the G+C content of its DNA is 68.4 mol%.

Description of *Acidovorax konjaci* (Goto 1983) comb. nov. The description of *Acidovorax konjaci* (kon.ja'ci. M.L. gen. n. *konjaci*, of the konjac plant, *Amorphophallus rivieri* cv. Konjac) is the same as that given above for the genus. Strains of this species cause bacterial leaf blight on *Amorphophallus rivieri* cv. Konjac. Additional characteristics are shown in Tables 4 and 6. The G+C contents of the DNAs range from 67.7 to 68.4 mol%. The type strain is strain ICMP 7733 (= LMG 5691); it was isolated from *Amorphophallus rivieri* cv. Konjac, and the G+C content of its DNA is 68.4 mol%.

ACKNOWLEDGMENTS

J.D.L., K.K., and M. Gillis are indebted to the National Fund for Medical Scientific Research, Belgium, for research and personnel grants. A.W. and M. Goor are indebted to the National Fund for Scientific Research, Belgium, for positions as research assistants.

REFERENCES

1. Ark, P. A., and H. E. Thomas. 1946. Bacterial leaf spot and bud rot of orchids caused by *Phytomonas cattleyae*. *Phytopathology* 36:695-698.
2. Baraoidan, M. R. 1981. Bacterial stripe of rice: occurrence, identification and sources of inoculum. M.S. thesis. University of the Philippines, Los Baños, The Philippines.
3. Byng, G. S., J. L. Johnson, R. J. Whitaker, R. L. Gherna, and R. A. Jensen. 1983. The evolutionary pattern of aromatic amino acid biosynthesis and the emerging phylogeny of pseudomonad bacteria. *J. Mol. Evol.* 19:262-282.
4. Christopher, W. N., and C. W. Edgerton. 1930. Bacterial stripe diseases of sugar-cane in Louisiana. *J. Agric. Res.* 41:259-267.
5. Cottrell-Dormer, W. 1932. Red stripe disease of sugar-cane in Queensland. *Bull. Div. Pathol. Sugarcane Exp. Stn. Queensland* 3:25.
6. De Ley, J. 1970. Reexamination of the association between melting point, buoyant density and chemical base composition of deoxyribonucleic acid. *J. Bacteriol.* 101:738-754.
7. De Ley, J. 1978. Modern molecular methods in bacterial taxonomy: evaluation, application, prospects, p. 347-357. In *Proceedings of the 4th International Conference of Plant Pathogenic Bacteria*, vol. 1. Gibert-Clarey, Tours, France.
8. De Ley, J., H. Cattoir, and A. Reynaerts. 1970. The quantitative measurement of DNA hybridization from renaturation rates. *Eur. J. Biochem.* 12:133-142.
9. De Ley, J., and J. De Smedt. 1975. Improvements of the membrane filter method for DNA:rRNA hybridization. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 41:287-307.
10. De Ley, J., and J. Van Muylem. 1963. Some applications of deoxyribonucleic acid base composition in bacterial taxonomy. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 29:344-358.
11. 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.
12. 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.
13. 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.
14. 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.
15. Doudoroff, M., and N. J. Palleroni. 1974. Genus *Pseudomonas*, p. 217-243. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
16. Dowson, W. J. 1943. On the generic names *Pseudomonas*, *Xanthomonas*, *Bacterium* for certain bacterial pathogens. *Trans. Br. Mycol. Soc.* 26:1-14.
17. Elliott, C. 1930. Bacterial streak disease of sorghums. *J. Agric. Res.* 40:963-976.
18. Goto, M. 1983. *Pseudomonas pseudoalcaligenes* subsp. *konjaci* subsp. nov., the causal agent of bacterial leaf blight of konjac (*Amorphophallus konjac* Koch.) *Int. J. Syst. Bacteriol.* 33:539-545.
19. Goto, M., and N. Okabe. 1952. Studies on the causal organisms of bacterial stripe disease of millet and the brown stripe disease of Italian millet. *Bull. Fac. Agric. Shizuoka Univ.* 2:15-24.
20. Goto, M., and M. P. Starr. 1971. A comparative study of *Pseudomonas andropogonis*, *P. stizolobii* and *P. albobrevipetans*. *Ann. Phytopathol. Soc. Jpn.* 37:233-241.
21. Haynes, W. C. 1957. Genus I. *Pseudomonas* Migula 1894, p. 89-152. In R. S. Breed, E. G. D. Murray, and N. R. Smith (ed.), *Bergey's manual of determinative bacteriology*, 7th ed. The Williams & Wilkins Co., Baltimore.
22. Hayward, A. C. 1962. Studies on bacterial pathogens of sugar cane. II. Differentiation, taxonomy and nomenclature of the bacteria causing red stripe and mottled stripe diseases. *Mauritius Sugar Ind. Res. Inst. Occas. Pap.* 13:13-17.
23. 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.
24. Johnson, A. G., A. L. Robert, and L. Cash. 1949. Bacterial leaf blight and stalk rot of corn. *J. Agric. Res.* 78:719-732.
25. Johnson, J. L., and N. J. Palleroni. 1989. Deoxyribonucleic acid similarities among *Pseudomonas* species. *Int. J. Syst. Bacteriol.* 39:230-235.
26. Kersters, K., and J. De Ley. 1984. Genus *Alcaligenes* Castellani and Chalmers 1919, 936^{AL}, p. 361-373. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
27. Kersters, K., K.-H. Hinz, A. Hertle, P. Segers, A. Lievens, O. Siegmann, and J. De Ley. 1984. *Bordetella avium* sp. nov., isolated from the respiratory tracts of turkeys and other birds. *Int. J. Syst. Bacteriol.* 34:56-70.
28. Kiredjian, M., B. Holmes, K. Kersters, I. Guilvout, and J. De Ley. 1986. *Alcaligenes piechaudii*, a new species from human clinical specimens and the environment. *Int. J. Syst. Bacteriol.* 36:282-287.
29. Lee, H. A., H. A. Purdy, C. C. Barnum, and J. P. Martin. 1925. A comparison of red-stripe of sugar cane and other grasses. In *Red-stripe disease studies*, *Bull. Exp. Stn. Hawaiian Sugar*

- Planters' Assoc., 1-99.
30. Manns, T. F. 1909. The blade blight of oats—a bacterial disease. Ohio Agric. Res. Stn. Res. Bull. **210**:91-167.
 31. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3**:208-218.
 32. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109-118.
 33. Okabe, N. 1934. Bacterial diseases of plants occurring in Formosa. IV. J. Soc. Trop. Agric. Taiwan **6**:54-63.
 34. Ou, S. H. 1972. Rice diseases. Commonwealth Mycological Institute, Kew, England.
 35. Palleroni, N. J. 1984. Genus I. *Pseudomonas* Migula 1894, 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.
 36. Palleroni, N. J. 1986. Taxonomy of the pseudomonads, p. 3-25. In J. R. Sokatch and L. N. Ornston (ed.), The bacteria. A treatise on structure and function, vol. 10. The biology of *Pseudomonas*. Academic Press Inc., London.
 37. 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.
 38. Pavarino, G. L. 1911. Malattie causate da bacteri nelle Orchidee. Atti Accad. Lincei **20**:233-237.
 39. Pot, B., A. Willems, M. Gillis, and J. De Ley. 1991. Intra- and intergeneric relationships of the genus *Aquaspirillum*: *Prolinoborus*, a new genus for *Aquaspirillum fasciculus*, with the species *Prolinoborus fasciculus* comb. nov. Int. J. Syst. Bacteriol. **42**:44-57.
 40. Quimio, A. J., and H. Tabei. 1979. Identity of the bacterium associated with bacterial brown spot of *Phalaenopsis* orchids. Philipp. Phytopathol. **15**:76-80.
 41. 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.
 42. Rosen, H. R. 1922. A bacterial disease of foxtail (*Chaetochloa lutescens*). Ann. Mo. Bot. Gard. **9**:333-402.
 43. Rossau, R., K. Kersters, E. Falsen, E. Jantzen, P. Segers, A. Union, L. Nehls, and J. De Ley. 1987. *Oligella*, a new genus including *Oligella urethralis* comb. nov. (formerly *Moraxella urethralis*) and *Oligella ureolytica* sp. nov. (formerly CDC group IVe): relationship to *Taylorella equigenitalis* and related taxa. Int. J. Syst. Bacteriol. **37**:198-210.
 44. Savulescu, T. 1947. Contribution à la classification des bactéries phytopathogènes. An. Acad. Romane Ser. III **22**:135-160.
 45. Schaad, N. W., C. I. Kado, and D. R. Sumner. 1975. Synonymy of *Pseudomonas avenae* Manns 1905 and *Pseudomonas albo-precipitans* Rosen 1922. Int. J. Syst. Bacteriol. **25**:133-137.
 46. Schaad, N. W., G. Sowell, Jr., R. W. Goth, R. R. Colwell, and R. E. Webb. 1978. *Pseudomonas pseudoalcaligenes* subsp. *citrulli* subsp. nov. Int. J. Syst. Bacteriol. **28**:117-125.
 47. 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.
 48. Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. The principles and practice of numerical classification. W. H. Freeman and Co., San Francisco.
 49. Stackebrandt, E., R. G. E. Murray, and H. G. Trüper. 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.
 50. Stackebrandt, E., and C. R. Woese. 1981. The evolution of prokaryotes, p. 1-31. In M. J. Carlile, J. F. Collins, and B. E. B. Moseley (ed.), Molecular and cellular aspects of microbial evolution. Cambridge University Press, Cambridge.
 51. Stanier, R. Y., N. J. Palleroni, and M. Doudoroff. 1966. The aerobic pseudomonads: a taxonomic study. J. Gen. Microbiol. **43**:159-271.
 52. Starr, M. P., and W. H. Burkholder. 1942. Lipolytic activity of phytopathogenic bacteria determined by means of spirit blue agar and its taxonomic significance. Phytopathology **32**:598-604.
 53. Swings, J., P. De Vos, M. Van den Mooter, and J. De Ley. 1983. Transfer of *Pseudomonas maltophilia* Hugh 1981 to the genus *Xanthomonas* as *Xanthomonas maltophilia* (Hugh 1981) comb. nov. Int. J. Syst. Bacteriol. **33**:409-413.
 54. Tamaoka, J., D.-M. 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.
 55. Van Landschoot, A., and J. De Ley. 1983. Intra- and intergeneric similarities of the rRNA cistrons of *Alteromonas*, *Marinomonas* (gen. nov.) and some other Gram-negative bacteria. J. Gen. Microbiol. **129**:3057-3074.
 56. Van Zyl, E., and P. L. Steyn. 1991. Taxonomy of phytopathogenic *Pseudomonas* species belonging to the acidovorans rRNA complex. Syst. Appl. Microbiol. **14**:165-168.
 57. Webb, R. E., and R. W. Goth. 1965. A seed-borne bacterium isolated from watermelon. Plant Dis. Rep. **49**:818-821.
 58. 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.
 59. Willems, A., J. De Ley, M. Gillis, and K. Kersters. 1991. *Comamonadaceae*, a new family encompassing the acidovorans rRNA complex, including *Variovorax paradoxus* gen. nov., comb. nov., for *Alcaligenes paradoxus* (Davis 1969). Int. J. Syst. Bacteriol. **41**:445-450.
 60. 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.
 61. Willems, A., M. Gillis, K. Kersters, L. Van den Broecke, and J. De Ley. 1987. Transfer of *Xanthomonas ampelina* Panagopoulos 1969 to a new genus, *Xylophilus* gen. nov., as *Xylophilus ampelinus* (Panagopoulos 1969) comb. nov. Int. J. Syst. Bacteriol. **37**:422-430.
 62. 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.
 63. Wishart, D. 1978. Clustan user manual, 3rd ed. Program Library Unit, Edinburgh University, Edinburgh, Scotland.
 64. Woese, C. R. 1987. Bacterial evolution. Microbiol. Rev. **51**:221-271.
 65. Young, J. M. (DSIR Plant Protection, New Zealand). 1991. Personal communication.
 66. Young, J. M., D. W. Dye, and J. P. Wilkie. 1978. Genus VII. *Pseudomonas* Migula 1894. In J. M. Young, D. W. Dye, J. F. Bradbury, C. G. Panagopoulos, and C. F. Robbs (ed.), A proposed nomenclature and classification for plant pathogenic bacteria. N. Z. J. Agric. Res. **21**:153-177.