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*)

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The relationships of the yellow-pigmented hydrogen-oxidizing species *Pseudomonas flava*, *Pseudomonas pseudoflava*, *Pseudomonas palleronii*, *Pseudomonas taeniospiralis*, and “*Pseudomonas carboxydoflava*,” which are all members of the acidovorans ribosomal ribonucleic acid (rRNA) complex in rRNA superfamily III, were studied by using deoxyribonucleic acid (DNA):rRNA hybridization, immunotyping, numerical analysis of biochemical and auxanographic features, polyacrylamide gel electrophoresis of cellular proteins, numerical analysis of fatty acid patterns, and DNA:DNA hybridization. Our results show that these five yellow-pigmented hydrogen-oxidizing *Pseudomonas* species are more closely related to each other than to other taxa belonging to the acidovorans rRNA complex. We propose the transfer of these species to a new genus, *Hydrogenophaga*, with the following four species: *Hydrogenophaga flava* (formerly *Pseudomonas flava*), *Hydrogenophaga pseudoflava* (to accommodate both *Pseudomonas pseudoflava* and “*Pseudomonas carboxydoflava*”), *Hydrogenophaga taeniospiralis* (formerly *Pseudomonas taeniospiralis*), and *Hydrogenophaga palleronii* (formerly *Pseudomonas palleronii*). The type species is *H. flava*, with monotype strain DSM 619 (= LMG 2185 = CCUG 1658). Because *H. flava* grows slowly and unreliably, but is genotypically and protein electrophoretically very similar to *H. pseudoflava*, the latter species can be used as an alternative reference taxon for the new genus. The type strains of *H. pseudoflava*, *H. taeniospiralis*, and *H. palleronii* are strains GA3 (= LMG 5945 = CCUG 13799), DSM 2082 (= LMG 7170 = CCUG 15921), and Stanier 362t1 (= LMG 2366t1 = CCUG 20334), respectively.

The genus *Hydrogenomonas* was originally created to shelter gram-negative, facultatively autotrophic hydrogen bacteria (34). In 1969 Davis et al. (7) proposed that this genus should be abandoned and transferred the various species to other genera. Because of their deoxyribonucleic acid (DNA) base compositions, their polar flagellation, and their overall phenotypic properties, the yellow-pigmented organism *Hydrogenomonas flava* and the nonpigmented organisms *Hydrogenomonas facilis* and *Hydrogenomonas ruhlandii* were assigned to the genus *Pseudomonas*. Later, *Pseudomonas palleronii* was described as an additional yellow-pigmented hydrogen-oxidizing species, differing from *Pseudomonas flava* in the nature of its carotenoid pigments and its inability to grow on several carbohydrates (8).

Within the phenotypically defined genus *Pseudomonas*, five ribosomal ribonucleic acid (rRNA) homology groups were delineated by means of DNA:rRNA hybridization (37). Later, De Vos and De Ley (14) extended these five rRNA groups considerably and showed that they are only very remotely interrelated and cannot be maintained in a single genus. This conclusion was confirmed by results from several other techniques (6, 46). Most *Pseudomonas* species are

located on three rRNA branches; only the *Pseudomonas fluorescens* rRNA branch in rRNA superfamily II represents the true pseudomonads (14). We consider all of the other *Pseudomonas* species to be generically misnamed (14). Below all misnamed taxa are indicated by brackets. The yellow-pigmented hydrogen-oxidizing species [*Pseudomonas*] *flava*, [*Pseudomonas*] *palleronii*, [*Pseudomonas*] *pseudoflava*, “[*Pseudomonas*] *carboxydoflava*,” and [*Pseudomonas*] *taeniospiralis* belong to the acidovorans rRNA complex, i.e., rRNA homology group III sensu Palleroni (36). This rRNA complex belongs to rRNA superfamily III (sensu De Ley [10]), which corresponds to the beta subclass of the *Proteobacteria* (41). In addition, this rRNA complex contains the following species: [*Pseudomonas*] *acidovorans*, [*Pseudomonas*] *avenae*, [*Pseudomonas*] *cattleyae*, [*Pseudomonas*] *delafieldii*, [*Pseudomonas*] *facilis*, [*Pseudomonas*] *pseudoalcaligenes* subsp. *citrulli*, [*Pseudomonas*] *pseudoalcaligenes* subsp. *konjaci*, [*Pseudomonas*] *rubrilineans*, [*Pseudomonas*] *saccharophila*, “[*Pseudomonas*] *setariae*,” [*Pseudomonas*] *testosteroni*, *Comamonas terrigena*, [*Alcaligenes*] *paradoxus*, [*Alcaligenes*] *latus*, [*Rhodocyclus*] *gelatinosus*, and *Xylophilus ampelinus* (15, 16, 44). [*Pseudomonas*] *acidovorans* and [*Pseudomonas*] *testosteroni* were recently transferred to the genus *Comamonas* (42).

So far, within the acidovorans rRNA complex, labeled

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rRNAs have been available from the type strains of only *Comamonas acidovorans*, [*Alcaligenes*] *paradoxus*, and *Xylophilus ampelinus*. Hybridization of these rRNAs with DNAs from strains belonging to the acidovorans rRNA complex resulted in delineation of three rRNA subbranches linked at a $T_{m(e)}$ level [$T_{m(e)}$ is the temperature, in degrees Celsius, at which one-half of the DNA:rRNA duplex is denatured] of $76.2 \pm 0.7^\circ\text{C}$ and containing one species each. All of the other species of the acidovorans rRNA complex are presently located on the $T_{m(e)}$ dendrogram at the branching level of these three rRNA subbranches (16, 44). Within this phenotypically very diverse group, some taxa may be more closely related to each other. We are in the process of revealing these possible subgroups by using a repeated process of rRNA preparation from new strains and DNA:rRNA hybridization. The combination of DNA:rRNA hybridization data with the results of other techniques should provide a clearer view of the relationships within the acidovorans rRNA complex. In this study, we used a broad spectrum of methods to investigate the taxonomy of the yellow-pigmented hydrogen-oxidizing [*Pseudomonas*] species belonging to the acidovorans rRNA complex. We propose the creation of a new genus, *Hydrogenophaga*, for these organisms.

MATERIALS AND METHODS

Bacterial strains. The strains which we used are listed in Table 1. "[*Pseudomonas*] *gazotroph*" was grown on PSS medium, which contained 1% (wt/vol) neutralized bacteriological peptone (type L34; Oxoid Ltd., London, England), 0.1% (wt/vol) succinic acid, 0.1% (wt/vol) $(\text{NH}_4)_2\text{SO}_4$, 0.1% (wt/vol) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0002% (wt/vol) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and 0.0002% (wt/vol) $\text{MnSO}_4 \cdot \text{H}_2\text{O}$; the pH was adjusted to 7.0 with 1 M KOH. The media used for growth of [*Rhodocyclus*] *gelatinosus* and *Xylophilus ampelinus* have been described previously (44); all other strains were grown 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). For fatty acid analysis, cells were grown for 40 h on Columbia agar (Oxoid) containing 5% (vol/vol) defibrinated horse blood. All strains except *Xylophilus ampelinus* NCPPB 2217^T (T = type strain) were grown at 28°C; *Xylophilus ampelinus* NCPPB 2217^T was grown at 24°C.

Preparation of high-molecular-weight DNA. Cells were grown in Roux flasks for 2 to 3 days. DNA was isolated by the method of Marmur (29).

DNA base composition. The average guanine-plus-cytosine (G+C) contents were determined by the thermal denaturation method (13) and were calculated by using the equation of Marmur and Doty (30), as modified by De Ley (9).

DNA:rRNA hybridization. DNA was further purified by CsCl gradient centrifugation (12). Fixation of thermally denatured DNA on cellulose nitrate filters and chemical estimation of the amount of filter-fixed DNA were performed as described previously (12, 43). [³H]rRNAs from [*Pseudomonas*] *palleronii* Stanier 362t1^T and *Comamonas terrigena* NCIB 8193^T and [¹⁴C]rRNA from [*Pseudomonas*] *pseudoflava* GA3^T were isolated and purified as described by De Ley and De Smedt (12). Purified 23S [³H]rRNA from *Comamonas acidovorans* Stanier 14^T and 16S [¹⁴C]rRNA from [*Alcaligenes*] *paradoxus* ATCC 17713t1^T were available in our research group (15, 16). Hybridizations between labeled 16S or 23S rRNA and filter-fixed DNA were carried out as described previously (12). Each hybrid was characterized by its $T_{m(e)}$ and by the percentage of rRNA binding (the amount

of rRNA [in micrograms] bound to 100 µg of filter-fixed DNA after ribonuclease treatment).

DNA:DNA hybridization. The degree of binding, expressed as a percentage, was determined spectrophotometrically by using the initial renaturation rate method (11). We used a model 2600 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) equipped with a thermostatically controlled cuvette chamber and a model 7225A plotter (Hewlett-Packard Co., Palo Alto, Calif.). 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 80.9°C and with a total DNA concentration of 0.101 mM base pairs. Degrees of binding of 25% or less indicate no significant DNA homology.

Taxonomic immunotyping. The preparation of antisera by immunizing rabbits with bacterial extracts in Freund incomplete adjuvant and the immunodiffusion technique have been described previously (16, 17).

Morphological and biochemical features. To determine morphological and biochemical features, we used the methods described by De Vos et al. (16). Nitrite reduction was tested as described by Rossau et al. (39).

Carbon substrate assimilation tests. API 50CH, API 50AO, and API 50AA galleries (API System S.A., Montalieu-Vercieu, France) were used to test the assimilation of 147 organic compounds as sole carbon sources. The experimental procedure which we used has been described previously (23). For "[*Pseudomonas*] *gazotroph*" 0.1 M potassium phosphate buffer (pH 7) was used to prepare the medium for the auxanographic tests instead of sodium phosphate buffer, and the API galleries were incubated at 28°C instead of 30°C.

Numerical analysis of phenotypic features. The scoring of the auxanographic and biochemical tests has been described previously (39). Of the 251 features tested, 192 were used in the numerical analysis. The API 20NE tests (21 features) were not included in the computer analysis as they duplicated other tests, and 38 other features were omitted because all 38 strains tested were either negative or displayed the same positive value. The levels of interstrain similarity were calculated by using the Gower similarity coefficient (S_G) (40). Cluster analysis by the unweighted average pair group method (40) was performed with the CLUSTAN 2.1 program of Wishart (45) on the Siemens model 7551 computer of the Centraal Digitaal Rekencentrum, Rijksuniversiteit Ghent, Ghent, Belgium. The reproducibility of the clustering was estimated by including duplicate tests for seven strains.

Polyacrylamide gel electrophoresis of proteins. All strains were grown on nutrient agar at 28°C for 40 h in Roux flasks. Whole-cell protein extracts were prepared, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by using small modifications of the procedure of Laemmli (27), as described previously (24). The normalized densitometric traces of the protein electrophoretic patterns were grouped by numerical analysis, using the Pearson product moment correlation coefficient (r) and the techniques described by Pot et al. (38).

Gas chromatography of cellular fatty acids. The following strains were examined for cellular fatty acid composition: [*Pseudomonas*] *flava* DSM 619^T; [*Pseudomonas*] *palleronii* Stanier 362t1^T, Stanier 362t2^T, Stanier 366, RH2, Z14, Z17, and Z32; [*Pseudomonas*] *pseudoflava* GA3^T, GA2, and GA4; "[*Pseudomonas*] *carboxydoflava*" DSM 1084^T; [*Pseudomonas*] *taeniospiralis* DSM 2082^T; [*Pseudomonas*] *delafeldii* ATCC 17505^T; [*Pseudomonas*] *facilis* ATCC 11228^T; [*Pseudomonas*] *avenae* NCPPB 1011^T; "[*Pseudomonas*] *gazotro-*

Species	Strain ^a	Other strain designation(s)	Source and place and year of isolation
Organisms assigned to the genus			
<i>Hydrogenophaga</i>			
“[<i>Pseudomonas</i>] <i>carboxydo-flava</i> ”	DSM 1084 ^T	LMG 7584 ^T , CCUG 20741 ^T , Z-1107 ^T (= LMG 8355 ^T = CCUG 22764 ^T)	Mud and soil, River Moskwa, USSR
“[<i>Pseudomonas</i>] <i>carboxydo-flava</i> ”	Z-1607	LMG 8356, CCUG 22765	Soil near River Nischenka, Moscow area, USSR
[<i>Pseudomonas</i>] <i>flava</i>	DSM 619 ^T	LMG 2185 ^T , CCUG 1658 ^T , ATCC 33667 ^T	Mud from ditch, 1942
[<i>Pseudomonas</i>] <i>palleronii</i>	Stanier 362t1 ^{Tb}	LMG 2366t1 ^{Tb} (= CCUG 20334 ^T), ATCC 17724 ^T (= CCUG 1780 ^T)	Water enriched for hydrogen bacteria in an atmosphere containing 6% O ₂
[<i>Pseudomonas</i>] <i>palleronii</i>	Stanier 362t2 ^{Tb}	LMG 2366t2 ^{Tb} (= CCUG 20335 ^T), ATCC 17724 ^T (= CCUG 1780 ^T)	Water enriched for hydrogen bacteria in an atmosphere containing 6% O ₂
[<i>Pseudomonas</i>] <i>palleronii</i>	Stanier 366	LMG 6346, CCUG 17387, CCUG 20336, ATCC 17728	Water enriched for hydrogen bacteria in an atmosphere containing 6% O ₂
[<i>Pseudomonas</i>] <i>palleronii</i>	RH2	LMG 6348, CCUG 20338	Göttingen, Federal Republic of Germany
[<i>Pseudomonas</i>] <i>palleronii</i>	Z14	LMG 6349, CCUG 21796	Russia
[<i>Pseudomonas</i>] <i>palleronii</i>	Z17	LMG 6350, CCUG 21797	Russia
[<i>Pseudomonas</i>] <i>palleronii</i>	Z32	LMG 6347, CCUG 17388, CCUG 20337	Russia
[<i>Pseudomonas</i>] <i>pseudoflava</i>	GA3 ^T	LMG 5945 ^T , CCUG 13799 ^T , ATCC 33668 ^T	Water, River Weende, Federal Republic of Germany
[<i>Pseudomonas</i>] <i>pseudoflava</i>	GA2	LMG 6351, CCUG 17389, CCUG 20339	
[<i>Pseudomonas</i>] <i>pseudoflava</i>	GA4	LMG 6352, CCUG 17390, CCUG 20340	
[<i>Pseudomonas</i>] <i>taeniospiralis</i>	DSM 2082 ^T	LMG 7170 ^T , CCUG 15921 ^T	Soil, Spain
Reference organisms			
[<i>Pseudomonas</i>] <i>avenae</i>	NCPPB 1011 ^T	LMG 2117 ^T , CCUG 15838 ^T , ATCC 19860 ^T	<i>Zea mays</i> , United States, 1958
[<i>Pseudomonas</i>] <i>cattleyae</i>	NCPPB 961 ^T	LMG 5286 ^T , CCUG 21975 ^T , ATCC 33619 ^T	
[<i>Pseudomonas</i>] <i>delafieldii</i>	ATCC 17505 ^T	LMG 5943 ^T , CCUG 1779 ^T	Soil with PHB as the sole carbon source ^c
[<i>Pseudomonas</i>] <i>delafieldii</i>	Stanier 134t1 ^b	LMG 1792t1 ^b , CCUG 14277, ATCC 17506	Soil with PHB as the sole carbon source
[<i>Pseudomonas</i>] <i>delafieldii</i>	ATCC 17508	LMG 5944, CCUG 14478	Soil with PHB as the sole carbon source
[<i>Pseudomonas</i>] <i>facilis</i>	ATCC 11228 ^T	LMG 2193 ^T , CCUG 2113 ^T	Lawn soil, United States
[<i>Pseudomonas</i>] <i>facilis</i>	ATCC 15376	LMG 2194, CCUG 14278	
[<i>Pseudomonas</i>] <i>facilis</i>	DSM 550	LMG 6598, CCUG 15919, ATCC 17695	Lawn soil
“[<i>Pseudomonas</i>] <i>gazotropha</i> ”	DSM 1085 ^T	LMG 7583 ^T , CCUG 20742 ^T , CCUG 21978 ^T	Mud, River Moskwa, USSR
[<i>Pseudomonas</i>] <i>rubrilineans</i>	NCPPB 920 ^T	LMG 2281 ^T , CCUG 15837 ^T , ATCC 19307 ^T	<i>Saccharum officinarum</i> cv. R445, Réunion, 1960
[<i>Pseudomonas</i>] <i>saccharophila</i>	ATCC 15946 ^T	LMG 2256 ^T , LMG 7831 ^T	Mud from a stagnant pool
“[<i>Pseudomonas</i>] <i>setariae</i> ”	NCPPB 1392 ^T	LMG 1806 ^T , CCUG 15836 ^T , ATCC 19882 ^T	<i>Oryza sativa</i> , Japan, 1955
<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>Comamonas acidovorans</i>	ATCC 17406	LMG 1790, CCUG 15340	Soil enriched with <i>p</i> -hydroxybenzoate
<i>Comamonas acidovorans</i>	ATCC 17476	LMG 1791, CCUG 15337	Great Britain
<i>Comamonas acidovorans</i>	CCUG 274B	LMG 7098	Urine, male, Göteborg, Sweden, 1968
<i>Comamonas terrigena</i>	NCIB 8193 ^T	LMG 1253 ^T , CCUG 2185 ^T (= LMG 5929 ^T), CCUG 15327 ^T , ATCC 8461 ^T	Hay infusion filtrate, United States
<i>Comamonas terrigena</i>	NCIB 2581	LMG 1249, CCUG 2474	Soil
<i>Comamonas terrigena</i>	CCUG 12940	LMG 5520	Blood, 1982
<i>Comamonas terrigena</i>	NCIB 2582	LMG 1251, CCUG 2475, ATCC 14636	Soil
<i>Comamonas testosteroni</i>	NCTC 10698 ^T	LMG 1786 ^T , CCUG 1426 ^T , ATCC 11996 ^T	Soil, Berkeley, Calif.
<i>Comamonas testosteroni</i>	ATCC 17407	LMG 1787, CCUG 15341	Soil enriched with anthranilate
<i>Comamonas testosteroni</i>	ATCC 17409	LMG 1788, CCUG 15339	Soil enriched with kynurenate, Berkeley, Calif., 1963
[<i>Alcaligenes</i>] <i>latus</i>	Palleroni H-4 ^T	LMG 3321 ^T , CCUG 10983 ^T , ATCC 29712 ^T	Soil, California
[<i>Alcaligenes</i>] <i>paradoxus</i>	ATCC 17713t1 ^{Tb}	LMG 1797t1 ^{Tb} , CCUG 1777 ^T	Soil in mineral medium under an atmosphere containing 91% H ₂ , 4% O ₂ , and 5% CO ₂
[<i>Alcaligenes</i>] <i>paradoxus</i>	ATCC 17712	LMG 3572, CCUG 15916	Soil in atmosphere containing H ₂ , 6% O ₂ and 5% CO ₂
[<i>Alcaligenes</i>] <i>paradoxus</i>	ATCC 17549t1 ^b	LMG 1796t1 ^b , CCUG 1778	Soil enriched with panthotenate
[<i>Rhodocyclus</i>] <i>gelatinosus</i>	NCIB 8290 ^T	LMG 4311 ^T , ATCC 17011 ^T , CCUG 15841 ^T , CCUG 21977 ^T	Acetate enrichment, pH 6.6
<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, 1966

^a Strain number as received. ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Federal Republic of 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; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England.

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

^c PHB, Poly-β-hydroxybutyrate.

pha^T DSM 1085^T; *Comamonas acidovorans* Stanier 14^T, ATCC 17406, and ATCC 17476; *Comamonas terrigena* NCIB 8193^T, NCIB 2581, NCIB 2582, and CCUG 12940; *Comamonas testosteroni* NCTC 10698^T, ATCC 17407, and ATCC 17409; [*Alcaligenes*] *paradoxus* ATCC 17713t1^T, ATCC 17712, and ATCC 17549t1; [*Alcaligenes*] *latus* Palleroni H-4^T; and [*Rhodocyclus*] *gelatinosus* NCIB 8290^T. The strains were grown on Columbia blood agar as described above, washed, harvested, freeze-dried, and methanolized (both alkaline and acidic) as described previously (18, 19). The resulting fatty acid methyl esters were analyzed on a gas chromatograph equipped with a flame ionization detector as described previously (39). The hydroxylated fatty acids were separated from the nonhydroxylated fatty acids on a silica BOND ELUT extraction column (Analytichem International, Harbor City, Calif.) and analyzed and identified separately by gas chromatography-mass spectrometry (Ion-trap 700 instrument; Finnigan, San Jose, Calif.) after trifluoroacetylation and trimethylsilylation (31).

Numerical analysis of gas chromatographic data. Principal component analysis was carried out by using the soft independent modeling of class analogy method (47), as previously described (20). The software program (in a PC-DOS version) was obtained from Pattern Recognition Systems, Bergen, Norway.

Determination of quinone and polyamine contents. Quinones were analyzed by thin-layer chromatography as described by Auling et al. (3), and polyamines were analyzed by high-performance liquid chromatography as described by Busse and Auling (5).

RESULTS

DNA base composition. The average G+C values of the strains studied are shown in Table 2.

DNA:rRNA hybridizations. The specific activities of the 23S [¹⁴C]rRNA fraction from [*Pseudomonas*] *pseudoflava* GA3^T, the 23S [³H]rRNA fraction from [*Pseudomonas*] *palleronii* Stanier 362t1^T, and the 16S and 23S [³H]rRNA fractions from *Comamonas terrigena* NCIB 8193^T were 1,757, 82,684, 14,236, and 21,426 cpm/μg of rRNA, respectively. The results of the DNA:rRNA hybridizations are shown in Table 2 and are presented as a dendrogram based on $T_{m(e)}$ values in Fig. 1. Our results allow the delineation of three new rRNA groups within the acidovorans rRNA complex. The first group, formed by the four *Comamonas terrigena* strains, is linked to the other taxa in the acidovorans rRNA complex at the branching level of the three existing rRNA subbranches [$T_{m(e)}$, 76.1 ± 1.1°C] and thus constitutes a new rRNA subbranch. The second rRNA group exclusively comprises [*Pseudomonas*] *palleronii* strains, and the third one is formed by [*Pseudomonas*] *pseudoflava*, "[*Pseudomonas*] *carboxydo-flava*," and [*Pseudomonas*] *flava* strains. Reciprocal hybridizations between the [*Pseudomonas*] *palleronii* and [*Pseudomonas*] *pseudoflava* rRNA groups yielded a mean clustering level of 78.0 ± 0.7°C. Hybridizations between rRNAs from both [*Pseudomonas*] *palleronii* Stanier 362t1^T and [*Pseudomonas*] *pseudoflava* GA3^T and DNAs from reference strains belonging to the acidovorans rRNA complex showed that these rRNAs are linked to the other four rRNA subbranches at the branching level of 76.1 ± 1.1°C. Therefore we consider the [*Pseudomonas*] *palleronii* and [*Pseudomonas*] *pseudoflava* rRNA groups to be a single bifurcated rRNA subbranch. [*Pseudomonas*] *taeniospiralis* DSM 2082^T is located on this rRNA subbranch at the bifurcation level (Table 2).

DNA:DNA hybridizations. The results of our DNA:DNA hybridizations are summarized in Fig. 2. We distinguished two DNA homology groups, one formed by the [*Pseudomonas*] *palleronii* strains and the other formed by the [*Pseudomonas*] *pseudoflava*, "[*Pseudomonas*] *carboxydo-flava*," and [*Pseudomonas*] *flava* strains. Within the latter DNA homology group, [*Pseudomonas*] *flava* DSM 619^T was the most aberrant strain (degree of binding, 48 to 62%). The members of the [*Pseudomonas*] *palleronii* DNA homology group showed no significant DNA binding with the type strains of [*Pseudomonas*] *pseudoflava*, "[*Pseudomonas*] *carboxydo-flava*," [*Pseudomonas*] *flava*, and [*Pseudomonas*] *taeniospiralis*. [*Pseudomonas*] *taeniospiralis* showed no significant DNA binding with representative strains of any of the other yellow-pigmented hydrogen-oxidizing [*Pseudomonas*] species.

Immunotyping properties. Our immunotyping results are summarized in Table 3. [*Pseudomonas*] *flava*, [*Pseudomonas*] *pseudoflava*, "[*Pseudomonas*] *carboxydo-flava*," and [*Pseudomonas*] *taeniospiralis* were very similar to each other and could easily be separated from [*Pseudomonas*] *palleronii*. [*Pseudomonas*] *pseudoflava* and [*Pseudomonas*] *flava* gave different precipitation patterns of similar magnitude. The antiserum of [*Pseudomonas*] *pseudoflava* revealed a fairly high level of relatedness between this species and [*Pseudomonas*] *palleronii*. By extending the time of diffusion, the antiserum of [*Pseudomonas*] *palleronii* confirmed this observation. [*Pseudomonas*] *delafeldii* appeared to be the closest immunological neighbor of the yellow-pigmented hydrogen oxidizers.

Numerical analysis of phenotypic features. A total of 13 representative strains of the yellow-pigmented hydrogen-oxidizing [*Pseudomonas*] species and a number of reference strains belonging to the acidovorans rRNA complex were compared by performing a numerical analysis of auxanographic and biochemical features. Under our test conditions [*Alcaligenes*] *latus* Palleroni H-4^T did not grow on any of the substrates of the auxanographic galleries and was therefore excluded from the numerical analysis. *Xylophilus ampelinus* was not included either because this organism requires special incubation conditions which would have produced incompatible results. Strains tested in duplicate clustered, on the average, at an S_G value of 95% (10 sets of comparisons, with one exceptional minimum having an S_G value of 90%). The following phenon separated at S_G values of more than 79% (Fig. 3): [*Pseudomonas*] *pseudoflava* and "[*Pseudomonas*] *carboxydo-flava*"; [*Pseudomonas*] *palleronii*; *Comamonas* spp.; [*Pseudomonas*] *facilis* and [*Pseudomonas*] *delafeldii*; a phenon formed by the type strains of [*Pseudomonas*] *avenae*, [*Pseudomonas*] *rubrilineans*, [*Pseudomonas*] *cattleyae*, and "[*Pseudomonas*] *setariae*"; and [*Alcaligenes*] *paradoxus*. [*Pseudomonas*] *flava* and [*Pseudomonas*] *taeniospiralis* were linked to the [*Pseudomonas*] *pseudoflava*-"[*Pseudomonas*] *carboxydo-flava*" phenon at an S_G value of 74%. "[*Pseudomonas*] *gazotropha*" and [*Rhodocyclus*] *gelatinosus* occupy separate positions on the dendrogram.

Protein electrophoresis. Above an r value of 0.8 the following two electrophoretic groups of yellow-pigmented hydrogen-oxidizing species could be delineated: one formed by the [*Pseudomonas*] *palleronii* strains and the other formed by the [*Pseudomonas*] *pseudoflava* and "[*Pseudomonas*] *carboxydo-flava*" strains (Fig. 4). [*Pseudomonas*] *flava* DSM 619^T and [*Pseudomonas*] *taeniospiralis* DSM 2082^T occupy separate positions on the dendrogram, although the pattern of [*Pseudomonas*] *flava* DSM 619^T has some overall visual

TABLE 2. DNA base compositions of strains and parameters of hybrids of DNAs with labeled rRNAs from [*Pseudomonas*] *pseudoflava* GA3^T, [*Pseudomonas*] *palleronii* Stanier 362t1^T, *Comamonas terrigena* NCIB 8193^T, *Comamonas acidovorans* Stanier 14^T, and [*Alcaligenes*] *paradoxus* ATCC 17713t1^T

DNA from strain:	G+C content (mol%)	Hybridized with:									
		[¹⁴ C]rRNA from [<i>Pseudomonas</i>] <i>pseudoflava</i> GA3 ^T		[³ H]rRNA from [<i>Pseudomonas</i>] <i>palleronii</i> Stanier 362t1 ^T		[³ H]rRNA from <i>Comamonas terrigena</i> NCIB 8193 ^T		[³ H]rRNA from <i>Comamonas acidovorans</i> Stanier 14 ^T		[¹⁴ C]rRNA from [<i>Alcaligenes</i>] <i>paradoxus</i> ATCC 17713t1 ^T	
		<i>T_m(e)</i> (°C)	rRNA binding (%)	<i>T_m(e)</i> (°C)	rRNA binding (%)	<i>T_m(e)</i> (°C)	rRNA binding (%)	<i>T_m(e)</i> (°C)	rRNA binding (%)	<i>T_m(e)</i> (°C)	rRNA binding (%)
[<i>Pseudomonas</i>] <i>flava</i> DSM 619 ^T	66.7 ^a	79.6	0.08	78.2	0.07			75.5 ^a	0.02 ^a	76.0 ^b	0.04 ^b
[<i>Pseudomonas</i>] <i>pseudoflava</i> GA3 ^T	66.4	80.8	0.04	77.4	0.09	75.2	0.09	76.0	0.08		
[<i>Pseudomonas</i>] <i>pseudoflava</i> GA2	68.6	81.1	0.06	77.7	0.08						
[<i>Pseudomonas</i>] <i>pseudoflava</i> GA4	66.2	81.1	0.07	78.0	0.09						
“[<i>Pseudomonas</i>] <i>carboxydoflava</i> ” DSM 1084 ^T	66.9	80.8	0.07	77.3	0.04			75.3	0.05		
“[<i>Pseudomonas</i>] <i>carboxydoflava</i> ” Z-1607	66.8	79.2	0.04	76.7	0.06						
[<i>Pseudomonas</i>] <i>palleronii</i> Stanier 362t1 ^T	67.3	78.1	0.06	80.4	0.07	73.4	0.06	76.0 ^a	0.05 ^a	77.5 ^b	0.06 ^b
[<i>Pseudomonas</i>] <i>palleronii</i> Stanier 362t2 ^T	67.3	78.4	0.04	79.1	0.07						
[<i>Pseudomonas</i>] <i>palleronii</i> Stanier 366	67.5	77.4	0.06	79.1	0.06						
[<i>Pseudomonas</i>] <i>palleronii</i> RH2	68.3	79.0	0.05	79.5	0.06						
[<i>Pseudomonas</i>] <i>palleronii</i> Z14	68.5	77.8	0.06	79.1	0.09						
[<i>Pseudomonas</i>] <i>palleronii</i> Z17	68.3	77.7	0.05	78.6	0.05						
[<i>Pseudomonas</i>] <i>palleronii</i> Z32	68.0	79.1	0.04	79.0	0.06						
[<i>Pseudomonas</i>] <i>taeniospiralis</i> DSM 2082 ^T	64.8	78.5	0.09	79.0	0.05			75.0	0.04		
[<i>Pseudomonas</i>] <i>facilis</i> ATCC 11228 ^T	64.7 ^a	76.8	0.06	76.3	0.06	75.0	0.08	77.0 ^a	0.09 ^a	77.5 ^b	0.07 ^b
[<i>Pseudomonas</i>] <i>facilis</i> ATCC 15376	63.7 ^a	76.1	0.06	76.0	0.06			76.5	0.09	78.0	0.08
[<i>Pseudomonas</i>] <i>delafeldii</i> ATCC 17505 ^T	65.6 ^b	75.7	0.04	75.6	0.06	75.1	0.05	77.2 ^b	0.11 ^b	76.6 ^b	0.02 ^b
[<i>Pseudomonas</i>] <i>delafeldii</i> Stanier 134t1	65.3	77.0	0.07	75.3	0.09			78.0	0.10	77.5	0.09
[<i>Pseudomonas</i>] <i>avenae</i> NCPPB 1011 ^T	69.8	76.8	0.04	75.6	0.07	75.7	0.09	76.6 ^b	0.12 ^b	76.5 ^b	0.09 ^b
[<i>Pseudomonas</i>] <i>saccharophila</i> ATCC 15946 ^T	69.1	72.5	0.07	73.4	0.05	71.8	0.05	72.6 ^b	0.05 ^b	73.4	0.04
“[<i>Pseudomonas</i>] <i>gazotroph</i> ” DSM 1085 ^T	58.0	67.3		68.0							
<i>Comamonas terrigena</i> NCIB 8193 ^T	64.0 ^c	74.2	0.41	73.9	0.14	81.1	0.32	75.9 ^c	0.19 ^c	75.5 ^c	0.16 ^c
<i>Comamonas terrigena</i> NCIB 2581	65.8 ^c					79.4	0.27	75.5 ^c	0.16 ^c	76.0 ^c	0.20 ^c
<i>Comamonas terrigena</i> CCUG 12940	66.1					80.5	0.23	76.9	0.14		
<i>Comamonas terrigena</i> NCIB 2582	66.1 ^c					81.2	0.30	76.0 ^c	0.19 ^c	76.0 ^c	0.19 ^c
<i>Comamonas acidovorans</i> Stanier 14 ^T	66.6 ^a	73.6	0.04	73.6	0.07	75.0	0.15	80.6 ^a	0.12 ^a	77.0 ^c	0.10 ^c
<i>Comamonas acidovorans</i> ATCC 17406	68.4 ^a					76.2	0.16	79.5 ^a	0.12 ^a	76.5 ^c	0.10 ^c
<i>Comamonas acidovorans</i> ATCC 17476	68.4 ^a					75.8	0.16	81.0 ^a	0.10 ^a	77.0 ^c	0.10 ^c
<i>Comamonas testosteroni</i> NCTC 10698 ^T	62.5 ^a	74.5	0.36	75.6	0.17	76.7	0.13	76.5 ^a	0.17 ^a	76.5 ^c	0.18 ^c
<i>Comamonas testosteroni</i> ATCC 17407	64.5 ^a					75.9	0.14	77.5 ^a	0.17 ^a		
<i>Comamonas testosteroni</i> ATCC 17409	63.0 ^a					77.7	0.24	77.5 ^a	0.15 ^a	77.0 ^c	0.17 ^c
[<i>Alcaligenes</i>] <i>paradoxus</i> ATCC 17713t1 ^T	67.0 ^a	76.0	0.11	76.0	0.03	74.8	0.05	76.5 ^a	0.03 ^a	81.0 ^c	0.06 ^c
[<i>Alcaligenes</i>] <i>latus</i> Palleroni H-4 ^T	70.0 ^d	70.2	0.05	73.4	0.10	71.6	0.15	74.5 ^b	0.12 ^b	70.0 ^b	0.11 ^b
[<i>Rhodocyclus</i>] <i>gelatinosus</i> NCIB 8290 ^T	70.7	70.8	0.02	71.9	0.01	73.5	0.06	74.5 ^b	0.05 ^b	71.0 ^b	0.02 ^b
<i>Xylophilus ampelinus</i> NCPPB 2217 ^T	68.4 ^b	75.5	0.06	75.8	0.06	75.6	0.10	76.0 ^b	0.09 ^b	76.2	0.06

^a Data from reference 14.

^b Data from reference 44.

^c Data from reference 16.

^d Data from reference 22.

similarities to the patterns of [*Pseudomonas*] *pseudoflava* strains. The protein electropherogram of [*Pseudomonas*] *taeniospiralis* DSM 2082^T is also visually unique. To compare representative reference strains from the acidovorans rRNA complex, we included the following three strains from which labeled rRNAs are available: *Comamonas acidovorans* Stanier 14^T, *Comamonas terrigena* NCIB 8193^T, and [*Alcaligenes*] *paradoxus* ATCC 17713t1^T. Also included were the type strains of the following representative species that are equidistantly related to each of the three above-mentioned rRNA subbranches: *Comamonas testosteroni*, [*Pseudomonas*] *avenae*, [*Pseudomonas*] *facilis*, and [*Pseudomonas*] *delafeldii*. *Xylophilus ampelinus* NCPPB 2217^T cannot be grown on nutrient agar and therefore was not included. There was no resemblance between the protein patterns of the yellow-pigmented hydrogen-oxidizing [*Pseu-*

domonas] species and the pattern of any of the reference strains from the acidovorans rRNA complex investigated (Fig. 4).

Numerical analysis of gas chromatographic cellular fatty acid patterns. Table 4 shows the cellular fatty acid compositions of the species examined. The common pattern was characterized by unbranched fatty acids with chain lengths ranging from 12 to 18 carbon atoms, with palmitoleic acid (16:1), palmitic acid (16:0), and *cis*-vaccenic acid (Δ11-18:1) as the most abundant constituents. Generally present, but less abundant, were also lauric acid (12:0), myristic acid (14:0), *n*-pentadecanoic acid (15:0), *n*-heptadecanoic acid (17:0), stearic acid (18:0), and cyclopropane-substituted methylene-hexadecanoic acid (17:cyc). The two hydroxylated acids, 3-hydroxy-octanoic acid (3-OH-8:0) and 3-hydroxy-decanoic acid (3-OH-10:0), were more unevenly dis-

TABLE 3. Immunodiffusion analyses

Antigen from:	Antiserum against:				
	[<i>Pseudomonas</i>] <i>palleronii</i> CCUG 1780 ^T	[<i>Pseudomonas</i>] <i>pseudoflava</i> CCUG 13799 ^T	<i>Comamonas</i> <i>acidovorans</i> CCUG 274B	<i>Comamonas</i> <i>terrigena</i> CCUG 2185 ^T	[<i>Alcaligenes</i>] <i>paradoxus</i> CCUG 1778
[<i>Pseudomonas</i>] <i>flava</i> DSM 619 ^T	0 ^a	5	1	1	0
[<i>Pseudomonas</i>] <i>pseudoflava</i> GA3 ^T	2	7	0	0	0
[<i>Pseudomonas</i>] <i>pseudoflava</i> GA2	2	6	0	0	0
[<i>Pseudomonas</i>] <i>pseudoflava</i> GA4	1	6	0	0	0
“[<i>Pseudomonas</i>] <i>carboxydoxiflava</i> ” DSM 1084 ^T	2	6	0	0	0
“[<i>Pseudomonas</i>] <i>carboxydoxiflava</i> ” Z-1607	2	6	0	0	0
[<i>Pseudomonas</i>] <i>palleronii</i> CCUG 1780 ^T	5	3	0	0	0
[<i>Pseudomonas</i>] <i>palleronii</i> Stanier 362t1 ^T	6	2	0	0	0
[<i>Pseudomonas</i>] <i>palleronii</i> Stanier 362t2 ^T	6	3	0	0	0
[<i>Pseudomonas</i>] <i>palleronii</i> Stanier 366	6	2	0	0	0
[<i>Pseudomonas</i>] <i>palleronii</i> RH2	6	3	0	0	0
[<i>Pseudomonas</i>] <i>palleronii</i> Z14	6	3	0	1	1
[<i>Pseudomonas</i>] <i>palleronii</i> Z17	6	4	1	1	1
[<i>Pseudomonas</i>] <i>palleronii</i> Z32	6	4	0	0	1
[<i>Pseudomonas</i>] <i>taeniospiralis</i> DSM 2082 ^T	1	5	0	0	0
[<i>Pseudomonas</i>] <i>facilis</i> ATCC 11228 ^T	1	3	1	0	1
[<i>Pseudomonas</i>] <i>facilis</i> ATCC 15376	1	3	2	1	1
[<i>Pseudomonas</i>] <i>delafeldii</i> ATCC 17505 ^T	0	3	0	0	0
[<i>Pseudomonas</i>] <i>delafeldii</i> Stanier 134t1	0	4	1	1	1
[<i>Pseudomonas</i>] <i>delafeldii</i> ATCC 17508	0	2	1	1	0
[<i>Pseudomonas</i>] <i>avenae</i> NCPPB 1011 ^T	0	2	1	1	1
[<i>Pseudomonas</i>] <i>cattleyae</i> NCPPB 961 ^T	0	1	0	0	1
[<i>Pseudomonas</i>] <i>rubrilineans</i> NCPPB 920 ^T	0	2	0	1	1
“[<i>Pseudomonas</i>] <i>setariae</i> ” NCPPB 1392 ^T	0	1	1	0	1
“[<i>Pseudomonas</i>] <i>gazotroph</i> ” DMS 1085 ^T	0	0	0	0	0
<i>Comamonas testosteroni</i> NCTC 10698 ^T	0	2	2	3	0
<i>Comamonas testosteroni</i> ATCC 17407	0	2	1	3	0
<i>Comamonas testosteroni</i> ATCC 17409	0	1	1	2	0
<i>Comamonas terrigena</i> NCIB 8193 ^T	0	0	3	6	0
<i>Comamonas terrigena</i> NCIB 2581	0	1	3	5	0
<i>Comamonas terrigena</i> CCUG 12940	0	2	3	5	1
<i>Comamonas terrigena</i> NCIB 2582	0	1	2	5	0
<i>Comamonas acidovorans</i> Stanier 14 ^T	1	2	7	1	0
<i>Comamonas acidovorans</i> ATCC 17406	0	1	5	3	0
<i>Comamonas acidovorans</i> ATCC 17476	0	2	6	2	0
<i>Comamonas acidovorans</i> CCUG 274B	1	2	8	4	1
[<i>Alcaligenes</i>] <i>paradoxus</i> ATCC 17713t1 ^T	0	1	0	0	4
[<i>Alcaligenes</i>] <i>paradoxus</i> ATCC 17712	1	1	1	1	5
[<i>Alcaligenes</i>] <i>paradoxus</i> CCUG 1778	0	1	0	0	6
[<i>Alcaligenes</i>] <i>latus</i> Palleroni H-4 ^T	0	1	2	0	0

^a 0, No precipitate; 1 or 2, weak reaction with uncertain interpretation; 3, weak reaction usually revealing relatedness when serum specificity was high; 4 or 5, moderate reaction revealing relatedness or identity with unsatisfactory serum; 6, 7, or 8, strong reaction observed only with closely related strains. Each value is the average of at least two successful immunodiffusion analyses performed under similar conditions.

clusters; however, both the interspecies and intergeneric distances appear to be uncorrelated with the genetic affiliations observed.

Quinone and polyamine contents. A ubiquinone with eight isoprenoid units in the side chain was found in [*Pseudomonas*] *flava* DSM 619^T, [*Pseudomonas*] *pseudoflava* GA3^T, “[*Pseudomonas*] *carboxydoxiflava*” DSM 1084^T, [*Pseudomonas*] *taeniospiralis* DSM 2082^T, [*Pseudomonas*] *palleronii* Stanier 362t1^T, and “[*Pseudomonas*] *gazotroph*” DSM 1085^T. A quantitative analysis of the polyamines of these strains showed nearly identical patterns. The following data for [*Pseudomonas*] *flava* DSM 619^T are representative: 1 g (dry weight) of cells contained 36.3 μmol of hydroxyputrescine and 35.6 μmol of putrescine. [*Pseudomonas*] *pseudoflava* GA3^T and “[*Pseudomonas*] *carboxydoxiflava*” DSM 1084^T contained low amounts of spermidine and spermine in addition. This polyamine pattern is typical for members of superfamily III (5).

DISCUSSION

Our results show that [*Pseudomonas*] *flava*, [*Pseudomonas*] *palleronii*, [*Pseudomonas*] *pseudoflava*, “[*Pseudomonas*] *carboxydoxiflava*,” and [*Pseudomonas*] *taeniospiralis* form a separate group within the acidovorans rRNA complex. Our arguments are given below. (i) The hybrids between rRNAs from [*Pseudomonas*] *pseudoflava* and [*Pseudomonas*] *palleronii* and DNAs from strains of [*Pseudomonas*] *flava*, [*Pseudomonas*] *pseudoflava*, “[*Pseudomonas*] *carboxydoxiflava*,” [*Pseudomonas*] *palleronii*, and [*Pseudomonas*] *taeniospiralis* have $T_{m(e)}$ values ranging from 77.3 to 81.1°C. Hybrids between the same rRNAs and DNAs from representative strains of other taxa belonging to the acidovorans rRNA complex have distinctly lower $T_{m(e)}$ values ($76.1 \pm 1.1^\circ\text{C}$). Therefore, [*Pseudomonas*] *flava*, [*Pseudomonas*] *palleronii*, [*Pseudomonas*] *pseudoflava*,

FIG. 3. Dendrogram obtained by unweighted average linkage cluster analysis of S_G values. Each of the 38 strains was tested for 251 phenotypic features. For abbreviations see the legend to Fig. 1.

“[*Pseudomonas*] carboxydoflava,” and [*Pseudomonas*] taeniospiralis are more closely related to each other than to the other taxa belonging to the acidovorans rRNA complex. (ii) High levels of cross-reaction for the group of five species described above versus antisera from [*Pseudomonas*] palleronii and [*Pseudomonas*] pseudoflava and low levels of cross-reaction versus the other reference antisera from organisms belonging to the acidovorans rRNA complex were obtained. (iii) [*Pseudomonas*] flava, [*Pseudomonas*] pseudoflava, “[*Pseudomonas*] carboxydoflava,” and [*Pseudomonas*] taeniospiralis formed a separate phenon in our numerical analysis of phenotypic features (Fig. 3). The [*Pseudomonas*] palleronii phenon is linked with the phenon formed by the four yellow-pigmented hydrogen-oxidizing [*Pseudomonas*] species at an S_G value of only 66.8% (Fig. 3). This is due to the limited number of carbohydrates used for growth by [*Pseudomonas*] palleronii strains and even more to the great substrate versatility of the other species.

Information from the literature is not opposed to our data. No significant DNA homology was found between [*Alcaligenes*] paradoxus, [*Pseudomonas*] facilis, or [*Pseudomonas*] saccharophila and either [*Pseudomonas*] palleronii, [*Pseudomonas*] flava, or [*Pseudomonas*] pseudoflava (2). The 3-hydroxy fatty acid and quinone patterns were similar in [*Pseudomonas*] flava, [*Pseudomonas*] pseudoflava, [*Pseudomonas*] acidovorans, [*Pseudomonas*] testosteroni, and *Comamonas terrigena*; [*Pseudomonas*] palleronii was placed in a separate group (35). However, these data do not allow conclusions at the generic level.

The acidovorans rRNA complex consists of at least 10 rRNA subbranches, which are very heterogeneous phenotypically and genotypically (Willems, Gillis, and De Ley, manuscript in preparation). Several genera are needed to accommodate the component groups; two of these genera,

FIG. 4. Normalized sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns of representative strains of *Hydrogenophaga* species ([*Pseudomonas*] flava, [*Pseudomonas*] pseudoflava, “[*Pseudomonas*] carboxydoflava,” [*Pseudomonas*] palleronii, [*Pseudomonas*] taeniospiralis) and the type strains of some reference taxa. The data are presented in a correlation coefficient r dendrogram as calculated by the unweighted average pair group method. Each branch of the dendrogram faces the corresponding electrophoretic trace in the photograph. For abbreviations see the legend to Fig. 1.

TABLE 4. Cellular fatty acid compositions of *[Pseudomonas] flava*, *[Pseudomonas] palleronii*, *[Pseudomonas] pseudoflava*, "[*Pseudomonas*] carboxydoflava," [*Pseudomonas*] *taeniospiralis*, [*Pseudomonas*] *facilis*, [*Pseudomonas*] *delafieldii*, [*Pseudomonas*] *avenae*, *Comamonas terrigena*, *Comamonas acidovorans*, *Comamonas testosteroni*, [*Alcaligenes*] *paradoxus*, [*Alcaligenes*] *latus*, *Rhodocyclus gelatinosus*, and "[*Pseudomonas*] gazotropha"

Fatty acid	% of total in:														
	<i>[Pseudo- monas]</i> <i>flava</i> (1) ^a	<i>[Pseudo- monas]</i> <i>palleronii</i> (7)	<i>[Pseudo- monas]</i> <i>pseudo- flava</i> (3)	"[<i>Pseudo- monas]</i> <i>carboxy- doflava</i> " (1)	<i>[Pseudo- monas]</i> <i>taenio- spiralis</i> (1)	<i>[Pseudo- monas]</i> <i>facilis</i> (1)	<i>[Pseudo- monas]</i> <i>delafieldii</i> (1)	<i>[Pseudo- monas]</i> <i>avenae</i> (1)	<i>Comamo- nas terri- gena</i> (4)	<i>Comamo- nas aci- dovorans</i> (3)	<i>Comamo- nas test- osteroni</i> (3)	<i>[Alcali- genes]</i> <i>paradoxus</i> (3)	<i>[Alcali- genes]</i> <i>latus</i> (1)	<i>[Rhodo- cyclus]</i> <i>gelatino- sus</i> (1)	"[<i>Pseudo- monas]</i> <i>gazotro- pha</i> " (1)
12:0 ^b	0.2	0.0-tr ^c	tr	tr	1.4	2.7	2.7	2.3	2.7-6.1	2.6-3.4	3.6-6.5	2.7-3.0	1.1	3.7	1.8
14:0	2.8	0.2-0.4	4.5-4.7	3.3	3.1	2.7	4.1	2.4	3.0-3.9	0.7-1.1	0.5-0.6	0.6-1.0	2.3	4.3	3.2
15:1	tr	0.1-3.1	0.1-0.4	tr	0.2		tr				tr	0.1-0.7		0.2	tr
15:0	0.4	0.2-5.1	0.6-0.7	0.2	2.7	0.3	0.4	0.3	0.8-1.0	0.3-1.1	0.2-0.6	0.1-1.9	tr	0.4	tr
16:1	51.7	25.3-34.4	42.4-49.8	45.0	43.1	42.4	39.5	41.6	37.5-41.5	30.2-35.4	22.2-33.5	32.2-33.4	35.3	38.7	50.3
16:0	19.4	16.3-27.2	23.1-25.9	21.3	27.1	24.8	24.9	29.0	20.4-24.9	23.9-28.3	14.4-24.3	20.7-26.5	19.8	20.3	32.2
17:1	tr	0.0-0.7	tr	tr	0.7		tr		tr	tr		tr		0.2	tr
17:0	1.3	0.2-7.1	0.6-0.8	0.3	0.7	0.2	0.2	0.2	0.2-0.4	0.1-0.6	tr-6.8	0.2-0.9		0.2	tr
Δ ⁹ -18:1 ^d	0.4	0.1-0.2	0.2-0.7	0.2	0.7	0.7	0.5	0.5	0.4-0.5	0.4-0.6	0.0-0.8	0.6-0.8	0.9	0.6	
Δ ¹¹ -18:1 ^c	13.3	13.5-23.3	7.8-9.4	7.2	9.6	18.0	20.3	15.9	11.6-14.4	14.7-16.6	12.4-16.1	12.3-20.1	15.5	15.5	2.0
18:0	1.5	0.2-0.5	0.6-0.7	0.7	0.3	0.2	0.3	0.3	tr	0.2-0.5	0.2-0.8	0.3-0.5	2.7	0.2	0.2
17:cyc	tr	12.9-21.1	1.9-2.5	2.4	5.3	0.2	0.4	0.2	1.2-4.8	9.0-14.5	1.5-15.2	3.7-6.0	tr	0.2	1.7
19:cyc		tr								tr	tr				
3-OH-8:0	1.2	1.9-5.6	1.0-1.5	1.2	2.7	1.6	1.2			2.0-2.3		1.9-2.3			
3-OH-10:0	2.9		1.1-4.1	6.3		4.4	4.5	5.8	7.4-9.0	4.9-6.7	7.8-17.3	4.8-5.6	7.2	7.4	5.6
3-OH-18:0			tr												
2-OH-14:0											0.3-0.8	3.0			
2-OH-16:0				tr							3.9-6.9				
2-OH-18:0											0.2-1.7				

^a The numbers in parentheses are the numbers of strains investigated; the type strain was included for each taxon.

^b Number of carbon atoms; number of double bonds.

^c Range among strains. tr, Less than 0.1%.

^d Oleic acid.

^e *cis*-Vaccenic acid.

FIG. 5. Score plot of species on the basis of cellular fatty acid data. PC, principal component. Species: 1, [*Pseudomonas*] *flava*; 2, [*Pseudomonas*] *pseudoflava*; species 3, "[*Pseudomonas*] *carboxydo-flava*"; 4, [*Pseudomonas*] *palleronii*; 5, [*Pseudomonas*] *taenio-spiralis*; 6, [*Pseudomonas*] *avenae*; 7, [*Pseudomonas*] *delafeldii*; 8, [*Pseudomonas*] *facilis*; 9, *Comamonas terrigena*; 10, *Comamonas acidovorans*; 11, *Comamonas testosteroni*; 12, [*Alcaligenes*] *paradoxus*; 13, [*Alcaligenes*] *latus*; and 14, [*Rhodocyclus*] *gelatinosus*.

Xylophilus (44) and *Comamonas* (16, 42), exist already. Inclusion of our five species in the genus *Xylophilus* is ruled out because of too many phenotypic differences (44). Transfer to the genus *Comamonas* is not acceptable either. Tamaoka et al. (42) proposed emendation of the genus *Comamonas* by including [*Pseudomonas*] *acidovorans* and [*Pseudomonas*] *testosteroni* in this genus. This proposal was based on a combination of data from several techniques, although no other taxa belonging to the acidovorans rRNA complex were included. Our DNA:rRNA hybridization data show that *Comamonas acidovorans* and *Comamonas testosteroni* do not belong to the *Comamonas terrigena* rRNA subbranch, but are located at the branching level, together with all other taxa in the acidovorans rRNA complex. This shows that a genus can consist of one or more rRNA subbranches, but the acidovorans rRNA complex is too heterogeneous to be included in one genus. For taxonomic conclusions DNA:rRNA hybridization data should be evaluated in combination with the results from other techniques (44). There are important genotypic and immunotypic differences between the members of the genus *Comamonas* and the five yellow-pigmented hydrogen-oxidizing species (Fig. 1 and Table 3). Moreover, phenotypic differentiation of the species belonging to the genus *Comamonas* is clearly possible (Table 5), although in some features [*Pseudomonas*] *palleronii* is aberrant from its four neighbors. The separate position of the five yellow-pigmented hydrogen-oxidizing species should be reflected by creation of a new genus. We propose to name the new genus *Hydrogenophaga*. Features differentiating the genus *Hydrogenophaga* from the other hydrogen-oxidizing taxa of the acidovorans rRNA complex and from the genus *Comamonas* are given in Table 5.

The internal taxonomy of the genus *Hydrogenophaga* was

TABLE 5. Differentiating features for the genus *Hydrogenophaga*, the genus *Comamonas*, [*Pseudomonas*] *facilis*, [*Alcaligenes*] *paradoxus*, and [*Pseudomonas*] *saccharophila*

Feature	<i>Hydrogenophaga</i>	<i>Comamonas</i> ^a	[<i>Pseudomonas</i>] <i>facilis</i> ^a	[<i>Alcaligenes</i>] <i>paradoxus</i> ^a	[<i>Pseudomonas</i>] <i>saccharophila</i> ^b
Yellow nondiffusible pigment	+ ^c	-	-	+	-
Autotrophic growth with H ₂	+	-	+	d	+
Growth on:					
D-Glucose	+	-	+	+	+
L-Arabinose, D-galactose	D	-	d	+	+
Maltose, sucrose	D	-	-	+	+
Ribose	-	-	-	-	+
D-Cellobiose	D	-	-	d	+
Adonitol	-	-	-	d	+
Isobutyrate	-	-	-	d	-
Glycolate	D	d	-	d	+
Citrate	-	d	-	d	-
Mesaconate	-	d	-	d	-
<i>T_{me}</i> range vs rRNAs from strains GA3 ^T and Stanier 3621 ^T (°C)	77.3 - 81.1	76.1 ± 1.1	76.1 ± 1.1	76.1 ± 1.1	73.1 ± 1.4
Occurrence of 2-hydroxy-substituted fatty acids	-(13) ^d	d(10)	-(1)	+(3)	-

^a Our own unpublished data and data from references 16, 22, and 36.

^b Phenotypic features from reference 36.

^c +, Present in 90% or more of the strains; -, present in 10% or less of the strains; d, 11 to 89% of all strains positive; D, different reactions for different *Hydrogenophaga* species.

^d The numbers in parentheses are numbers of strains investigated.

studied by using several suitable techniques. "[*Pseudomonas*] *carboxydoflava*" and [*Pseudomonas*] *pseudoflava* strains produce very similar protein electropherograms (Fig. 4) and have very similar fatty acid compositions (Table 4 and Fig. 5) and phenotypic features (S_G value, 82.3%) (Fig. 3). A DNA-binding level of at least 79% (Fig. 2) and $T_{m(e)}$ values for hybridizations between "[*Pseudomonas*] *carboxydoflava*" DNA and rRNA of [*Pseudomonas*] *pseudoflava* GA3^T of 79.2 to 80.8°C (Table 2) justify the fusion of these two taxa into one species, *Hydrogenophaga pseudoflava*. The ability of "[*Pseudomonas*] *carboxydoflava*" to grow at the expense of carbon monoxide (33) should not be an argument for maintaining it as a separate species. Plasmids have been found in "[*Pseudomonas*] *carboxydoflava*" DSM 1084^T, and carbon monoxide oxidation has been determined to be a plasmid-encoded, and thus not stable, trait in other gram-negative bacteria (26).

Our DNA:DNA hybridization results indicate a close relationship among [*Pseudomonas*] *flava*, [*Pseudomonas*] *pseudoflava*, and "[*Pseudomonas*] *carboxydoflava*" (Fig. 2) (degree of binding, 48 to 62%). The protein electrophoretic pattern of [*Pseudomonas*] *flava* resembles the patterns of the [*Pseudomonas*] *pseudoflava* strains, but it is clearly different from them in the central part of the gel (Fig. 4). On the other hand, the fatty acid compositions did not reveal any significant differences between [*Pseudomonas*] *flava* and [*Pseudomonas*] *pseudoflava*. As Fig. 5 shows, these species form a uniform cluster without any visible subgrouping, but distinctly separated from the cluster formed by [*Pseudomonas*] *palleronii* and [*Pseudomonas*] *taeniospiralis*. However, because of the considerable phenotypic and other differences with [*Pseudomonas*] *flava*, Auling et al. (4) created [*Pseudomonas*] *pseudoflava* as a separate species. This is also reflected in the results of our phenotypic analysis (Fig. 3), and we therefore prefer to maintain the two taxa as separate species, *Hydrogenophaga flava* and *Hydrogenophaga pseudoflava*.

All [*Pseudomonas*] *palleronii* strains form a homogeneous and separate group as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 4), as well as by phenotypic analyses (Fig. 3), numerical analysis of fatty acid patterns (Fig. 5), and DNA:DNA hybridization (Fig. 2). These strains are transferred to the genus *Hydrogenophaga* as a separate species, *Hydrogenophaga palleronii*. Auling et al. (2) also reported very low DNA:DNA hybridization values between [*Pseudomonas*] *palleronii* and both [*Pseudomonas*] *pseudoflava* and [*Pseudomonas*] *flava* (degrees of binding, 25 and 34%, respectively).

[*Pseudomonas*] *taeniospiralis*, containing only one strain, occupies gel electrophoretically (Fig. 4), genotypically (Fig. 2), and phenotypically (Fig. 3) a separate position among the yellow-pigmented hydrogen-oxidizing [*Pseudomonas*] species. From the numerical analysis of fatty acid patterns (Fig. 5) a closer relationship with [*Pseudomonas*] *palleronii* can be presumed, but this could not be confirmed by DNA:DNA hybridizations (Fig. 2). [*Pseudomonas*] *taeniospiralis* is transferred to the genus *Hydrogenophaga* as *Hydrogenophaga taeniospiralis*. Differentiating features for the four *Hydrogenophaga* species are shown in Table 6.

"[*Pseudomonas*] *gazotropha*" was included in this study because its facultatively lithoautotrophic growth at the expense of hydrogen or carbon monoxide (48) could indicate a closer relationship to [*Pseudomonas*] *pseudoflava*. Our results (Tables 2 and 3) prove that "[*Pseudomonas*] *gazotropha*" is a member of rRNA superfamily III, but its exact position within this group is still unknown. The presence of

hydroxyputrescine also indicates that it belongs in rRNA superfamily III (5). It is not related to [*Pseudomonas*] *pseudoflava* and does not belong to the acidovorans rRNA complex. Furthermore, the fatty acid pattern of "[*Pseudomonas*] *gazotropha*" DSM 1085^T does not contain oleic acid (Δ^9 -18:1), which is present in all of the strains belonging to the acidovorans rRNA complex which we tested.

Description of *Hydrogenophaga* gen. nov. *Hydrogenophaga* (Hy.dro.ge.no'pha.ga. Gr. n. *hydroo*, water; Gr. n. *gennaoo*, to create; M.L. *hydrogenum*, hydrogen, that which produces water; Gr. v. *phagein*, to eat; M.L. fem. n. *Hydrogenophaga*, eater of hydrogen). Cells are straight to slightly curved rods (0.3 to 0.6 by 0.6 to 5.5 μ m); they occur singly or in pairs. Motile by means of one, rarely two, polar to subpolar flagella. Gram negative. Oxidase positive. Catalase variable. A nondiffusible yellow pigment is produced. Aerobic. Chemoorganotrophic or chemolithoautotrophic, using the oxidation of hydrogen as an energy source. Oxidative carbohydrate metabolism with oxygen as a terminal electron acceptor or heterotrophic denitrification of nitrate (by *Hydrogenophaga pseudoflava* and *Hydrogenophaga taeniospiralis*). Cells grow well on media containing organic acids, amino acids, or peptone, but are less versatile in the use of carbohydrates. A cyclopropane-substituted fatty acid (17: cyc) is present; 3-hydroxy-octanoic acid (3-OH-8:0) is present alone or together with 3-hydroxy-decanoic acid (3-OH-10:0). 2-Hydroxy-substituted fatty acids are absent. Ubiquinone Q-8 is present as the main quinone. 2-Hydroxyputrescine and putrescine are present in roughly equimolar concentrations either exclusively or as the dominant polyamine compounds. The mean G+C content of the DNA ranges from 65 to 69 mol%. In DNA:rRNA hybridizations $T_{m(e)}$ values of 77.3 to 81.1°C are obtained versus rRNA from *Hydrogenophaga pseudoflava* GA3^T or *Hydrogenophaga palleronii* Stanier 3621^T. The genus *Hydrogenophaga* belongs to the acidovorans rRNA complex. It is equidistantly related to the following other taxa in this complex: the genera *Comamonas* and *Xylophilus* and the generically misnamed species [*Pseudomonas*] *facilis*, [*Pseudomonas*] *delafeldii*, [*Pseudomonas*] *avenae*, and [*Alcaligenes*] *paradoxus*. The type species is *Hydrogenophaga flava* (Niklewski 1910) comb. nov. This species grows slowly and easily loses its chemolithoautotrophic ability (4). Because of its high genotypic and protein electrophoretic similarity to the type species, *Hydrogenophaga pseudoflava* can be used as an alternative reference species for the genus.

Description of *Hydrogenophaga flava* (Niklewski 1910) comb. nov. The description of *Hydrogenophaga flava* (fla'va. L. fem. adj. *flava*, yellow) is the same as that for the genus. The ability to grow lithoautotrophically at the expense of hydrogen is easily lost (4). Aragno et al. (1) reported the presence of pili spread over the total cell surface. Morphological, biochemical, and nutritional features have been described by Davis et al. (8) and by Palleroni (36). Our findings, comprising more than 250 features, corroborate these data fairly well except for the following features (our results are indicated in parentheses): amylase (negative) and the use of the following substrates as sole carbon sources—D-arabinose (positive), salicin (positive), levulinate (positive), L-isoleucine (positive), L-histidine (positive), butyrate (late [7 to 15 days]), amylamine (late), L-tartrate (negative), 2-ketoglutarate (negative), and L- α -alanine (negative). The hydrolysis of esculin and the urease reaction are positive. *Hydrogenophaga flava* was isolated from mud. The mean G+C content of the DNA is 67 mol%. The monotype strain is strain DSM 619 (= LMG 2185 = CCUG 1658 = ATCC

TABLE 6. Differentiation among the four *Hydrogenophaga* species

Feature	<i>Hydrogenophaga flava</i>	<i>Hydrogenophaga palleronii</i>	<i>Hydrogenophaga pseudo-flava</i>	<i>Hydrogenophaga taeniospiralis</i>
Growth on:				
Glycolate	+	+	+	—
L-Arabinose, sucrose, D-galactose, D-fructose, D-mannose, mannitol, D-arabitol, sorbitol, D-cellobiose, butylamine, ethanolamine	+	—	+	+
Maltose, D-turanose, L-histidine	+	—	+	—
L-Fucose	+	—	—	+
Azelate	—	+	+	+
D-Xylose, DL-5-aminovalerate	—	—	+	+
Lactose	—	—	+	—
DL-3-Aminobutyrate	—	—	—	+
Denitrification	—	—	+	+
Reduction of nitrate	+	—	+	+
Reduction of nitrite	—	—	+	+
Presence of 3-hydroxy-decanoic acid	+	—	+	—

33667). This strain is the neotype strain proposed by Klyuver and Manten (25) to replace the original [*Pseudomonas*] *flava* type strain of Niklewski (32), which has been lost.

Description of *Hydrogenophaga palleronii*. (Davis, Stanier, Doudoroff, and Mandel 1970) *comb. nov.* The description of *Hydrogenophaga palleronii* (pal.le.ro'ni.i. M.L. gen. n. *palleronii*, of Palleroni, named after N. J. Palleroni, who first isolated the type strain) is the same as that for the genus. Two strains, including the type strain, have been reported to bear pili at the polar caps of the cells (1). Biochemical and nutritional features have been described by Davis et al. (8) and by Palleroni (36). The findings of these authors are in good agreement with our own extensive findings, except for the auxanographic features listed below. All of the strains tested use isovalerate and sebacate as sole carbon sources. Only some strains (listed in parentheses) use the following substrates as sole carbon sources: L- α -alanine, L-leucine, and L-phenylalanine (Stanier 362t1^T, RH2, Z14, Z17, and Z32); pimelate (Stanier 362t1^T, Stanier 366, RH2, Z32, and Z17); DL-norleucine (Stanier 362t1^T, Z14, Z17, and Z32); L-norleucine and D- α -alanine (Stanier 362t1^T, RH2, and Z32); L-tyrosine and L-tryptophan (RH2, Z14, and Z17); *n*-valerate (Stanier 362t1^T, Stanier 366, and RH2); terphenylate (Stanier 362t1^T and Stanier 366); L-isoleucine (Stanier 362t1^T and Z32); propionate (Stanier 366 and RH2); and itaconate (Stanier 362t1^T). None of the strains tested use the following organic acids as sole carbon sources: acetate, L-tartrate, 2-ketoglutarate, aconitate, and citrate. For four of the seven strains tested, weak production of acid in oxidative-fermentative medium containing D-glucose occurs; the type strain does not produce acid, in agreement with the report by Auling et al. (4). *Hydrogenophaga palleronii* strains were isolated from water enriched for hydrogen bacteria. The mean G+C content of the DNA ranges from 67 to 68 mol%. The type strain is strain Stanier 362t1 (= LMG 2366t1 = CCUG 20334 = ATCC 17724). Its characteristics are the same as those listed above for the species, and the mean G+C content of its DNA is 67 mol%.

Description of *Hydrogenophaga pseudo-flava* (Auling, Reh, Lee, and Schlegel 1978) *comb. nov.* The description of

Hydrogenophaga pseudo-flava (pseu.do fla'va. Gr. adj. *pseudes*, false; L. fem. adj. *flava*, yellow; M. L. fem. adj. *pseudo-flava*, not the true [*Pseudomonas*] *flava*) is the same as that for the genus. Some strains of this species (originally described as "[*Pseudomonas*] *carboxydo-flava*") are facultative autotrophs, growing at the expense of carbon monoxide oxidation (33). Aragno et al. (1) reported the presence of pili over the total cell surface for two strains, including the type strain. Since this species includes strains of both the former species [*Pseudomonas*] *pseudo-flava* and the former species "[*Pseudomonas*] *carboxydo-flava*," we provide a new description for *Hydrogenophaga pseudo-flava* based on our own results, which are generally in good agreement with the original species descriptions (4, 48). Nutritional and enzymatic features are listed in Table 7. The following biochemical and physiological characteristics are present in all *Hydrogenophaga pseudo-flava* strains: growth at 30 and 37°C, growth in the presence of 0.5% NaCl, hydrolysis of Tween 80 and esculin, reduction of nitrate and nitrite, denitrification, and β -galactosidase. The following features are lacking in all *Hydrogenophaga pseudo-flava* strains: catalase, hemolysis, resistance to penicillin (10 μ g per disk), growth on Drigalski-Conradi agar at 30°C, growth at 42°C, growth in the presence of 1.5, 3, 4.5, or 6.5% NaCl, production of acid in 10% lactose, triple sugar iron medium, and oxidative-fermentative medium containing D-glucose, D-fructose, D-xylose, maltose, or adonitol, fluorescence, growth on cetrimide, production of H₂S in triple sugar iron medium, amylase, lysine and ornithine decarboxylase, arginine dihydrolase, urease, and hydrolysis of gelatin. Hydrolysis of DNA and acetamide and production of acid from indole are absent in all strains except the type strain, which was not tested. Recently, Jenni et al. (21) reported nitrogen fixation for a strain (strain NEU 2226) which they assigned to [*Pseudomonas*] *pseudo-flava*, although this feature could not be detected in strain GA3^T. *Hydrogenophaga pseudo-flava* was isolated from soil, mud, and water by enrichment for hydrogen bacteria (4, 33). The mean G+C content of the DNA is 66 to 69 mol%. The type strain is strain GA3 (= LMG 5945 = CCUG 13799 = ATCC 33668). Its characteristics are the same as those listed above for the species, and the mean G+C content of its DNA is 66 mol%.

Description of *Hydrogenophaga taeniospiralis* (Lalucat, Parés, and Schlegel 1982) *comb. nov.* The description of *Hydrogenophaga taeniospiralis* (tae.ni.o.spi.ra'lis. Gr. n. *taenia*, ribbon; L. adj. *spiralis*, coiled; M.L. adj. *taeniospiralis*, ribbon coiled, named after *Caedibacter taeniospiralis*, an organism also containing R bodies) is the same as that for the genus. The original description by Lalucat et al. (28) was verified. This description was found to be in good agreement with our own results, except for the following features (our results are indicated in parentheses): reduction of nitrite (positive), hydrolysis of esculin (positive), urease (weak), catalase (negative), growth at 37°C (negative), and use of the following substrates as sole carbon sources—butyrate (positive), trehalose (negative), and L-lysine (negative). Additions to the original description are presented below. Nutritional and enzymological features are listed in Table 7. *Hydrogenophaga taeniospiralis* grows at 30°C, hydrolyzes Tween 80, and possesses β -galactosidase. The following features are lacking: growth at 42°C, growth on Drigalski-Conradi agar at 30°C, growth in the presence of 0.5, 1.5, 3, 4.5, or 6.5% NaCl, resistance to penicillin (10 μ g per disk), hemolysis, production of acid in oxidative-fermentative medium containing D-glucose, maltose, or adonitol, growth on cetrimide, lysine and ornithine decarboxylase, arginine di-

TABLE 7. Nutritional and enzymatic features of *Hydrogenophaga pseudoflava* and *Hydrogenophaga taeniospiralis*

Feature	<i>Hydrogenophaga pseudoflava</i> (5 strains)	<i>Hydrogenophaga taeniospiralis</i> (1 strain)
Carbon sources for growth ^a :		
D-Turanose, maltose, lactose, trehalose, salicin, acetate, propionate, <i>n</i> -valerate, glycolate, L- α -alanine, L-serine, L-lysine, L-histidine, amylamine, benzylamine	+	—
L-Fucose, glutarate, 2-ketoglutarate, DL-3-aminobutyrate	—	+
L-Isoleucine, diaminobutane	GA3 ^T , GA4, DSM 1084, Z-1607	+
<i>m</i> -Hydroxybenzoate	GA3 ^T , GA2, GA4, DSM 1084	—
<i>n</i> -Caproate	GA3 ^T , GA2, GA4, Z-1607	—
L-Valine	GA3 ^T , GA2, DSM 1084, Z-1607	—
L-Mandelate, inositol, L-aspartate	GA3 ^T , GA2, GA4	+
D- α -Alanine	GA3 ^T , GA2, GA4	—
<i>p</i> -Hydroxybenzoate	GA3 ^T , GA2, DSM 1084	—
Spermine	GA3 ^T , DSM 1084, Z-1607	+
L-Threonine, isovalerate	GA3 ^T , DSM 1084, Z-1607	—
L-Norleucine, DL-norvaline	GA3 ^T , GA2	—
L-Arginine	GA3 ^T , GA4	+
DL-2-Aminobutyrate	GA3 ^T , GA4	—
D-Lyxose	DSM 1084, Z-1607	—
Citrate	GA4, DSM 1084	—
L-Rhamnose	GA2	+
L-Tartrate	GA2	—
DL-Norleucine	GA4	—
Hydrolysis of ^c :		
2-Naphthylphosphate (pH 8.5), 2-naphthyl- α -D-glucopyranoside	GA2, GA4, DSM 1084	—
6-Bromo-2-naphthyl- β -D-glucopyranoside	GA2, GA4	+
2-Naphthylmyristate	GA2	—

^a All *Hydrogenophaga pseudoflava* strains and *Hydrogenophaga taeniospiralis* DSM 2082^T use the following substrates as sole carbon sources: D-xylose, L-arabinose, D-galactose, D-glucose, D-fructose, D-mannose, sucrose, D-cellobiose, glycerol, sorbitol, D-arabitol, mannitol, gluconate, pyruvate, butyrate, succinate, fumarate, suberate, azelate, levulinate, sebacate, DL-lactate, DL-glycerate, DL-3-hydroxybutyrate, D-malate, L-malate, L-leucine, L-phenylalanine, L-tyrosine, L-tryptophan, L-glutamate, L-ornithine, L-proline, DL-4-aminobutyrate, DL-5-aminovalerate, butylamine, and ethanolamine. All strains use DL-lactate as a sole carbon source in the presence of L-methionine. The following substrates are not used by either species: D-ribose, L-xylose, methyl- β -D-xyloside, D-fucose, D-arabinose, L-sorbose, methyl- α -D-mannoside, methyl- α -D-glucoside, D-tagatose, D-melibiose, D-melezitose, D-raffinose, β -gentiobiose, amygdalin, arbutin, esculin, inulin, starch, glycogen, erythritol, adonitol, dulcitol, xylitol, L-arabitol, 2-ketogluconate, 5-ketogluconate, N-acetylglucosamine, isobutyrate, isovalerate, heptanoate, caprylate, pelargonate, caprate, oxalate, malonate, maleate, adipate, pimelate, D-tartrate, *meso*-tartrate, citraconate, itaconate, mesaconate, aconitate, phenylacetate, benzoate, *o*-hydroxybenzoate, D-mandelate, phthalate, isophthalate, terephthalate, glycine, L-cysteine, L-methionine, D-tryptophan, trigonellin, L-citrulline, DL-kynurenine, betaine, creatine, β -alanine, 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, urea, acetamide, sarcosine, ethylamine, histamine, tryptamine, and glucosamine.

^b +, Present in all strains tested; —, absent in all strains tested. For features present in only some strains, those strains are listed.

^c API ZYM galleries were used. All *Hydrogenophaga pseudoflava* strains and *Hydrogenophaga taeniospiralis* DSM 2082^T hydrolyze 2-naphthylcaprylate, 2-naphthylbutyrate, and L-leucyl-2-naphthylamide. The following substrates are not hydrolyzed by either species: L-cystyl-2-naphthylamide, L-valyl-2-naphthylamide, N-benzoyl-DL-arginine-2-naphthylamide, N-glutaryl-phenylalanine-2-naphthylamide, 2-naphthylphosphate (pH 5.4), 6-bromo-2-phosphodiamide-3-naphthoic acid-2-methoxyanilide (= naphthol-AS-BI-phosphodiamide), 6-bromo-2-naphthyl- α -D-galactopyranoside, 2-naphthyl- β -D-galactopyranoside, 6-bromo-2-hydroxy-3-naphthoic acid-2-methoxyanilide- β -D-glucuronate (= naphthol-AS-BI- β -D-glucuronate), 1-naphthyl-N-acetyl- β -D-glucosaminide, 6-bromo-2-naphthyl- α -D-mannopyranoside, and 2-naphthyl- α -L-fucopyranoside.

hydrolase, and hydrolysis of acetamide. *Hydrogenophaga taeniospiralis* was isolated from soil near Barcelona, Spain. The mean G+C content of the DNA is 65 mol%. The monotypic strain is strain DSM 2082 (= LMG 7170 = CCUG 15921).

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