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Transfer of *Rhodocyclus gelatinosus* to *Rubrivivax gelatinosus* gen. nov., comb. nov., and Phylogenetic Relationships with *Leptothrix*, *Sphaerotilus natans*, *Pseudomonas saccharophila*, and *Alcaligenes latus*

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The taxonomic relationships among *Rhodocyclus*, *Leptothrix*, and *Sphaerotilus* species, *Pseudomonas saccharophila*, and *Alcaligenes latus* were investigated by performing DNA-rRNA hybridization experiments, by determining DNA base compositions, and by performing protein gel electrophoresis experiments. These taxa have relatively high guanine-plus-cytosine contents (67.8 to 72.5 mol%) and form a separate group within rRNA superfamily III. *Rhodocyclus gelatinosus* and "*Leptothrix discophora*" form two separate rRNA branches, which are linked at a $T_{m(e)}$ level of 74.8 ± 0.7°C [$T_{m(e)}$, temperature at which 50% of an DNA-rRNA hybrid is denatured]. Also situated at this $T_{m(e)}$ level and therefore equidistantly related to *Rhodocyclus gelatinosus* and "*Leptothrix discophora*" are *Sphaerotilus natans*, *P. saccharophila*, and *A. latus*. *Leptothrix cholodnii* is located on the "*L. discophora*" rRNA branch but produces a protein pattern which is different from that of "*L. discophora*." All of the other taxa which we investigated also have unique protein patterns. Since *Rhodocyclus gelatinosus* is only very distantly related to the type species of the genus *Rhodocyclus* (*Rhodocyclus purpureus*) and to *Rhodocyclus tenuis* and because it is clearly different from its nearest phylogenetic neighbors, we propose to rename this species as *Rubrivivax gelatinosus* gen. nov., comb. nov. The type strain remains strain NCIB 8290 (= LMG 4311).

In 1978 Pfennig (33) created the genus *Rhodocyclus* as a new genus within the family *Rhodospirillaceae* (34); he described *Rhodocyclus purpureus*, a phototrophic half-ring-shaped bacterium isolated from a red-colored swine waste lagoon, as the only species. When Imhoff et al. rearranged the *Rhodospirillaceae* (16), they transferred *Rhodopseudomonas gelatinosa* and *Rhodospirillum tenue* to this genus as *Rhodocyclus gelatinosus* and *Rhodocyclus tenuis*, respectively, because of several chemotaxonomic properties which these taxa have in common and because of the morphological similarity of their photosynthetic membrane systems. This transfer was proposed as a provisional solution until more detailed data for evaluation of the taxonomic relationships at the generic level became available.

Within the traditional family *Rhodospirillaceae*, the three *Rhodocyclus* species form a separate group that is different from all other genera (15, 35). Phylogenetically, the *Rhodocyclus* species belong to rRNA superfamily III sensu De Ley (6) (the beta subclass of the *Proteobacteria* [38, 49]), while all other members of the *Rhodospirillaceae* belong to rRNA superfamily IV (12c) (the alpha subclass of the *Proteobacteria* [38, 48]). Furthermore, most groups of photosynthetic bacteria have been found to be more closely related to nonphotosynthetic bacteria than to other photosynthetic taxa. Therefore, the family *Rhodospirillaceae* is taxonomically unsound. This is recognized in *Bergey's Manual of Systematic Bacteriology*, where the use of the name *Rhodospirillaceae* has been abandoned, but the separate taxa are on practical grounds still treated as a group, which is now named the purple nonsulfur bacteria (14).

On the basis of DNA-rRNA hybridization data, we have previously reported that *Rhodocyclus gelatinosus*, *Pseudomonas saccharophila*, and *Alcaligenes latus* are closely related to the acidovorans rRNA complex in rRNA superfamily III (46, 47). The acidovorans rRNA complex, as defined by DNA-rRNA hybridization data (46), corresponds to part of the beta-1 subcluster as defined by Woese et al. (49) on the basis of 16S rRNA cataloging data. Woese et al. (49) reported that there is a close relationship between *Rhodocyclus gelatinosus* and the sheathed species *Sphaerotilus natans* within the beta-1 subcluster. Another sheathed strain, "*Leptothrix discophora*" Stokes, and two strains of the gliding bacterium *Vitreoscilla filiformis*, strains ATCC 15551 and L1401-7, were shown to be relatively closely related to *Rhodocyclus gelatinosus* on the basis of 5S rRNA sequencing data (39). Since all of these taxa have never been included previously together in the same study, it is difficult to evaluate their precise taxonomic relationships. In this paper we describe the phylogenetic relationships of *Rhodocyclus*, *Sphaerotilus*, and *Leptothrix* species and the facultatively autotrophic species *Pseudomonas saccharophila* and *Alcaligenes latus*. We examined these taxa by determining DNA base compositions and by performing DNA-rRNA hybridization and whole-cell protein electrophoresis experiments.

Below, the name *Rubrivivax gelatinosus* is used for the taxon formerly called *Rhodocyclus gelatinosus* and taxa which are presently misnamed according to phylogenetic data are indicated by brackets.

MATERIALS AND METHODS

Bacterial strains. To study their taxonomic relationships, we tried to obtain as many *Leptothrix* strains as possible from various culture collections but ended up with only six strains belonging to two species. The strains which we studied are shown in Table 1. Most of the strains were grown

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TABLE 1. Strains used

Taxon	Strain[a]	Other designation(s)	Isolation source (year)
[*Alcaligenes*] *latus*	Palleroni H-4^{T}	LMG 3321^{T}, ATCC 29712^{T}	Soil, California
Leptothrix cholodnii	CCM 1827	LMG 9467	Active sludge
"*Leptothrix discophora*"	SP-6(s)	LMG 8142	Artificial iron seep in laboratory, Ithaca, N.Y.
"*Leptothrix discophora*"	SP-6(sl)[b]	LMG 8143	Artificial iron seep in laboratory, Ithaca, N.Y.
"*Leptothrix discophora*"	SS-1	LMG 8141	Surface film of a swamp, Ithaca, N.Y.
"*Leptothrix discophora*"	SS10A	LMG 9068	Surface film of a swamp, Ithaca, N.Y.
"*Leptothrix discophora*"	EH1	LMG 9069	Natural iron seep near Ithaca, N.Y.
[*Pseudomonas*] *saccharophila*	ATCC 15946^{T}	LMG 7831^{T}, LMG 2256^{T}	Mud, Bay of San Francisco, Calif. (1940)
[*Pseudomonas*] sp.	DSM 2583	LMG 8144	Berkeley, Calif. (1971)
Rubrivivax gelatinosus	NCIB 8290^{T}	LMG 4311^{T}, ATCC 17011^{T}	Acetate enrichment, pH 6.6 (1944)
Rubrivivax gelatinosus	Pfennig 2850	LMG 4309	
Rubrivivax gelatinosus	Pfennig 2150	LMG 4308, DSM 149	Greenhouse water basin
Rubrivivax gelatinosus	DSM 150	LMG 4310	Pond
Rubrivivax gelatinosus	DSM 151	LMG 4306	Ditch mud
Rhodocyclus purpureus	DSM 168^{T}	LMG 7759^{T}	Slaughterhouse waste lagoon
Rhodocyclus tenuis	DSM 109^{T}	LMG 4367^{T}, ATCC 25093^{T}	Muddy freshwater pond in forest, Grünenplan, Federal Republic of Germany
Rhodocyclus tenuis	DSM 110	LMG 7757	Dystrophic pond
Rhodocyclus tenuis	DSM 112	LMG 4369	Mountain peat bog
Rhodocyclus tenuis	Pfennig 1/67	LMG 4371	
Rhodocyclus tenuis	Biebl Fa2a	LMG 4372	
Sphaerotilus natans	NCIB 11197t$_1^{T}$	LMG 7172t$_1^{T}$	Hay infusion
Thiobacillus perometabolis	ATCC 23370^{T}	LMG 8564^{T}	Soil, Los Angeles, Calif.
Reference strains			
Acidovorax delafieldii	ATCC 17505^{T}	LMG 6832^{T}	Soil enriched with poly-β-hydroxybutyrate as a sole carbon source
Acidovorax facilis	ATCC 11228^{T}	LMG 2193^{T}	Soil, United States
Acidovorax temperans	CIP 239.74	LMG 3332	Blood culture, France
Acidovorax temperans	CIP 471.74	LMG 3334	Unknown
Alcaligenes faecalis subsp. *faecalis*	NCIB 8156^{T}	LMG 1229^{T}, CCUG 1814^{T}	Unknown
[*Alcaligenes*] *paradoxus*	ATCC 17713t$_1^{T}$	LMG 1797t$_1^{T}$	Soil in mineral medium under a 91% H_2–4% O_2–5% CO_2 atmosphere
[*Aquaspirillum*] *aquaticum*	ATCC 11330^{T}	LMG 2370^{T}, CCUG 17395^{T}	Freshwater
Chromobacterium violaceum	NCTC 9757^{T}	LMG 1267^{T}, ATCC 12472^{T}	Freshwater, Mentekab, Malaya (1952)
Comamonas acidovorans	Stanier 14^{T}	LMG 1226^{T}	Soil enriched with acetamide, Delft, The Netherlands (1926)
Comamonas terrigena	NCIB 8193^{T}	LMG 1253^{T}, ATCC 8461^{T}	Hay infusion, United States
Comamonas terrigena	CCUG 12940	LMG 5520	Blood, United States (1982)
Comamonas testosteroni	NCTC 10698^{T}	LMG 1786^{T}, ATCC 11996^{T}	Soil enriched with testosterone, Berkeley, Calif. (1953)
Hydrogenophaga flava	DSM 619^{T}	LMG 2185^{T}, ATCC 33667^{T}	Mud from ditch (1942)
Hydrogenophaga palleronii	Stanier 362t$_1^{T}$[c]	LMG 2366t$_1^{T}$[c], ATCC 17724^{T}	Water enriched for the isolation of hydrogen bacteria under an atmosphere containing 6% O_2
Hydrogenophaga pseudoflava	GA3^{T}	LMG 5945^{T}, ATCC 33668^{T}	Water from the River Weende, Federal Republic of Germany
Hydrogenophaga taeniospiralis	DSM 2082^{T}	LMG 7170^{T}	Soil, Spain
Janthinobacterium lividum	Sneath HB^{T}	LMG 2892^{T}, ATCC 12473^{T}	Soil, Michigan (1952)
Oligella urethralis	WM6	LMG 5304, CCUG 994	Cervix, Aarhus, Denmark (1961)
[*Pseudomonas*] *avenae*	NCPPB 1011^{T}	LMG 2117^{T}, ATCC 19860^{T}	*Zea mays*, United States (1958)
[*Pseudomonas*] *solanacearum*	NCPPB 325^{T}	LMG 2299^{T}, ATCC 11696^{T}	*Lycopersicon lycopersicum*, United States
Rhodobacter sphaeroides	NCIB 8253^{T}	LMG 2827^{T}, ATCC 11167^{T}	
Rhodospirillum rubrum	ATCC 11170^{T}	LMG 4362^{T}	
Xylophilus ampelinus	NCPPB 2217^{T}	LMG 5856^{T}, ATCC 33914^{T}	*Vitis vinifera* var. *sultana*, Crete (1966)

[a] Strain number as received. ATCC, American Type Culture Collection, Rockville, Md.; CCM, Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; CIP, Culture Collection of the Institut Pasteur, Paris, France; 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] Strain SP-6(sl) is a sheathless mutant of strain SP-6(s); it arose spontaneously after several transfers on a peptone-yeast extract-glucose liquid medium (12a).

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

aerobically in the dark at 28°C; the exceptions were the *Xylophilus ampelinus* strain, which was grown at 24°C, and *Rhodocyclus tenuis* and *Rhodocyclus purpureus* strains, which were grown at room temperature under constant light either in a jar (type HP11; Oxoid), in which anaerobiosis was created by using a gas-generating kit (type BR38; Oxoid) and a Pd catalyst (type BR42; Oxoid), or in completely filled and tightly closed vials. The *Leptothrix* and *Sphaerotilus* strains were maintained on PYG medium (1) or medium 12 (19). For DNA and rRNA preparation, PYG medium was used for growth. All *Rhodocyclus* and *Rubrivivax* strains were grown on medium 27 (4). For *Rhodocyclus tenuis* Pfennig 1/67, the medium was supplemented with 0.5% (wt/vol) yeast extract and, after autoclaving, with 1 ml of a filter-sterilized (type Minisart SM165 34 filter; pore diameter, 0.2 μm; Sartorius, Göttingen, Federal Republic of Germany) vitamin B_{12} solution (20 μg/liter; Merck, Darmstadt, Federal Republic of Germany) per liter. For *Rhodocyclus purpureus* DSM 168^T (T = type strain), the same amount of vitamin B_{12} solution and 0.05% (wt/vol) sodium pyruvate were added. Most reference strains were grown on nutrient agar (0.1% beef extract, 0.2% yeast extract, 0.5% peptone, 0.5% NaCl, 2% agar, pH 7.4); the exception was the *Xylophilus ampelinus* strain, which was grown on GYCA medium (47).

DNA preparation. The method of Marmur (23) was used to prepare high-molecular-weight DNA, but instead of 1 M sodium perchlorate, we used 1 M NaCl to separate nucleic acids and proteins after lysis of the cells. For the *Leptothrix*, *Sphaerotilus*, and *Thiobacillus* strains, additional slight modifications of this method were necessary to obtain a sufficient amount of DNA; after harvesting, the cells were washed and resuspended in saline-EDTA (0.15 M NaCl plus 0.1 M EDTA, pH 8) in the presence of proteinase K (50 μg/ml; Merck). When the viscosity of the lysed cells was low, shaking during the isolation procedure was restricted to a minimum; the lysate was gently shaken in 1 M NaCl, and for deproteinization the mixture containing chloroform was slowly shaken by hand for 2 to 5 min. Sometimes chloroform was replaced by a mixture containing phenol (50%, vol/vol), chloroform (48%, vol/vol), and isoamyl alcohol (2%, vol/vol), which is a more effective but also a more aggressive deproteinizing agent.

DNA base composition. The average guanine-plus-cytosine (G+C) values of the DNAs were determined by the thermal denaturation method (10) and were calculated by using the equation of Marmur and Doty (24) as modified by De Ley (5).

DNA-rRNA hybridizations. DNA was further purified by CsCl gradient centrifugation and fixed on cellulose nitrate filters (type SM11358; Sartorius) as described previously (7). The amount of filter-fixed DNA was estimated chemically (7, 41). [^{3}H]rRNAs from *Rubrivivax gelatinosus* NCIB 8290^T and "*Leptothrix discophora*" SP-6(s) were isolated and fractionated as described by De Ley and De Smedt (7). We used 5,6-[^{3}H]uracil and 2,8-[^{3}H]adenine (New England Nuclear Research Products, Boston, Mass.) as precursors for in vivo labeling of the rRNAs in *Rubrivivax gelatinosus* NCIB 8290^T and "*Leptothrix discophora*" SP-6(s), respectively. Purified 23S [^{3}H]rRNAs from *Comamonas acidovorans* Stanier 14^T, *Janthinobacterium lividum* Sneath HB^T, *Oligella urethralis* WM6, and *Rhodospirillum rubrum* ATCC 11170^T, 16S [^{3}H]rRNAs from [*Pseudomonas*] *solanacearum* NCPPB 325^T and *Chromobacterium violaceum* NCTC 9757^T, and 16S [^{14}C]rRNAs from *Rhodobacter sphaeroides* NCIB 8253^T, *Alcaligenes faecalis* subsp. *faecalis* NCIB 8156^T, and [*Alcaligenes*] *paradoxus* ATCC 17713t1^T were available from members of our research group (8, 9, 11, 12b, 17, 36). Hybridizations between labeled 23S or 16S rRNA and single-stranded filter-fixed DNA were performed as described previously (7, 37). Each hybrid was characterized by its $T_{m(e)}$ (the temperature [in degrees Celsius] at which one-half of the DNA-rRNA duplex was denatured) and by its percentage of rRNA binding (the amount of rRNA [in micrograms] bound to 100 μg of filter-fixed DNA after RNase treatment).

SDS-PAGE. Only strains belonging to the *Rubrivivax-Leptothrix* rRNA cluster were compared by using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). Each of these organisms was grown on PYG medium for 48 h at 28°C in one or two Roux flasks. Whole-cell protein extracts were prepared by heating 50 to 60 mg (wet weight) of cells in 1 ml of sample treatment buffer (20) at 95°C for 10 min. The extracts were centrifuged at 10,000 × *g* for 8 min. For the sheathed *Leptothrix* and *Sphaerotilus* strains this standard procedure was modified slightly to obtain complete suspension of the cells; the cells were sonicated for 30 s in 0.9 ml of sample treatment buffer (20) without SDS by using a model Labsonic 2000 apparatus (B. Braun, Melsungen, Federal Republic of Germany) equipped with a needle probe tip (length, 127 mm; diameter, 4 mm; output, 50 W). A 0.1-ml portion of 10% SDS was added, and the extracts were prepared as described above. SDS-PAGE was performed by the method of Laemmli (20), using the slight modifications described by Kiredjian et al. (18).

RESULTS

DNA base composition. The average G+C values of the strains which we used are shown in Tables 2 and 3.

DNA-rRNA hybridizations. The specific activities of the 23S [^{3}H]rRNA from *Rubrivivax gelatinosus* NCIB 8290^T and of the 23S [^{3}H]rRNA from "*Leptothrix discophora*" SP-6(s) were 26,574 and 27,376 cpm/μg of rRNA, respectively. The results from hybridizations performed with these rRNAs and with rRNAs from *Comamonas acidovorans* Stanier 14^T and [*Alcaligenes*] *paradoxus* ATCC 17713t1^T are shown in Table 2. Together with previously published data from members of our research group (11, 12, 36, 37, 46), these data were combined to produce the $T_{m(e)}$ dendrogram shown in Fig. 1.

Rubrivivax gelatinosus NCIB 8290^T and "*Leptothrix discophora*" SP-6(s) form two separate rRNA branches within rRNA superfamily III and are linked at a $T_{m(e)}$ level of 74.8 ± 0.7°C. All *Rubrivivax gelatinosus* strains have $T_{m(e)}$ values of 80.0 to 81.4°C versus rRNA from type strain NCIB 8290, and no other taxa belong to this rRNA branch. All "*Leptothrix discophora*" strains have $T_{m(e)}$ values of 77.6 to 81.3°C versus rRNA from strain SP-6(s). With a $T_{m(e)}$ of 79.0°C versus the latter rRNA, *Leptothrix cholodnii* CCM 1827 also belongs to this rRNA branch. [*Pseudomonas*] *saccharophila*, [*Pseudomonas*] sp. strain DSM 2583, and [*Alcaligenes*] *latus* are located at the bifurcation level of the two rRNA branches discussed above [$T_{m(e)}$, 74.8 ± 0.7°C]. Within rRNA superfamily III, the nearest neighbor of this group of taxa is the acidovorans rRNA complex [$T_{m(e)}$, 73.0 ± 1.4°C].

The results from hybridizations of DNAs from *Rhodocyclus purpureus* and *Rhodocyclus tenuis* strains with various representative rRNAs from members of rRNA superfamilies III and IV are shown in Table 3. As shown in Fig. 1, both of these species are located at the base of rRNA superfamily III.

TABLE 2. DNA base compositions of strains belonging to the acidovorans rRNA complex and parameters of the hybrids of their DNAs with labeled rRNAs from *Rubrivivax gelatinosus* NCIB 8290$^{\mathrm{T}}$, "*Leptothrix discophora*" SP-6(s), *Comamonas acidovorans* Stanier 14$^{\mathrm{T}}$, and [*Alcaligenes*] *paradoxus* ATCC 17713t$_{1}^{\mathrm{T}}$

DNA from strain:	G+C content (mol%)	Hybridized with:							
		23S [^{3}H]rRNA from *Rubrivivax gelatinosus* NCIB 8290$^{\mathrm{T}}$		23S [^{3}H]rRNA from "*Leptothrix discophora*" SP-6(s)		23S [^{3}H]rRNA from *Comamonas acidovorans* Stanier 14$^{\mathrm{T}}$		16S [^{14}C]rRNA from [*Alcaligenes*] *paradoxus* ATCC 17713t$_{1}^{\mathrm{T}}$	
		$T_{m(e)}$ (°C)	% of rRNA binding	$T_{m(e)}$ (°C)	% of rRNA binding	$T_{m(e)}$ (°C)	% of rRNA binding	$T_{m(e)}$ (°C)	% of rRNA binding
Rubrivivax gelatinosus NCIB 8290$^{\mathrm{T}}$	71.9	81.4	0.05	74.0	0.04	74.5^{a}	0.05^{a}	71.0^{a}	0.02^{a}
Rubrivivax gelatinosus Pfennig 2850	70.2	80.0	0.05	73.7	0.04	72.6	0.03		
Rubrivivax gelatinosus Pfennig 2150	71.0	81.3	0.09	75.1	0.05	75.5	0.07	74.0	0.06
Rubrivivax gelatinosus DSM 150	72.0	80.9	0.09	74.1	0.07				
Rubrivivax gelatinosus DSM 151	71.0					75.5	0.06	74.0	0.05
Leptothrix cholodnii CCM 1827	69.6^{b}	74.6	0.02	79.0	0.06	72.2	0.05	73.2	0.04
"*Leptothrix discophora*" SP-6(s)	69.4	74.3	0.07	80.3	0.04	73.4	0.06		
"*Leptothrix discophora*" SP-6(sl)	68.1	75.8	0.08	81.3	0.07				
"*Leptothrix discophora*" SS-1	71.1	75.0	0.09	77.6	0.05				
"*Leptothrix discophora*" EH1	67.8	75.1	0.07	78.7	0.07				
[*Alcaligenes*] *latus* Palleroni H-4$^{\mathrm{T}}$	70.0^{c}	75.8	0.12	75.5	0.07	74.5^{a}	0.12^{a}	73.2	0.10
[*Pseudomonas*] *saccharophila* ATCC 15946$^{\mathrm{T}}$	69.1	75.8	0.04	74.9	0.05	72.6^{a}	0.05^{a}	73.4^{a}	0.04^{a}
[*Pseudomonas*] sp. strain DSM 2583	69.7	75.1	0.05	73.6	0.04				
Sphaerotilus natans NCIB 11197t$_{1}^{\mathrm{T}}$	69.9	75.2	0.13	74.8	0.12	72.8	0.13	74.7	0.06
[*Thiobacillus*] *perometabolis* ATCC 23370$^{\mathrm{T}}$	64.8	71.4	0.03	70.4	0.03				
Reference strains									
Acidovorax delafieldii ATCC 17505$^{\mathrm{T}}$	65.6^{a}	72.3	0.08			77.2^{a}	0.11^{a}	76.6^{a}	0.02^{a}
Acidovorax facilis ATCC 11228$^{\mathrm{T}}$	64.7^{d}	73.2	0.09	71.6	0.08	77.0^{d}	0.09^{d}	77.5^{a}	0.07^{a}
Acidovorax temperans CIP 239.74	63.0	73.3	0.09			76.9	0.11	75.8	0.07
Acidovorax temperans CIP 471.74	63.3	73.5	0.14			76.8	0.10	75.9	0.07
[*Alcaligenes*] *paradoxus* ATCC 17713t$_{1}^{\mathrm{T}}$	67.0^{d}	72.6	0.04			76.5^{d}	0.03^{d}	81.0^{e}	0.06^{e}
[*Aquaspirillum*] *aquaticum* ATCC 11330$^{\mathrm{T}}$	64.0	72.4	0.19	69.8	0.16	74.8	0.25	75.1	0.10
Comamonas acidovorans Stanier 14$^{\mathrm{T}}$	66.6^{d}	73.6	0.09	73.4	0.11	80.6^{d}	0.12^{d}	77.0^{e}	0.10^{e}
Comamonas terrigena NCIB 8193$^{\mathrm{T}}$	64.0^{e}	72.1	0.18	72.5	0.17	75.9^{e}	0.19^{e}	75.5	0.16
Comamonas terrigena CCUG 12940	66.1^{f}	75.0	0.16			76.9^{f}	0.14^{f}		
Comamonas testosteroni NCTC 10698$^{\mathrm{T}}$	62.5^{d}	72.2	0.20	71.2	0.17	76.5^{d}	0.17^{d}	76.5^{e}	0.18^{e}
Hydrogenophaga flava DSM 619$^{\mathrm{T}}$	66.7^{d}	73.1	0.15			75.5^{d}	0.02^{d}	76.0^{a}	0.04^{a}
Hydrogenophaga taeniospiralis DSM 2082$^{\mathrm{T}}$	64.8^{f}	72.9	0.15			75.0^{f}	0.04^{f}		
Hydrogenophaga palleronii Stanier 362t$_{1}^{\mathrm{T}}$	67.3^{f}	74.9	0.04			76.0^{d}	0.05^{d}	77.5^{a}	0.06^{a}
Hydrogenophaga pseudoflava GA3$^{\mathrm{T}}$	66.4^{f}	73.7	0.06	72.5	0.07	76.0^{f}	0.08^{f}		
[*Pseudomonas*] *avenae* NCPPB 1011$^{\mathrm{T}}$	69.8^{f}	73.5	0.06			76.6^{a}	0.12^{a}	76.5	0.09
Xylophilus ampelinus NCPPB 2217$^{\mathrm{T}}$	68.5^{a}	73.1	0.10			76.0^{a}	0.09^{a}	76.2^{f}	0.06^{f}

[a] Data from reference 47.
[b] Data from reference 42.
[c] Data from reference 17.
[d] Data from reference 11.
[e] Data from reference 12.
[f] Data from reference 46.

Protein gel electrophoresis. The results of SDS-PAGE are shown in Fig. 2. All of the taxa which we investigated produced unique protein banding patterns. A common feature for all strains was the very small number of protein bands in the lower-molecular-weight zone (molecular weights up to 22,000). Two of the three *Rubrivivax gelatinosus* strains tested had almost identical protein patterns (strains NCIB 8290$^{\mathrm{T}}$ and Pfennig 2150 [Fig. 2]), while the third strain (strain DSM 150) was clearly different despite some similarities, mainly in the 21,100- to 29,000-dalton region. Of the five "*Leptothrix discophora*" strains studied, strains SP-6(s) and SP-6(sl) had almost identical protein patterns (Fig. 2). This was not unexpected, as strain SP-6(sl) was isolated from a culture of strain SP-6(s) as a spontaneous nonsheathed mutant (12a). Strains SS10A and EH1 produced very similar but not identical electrophoregrams, which resembled those of strains SP-6(s) and SP-6(sl). The protein pattern of strain SS-1 had some overall similarities with the patterns of the other "*Leptothrix discophora*" strains, but was clearly the most aberrant pattern (Fig. 2). *Leptothrix cholodnii* CCM 1827 had a protein pattern that at molecular weights of 52,000 and above resembled the patterns of the "*Leptothrix discophora*" strains (Fig. 2).

DISCUSSION

Our hybridization data (Tables 2 and 3) confirm that [*Rhodocyclus*] *gelatinosus*, *Rhodocyclus tenuis*, and *Rhodocyclus purpureus* belong to rRNA superfamily III and not to rRNA superfamily IV, to which all other purple nonsulfur bacteria belong. [*Rhodocyclus*] *gelatinosus* forms a separate rRNA branch within rRNA superfamily III. Its nearest neighbors, at a $T_{m(e)}$ level of 74.8 ± 0.7°C, are the sheathed bacteria belonging to the genera *Leptothrix* and *Sphaerotilus* and the facultatively autotrophic species [*Pseudomonas*] *saccharophila* and [*Alcaligenes*] *latus*. Because of the low level of rRNA cistron similarity and also because of their phenotypic diversity (Table 4), none of these taxa can be related to [*Rhodocyclus*] *gelatinosus* at the generic level. In addition, all of the strains belonging to these taxa have protein patterns that are different from the pattern of the [*Rhodocyclus*] *gelatinosus* strains (Fig. 2). [*Rhodocyclus*] *gelatinosus* is related to the acidovorans rRNA complex at a $T_{m(e)}$ level of 73.0 ± 1.4°C (mean value calculated from Table 2 and our unpublished data); it is even more distantly related to the type species of the genus *Rhodocyclus* (*Rhodocyclus purpureus*) and to *Rhodocyclus tenuis* ($T_{m(e)}$ versus strain NCIB 8290^T, 65.8 to 69.2°C [Table 3]) and therefore should certainly be removed from the genus *Rhodocyclus*. *Rhodocyclus tenuis* and *Rhodocyclus purpureus* could not be assigned to any of the large rRNA clusters in rRNA superfamily III (Table 3 and Fig. 1). Therefore, they are at present situated at the base of rRNA superfamily III ($T_{m(e)}$, 68.6 ± 2.2°C), in which they probably form either one or two new rRNA branches. For all of the reasons described above, we propose to transfer [*Rhodocyclus*] *gelatinosus* to a new genus, *Rubrivivax*, as *Rubrivivax gelatinosus*. Additional differences among *Rubrivivax gelatinosus*, *Rhodocyclus tenuis*, and *Rhodocyclus purpureus* are summarized in Table 5. The species *Rubrivivax gelatinosus* contains at least two protein electrophoretic groups (Fig. 2). This apparent heterogeneity within *Rubrivivax gelatinosus* has been demonstrated before; two subgroups were described previously on the basis of morphology, spectral analysis of pigments, and auxanographic features (3, 43), and two serotypes, not corresponding to the subgroups described above, were described later (44).

Hybridizations with rRNA from "*Leptothrix discophora*" SP-6(s) showed that four "*Leptothrix discophora*" strains and one *Leptothrix cholodnii* strain are closely related. These organisms form a new rRNA branch that is linked to the *Rubrivivax gelatinosus* rRNA branch at a $T_{m(e)}$ level of 74.8 ± 0.7°C. The sheathed bacteria belonging to the genera *Leptothrix* and *Sphaerotilus* are able to oxidize iron and, in the case of *Leptothrix* spp., also manganese and deposit the produced $Fe(OH)_3$, Fe_2O_3, and MnO_2 on the sheaths (26).

In *Bergey's Manual of Systematic Bacteriology* the following *Sphaerotilus* and *Leptothrix* species are described: *Sphaerotilus natans*, *Leptothrix ochracea*, *Leptothrix cholodnii*, *Leptothrix lopholea*, "*Leptothrix pseudoochracea*," and "*Leptothrix discophora*" (26, 27). Most of these taxa are differentiated on morphological grounds only and are biochemically poorly characterized. It is most unfortunate that only a few of the *Leptothrix* strains described in the past have been preserved. Furthermore, strains of the type species of the genus *Leptothrix*, *Leptothrix ochracea*, have never been grown in pure culture; therefore, the choice of this morphological taxon as the type species is questionable. The protein patterns of all available *Leptothrix* strains are similar, but clearly have some differences (Fig. 2). There does not seem to be a close relationship between the genera *Leptothrix* and *Sphaerotilus*, since *Sphaerotilus natans* together with [*Pseudomonas*] *saccharophila* and [*Alcaligenes*] *latus* is located at the $T_{m(e)}$ level of *Rubrivivax gelatinosus*. This finding was also confirmed by the different protein patterns which we obtained for all of these taxa (Fig. 2).

[*Pseudomonas*] sp. strain DSM 2583 was included in our study because Palleroni (31) reported that this strain resem-

TABLE 3. DNA base compositions and parameters of the hybrids between DNAs from *Rhodocyclus purpureus* and *Rhodocyclus tenuis* strains and labeled rRNAs from various reference strains belonging to rRNA superfamilies III and IV

DNA from strain:	G+C content (mol%)	rRNA superfamily IV				rRNA superfamily III													
		16S [^{14}C]rRNA from *Rhodobacter sphaeroides* NCIB 8253^T		23S [^{3}H]rRNA from *Rhodospirillum rubrum* ATCC 11170^T		16S [^{3}H]rRNA from [*Pseudomonas*] *solanacearum* NCPPB 325^T		23S [^{3}H]rRNA from *Janthinobacterium lividum* Sneath HB^T		16S [^{3}H]rRNA from *Chromobacterium violaceum* NCTC 9757^T		23S [^{3}H]rRNA from *Rubrivivax gelatinosus* NCIB 8290^T		23S [^{3}H]rRNA from *Comamonas acidovorans* Stanier 14^T		16S [^{14}C]rRNA from *Alcaligenes faecalis* subsp. *faecalis* NCIB 8156^T		23S [^{3}H]rRNA from *Oligella urethralis* WM6	
		$T_{m(e)}$ (°C)	% of rRNA binding	$T_{m(e)}$ (°C)	% of rRNA binding	$T_{m(e)}$ (°C)	% of rRNA binding	$T_{m(e)}$ (°C)	% of rRNA binding	$T_{m(e)}$ (°C)	% of rRNA binding	$T_{m(e)}$ (°C)	% of rRNA binding	$T_{m(e)}$ (°C)	% of rRNA binding	$T_{m(e)}$ (°C)	% of rRNA binding	$T_{m(e)}$ (°C)	% of rRNA binding
Rhodocyclus purpureus DSM 168^T	67.7	60.4	0.09			68.8	0.29	68.6	0.08	69.6	0.10	67.4	0.10	70.0	0.09	65.5	0.08	65.1	0.04
Rhodocyclus tenuis DSM 109^T	66.1			61.8	0.04							68.3	0.08	70.4	0.07	65.2	0.07	65.4	0.06
Rhodocyclus tenuis DSM 110	64.4	57.5	0.10									68.3	0.10	69.5	0.08				
Rhodocyclus tenuis DSM 112	65.3	59.3	0.10									69.2	0.09	70.0	0.16				
Rhodocyclus tenuis Pfennig 1/67	67.2											66.4	0.06	71.8	0.06				
Rhodocyclus tenuis Biebl Fa2a	66.4							70.7	0.09	71.2	0.13	65.8	0.04	71.1	0.08				

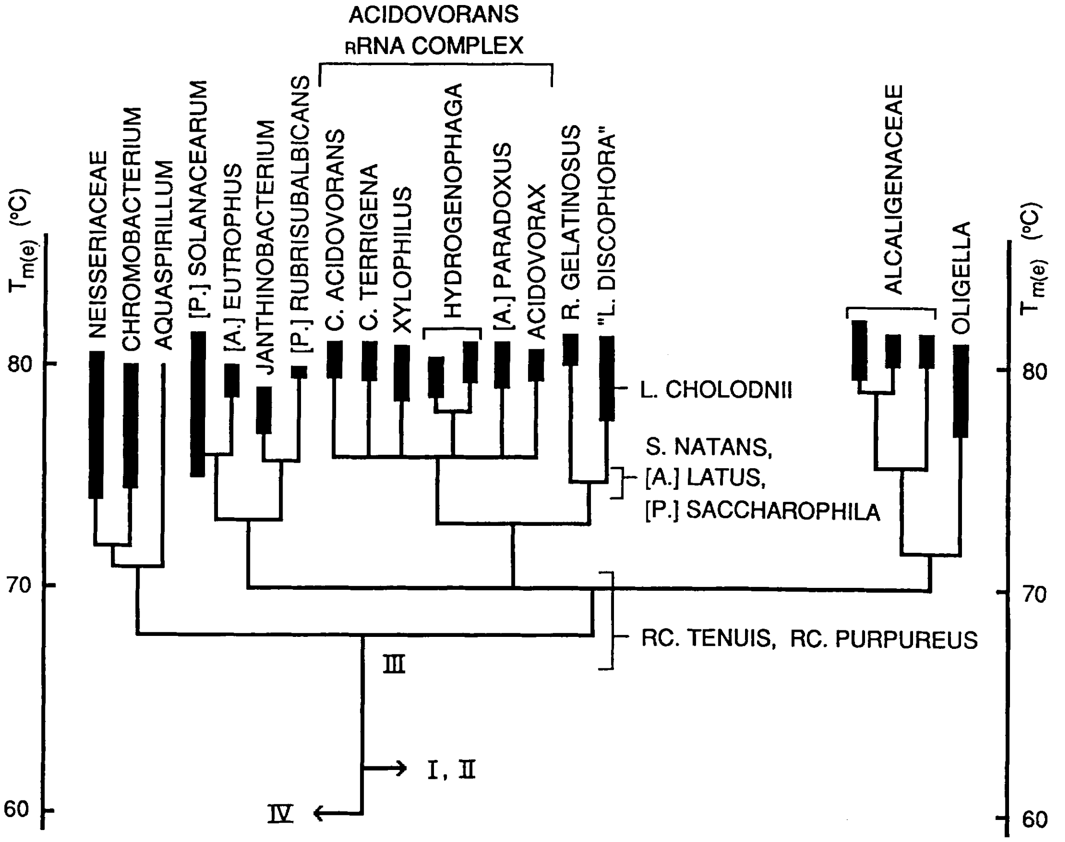

FIG. 1. Simplified rRNA cistron similarity dendrogram of rRNA superfamily III. The $T_{m(e)}$ values are from Tables 2 and 3 and from previously published work (11, 12, 36, 37). The bars indicate $T_{m(e)}$ ranges within individual rRNA branches. The roman numerals indicate the roots of the rRNA superfamilies sensu De Ley (6); I+II, III, and IV correspond to the gamma, beta, and alpha subclasses, respectively, of the *Proteobacteria* (38). A., *Alcaligenes*; C., *Comamonas*; L., *Leptothrix*; P., *Pseudomonas*; R., *Rubrivivax*; RC., *Rhodocyclus*; S., *Sphaerotilus*.

bles [*Pseudomonas*] *saccharophila* phenotypically. Protein gel electrophoresis data show that these two organisms are different except for the presence of a few homologous protein bands in the molecular weight range from 32, 000 to 33,000 (Fig. 2).

The relationships among the taxa at the base of the bifurcation of the *Leptothrix* and *Rubrivivax* rRNA branches (e.g., [*Pseudomonas*] *saccharophila*, [*Alcaligenes*] *latus*, *Sphaerotilus natans*, and unidentified [*Pseudomonas*] sp. strain DSM 2583) are still obscure. Since all of these organisms have unique protein electrophoretic patterns (Fig. 2) and because of their large phenotypic differences (Table 4),

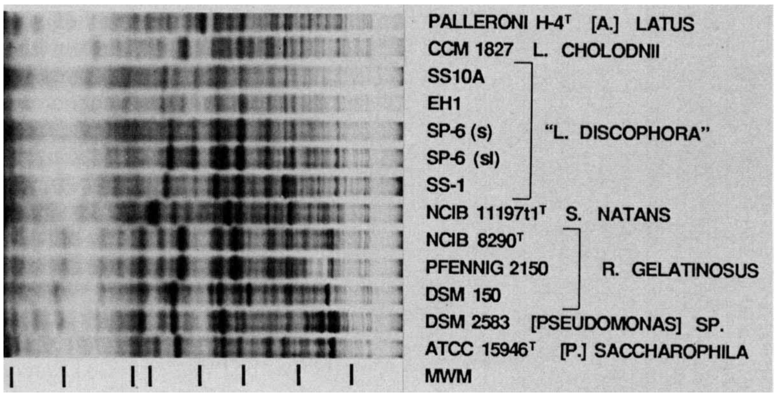

FIG. 2. Normalized protein electrophoregrams of strains belonging to the *Rubrivivax gelatinosus* and "*Leptothrix discophora*" rRNA branches. The positions of the molecular weight markers are indicated on the trace labeled MWM; they are, from left to right, lysozyme (molecular weight, 14,200), trypsin inhibitor (21,100), trypsinogen (24,000), carbonic anhydrase (29,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), egg albumin (45,000), bovine albumin (66,000), and β-galactosidase (116,000). For abbreviations see the legend to Fig. 1.

TABLE 4. Characteristics of the species belonging to the *Rubriviax gelatinosus*-"*Leptothrix discophora*" rRNA cluster[a]

Characteristic	*Rubrivivax gelatinosus*	"*Leptothrix discophora*"	*Leptothrix cholodnii*	*Sphaerotilus natans*	[*Pseudomonas*] *saccharophila*	[*Pseudomonas*] sp. strain DSM 2583	[*Alcaligenes*] *latus*
Flagellation	1, Polar	1, Polar	1, Polar	Polar bunch	1, Polar	1, Polar	Peritrichous
Formation of sheaths	−	+	+	+	−	−	−
Photoautotrophic growth	+	−	−	−	−	−	−
Autotrophic growth with hydrogen	+				+	+	+
Oxidation of:							
Carbon monoxide	+						
Manganese (Mn^{2+})		+	+	−			
Accumulation of Fe_2O_3 on the sheaths		+	+	+			
Nitrogen fixation	+				+		+
Gelatinase	+			Slow	+	+	+
Accumulation of:							
Poly-β-hydroxybutyrate		+	+	+	+	+	+
Polysaccharides		−	−	+			
Carotenoid pigments	+	−	−	−	−	−	−
Growth factors required	Biotin, thiamine	Vitamin B_{12}[b]	Vitamin B_{12}[b]	Vitamin B_{12}		−	
Carbon sources used for growth							
Acetate	+	−	+	+	+	−	−
Pyruvate	+	+		+	+	Weak	−
Butyrate	d			+	+	−	+
Lactate	+	−	+	+	+	+	+
L-Malate	+	+	+	+	+	+	+
Succinate	+	+		+	+	+	+
Fumarate	+	−		+	+	Weak	+
Citrate	+	−	−	+	+	+	d
D-Ribose		−			+	−	−
Glucose	+	+[c]	+	+	+[d]	Late	+
Fructose	+	−	+	+	+[d]	Late	+
D-Galactose				+	+	+	−
Sucrose		+[e]	+	+	+	+	+
Glycerol	−	+	+	+	−	−	+
Mannitol	−			+	−	−	−
Sorbitol	−			+	−	−	−
Isolation source	Mud	Water	Water	Water	Mud		Soil
G+C content (mol%)	70.0−72.5	67.8−71.1	69.6	69.9	69.1	69.7	69.1−71.1

[a] Data from references 1, 2, 15, 17, 26–32, and 42. +, Present in 90% or more of the strains; −, absent in 90% or more of the strains; d, present in 11 to 89% of the strains. Most G+C values were determined by us from thermal denaturation curves; the exception was the value for *Leptothrix cholodnii*, which was taken from reference 42.

[b] Thiamine and biotin may be required by some strains (27).

[c] Glucose is used as a carbon source but does not support growth as a sole energy source (1).

[d] Growth may require mutation.

[e] Data from reference 28. Adams and Ghiorse (1) reported that "*Leptothrix discophora*" SS-1 cannot use glucose.

a close relationship seems unlikely. The strains belonging to the *Rubrivivax* and *Leptothrix* rRNA branches and all of the strains located at the branching level of these groups [$T_{m(e)}$, 74.8 ± 0.7°C] have G+C values of 67.8 to 72.5 mol%. Although [*Pseudomonas*] *saccharophila* and [*Alcaligenes*] *latus* are generically misnamed and should be removed from their present genera, we prefer not to do so at this time. Further taxonomic changes require a more thorough phenotypic and chemotaxonomic study of all of the taxa belonging to the *Leptothrix* and *Rubrivivax* rRNA branches. The relationships with *Vitreoscilla filiformis* (39) should also be studied in further detail.

On the basis of the results of 5S rRNA sequencing experiments, Lane et al. (21) reported that *Thiobacillus perometabolis* and *Thiobacillus intermedius* represent an earlier offshoot on the same phylogenetic lineage as *Rubrivivax gelatinosus*. With $T_{m(e)}$ values of 71.4 and 70.8°C versus rRNAs from *Rubrivivax gelatinosus* and "*Leptothrix discophora*," respectively (Table 2), *Thiobacillus perometabolis* ATCC 23370^T is indeed only very distantly related to these rRNA branches. It belongs to rRNA superfamily III, but its exact position relative to the other rRNA branches remains to be determined.

Description of *Rubrivivax* gen. nov. *Rubrivivax* (Ru.bri. vi'vax. L. adj. *ruber*, red; L. adj. *vivax*, living; M. L. masc. n. *Rubrivivax*, red living organism). The description below is based mainly on previously published descriptions by Weckesser et al. (43) and Imhoff and Trüper (15). Cells are straight to slightly curved rods, 0.4 to 0.7 by 1 to 3 μm. Irregularly curved cells up to 15 μm long may occur in older cultures. Sheaths are not formed. Cells are motile by means of a polar flagellum. Gram negative. Under anaerobic conditions in the light, photoheterotrophic growth with various organic substrates as electron donors or photoautotrophic growth in the presence of H_2, CO_2, and growth factors is possible. In the dark, chemoautotrophic growth with CO as a sole carbon and energy source (40) and aerobic chemoheterotrophic growth with various organic substrates are also possible.

TABLE 5. Characteristics that differentiate *Rubrivivax gelatinosus*, *Rhodocyclus purpureus*, and *Rhodocyclus tenuis*

Characteristic	*Rubrivivax gelatinosus*	*Rhodocyclus purpureus*	*Rhodocyclus tenuis*
Motility[a]	+	−	+
Gelatinase[a]	+	−	−
Growth on fatty acids with chain lengths ranging from 10 to 18 C atoms[b]	+	−	+
Use of the following substrates as electron and carbon sources: citrate, aspartate, glutamate, glucose, fructose, mannose[a]	+	−	−
Characteristics of lipopolysaccharides[c]			
Presence of arabinose, 4-amino-arabinose, and myristic acid (14:0) in lipid A	−	+	+
Presence of lauric acid (12:0) in lipid A	+	−	−
Presence of L-glycero-D-mannoheptose in polysaccharide fraction	−	+	+
Presence of O-specific chains	−	+	+
Major carotenoids[a]	Spheroidene, hydroxyspheroidene, spirilloxanthin	Rhodopin, rhodopinal	Rhodopin, rhodopinal, lycopene
N_2 fixation[d]	+	−	+
$T_{m(e)}$ vs rRNA from *Rubrivivax gelatinosus* NCIB 8290^T (°C)	80.0−81.4	67.4	65.8−69.2
16S rRNA subcluster[e]	Beta-1	Beta-2	Beta-2
G+C content (mol%)[f]	70.0−72.5	67.7	64.4−67.2

[a] Data from reference 15.
[b] Data from reference 13.
[c] Data from reference 45.
[d] Data from reference 22.
[e] Data from reference 49.
[f] Determined by the thermal denaturation method.

Bacteriochlorophyll *a* and carotenoids are present as photosynthetic pigments. Small fingerlike invaginations of the cytoplasmic membrane represent photosynthetic membranes. Nitrogen may be fixed under photoautotrophic growth conditions. Colonies are pink to deep red because of photosynthetic pigments; heterotrophically grown colonies are initially faintly pink. The G+C values of the DNA range from 70.0 to 72.5 mol%. In DNA-rRNA hybridizations, $T_{m(e)}$ values of 77.0 to 81.5°C are obtained versus rRNA of *Rubrivivax gelatinosus* NCIB 8290^T. The type species is *Rubrivivax gelatinosus*.

Description of *Rubrivivax gelatinosus* (Molisch 1907) comb. nov. The description of *Rubrivivax gelatinosus* is the same as that given above for the genus. A detailed description of phenotypic features has been published by Imhoff and Trüper (15). *Rubrivivax gelatinosus* strains have been isolated from water and mud. The type strain remains strain NCIB 8290 (= LMG 4311); it was isolated from an acetate enrichment (pH 6.6) in 1944 and has a mean DNA base composition of 71.9 mol% G+C.

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