Intragenetic Structure of the Genus *Rhodobacter*: Transfer of *Rhodobacter sulfidophilus* and Related Marine Species to the Genus *Rhodovulum* gen. nov.

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Phylogenetic relationships among species of the genus *Rhodobacter* and related taxa were elucidated by studying 16S rRNA sequence information and genomic DNA homology data. The 16S rRNA gene was amplified by the PCR and was sequenced directly by a combined method consisting of cycle sequencing and automated fluorescence detection. Pairwise sequence comparisons and a distance matrix analysis showed that the *Rhodobacter* species could be divided into two major clusters; one cluster included the freshwater and terrestrial species, and the other cluster contained the marine species. The cluster containing the freshwater *Rhodobacter* species also included *Rhodopseudomonas blastica* and was linked more closely to the chemotroph *Paracoccus denitrificans* and the aerobic phototroph *Roseobacter denitrificans* than to the cluster containing the marine *Rhodobacter* species. Genomic DNA-DNA hybridization data supported the results of 16S ribosomal DNA sequence comparisons. With few exceptions, the marine *Rhodobacter* species can be differentiated phenotypically from the freshwater species on the basis of salt requirement for optimal growth, sulfide tolerance, final oxidation product of sulfide, and polar lipid composition. Thus, we propose that all marine *Rhodobacter* species should be transferred to the genus *Rhodovulum* gen. nov.; *Rhodovulum sulfidophilum* comb. nov. is the type species of this genus.

In 1984 Imhoff et al. (25) described a rearrangement of the genera and species of phototrophic purple nonsulfur bacteria and proposed creation of the new genus *Rhodobacter* and the new combinations *Rhodobacter adriaticus*, *Rhodobacter capsulatus* (type species), *Rhodobacter sphaeroides*, and *Rhodobacter sulfidophilus*, all of which had been included previously in the genus *Rhodopseudomonas*. Since then, two new species, *Rhodobacter veldkampii* (12) and *Rhodobacter euryhalinus* (31), have been added to this genus. Strains of *Rhodobacter* species are gram negative and facultatively photoheterotrophic and are characterized by their ovoid to rod-shaped morphology, their formation of vesicular intracytoplasmic membranes together with carotenoids of the spheroidene series, and their ability to grow anaerobically and phototrophically in the presence of sulfide as an electron donor (23). They are also similar in their chemotaxonomic properties, such as quinone compositions (18, 19, 22), fatty acid profiles (24, 28), and lipid A structures (42, 43). Although *Rhodobacter* species share many common characteristics, as noted above, they can be divided clearly into two groups on the basis of their natural habitats. Freshwater and terrestrial environments are common sources of *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, and *Rhodobacter veldkampii*, while *Rhodobacter adriaticus*, *Rhodobacter euryhalinus*, and *Rhodobacter sulfidophilus* are found only in marine and hypersaline environments. As suggested by their natural habitats, strains of the latter three species require 0.5 to 7.5% NaCl for optimal growth. There are also differences in polar lipid composition (24) and some other phenotypic traits among *Rhodobacter* species.

The phylogenetic affiliations of members of the genus *Rhodobacter*, as well as other phototrophic bacteria and their nonphototrophic relatives, have been estimated on the basis of structures and amino acid sequences of cytochrome *c* (1, 5), 16S rRNA oligonucleotide catalogs (11, 46, 47) and sequences (29, 32, 45), rRNA operon structures (6), and rRNA cistron similarities (4). An important conclusion deduced from these molecular approaches is that *Rhodobacter* species are more closely related to the chemotrophic bacterium *Paracoccus denitrificans* than to other members of the phototrophic bacteria belonging to the alpha group of the class *Proteobacteria* (39). It is also noteworthy that there is a close phylogenetic relationship between *Rhodobacter* species and a phototroph with lamellar membranes, *Rhodopseudomonas blastica*, as shown by the results of DNA-rRNA reassociation assays (4), immunological analyses of malate dehydrogenase (40), and 16S rRNA sequence comparisons (29). These findings have led some workers to propose that *Rhodopseudomonas blastica* should be transferred to the genus *Rhodobacter* (4, 29) and to suggest that the types of intracytoplasmic membrane systems are of questionable value as generic criteria (29). Moreover, DNA-rRNA pairing studies have shown that there are closer affinities among some freshwater *Rhodobacter* species, *Rhodopseudomonas blastica*, and *Paracoccus denitrificans* than between the freshwater *Rhodobacter* species and the marine species *Rhodobacter sulfidophilus* (4). Genomic DNA-DNA hybridization studies have provided some information about the intragenetic structure of and interspecies relationships within the genus *Rhodobacter* (3, 26). At present, the genus *Rhodobacter* is considered the taxonomically best characterized genus among genera belonging to the alpha subclass of phototrophic proteobacteria (23). However, circumstantial evidence suggests that there is still phylogenetic and phenotypic diversity among members of this genus.

The present study was undertaken to investigate more

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TABLE 1. Bacterial strains studied and accession numbers for their 16S rDNA sequences

Species	Strain designation or received as strain ^a	Other designation(s) or source ^a	Reference	Nucleotide sequence accession no.
Freshwater species				
<i>Rhodobacter capsulatus</i>	ATCC 11166 ^{Tb} C5	DSM 1710 ^T , C. B. van Niel ATH 2.3.1 ^T Collection of A.H., isolated from a paddy field	36	D16428 D16427
<i>Rhodobacter sphaeroides</i>	IFO 12203 ^T IL106	ATCC 17023 ^T , DSM 158 ^T , C. B. van Niel ATH 2.4.1 ^T T. Satoh	36 38	D16425 D16424
<i>Rhodobacter veldkampii</i>	ATCC 35703 ^T	T. A. Hansen 51 ^T	12	D16421
<i>Rhodopseudomonas blastica</i>	ATCC 33485 ^T	DSM 2131 ^T , NCIB 11576 ^T	7	D16429
Marine species				
<i>Rhodobacter adriaticus</i>	DSM 2781 ^T	J. F. Imhoff 6II ^T	35	D16418
<i>Rhodobacter euryhalinus</i>	DSM 4868 ^T	E. I. Kompantseva KA-65 ^T	31	D16426
<i>Rhodobacter sulfidophilus</i>	DSM 1374 ^T DSM 2351 TW13	T. A. Hansen W4 ^T T. A. Hansen W12 Collection of A.H., isolated from a tide pool	14 14 19	D16423 D16430 D16422
<i>Rhodobacter</i> sp.	MB260	Collection of A.H., isolated from a tide pool		D16420
<i>Rhodobacter</i> sp.	MB-G2	Collection of A.H., isolated from a tide pool		D16419

^a ATCC, American Type Culture Collection, Rockville, Md.; IFO, Institute for Fermentation, Osaka, Japan; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Germany; NCIB, National Collection of Industrial Bacteria, Aberdeen, United Kingdom; A.H., A. Hiraishi, Laboratory of Environmental Biotechnology, Konishi Co., Tokyo, Japan.

^b T = type strain.

thoroughly the intragenomic structure of the genus *Rhodobacter* by means of direct automated sequencing of PCR-amplified 16S rRNA genes and genomic DNA-DNA pairing. On the basis of the results of these molecular approaches and previous phenotypic information, we propose that *Rhodobacter sulfidophilus* and related marine species should be transferred to a new genus, *Rhodovulum*.

MATERIALS AND METHODS

Bacterial cultures and cultivation. The test bacteria included 10 strains belonging to six previously described species of the genus *Rhodobacter*, 2 strains belonging to unknown *Rhodobacter* species, and one strain of *Rhodopseudomonas blastica* (Table 1). *Rhodobacter capsulatus* C5 and the two strains belonging to the unknown *Rhodobacter* species, MB260 and MB-G2, were new strains isolated by one of us (A.H.) from the environment. MYS medium (18) and modifications of this medium were used to cultivate the organisms. The medium was modified by adding 2 mM cysteine (filter sterilized) for the growth of *Rhodobacter veldkampii*. For most marine species, 0.5 mM sodium thiosulfate, 0.5 mM sodium sulfide, and 3% NaCl were added to the medium; the concentration of NaCl was reduced to 1% for *Rhodobacter euryhalinus* and *Rhodobacter* sp. strain MB-G2. The organisms were grown anaerobically at 30°C in screw-cap test tubes or bottles filled with medium under incandescent illumination (ca. 5,000 lux). Cells were harvested by centrifugation from cultures at the mid-exponential phase of growth, washed with sterilized 1% saline, resuspended either in pure water (for PCR experiments) or in EDTA-saline (for DNA hybridization studies), and stored at -20°C until they were used.

PCR amplification and sequencing of 16S rDNA. Crude lysates were prepared from stock cell suspensions by protease digestion, heat treatment, and centrifugation. 16S ribosomal DNA (rDNA) fragments were amplified by the PCR directly from the crude extract, using a commercial PCR kit, *Taq* DNA polymerase, and a pair of eubacterial consensus primers homologous or complementary to posi-

tions 8 to 27 and 1492 to 1510 (44) (all positions indicated for the 16S rRNA molecule refer to the *Escherichia coli* numbering system [2]). Detailed information concerning the procedures used has been given elsewhere (16, 17). PCR products were extracted with chloroform-isoamyl alcohol (24:1 [vol/vol]), precipitated with ammonium acetate and ethanol, and purified by agarose gel electrophoresis. Separated fragments were cut from the gel and withdrawn with a Sepaglas BandPrep kit (Pharmacia LKB Biotechnology, Uppsala, Sweden) according to the manufacturer's instructions. PCR-amplified double-stranded 16S rDNA was sequenced directly by the linear PCR amplification (cycle sequencing) method (8, 34) with a Pharmacia AutoCycle sequencing kit and was analyzed with a Pharmacia A.L.F. laser fluorescent DNA sequencer as described previously (16, 17).

Phylogenetic analysis. Pairs of sequences were aligned, and similarities were calculated with the GENETYX computer program (Software Development Co., Tokyo, Japan). Nucleotide substitution rates (K_{nuc}) (30) were determined, and a distance matrix tree was constructed by the neighbor-joining method (37), using the CLUSTAL V program (15). Alignment gaps and unidentified base positions were not taken into consideration for the calculations. The topology of the phylogenetic tree was evaluated by performing a bootstrap analysis (10) with 1,000 bootstrapped trials.

DNA base composition and DNA-DNA homology. Genomic DNAs were extracted and purified by the method of Marmur (33). DNA base compositions (G+C contents) were determined by high-performance liquid chromatography (HPLC) of nuclease P1 hydrolysates of genomic DNAs, using external standards obtained from Yamasa Shoyu Co., Choshi, Japan (20). DNA-DNA pairing studies were performed by the quantitative dot blot hybridization method with photobiotin labeling and colorimetric detection (9, 20).

Nucleotide sequence accession numbers. The 16S rDNA sequences determined in this study have been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence data bases under accession numbers D16418 to D16430 (Table 1).

The accession numbers of the sequences used as references are as follows: *Erythrobacter longus*, M59062; *Escherichia coli*, J01859; *P. denitrificans*, X69159; *Rhodomicrobium vannielii*, M34127; *Rhodopila globiformis*, M59066; *Rhodopseudomonas acidophila*, M34128; *Rhodopseudomonas marina*, M27534; *Rhodopseudomonas palustris*, M59068; *Rhodospirillum molischianum*, M59067; *Rhodospirillum rubrum*, M32020; *Rhodospirillum salexigens*, M59070; *Roseobacter denitrificans* (formerly *Erythrobacter* sp. strain OCh 114), M59063.

RESULTS AND DISCUSSION

The PCR-amplified 16S rDNA sequences of 12 strains of *Rhodobacter* species and one strain of *Rhodopseudomonas blastica* were determined by the combined method consisting of cycle sequencing and automated fluorescence detection. All of the fragments, except the PCR primer-annealing regions, made up a continuous nucleotide region stretching from position 28 to position 1491 (length, 1,386 to 1,390 residues). With few exceptions, the 16S rRNA structures of all of the test strains deduced from the rDNA sequence information included the definitive signatures of the alpha subdivision of the *Proteobacteria* (45), and the 16S rRNAs had nucleotide deletions in loop helices at positions 74 to 94, 201 to 215, 452 to 479, and 1258 to 1278. Also, as described previously for *Rhodobacter sphaeroides* 16S rRNA (6), the unusual large stem-loop around position 1,450 which contained one to four bases more than the stem-loop of *E. coli* was observed in all test organisms. Characteristic differences in 16S rRNA nucleotide signatures between the freshwater and marine *Rhodobacter* species were also found (see below).

From the sequence data obtained binary sequence similarity and evolutionary distance (K_{nuc}) values were calculated for the 13 test strains and some reference organisms. To have as many alignable positions as possible for the calculations, we first attempted to add only two reference sequences, the reference sequences from *P. denitrificans* and *Roseobacter denitrificans*, to the data set. The results of this experiment are shown in Table 2, which shows the overall percentages of similarity obtained after pairwise sequence alignment, as well as the corrected distance values calculated for the alignable 1,346 positions of the entire set. The levels of sequence similarity between the *Rhodobacter* test strains ranged from 91.6 to 100%. It is important to note that the freshwater species *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, and *Rhodobacter veldkampii* are more similar to each other (level of similarity, >94.8%) than to the marine species *Rhodobacter adriaticus*, *Rhodobacter euryhalinus*, and *Rhodobacter sulfidophilus*. In contrast, the marine species are more similar to each other (level of similarity, >93.9%) than to the freshwater species. The levels of similarity between the freshwater and marine species range from 91.6 to 94.4%. As expected from the previously published molecular information (4, 29), *Rhodopseudomonas blastica* exhibits relatively high levels of sequence similarity (ca. 95%) to the freshwater *Rhodobacter* species.

A distance matrix tree was reconstructed by the neighbor-joining method by using the corrected values for the 13 test strains and the 2 reference organisms (Fig. 1). The intragenetic structure of the genus *Rhodobacter* is clear on the phylogenetic tree, as the freshwater and marine species of this genus are separated into two major clusters. A bootstrap analysis confirmed the monophyly of the cluster that in-

cluded the marine *Rhodobacter* species in 99.8% of the 1,000 trees generated. Newly isolated *Rhodobacter* sp. strains MB260 and MB-G2 were included in the cluster containing the marine species. The tree topography suggests that the former strain is related to *Rhodobacter sulfidophilus*, while the latter may be a member of a new marine species. The cluster containing the freshwater *Rhodobacter* species also included *Rhodopseudomonas blastica* and was linked more closely to the chemotroph *P. denitrificans* than to the marine *Rhodobacter* group. These results not only confirm the previous findings concerning the genealogical relationships among *Rhodobacter* species, *Rhodopseudomonas blastica*, and *P. denitrificans*, but also demonstrate that there is still phylogenetic diversity within the genus *Rhodobacter*.

The 16S rDNA sequences of the six established species and one unknown species (strain MB-G2) belonging to the genus *Rhodobacter* were also compared with the sequences of nine reference species of alpha group phototrophs in addition to the sequences of *P. denitrificans* and *Roseobacter denitrificans*, and another phylogenetic tree was constructed by using the evolutionary distance values calculated for the 1,209 positions that could be aligned (Fig. 2). This tree shows that the species of the genus *Rhodobacter*, together with *P. denitrificans* and *Roseobacter denitrificans*, form a major cluster which is relatively distant from the lineages containing other members of the alpha group of phototrophic bacteria. Within the *Rhodobacter* branch, this tree has almost the same topography as the tree shown in Fig. 1. In particular, the *Rhodobacter* cluster consists of two separate lineages; one lineage includes the freshwater *Rhodobacter* species linked to *P. denitrificans* and *Roseobacter denitrificans* as the nearest neighbors, and the other lineage includes the marine *Rhodobacter* species.

The results of genomic DNA-DNA hybridization studies were consistent with those of 16S rDNA sequence comparisons (Table 3). The freshwater *Rhodobacter* species and *Rhodopseudomonas blastica* exhibited DNA-DNA homology levels of more than 15% in comparisons with the type strain of *Rhodobacter capsulatus*. On the other hand, the homology levels were somewhat lower (9 to 14%) between the marine species and the type species, *Rhodobacter capsulatus*. The reverse was true when DNAs were paired with labeled DNA from the marine species *Rhodobacter sulfidophilus*. The DNA-DNA homology values obtained indicate that the levels of genetic relatedness between the freshwater and marine *Rhodobacter* species are relatively low for members of a genus. DNA-DNA hybridization studies also provided some interesting information about the taxonomic position of new isolate MB260. This strain has been shown to be related to *Rhodobacter sulfidophilus* on the basis of 16S rDNA sequence data, as noted above, but exhibited 53% DNA homology to the type strain of *Rhodobacter sulfidophilus*, suggesting that the two taxa differ at the species level. Our DNA base composition data for some species were different from data obtained previously. In particular, this was true for *Rhodobacter sulfidophilus*, for which we found G+C contents of 66.3 to 66.6 mol% (as determined by HPLC), values that were much lower than the previously reported values (3, 14).

While there has been no general agreement regarding the phylogenetic definition of a genus (41), Johnson (27) has stated that the minimum DNA-DNA homology level for members of a genus should be 20%. Plotting the 16S rDNA sequence similarity values against the DNA-DNA homology values for each pair of test organisms revealed that 20% DNA-DNA homology corresponded to a level of 16S rRNA

TABLE 2. Levels of sequence similarity and evolutionary distances for 16S rRNAs and rDNAs of *Rhodobacter* species, *Rhodopseudomonas blautica*, and two reference species, *Paracoccus denitrificans* and *Roseobacter denitrificans*

Organism	Level of sequence similarity (%) or evolutionary distance (K_{unc}) ^a														
	<i>Rhodobacter capsulatus</i> ATCC 11166 ^T	<i>Rhodobacter capsulatus</i> C5	<i>Rhodobacter sphaeroides</i> IFO 12203 ^T	<i>Rhodobacter sphaeroides</i> IL106	<i>Rhodobacter veldkampii</i> ATCC 35703 ^T	<i>Rhodopseudomonas blautica</i> ATCC 33486 ^T	<i>Rhodobacter sulfidophilus</i> DSM 1374 ^T	<i>Rhodobacter sulfidophilus</i> TW13	<i>Rhodobacter sulfidophilus</i> DSM 2351	<i>Rhodobacter</i> sp. strain MB260	<i>Rhodobacter</i> sp. strain MB-G2	<i>Rhodobacter adriaticus</i> DSM 2781 ^T	<i>Rhodobacter euryhalinus</i> DSM 4868 ^T	<i>Paracoccus denitrificans</i>	<i>Roseobacter denitrificans</i>
<i>Rhodobacter capsulatus</i> ATCC 11166 ^T															
<i>Rhodobacter capsulatus</i> C5	0.0059														
<i>Rhodobacter sphaeroides</i> IFO 12203 ^T	0.0446	0.0401													
<i>Rhodobacter sphaeroides</i> IL106	0.0453	0.0409	0.0007												
<i>Rhodobacter veldkampii</i> ATCC 35703 ^T	0.0520	0.0475	0.0490	0.0498											
<i>Rhodopseudomonas blautica</i> ATCC 33486 ^T	0.0513	0.0483	0.0498	0.0490	0.0527										
<i>Rhodobacter sulfidophilus</i> DSM 1374 ^T	0.0646	0.0609	0.0669	0.0676	0.0594	0.0706									
<i>Rhodobacter sulfidophilus</i> DSM 2351	0.0661	0.0624	0.0684	0.0691	0.0594	0.0721	0.0000								
<i>Rhodobacter</i> sp. strain MB260	0.0691	0.0654	0.0698	0.0706	0.0632	0.0743	0.0082	0.0082							
<i>Rhodobacter</i> sp. strain MB-G2	0.0706	0.0661	0.0654	0.0661	0.0565	0.0572	0.0312	0.0312	0.0082						
<i>Rhodobacter adriaticus</i> DSM 2781 ^T	0.0788	0.0758	0.0624	0.0632	0.0661	0.0788	0.0423	0.0423	0.0409	0.0342					
<i>Rhodobacter euryhalinus</i> DSM 4868 ^T	0.0676	0.0639	0.0594	0.0602	0.0535	0.0602	0.0498	0.0498	0.0498	0.0475	0.0579				
<i>Paracoccus denitrificans</i>	0.0624	0.0602	0.0632	0.0639	0.0579	0.0713	0.0743	0.0743	0.0743	0.0765	0.0832	0.0758			
<i>Roseobacter denitrificans</i>	0.0936	0.0892	0.0862	0.0869	0.0728	0.0958	0.0958	0.0958	0.0944	0.0966	0.1062	0.0988	0.0713		
														0.0817	

^a The values on the upper right are levels of sequence similarity, and the values on the lower left are corrected evolutionary distances.

FIG. 1. Unrooted distance matrix tree showing phylogenetic relationships among the test strains of *Rhodobacter* species and *Rhodopseudomonas blastica*, as well as *P. denitrificans* and *Roseobacter denitrificans*. The bacteria examined for 16S rDNA sequences in this study are indicated by boldface type. Bootstrap confidence values are given at branching points of interest. Bar = 0.01 K_{nuc} .

sequence similarity of ca. 96% (Fig. 3). If these criteria are used to define a generic boundary, the *Rhodobacter* species can be separated into two or more genera. In addition, the freshwater and marine *Rhodobacter* species may be related phylogenetically above the genus level.

The current genus *Rhodobacter* was proposed on the basis of phenotypic, chemotaxonomic, and molecular data when the purple nonsulfur bacteria were reclassified (25). This genus is more coordinated than the other genera of phototrophic bacteria belonging to the alpha subclass of the *Proteobacteria*. Nevertheless, as reported in this paper, our detailed phylogenetic analyses performed with information on 16S rRNA gene sequences and levels of genomic DNA homology have revealed that there is phylogenetic diversity among members of the genus *Rhodobacter* and that there are two separate lineages, one of which includes the freshwater species and one of which includes the marine species. The 16S rRNA-based tree topography showing the marine species cluster as a monophyletic group is supported by a high level of bootstrap confidence. The molecular data also suggest that the freshwater *Rhodobacter* species are more closely related to the nonphototrophic bacterium *P. denitrificans* than to the marine species. From a phylogenetic standpoint, therefore, it is logical to conclude that all of the marine species *Rhodobacter adriaticus*, *Rhodobacter euryhalinus*, and *Rhodobacter sulfidophilus* should be removed from the genus *Rhodobacter* and placed in a new genus. We propose that all marine *Rhodobacter* species should be transferred to the genus *Rhodovulum* gen. nov.; *Rhodovulum sulfidophilum* comb. nov. is the type species of this genus. Although in the new genus *Rhodovulum* there are still variations in levels of interspecific 16S rRNA and genomic DNA homology within a relatively broad range, it is appro-

priate, at present, to place the three marine species in a single genus on the basis of their phenotypic homogeneity, as described below.

In the taxonomy of *Rhodobacter* species, as well as in the taxonomy of many other bacterial taxa, a salt requirement for growth and natural habitats (freshwater or marine origin) are important traits. Our proposal for creation of the new genus *Rhodovulum* clarifies the taxonomic problem concerning the heterogeneity of the *Rhodobacter* species in these respects. Following the transfer of the marine species to the genus *Rhodovulum*, the genus *Rhodobacter* should include only freshwater species. There are some other phenotypic characteristics that are useful for differentiating the genus *Rhodovulum* from the genus *Rhodobacter* sensu stricto. Sulfide tolerance is considerably higher in the marine species than in the freshwater species, and the final oxidation product of sulfide is sulfate in the former species and sulfur in the latter species (12, 14, 23, 31, 35); *Rhodobacter veldkampii* is an exception to these general statements (12). In tests for photoassimilation of organic substrates, utilization of the one-carbon compound formate has been found mainly in the marine species (14, 31, 35); this characteristic has also been found in new isolates MB260 and MB-G2 (21). Polar lipid profiles may also be useful as diagnostic characteristics. The marine species contain sulfolipids (probably sulfoquinovosyldiglyceride) but not phosphatidylcholine, while the freshwater species are heterogeneous for these characteristics (24). Characteristic differences in 16S rRNA nucleotide signatures are found for the genera *Rhodovulum* and *Rhodobacter* sensu stricto, indicating the usefulness of molecular information, as well as phenotypic traits, for identification and classification of strains of the two genera.

FIG. 2. Unrooted distance matrix tree showing phylogenetic relationships of *Rhodobacter* species (represented by their type strains [except strain MB-G2]) and other phototrophic and nonphototrophic bacteria belonging to the alpha subclass of the *Proteobacteria*. The *Escherichia coli* sequence was used as an outlier sequence to root the tree. The lengths of the horizontal lines are proportional to the evolutionary distances, while the lengths of the vertical lines are meaningless. The species examined for 16S rDNA sequences in this study are indicated by boldface type. Bar = $0.02 K_{nuc}$.

The differential characteristics of the two genera are summarized in Table 4.

Because of the transfer of the marine species to the genus *Rhodovulum*, the description of the genus *Rhodobacter* must be emended, and *Rhodopseudomonas blastica* must be reclassified as a member of the latter genus. However, some taxonomic problems concerning the circumscription of the genus *Rhodobacter* remain. Compared with the species of the genus *Rhodovulum*, the members of the genus *Rhodo-*

bacter sensu stricto seem to exhibit more phenotypic diversity in key characteristics (for example, in sulfur metabolism and polar lipid profiles) (Table 4). Also, the proposal to incorporate *Rhodopseudomonas blastica* into the genus *Rhodobacter* (29) complicates the problems because of the resulting rejection of some important phenotypic characteristics (i.e., mode of cell division, photosynthetic membrane systems, and sulfide utilization) as generic criteria. We prefer to transfer *Rhodopseudomonas blastica* and *Rhodo-*

TABLE 3. Levels of genomic DNA relatedness for test strains of *Rhodobacter* species and *Rhodopseudomonas blastica*

Organism	G+C content (mol%)	% Homology with labeled DNA from:	
		<i>Rhodobacter capsulatus</i> ATCC 11166 ^T	<i>Rhodobacter sulfidophilus</i> DSM 1374 ^T
Freshwater strains			
<i>Rhodobacter capsulatus</i> ATCC 11166 ^T	65.9	100	14
<i>Rhodobacter capsulatus</i> C5	66.3	60	14
<i>Rhodobacter sphaeroides</i> IFO 12203 ^T	69.0	20	13
<i>Rhodobacter sphaeroides</i> IL106	69.2	22	12
<i>Rhodobacter veldkampii</i> ATCC 35703 ^T	66.5	15	10
<i>Rhodopseudomonas blastica</i> ATCC 33486 ^T	65.6	16	8
Marine strains			
<i>Rhodobacter adriaticus</i> DSM 2781 ^T	65.0	9	16
<i>Rhodobacter euryhalinus</i> DSM 4868 ^T	65.5	10	14
<i>Rhodobacter sulfidophilus</i> DSM 1374 ^T	66.3	14	100
<i>Rhodobacter sulfidophilus</i> DSM 2351	66.6	13	73
<i>Rhodobacter sulfidophilus</i> TW13	66.4	11	95
<i>Rhodobacter</i> sp. strain MB260	66.6	11	53
<i>Rhodobacter</i> sp. strain MB-G2	67.5	11	20

FIG. 3. Relationship between level of 16S rDNA sequence homology and level of genomic DNA homology for pairs of *Rhodobacter* strains. Symbols: ●, homology values determined with *Rhodobacter capsulatus* ATCC 11166^T; ▲, homology values determined with *Rhodobacter sulfidophilus* DSM 1374^T.

bacter veldkampii for practical reasons to separate new genera, but will not present nomenclatural and taxonomic proposals to do this until more information and more strains related to these taxa become available.

Description of *Rhodovulum* gen. nov. *Rhodovulum* (Rho.do'vu.lum. Gr. n. *rhodos*, rose; L. dim. n. *ovulum*, small egg; M. L. neut.n. *Rhodovulum*, small red egg). The

characteristics described below are based on information obtained from Imhoff et al. (23–25) and this study. Cells are ovoid to rod shaped, 0.5 to 0.9 μm wide, and 0.9 to 2.0 μm long. Motile by means of a single polar flagellum or nonmotile. Cells divide by binary fission. Gram negative. Facultatively anaerobic phototrophs which grow under anaerobic conditions in the light or under aerobic conditions in the dark. Phototrophically grown cells form vesicular intracytoplasmic membranes together with bacteriochlorophyll *a* (esterified with phytol) and carotenoids of the spheroidene series. The color of phototrophic cultures is yellow-green to yellow-brown, while aerobic cultures are pink to red. Mesophilic and halophilic. Sodium chloride at a concentration of 0.5 to 7.5% is required for optimal growth. Photoorganotrophy with various organic compounds is the preferred mode of growth. Good carbon sources are pyruvate, lactate, lower fatty acids, intermediates of the citric acid cycle, and some sugars. Growth on formate also occurs. Photoautotrophic or photoheterotrophic growth occurs in the presence of sulfide or thiosulfate as the electron donor. Growth occurs in the presence of high sulfide concentrations (2 mM or more). The final oxidation product of sulfide is sulfate. Ubiquinone 10 is the major quinone. The major fatty acid is C_{18:1}. Membrane lipids contain sulfolipids but not phosphatidylcholine. The G+C content of the genomic DNA ranges from 62 to 69 mol%. The phylogenetic position is the alpha subclass (α -3) of the *Proteobacteria*. Natural habitats are marine and hypersaline environments. The type species is *Rhodovulum sulfidophilum*.

Description of *Rhodovulum sulfidophilum* comb. nov. *Rhodovulum sulfidophilum* (*Rhodobacter sulfidophilus* (Hansen and Veldkamp 1973) Imhoff, Trüper, and Pfennig 1984) (sul.fi.do'phi.lum. N. L. n. *sulfidum*, sulfide; Gr. adj. *philus*, loving; M. L. adj. *sulfidophilum*, sulfide loving). The characteristics are the same as the characteristics described above for the genus. Additional properties have been described previously (14, 23–25). The G+C content of the

TABLE 4. Differential phenotypic characteristics and 16S rRNA nucleotide signatures of the genera *Rhodovulum* gen. nov. and *Rhodobacter* sensu stricto^a

Characteristic	<i>Rhodovulum</i>	<i>Rhodobacter</i>
Salt required for optimal growth	+ ^b	–
Final oxidation product of sulfide	SO ₄ ^{2–}	S ⁰ /SO ₄ ^{2–}
Sulfide tolerance (>2 mM)	+	–/+
Utilization of:		
Thiosulfate	+	–/+
Formate	+	–/+
Polar lipid composition:		
Sulfolipid	+	–/+
Phosphatidylcholine	–	+/-
G+C content of genomic DNA (mol%)	62–69	64–70
Natural habitat	Hypersaline and marine environments	Freshwater and terrestrial environments
16S rRNA signature(s) at position(s):		
241–285	A–U/U–A	G–C
278	T	G
360	A	G
579	G	A
653	A/G	T
1310–1327	U–G	G–C
1311–1326	T–A/C–G	G–C
1312–1325	C–G	G–C
1426–1474	T–A	C–G

^a Data from references 12, 14, 23, 24, 31, and 35 and this study.

^b +, positive; –, negative; –/+ and +/–, variable reaction (the first sign indicates the most frequent result).

DNA ranges from 66.3 to 66.6 mol% (as determined by HPLC). The type strain is DSM 1374 (= ATCC 35886 = Hansen W4).

Description of *Rhodovulum adriaticum* comb. nov. *Rhodovulum adriaticum* (*Rhodobacter adriaticus* (Neutzing, Imhoff, and Trüper 1984) Imhoff, Trüper, and Pfennig 1984) (a.dri.a'ti.cum. M.L. masc. adj. *adriaticum* pertaining to the Adriatic Sea). The characteristics are the same as the characteristics described above for the genus. Additional properties have been described previously (23–25, 35). The G+C content of the DNA ranges from 64.9 to 66.7 mol% (as determined by the thermal denaturation method). The type strain is DSM 2781 (= ATCC 35885 = Imhoff 6II).

Description of *Rhodovulum euryhalinum* comb. nov. *Rhodovulum euryhalinum* (*Rhodobacter euryhalinus* Kompantseva 1985) (eu.ry.ha'li.num. Gr. adj. *eury*s, wide; Gr. n. *halts*, salt; M. L. masc. adj. *euryhalinum*, wide range salinity living). The characteristics are the same as the characteristics described above for the genus. Additional properties have been described previously (31). The G+C content of the DNA was reported to range from 62.1 to 68.6 mol% in the original description (31), but this broad range of base ratios may have resulted from heterogeneity of the test strains at the species level. The base composition of the type strain is 65.5 mol% G+C (as determined by HPLC) or 66.3 mol% G+C (as determined by the thermal denaturation method). The type strain is DSM 4868 (= Kompantseva KA-65).

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