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A New Genus of Marine Budding Phototrophic Bacteria, *Rhodobium* gen. nov., Which Includes *Rhodobium orientis* sp. nov. and *Rhodobium marinum* comb. nov.

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Strains of a previously undescribed species of purple nonsulfur phototrophic bacteria were isolated from coastal seawater in Japan. These new isolates were gram-negative, motile, budding rods that contained lamellar intracytoplasmic membranes and produced pink to red cultures. Cell extracts of photosynthetic cultures exhibited absorption maxima at 377, 468, 500, 530, 591, 802, and 870 nm, indicating that bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series were present. The new isolates were halophilic, facultatively aerobic photoheterotrophs that grew anaerobically in the light or aerobically in the dark. Maximum growth occurred in the presence of 4 to 5% NaCl. Anaerobic growth in the dark with nitrate as a terminal electron acceptor also occurred. Various organic compounds were used as photosynthetic electron donors and carbon sources. Sulfate was used as a sulfur source. Both menaquinone 10 and ubiquinone 10 were produced; these quinones were the major quinones. A phylogenetic analysis based on 16S rRNA gene sequences revealed that strain MB312[T] (T = type strain), a representative of the new phototrophs, was a member of a lineage that was distinct from members of the genus *Rhodopseudomonas*; *Rhodopseudomonas marina* was the closest relative. On the basis of the data described above, we propose the name *Rhodobium orientis* gen. nov., sp. nov. for the new isolates. We also propose that *Rhodopseudomonas marina* Imhoff 1983 should be transferred to the genus *Rhodobium* as *Rhodobium marinum* comb. nov.

Phototrophic purple nonsulfur bacteria with budding morphology and lamellar intracytoplasmic membranes have been classified previously in the genus *Rhodopseudomonas* (21, 35). However, the current taxonomic status of the genus *Rhodopseudomonas* is unsatisfactory, as members of this genus are diverse with respect to phenotypic and chemotaxonomic characteristics, natural habitats, and phylogenetic positions. Molecular phylogenetic analyses based on DNA-rRNA cistron similarities (4) and 16S rRNA catalogs (33, 37) and sequences (17, 29, 36, 38) have proven that the type species, *Rhodopseudomonas palustris*, is more closely related to some species of chemotrophic bacteria (e.g., *Blastobacter denitrificans*, *Bradyrhizobium japonicum*, and *Nitrobacter winigradskyi*) than to any other *Rhodopseudomonas* species in the alpha-2 subclass of the *Proteobacteria*. Chemotaxonomic analyses in which lipoquinone profiles were determined have revealed that ubiquinones are the only quinones produced by *Rhodopseudomonas palustris*, whereas other *Rhodopseudomonas* species also produce menaquinones and/or rhodoquinones (11, 12, 19). Molecular and chemotaxonomic heterogeneity among *Rhodopseudomonas* species is also found in cytochrome *c* size (2, 5), the lipid A structures of the outer membranes (18, 30, 34), and whole-cell fatty acid profiles (23). While the type species, *Rhodopseudomonas palustris*, occurs in freshwater habitats, the natural habitats of other *Rhodopseudomonas* species differ depending on the species and include freshwater, terrestrial, and marine environments.

Recently, workers have begun to clarify the unsatisfactory taxonomy of the genus *Rhodopseudomonas* in part on the basis of 16S rRNA sequence information and phenotypic and chemotaxonomic data. It has been suggested that *Rhodopseudomonas blastica* (6) should be reclassified as a member of the alpha-3 subclass and is closely related to *Rhodobacter* species (16, 24). It has been proposed that *Rhodopseudomonas rosea* (22) should be transferred to the new genus *Rhodoplanes* (17). The accumulated phenotypic and phylogenetic data suggest that all other previously described *Rhodopseudomonas* species except the type species, *Rhodopseudomonas palustris*, should be reclassified and placed in different new genera.

In this paper we describe the isolation and characterization of a new marine phototrophic purple nonsulfur bacterium which has budding morphology and a lamellar type of intracytoplasmic membrane arrangement. On the basis of the phenotypic criteria that have been used previously for the taxonomy of the phototrophic bacteria, this bacterium should have been classified as a marine species belonging to the genus *Rhodopseudomonas*. Since *Rhodopseudomonas marina* (18) is the only marine species among the previously described members of this genus, we compared the phenotypic, genotypic, and phylogenetic characteristics of the new marine phototrophic bacterium with the characteristics of *Rhodopseudomonas marina*. On the basis of our results, we propose that a new genus and species, *Rhodobium orientis*, should be created for the new phototrophic bacterium; strain MB312 is the type strain of this species. We also propose that *Rhopseudomonas marina* should be transferred to the new genus *Rhodobium*.

MATERIALS AND METHODS

Enrichment samples and isolation. The samples used for enrichment and isolation of phototrophic bacteria were seawater samples with sediment mud that were obtained from two different areas in Japan. One of the samples was collected from the bottom of a tidal pool (depth, ca. 30 to 50 cm) in Goto Island, Nagasaki Prefecture, in August 1989, and the other was obtained from the

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bottom of a tidal pool (depth, ca. 50 to 70 cm) in Makurazaki, Kagoshima Prefecture, in October 1992. Screw-cap 20-ml test tubes which contained 10 ml of an enrichment medium consisting of mineral base RM2 (14) supplemented with 10 mM acetate, 10 mM succinate, 2 mM thiosulfate, 2 mM sodium ascorbate, 2.5% NaCl, 0.01% yeast extract, and vitamin solution VA (1 ml/liter) (20) were inoculated with 0.1- or 1-ml portions of seawater samples with mud, completely filled with the same medium, and then incubated at 28°C in the presence of 2,000 lx of incandescent illumination. The agar shake technique (20) was used to isolate colonies from the enrichment cultures on the medium described above containing 0.8% agar. Single colonies that were pink to red were picked from the tubes and subjected to standard purification procedures by using agar plates and the GasPak anaerobic system.

Bacterial strains and cultivation. We studied three new isolates (strains MB303, MB312^T [T = type strain], and MB314) of purple nonsulfur bacteria isolated from coastal seawater samples. Some strains of *Rhodopseudomonas marina* were used in comparative studies. *Rhodopseudomonas marina* DSM 2698^T and DSM 2780 were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany, whereas *Rhodopseudomonas marina* subsp. *agilis* ATCC 35601 was obtained from the American Type Culture Collection, Rockville, Md. *Rhodopseudomonas marina* E2-A was isolated by one of us (A.H.) from a marine environment. A chemically defined medium, designated MMYS-III medium, was routinely used to grow the new isolates for various tests. This medium contained mineral base RM2, 20 mM sodium DL-malate as the carbon source, 2 mM thiosulfate, 5% NaCl, and 0.1% yeast extract as the growth factor. MYS medium (14) supplemented with 2.5% NaCl was also used to grow *Rhodopseudomonas marina* strains. All media contained 1.5% agar when the organisms were grown on agar slants or plates. The strains were routinely cultivated anaerobically in the light in screw-cap test tubes or bottles filled with medium. For genetic studies, cells were harvested by centrifugating 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.

Morphology and ultrastructure. Morphology and related properties were studied by using intact cells and an Olympus phase-contrast microscope; negatively stained cells were examined with a JEOL transmission electron microscope. Gram reactions were determined by using a commercial Gram stain reagent kit (Wako Pure Chemicals, Kyoto, Japan). Intracellular photosynthetic membrane arrangement was determined by electron microscopy of ultrathin sections prepared by a standard fixation method, as previously described (13). The rapid-freezing and substitution fixation method (1) was also used to prepare samples for ultrathin-section electron microscopy. The cells in a light-limited culture were pelleted by centrifugation, immediately immobilized with agar spread on a glass slide, and frozen with a model RF-2 rapid-freezing device (Eiko Engineering Corp., Tokyo, Japan). The frozen sample was then fixed with acetone containing 2% OsO$_4$ in dry ice-acetone and embedded in Epon 812. Thin sections were cut with an ultramicrotome equipped with a glass knife, stained with uranyl acetate and lead citrate, and examined with a JEOL transmission electron microscope.

Phenotypic characterization. Unless indicated otherwise, all test media were incubated at 30°C in the presence of 5,000 lx of incandescent illumination. Aerobic growth in the dark was studied by using 100-ml flasks containing 10 ml of MMYS-III medium on a reciprocal shaker. Anaerobic growth with nitrate respiration was studied by using screw-cap test tubes that were completely filled with MMYS-III medium supplemented with either 10 mM fructose or 10 mM glucose and 20 mM potassium nitrate. Tests to determine photoassimilation of organic substrates were performed in screw-cap test tubes containing mineral medium RM2 supplemented with 5% NaCl, a neutralized, filter-sterilized organic compound as the carbon source, and 0.01% yeast extract as the growth factor. Photoautotrophic growth was studied by using the 5% NaCl-containing mineral medium supplemented with either 0.5 mM sodium sulfide (neutralized) or 2 mM sodium thiosulfate as the electron donor, 20 mM sodium bicarbonate (filter sterilized) as the carbon source, and 0.01% yeast extract. In all growth response tests, growth was monitored turbidometrically at 660 nm with a model ANA-75 spectrophotometer (Tokyo Photoelectric Co., Tokyo, Japan); the final data were obtained after 2 weeks of incubation. Test tubes in which the optical density was higher than the optical density in a negative control containing no substrate were considered positive. Absorption spectra of intact cells or cell extracts were determined with a Hitachi model U-2000 spectrophotometer. Quinones were extracted with an organic solvent mixture, purified by thin-layer chromatography, and analyzed by UV spectrophotometry and high-performance liquid chromatography (HPLC) (11, 12).

DNA base composition and DNA-DNA hybridization. Chromosomal DNA was extracted and purified by the method of Marmur (28). The guanine-plus-cytosine (G+C) content of DNA was determined by subjecting nuclease P1 hydrolysates of the DNA to HPLC, using external nucleotide standards as described previously (13). DNA-DNA reassociation studies were performed by the quantitative dot blot hybridization method with photobiotin labeling and colorimetric detection (7, 13).

Sequencing of 16S rRNA genes and phylogenetic analysis. 16S ribosomal DNA (rDNA) fragments that corresponded to positions 8 to 1510 on the *Escherichia coli* 16S rRNA sequence (3) were amplified by PCR from crude cell

lysates and were sequenced directly by the cycle sequencing method modified for automated fluorescence detection with a Pharmacia laser fluorescent DNA sequencer. Detailed information concerning the PCR and sequencing procedures which we used has been given previously (10, 15). Sequence data were compiled and levels of binary sequence similarity were calculated with the GENETYX program package (Software Development Co., Tokyo, Japan). The CLUSTAL V program (9) was used to align multiple sequences, to calculate nucleotide substitution rates (K_{nuc} values) (25), to construct a neighbor-joining phylogenetic tree (31), and to statistically evaluate the tree topology by bootstrapping (8). Alignment positions that included gaps and/or unidentified bases were not taken into consideration for the calculations.

Nucleotide sequence accession numbers. The 16S rDNA sequences determined in this study have been deposited in the DDBJ, EMBL, GSDB, and NCBI nucleotide sequence databases under accession numbers D30790 to D30792. The accession number for the *Rhodopseudomonas marina* subsp. *agilis* nucleotide sequence described previously is M27534. The accession numbers for the sequences used in the phylogenetic analysis are as follows: strain MB312^T, D30792; *Rhodopseudomonas marina* DSM 2698^T, D30790; *Rhodopseudomonas marina* ATCC 35601, D30791; *Rhodopseudomonas acidophila*, M34128; *Rhodomicrobium vannielii*, M34127; *Rhodoplanes roseus*, D25313; *Rhodopseudomonas viridis*, D25314; *Rhodopseudomonas palustris*, D25312; *Bradyrhizobium japonicum*, X66024; *Rhodobacter capsulatus*, D16428; *Rhodobacter blasticus*, D16429; *Rhodovulum sulfidophilum*, D16423; *Erythrobacter longus*, M59062; *Rhodospirillum salexigens*, M59070; *Rhodospirillum rubrum*, M32020; and *Rhodopila globiformis*, M59066. The sequence of *Methylobacterium organophilum* has been determined but has not been deposited in a database (Hiraishi, unpublished data).

RESULTS

Enrichment and isolation. Most of the test tubes inoculated with seawater samples exhibited positive results for enrichment of phototrophic bacteria after 1 to 3 weeks of incubation, as indicated by pink to red growth. An agar shake dilution series was prepared with 0.1-ml portions of each of the enrichment cultures, and separated pink colonies were obtained in the test tubes at high dilutions. Three representative colonies recovered from each of the two seawater samples were purified for taxonomic studies. However, one-half of the isolates were lost during subculturing, perhaps because of unfavorable growth conditions (e.g., low salinity) at the initial stage of the study. As a result of these procedures, one isolate obtained from Goto Island (isolate MB303) and two isolates obtained from Makurazaki (isolates MB312^T and MB314) were successfully used for further studies.

Morphology and ultrastructure. The cells of all three isolates were gram negative, rod shaped, 0.7 to 0.9 μm wide, and 1.5 to 3.2 μm long (Fig. 1A). These organisms reproduced by budding and exhibited polar growth. The mode of budding appeared to be the sessile type and was similar to the mode of budding of *Rhodopseudomonas marina* and *Rhodoplanes roseus*. All isolates were motile by means of flagella. However, it was rather difficult to determine the type of flagella definitely, because electron microscopy revealed only a few flagellated cells and many loose flagella. Although the cells had single polar flagella, we could not eliminate the possibility that the isolates have subpolar, lateral, or randomly distributed flagella, like *Rhodopseudomonas marina* and *Rhodoplanes elegans*. Ultrathin-section electron microscopy revealed that the intracytoplasmic membrane arrangement in the cells of the isolates was lamellar (Fig. 1B, arrowhead) and resembled the arrangement found in *Rhodopseudomonas marina*. The photosynthetic membranes of the isolates were difficult to find when we examined thin sections prepared by the standard fixation method because of degradation of structures during the slow fixation procedure. The use of the rapid-freezing and substitution fixation technique (1) overcame this problem.

Photopigments and quinones. Mass cultures of the isolates were pink to red when they were grown anaerobically in the light. In this respect, the new isolates are similar to the marine phototroph *Rhodopseudomonas marina*. However, the isolates were easily differentiated from the latter species by their ab-
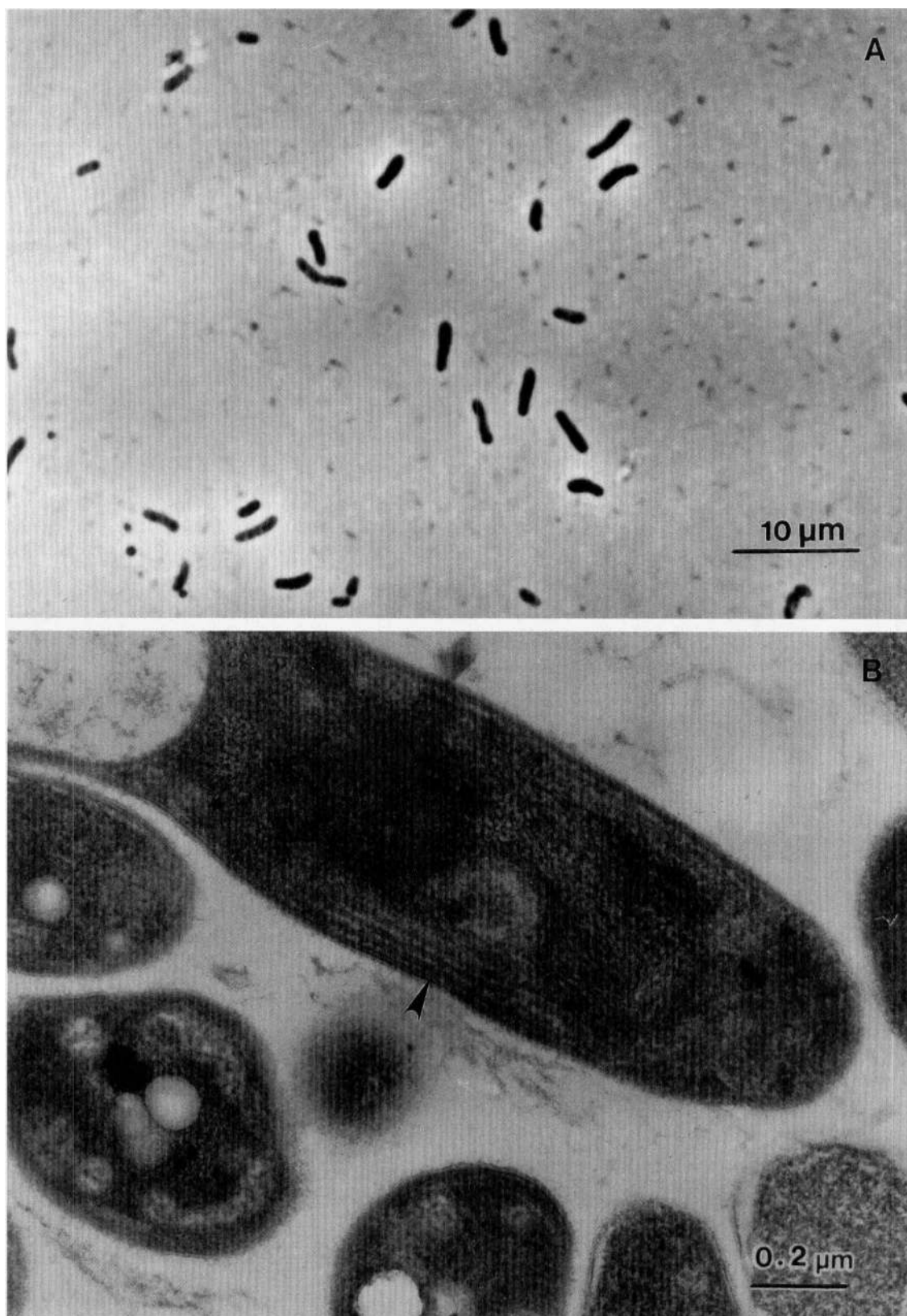
FIG. 1. Phase-contrast photomicrograph of intact cells (A) and transmission electron micrograph of an ultrathin section of cells (B), showing the general morphology and ultrastructure of a representative of the new isolates (strain MB312^T).
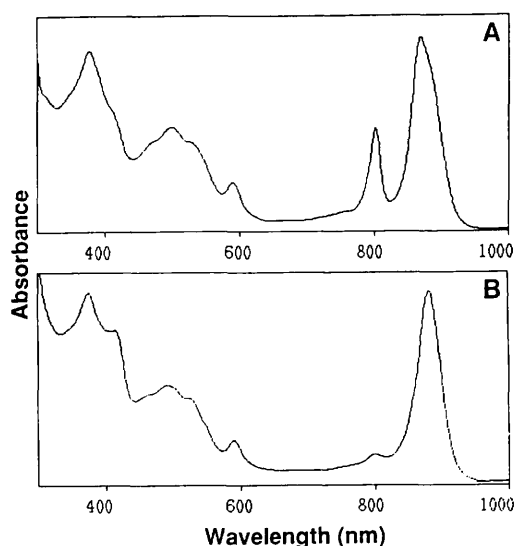
FIG. 2. Absorption spectra of cell extracts of strain MB312^T (A) and *Rhodopseudomonas marina* DSM 2698^T (B).

TABLE 1. Utilization of various compounds as electron donors and carbon sources by the new isolates[a]

Electron donor and carbon source[b]	Utilization by:		
	Strain MB303	Strain MB312^T	Strain MB314
Organic acids			
Formate	−	−	−
Acetate	+++	+++	+++
Propionate (0.05%)	−	−	−
Butyrate (0.05%)	++	+	+
Valerate (0.05%)	+	+	+
Caproate (0.05%)	+	++	+
Caprylate (0.05%)	−	−	−
Lactate	+++	+++	+++
Pyruvate	+++	+++	+++
Citrate	±	−	−
Succinate	+++	+++	+++
Fumarate	+++	+++	+++
Malate	+++	+++	+++
Tartrate	−	−	−
Benzoate (0.05%)	−	−	−
Sugars and alcohols			
L-Arabinose	±	−	−
D-Xylose	+++	+	+
Fructose	+++	++	++
Glucose	+++	+++	+++
Galactose	±	±	±
Mannose	±	±	±
Mannitol	±	+	+
Sorbitol	+	++	+
Glycerol	−	−	−
Methanol	−	−	−
Ethanol	−	−	−
Other compounds			
Glutamate	+	−	−
Casamino Acids	±	±	±
Peptone	++	++	++
Yeast extract	+++	+++	++
Thiosulfate (2 mM)	++	+	+
Sulfide (0.5 mM)	±	±	±

[a] +++, good growth (optical density at 660 nm, >0.5); ++, moderate growth (optical density at 660 nm, 0.2 to 0.5); +, poor growth (optical density at 660 nm, 0.1 to 0.2); ±, negligible growth (optical density at 660 nm, <0.1); −, no growth.

[b] All substrates were added at a concentration of 0.2%, unless indicated otherwise.

sorption spectra. A cell extract of strain MB312^T, a representative of the new isolates, had absorption maxima at 377, 468, 500, 530, 591, 802, and 870 nm, indicating that bacteriochlorophyll *a* and carotenoids belonging to the spirilloxanthin group were present (Fig. 2A). A cell extract of *Rhodopseudomonas marina* was characterized by its low level of absorption at 803 nm and the presence of an absorption maximum at 883 nm (Fig. 2B), like the absorption spectrum pattern of *Rhodospirillum rubrum*.

Thin-layer chromatography and spectrophotometry of the lipid extracts revealed that both ubiquinones and menaquinones were present; the molar ratio of menaquinones to ubiquinones was 1.0 to 1.5 in all isolates. The major homologs were ubiquinone 10 and menaquinone 10, as determined by HPLC.

Growth and physiological characteristics. All isolates were facultatively aerobic photoheterotrophs that were able to grow anaerobically in the light or aerobically in the dark at the full oxygen tension found in air. When the organisms were grown phototrophically in MMYS-III medium, they exhibited a doubling time of 5 to 9 h. It was interesting that the isolates were also able to grow under anaerobic conditions in the dark, provided that nitrate was added as a terminal electron acceptor. However, the denitrifying growth of the new isolates was much slower and weaker than the denitrifying growth of *Rhodobacter sphaeroides* IL106, the first strain reported to be a photodenitrifier (32), and seemed to be comparable to the denitrifying growth of denitrifying *Rhodopseudomonas palustris* strains (26) and *Rhodoplanes* species (17). Fermentative growth in the dark with glucose or fructose was not observed. Concurrent studies showed that all of the *Rhodopseudomonas marina* strains tested, including strain ATCC 35601, were able to grow fermentatively on fructose but not anaerobically by nitrate respiration. Our results concerning fermentative growth of *Rhodopseudomonas marina* subsp. *agilis* ATCC 35601 differ from the results of Mangels et al. (27).

Our new isolates were mesophilic, neutrophilic, and halophilic. The optimal growth temperature ranged from 30 to 35°C. Growth occurred at pH values between 6 and 8.5 (optimum pH, 7.0 to 7.5) but not at pH 5 or 9. The isolates grew in the presence of 2 to 8% NaCl, and maximum growth occurred

in the presence of 4 to 5% NaCl. The isolates required biotin and *p*-aminobenzoic acid as growth factors, but these compounds could be replaced by 0.01% yeast extract. Addition of 0.05% yeast extract to the medium enhanced growth considerably.

The results of tests to determine utilization of electron donors and carbon sources under phototrophic growth conditions are shown in Table 1. The good carbon sources were acetate, lactate, pyruvate, succinate, fumarate, malate, fructose, glucose, peptone, and yeast extract. Moderate growth occurred with butyrate, valerate, caproate, xylose, mannitol, and sorbitol. When 2 mM thiosulfate was added to the basal medium consisting of mineral salts and 0.01% yeast extract, weak but significant phototrophic growth of the isolates occurred. This observation suggests that our isolates were able to grow autotrophically with thiosulfate as an electron donor, although we did not confirm that these organisms oxidized thiosulfate. It was not clear whether the isolates were able to grow photoautotrophically with sulfide as the electron donor when the test was performed with 0.5 mM sodium sulfide in batch cultures.

Our isolates used ammonium salts as nitrogen sources and sulfate and thiosulfate as sulfur sources.

Phylogenetic relationships. In the phylogenetic analysis, 16S rRNA genes from three strains, new isolate MB312^T, *Rhodopseudomonas marina* DSM 2698^T, and *Rhodopseudomonas marina* subsp. *agilis* ATCC 35601, were amplified by the PCR and sequenced directly by a combined method consisting of cycle sequencing and automated fluorescence detection. Our sequencing strategy generated almost complete 16S rDNA sequences for ca. 95% of the entire molecule (i.e., 1,408 bases for strain MB312^T and 1,390 bases for the two *Rhodopseudomonas marina* strains). The shorter primary sequence of *Rhodopseudomonas marina* compared with the primary sequence of strain MB312^T was due to a 21-base deletion in the conserved region between positions 1256 and 1281 (*E. coli* numbering), like the deletion found in the phototrophic members of the alpha-3 subclass (16) of the *Proteobacteria*. The primary sequences of the two *Rhodopseudomonas marina* strains examined were identical, and our data confirmed the sequence of *Rhodopseudomonas marina* subsp. *agilis* reported previously, except at a few positions.

The new 16S rDNA sequences which we determined were aligned and compared with a data set consisting of 14 sequences available from databases, including the sequences of 11 anaerobic phototrophic bacterial species and 3 related species belonging to the alpha subclass of the *Proteobacteria*. Table 2 shows the levels of binary sequence similarity and the evolutionary distances (K_{nuc} values) based on the 1,265 positions of the entire sequence set that could be aligned. Strain MB312^T exhibited the highest level of sequence similarity to *Rhodopseudomonas marina* (93.8%) among the organisms compared. The levels of sequence similarity between strain MB312^T and the other species were much lower (less than 91%).

Figure 3 shows a phylogenetic tree which was reconstructed on the basis of the distance matrix values shown in Table 2. Strain MB312^T formed a cluster with *Rhodopseudomonas marina*, and this cluster was distinct from the previously described species of phototrophic bacteria and their relatives belonging to the alpha subclass of the *Proteobacteria*. However, the monophyletic relationship between the new isolate and *Rhodopseudomonas marina* seemed to be unstable, as suggested by the deep branching of the two taxa within the cluster and by the relatively low level (61.3%) of bootstrap confidence that supported this node (Fig. 3, arrow).

We determined DNA base ratios and performed DNA-DNA hybridization assays to obtain more detailed information about the genetic relationship between the new marine group and *Rhodopseudomonas marina* (Table 3). The strains belonging to the new marine group had DNA G+C contents of 65.2 to 65.7 mol%; these values were 2 to 3 mol% higher than the values obtained for *Rhodopseudomonas marina*. The levels of genomic DNA hybridization between strains belonging to the same group were more than 70%, but the levels of genomic DNA hybridization between strains belonging to different groups were low (<16%), suggesting that the organisms belonging to the two taxa represent distinct species.

DISCUSSION

We successfully characterized new marine isolates of budding purple nonsulfur bacteria and compared these organisms with *Rhodopseudomonas marina*, which is the only previously described marine species of budding phototrophic bacteria. Our new isolates, which are genetically conspecific, are facultatively aerobic, halophilic photoheterotrophs that produce

pink to red cultures, form lamellar photosynthetic membranes, produce bacteriochlorophyll *a* and carotenoids belonging to the spirilloxanthin group, and contain ubiquinone 10 and menaquinone 10 as their major quinones. In these respects, the new isolates resemble *Rhodopseudomonas marina*. On the basis of our results and previous data for *Rhodopseudomonas marina* (18), however, we identified differences between the two taxa in a number of phenotypic characteristics, including NaCl range for growth, absorption spectrum pattern, denitrification, fermentative growth on hexoses, and photoassimilation of some organic compounds, including propionate and ethanol. It is also easy to differentiate our isolates from *Rhodopseudomonas* species (35) and *Rhodoplanes* species (17) on the basis of their natural habitats and basic phenotypic traits.

The molecular genetic data based on 16S rRNA gene sequences and DNA-DNA hybridization results demonstrated that the new isolates are most closely related to, but are genetically different from *Rhodopseudomonas marina*. These data led us to conclude unequivocally that the new isolates belong to a previously undescribed species of marine phototrophic bacteria.

The available molecular and chemotaxonomic information strongly suggests that most *Rhodopseudomonas* species, including the marine species *Rhodopseudomonas marina*, should be reclassified and placed in different new genera (17). We determined that the new marine isolates should also be classified in a genus other than the genus *Rhodopseudomonas*. The remaining problem which we had to consider was whether our isolates should be united with *Rhodopseudomonas marina* in a single new genus. The primary 16S rDNA structure of strain MB312^T, a representative of the new isolates, is most similar to the primary 16S rDNA structure of *Rhodopseudomonas marina*, but the level of sequence similarity between these two organisms (93.8%) appears to be relatively low for members of the same genus. Also, the secondary 16S rRNA structure of *Rhodopseudomonas marina* differs from the secondary 16S rRNA structure of the new isolate in that the conserved helix region between positions 1256 and 1281 (*E. coli* numbering) is largely deleted. Our phylogenetic analysis with bootstrapping revealed that strain MB312^T forms a cluster with *Rhodopseudomonas marina*, but the relationship is not very stable as a monophyletic unit. Therefore, we thought that it would be possible to classify the new isolates and *Rhodopseudomonas marina* in two new genera as long as only molecular data were used for generic assignment.

However, we concluded that the new isolates and *Rhodopseudomonas marina*, should be placed in one genus at this time, because the phenotypic differences between the two taxa found so far seem to be too small to warrant generic separation. Below we propose the name *Rhodobium orientis* gen. nov., sp. nov. to accommodate the new marine phototrophs; the type strain is strain MB312. We also propose that *Rhodopseudomonas marina* Imhoff 1983 should be transferred to the new genus *Rhodobium* as *Rhodobium marinum* comb. nov. Table 4 summarizes characteristics which differentiate *Rhodobium orientis*, *Rhodobium marinum*, and some other species of budding phototrophic bacteria.

Description of *Rhodobium* gen. nov. *Rhodobium* (Rho.do'bi.um. Gr. n. *rhodos*, rose; Gr. n. *bios*, life; M.L. n. *Rhodobium*, red life). Cells are ovoid to rod shaped, 0.7 to 0.9 μm wide, and 1.0 to 3.2 μm long. Motile by means of polar or randomly distributed flagella. Cells multiply by budding and asymmetric cell division. Rosette formation is rare. Gram negative. Phototrophically grown cells contain intracytoplasmic membranes of the lamellar type together with bacteriochlorophyll *a* and carotenoids belonging to the spirilloxanthin series.

TABLE 2. Levels of sequence similarity and evolutionary distances for 16S rRNAs and 16S rDNAs of new isolate MB312^T, Rhodopseudomonas marina, and other species of phototrophic bacteria and their relatives belonging to the alpha subclass of the Proteobacteria

Taxon	% Sequence similarity or evolutionary distance (K_{nuc} value) with^a:															
	Strain MB312^T	Rhodopseudomonas marina	Rhodopseudomonas acidophila^b	Rhodomicrobium vannielii	Rhodoplanes roseus	Rhodopseudomonas viridis	Rhodopseudomonas palustris	Bradyrhizobium japonicum	Methylobacterium organophilum	Rhodobacter capsulatus	Rhodobacter blasticus	Rhodovulum sulfidophilum	Erythrobacter longus	Rhodospirillum salexigens	Rhodospirillum rubrum	Rhodopila globiformis
Strain MB312^T		93.8	89.6	90.5	90.9	91.0	88.1	88.1	89.5	89.7	89.6	91.0	88.6	89.9	88.3	86.8
Rhodopseudomonas marina^b	0.0601		88.8	90.2	90.4	91.3	89.5	89.5	89.7	88.7	88.3	89.1	87.2	89.4	89.2	86.7
Rhodopseudomonas acidophila	0.0909	0.0996		89.3	91.9	91.1	91.6	92.1	90.2	86.5	86.4	86.4	86.3	89.6	86.0	84.3
Rhodomicrobium vannielii	0.0783	0.0798	0.1004		89.9	89.7	88.5	88.9	89.1	87.1	87.3	87.3	86.1	88.9	87.4	85.8
Rhodoplanes roseus	0.0814	0.0877	0.0727	0.0885		94.0	91.9	92.3	90.6	88.1	88.1	87.3	86.9	88.0	85.5	85.5
Rhodopseudomonas viridis	0.0877	0.0798	0.0798	0.0949	0.0538		98.4	90.7	89.9	87.0	87.0	86.8	86.8	86.8	86.8	86.6
Rhodopseudomonas palustris	0.0798	0.0798	0.0798	0.0949	0.0704	0.0134		90.0	90.3	86.1	86.1	86.2	86.3	86.2	86.1	84.3
Bradyrhizobium japonicum	0.0798	0.0957	0.0806	0.1028	0.0538	0.0806	0.0877		90.4	86.1	86.7	86.7	86.3	85.8	86.8	84.8
Methylobacterium organophilum	0.1036	0.0949	0.0751	0.1004	0.0656	0.0806	0.0806	0.0822		85.8	86.7	87.0	86.3	86.3	86.8	84.3
Rhodobacter capsulatus	0.1036	0.0941	0.0917	0.0996	0.0877	0.0917	0.1257	0.1209	0.0814		94.9	93.2	86.0	86.0	86.7	85.8
Rhodobacter blasticus	0.0972	0.1067	0.1273	0.1233	0.1146	0.1233	0.1209	0.1225	0.1344	0.0474		92.6	85.9	86.3	86.6	83.4
Rhodovulum sulfidophilum	0.1020	0.1091	0.1241	0.1209	0.1170	0.1265	0.1225	0.1202	0.1209	0.0617	0.0672		87.9	88.7	87.6	83.9
Erythrobacter longus	0.0972	0.1012	0.1257	0.1178	0.1138	0.1225	0.1281	0.1281	0.1312	0.1383	0.1344	0.1241		88.4	86.5	83.1
Rhodospirillum salexigens	0.0838	0.1178	0.1312	0.1336	0.1186	0.1186	0.1399	0.1328	0.1312	0.1217	0.1194	0.1138	0.1138		87.7	82.9
Rhodospirillum rubrum	0.1162	0.1012	0.1328	0.1281	0.1194	0.1241	0.1225	0.1233	0.1249	0.1383	0.1304	0.1202	0.1360	0.1257		86.4
Rhodopila globiformis	0.1296	0.1075	0.1534	0.1375	0.1241	0.1257	0.1549	0.1526	0.1375	0.1644	0.1605	0.1628	0.1621	0.1383	0.1320	

^{a} The values on the upper right are percentages of sequence similarity, and the values on the lower left are evolutionary distances (K_{nuc} values).

^{b} Two Rhodopseudomonas marina strains, strains DSM 2698^T and ATCC 35601, were used in the analyses.
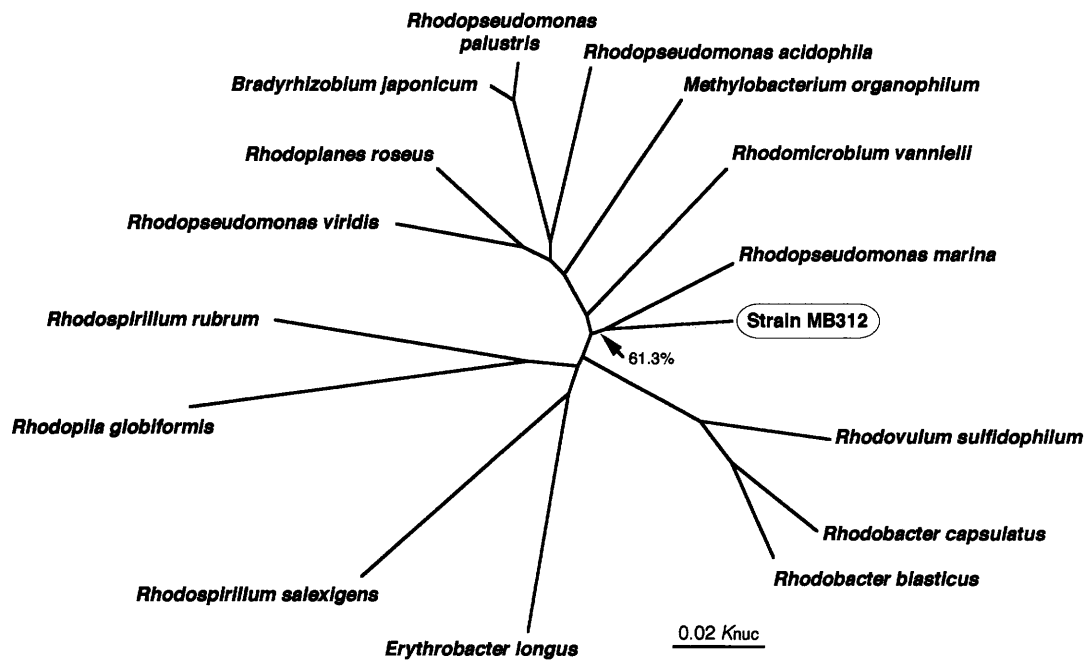
FIG. 3. Unrooted distance matrix tree showing phylogenetic affiliations of strain MB312^T, *Rhodopseudomonas marina*, and other species of phototrophic bacteria and their relatives in the alpha subclass of the *Proteobacteria*. A bootstrap confidence value (obtained by using the results of 1,000 bootstrapped trials) is given at the node which separates strain MB312^T and *Rhodopseudomonas marina* as a monophyletic fraction.

Facultative photoheterotrophs that grow anaerobically in the light or aerobically in the dark. Photosynthetic cultures are pink to red, while aerobic chemotrophic cultures are colorless. Mesophilic and halophilic. Optimal growth occurs at 25 to 35°C, at pH 6.9 to 7.5, and in the presence of 1 to 5% NaCl. Photoorganotrophy with simple organic compounds as carbon sources is the preferred mode of growth. Ubiquinone 10 and menaquinone 10 are the major quinones. The G+C contents of the DNAs range from 61.5 to 65.7 mol%. The phylogenetic position is in the alpha-2 subclass of the *Proteobacteria*.

The type species is *Rhodobium orientis*.

Description of *Rhodobium orientis* sp. nov. *Rhodobium orientis* (or.i.en'tis. L. part. adj. *orientis*, of the orient). The basic morphological fine-structure, physiological, and chemotaxonomic characteristics are the same as those described above for the genus. Cells are rod shaped, 0.7 to 0.9 μm wide, and 1.5 to

TABLE 3. Levels of genomic DNA relatedness among the new isolates and *Rhodopseudomonas marina* strains

Organism	G+C content (mol%)	% Hybridization with labeled DNA from:	
		Strain MB312^T	Strain DSM 2698^T
New isolates			
MB303	65.7	72	16
MB312^T	65.3	100	14
MB314	65.2	104	14
Rhodopseudomonas marina strains			
DSM 2698^T	63.4 (63.6)^a	10	100
DSM 2780	62.4 (61.5)^a	10	91
E2-A	63.8	11	82
ATCC 35601^b	64.1 (64.1)^c	12	74

^{a} The value in parentheses is from reference 18.
^{b} *Rhodopseudomonas marina* subsp. *agilis*.
^{c} The value in parentheses is from reference 27.

3.2 μm long. The absorption maxima of cell extracts are at 377, 468, 500, 530, 591, 802, and 870 nm. Biotin and *p*-aminobenzoic acid are required as growth factors, and growth is stimulated considerably by 0.05% yeast extract. Optimal growth occurs at 30 to 35°C, at pH 7.0 to 7.5, and in the presence of 4 to 5% NaCl; growth occurs in the presence of 2 to 8% NaCl. Anaerobic growth in the dark with nitrate as a terminal electron acceptor is possible; denitrification occurs. Good carbon sources are acetate, lactate, pyruvate, succinate, fumarate, malate, fructose, glucose, peptone, and yeast extract. Moderate growth occurs with butyrate, valerate, caproate, xylose, mannitol, and sorbitol. Formate, propionate, caprylate, citrate, tartrate, benzoate, arabinose, galactose, mannose, glycerol, methanol, ethanol, and Casamino Acids are not utilized. Photoautotrophic growth with thiosulfate as the electron donor occurs in the presence of 0.01% yeast extract. Ammonium salts are used as nitrogen sources. Sulfate is assimilated. The G+C contents of the DNAs range from 65.2 to 65.7 mol% (as determined by HPLC).

Habitats: seawater pools and similar marine environments.

The type strain is strain MB312, which has been deposited in the Japan Collection of Microorganisms, RIKEN, Wako, Japan, as *Rhodobium orientis* JCM 9337.

Description of *Rhodobium marinum* comb. nov. *Rhodobium marinum* (*Rhodopseudomonas marina* Imhoff 1983) (ma.ri' num. L. adj. *marinum*, of the sea, marine). The characteristics described below are based on information obtained from previous reports (18, 27) and in this study. The basic morphological fine-structure, physiological, and chemotaxonomic characteristics are the same as those described above for the genus. Cells are ovoid to rod shaped, 0.7 to 0.9 μm wide, and 1.0 to 2.5 μm long. Rosette formation is not observed. The absorption maxima of living cells or cell extracts are at 375, 483, 516, 533, 590, 803, and 883 nm, and there is a low absorption maximum at around 800 nm. Growth factors are required. Optimal growth occurs at 25 to 30°C, at pH 6.9 to 7.1, and in the

TABLE 4. Differential characteristics of *Rhodobium orientis* gen. nov., sp. nov., *Rhodobium marinum* comb. nov., and some other budding phototrophic bacteria[a]

Characteristic	*Rhodobium orientis*	*Rhodobium marinum*	*Rhodoplanes roseus*	*Rhodoplanes elegans*	*Rhodopseudomonas palustris*	*Rhodopseudomonas acidophila*
Cell diam (μm)	0.7–0.9	0.7–0.9	1.0	0.8–1.0	0.6–0.8	1.0–1.2
Type of budding	Sessile	Sessile	Sessile	Tube	Tube	Sessile
Rosette formation	−/+	−	−	+	+	−
Color(s) of cultures	Pink, red	Pink, red	Pink	Pink	Red	Red
Low absorption maximum at ca. 800 nm	−	+	−	−	−	−
Salt requirement	+	+	−	−	−	−
Acidophilic	−	−	−	−	−	+
Denitrification	+	−	+	+	−/+	−
Fermentative growth on fructose	−	+	−	−	−	−
Vitamin(s) required	*p*-ABA, biotin	Complex	Biotin	*p*-ABA, biotin	*p*-ABA (biotin)	None
Utilization of:						
Benzoate	−	−	−	−	+	−
Citrate	−	−/+	+	+	+	−
Formate	−	+	−	−	−	−
Tartrate	−	−	+	+	−	−
Glucose	+	+	−	−	−	−
Thiosulfate	+	−	+	+	+	−
Major quinone(s)	Q-10, MK-10	Q-10, MK-10	Q-10, RQ-10	Q-10, RQ-10	Q-10	Q-10, MK-10, RQ-10
G+C content (mol%)	65.2–65.7	61.5–64.1	66.5	69.6–69.7	64.1–66.3	62.2–66.8
Habitat	Seawater	Seawater	Freshwater	Freshwater	Freshwater	Freshwater

[a] +, positive; −, negative; −/+, most strains are negative but some strains are positive; *p*-ABA, *p*-aminobenzoic acid; (biotin), biotin is required by some strains; Q-10, ubiquinone 10; MK-10, menaquinones 10; RQ-10, rhodoquinone 10. Information was obtained from references 11, 17, 18, 22, and 35.

presence of 1 to 5% NaCl. Fermentative growth in the dark is possible at the expense of fructose. Denitrification does not occur. All or most strains use the following compounds as electron donors and carbon sources: acetate, propionate, butyrate, valerate, caproate, lactate, pyruvate, succinate, fumarate, malate, ascorbate, fructose, glucose, sucrose, mannitol, sorbitol, ethanol, propanol, and Casamino Acids. Weak growth occurs with formate, galactose, and glycerol. Benzoate, tartrate, and methanol are not utilized. Sulfide is used as an electron donor for phototrophic growth. No photoautotrophic growth occurs with thiosulfate as the electron donor. Ammonium salts are used as nitrogen sources. Sulfate is assimilated. The G+C contents of the DNAs range from 62.4 to 64.1 mol% (as determined by HPLC) or from 61.5 to 64.1 mol% (as determined by the thermal denaturation method).

Habitats: marine and moderately hypersaline waters and marine coastal sediments.

The type strain is DSM 2698 (= ATCC 35675 = Imhoff BN 126).

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