Rhodoplanes gen. nov., a New Genus of Phototrophic Bacteria Including *Rhodopseudomonas rosea* as *Rhodoplanes roseus* comb. nov. and *Rhodoplanes elegans* sp. nov.

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Two new strains (AS130 and AS140) of phototrophic purple nonsulfur bacteria isolated from activated sludge were characterized and compared with *Rhodopseudomonas rosea* and some other species of the genus *Rhodopseudomonas*. The new isolates produced pink photosynthetic cultures, had rod-shaped cells that divided by budding, and formed intracytoplasmic membranes of the lamellar type together with bacteriochlorophyll *a* and carotenoids of the normal spirilloxanthin series. They were also characterized by their capacity for complete denitrification and their production of both ubiquinone-10 and rhodoquinone-10 as major quinones. The isolates were phenotypically most similar to *R. rosea* but exhibited low levels of genomic DNA hybridization to this species and to all other *Rhodopseudomonas* species compared. Phylogenetic analyses on the basis of PCR-amplified 16S rRNA gene sequences showed that our isolates and *R. rosea* formed a cluster distinct from other members of the genus *Rhodopseudomonas*. The phenotypic, genotypic, and phylogenetic data show that the new isolates and *R. rosea* should be placed in a new single genus rather than included in the genus *Rhodopseudomonas*. Thus, we propose to transfer *R. rosea* to a new genus, *Rhodoplanes*, as *Rhodoplanes roseus* gen. nov., comb. nov. (type species) and to designate strains AS130 and AS140 a new species, *Rhodoplanes elegans* sp. nov.

Species of the genus *Rhodopseudomonas*, which constitute a major group of phototrophic purple nonsulfur bacteria belonging to the alpha subclass of the class *Proteobacteria*, are characterized by having a budding cell morphology and forming intracytoplasmic membranes of the lamellar type (16, 32). In spite of their morphological and structural similarities, *Rhodopseudomonas* species are phylogenetically quite diverse. Comparisons of rRNA, i.e., DNA-rRNA hybridization and 16S rRNA cataloging and sequencing, have demonstrated that these bacteria belong to several different lines of descent within the alpha-2 subclass of the *Proteobacteria* (2, 33, 34). Exceptionally, *Rhodopseudomonas blastica* is a member of the alpha-3 subclass, closely related to *Rhodobacter* species (14, 18), and this situation has led to the proposal for transfer of *R. blastica* to the genus *Rhodobacter* (18). The phenotypic and chemotaxonomic heterogeneity in the genus *Rhodopseudomonas* has been summarized by Imhoff et al. (16).

As suggested by their phylogenetic heterogeneity, the color of phototrophic cell suspensions of *Rhodopseudomonas* strains differs from species to species, depending upon bacteriochlorophyll types and carotenoid compositions. Thus, this trait is of value for the classification and identification of these species. Mass cultures of the type species *Rhodopseudomonas palustris* and *Rhodopseudomonas acidophila* (26) are red to brownish red; those of the species with bacteriochlorophyll *b*, *Rhodopseudomonas viridis* (3) and *Rhodopseudomonas sulfoviridis* (19), are green; and those of the marine species *Rhodopseudomonas marina* (15) and the recently described freshwater species *Rhodopseudomonas rosea* (17) are pink.

Activated sludge and other wastewater environments are constant sources of the purple nonsulfur bacteria (10, 12, 31). During the course of ecological studies on phototrophic bacteria in activated sludge, we isolated some strains of facultative

phototrophs that produced pink cultures and a cell morphology typical of the genus *Rhodopseudomonas*. The new isolates most closely resembled *R. rosea* with respect to general morphology, pigmentation, and natural habitats. In the present study, the new pink isolates were characterized phenotypically, genotypically, and phylogenetically and compared with *R. rosea* and some other known members of the genus *Rhodopseudomonas*. In this report, we propose to transfer *R. rosea* to a new genus, *Rhodoplanes*, and to designate a new species of this genus for our isolates.

MATERIALS AND METHODS

Bacterial strains and cultivation. Two pink-pigmented strains (designated AS130 and AS140) of the purple nonsulfur bacteria isolated from activated sludge were studied. *R. rosea* 941^T (T = type strain) was kindly provided by C. G. Harfoot, University of Waikato, Hamilton, New Zealand. *R. acidophila* ATCC 25092^T, *R. blastica* ATCC 33485^T, *R. marina* DSM 2698^T, *R. palustris* ATCC 17001^T, RS22, SA37, SA42, Tb, and HLS2, and *R. viridis* ATCC 19567^T were also used for comparison. The strains with ATCC and DSM numbers were obtained, respectively, from the American Type Culture Collection, Rockville, Md., and from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. The *R. palustris* strains other than the type strain were isolated from various environments, including sewage, waste sludge, pond water, and soil (10, 11). A chemically defined medium (designated PYS medium) which consisted of mineral base RM2 (pH 6.8) (10), 20 mM pyruvate (filter sterilized) as the sole carbon source, and 0.1% yeast extract as the growth factor was used for cultivation of the organisms to be subjected to various tests. In some cases, MYCA medium (9) was used for growth of organisms other than our new isolates and *R. rosea*. The culture media were modified by lowering the pH to 5.5 for *R. acidophila* and by adding 2.5% NaCl for *R. marina*. Routine cultivation was done anaerobically in screw-capped test tubes or bottles filled with medium;

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FIG. 1. Phase-contrast micrographs showing cell morphology of strain AS130 (A), *R. rosea* 941^T (B), and *R. palustris* ATCC 17001^T (C). Bar, 10 μ m.

incubation was at 30°C and 5,000 lx of incandescent illumination. For genetic studies, 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.

Phenotypic characterization. Morphology and related properties were studied under an Olympus phase-contrast microscope and a JEOL transmission electron microscope. The photosynthetic membrane arrangement of cells was determined by electron microscopy of ultrathin sections as described previously (9). Absorption spectra of intact cells or cell extracts were measured with a Hitachi U-2000 spectrophotometer. Aerobic growth in darkness was determined in 100-ml flasks containing 10 ml of PYS medium on a reciprocal shaker. Anaerobic growth by nitrate respiration was determined in screw-capped test tubes completely filled with PYS medium supplemented with 20 mM potassium nitrate. N₂ gas production by complete denitrification was observed in these test tubes, with Durham tubes compared with a negative control without nitrate. Tests for photoassimilation of organic substrates were performed in screw-capped test tubes containing mineral base medium RM2 supplemented with a neutralized, filter-sterilized, organic compound as the carbon source and 0.01% yeast extract as the growth factor. Photoautotrophic growth was determined with the mineral medium supplemented with either 0.5 mM sodium sulfide or 2 mM sodium thiosulfate as the electron donor, 20 mM sodium bicarbonate (filter sterilized), and 0.01% yeast extract. In all growth response tests, growth was monitored turbidometrically with an ANA-75 spectrophotometer (Tokyo Photoelectric Co., Tokyo, Japan), and the final reading was taken after 2 weeks of incubation. All other physiological and biochemical tests were performed as described elsewhere (10, 11). Quinones were extracted with an organic solvent mixture, purified by thin-layer chromatography, and analyzed by high-performance liquid chromatography (HPLC) (8).

DNA base composition and DNA-DNA hybridization.

Genomic DNA was extracted and purified according to the method of Marmur (23). DNA base composition was determined by HPLC of nuclease P1 hydrolysates of DNA with external nucleotide standards as previously reported (9). Genomic DNA reassociation studies were performed by the quantitative dot blot hybridization method with photobiotin labeling and colorimetric detection (4, 9).

PCR amplification and sequencing of 16S rRNA gene. 16S ribosomal DNA (rDNA) fragments that corresponded to positions 8 to 1510 of *Escherichia coli* 16S rRNA (1) were amplified by PCR from crude cell lysates and sequenced directly by the cycle sequencing method modified for automated fluorescence detection. The reactions were analyzed with a Pharmacia laser fluorescent DNA sequencer. Detailed information concerning the PCR and sequencing procedures used has been given previously (7, 13).

Phylogenetic analysis. Compiling of sequence data and calculation of levels of binary sequence similarities were performed with an Apple Macintosh personal computer with the GENETYX program package (Software Development Co., Tokyo, Japan). The CLUSTAL V program (6) was used for multiple sequence alignment, calculation of nucleotide substitution rates (K_{nuc}) (20), construction of a neighbor-joining phylogenetic tree (27), and statistical evaluation of the tree topology by bootstrap analysis (5). Alignment positions including gaps and/or unidentified bases were not taken into consideration for the calculations.

Nucleotide sequence accession numbers. The 16S rDNA sequences determined in the present study have been deposited in the DDBJ, EMBL, GSDB, and NCBI nucleotide sequence databases under accession numbers D25311 to D25314.

RESULTS

Isolation. A sample of activated sludge was taken from the main purification stage of a sewage treatment plant in Tokyo, Japan, and examined promptly upon return to the laboratory.

FIG. 2. Electron micrographs of negatively stained cells (A) and ultrathin sections (B) of strain AS130, showing the mode of flagellation and intracytoplasmic membranes (arrow), respectively. Bars, 1 μm (A) and 0.5 μm (B).

FIG. 3. Absorption spectrum of the cell extract of strain AS130.

Phototrophic purple nonsulfur bacteria were quantitatively isolated from the sludge by using the agar plating technique with the GasPak anaerobic system according to previously described procedures (10), in which malate and Casamino Acids were used as carbon sources. After 10 days of incubation at 28°C in the light, the sludge gave 1.3×10^5 CFU of the purple nonsulfur bacteria per mg (dry weight), a count similar to those previously recorded for activated sludge (10, 31). Most of the colonies detected were red or brown and appeared to resemble *R. palustris*, *R. blastica*, and *Rhodobacter sphaeroides* on the basis of their pigmentation and cell morphology observed under a phase-contrast microscope. Among the colonies detected, there were a few tiny pink colonies which interested us in their taxonomic aspects. Thus, two representatives of the single pink colonies were picked from the plate for standard purification and were designated AS130 and AS140.

Morphology and ultrastructure. Strains AS130 and AS140 had gram-negative, rod-shaped cells measuring 0.8 to 1.0 μm in width and 2.0 to 3.0 μm in length (Fig. 1A). The cells divided asymmetrically by budding with a division tube which occurred between the mother and daughter cells, and they formed rosette-like clusters in older cultures. In these respects, the isolates were similar to *R. palustris* (Fig. 1C) and *R. viridis*, but with respect to cell size they most closely resembled *R. rosea* (Fig. 1B) among members of this genus. Both of the new isolates were motile by means of polar, subpolar, or randomly distributed flagella; the majority of cells had two flagella located in subpolar areas (Fig. 2A). Electron microscopy of ultrathin sections revealed that phototrophically grown cells of the isolates formed intracytoplasmic membranes of the lamellar type typical of the genus *Rhodopseudomonas* (Fig. 2B).

Photopigments. The isolates produced pink, lens-shaped colonies in agar media and pink cell suspensions in liquid media when grown under anaerobic phototrophic growth conditions, like *R. rosea*. The cell extract of strain AS130 had absorption maxima at 373, 466, 491, 527, 592, 799, and 855 nm, indicating the presence of bacteriochlorophyll *a* and carotenoids of the normal spirilloxanthin series (Fig. 3). Similar absorption spectra were recorded for strain AS140 and *R. rosea* 941^T (data not shown). Our isolates as well as *R. rosea* were colorless when grown aerobically in darkness.

Growth. Strains AS130 and AS140 were facultatively aerobic phototrophs that were able to grow anaerobically in the light or aerobically in darkness at full atmospheric oxygen tension. Of particular interest was that the new isolates had the ability to grow anaerobically in darkness with nitrate as the terminal electron acceptor with concomitant production of nitrogen gas, thereby confirming their capacity for complete denitrification.

Anaerobic growth in darkness by nitrate respiration was also observed for *R. rosea* but not for any other *Rhodopseudomonas* strains tested. Comparative growth rates and cell yields of strain AS130 and *R. rosea* 941^T under different growth conditions are shown in Table 1. The doubling times of both test strains under phototrophic growth conditions were more than 6 h, suggesting that they grow phototrophically at relatively slow rates compared with other known species of the genus *Rhodopseudomonas*.

Physiological and biochemical characteristics. The isolates showed a strong tendency for photoorganotrophy with simple organic compounds as electron donor and carbon sources, and they resembled *R. rosea* in photoassimilation patterns with organic substrates. Good carbon sources were acetate, lactate, pyruvate, succinate, fumarate, malate, and yeast extract. Moderate growth occurred with propionate, butyrate, valerate, caproate, citrate, tartrate, Casamino Acids, and peptone. No significant growth was found with formate, glycolate, malonate, benzoate, fatty acids containing six or more carbon atoms, arabinose, xylose, rhamnose, fructose, glucose, mannose, galactose, dulcitol, mannitol, sorbitol, glycerol, methanol, ethanol, propanol, alanine, asparagine, aspartate, glutamate, or leucine. The isolates did not appear to utilize sulfide as the electron donor, because they grew more slowly in PYS medium supplemented with 0.1 mM sulfide than in the medium without sulfide. Photoautotrophic growth occurred with 2 mM thiosulfate but not with 0.5 mM sulfide as the electron donor in the presence of 0.01% yeast extract.

Other phenotypic characteristics which our isolates shared were as follows. The optimal temperature for growth was 30 to 35°C. The pH range for growth was 6.0 to 8.5, with a pH optimum of 7.0. Growth occurred in the absence of added NaCl. No or little growth was found at NaCl concentrations of 1% or more. Thiamine and *p*-aminobenzoic acid were required as growth factors, but growth was stimulated considerably by the addition of 0.01% yeast extract. Sulfate was assimilated as the sulfur source. The capacity for nitrogen fixation seemed to be absent, as suggested by the observation that no hydrogen gas was produced in ammonium-limited PYS medium under phototrophic growth conditions. Hydrolysis of gelatin and Tween 80 was negative.

We reexamined *R. rosea* 941^T for the physiological and biochemical properties noted above and found some differences between the results of Janssen and Harfoot (17) and ours. The original report for *R. rosea* mentioned that this organism was able to utilize glucose and glycerol as carbon sources, but our attempts to demonstrate this gave negative results. Also, the previous study showed that *R. rosea* did not photoassimilate valerate, while we found weak growth with valerate. Moreover, we noted that *R. rosea* failed to grow at a pH of less than 6.0, which differs from the results of Janssen and Harfoot (17), who reported it to grow at pH 5.5. The results of comparative phenotypic studies showed dissimilarities between our isolates and *R. rosea* in such physiological properties as growth at pH 6.0, vitamin requirement, and caproate utilization.

Quinone composition and genomic DNA relatedness. The quinone profiles of the isolates compared with that of *R. rosea* are shown in Table 2. Strains AS130 and AS140 and *R. rosea* 941^T produced both ubiquinones and rhodoquinones as primary quinone components, a chemotaxonomic feature distinct from those of other *Rhodopseudomonas* species. HPLC experiments showed that ubiquinone-10 and rhodoquinone-10 constituted more than 90% of the total ubiquinone and rhodoquinone fractions, respectively, in all these strains.

Table 2 also shows the DNA base compositions of and

TABLE 1. Growth responses to different environmental conditions by strain AS130 and *R. rosea*

Growth conditions	Addition of nitrate	Strain AS130		<i>R. rosea</i> 941 ^T	
		<i>t</i> _D ^a (h)	Relative cell yield ^b (%)	<i>t</i> _D	Relative cell yield (%)
Anaerobic, light	—	6.5	100	10	100
	+	7.0	100	11	63
Aerobic, dark	—	6.3	74	10	64
Anaerobic, dark	+	31	13	22	44

^a Doubling time.^b Measured turbidometrically.

genomic DNA relatedness among the test organisms. The G+C contents of the genomic DNAs of strains AS130 and AS140 were, respectively, 69.7 and 69.6 mol%, which are considerably higher than those recorded for *R. rosea* 941^T and other reference organisms. The two new isolates were highly related to each other but showed low levels of DNA-DNA hybridization (less than 40%) to *R. rosea* 941^T. Much lower levels of genomic DNA relatedness were found between our isolates and all other test strains of *Rhodopseudomonas* species.

Phylogenetic relationships. The continuous nucleotide sequences of 16S rDNA fragments amplified from one of the new isolates (strain AS130), *R. palustris*, *R. rosea*, and *R. viridis* were determined. The sequences determined ranged from 1,407 to 1,413 residues, representing 95% of the entire 16S rRNA gene. Strain AS130 differed from the other test organisms in having a 16S rRNA secondary structure with two-pair-shorter stems around position 80 (*E. coli* numbering). The 16S rDNA sequences of these organisms were compared with the sequences from representative species of phototrophs and their nonphototrophic relatives which belong to the alpha subclass of the *Proteobacteria*. Table 3 shows levels of sequence similarity for each pair of the organisms and evolutionary distance (K_{nuc}) values calculated for the alignable 1,238 positions of all sequences of the entire set. Strain AS130 was closely related to *R. rosea* at a similarity level of 98.3% (K_{nuc} = 0.0129) but showed much lower affinities to all other *Rhodopseudomonas* species compared (<95% similarity).

Almost complete sequences of the 16S rRNAs of *R. palustris* GH and DSM 123^T have been reported previously by Woese

(GenBank accession number M59068) and Orso et al. (24) (GenBank accession number L11664), respectively. As expected, the 16S rRNA gene sequence of *R. palustris* determined in this study was almost identical to the sequence for strain GH, except for a number of undetermined positions included in the latter sequence. On the other hand, it was strange that the sequence for ATCC 17001^T that we determined differed from the sequence for DSM 123^T in 38 positions of 1,411 residues compared, corresponding to 97.3% similarity. This similarity level is too low to be due to different sequencing methods used or to sequencing errors. The previous data on *R. palustris* might have resulted from mislabeling of the strain or sequencing sample.

On the basis of the corrected distance values shown in Table 3, a neighbor-joining phylogenetic tree was reconstructed (Fig. 4). Strain AS130 and *R. rosea* formed a tight cluster distinct from the type species *R. palustris* and all other species of *Rhodopseudomonas* compared. The monophyletic nature of this cluster was supported by bootstrapping in 100% of the 1,000 trees generated. The *R. rosea* cluster was linked to *R. viridis* as the phylogenetic neighbor, and the cluster including *R. viridis* as the sister group also proved to be stable (90.3% support by bootstrapping). The topology of the tree confirmed phylogenetic divergence of *Rhodopseudomonas* species within the alpha-2 subclass of the *Proteobacteria*.

DISCUSSION

The results of the present study have provided taxonomic and phylogenetic information concerning the new pink-pigmented isolates of the phototrophic bacteria and have expanded the previous results obtained with *R. rosea* (17). Our results show that the new isolates make up a phenotypically and genotypically coherent group that falls into a single species. They are facultatively photoheterotrophic, have a budding type of cell division, and produce photosynthetic membrane systems of the lamellar type together with bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series, features conforming to the definition of the genus *Rhodopseudomonas* (16, 32). Phenotypically, the new isolates most closely resemble *R. rosea* among species of this genus. The important characteristics that are common between the isolates and *R. rosea* are cell size (0.8 to 1.0 μm in width), pink pigmentation, relatively slow phototrophic growth, denitrifica-

TABLE 2. Quinone profiles of and genomic DNA relatedness between the isolates and known species of the genus *Rhodopseudomonas*

Organism	Major quinone homolog ^a			G+C (mol%) ^b	% Hybridization of labeled DNA from:			
	Q	RQ	MK		AS130	AS140	<i>R. rosea</i> 941 ^T	<i>R. palustris</i> ATCC 17001 ^T
Strain AS130	10	10		69.7	100	105	39	14
Strain AS140	10	10		69.6	98	100		
<i>R. rosea</i> 941 ^T	10	10		66.8	34	40	100	11
<i>R. palustris</i> ATCC 17001 ^T	10			65.0	11	10	11	100
<i>R. palustris</i> RS22	10			64.9	13			
<i>R. palustris</i> SA37	10			64.6	11			
<i>R. palustris</i> Tb	10			64.6	9			
<i>R. palustris</i> HLS2	10			64.8	11			
<i>R. viridis</i> ATCC 19567 ^T	9		9	66.5	17	15	20	
<i>R. acidophila</i> ATCC 25092 ^T	10	10	10	65.1	9	8	12	
<i>R. marina</i> DSM 2698 ^T	10		10	63.6	7	8	6	
<i>R. blastica</i> ATCC 33485 ^T	10			65.5	5	5	6	

^a Q, ubiquinone; RQ, rholoquinone; MK, menaquinone. Information for the new isolates and *R. rosea* is from this study, and that for the other organisms is from references 8 and 11.

^b Information for the new isolates and *R. rosea* is from this study, and that for the other organisms is from references 11 and 15.

TABLE 3. Levels of sequence similarities and evolutionary distances for 16S rRNAs and 16S rDNAs of the new isolate, *R. rosea*, and some other species of phototrophic bacteria and their nonphototrophic relatives belonging to the alpha subclass of *Proteobacteria*^a

No.	Organism	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
		Level of sequence similarity (%) or evolutionary distance (K_{unc})																	
1	Strain AS130	98.3	94.2	92.6	92.6	92.8	93.0	92.6	91.9	90.5	90.9	90.9	87.7	87.3	88.1	86.8	87.2	86.9	85.9
2	<i>Rhodopseudomonas rosea</i>	0.0129	0.0129	92.6	92.6	92.3	92.3	92.0	91.9	89.9	90.6	90.6	88.1	87.0	87.3	86.9	86.8	86.2	85.5
3	<i>Rhodopseudomonas viridis</i>	0.0525	0.0549	90.0	90.0	90.7	90.7	90.9	91.1	89.7	89.9	89.9	87.0	87.0	86.8	86.1	88.0	86.8	86.6
4	<i>Rhodopseudomonas palustris</i>	0.0654	0.0719	0.0897	0.0897	97.5	98.4	96.8	91.6	88.5	90.3	89.3	86.1	86.1	86.2	86.3	86.2	86.1	84.3
5	<i>Blastobacter denitrificans</i>	0.0622	0.0670	0.0800	0.0210	98.5	98.5	96.7	91.8	89.0	90.8	89.2	86.6	87.0	86.5	85.8	85.8	86.4	84.7
6	<i>Bradyrhizobium japonicum</i>	0.0630	0.0679	0.0816	0.0153	0.0105	98.0	98.0	92.1	88.9	90.4	89.6	86.8	86.9	86.7	86.3	85.8	86.6	84.8
7	<i>Azotobacter vinelandii</i>	0.0654	0.0695	0.0767	0.0275	0.0242	0.0170	0.0719	92.3	89.4	90.8	89.5	86.1	86.8	86.1	86.7	86.2	87.1	85.0
8	<i>Rhodopseudomonas acidiphila</i>	0.0759	0.0743	0.0800	0.0792	0.0767	0.0719	0.0711	0.0872	89.3	90.2	89.1	86.5	86.7	86.4	86.3	86.6	86.0	84.3
9	<i>Rhodospirillum rubrum</i>	0.0784	0.0840	0.0872	0.0985	0.0945	0.0945	0.0945	0.0969	88.7	89.1	89.6	85.8	86.7	87.0	85.3	86.3	86.7	85.8
10	<i>Methylobacterium organophilum</i>	0.0848	0.0872	0.0913	0.0792	0.0759	0.0767	0.0711	0.0872	0.0961	0.1010	0.1058	88.7	88.4	89.1	87.4	89.9	89.2	87.6
11	<i>Rhodopseudomonas marina</i>	0.0840	0.0864	0.0816	0.0945	0.0945	0.0945	0.0945	0.0969	0.0899	0.1010	0.1058	88.7	88.4	89.1	87.4	89.9	89.2	87.6
12	<i>Rhodobacter capsulatus</i>	0.1171	0.1163	0.1244	0.1276	0.1220	0.1236	0.1236	0.1284	0.1276	0.1349	0.1066	0.0444	0.0614	92.6	85.9	87.8	86.6	83.9
13	<i>Rhodopseudomonas blautia</i>	0.1147	0.1171	0.1131	0.1284	0.1236	0.1236	0.1236	0.1220	0.1212	0.1220	0.1066	0.0444	0.0614	92.6	85.9	87.8	86.6	83.9
14	<i>Rhodovulum sulfidophilum</i>	0.1082	0.1131	0.1187	0.1228	0.1187	0.1204	0.1220	0.1171	0.1212	0.1212	0.1026	0.0598	0.0614	92.6	85.9	87.8	86.6	83.9
15	<i>Erythrobacter longus</i>	0.1195	0.1171	0.1204	0.1268	0.1276	0.1268	0.1268	0.1252	0.1284	0.1317	0.1026	0.0598	0.0614	92.6	85.9	87.8	86.6	83.9
16	<i>Rhodospirillum salexigens</i>	0.1179	0.1163	0.1099	0.1381	0.1284	0.1300	0.1260	0.1260	0.1018	0.1284	0.1058	0.1228	0.1236	0.1171	0.1115	88.4	86.5	82.9
17	<i>Rhodospirillum rubrum</i>	0.1179	0.1228	0.1220	0.1220	0.1236	0.1236	0.1195	0.1317	0.1179	0.1244	0.1090	0.1276	0.1236	0.1187	0.1292	0.1195	87.7	86.4
18	<i>Rhodospila globiformis</i>	0.1341	0.1341	0.1276	0.1543	0.1494	0.1527	0.1502	0.1527	0.1365	0.1389	0.1276	0.1648	0.1599	0.1640	0.1559	0.1373	0.1292	

^a Accession numbers of sequences used for phylogenetic analysis: 1, D25311; 2, D25313; 3, D25314; 4, D25312; 5, X66025; 6, X66024; 7, M65248; 8, M34128; 9, M34127; 10, unpublished (Hiraishi); 11, M27534; 12, D16428; 13, D16429; 14, D16423; 15, M59062; 16, M59070; 17, M32020; 18, M59066. Sequence similarities are in the top right portion, and evolutionary distances are in the bottom left portion.

tion, photoassimilation of tartrate, utilization of thiosulfate but not sulfide, and production of ubiquinone-10 and rhodoquinone-10 as major quinones. By the combination of these features, our isolates and *R. rosea* are clearly differentiated from all other species of the genus *Rhodopseudomonas*.

To date, the capacity for complete denitrification has been found in some strains of *Rhodobacter sphaeroides* (25, 28, 30) and *R. palustris* (21) among the phototrophic bacteria. In the present study, however, we did not find denitrifying growth in any test strains of *R. palustris*. In view of the fact that the taxonomic properties of the denitrifying strains of *R. palustris* were not described in detail (21), it would be interesting to study the taxonomic relationships of these strains to our isolates and *R. rosea*.

Although the new isolates share a number of characteristics with *R. rosea*, as described above, the former group differs clearly from the latter in the following characteristics: budding types (tube or sessile), rosette formation, growth at pH 6.0, caproate utilization, vitamin requirement, and DNA base composition. In addition, the results of genomic DNA hybridization studies demonstrate that the isolates are genetically different from *R. rosea* at the species level and have more distant genealogical relationships to all other *Rhodopseudomonas* species compared. These phenotypic and genotypic data are sufficient to justify classification of our isolates as a new species related to *R. rosea*. In this study we did not examine some species of the genus *Rhodopseudomonas*, such as *R. julia* (22) and *R. sulfobacter* (19), from genotypic viewpoints. However, it is easy to differentiate the new isolates from these species by a number of phenotypic differences and by their natural habitats.

According to the phenotypic criteria for the current taxonomy of the purple nonsulfur bacteria (16, 32), our isolates may be classified as a new species of the genus *Rhodopseudomonas*. However, attention should be paid to the fact that the genus *Rhodopseudomonas* itself is an assemblage of phylogenetically heterogeneous species. The present study and other phylogenetic studies by 16S rRNA cataloging (29, 34) and sequencing (24, 33, 35) demonstrate that the type species *R. palustris* is more closely related to some chemotrophic taxa, i.e., *Azotobacter vinelandii*, *Bradyrhizobium japonicum*, *Blastobacter denitrificans*, and *Nitrobacter winogradskyi*, than to any of the other *Rhodopseudomonas* species for which sequence formation is available. Our data also show that the new isolates and *R. rosea* belong to a lineage distinct from that of *R. palustris* and all other *Rhodopseudomonas* species compared. Accordingly, the established species of the genus *Rhodopseudomonas* can no longer be placed in a single genus on phylogenetic grounds.

In view of the unsatisfactory taxonomic status of the genus *Rhodopseudomonas* and the resultant need for improvement of its taxonomy, it is more appropriate to incorporate our isolates together with *R. rosea* into a new genus than to place them in the genus *Rhodopseudomonas*. The present results have demonstrated that *R. viridis* is the closest relative of the phylogenetic group encompassing our isolates and *R. rosea*. However, phenotypic similarities and the level of sequence homology between the former species and the latter group are low enough to warrant different generic allocations. Here we propose to transfer *R. rosea* to the new genus *Rhodoplanes* as *Rhodoplanes roseus* gen. nov., comb. nov. (type species) and to designate strains AS130 and AS140 members of a new species of this genus, for which we propose the name *Rhodoplanes elegans* sp. nov. Diagnostic characteristics of members of the new genus *Rhodoplanes* and some species of the genus *Rhodopseudomonas* are summarized in Table 4.

The available phylogenetic and chemotaxonomic informa-

FIG. 4. Unrooted distance matrix tree showing phylogenetic affiliations of strain AS130, *R. rosea*, and other species of phototrophic bacteria and their nonphototrophic relatives within the alpha subclass of the *Proteobacteria*. For organism names and accession numbers for sequences used, see Table 3.

tion strongly suggests the need for rearrangement and nomenclatural revisions of *Rhodopseudomonas* species within the alpha-2 subclass of the *Proteobacteria*. Our proposal for the transfer of *R. rosea* to the new genus *Rhodoplanes* is the first step in this direction. Probably all other *Rhodopseudomonas* species, except the type species *R. palustris*, would be split up into different new genera via further systematic studies in the near future. Following this and taxonomic revisions for related chemotrophic taxa, e.g., *Blastobacter*, *Bradyrhizobium*, and *Nitrobacter*, the description of the genus *Rhodopseudomonas* should be emended.

Description of *Rhodoplanes* gen. nov. *Rhodoplanes* (Rho.do.plan'es. Gr. n. *rhodos*, rose; Gr. n. *planes*, a wanderer; Gr. n. *Rhodoplanes*, a red wanderer). Cells are gram-negative rods. Multiply by budding and asymmetric cell division. Motile by means of polar, subpolar, or randomly distributed flagella. Facultative phototrophs growing anaerobically in the light or aerobically in darkness. Anaerobic growth in darkness by nitrate respiration is also possible; denitrification positive. Photosynthetic cultures are pink, while aerobic chemotrophic cultures are colorless. Phototrophically grown cells contain intracytoplasmic membranes of the lamellar type together with bacteriochlorophyll *a* and carotenoids of the normal spirilloxanthin series. Mesophilic, neutrophic, and nonhalophilic. Growth in the presence of NaCl at a concentration of 1% or

more is inhibited. Photoorganotrophy with simple organic compounds is the preferred mode of growth. Photoautotrophic growth occurs with thiosulfate as the electron donor in the presence of 0.01% yeast extract. Sulfide may not be utilized. Hydrolysis of gelatin and Tween 80 is negative. Ubiquinone-10 and rhodoquinone-10 are present as major quinones. The G+C content of DNA ranges from 66 to 70 mol%. Phylogenetic position is the alpha-2 subclass of *Proteobacteria*.

Habitats: freshwater and wastewater environments.

Type species: *Rhodoplanes roseus*.

Description of *Rhodoplanes roseus* comb. nov. *Rhodoplanes roseus* (*Rhodopseudomonas rosea* Janssen and Harfoot 1991) (ro'se.us. L. adj. *roseus*, rose-colored, pink). The characteristics are the same as the characteristics described above for the genus. The following additional properties are based on the information from the previous report (17) and this study. Cells are 1.0 μm wide and 1.8 to 2.5 μm long. Reproduce by budding without a division tube. Rosette formation is not found. Absorption maxima of cell extracts are at 373, 468, 493, 530, 593, 801, and 854 nm. The pH range for growth is 6.5 to 8.0. No growth occurs at pH 6.0. Niacin is required as a growth factor, but growth is stimulated considerably by addition of 0.01% yeast extract. Utilizable electron donor and carbon sources for phototrophic growth are acetate, propionate, butyrate, valerate, lactate, pyruvate, succinate, fumarate, malate, citrate,

TABLE 4. Characteristics of members of *Rhodoplanes* gen. nov. and some species of the genus *Rhodopseudomonas*^a

Characteristic	<i>Rhodoplanes</i>		<i>Rhodopseudomonas</i>			
	<i>R. roseus</i>	<i>R. elegans</i>	<i>R. acidophila</i>	<i>R. marina</i>	<i>R. palustris</i>	<i>R. viridis</i>
Cell diameter (μm)	1.0	0.8–1.0	1.0–1.3	0.7–0.9	0.6–0.9	0.6–0.9
Type of budding	Sessile	Tube	Sessile	Sessile	Tube	Tube
Rosette formation	–	+	–	–	+	+
Color of cultures	Pink	Pink	Red	Pink	Red	Green
Bacteriochlorophyll	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>b</i>
Salt requirement	–	–	–	+	–	–
Denitrification	+	+	–	–	–/+	–
Vitamin required	Niacin	p-ABA + thiamine	None	ND	p-ABA (+ biotin)	p-ABA + biotin
Utilization of:						
Benzoate	–	–	–	–	+	–
Citrate	+	+	–	–/+	+	–
Tartrate	+	+	–	–	–	–
Sulfide	–	–	–	+	+	–
Major quinone(s)	Q-10, RQ-10	Q-10, RQ-10	Q-10, RQ-10, MK-10	Q-10, MK-10	Q-10	Q-9, MK-9
G+C (mol%)	66 (<i>T_m</i>), 66.8 (HPLC)	69.6–69.7 (HPLC)	62.2–66.8 (Bd)	61.5–63.8 (<i>T_m</i>)	64.5–65.5 (HPLC)	66.3–71.4 (Bd)
Habitat	Freshwater	Freshwater	Freshwater	Seawater	Freshwater	Freshwater

^a Symbols and abbreviations: +, positive; –, negative; –/+, most strains negative and some strains positive; p-ABA, *p*-aminobenzoic acid; ND, not determined; (+ biotin), biotin required by a few strains only; Q, ubiquinone; RQ, rhodoquinone; MK, menaquinone; Bd, buoyant density. Information is from references 3, 8, 11, 15, 17, 26, and 32 and this study.

tartrate, Casamino Acids, yeast extract, and peptone. The following organic compounds are not utilized: formate, long-chain fatty acids with more than five carbon atoms, glycolate, malonate, benzoate, arabinose, xylose, rhamnose, fructose, glucose, mannose, galactose, dulcitol, mannitol, sorbitol, glycerol, methanol, ethanol, propanol, alanine, aspartate, asparagine, glutamate, and leucine. The G+C content of the DNA is 66.8 mol% (by HPLC) and 66 mol% (by *T_m*).

Isolated from freshwater lake sediment.

The type strain is DSM 5909 (Janssen and Harfoot 941).

Description of *Rhodoplanes elegans* sp. nov. *Rhodoplanes elegans* (e'le.gans. L. adj. *elegans*, choice, elegant). The characteristics are the same as the characteristics described above for the genus. Additional properties are as follows. Cells are 0.8 to 1.0 μm wide and 2.0 to 3.5 μm long. Reproduce by budding with a division tube occurring between the mother and daughter cells. Rosettes and clusters are formed in older cultures. Absorption maxima of cell extracts are at 373, 466, 491, 527, 592, 799, and 855 nm. The pH range for phototrophic growth is 6.0 to 8.5. Thiamine and *p*-aminobenzoic acid are required as growth factors. The following organic compounds are photoassimilated: acetate, propionate, butyrate, valerate, caproate, lactate, pyruvate, succinate, fumarate, malate, citrate, tartrate, Casamino Acids, and yeast extract. The following organic compounds are not utilized: formate, long-chain fatty acids with more than six carbon atoms, glycolate, malonate, benzoate, arabinose, xylose, rhamnose, fructose, glucose, mannose, galactose, dulcitol, mannitol, sorbitol, glycerol, methanol, ethanol, propanol, alanine, aspartate, asparagine, glutamate, and leucine. The G+C content of the DNA is 69.6 to 69.7 mol% (by HPLC).

Isolated from activated sludge.

The type strain is AS130, which has been deposited in the Japan Collection of Microorganisms, RIKEN, Wako, Japan, as *R. elegans* JCM 9224.

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