Descriptions of *Rhodopseudomonas parapalustris* sp. nov., *Rhodopseudomonas harwoodiae* sp. nov. and *Rhodopseudomonas pseudopalustris* sp. nov., and emended description of *Rhodopseudomonas palustris*

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Four strains (JA310^T, JA531^T, JA447 and JA490) of red to reddish brown pigmented, rod-shaped, motile and budding phototrophic bacteria were isolated from soil and freshwater sediment samples from different geographical regions of India. All strains contained bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series. The major cellular fatty acid of strains JA310^T and JA531^T was C_{18:1}ω7c, the quinone was Q-10 and polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an aminohopanoid and an unidentified aminolipid. Phylogenetic analysis based on 16S rRNA gene sequences showed that all strains clustered with species of the genus *Rhodopseudomonas* in the class *Alphaproteobacteria*. Strains JA531^T, JA447 and JA490 were genotypically (>80 % related based on DNA–DNA hybridization) and phenotypically closely related to each other and the three strains were distinct from strain JA310^T (33 % related). Furthermore, all four strains had less than 48 % relatedness (DNA–DNA hybridization) with type strains of members of the genus *Rhodopseudomonas*, i.e. *Rhodopseudomonas palustris* ATCC 17001^T, *Rhodopseudomonas faecalis* JCM 11668^T and *Rhodopseudomonas rhenobacensis* DSM 12706^T. The genomic DNA G + C contents of strains JA310^T and JA531^T were 63.8 and 62.4 mol%, respectively. On the basis of phenotypic, chemotaxonomic and molecular genetic evidence, it is proposed that strains JA310^T (=NBRC 106083^T=KCTC 5839^T) and JA531^T (=NBRC 107575^T=KCTC 5841^T) be classified as the type strains of two novel species of the genus *Rhodopseudomonas*, *Rhodopseudomonas parapalustris* sp. nov. and *Rhodopseudomonas harwoodiae* sp. nov., respectively. In addition, we propose that strain DSM 123^T (=NBRC 100419^T) represents a novel species, *Rhodopseudomonas pseudopalustris* sp. nov., since this strain differs genotypically and phenotypically from *R. palustris* ATCC 17001^T and other members of the genus *Rhodopseudomonas*. An emended description of *R. palustris* is also provided.

Abbreviations: AH, aminohopanoid; AL, aminolipid; BChl, bacteriochlorophyll; DPG, diphosphatidylglycerol; PABA, *para*-aminobenzoic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains JA310^T, JA531^T, JA447 and JA490 are AM947938, FN813512, FN543491 and FN813513, respectively.

Two supplementary figures and additional supplementary data are available with the online version of this paper.

Ever since the first description of the genus *Rhodopseudomonas* (Czurda & Maresch, 1937), classification of members of this genus has been subject to significant changes including reclassification of species (Imhoff *et al.*, 1984; Hiraishi & Ueda, 1994; Hiraishi, 1997). Currently, there are only three species with validly published names remaining in this genus (<http://www.bacterio.cict.fr/q/r/rhodopseudomonas.html>): *Rhodopseudomonas palustris* ATCC 17001^T, *Rhodopseudomonas rhenobacensis* DSM 12706^T and

Rhodopseudomonas faecalis JCM 11668^T. In this study, we propose the description of two novel species, *Rhodopseudomonas harwoodiae* sp. nov. (with two additional strains) and *Rhodopseudomonas parapalustris* sp. nov., to accommodate four novel isolates from different geographical regions of India. We also propose that strain DSM 123^T (listed as a type strain of *R. palustris*) represents a novel species, *Rhodopseudomonas pseudopalustris* sp. nov., since this strain showed genotypic and phenotypic differences (Okamura *et al.*, 2009; this study) to *R. palustris* ATCC 17001^T and all other members of the genus *Rhodopseudomonas*.

Strain JA310^T was isolated from a soil sample collected below salt crystals near Pangong Lake, Jammu and Kashmir, India on 22 May 2006 (GPS position 33° 43' 04.59" N 78° 53' 48.48" E). Strain JA531^T was isolated from sediment of a saltpan at Kalambakam, Tamil Nadu, India (GPS position 12° 45.464' N 86° 12.728' E). Strains JA447 and JA490 were isolated from a freshwater pond near Jamnagar, Gujarat, India (GPS position 22° 23' 27.86" N 69° 55' 01.97" E) and sediment of a freshwater canal at Venkatapuram, Karimnagar District, India (GPS position 18° 38' 30.79" N 79° 27' 10.63" E), respectively. All strains were isolated from enrichments obtained in a previously described medium (Lakshmi *et al.*, 2009) with pyruvate (25 mM) and ammonium chloride (7 mM) as carbon and nitrogen sources, respectively, in 50 ml fully filled screw-capped bottles incubated at a light intensity of 2500 lx and 30 ± 2 °C for 7 days under anaerobic conditions. Cultures were purified by repeated streaking on agar slants (Srinivas *et al.*, 2007; Lakshmi *et al.*, 2009) and were maintained in pure culture under refrigeration at 4 °C and preserved as lyophilized vials. *R. palustris* DSM 123^T, *R. palustris* ATCC 17001^T, *R. faecalis* JCM 11668^T and *R. rhenobacensis* DSM 12706^T were obtained from the respective culture collection centres and used for comparative studies in our laboratory as recommended by Tindall *et al.* (2010).

Cell morphology (cell shape, cell division, cell size, flagella and visible internal or external structures) was observed by phase-contrast microscopy (Olympus BH-2) and transmission electron microscopy (Hitachi H-7500 model). For transmission electron microscopic examination, cells were subjected to negative staining with phosphotungstic acid and grids were examined under a Hitachi H-7500 electron microscope. Motility was examined by the hanging drop method. Growth at various NaCl concentrations [0–10 % (w/v) at intervals of 0.5 %], pH (pH 5.0–10.0, at intervals of 0.5 pH units) and temperatures (5–45 °C, at intervals of 5 °C) was investigated in the medium described above. Utilization of organic compounds as carbon sources/electron donors under photoheterotrophic growth was tested in the above medium containing the specific organic compound (0.35 %, w/v) in the presence of yeast extract (0.01 %, w/v). Na₂S·9H₂O (0.1, 0.5, 1.0 mM) and 1 mM each of MgSO₄, Na₂S₂O₃, S⁰, SO₃²⁻, cysteine and thioglycolate were tested for utilization as sulfur source. Nitrogen source utilization was tested by replacing ammonium

chloride with urea, nitrate, nitrite, glutamate and glutamine at 0.06 % (w/v). Vitamin requirement was tested by replacing yeast extract with different vitamins [vitamin B₁₂, biotin, niacin, *para*-aminobenzoic acid (PABA), pantothenate, pyridoxal phosphate, riboflavin, thiamine and a mixture of all the above vitamins (0.02 %, w/v)] as growth factors. Growth was followed turbidometrically by measuring the increase in OD₆₆₀.

In vivo absorption spectra were measured with a Spectronic Genesys 2 spectrophotometer in sucrose solution (Trüper & Pfennig, 1981). For pigment analysis, 1 l of 48 h grown culture was harvested by centrifugation (16 000 r.p.m. for 10 min). The supernatant was discarded and the pellet was washed twice with 0.9 % NaCl solution and lyophilized. Pigments were extracted from lyophilized cells with methanol/acetone (2 : 7, v/v). Carotenoid composition was analysed by using HPLC [solvent system, acetonitrile/methanol/ethyl acetate (50 : 40 : 10); flow rate, 1 ml min⁻¹; column, luna 5 µm, C18, 250 × 4.6 mm phenomenex; photo diode array detector (400–800 nm); wavelengths monitored, 450, 500 and 550 nm]. Cellular fatty acids were methylated, separated and identified according to the instructions for the Microbial Identification System (Microbial ID; MIDI 6.0 version) [Sasser (1990); revised, www.midi-inc.com] which was outsourced through the Royal Research Laboratories, Secunderabad, India. Cells of all strains of *Rhodopseudomonas* grown photoheterotrophically on pyruvate (25 mM) and ammonium chloride (7 mM) broth at 37 °C were harvested by centrifugation (10 000 × g for 15 min at 4 °C) on reaching cell densities of 70 % of the maximum OD and the lyophilized pellet was used for fatty acid analysis.

Polar lipids were extracted from 1 g freeze-dried cells with methanol/chloroform/saline (2 : 1 : 0.8, by vol.) as described by Kates (1986). Lipids were separated using silica gel TLC (Kieselgel 60 F₂₅₄; Merck) by two-dimensional chromatography using chloroform/methanol/water (75 : 32 : 4, by vol.) in the first dimension and chloroform/methanol/acetic acid/water (86 : 16 : 15 : 4, by vol.) in the second dimension (modified after Tindall, 1990a, b; Oren *et al.*, 1996). Total polar lipid profiles were detected by spraying with 5 % ethanolic molybdophosphoric acid and further characterized by spraying with ninhydrin (specific for amino groups), molybdenum blue (specific for phosphates), Dragendorff reagent (quaternary nitrogen) or α -naphthol (specific for sugars) (Kates, 1972; Oren *et al.*, 1996). Hopanoids were visualized at 360 nm after spraying with 0.1 % solution of barberine chlorohydrate in ethanol (Neunlist *et al.*, 1985). Quinones were extracted with a chloroform/methanol (2 : 1, v/v) mixture, purified by TLC and analysed by HPLC (Imhoff, 1984; Hiraishi & Hoshino, 1984; Hiraishi *et al.*, 1984).

Genomic DNA was extracted and purified according to the method of Marmur (1961) and the DNA G+C content was determined by HPLC (Mesbah *et al.*, 1989). Cell material for 16S rRNA gene sequencing was taken from a

Table 1. Differential characteristics of strains JA531^T and JA310^T and other members of the genus *Rhodopseudomonas*

Taxa: 1, *Rhodopseudomonas harwoodiae* sp. nov. JA531^T (includes data for the additional strains JA447 and JA490; data that varies between strains is separated by a solidus); 2, *Rhodopseudomonas parapaalustris* sp. nov. JA310^T; 3, *Rhodopseudomonas pseudopalastris* sp. nov. DSM 123^T; 4, *Rhodopseudomonas palustris* ATCC 17001^T; 5, *Rhodopseudomonas faecalis* JCM 11668^T; 6, *Rhodopseudomonas rhenobacensis* DSM 12706^T. Data are from this study. For all taxa, organic substrate utilization was tested during photoheterotrophic growth. Acetate, lactate and pyruvate are utilized by all strains. None of the strains could utilize citrate, fructose, mannitol, methanol or aspartate. Intracellular photosynthetic membranes are of the lamellae type for all strains. All strains have Bchl *a*, are tryptophanase-negative, have no salt requirement, assimilate sulfate, do not ferment glucose/fructose and have Q-10 as major quinone. +, Substrate utilized/present; –, substrate not utilized/absent; (+), weak growth; NA, not available.

Characteristic	1	2	3	4	5	6
Isolation source	Saltpan/pond sediment	Soil	Soil/pond water	Soil/sludge/pond water	Chicken faeces	Eutrophic pond
Cell shape	Rods	Rods	Rods	Rods with rounded ends	Vibroid	Rods
Cell size (µm)						
Width	0.8–1.0	0.8–1.2	0.5–1.0	0.4–1.0	0.6–0.8	0.4–0.6
Length	2.0–3.5	2.0–4.0	2.0–6.0	1.5–3.0	1.0–2.0	1.5–2.0
Type of budding	Tube	Sessile	Sessile	Tube	Sessile	Sessile
Colour of cultures*	R	RB	RB	RB	R	R
Growth pH						
Optimum	6.0–7.5	6.5–7.0	6.0–8.0	6.5–8.5	7.0	5.5
Range	5.0–9.0	6.0–9.0	7.0	6.0–9.0	6.5–7.0	5.0–7.0
Growth temperature (°C)						
Optimum	30	30	30	30	30	25–35
Range	25–40	15–35	20–35	25–40	25–35	30
Aerobic growth in the dark	+	+	+	+	–	+
Photoautotrophic growth	–	–	–	With H ₂ , thiosulfate, sulfide	With H ₂ , sodium bicarbonate	–
Growth factors	PABA	Thiamine, PABA	PABA	PABA†	Thiamine	PABA
Major polar lipids‡	DPG, PG, PE, PC, AL1–2, AL6, AL8, PL1, AH, L2–4, L6	DPG, PG, PE, PC, AL1, AL3, PL1, AH, L2–4	DPG, PG, PE, PC, AL1, AL3, PL1, AH, L1–5	DPG, PG, PE, PC, AL1–4, PL1, AH, L1–4	DPG, PG, PE, PC, AL1–7, PL1, AH, L1–5	DPG, PG, PE, PC, AL1, AL4, PL1, AH, L1–4
Fatty acid composition (%)						
C _{12:0}	–	1.1	2.4	1.6	1.7	0.2
C _{14:0}	–	–	–	1.8	–	–
C _{14:0} 2-OH	–	–	1.0	–	–	0.1
C _{14:0} 3-OH	0.7	0.6	1.7	–	–	–
C _{16:0}	7.6	17.8	18.2	20.8	14.9	13.4
C _{16:1} ω7c alcohol§	1.0	1.1	1.5	0.3	1.7	0.5
iso-C _{16:1} I	–	0.5	0.8	–	–	–
C _{16:1} ω7c/ω6c	2.8	2.5	3.3	10.2	6.2	7.8
C _{18:0}	6.9	5.3	5.0	4.0	2.8	3.5
C _{18:1} ω5c	–	0.7	–	0.1	1.1	0.5
C _{18:1} ω7c	74.2	53.2	56.5	51	63	69.6
11-Methyl C _{18:1} ω7c	3.2	7.5	4.0	4.3	5.8	1.8
iso-C _{19:0}	–	–	4.2	0.3	–	0.2
C _{20:0}	–	1.3	2.4	0.1	–	0.1
C _{20:1} ω7c	–	1.2	–	0.2	–	0.2
Organic substrates supporting growth						
Benzoate	+	–	+	+	–	–
Butyrate	+	–	+	+	+	+
Caproate	+	–	+	(+)	(+)	–
Caprylate	–	–	+	–	–	–
Ethanol	–	–	+	+	–	+
Formate	–	–	+	–	–	+

Table 1. cont.

Characteristic	1	2	3	4	5	6
Fumarate	(+)	+	+	+	(+)	+
D-Glucose	—	(+)	+	+	+	—
Glutamate	—	—	+	—	—	—
Glycerol	(+)/—	+	+	+	—	(+)
Glycolate	—	—	+	NA	—	—
Malate	—	+	+	+	+	+
Malonate	(+)/—	—	+	+	—	—
Propanol	—	—	+	NA	—	—
Propionate	(+)	—	+	+	(+)	—
Sorbitol	—	+	+	NA	—	—
Succinate	(+)	+	+	+	+	+
Tartrate	—	—	—	—	—	+
Valerate	+	—	+	NA	—	—
DNA–DNA relatedness (%) to strains:¶						
1	100					
2	33.1 ± 5.2	100				
3	45 ± 9.2	48 ± 4.2	100			
4	47.5 ± 4.4	45.3 ± 2.8	32.8 ± 3.8 (25)	100		
5	28.4 ± 2.9	32.1 ± 2.2	41 ± 8.3 (25)	40.7 ± 2 (18)	100	
6	27 ± 4.6	24.1 ± 3.8	32.5 ± 14.5 (20)	38.6 ± 4.4 (29)	43.5 ± 4.2 (25)	100
DNA G + C content (mol%; HPLC)	62.4	63.8	65.8	64.9	64.0	65.4

*R, Red; RB, red–brown.

†A few strains require biotin.

‡AH, Aminohopanoid; AL1–8, unknown aminolipids; DPG, diphosphatidylglycerol; L1–6, unknown lipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; and PL, unknown phospholipid. Numbering of polar lipids is according to the convention adopted by Kämpfer *et al.* (2006).

§Alcohol (not a fatty acid).

||Tested at 0.5 and 1.0 mM.

¶Data given are the mean and standard deviation values of three independent experiments performed in triplicate for each sample (control: reversal of strains was used for binding and labelling). Values given in parentheses are from Okamura *et al.* (2009).

colony. DNA was extracted and purified by using a Qiagen genomic DNA extraction kit. Recombinant *Taq* polymerase (Genei) was used for PCR. The complete length of the 16S rRNA gene sequence was obtained by sequencing with four primers, F'-27 (5'-GTTTGATCCTGGCTCAG-3'), F'-372 (5'-TACGGGAGGCAGCAG-3'), F'-790 (5'-GGTTACCTTGTTACGACT-3') and R'-1489 (5'-TACCTTGTTACGACTTCA-3') [positions 11–27 and 1489–1506 for F'-27 and R'-1489, respectively, according to the *Escherichia coli* 16S rRNA numbering system of the International Union of Biochemistry (Brosius *et al.*, 1978; Lane *et al.*, 1985)]. PCR amplification was done as described previously (Imhoff & Pfennig, 2001; Imhoff *et al.*, 1998) and 16S rRNA gene sequences were obtained (without gene cloning) by cycle sequencing with a SequiTherm sequencing kit (Biozym) and chain termination reaction (Sanger *et al.*, 1977) using an automated laser fluorescence sequencer (Pharmacia). The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved using an

NCBI BLAST search (Altschul *et al.*, 1990) and the EzTaxon server (Chun *et al.*, 2007). The CLUSTAL W algorithm of MEGA4 was used for sequence alignments and MEGA4 (Tamura *et al.*, 2007) software was used for phylogenetic analysis of the individual sequences. Distances were calculated by using the Kimura two-parameter model (Kimura, 1980). Neighbour-joining and minimum evolution methods in the MEGA4 software (<http://www.mega-software.net/mega4/mega4.pdf>) were used to reconstruct phylogenetic trees. Support values (%) were obtained using a bootstrap procedure. The taxonomic relationship between strains of the genus *Rhodopseudomonas* was examined using genome DNA–DNA hybridization. Genomic relatedness was determined by a membrane filter technique (Seldin & Dubnau, 1985; Tourova & Antonov, 1988) using a DIG High Prime DNA labelling and detection starter kit II (Roche). Hybridization was performed with three replications for each sample (control: reversal of strains used for binding and labelling) and the mean values are quoted as DNA–DNA relatedness values.

Four strains (JA310^T, JA531^T, JA447 and JA490) belonging to the genus *Rhodopseudomonas* were isolated from different geographical regions of India. Cells of all strains were rod-shaped, 0.8–1.2 µm wide and 2.0–3.0 µm long, and they multiplied by budding. Rosette formation was observed in old cultures of all strains. Cells of all strains were motile by means of polar flagella. Transmission electron micrographs of ultrathin sections of cells of the four strains revealed a lamellar type of internal membrane structure parallel to the cytoplasmic membrane. All strains were able to grow photo-organoheterotrophically [anaerobically in the light (2400 lx) with sodium pyruvate (0.3 % w/v) as carbon source] and chemo-organoheterotrophically [aerobically in the dark with sodium pyruvate (0.3 % w/v) as carbon source]. Photolithoautotrophic [anaerobically in the light (2400 lx)] and chemolithoautotrophic [aerobically in the dark] growth with Na₂S₂O₃ · 5H₂O (0.5 mM) as electron donor and NaHCO₃ (0.1 % w/v) as carbon source could not be demonstrated in any of the isolates. Fermentative growth [anaerobic, dark with glucose/fructose/pyruvate (0.3 % w/v)] could not be demonstrated in any of the strains. Organic substrates utilized/not utilized for growth by all four strains are given in Table 1. Benzoate (0.5 and 1.0 mM) could not support growth of strain JA310^T, although it supported growth of strains JA531^T, JA447 and JA490. Ammonium chloride, nitrate and asparagine supported growth of JA310^T, but urea, glutamate, glutamine and nitrite could not. Strains JA531^T, JA447 and JA490 grew with ammonium chloride, glutamine and urea as nitrogen sources, but nitrite, nitrate and glutamate could not support growth. Strains JA531^T, JA447 and JA490 exhibited diazotrophic growth, which was not observed in strain JA310^T.

Both thiamine and PABA were required by strain JA310^T, whereas strains JA531^T, JA447 and JA490 had a requirement for PABA alone. Sulfate, MgSO₄ · 7H₂O, Na₂S · 9H₂O, Na₂S₂O₃, thioglycolate and cysteine were used as sulfur sources, but sulfite did not support phototrophic growth of any of the four strains. NaCl was not obligatory for growth of the strains; strain JA531^T could tolerate up to 4 % NaCl, whereas the other three strains tolerated up to 0.5 % NaCl. The pH range for growth of strain JA310^T was 6.0–9.0 (optimum pH 6.5–7.0), whereas strains JA531^T, JA447 and JA490 grew at pH 5.0–9.0 (optimum pH 6.0–7.5). All four strains were mesophilic, with optimal growth at 30 °C.

The colour of phototrophically-grown cell suspensions of strain JA310^T was reddish brown, whereas those of strains JA531^T, JA447 and JA490 were red. Whole cell absorption spectra of all four strains showed absorption maxima at 524–528, 590–593, 806 and 863–869 nm (Fig. S1, available in IJSEM Online) confirming the presence of bacteriochlorophyll (Bchl) *a* and carotenoids. Acetone extracts of all strains had absorption maxima at 445, 475 and 502 nm, indicating the presence of carotenoids of the spirilloxanthin series (Britton *et al.*, 2004). Carotenoid composition of strains JA310^T, JA531^T, JA447 and JA490 as detected by HPLC analysis was: rhodopin (21–25 %),

3,4-dihydrorhodopin (36–44 %), anhydrorhodovibrin (13–16 %), lycopene (3–4 %), rhodovibrin (5–7 %) and spirilloxanthin (5–6 %). Whole-cell fatty acid analysis revealed that C_{18:1}ω7c (51–74 %) was the major fatty acid for all the strains tested and differences in the fatty acid composition among members of the genus *Rhodopseudomonas* are shown in Table 1. All strains contained ubiquinone-10 (Q-10) as the primary quinone component.

The polar lipid profile common to all *Rhodopseudomonas* strains tested consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) as major components, with moderate amounts of an unidentified phospholipid (PL), unidentified lipids (L2–4), an aminohopanoid (AH) and an unidentified aminolipid (AL1) (Table 1; Fig. S2). The major polar lipid composition observed in this study is in agreement with those of previous reports for members of the genera *Rhodopseudomonas*/Bradyrhizobium/Nitrobacter/Afipia (Hougardy *et al.*, 2000; Imhoff *et al.*, 1982). The presence of AH is also in agreement with members of the genus *Rhodopseudomonas* (Neunlist *et al.*, 1985; Hougardy *et al.*, 2000). The absence of a few unidentified ALs (AL5–8) and other lipids (L5, 6) differentiated *R. palustris* ATCC 17001^T from other type strains in the genus *Rhodopseudomonas*. Unidentified ALs (AL2, 4–8) and other lipids (L1, 5, 6) were absent in strain JA310^T. The presence of AL8 and L6 was unique to strain JA531^T. *R. rhenobacensis* DSM 12706^T could be differentiated from other members by the absence of unidentified AL2–8 and other lipids (L5, 6). *R. faecalis* JCM 11668^T possessed unidentified AL5 and AL7, which were not observed in other members of the genus. An unidentified lipid (L5) was present in strain DSM 123^T alone, whereas unidentified AL2, AL4–8 and L6 were absent in this strain. A few unidentified polar lipids (PL, L1–4) were not previously reported in *R. rhenobacensis* DSM 12706^T (Hougardy *et al.*, 2000).

The genomic DNA G+C content of strain JA310^T was 63.8 mol% and those of strains JA531^T, JA447, JA490 were 62.4–63.3 mol% (by HPLC). The phylogenetic relationship (Fig. 1) between strains JA310^T, JA531^T, JA447 and JA490 and other closely related bacteria was examined using 16S rRNA gene sequence analysis. The data obtained revealed that the novel isolates clustered with the type strains of species of the genus *Rhodopseudomonas*, but were distinct from phototrophic and chemotrophic members of other genera. The neighbour-joining tree (Fig. 1; tree topology was similar in minimum evolution, unweighted pair group method with arithmetic averages and maximum-likelihood trees and a multiple sequence alignment file is available in IJSEM Online) revealed three distinct subclades within the genus *Rhodopseudomonas*. Strains JA531^T, JA447 and JA490 formed a distinct microclade. *R. palustris* ATCC 17001^T and *R. faecalis* JCM 11668^T clustered together, but strain JA310^T, along with strain DSM 123^T and *R. rhenobacensis* DSM 12706^T were separate from the rest of the members of the genus. Strains JA310^T and JA531^T

Fig. 1. Dendrogram based on 16S rRNA gene sequences depicting the phylogenetic relationships of strains JA310^T, JA531^T, JA447, JA490 and DSM 123^T with members of the genus *Rhodopseudomonas* and other chemotrophic genera. The tree was reconstructed (1350 gap-free sites were compared) by the neighbour-joining method using the MEGA4 software. Bootstrap values (percentages from 500 replicates) are shown next to the corresponding branch. Bar, 2 nucleotide substitutions per 100 nt.

showed the highest gene sequence similarities to *R. palustris* ATCC 17001^T (96.3 and 97.5 %, respectively), *R. faecalis* JCM 11668^T (96.7 and 97.3 %) and *R. rhenobacensis* DSM 12706^T (97.4 and 97.1 %). Strains JA310^T and JA531^T had about 96.3 % 16S rRNA gene sequence similarity.

DNA–DNA hybridization of strains JA310^T and JA531^T (JA447 and JA490) with the most closely related type strains of members of the genus *Rhodopseudomonas* revealed relatedness values of 24–48 %, whereas strains JA310^T and JA531^T were about 33.0 % related (Table 1). Furthermore, strains JA447 and JA490 showed 79–89 % DNA relatedness to strain JA531^T and phenotypic traits were very similar to those of strain JA531^T; thus, we propose that strains JA447 and JA490 are additional strains of a novel species represented by JA531^T. Relatedness levels among other members of the genus *Rhodopseudomonas* were also determined; as suggested by Okamura *et al.* (2009), data confirmed that strains DSM 123^T and ATCC 17001^T are not members of the same species. DNA–DNA hybridization data were supported by differences in whole-cell fatty acid profile, polar lipid composition, vitamin requirements, pH optima and range, temperature range, organic substrate and electron donor utilization (Table 1; particularly benzoate utilization), nitrogen, sulfur source utilization and cell size (strain DSM 123^T has long cells). Our data justify the description of strains JA310^T, JA531^T and DSM 123^T as representatives of three novel species, for which the names *Rhodopseudomonas*

parapaalustris sp. nov., *Rhodopseudomonas harwoodiae* sp. nov. and *Rhodopseudomonas pseudopaalustris* sp. nov. are proposed, respectively.

Description of *Rhodopseudomonas parapaalustris* sp. nov.

Rhodopseudomonas parapaalustris (pa.ra.pa.lus'tris. Gr. prep. *para* alongside of, resembling; L. fem. adj. *palustris* marshy, and also a bacterial epithet; N.L. fem. adj. *parapaalustris* resembling *Rhodopseudomonas palustris*).

Cells are rod-shaped, 0.8–1.2 µm wide and 2.0–4.0 µm long. Cells are motile by means of polar flagella and multiply by budding. Cells of old cultures form rosettes. Internal photosynthetic membranes are present as lamellar stacks parallel to the cytoplasmic membrane. Phototrophically grown cultures are reddish brown. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 497, 590, 800 and 863 nm. Bchl *a* and carotenoids of the spirilloxanthin series are present. The type strain is mesophilic (range 15–35 °C). No NaCl is required for growth (tolerates up to 0.5 %, w/v) and growth pH optimum is 6.5–7.0 (range pH 6.0–9.0). The preferred mode of growth is photo-organoheterotrophy with a few organic compounds. Good growth is observed with malate, succinate, pyruvate, glycerol, fumarate and sorbitol as carbon sources. Phototrophic growth also occurs on

D-glucose. Benzoate, butyrate, caproate, caprylate, ethanol, formate, glutamate, glycolate, malonate, propanol, propionate, tartrate and valerate are not utilized as carbon sources. Photoautotrophic and chemoautotrophic growth with thiosulfate/H₂/sulfide/sulfur/sulfite/thioglycolate as electron donor and NaHCO₃ as carbon source is not observed. Fermentative growth does not occur in the presence of glucose, fructose or pyruvate. Nitrate and aspartate are used as nitrogen sources. Diazotrophy is absent. Sulfate, thiosulfate, thioglycolate and cysteine are used as sulfur sources. Thiamine and PABA are required as growth factors. C_{18:1}ω7c is the major fatty acid, with moderate amounts of C_{12:0}, C_{16:0}, 11-methyl C_{18:1}ω7c, C_{18:0} and C_{16:1}ω7c/ω6c. Ubiquinone-10 is the major quinone. DPG, PG, PE, PC and AL1 are the major polar lipids. Moderate amounts of AL3, PL and other lipids (L2–4) are also present. AH is present.

The type strain is JA310^T (=NBRC 106083^T=KCTC 5839^T), isolated from a sediment soil sample taken near Pangong Lake at Leh, Jammu and Kashmir, India. The genomic DNA G+C of the type strain is 63.8 mol% (by HPLC). Natural habitats are surface soils near lakes at high altitudes.

Description of *Rhodopseudomonas harwoodiae* sp. nov.

Rhodopseudomonas harwoodiae [har.wo.o.di'a.e. N.L. fem. gen. n. *harwoodiae* of Harwood, named after Professor Dr C. S. Harwood, a microbiologist who has significantly contributed to the knowledge of aromatic hydrocarbon metabolism of *Rhodopseudomonas palustris* strain CGA009, the genome sequence of which is available from GenBank/EMBL/DBJ under accession numbers BX571963 (chromosome) and BX571964 (plasmid) (Larimer *et al.*, 2004)].

Cells are rod-shaped, 0.8–1.0 µm wide and 2.0–3.5 µm long, and motile; division is by budding and cells have lamellar internal cytoplasmic membrane structures. Phototrophic cultures are red. *In vivo* absorption spectra of intact cells exhibit maxima at 497, 590, 605 and 863 nm. Bchl *a* and carotenoids of the spirilloxanthin series are present. No NaCl is required for growth; the type strain tolerates up to 4 % NaCl, whereas other strains tolerate up to 0.5 % (w/v). Mesophilic (range 25–40 °C) with optimum growth at pH 6.0–7.5 (range pH 5.0–9.0). The preferred mode of growth is photo-organoheterotrophy with a few organic compounds. Good growth occurs on benzoate, butyrate, caproate and valerate. Feeble growth occurs with fumarate, glycerol, malonate, propionate and succinate as carbon sources. Caprylate, ethanol, formate, D-glucose, glutamate, glycolate, malate, propanol, sorbitol and tartrate do not support growth. Photoautotrophic and fermentative growth is absent. PABA is required as growth factor. Diazotrophy is present. C_{18:1}ω7c is the major fatty acid, with moderate amounts of C_{16:0}, C_{18:0}, 11-methyl C_{18:1}ω7c and C_{16:1}ω7c/ω6c. Ubiquinone-10 is the major quinone. DPG, PG, PE, AL1 and PC are the major polar lipids. Moderate amounts of

unidentified ALs (AL2, 6, 8), PL and other lipids (L2–4, 6) are also present. AH is present.

The type strain is JA531^T (=NBRC 107575^T=KCTC 5841^T), isolated from sediment of a saltpan at Kalambakam, Tamil Nadu, India. The DNA G+C content of the type strain is 62.4 mol% (by HPLC). Additional strains JA447 and JA490, which were isolated from freshwater sediments from different geographical regions of India, have properties similar to the type strain JA531^T. Natural habitats are salterns of coastal ecosystems and freshwater sediments.

Description of *Rhodopseudomonas pseudopalustris* sp. nov.

Rhodopseudomonas pseudopalustris (pseu.do.pa'lus.tris. Gr. adj. *pseudēs* false; L. fem. adj. *palustris* marshy, and also a bacterial epithet; N.L. fem. adj. *pseudopalustris* the false [*Rhodopseudomonas*] *palustris*).

Cells are rod-shaped, 0.8–1.0 µm wide and 2.0–6.0 µm long, and motile; division is by budding and cells have lamellar internal cytoplasmic membrane structures. Photo-trophic cultures are reddish brown. *In vivo* absorption spectra of intact cells exhibit maxima at 497, 590, 605 and 865 nm. Bchl *a* and carotenoids of the spirilloxanthin series are present. No NaCl is required for growth (tolerates up to 0.5 %, w/v). Mesophilic (range 20–35 °C) with optimum growth at pH 7.0 (range pH 6.0–8.0). The preferred mode of growth is photo-organoheterotrophy with a few organic compounds. Good growth occurs on benzoate, formate, butyrate, caproate, caprylate, fumarate, glutamate, glycerol, glycolate, malate, malonate, propanol, propionate, sorbitol, succinate and valerate. Feeble growth occurs with ethanol and D-glucose. Tartrate does not support growth. Photoautotrophic and fermentative growth is absent. PABA is required as a growth factor. C_{18:1}ω7c is the major fatty acid with moderate amounts of C_{12:0}, C_{16:0}, C_{18:0}, 11-methyl C_{18:1}ω7c, C_{16:1}ω7c/ω6c, iso-C_{19:0} and C_{20:0}. Ubiquinone-10 is the major quinone. DPG, PG, PE, AL1 and PC are the major polar lipids. Moderate amounts of AL3, PL and other lipids (L1–5) are also present. AH is present.

The type strain is DSM 123^T (=NBRC 100419^T; confirmed by Okamura *et al.*, 2009), which was isolated by van Niel (1944). The DNA G+C content of the type strain is 65.8 mol% (by HPLC).

Emended description of *Rhodopseudomonas palustris*

The description of the species is as given previously (Imhoff, 2005). DPG, PG, PE, AL1 and PC are the major polar lipids. Moderate amounts of AL2–4, PL1 and other lipids (L1–4) are also present. AH is present.

The type strain is ATH2.1.6^T (=ATCC 17001^T). The DNA G+C content of the type strain is 64.9 mol% (by HPLC).

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