Rhodoplanes pokkaliisoli sp. nov., a phototrophic alphaproteobacterium isolated from a waterlogged brackish paddy soil

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A Gram-negative, rod-shaped, purple non-sulfur bacterial strain, designated JA415^T, was isolated from the mud of a pokkali rice field located on Vypeen Island, Ernakulam, Kerala, India. Strain JA415^T was motile by means of a single polar flagellum. Photo- and chemo-organoheterotrophic growth was observed using organic compounds as carbon sources and electron donors. Photo- and chemolithoautotrophic growth using thiosulfate as electron donor did not occur. Fermentative growth could not be demonstrated. Intracellular photosynthetic membranes were lamellar stacks parallel to the cytoplasmic membrane. Bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series were present as photosynthetic pigments. Niacin, pantothenate and *p*-aminobenzoate were required as growth factors. C_{18:1}ω7c was the predominant cellular fatty acid component. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain JA415^T clustered with members of the genus *Rhodoplanes* in the class *Alphaproteobacteria*. On the basis of phenotypic and molecular genetic evidence, it is proposed that strain JA415^T be classified as a representative of a novel species of the genus *Rhodoplanes*, family *Hyphomicrobiaceae*, with the name *Rhodoplanes pokkaliisoli* sp. nov. The type strain is JA415^T (=KCTC 5711^T =NBRC 104972^T).

Pokkali is a unique variety of rice that is cultivated organically in waterlogged brackish regions of Ernakulam, Kerala, India. This variety of rice, which gets no fertilizer or manure, draws nutrients from prawn excrement and other remnants. During our studies on the distribution of purple bacteria from paddy soils of India, strain JA415^T was isolated from the pokkali fields. Based on 16S rRNA gene sequencing analysis, strain JA415^T belonged to the genus *Rhodoplanes*, family *Hyphomicrobiaceae*, class *Alphaproteobacteria*. At the time of writing, the genus *Rhodoplanes* comprised three species with validly published names: *Rhodoplanes roseus*, *Rhodoplanes elegans* (Hiraishi & Ueda, 1994) and *Rhodoplanes serenuss* (Okamura *et al.*, 2009). The name of a further species, '*Rhodoplanes cryptolactis*' (Okamura *et al.*, 2007), has not been validly published. The type strains of these species were isolated from activated sludge from a sewage-treatment plant (*Rpl.*

elegans) and sediment (*Rpl. roseus*) and water (*Rpl. serenuss*) from freshwater ponds. The type strain of '*Rpl. cryptolactis*' was isolated from a hot spring.

Strain JA415^T was isolated from a soil sample of a pokkali rice field (pH 7) on Vypeen Island, Ernakulam, Kerala, on the west coast of India (10° N 76° E), on 17 November 2007. Original enrichment of the strain was from a photoheterotrophic medium (pH 7.0) in 50 ml fully filled screw-capped bottles incubated at 2000 lx, 28–30 °C for 7 days. The medium contained (g l⁻¹, except where indicated otherwise): KH₂PO₄, 0.5; MgSO₄·7H₂O, 0.2; NaCl, 0.4; NH₄Cl, 0.6; CaCl₂·2H₂O, 0.05; sodium pyruvate, 3.0; yeast extract, 0.3; ferric citrate, 5 ml l⁻¹ from 0.1 % (w/v) stock; and trace element solution SL7, 1 ml l⁻¹. Trace element solution SL7 (Biebl & Pfennig, 1981) contained (l⁻¹) HCl, 1 ml of a 25 % (v/v) solution; ZnCl₂, 70 mg; MnCl₂·4H₂O, 100 mg; H₃BO₃, 60 mg; CoCl₂·6H₂O, 200 mg; CuCl₂·H₂O, 20 mg; NiCl₂·6H₂O, 20 mg; and NaMoO₄·2H₂O, 40 mg.

Purification was done by repeated streaking on agar slants (25 × 150 mm test tubes sealed with suba seals and the gas phase replaced with argon) and the purified culture was grown in the same medium as above. Taxonomic studies

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA415^T is FM202448.

A phase-contrast micrograph, electron micrographs of ultrathin cell sections showing negatively stained cells with a single polar flagellum and the lamellar nature of the photosynthetic membranes and the whole-cell absorption spectrum of strain JA415^T are available as supplementary material with the online version of this paper.

based on a polyphasic approach were carried out as described previously (Srinivas *et al.*, 2007). Anaerobic growth (in the dark) by nitrate respiration was determined in screw-capped test tubes filled completely with the above growth medium supplemented with 20 mM potassium nitrate. N₂ gas production by denitrification was investigated in these test tubes using Durham tubes; results were compared with those of a negative control that lacked nitrate. Spectral analysis was carried out as described previously (Anil Kumar *et al.*, 2008). Carotenoid composition was analysed by S. Takaichi (personal communication) using HPLC. Fatty acid methyl esters were prepared, separated and identified according to the instructions of the Microbial Identification System (Microbial ID; MIDI; Agilent 6850) (Sasser, 1990) (outsourced to the Royal Research Laboratories, Secunderabad, India). Quinones were extracted with a chloroform/methanol mixture, purified by TLC and analysed by HPLC (Imhoff, 1984; Hiraishi & Hoshino, 1984). Genomic DNA was obtained from 1–2 ml well-grown liquid culture using a Genomic DNA Extraction kit (Qiagen). Recombinant *Taq* polymerase was used for PCR. The PCR used the primers 5'-GTTTGATCCTGGCTCAG-3' (27F) and 5'-TACCTTGT-TACGACTTCA-3' (1489R), corresponding to positions 11–27 and 1489–1506, respectively, according to the *Escherichia coli* 16S rRNA numbering system of the International Union of Biochemistry. PCR amplification was done as described previously (Imhoff *et al.*, 1998) and 16S rRNA gene sequencing was outsourced to MWG Biotech, Bangalore, India. Sequences were aligned using the program CLUSTAL_X (Thompson *et al.*, 1997) and the alignment was corrected manually. The dendrogram was constructed with the program PHYL (Guindon & Gascuel, 2003) using 100 replicates of non-parametric bootstrap analysis, the GTR model of nucleotide substitution and four substitution rate categories.

Cells of strain JA415^T were rod-shaped, 1.0–1.2 µm wide and 3.0–5.0 µm long. Strain JA415^T multiplied by budding and asymmetrical cell division without forming prosthecae. Cells associated to form characteristic T, Y and V shapes along with the formation of short chains and rosettes (Supplementary Fig. S1, available in IJSEM Online). Strain JA415^T was motile by means of a single polar flagellum (Supplementary Fig. S2a). Transmission electron micrographs of ultrathin sections of the strain revealed lamellar internal membrane structures parallel to the cytoplasmic membrane (Supplementary Fig. S2b). Strain JA415^T was able to grow photo-organoheterotrophically [anaerobic, light (2400 lx), with pyruvate (0.03 % w/v) as carbon source] and chemo-organoheterotrophically [aerobic, dark, with pyruvate (0.3 % w/v) as carbon source]. Photolithoautotrophy [anaerobic, light (2400 lx), Na₂S·9H₂O (0.5 mM), Na₂S₂O₃·5H₂O (0.5 mM) and NaHCO₃ (0.1 %, w/v)], chemolithoautotrophy [aerobic, dark, Na₂S₂O₃·5H₂O (0.5 mM) and NaHCO₃ (0.1 %, w/v)] and fermentative growth [anaerobic, dark, with glucose, fructose or pyruvate (0.3 %, w/v)] could not be

demonstrated. Organic substrates utilized/not utilized by strain JA415^T are given in Table 1. Strain JA415^T could utilize ammonium salts and glutamate as nitrogen sources, but nitrite and nitrate did not support phototrophic growth. Denitrifying growth was observed without visible dinitrogen gas production. Diazotrophic growth and acetylene reduction (protocol of Sasikala *et al.*, 1990) could not be demonstrated. Niacin, pantothenate and *p*-aminobenzoate were required as growth factors. Sulfate, sulfite, thiosulfate and methionine were used as sulfur sources, but cysteine did not support phototrophic growth. Strain JA415^T did not require salt (NaCl) for growth, but the growth yield increased (~30 %) in the presence of NaCl (range 0–2.5 %, w/v; optimum 1.5 %, w/v). The pH range for growth of strain JA415^T was 6.0–7.5, with optimum growth at pH 7. The temperature range was 25–30 °C, with optimum growth at 30 °C.

Cell suspensions of strain JA415^T were yellowish brown when grown phototrophically and white when grown chemotrophically (aerobic, dark). The whole-cell absorption spectrum of strain JA415^T (Supplementary Fig. S3) showed absorption maxima at 380, 464, 491, 527, 596, 800 and 866 nm. Cell extracts of strain JA415^T in methanol showed an absorption maximum at 770 nm, confirming the presence of bacteriochlorophyll *a*. Acetone extracts showed absorption maxima at 359, 473, 503 and 570 nm, thus confirming the presence of carotenoids of the spirilloxanthin series (Britton *et al.*, 2004). The major carotenoid of strain JA415^T as detected by HPLC analysis was rhodopin (77 %); minor components were methyl rhodopin, lycopene and unidentified spirilloxanthins (S. Takaichi, personal communication). No differences were observed in the whole-cell absorption spectra of cells of strain JA415^T grown at high [5000 lx (incandescent)] and low [500 lx (incandescent)] light intensities, as observed previously for '*Rpl. cryptolactis*' (Okamura *et al.*, 2007). Whole-cell fatty acid analysis revealed C_{18:1ω7c} as the predominant fatty acid (61 %) in strain JA415^T (Table 1). Significant proportions of C_{16:0} (9 %), C_{18:0} (3.8 %), C_{18:0} 2-OH (5.2 %) and C_{19:0} cyclo ω8c (5.5 %) were also detected. Strain JA415^T contained ubiquinone-10 and rhodoquinone-10 as primary quinone components (molar ratio approximately 0.5). Menaquinones were absent. The combination of the presence of ubiquinone-10 and rhodoquinone-10 and the absence of menaquinones is specific for members of the genus *Rhodoplanes* (Hiraishi & Ueda, 1994).

The genomic DNA G+C content of strain JA415^T was 67.2 mol% (by HPLC). The phylogenetic relationship between strain JA415^T and other purple non-sulfur bacteria was examined using 16S rRNA gene sequence analysis. The data obtained revealed that the novel isolate clustered with the type strains of species of the genus *Rhodoplanes*, but was distinct from members of other genera of purple non-sulfur bacteria. Strain JA415^T showed the highest sequence similarities to the type or proposed type strains of *Rpl. roseus* (95.85 %), *Rpl. elegans* (95.70 %), '*Rpl. cryptolactis*'

Table 1. Differential characteristics of members of the genus *Rhodoplanes*

Strains/species: 1, JA415^T; 2, *Rpl. roseus* (data from this study and Hiraishi & Ueda, 1994); 3, *Rpl. elegans* (this study and Hiraishi & Ueda, 1994); 4, '*Rpl. cryptolactis*' (Okamura *et al.*, 2007); 5, *Rpl. serenus* (Okamura *et al.*, 2009). Organic substrate utilization was tested during photoheterotrophic growth for all species. Butyrate, malate, propionate, pyruvate, succinate and acetate are utilized by all species, but aspartate, benzoate, formate, fructose, glucose, glycolate, mannitol, methanol, sorbitol, sulfide, alanine and leucine are not utilized. Cells of all species are motile and have a lamellar internal membrane system. The colour of phototrophically grown cultures is pink, apart from that of strain JA415^T, which is yellowish brown; aerobically grown cultures of all species are white. Bacteriochlorophyll *a* is present in all species. Salt is not required by any of the species. All species can grow aerobically in the dark. The major quinones for all species are ubiquinone-10 and rhodoquinone-10. ND, No data available.

Characteristic	1	2	3	4	5
Isolation source	Paddy soil	Pond sediment	Activated sludge	Hot spring	Pond water
Cell size (µm)	1.0–1.2 × 3.0–5.0	1.0 × 2.0–2.5	0.8–1.0 × 2.0–3.0	1.0 × 2.5–4.0	0.8–1.0 × 2.0–3.0
Type of budding	Sessile	Sessile	Tube	Tube	Tube
Rosette formation	+	–	+	+	+
<i>In vivo</i> infrared absorption maxima (nm)	800, 866	801, 854	799, 855	801–802, 822–823, 857	800, 850
Optimal pH	7.0	7.0–7.5	7.0	6.8–7.2	5.5–9.0
Optimal temperature (°C)	30	30	30–35	40	35
Upper limit of temperature (°C)	35	38	40	45–46	43
Growth factors*	N, PABA, P	N	T, PABA	N, PABA, B ₁₂	None
Gas from nitrate	–†	+	+	+	+
DNA G + C content (mol%; HPLC)	67.2	66.8	69.6–69.7	68.8–69.0	69.5
Utilization of carbon/electron source (%)‡					
Caproate (0.1)	–	–	+ / –	ND	+
Ethanol (0.1)	–	–	–	ND	+
Glutamate (0.3)	–	–	–	–	+
Glycerol (0.3)	–	–	–	–	+
Yeast extract (0.1)	–	+	+	+	–
Propanol (0.1)	–	–	–	ND	–
Lactate (0.1)	+	+	+	–	+
Citrate (0.3)	+	+	+	–	+
Fumarate (0.3)	+	+	+	ND	+
Valerate (0.1)	–	+	+	ND	+
Tartrate (0.1)	–	+	+	–	+
Casamino acids (0.05)	+	+	+	ND	+
Peptone (0.05)	–	+	–	ND	+
Use of thiosulfate (2 mM) as an electron donor	–	+	+	–	+
Cellular fatty acids (%)§					
C _{16:0}	9.0	17.8	15.8	18.1	14.4
C _{16:1} ω7c alcohol	–	0.3	0.7	–	tr
C _{16:1} ω7c	1.9	4.0	1.6	tr	2.5
C _{18:0}	3.8	0.3	3.8	3.5	5.0
C _{18:1} ω7c	61.0	74.2	78.1	73.3	75.7
11-Methyl C _{18:1} ω7c	–	2.8	0.4	3.1	tr
C _{19:0} cyclo ω8c	5.5	–	–	2.1	–
anteiso-C _{19:0}	–	0.2	–	tr	2.3
C _{16:0} 3-OH	–	1.3	0.2	tr	–

*B₁₂, Vitamin B₁₂; N, niacin; P, pantothenate; PABA, *p*-aminobenzoate; T, thiamine.

†Denitrifying growth was observed without visible dinitrogen gas production.

‡+, Substrate utilized (OD 0.65–1.20); –, substrate not utilized (OD < 0.25); + / –, different results in different strains.

§tr, Trace amount (<0.5%); –, not detected/not reported.

(95.70%) and *Rpl. serenus* (94.47%) (Fig. 1). In addition to showing 16S rRNA gene sequence divergence, strain JA415^T showed clear phenotypic differences from recognized *Rhodoplanes* species, i.e. in morphology (forms

characteristic shapes), colour of cell suspension (brown, whereas others are red–pink), carotenoids (the presence of certain unidentified carotenoids), the requirement for pantothenate as a growth factor, substrate utilization and

Fig. 1. Dendrogram constructed using the PHYLIM method depicting the phylogenetic relationships between strain JA415^T and photosynthetic members of the order *Rhodospirillales* determined using 16S rRNA gene sequence analysis. Bootstrap values (%) are given at branch points. Bar, 2 substitutions per 100 nucleotide positions.

fatty acid composition (Table 1). These results justify the description of this strain as a representative of a novel species, for which the name *Rhodoplanes pokkaliisoli* sp. nov. is proposed.

Description of *Rhodoplanes pokkaliisoli* sp. nov.

Rhodoplanes pokkaliisoli (pok.kali.i.so'li. N.L. n. *pokkali*um pokkali (a variety of rice); L. n. *solum* soil; N.L. gen. n. *pokkaliisoli* of/from the soil of a pokkali field, from where the type strain was isolated).

Cells are rod-shaped, 1.0–1.2 µm wide and 3.0–5.0 µm long. Cells are motile by means of a single polar flagellum and multiply by budding and asymmetrical cell division without forming prosthecae. Cells form chains, but are also capable of associating with each other such that they form characteristic T, Y and V shapes and rosettes. Internal photosynthetic membranes are present as lamellar stacks parallel to the cytoplasmic membrane. Phototrophically grown cultures are yellowish brown, whereas aerobic cultures are white. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 380, 464, 491, 527, 596, 800 and 866 nm. Bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series (rhodopin, methyl rhodopin, lycopene and a few unidentified spirilloxanthins) are present. The type strain is mesophilic (range 25–30 °C) and grows at an optimum salinity (NaCl) of 1.5 % (range 0–2.5 %, w/v) and pH 7 (range pH 6.0–7.5). Growth occurs under anaerobic conditions in the light (photo-organoheterotrophy) or under aerobic conditions in the dark (chemo-organoheterotrophy). The preferred mode of growth is photo-organoheterotrophy with a few organic compounds. Acetate, butyrate, lactate, pyruvate, malate, fumarate, succinate, glycerol and crotonate are good carbon sources. 2-Oxoglutarate, valerate, caproate, ethanol, butanol, propanol, formate, caprylate, propionate, citrate, glycolate, benzoate, glucose, fructose, gluconate, mannitol, tartrate, aspartate, sucrose, methanol, sorbitol,

leucine, alanine, peptone, Casamino acids and yeast extract are not utilized. Photoautotrophic and chemoautotrophic growth do not occur with thiosulfate, H₂, sulfide, sulfur, sulfite or thioglycolate as the electron donor. Chemotrophic growth by denitrification under anoxic conditions in the dark in the presence of nitrate is observed. Fermentative growth does not take place in the presence of glucose, fructose or pyruvate as fermentable carbon source. Ammonium salts and glutamate are used as good nitrogen sources. Sulfate, sulfite, thiosulfate and methionine are used as sulfur sources. Niacin, pantothenate and *p*-aminobenzoate are required as growth factors. The predominant cellular fatty acid component is C_{18:1}ω7c. Ubiquinone-10 and rhodoquinone-10 are present as major quinones.

The type strain is JA415^T (=NBRC 104972^T =KCTC 5711^T), which was isolated from a soil sample from a pokkali rice field located on Vypeen Island, Ernakulam, Kerala, on the west coast of India. The genomic DNA G + C content of the type strain is 67.2 mol% (HPLC).

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