Rhodoplanes serenus sp. nov., a purple non-sulfur bacterium isolated from pond water

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A bright pink to red-coloured, phototropic, purple non-sulfur bacterium, designated strain TUT3530^T, was isolated from pond water. Cells of the novel isolate were found to be Gram-negative, motile, budding rods. Cell extracts from phototrophically grown cultures had absorption maxima at 378, 482, 512, 550, 590, 800 and 850 nm, indicating the presence of bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series. The intracytoplasmic membrane system was of the lamellar type. Anaerobic photo-organotrophy with simple organic acids such as pyruvate was the preferred mode of growth. Aerobic growth at full atmospheric oxygen tension and anaerobic denitrifying growth in darkness were also possible. Photolithotrophic growth occurred with thiosulfate, but not with sulfide or hydrogen, as the electron donor. No growth factors were required. The major whole-cell fatty acid was C_{18:1ω7c}. The major quinones were ubiquinone-10 and rhodoquinone-10. A phylogenetic analysis based on 16S rRNA gene sequences and studies involving DNA–DNA hybridization demonstrated that strain TUT3530^T occupies a distinct taxonomic position within the genus *Rhodoplanes*. On the basis of these data, strain TUT3530^T represents a novel species of the genus *Rhodoplanes*, for which the name *Rhodoplanes serenus* sp. nov. is proposed. The type strain is TUT3530^T (=DSM 18633^T=NBRC 102049^T).

The genus *Rhodoplanes* comprises a group of purple non-sulfur bacteria belonging to the *Alphaproteobacteria* and is characterized by the following: budding cell morphology, lamellar intracytoplasmic membranes, nitrate-respiring activity and the presence of ubiquinone-10 (Q-10) and rhodoquinone-10 (RQ-10) as the major quinones (Hiraishi & Ueda, 1994; Okamura *et al.*, 2007). This genus was created with the reclassification of *Rhodopseudomonas rosea* (Janssen & Harfoot, 1991) as the type species, *Rhodoplanes roseus*, and the description of another species, *Rhodoplanes elegans*. Recently, we proposed the transfer of '*Rhodopseudomonas cryptolactis*' (Stadtward-Demchick *et al.*, 1990) to the genus *Rhodoplanes* as '*Rhodoplanes cryptolactis*' (Okamura *et al.*, 2007). In this paper, we describe the taxonomic characteristics of a novel denitrifying, phototropic, purple non-sulfur bacterium, designated strain TUT3530^T, isolated from pond water, and propose that it represents a novel species of the genus *Rhodoplanes*.

Strain TUT3530^T was isolated from a water sample (containing mud sediment) collected from the edge of the Sanshiro-ike pond at The University of Tokyo (Tokyo, Japan) in April 1996. The pond water had a pH of 7.2 and a temperature of 24 °C when sampled. A small portion of the sample was inoculated into 20 ml screw-capped test tubes completely filled with PE, PYS or SAYS medium (Hanada *et al.*, 1995; Hiraishi & Ueda, 1994; Hisada *et al.*, 2007) and incubated at 30 °C under incandescent illumination (approx. 8 W m⁻²). After 2 weeks incubation, all of the enrichment cultures turned pink. A pink-coloured organism from an enrichment culture with PYS medium was then purified by means of the standard agar shake dilution method and repeated streaking of agar plates. The isolate thus obtained was designated strain TUT3530^T and was maintained routinely in PYS agar stabs and liquid medium. For comparison, *Rhodoplanes elegans* strain AS130^T (=JCM 9924^T), *Rhodoplanes roseus* strain DSM 5909^T and '*Rhodoplanes cryptolactis*' strain DSM 9987 were used. The strains with DSM numbers were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany), whereas *Rhodoplanes elegans* strain AS130^T came from our freezer stocks (Hiraishi & Ueda, 1994). For cultivation of the test organisms, PYS medium, which contained 20 mM pyruvate (filter-sterilized)

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TUT3530^T is AB087717.

Phase-contrast and transmission electron micrographs of cells and absorption spectra of cell extracts of strain TUT3530^T are available as supplementary material with the online version of this paper.

as the sole carbon source (Hiraishi & Ueda, 1994), was used. For growth of '*Rhodoplanes cryptolactis*' strain DSM 9987, the medium was modified by supplementation with vitamin B₁₂, nicotinic acid, *p*-aminobenzoic acid and Na₂S₂O₃·5H₂O (Hisada *et al.*, 2007). All media were adjusted to pH 6.8. Routine cultivation was performed anaerobically at 30 °C under incandescent illumination (10 W m⁻²). When grown under anaerobic conditions in the light, strain TUT3530^T produced pink, lens-shaped colonies on agar media and bright pink to red cell suspensions in liquid media.

Morphology and related properties were studied under an Olympus phase-contrast microscope and a JEOL transmission electron microscope. Intracytoplasmic structures were observed in ultrathin sections subjected to transmission electron microscopy, as described previously (Matsuzawa *et al.*, 2000). Strain TUT3530^T had Gram-negative, rod-shaped cells that were 0.8–1.0 µm wide and 2.0–3.0 µm long (see Supplementary Fig. S1a, available in IJSEM Online). Cells divided asymmetrically by budding and formed rosette-like clusters in older cultures. Cells were motile by means of single polar flagella (Supplementary Fig. S1b). Electron microscopy of ultrathin sections revealed that phototrophically grown cells of strain TUT3530^T had lamellar-type intracytoplasmic membranes parallel to the cytoplasmic membrane (Supplementary Fig. S1c) typical of phototrophic, purple non-sulfur bacteria belonging to the order *Rhizobiales* (Imhoff *et al.*, 2005). The presence of bacteriochlorophyll *a* and the carotenoids of the normal spirilloxanthin series was demonstrated by the absorption spectra obtained from a cell extract from phototrophic cultures, analysed using a Shimadzu Biospec 1600 spectrophotometer. Cell-free extracts from cultures grown at a low light intensity (2 W m⁻²) had absorption maxima at 378, 482, 512, 550, 590, 800 and 846 nm and a lower peak at 824–828 nm, whereas those from cultures grown with high light levels (20 W m⁻²) showed major peaks at 800 and 850 nm in the near-infrared region (see Supplementary Fig. S2, available in IJSEM Online). These spectroscopic features of strain TUT3530^T were similar to those of '*Rhodoplanes cryptolactis*' strain DSM 9987 (Okamura *et al.*, 2007; Stadtwald-Demchick *et al.*, 1990).

All tests for growth and physiological characteristics were performed as described previously (Hiraishi & Ueda, 1994; Okamura *et al.*, 2007), unless otherwise specified. Growth was checked turbidometrically at 660 nm, and the final reading was taken after 2 weeks incubation. Strain TUT3530^T was a mesophilic, neutrophilic, freshwater bacterium that exhibited best growth under anaerobic, photo-organotrophic growth conditions. Aerobic, chemo-organotrophic growth at full atmospheric oxygen tension was also possible. The temperature range for growth was 20–43 °C, with an optimum at 35 °C. The pH range for growth was 5.5–9.0, with an optimum at pH 8.0. Little or no growth was found with ≥1.0% NaCl. No growth factors were required. Photolithotrophic and chemolithotrophic growth was determined using PYS medium in which pyruvate was replaced with 0.5 mM Na₂S, 0.5 mM

Na₂S₂O₃ or 20% hydrogen (CO₂/H₂ at 101 kPa) as the electron donor. All of the test media used for lithotrophic growth were supplemented with filter-sterilized NaHCO₃ (0.1%, w/v) as the carbon source. Photolithotrophic growth occurred with thiosulfate, but not with sulfide or H₂, as the electron donor in the presence of 0.01% yeast extract. Neither significant aerobic, chemolithotrophic growth with thiosulfate nor anaerobic, fermentative growth with glucose was demonstrated. Anaerobic growth by nitrate respiration in darkness was determined using screw-capped test tubes completely filled with PYS medium supplemented with 20 mM KNO₃. N₂ gas production from complete denitrification was observed using these test tubes containing Durham tubes. As is common for *Rhodoplanes* species (Hiraishi & Ueda, 1994), strain TUT3530^T was able to grow anaerobically in darkness with nitrate as the terminal electron acceptor. Nitrate-respiring cells produced N₂ gas, thereby confirming their capacity to perform complete denitrification. The doubling time for strain TUT3530^T under denitrifying growth conditions was 15 h, a growth rate 1.5–2.0-fold greater than those for previously described species of *Rhodoplanes* (Hiraishi & Ueda, 1994).

Photoassimilation of organic substrates was investigated in screw-capped test tubes containing PYS medium in which pyruvate was replaced with an organic carbon source at a concentration recommended by Imhoff & Caumette (2004). Nitrogen-source utilization was determined in PYS medium by replacing the (NH₄)₂SO₄ with different nitrogen sources at a concentration of 0.1% (w/v). Strain TUT3530^T utilized a number of simple organic compounds as electron donor and carbon sources under phototrophic growth conditions, including acetate, propionate, butyrate, valerate, caproate, pyruvate, lactate, citrate, succinate, fumarate, malate, malonate, tartrate, glycerol, ethanol, asparagine, aspartate, glutamate, glutamine, yeast extract, peptone and Casamino acids. The following were not utilized: formate, caprylate, pelargonate, palmitate, gluconate, benzoate, nicotinate, D-xylose, L-arabinose, L-rhamnose, fructose, glucose, galactose, mannose, sucrose, trehalose, mannitol, sorbitol, dulcitol, methanol, propanol, alanine, leucine and methionine. Ammonium, molecular nitrogen, nitrate, glutamate, glutamine and urea were utilized as nitrogen sources, whereas nitrite did not support growth. A capacity for nitrogen fixation was also suggested by the observation that hydrogen gas was produced in test tubes with Durham tubes containing ammonium-starved PYS medium under phototrophic growth conditions. Sulfate was assimilated as a sulfur source.

Whole-cell fatty acids were extracted and their methyl esters were analysed and identified by means of GLC using Sherlock Microbial Identification System software (MIS; MIDI). The analysis was performed by TechnoSuruga Lab (Shizuoka, Japan). Like recognized *Rhodoplanes* species (Okamura *et al.*, 2007), strain TUT3530^T contained C_{18:1}ω7c as the main fatty acid component (75.7% of the total content). C_{16:0} (14.4%) constituted a significant proportion of the total. C_{18:0}, (5.0%), C_{16:1}ω7c (2.5%),

anteiso- $C_{19:0}$ (2.3 %), $C_{16:1\omega7c}$ alcohol (<0.5 %) and 11-methyl $C_{18:1\omega7c}$ (<0.5 %) constituted minor components. Quinones were extracted from cells with a chloroform/methanol mixture, purified by TLC and analysed using HPLC (Hiraishi & Hoshino, 1984). Strain TUT3530^T contained both Q-10 and RQ-10 as major quinone components; menaquinones were absent.

The 16S rRNA gene sequence of strain TUT3530^T was PCR-amplified and determined as described previously (Hiraishi, 1992; Hisada *et al.*, 2007). The sequence was compared with those retrieved from the GenBank/EMBL/DBJ database. Multiple alignment of the sequences, calculation of the corrected evolutionary distance (Kimura, 1980) and construction of a neighbour-joining phylogenetic tree (Saitou & Nei, 1987) were performed using CLUSTAL W, version 1.83 (Thompson *et al.*, 1994). The topology of the tree was evaluated using bootstrapping based on 1000 resamplings (Felsenstein, 1985). The phylogenetic analysis showed that strain TUT3530^T formed a separate branch within the cluster of the genus *Rhodoplanes* (Fig. 1). This novel strain showed a sequence similarity of 96.6 % with respect to *Rhodoplanes elegans* AS130^T, 95.6 % with respect to *Rhodoplanes roseus* DSM 5909^T and 95.8 % with respect to '*Rhodoplanes cryptolactis*' DSM 9987. Genomic DNA was extracted and purified according to the method of Marmur (1961) and its G+C content was determined using the HPLC method with external nucleotide standards (Mesbah *et al.*, 1989). The G+C content of the genomic DNA of strain TUT3530^T was 69.5 mol%, similar to those of *Rhodoplanes elegans* and '*Rhodoplanes cryptolactis*'. Genomic DNA–DNA hybridization studies were performed using the dot-blot hybridization method with alkaline phosphatase labelling and chemiluminescence detection with an Amersham-Pharmacia AlkalPhos kit (Hiraishi *et al.*, 2002). Strain TUT3530^T had hybridization levels of 30 % with respect to

Rhodoplanes elegans AS130^T, 20 % with respect to *Rhodoplanes roseus* DSM 5909^T and 35 % with respect to '*Rhodoplanes cryptolactis*' DSM 9987.

As reported here, it is clear that strain TUT3530^T represents a species of the genus *Rhodoplanes* that is genotypically and phenotypically distinguishable from the established species of this genus (i.e. *Rhodoplanes elegans*, *Rhodoplanes roseus* and '*Rhodoplanes cryptolactis*'). As shown in Table 1, one of the most characteristic features of strain TUT3530^T is that its spectrum of usable carbon sources is much wider than those of the known *Rhodoplanes* species. On the basis of the data from this study, strain TUT3530^T represents a novel species of the genus *Rhodoplanes*, for which the name *Rhodoplanes serenus* sp. nov. is proposed.

Description of *Rhodoplanes serenus* sp. nov.

Rhodoplanes serenus (se're.nus. L. adj. *serenus* clear, bright, pertaining to the bright-pink culture).

Cells are Gram-negative, rod-shaped, 0.8–1.0 µm wide and 2.0–3.0 µm long. Multiplication is by budding and asymmetrical cell division during which a division tube occurs between the mother and daughter cells. Motile by means of single polar flagella. Rosette-like clusters are formed in older cultures. Intracytoplasmic photosynthetic membranes are of the lamellar type and are parallel to the cytoplasmic membrane. Phototrophic cultures are bright pink to red in colour, whereas aerobic cultures are pale pink. Phototrophically grown cells at high light intensities have absorption maxima at 378, 482, 512, 550, 590, 800 and 850 nm. Anaerobic photo-organotrophy is the preferred mode of growth. Aerobic growth at full atmospheric oxygen tension is detected. Anaerobic growth in darkness with nitrate as the terminal electron acceptor is also possible and denitrification is detected. The temperature range for growth is 20–43 °C (optimum, 35 °C). The pH range for phototrophic growth is 5.5–9.0 (optimum, pH 8.0). Little or no growth occurs with ≥1 % NaCl. No growth factors are required. Photolithotrophic growth occurs with thiosulfate, but not with sulfide or H₂, as the electron donor. Photoassimilates the following organic substrates: acetate, propionate, butyrate, valerate, caproate, glycerol, ethanol, pyruvate, lactate, citrate, malate, malonate, fumarate, succinate, tartrate, asparagine, aspartate, glutamate, glutamine, yeast extract, peptone and Casamino acids. The following are not utilized: formate, caprylate, pelargonate, palmitate, gluconate, benzoate, nicotinate, L-arabinose, D-xylose, L-rhamnose, fructose, glucose, sucrose, mannose, galactose, trehalose, dulcitol, mannitol, sorbitol, methanol, propanol, alanine, methionine and leucine. Ammonium, nitrate, glutamate, glutamine, urea and molecular nitrogen are utilized as nitrogen sources, but nitrite is not. Sulfate is assimilated as a sulfur source. $C_{18:1\omega7c}$ is the major cellular fatty acid. The major quinones are Q-10 and RQ-10. The DNA G+C content of the type strain is 69.5 mol% (by HPLC).

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain TUT3530^T and related strains of purple non-sulfur bacteria. Accession numbers are given in parentheses. Bootstrap percentages (based on 1000 trials) are given at branch points. *Rhodoblastus acidophilus* ATCC 25092^T was used as an outgroup to root the tree. Bar, 1 % sequence divergence (K_{nuc}).

Table 1. Differential characteristics of strain TUT3530^T and the type strains of recognized species of the genus *Rhodoplanes*

Strains: 1, TUT3530^T (*Rhodoplanes serenus* sp. nov.); 2, *Rhodoplanes roseus* DSM 5909^T; 3, *Rhodoplanes elegans* AS130^T; 4, '*Rhodoplanes cryptolactis*' DSM 9987. Data are from Hiraishi & Ueda (1994), Janssen & Harfoot (1991), Okamura *et al.* (2007), Stadtwald-Demchick *et al.* (1990) and this study. All strains utilize each of the following compounds as an electron donor and carbon source: acetate, butyrate, pyruvate, succinate and malate. The following are not utilized by any of the strains: benzoate, L-arabinose, D-xylose, L-rhamnose, D-fructose, D-glucose, D-galactose, D-mannose, D-mannitol, D-sorbitol, D-dulcitol and L-alanine. +, Positive; (+), weakly positive; –, negative or absent; ND, not determined; tr, trace amount (<0.5 %).

Characteristic	1	2	3	4
Cell width (µm)	0.8–1.0	1.0	0.8–1.0	1.0
Cell length (µm)	2.0–3.0	1.8–2.5	2.0–3.5	2.5–4.0
Type of budding	Tube	Sessile	Tube	Tube
Rosette formation	+	–	+	+
<i>In vivo</i> absorption maxima in near-infrared region (nm)	800, 850	801, 854	799, 855	801–802, 822–823, 857
pH range (optimum) for growth	5.5–9.0 (7.5–8.0)	6.5–8.0 (7.0–7.5)	6.0–8.5 (7.0)	6.4–8.5 (7.0)
Temperature range (optimum) for growth (°C)	20–43 (35)	(30)	(30–35)	25–46 (40)
Vitamin requirement(s)	None	Niacin	Thiamine, <i>p</i> -aminobenzoic acid	Vitamin B ₁₂ , <i>p</i> -aminobenzoic acid, niacin
Sulfate assimilation	+	+	+	–
Electron donor and carbon source				
Organic acids				
Formate	–	–	–	ND
Propionate	+	+	+	(+)
Valerate	+	+	+	ND
Caproate	+	–	+	ND
Caprylate	–	ND	ND	ND
Pelargonate	–	ND	ND	ND
Palmitate	–	ND	ND	ND
Lactate	+	+	+	–
Citrate	+	+	+	–
Fumarate	+	+	+	ND
Tartrate	+	+	+	–
Nicotinate	–	ND	ND	ND
Malonate	+	–	–	ND
Glycolate	–	–	–	ND
Gluconate	–	ND	ND	ND
Sugars and alcohols				
Sucrose	–	ND	ND	ND
Trehalose	–	ND	ND	ND
Glycerol	+	–	–	ND
Methanol	–	–	–	ND
Ethanol	+	–	–	ND
Propanol	–	–	–	ND
Other compounds				
Asparagine	+	–	–	ND
Aspartate	+	–	–	–
Glutamate	+	–	–	–
Glutamine	+	ND	ND	–
Leucine	–	–	–	ND
Methionine	–	ND	ND	ND
Peptone	+	+	+	ND
Casamino acids	+	+	+	ND
Thiosulfate	+	+	+	–
Major fatty acid composition (%)				
C _{16:0}	14.4	17.8	15.8	18.1
C _{16:1} ω7c alcohol	tr	tr	0.7	–

Table 1. cont.

Characteristic	1	2	3	4
C _{16:1} ω7c	2.5	4.0	1.6	tr
C _{18:0}	5.0	tr	3.8	3.5
C _{18:1} ω7c	75.7	74.2	78.1	73.3
11-Methyl C _{18:1} ω7c	tr	2.8	tr	3.1
C _{19:0} ω8c cyclo	—	—	—	2.1
anteiso-C _{19:0}	2.3	tr	—	tr
C _{16:0} 3-OH	—	1.3	tr	tr
Quinone composition (%)				
Q-10	85	+	+	75
RQ-10	14	+	+	24
DNA G + C content (mol%)	69.5	66.8	69.7	69.9
Isolation source	Pond water	Freshwater lake sediment	Activated sludge	Hot spring

The type strain, TUT3530^T (=DSM 18633^T=NBRC 102049^T), was isolated from pond water.

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