Rhodoblastus sphagnicola sp. nov., a novel acidophilic purple non-sulfur bacterium from *Sphagnum* peat bog

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An isolate of purple non-sulfur bacteria was obtained from an acidic *Sphagnum* peat bog and designated strain RS^T. The colour of cell suspensions of this bacterium growing in the light under anaerobic conditions is purplish red. Cells of strain RS^T are rod-shaped, 0.8–1.0 µm wide and 2.0–6.0 µm long, motile by means of polar flagella, reproduce by budding and have a tendency to form rosette-like clusters in older cultures. The cells contain lamellar intracytoplasmic membranes underlying, and parallel to, the cytoplasmic membrane. The photosynthetic pigments are bacteriochlorophyll *a* and carotenoids; the absorption spectrum of living cells shows maxima at 377, 463, 492, 527, 592, 806 and 867 nm. The cells grow photoheterotrophically under anaerobic or microaerobic conditions with various organic carbon sources or grow photolithoautotrophically with H₂ and CO₂. Strain RS^T is a moderately acidophilic organism exhibiting growth at pH values between 4.8 and 7.0 (with an optimum at pH 5.2–5.5). The major fatty acids are 16:1 ω 7c and 18:1 ω 7c; the major quinones are Q-10 and Q-9. The DNA G + C content of strain RS^T is 62.6 mol%. Analysis of the 16S rRNA gene sequence revealed that the novel isolate is most closely related (97.3 % sequence similarity) to the type strain ATCC 25092^T of the moderately acidophilic purple non-sulfur bacterium *Rhodoblastus acidophilus*, formerly named *Rhodopseudomonas acidophila*. However, in contrast to *Rbl. acidophilus*, strain RS^T is not capable of aerobic growth in the dark, has no spirilloxanthin among the carotenoids and differs in the pattern of substrate utilization. The value for DNA–DNA hybridization between strain RS^T and *Rbl. acidophilus* ATCC 25092^T is only 22 %. Thus, strain RS^T represents a novel species of the genus *Rhodoblastus*, for which the name *Rhodoblastus sphagnicola* sp. nov. is proposed. Strain RS^T (=DSM 16996^T = VKM B-2361^T) is the type strain.

The purple non-sulfur bacteria (PNSB) are a highly diverse and metabolically flexible group of anoxygenic phototrophic bacteria that grow phototrophically under anoxic conditions in the light or chemotrophically under micro-oxic to oxic conditions in the dark (Imhoff, 2001a). These phototrophic micro-organisms belong to the *Alphaproteobacteria* and *Betaproteobacteria* but do not form monophyletic groups within these phyla (Woese *et al.*, 1984;

Stackebrandt *et al.*, 1988; Kawasaki *et al.*, 1993; Imhoff & Trüper, 1992; Imhoff, 2001a). Instead, many representatives of the PNSB are closely related to non-phototrophic, strictly chemotrophic bacteria.

PNSB are widely distributed in various aquatic ecosystems as well as in sediments, moist soils, natural wetlands and paddy fields (Pratt & Gorham, 1970; Burke *et al.*, 1974; Imhoff, 2001a). Stagnant water bodies with significant amounts of soluble organic matter and low oxygen tension are the preferred habitats for these bacteria. *Sphagnum* peat bogs exemplify this kind of environment. However, in contrast to other freshwater habitats, peat bogs are rarely reported as sources of PNSB. The low pH (3.5–5.5) and extremely low mineral salt contents (5–50 mg l⁻¹ in peat water) are possible reasons for this. So far, taxonomically

Abbreviation: PNSB, purple non-sulfur bacteria.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RS^T is AM040096.

The absorption spectrum of a cell homogenate of phototrophically grown strain RS^T and a graph showing the influence of pH on growth of strain RS^T are available as supplementary material in IJSEM Online.

characterized PNSB from this acidic habitat are represented by only one strain, strain 3251, which was isolated from a *Sphagnum* peat bog near Kolshorn/Hannover (Germany) and subsequently described as a strain of *Rhodopseudomonas acidophila* (Pfennig, 1969). Later, this taxon was reclassified as *Rhodoblastus acidophilus* (Imhoff, 2001b). Here, we describe another example of PNSB isolation from acidic *Sphagnum* peat.

In the course of a study on cellulose degradation in *Sphagnum* peat, we detected intensive development of some phototrophic purple bacteria in anaerobic cellulolytic enrichments incubated under light. Several strains of PNSB that were morphologically and phenotypically similar to *Rbl. acidophilus* were isolated from these enrichments. These strains had the same morphology and physiological traits and possessed identical 16S rRNA gene sequences: thus, only one of these isolates was studied in detail. These phenotypic and genotypic studies revealed a number of features that distinguished *Rbl. acidophilus* from the newly isolated strain. Thus, we conclude that it represents a novel species of the genus *Rhodoblastus*.

The sample used in our study was collected from 5–10 cm below the surface of an acidic peat (pH 3.5–4.2) underlying the *Andromeda–Eriophorum–Sphagnum* plant community in the raised centre of the Sosvyatskoe ombrotrophic bog located in Tver Region, West Dvinskiy district, at the field station of the Institute of Forestry, Russian Academy of Sciences (56° 10' N 32° 12' E).

Anaerobic cellulolytic communities were enriched using screw-cap 120 ml serum bottles containing 30 ml liquid mineral medium of the following composition [g (l distilled water)⁻¹]: KH₂PO₄, 0.04; NH₄Cl, 0.1; MgCl₂·6H₂O, 0.01; CaCl₂·2H₂O, 0.05, with the addition of 0.1% (w/v) cellulose, 0.5% (v/v) trace element stock solution (Pfennig & Lippert, 1966) and 0.1% (v/v) vitamin stock solution (Wolin *et al.*, 1963). The initial pH of the medium was 5.5. The inoculation was done with 100 mg peat, and the bottles were closed with silicone-rubber septa, flushed with sterile N₂ and incubated at 25 °C in the light (2000 lx). As soon as visual development of purple phototrophic bacteria in these enrichments occurred, an aliquot was taken for pure-culture isolation. This was achieved by means of repeated deep-agar (0.8%) dilution series on succinate mineral medium (Pfennig, 1969). An isolate obtained was designated strain RS^T. Culture purity was ensured by examination under phase-contrast microscopy and by the absence of colourless colonies in a column of agar succinate mineral medium. Physiological tests were performed in liquid medium no. 27 recommended by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) under anoxic conditions in the light (2000 lx). *Rbl. acidophilus* ATCC 25092^T was used as a reference strain. Growth of strain RS^T was monitored by nephelometry at 600 nm for 7–10 days under a variety of conditions, including temperatures of 10–37 °C, pH 4.0–7.5 and NaCl concentrations of 0.01–3.0% (w/v). Variations in

the pH were achieved by mixing 0.1 M solutions of HCl and KOH. The range of potential growth substrates for strain RS^T was examined using 0.02–0.05% (w/v) concentrations of the following carbon sources: acetate, arginine, benzoate, butyrate, caproate, citrate, ethanol, formate, fructose, glucose, glutamate, glycerol, lactate, malate, malonate, mannitol, propionate, pyruvate, succinate, tartrate and valerate. The capacity to utilize methanol was determined at a range of concentrations from 0.01 to 3% (v/v). Tests for photolithotrophic growth with molecular hydrogen and carbon dioxide were performed in screw-capped 120 ml serum bottles containing 30 ml mineral medium no. 27 with a gas phase of H₂/CO₂ (80:20, v/v). Growth was examined after 7–10 days incubation under light by comparison with a negative control. Tests for the ability to grow in the dark under aerobic conditions were performed using both liquid and agar succinate mineral medium (Pfennig, 1969). The capacity to develop in nitrogen-free media was tested using liquid mineral medium no. 27 without yeast extract or NH₄Cl.

For the preparation of ultrathin sections, cells of exponentially growing cultures were collected by centrifugation and pre-fixed with 1.5% (w/v) glutaraldehyde in 0.05 M cacodylate buffer (pH 6.5) for 1 h at 4 °C and then fixed in 1% (w/v) OsO₄ in the same buffer for 4 h at 20 °C. After dehydration in an ethanol series, the samples were embedded in a Spurr epoxy resin. Thin sections were cut on an LKB-4800 microtome and then stained with 3% (w/v) uranyl acetate in 70% (v/v) ethanol. The specimen samples were examined with a JEM-100C transmission electron microscope (JEOL).

The absorption spectra of living cells were recorded with a LOMO SPH-56 spectrophotometer. For these measurements, the cells were suspended in 50% glycerol. In addition, pigments were extracted with acetone/methanol (1:1, v/v) and the absorption spectra of these extracts were recorded. For estimation of the pigment content, cells were harvested at approximately early stationary growth phase by centrifugation. Carotenoids were analysed by HPLC as described previously (Sidorova *et al.*, 1998). Satisfactory separation of the pigments was achieved using a Spherisorb ODS2 column (250 × 4.6 mm, 5 µm; Waters). The solvent flow rate was 1.0 ml min⁻¹. The column was equilibrated with a mixture composed of 77% acetonitrile/water (9:1, v/v) and 23% ethyl acetate for 5 min. The mixture was then linearly substituted by ethyl acetate. The total separation time varied from 25 to 30 min depending on the carotenoid composition. Pigment fractions were collected automatically and their absorption spectra were recorded on a Shimadzu UV-160 spectrophotometer (Toropygina *et al.*, 2003). Quantitative estimations of the individual carotenoids were made using the absorbance coefficients given by Davies (1976) and Heinemeyer & Schmidt (1983). For fatty acid and respiratory quinone analyses, cells of strains RS^T and *Rbl. acidophilus* ATCC 25092^T were grown on succinate mineral medium and harvested in the late exponential

Fig. 1. (a) Phase-contrast micrograph of cells of strain RS^T grown anaerobically in the light for 2 weeks. (b) Negatively stained young cell of strain RS^T showing a single polar flagellum. (c) Electron micrograph of an ultrathin section showing lamellar intracytoplasmic membranes. Bars: 10 μm (a) and 0.5 μm (b, c).

growth phase. Analyses were performed by the Identification Service of the DSMZ. Genomic DNA from strain RS^T was extracted by using the method of Marmur (1961). The DNA G+C content was determined by means of thermal denaturation using a Unicam SP1800 spectrophotometer (at a heating rate of $0.5\text{ }^{\circ}C\text{ min}^{-1}$) and calculated according to Owen *et al.* (1969). DNA–DNA hybridization of strain RS^T and *Rbl. acidophilus* ATCC 25092^T was performed as described by De Ley *et al.* (1970). PCR-mediated amplification of the 16S rRNA gene from position 28 to 1491 (numbering according to the International Union of Biochemistry nomenclature for *Escherichia coli* 16S rRNA) was performed using primers Eub9f and Eub1492r and the reaction conditions described by Weisburg *et al.* (1991). The 16S rRNA gene amplicons were purified using QIAquick spin columns (Qiagen) and sequenced on an ABI Prism 377 DNA sequencer using BigDye terminator chemistry, as specified by the manufacturer (PE Applied Biosystems). Phylogenetic analysis was carried out using the ARB program package (Ludwig *et al.*, 2004).

Photosynthetically grown liquid cultures of strain RS^T were purplish red in colour. Cells of this isolate were Gram-negative, large rods, $0.8\text{--}1.0\text{ }\mu m$ in width and $2.0\text{--}6.0\text{ }\mu m$ in length (Fig. 1a, b). They showed polar growth and they reproduced by budding. No tube or filament was formed between mother and daughter cells. Young cells were motile by means of a single polar flagellum, while in older cultures cells were non-motile and had a tendency to form rosette-like clusters. Electron microscopy of ultrathin sections revealed the presence of internal photosynthetic membranes appearing as lamellae underlying, and parallel to, the cytoplasmic membrane (Fig. 1c).

The photosynthetic pigments of strain RS^T were bacteriochlorophyll *a* and carotenoids. The absorption spectrum of

living cells showed maxima at 377, 463, 492, 527, 592, 806 and 867 nm. The latter two peaks and the peak at 377 nm are characteristic of bacteriochlorophyll *a* (see Supplementary Fig. S1 in IJSEM Online). The main absorption maxima detected in acetone/methanol extracts were at 803 and 863 nm (data not shown). Carotenoid analysis performed using HPLC identified rhodopin and rhodopinal as the major carotenoids (57.7% of total carotenoid content) of strain RS^T (Table 1). Lycopene was the next greatest component (12.9%), followed by rhodopinol (10.9%), lycopenal (6.4%), rhodopinol glucoside (5.6%) and lycopenal glucoside (5.1%). A high content of carotenoid glucosides (38–40%) was shown to be characteristic of *Rbl. acidophilus* (Schmidt, 1971; Heinemeyer & Schmidt, 1983). However, in contrast to *Rbl. acidophilus* ATCC 25092^T, our isolate did not contain spirilloxanthin.

Table 1. Carotenoid compositions of acidophilic phototrophic isolate RS^T and *Rbl. acidophilus* ATCC 25092^T

Values are percentages of total carotenoids; ND, not determined. Data for *Rbl. acidophilus* ATCC 25092^T were taken from Heinemeyer & Schmidt (1983).

Carotenoid	Strain RS^T	<i>Rbl. acidophilus</i> ATCC 25092 ^T
Rhodopin	28.7	25.0
Rhodopinol	10.9	0.5
Rhodopinal	29.0	5.0
Rhodopinol glucoside	5.6	38.0
Lycopene	12.9	5.0
Lycopenal	6.4	1.0
Lycopenal glucoside	5.1	ND
Spirilloxanthin	0	5.0

Table 2. Cellular fatty acid composition of strain RS^T and *Rbl. acidophilus* ATCC 25092^T

Values are percentages of total fatty acids; ND, not detected.

Fatty acid	Strain RS ^T	<i>Rbl. acidophilus</i> ATCC 25092 ^T
14:0	0.35	0.45
15:0	0.60	ND
16:1 ω 7c	45.40	46.78
16:1 ω 5c	0.31	0.17
16:0	8.68	11.36
16:0 3-OH	0.79	3.08
17:0	0.23	ND
iso-17:0 3-OH	0.15	0.45
18:1 ω 7c	42.16	35.02
18:0	0.87	0.63
20:1 ω 7c	0.19	0.16
20:0	0.35	0.22

Strain RS^T grew under anaerobic or microaerobic conditions in the light. No growth occurred under aerobic conditions in the dark. The following organic compounds served as carbon sources: formate, acetate, propionate, butyrate, pyruvate, valerate, malate, succinate, glycerol, methanol and ethanol. Best growth (OD₆₀₀ up to 0.7–0.9) was observed on butyrate and propionate. Methanol was utilized over a concentration range of 0.01–1 % (v/v), with the optimum between 0.05 and 0.1 %. Slow growth was also detected on caproate, lactate and malonate. No growth occurred on glucose, fructose, citrate, benzoate, tartrate, mannitol, glutamate or arginine. Growth factors were not required, but the presence of yeast extract slightly increased the growth rate. Sulfate was assimilated. Good growth was

observed on nitrogen-free medium. Thus, the nitrogen sources were ammonia and dinitrogen; nitrate was not utilized. Strain RS^T was capable of photolithoautotrophic growth with molecular hydrogen and carbon dioxide. Hydrogen sulfide and thiosulfate were not utilized.

The isolate grew in the pH range 4.8–7.0, with a pH optimum of 5.2–5.5 (Supplementary Fig. S2). The temperature optimum for growth was 25–30 °C. Growth inhibition of 50 % was observed in the presence of 1 % (w/v) NaCl in the medium, whereas 2 % NaCl inhibited growth completely.

The cellular fatty acid composition of strain RS^T is shown in Table 2. Similarly to *Rbl. acidophilus*, the major components of the phospholipid fatty acid profile of strain RS^T were straight-chain, monounsaturated 9-*cis*-hexadecenoic acid (16:1 ω 7c) and 11-*cis*-octadecenoic acid (18:1 ω 7c), which comprised 45.4 and 42.2 %, respectively, of the total phospholipid fatty acid content. However, in comparison with that of *Rbl. acidophilus* ATCC 25092^T, the phospholipid fatty acid profile of the novel isolate contained a smaller percentage of 16:0 fatty acids and contained minor amounts (<1 %) of some other saturated fatty acids, such as 15:0 and 17:0. Cells of strain RS^T contained ubiquinones Q-10 and Q-9, which comprised 92 and 8 %, respectively, of the total quinone content. In contrast to *Rbl. acidophilus*, strain RS^T did not contain any menaquinones or rhodoquinones (Table 3).

Comparative sequence analysis of the 16S rRNA gene revealed that strain RS^T belongs to the *Alphaproteobacteria* (Fig. 2). This novel phototrophic isolate is most closely related to the acidophilic phototrophic non-sulfur bacterium *Rbl. acidophilus* ATCC 25092^T (97.3 % sequence similarity). Other closely related micro-organisms, exhibiting

Table 3. Major characteristics that differentiate *Rhodoblastus sphagnicola* sp. nov. and *Rbl. acidophilus*

Data for *Rbl. acidophilus* are from Pfennig (1969), Imhoff & Trüper (1992) and Imhoff (2001b).
+, Positive; –, negative; W, weakly positive.

Characteristic	<i>Rbl. sphagnicola</i> RS ^T	<i>Rbl. acidophilus</i>
Colour of culture	Purplish red	Red to orange-red
Cell diameter (µm)	0.8–1.0	1.0–1.3
pH optimum	5.2–5.5	5.5–6.0
DNA G+C content (mol%)	62.6	65.3* (62.2–66.8)
Aerobic growth in the dark	–	+
Spirilloxanthin present in carotenoids	–	+
Major quinones†	Q-10, Q-9	Q-10, MK-10, RQ-10
Citrate photoassimilation	–	W
Glycerol photoassimilation	+	–
Phototrophic growth on butyrate	Good	Poor

*Value for the type strain, *Rbl. acidophilus* ATCC 25092^T. The range of values reported for other strains is given in parentheses.

†MK, Menaquinone; Q, ubiquinone; RQ, rhodoquinone.

butyrate and propionate. Methanol is utilized at a wide range of concentrations from 0.01 to 1% (v/v). No growth occurs with benzoate, glucose, fructose, tartrate, citrate or glutamate. Nitrogen sources are N₂ and ammonia. Photoautotrophic growth is possible with hydrogen as electron donor; sulfide and thiosulfate cannot be used. Growth factors are not required, though yeast extract increases the growth rate. No aerobic growth occurs in the dark. The major phospholipid fatty acids are 16:1 ω 7c and 18:1 ω 7c. Contains ubiquinones Q-10 and Q-9. Mesophilic, moderately acidophilic, with optimum growth at 25–30 °C and pH 5.2–5.5. NaCl inhibits growth at concentrations above 1% (w/v). The DNA G + C content is 62.6 mol%.

The type strain, RS^T (=DSM 16996^T=VKM B-2361^T), was isolated from an acidic *Sphagnum* peat bog (Sosvyatskoe), Tver Region, Russia.

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