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 RST. The colour of cell suspensions of this bacterium growing in the light under anaerobic conditions is purplish red. Cells of strain RST 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 RST 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 16:1ω7c and 18:1ω7c; the major quinones are Q-10 and Q-9. The DNA G+C content of strain RST 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 25092T of the moderately acidophilic purple non-sulfur bacterium Rhodoblastus acidophilus, formerly named Rhodopseudomonas acidophila. However, in contrast to Rbl. acidophilus, strain RST 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 RST and Rbl. acidophilus ATCC 25092T is only 22%. Thus, strain RST represents a novel species of the genus Rhodoblastus, for which the name Rhodoblastus sphagnicola sp. nov. is proposed. Strain RST (= DSM 16996T = VKM B-2361T) 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 microaerobic 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...
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 (Pfenning, 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 (1 distilled water)]: 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 (Pfenning & 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 enrichment. Several strains of phototrophic purple bacteria in anaerobic cellulolytic enrichments incubated under light. 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.

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For the preparation of ultrathin sections, cells of exponen
tially 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).
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 °C min⁻¹) and calculated according to Owen et al. (1969). DNA–DNA hybridization of strain RS*T and *Rbl. acidophilus* ATCC 25092T 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, 0·8–1·0 μm in width and 2·0–6·0 μm 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 %), lycopental (6·4 %), rhodopinal 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 25092T, our isolate did not contain spirilloxanthin.

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

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Strain RS*T</th>
<th>Rbl. acidophilus ATCC 25092T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodopin</td>
<td>28·7</td>
<td>25·0</td>
</tr>
<tr>
<td>Rhodopinol</td>
<td>10·9</td>
<td>0·5</td>
</tr>
<tr>
<td>Rhodopinal</td>
<td>29·0</td>
<td>5·0</td>
</tr>
<tr>
<td>Rhodopinal glucoside</td>
<td>5·6</td>
<td>38·0</td>
</tr>
<tr>
<td>Lycopene</td>
<td>12·9</td>
<td>5·0</td>
</tr>
<tr>
<td>Lycopenal</td>
<td>6·4</td>
<td>1·0</td>
</tr>
<tr>
<td>Lycopenal glucoside</td>
<td>5·1</td>
<td>ND</td>
</tr>
<tr>
<td>Spirilloxanthin</td>
<td>0</td>
<td>5·0</td>
</tr>
</tbody>
</table>
Table 2. Cellular fatty acid composition of strain RST and Rbl. acidophilus ATCC 25092T

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

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Strain RST</th>
<th>Rbl. acidophilus ATCC 25092T</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.35</td>
<td>0.45</td>
</tr>
<tr>
<td>15:0</td>
<td>0.60</td>
<td>ND</td>
</tr>
<tr>
<td>16:1o7c</td>
<td>45.70</td>
<td>46.78</td>
</tr>
<tr>
<td>16:1o5c</td>
<td>0.31</td>
<td>0.17</td>
</tr>
<tr>
<td>16:0</td>
<td>8.68</td>
<td>11.36</td>
</tr>
<tr>
<td>16:0 3-OH</td>
<td>0.79</td>
<td>3.08</td>
</tr>
<tr>
<td>17:0</td>
<td>0.23</td>
<td>ND</td>
</tr>
<tr>
<td>iso-17:0 3-OH</td>
<td>0.15</td>
<td>0.45</td>
</tr>
<tr>
<td>18:1o7c</td>
<td>42.16</td>
<td>35.02</td>
</tr>
<tr>
<td>18:0</td>
<td>0.87</td>
<td>0.63</td>
</tr>
<tr>
<td>20:1o7c</td>
<td>0.19</td>
<td>0.16</td>
</tr>
<tr>
<td>20:0</td>
<td>0.35</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Strain RST 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) 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 growth. Sulfate was assimilated. Good growth was observed on nitrogen-free medium. Thus, the nitrogen sources were ammonia and dinitrogen; nitrate was not utilized. Strain RST 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 RST is shown in Table 2. Similarly to Rbl. acidophilus, the major components of the phospholipid fatty acid profile of strain RST were straight-chain, monounsaturated 9-cis-hexadecenoic acid (16:1o7c) and 11-cis-octadecenoic acid (18:1o7c), which comprised 45.4 and 42.2 %, respectively, of the total phospholipid fatty acid content. However, in comparison with that of Rbl. acidophilus ATCC 25092T, 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 RST contained ubiquinones Q-10 and Q-9, which comprised 92 and 8 %, respectively, of the total quinone content. In contrast to Rbl. acidophilus, strain RST did not contain any menaquinones or rhodopinones (Table 3).

Comparative sequence analysis of the 16S rRNA gene revealed that strain RST belongs to the Alphaproteobacteria (Fig. 2). This novel phototrophic isolate is most closely related to the acidophilic phototrophic non-sulfur bacterium Rbl. acidophilus ATCC 25092T (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.

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

†Menaquinone; Q, ubiquinone; RQ, rhodoquinone.

* Value for the type strain, Rbl. acidophilus ATCC 25092T. The range of values reported for other strains is given in parentheses.
overall 16S rRNA gene sequence similarity of 94 % with respect to strain RST, are acidophilic methanotrophic bacteria of the genera Methylocella and Methylocapsa and acidophilic heterotrophic bacteria of the genus Beijerinckia. Interestingly, most known representatives of the genera Methylocella and Methylocapsa were also isolated from acidic Sphagnum peatlands (Dedysh et al., 2000, 2002, 2004). Together, these phototrophic, heterotrophic and methano-
trophic bacteria of the genera Rhodoblastus, Beijerinckia, Methylocella and Methylocapsa form a monophyletic cluster, which is supported by a bootstrap value of 97 % (Fig. 2). All members of this phylogenetic cluster are moderately acidophilic organisms that inhabit acidic, boggy waters and soils and have a pH optimum for growth of about 5-5. A meta-
bolic trait common to all organisms of this phylogenetic group, including those in the genus Beijerinckia (Dedysh et al., 2005), is the capacity for methylotrophic growth.

The DNA G+C content of strain RST was found to be 62.6 mol%. The corresponding value for the type strain (ATCC 25092\textsuperscript{T}) of Rbl. acidophilus, reported previously (Mandel et al., 1971) and also determined in our experiments, is 65.3 mol%, while the DNA G+C contents of other strains of this species range from 62.6 to 66.8 mol% (Pfenng, 1969; Imhoff, 2001b). The DNA–DNA hybridization value for strain RST and Rbl. acidophilus ATCC 25092\textsuperscript{T} was only 22 %. This low level of genomic DNA relatedness suggests a differentiation of the two strains at the species level, which is also supported by the results of some physiological tests. Strain RST should therefore be assigned to a novel species of the genus Rhodoblastus, for which the name

Rhodoblastus sphagnicola sp. nov.

Rhodoblastus sphagnicola (sphag.ni’co.la. N.L. n. Sphagnum generic name of sphagnum moss; L. suff. -cola from L. n. incola inhabitant, dweller; N.L. n. sphagnicola inhabitant of Sphagnum).

Cells are rod-shaped, straight or slightly curved, 0.8–1.0 µm wide and 2.0–6.0 µm long, motile by polar flagella in young cultures and have a tendency to form rosette-like clusters in older cultures. Cells reproduce by budding. No tube or filament is formed between mother and daughter cells. Cells contain lamellar intracytoplasmic photosynthetic membranes underlying, and parallel to, the cytoplasmic membrane. The colour of anaerobic liquid cultures is pur-

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**Fig. 2.** Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain RST in relation to Rbl. acidophilus ATCC 25092\textsuperscript{T}, acidophilic methanotrophs of the genera Methylocella and Methylocapsa, acidophilic heterotrophic bacteria of the genus Beijerinckia and some other representative members of the Alphaproteobacteria. The root was determined by using the 16S rRNA gene sequence of Pseudomonas stutzeri A1501 (GenBank accession no. AF143245) as the outgroup (not shown). Bootstrap percentages (from 1000 data resamplings) greater than 50 % are shown. Bar, 0.1 substitutions per nucleotide position.
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. Photosynthetic 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 16:1ω7c and 18:1ω7c. 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, RSᵀ (= DSM 16996ᵀ = VKM B-2361ᵀ), was isolated from an acidic *Sphagnum* peat bog (Sosvatskoe), Tver Region, Russia.

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References


