Sandarakinorhabdus limnophila gen. nov., sp. nov., a novel bacteriochlorophyll \(a\)-containing, obligately aerobic bacterium isolated from freshwater lakes

Frederic Gich and Jörg Overmann

Three strains (so36, so42\(^T\) and wo26) representing a novel Gram-negative, obligately aerobic, bacteriochlorophyll \(a\)-containing species of the \(\alpha\)-4 subgroup of the Proteobacteria were isolated from freshwater lakes using a high-throughput cultivation technique. The non-motile and slender rod-shaped cells formed orange–red-pigmented colonies. The main carotenoids were nostoxanthin and keto-nostoxanthin. According to the absorption spectrum, two different photosynthetic light-harvesting complexes, an LHI complex and a B800–830-type peripheral LHII complex, were present in the cells. The predominant fatty acids of strain so42\(^T\) were hexadecenoic acid (16 : 1\(v_7\)) and octadecenoic acid (18 : 1\(o_7\)), whereas 17 : 1\(o_6\)c and 14 : 0 iso 2-OH were present in smaller amounts. The main polar lipids were phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, diphosphatidylglycerol, glycolipid and sphingoglycolipids. The major respiratory lipoquinone was ubiquinone-10, whereas ubiquinone-9 was present in smaller amounts. The three strains were cytochrome oxidase-negative and catalase-positive and formed alkaline and acid phosphatases. The strains grew chemoorganoheterotrophically in mineral media supplemented with various organic acids, amino acids or complex substrates such as peptone and yeast extract. The G+C content of the genomic DNA of strain so42\(^T\) was 64.3 mol%. The three novel isolates contained the same 16S rRNA gene sequence. The 16S rRNA gene sequence similarity to the closest phylogenetic relative Sandaracinobacter sibiricus was only 92.8%. Accordingly, the three strains represent a new genus and species, for which the name Sandarakinorhabdus limnophila gen. nov., sp. nov., is proposed, with strain so42\(^T\) (=DSM 17366\(^T\) = CECT 7086\(^T\)) as the designated type strain.

Aerobic, phototrophic, bacteriochlorophyll (BChl) \(a\)-containing bacteria have been isolated from a wide range of different environments and the spectrum of their ecological niches ranges from cryptoendolithic communities in Antarctica to symbiotic associations with microalgae (Yurkov & Beatty, 1998; Allgaier et al., 2003; de la Torre et al., 2003). Despite their broad geographical distribution, the ecology and biogeochemical significance of aerobic, anoxygenic, phototrophic bacteria have only been studied in the marine environment, where they can constitute 10–30% of the total bacterial community and account for up to 5% of the photosynthetic electron transport (Shiba et al., 1991; Kolber et al., 2001; Béjà et al., 2002). In comparison, much less is known about the diversity and function of this bacterial group in freshwater lakes (Kolber et al., 2000; Page et al., 2004). Until very recently, freshwater representatives of aerobic, anoxygenic, phototrophic bacteria had been isolated exclusively from nutrient-rich sediment environments (Yurkov & Beatty, 1998), such as cyanobacterial mats in warm or hot springs (Sandaracinobacter, Erythromonas, Erythromicrobium, Roseococcus and Porphyrobacter) or from the surfaces of sub-tropical ponds (Porphyrobacter), river water (Roseateles) and acidic mineral environments and mine drainages (Acidiphilium and Acidisphaera). All planktonic species of marine and freshwater, aerobic, phototrophic bacteria isolated to date are affiliated with either the Rhodobacteraceae (\(\alpha\)-3 subgroup of the Proteobacteria) or the \(\beta\)-1 subgroup of the Proteobacteria (Yurkov, 2001). Very recently, however, two planktonic BChl \(a\)-containing species (Rhodobacter sp. HTCC515 and the betaproteobacterium HTCC2528) were isolated from the ultraoligotrophic Crater Lake, OR, USA (Page et al., 2004). Freshwater environments may therefore harbour a greater diversity and abundance of aerobic, anoxygenic, phototrophic bacteria isolated from freshwater lakes using a high-throughput cultivation technique. The non-motile and slender rod-shaped cells formed orange–red-pigmented colonies. The main carotenoids were nostoxanthin and keto-nostoxanthin. According to the absorption spectrum, two different photosynthetic light-harvesting complexes, an LHI complex and a B800–830-type peripheral LHII complex, were present in the cells. The predominant fatty acids of strain so42\(^T\) were hexadecenoic acid (16 : 1\(v_7\)) and octadecenoic acid (18 : 1\(o_7\)), whereas 17 : 1\(o_6\)c and 14 : 0 iso 2-OH were present in smaller amounts. The main polar lipids were phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, diphosphatidylglycerol, glycolipid and sphingoglycolipids. The major respiratory lipoquinone was ubiquinone-10, whereas ubiquinone-9 was present in smaller amounts. The three strains were cytochrome oxidase-negative and catalase-positive and formed alkaline and acid phosphatases. The strains grew chemoorganoheterotrophically in mineral media supplemented with various organic acids, amino acids or complex substrates such as peptone and yeast extract. The G+C content of the genomic DNA of strain so42\(^T\) was 64.3 mol%. The three novel isolates contained the same 16S rRNA gene sequence. The 16S rRNA gene sequence similarity to the closest phylogenetic relative Sandaracinobacter sibiricus was only 92.8%. Accordingly, the three strains represent a new genus and species, for which the name Sandarakinorhabdus limnophila gen. nov., sp. nov., is proposed, with strain so42\(^T\) (=DSM 17366\(^T\) = CECT 7086\(^T\)) as the designated type strain.

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aerobic, anoxygenic, phototrophic bacteria than that appre-
ciated to date.

In the present study, we describe the first phototrophic,
planktonic freshwater species representing a novel lineage
within the α-4 subgroup of the Proteobacteria to be isolated
in pure culture. The novel phylotype was also detected in situ
by PCR-denaturing gradient gel electrophoresis fingerprinting in a previous study (Gich et al., 2005). Dot-blot
hybridization with genomic probes demonstrated that the
isolates constitute 0.5–2.3% of the natural bacterioplank-
ton community (Gich et al., 2005). Furthermore, the 16S
rRNA gene sequence of the isolates was detected in lakes of all
trophic states (oligotrophic to eutrophic) (Gich et al.,
2005). The novel type of bacterium thus appears to represent
a typical and widely distributed constituent of freshwater
bacterioplankton. Future studies will reveal whether aerobic,
anoxygenic, phototrophic bacteria of the α4-subgroup of the
Proteobacteria are of significance for the carbon cycle in
freshwater lakes.

Bacterial strains were isolated from the mesotrophic pre-
alpine Starnberger See (25 km south-west of Munich,
Germany) and from the oligotrophic alpine Walchensee
(near Garmisch-Partenkirchen, 50 km south of Munich), as
described previously (Bruns et al., 2003; Gich et al.,
2005). Purification of the colonies was performed on agar plates
containing synthetic freshwater medium and a ten-vitamin
mixture (Bartscht et al., 1999) and 1% (w/v) peptone. Cell
morphology was examined by using an inverse phase-
contrast microscope (Axiovert 200; Zeiss). The three isolates
grew as single cells and formed orange–red-pigmented
colonies with a smooth surface on synthetic freshwater agar
medium. Colonies of strain so42T were bigger (1–3 mm)
than those of strains so36 and wo26 (usually ≤0.5 mm). In
addition, the colonies of strain so42T had a mucilaginous
texture because of the production of extracellular slime. The
strains could be stored at −80°C in 50% glycerol or 7%
DMSO for at least 7 months. The cells were non-motile
rods and reproduced by binary fission (Fig. 1a). During
exponential growth, cells were 0.32 ± 0.09 × 1.55 ± 0.44 μm
(strain so36), 0.31 ± 0.06 × 0.80 ± 0.19 μm (strain so42T)
and 0.26 ± 0.05 × 2.33 ± 0.83 μm (strain wo26) (Table 1).
Cells of strain so42T elongated upon entry into the stationary
phase (Fig. 1b), becoming 0.24 ± 0.03 × 3.24 ± 0.81 μm.
No change in cell volume occurred upon transfer of cultures
from low-nutrient artificial freshwater medium to media
containing high concentrations of complex substrates.
Electron microscopy of ultrathin sections indicated that
the cells possessed a typical Gram-negative cell wall (Fig. 1c).
No intracytoplasmic membrane systems were detected. Many
cells contained a single electron-dense polar granule, most
probably consisting of polyphosphate.

Absorption spectra were monitored with a Lambda 25 UV/
VIS spectrophotometer (Perkin Elmer). Absorption spectra
of whole cells in sucrose (Fig. 2) were similar for all three
strains and exhibited maxima at 430–490 and 800–865 nm,
indicating the presence of carotenoids and BChl a,
respectively. Absorption maxima at 800 and 865 nm and
the shoulder at 837 nm (Fig. 2b) are characteristic for two

![Phase-contrast photomicrographs of cells of isolate so42T during exponential growth (a) and in stationary phase (b). (c) Ultrathin section of exponentially grown cells of strain so42T stained with 1% uranyl acetate. The outer membrane (OM), cytoplasmic membrane (CM) and intracellular granules (G) can be distinguished. Bars, 5 μm (a, b) and 0.5 μm (c).](https://www.microbiologyresearch.org/article/56/848)
different photosynthetic, light-harvesting complexes, LHI (865 nm) and a B800-830-type LHII (800–837 nm). LHII is present in only a few and very distantly related aerobic phototrophic bacteria, namely *Erythromicrobium ramosum*, *Erythromicrobium ezovicum* and *Erythromicrobium hydrolyticum*, and is missing from *Sandaracinobacter sibiricus* and other species (Yurkov *et al.*, 1997; Yurkov & Beatty, 1998). Because the novel type of bacterium was found in lakes of all trophic states, the presence of a photosynthetic apparatus is not simply an adaptation to oligotrophic environments. This is supported by the observation that the isolates grew in mineral media supplemented with up to 1 % peptone and

### Table 1. Main morphological, biochemical and physiological characteristics of the three isolates of *Sandarakinorhabdus limnophila* gen. nov., sp. nov., compared with *Sandaracinobacter sibiricus* RB16-17<sup>T</sup>

Data for *Sandaracinobacter sibiricus* taken from Yurkov & Gorlenko (1990). ND, Not determined. All strains were isolated from a freshwater environment and are Gram-negative with rod-shaped cells. No information on enzymic activities (other than those involved in glycolysis and the tricarboxylic acid cycle), polar lipids or fatty acid composition is available for *Sandaracinobacter sibiricus* RB16-17<sup>T</sup>.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>so36</th>
<th>so42&lt;sup&gt;T&lt;/sup&gt;</th>
<th>wo26</th>
<th><em>Sandaracinobacter sibiricus</em> RB16-17&lt;sup&gt;T&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>Planktonic</td>
<td>Planktonic</td>
<td>Planktonic</td>
<td>Benthic</td>
</tr>
<tr>
<td>Cell size (exp. phase):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Width (μm)</td>
<td>0·32 ± 0·09</td>
<td>0·31 ± 0·06</td>
<td>0·26 ± 0·05</td>
<td>0·3–0·5</td>
</tr>
<tr>
<td>Length (μm)</td>
<td>1·55 ± 0·44</td>
<td>0·80 ± 0·19</td>
<td>2·33 ± 0·83</td>
<td>1·5–2·5</td>
</tr>
<tr>
<td>Volume (μm³)</td>
<td>0·15 ± 0·09</td>
<td>0·08 ± 0·05</td>
<td>0·14 ± 0·06</td>
<td>ND</td>
</tr>
<tr>
<td>Capsule formation</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ (3 subpolar flagella)</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Colour</td>
<td>Orange–red</td>
<td>Orange–red</td>
<td>Orange–red</td>
<td>Yellow–orange</td>
</tr>
<tr>
<td>In vivo absorption maxima of carotenoids (nm)</td>
<td>420, 460, 480</td>
<td>420, 460, 480</td>
<td>420, 460, 480</td>
<td>424, 450, 474</td>
</tr>
<tr>
<td>Carotenoid composition</td>
<td>Nostoxanthin, keto-nostoxanthin, 2 unidentified</td>
<td>Nostoxanthin, keto-nostoxanthin, 2 unidentified</td>
<td>Nostoxanthin, keto-nostoxanthin, 2 unidentified</td>
<td>ND</td>
</tr>
<tr>
<td>LHI (nm)</td>
<td>865</td>
<td>865</td>
<td>865</td>
<td>867</td>
</tr>
<tr>
<td>LHII (nm)</td>
<td>800, 837 (shoulder)</td>
<td>800, 837 (shoulder)</td>
<td>800, 837 (shoulder)</td>
<td>–</td>
</tr>
<tr>
<td>Quinone</td>
<td>ND</td>
<td>Ubiquinone-9 (8 %), ubiquinone-10 (92 %)</td>
<td>ND</td>
<td>Ubiquinone-9, ubiquinone-10</td>
</tr>
<tr>
<td>Main polar lipids*</td>
<td>ND</td>
<td>DPG, PG, PE, PC, SGL, GL</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>ND</td>
<td>64·3</td>
<td>ND</td>
<td>68·5 (facultative photoheterotroph)</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Strictly aerobic</td>
<td>Strictly aerobic</td>
<td>Strictly aerobic</td>
<td>Strictly aerobic (facultative photoheterotroph)</td>
</tr>
<tr>
<td>Peptone, optimum (% w/v)</td>
<td>0·1</td>
<td>1·0</td>
<td>1·0</td>
<td>ND</td>
</tr>
<tr>
<td>Yeast extract, optimum (% w/v)</td>
<td>0·1</td>
<td>0·1</td>
<td>0·1</td>
<td>ND</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose (5/2·5 mM)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Fructose (5/5·5 mM)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acetate (5/12 mM)</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Propionate (5/10 mM)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Pyruvate (5/9 mM)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Butyrate (2·5/9 mM)</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Succinate (10/4 mM)</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lactate (10/9 mM)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fumarate (5/6 mM)</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Methanol (5/31 mM)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*DPG, Diphostatidglycerol; GL, glycolipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; SGL, sphingoglycolipids.

†Concentrations used for the substrate test: isolates *Sandaracinobacter sibiricus*.
hence are not obligate oligotrophs. Carotenoid analysis was performed by HPLC according to method B of Airs et al. (2001). Individual carotenoids were identified according to their retention time and absorption spectra by co-chromatography of pigment extracts of the reference strains Erythrobacter longus DSM 6997T and Erythromicrobium ramosum DSM 8510T. The strains contained BChl a esterified with phytol (BChl aP). Based on HPLC analyses, three major carotenoids were present. All three strains contained nostoxanthin as a major carotenoid. Strains so36 and wo26 had an almost identical pigment composition and contained keto-nostoxanthin as a second major carotenoid. In addition, the three strains contained two unidentified carotenoids that are novel among the aerobic, anoxygenic phototrophic bacteria, an unidentified carotenoid. In addition, the three strains contained two unidentified carotenoids that are novel among the aerobic, anoxygenic phototrophic bacteria, an unidentified carotenoid.

Results of the chemotaxonomic analyses are given in the species description. The following analytical procedures were performed. Respiratory lipoquinones and polar lipids were extracted from 2 and 0·1 g, respectively, freeze-dried cell material and analysed according to Tindall (1990a, b). Polar lipid analyses were carried out by the Identification Services and B. J. Tindall, DSMZ. For fatty acid analysis, 40 mg (wet weight) of cells was scraped from Petri dishes and the fatty acid methyl esters were extracted by using the method of Miller (1982) and Kuykendall et al. (1988). DNA G+C content was analysed according to Mesbah et al. (1989). The polar lipid fingerprints of the isolates so36, so42T and wo26 obtained by two-dimensional TLC were similar to those of Sphingomonas phyllosphaerae FA2T (Rivas et al., 2004) and were identified by their chromatographic behaviour and staining characteristics (Fig. 3). The fatty acid composition is summarized in Table 2. Interestingly, only one saturated fatty acid without hydroxy groups was detected in strain so42T (16 : 0), whereas a variety of saturated fatty acids are commonly found in the alphaproteobacteria (Denner et al., 2002; Rainey et al., 2003; Yoon et al., 2003, 2004). The similarity index of the fatty acid patterns of strain so42T and the closest related species present in the MIDI database, Novosphingobium aromaticivorans F199T, was 0·04. Since a similarity value of >0·30 is indicative of strains belonging to the same species, the fatty acid analyses provide independent evidence for an isolated phylogenetic position of the three strains.

Gram-staining and catalase and oxidase tests were performed by using conventional methods (Gerhardt, 1994). Strains so36, so42T and wo26 were Gram-negative and tested negative for cytochrome oxidase and were catalase-positive. Enzymic activities were determined with the API ZYM test system (bioMérieux), according to the manufacturer’s protocol. The three isolates produced the same spectrum of enzyme activities (Table 3) except for trypsin and β-galactosidase, which were only detected as weak activities in the isolates so42T and wo26, respectively. The three isolates showed a strong positive reaction in tests for alkaline and acid phosphatases, which is consistent with an adaptation to limiting phosphate concentrations in Walchensee and Starnberger See (Chrost, 1991; Gich et al., 2005).

The strains were characterized further by using aerobic growth tests in microtitre plates using synthetic freshwater medium supplemented with 69 individual carbon substrates.
Table 2. Cellular fatty acid composition (%) of isolate so42T, N. aromaticivorans F199T, Erythrobacter citreus DSM 14432T, Erythromicrobium ramossum DSM 8510T, Roseisalinus antarcticus DSM 11466T and Porphyrobacter cryptus DSM 12079T

<table>
<thead>
<tr>
<th>Compound</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated fatty acids</td>
<td>16:0</td>
<td>6.99</td>
<td>3.3</td>
<td>8.2</td>
<td>3.0</td>
<td>15.1</td>
</tr>
<tr>
<td>Unsaturated fatty acids</td>
<td>16:1o5c</td>
<td>2.33</td>
<td>0.3</td>
<td>3.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydroxy fatty acids</td>
<td>14:0 iso 2-OH</td>
<td>9.32</td>
<td>26.7</td>
<td>0.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Summed feature*</td>
<td>35.07</td>
<td>2.8</td>
<td>-</td>
<td>-</td>
<td>2.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*Summed features represent a group of fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 contained 16:1o7c and/or 15:0 iso 2-OH. Feature 2 contained 18:2o6,9c, 18:0 anteiso and/or 18:2o6,9c.

Table 3. Enzymes detected in strains so36, so42T and wo26

<table>
<thead>
<tr>
<th>Enzyme assay</th>
<th>so36</th>
<th>so42T</th>
<th>wo26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatase</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Esterase lipase (C8)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Leucine arylamidase</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2-Chymotrypsin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Results of API ZYM tests. ++ +, Very strong positive reaction; ++, strong positive reaction; +, weakly positive reaction. The following tests were negative for all strains: lipase (C14), z-galactosidase, z-glucuronidase, z-glucosidase, z-glucosidase, N-acetyl-z-glucosaminidase, z-mannosidase, z-fucosidase.

in two parallels. Negative controls devoid of substrates or without inoculum were used throughout. After incubation for 8 weeks in the dark at 15 °C, substrate utilization was assessed by measuring the optical density at 620 nm in each well using a microtitre plate reader (TECAN Sunrise Remote Control). Fermentative metabolism and anaerobic respiration with nitrate or sulfate were studied using standard anaerobic culturing techniques (Miller & Wolin, 1974). The isolates grew as obligately aerobic chemoorganoheterotrophs. Fermentation or anaerobic growth with nitrate or sulfate were not detected. However, the three strains differed with respect to carbon utilization (see Supplementary Table S1 in IJSEM Online). Since our strains were isolated from oligotrophic freshwater lakes with low organic carbon content, two concentrations of fatty acids and complex substrates were tested with respect to possible inhibition of growth. All three strains grew readily with crotonate, propionate and valerate, but did not utilize most alcohols (nine of ten) or sugars (29 of 31) tested. The only commonly used sugar and amino acid in all three strains were glucose and ( + )-1-cysteine, respectively. Strain so36 did not use any of the tested oxo-acids. All three strains were capable of growing on complex organic substrates such as Casamino acids, yeast extract and peptone, but no growth was observed when fermented rumen extract was used, even at low concentrations (0.001 %). Maximum growth rates were achieved for all three strains at 0.1 % [460 (mg C) l-1] yeast extract. Strains so42T and wo26 showed a higher tolerance towards peptone [maximum growth rates observed at 1 % peptone, corresponding to 4·3 g (mg C) l-1], whereas the maximum growth rate of strain so36 was observed at 0·1 % peptone [0·4 (g C) l-1]. The minimum doubling times recorded for the strains were between 12·1 and 16·8 h. When precultures grown at low substrate concentrations were inoculated into these complex media with high substrate concentrations, lag phases of up to 80 h occurred.

The 16S rRNA genes were amplified for each of the three strains using primers 8f and 1492r (Lane, 1991). The PCR products were separated from free PCR primers, purified using the QiAquick Spin kit (Qiagen) and sequenced directly employing eight primers to cover the entire 16S rRNA gene (Gich et al., 2005), using an ABI Prism BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems) and an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Sequences were analysed using the ARB software package (Ludwig et al., 2004). Additional sequences of close relatives of other more recently described BChl a-containing alphaproteobacteria present in GenBank were retrieved from the database employing BLAST 2.0.4 (Altschul et al., 1997) and imported into the ARB database. The Fast Aligner V1.03 tool was used for automatic sequence alignment with previously aligned sequences from the ARB database. The alignment was subsequently checked and corrected manually based on secondary-structure information. Phylogenetic trees were constructed using maximum-likelihood and neighbour-joining methods and bootstrap values were calculated to determine the robustness of the clusters. Sequence similarities were calculated using the ARB
distance matrix. A comparison of the almost-complete 16S rRNA gene sequences (positions 60–1458, *Escherichia coli* numbering) revealed that all three isolates contained identical sequences. Maximum-likelihood and neighbour-joining analyses led to the same results (Fig. 4). Based on the phylogenetic analysis, the isolates belong to the family *Sphingomonadaceae*, with *Sandaracinobacter sibiricus* strain RB16-17T, an aerobic BChl *a*-containing bacterium that forms an isolated phylogenetic branch among non-photosynthetic members of the *α*-4 subgroup of the *Proteobacteria*, representing the most closely related species with a validly published name. The latter was isolated from a microbial mat in freshwater near hydrothermal sulfide-containing vents on the bottom of the Bol’shoi river (Yurkov & Gorlenko, 1990). The sequence similarity of isolates so36, so42T and wo26 to *Sandaracinobacter sibiricus* was 92 ± 8%, which clearly indicates an independent phylogenetic lineage. A 16S rRNA gene sequence divergence of 3% is commonly used as a criterion for the separation of two bacterial species (Stackebrandt & Goebel, 1994). Bacterial genera typically show ≤ 93% sequence similarity of the 16S rRNA gene. Numerous cytological, biochemical and physiological characteristics (Table 1) support the conclusion that the three isolates represent a novel bacterial genus. Accordingly, a new genus and species, *Sandarakinorhabdus limnophila* gen. nov., sp. nov., is proposed. Genomic fingerprints generated with ERIC-PCR or repetitive extragenic palindromic DNA (REP)-PCR primers (de Bruijn, 1992) ranged from 6000 to 400 bp (Fig. 5). According to both analyses, strains so36 and wo26 are more similar to each other than to strain so42T.

**Description of *Sandarakinorhabdus* gen. nov.**

*Sandarakinorhabdus* (San.da.ra’ki.no.rhab.dus. Gr. adj. sandarakinos of orange colour; Gr. fem. n. rhabdos rod; N.L. fem. n. *Sandarakinorhabdus* orange-coloured rod).

Cells are non-spore-forming, non-motile rods that do not form a capsule and are Gram-negative. They reproduce by binary fission and form orange–red-pigmented colonies with smooth surfaces on agar plates. Cells may contain polyphosphate granules and exhibit strong enzymic activity for acid and alkaline phosphatases. Cytochrome oxidase-negative and catalase-positive. Obligately aerobic chemooorganoheterotrophs. Fermentation or anaerobic growth with nitrate or sulfate are not detected. Produce Bchl *a*. Grow in the presence of a number of short-chain organic acids and amino acids as electron donors and carbon sources. Grow well in media containing 0–1% peptone or yeast extract. 16S rRNA gene sequence information places the genus within the *α*-4 subgroup of the *Proteobacteria*, with *Sandaracinobacter* as its phylogenetic neighbour. The type species is *Sandarakinorhabdus limnophila*.
Description of *Sandarakinorhabdus limnophila* sp. nov.

*Sandarakinorhabdus limnophila* [lim.no ‘phi.la. Gr. n. limnos (or limné) lake, pool of standing water; Gr. adj. philos loving; N.L. fem. adj. limnophila lake-loving, isolated from a freshwater lake].

General characteristics are the same as those given in the description of the genus. Rods are 0.31 ± 0.06 x 0.80 ± 0.19 μm with a biovolume of 0.08 μm³. Facultatively oligotrophic, grows as single cells and forms orange–red colonies of 1–3 mm with mucilaginous texture on agar plates. Cells absorb at 430–490 nm and at 800, 837 and 865 nm, because of the presence of carotenoids and BChl absorb at 430–490 nm and at 800, 837 and 865 nm, respectively. Nostoxanthin is the main carotenoid. In addition, two unidentified carotenoids containing keto groups are present. Kedo-nostoxanthin may also be present in some strains. Identified carotenoids containing keto groups are present. Nostoxanthin is the main carotenoid. In addition, two unidentified carotenoids containing keto groups are present. Keto-nostoxanthin may also be present in some strains.

The type strain is so42T (= DSM 17366T = CECT 7086T), which was isolated from the mesotrophic freshwater lake Starnberger See (Bavaria, Germany).

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**References**


Porphyrobacter cryptus

Porphyrobacter doghaensis

Porphyrobacter japonicum

Porphyrobacter sibiricus

Porphyrobacter ubique

Porphyromonas deficiens

Porphyromonas gingivalis