Rhodoferax saidenbachensis sp. nov., a psychrotolerant, very slowly growing bacterium within the family *Comamonadaceae*, proposal of appropriate taxonomic position of *Albidiferax ferrireducens* strain T118^T in the genus *Rhodoferax* and emended description of the genus *Rhodoferax*

René Kaden,[1] Cathrin Spröer,[2] Daniel Beyer[3] and Peter Krolla-Sidenstein[4]

Correspondence
Rene Kaden
rene.kaden@sva.se

[1]National Veterinary Institute SVA, Ulls väg 2b, 75189 Uppsala, Sweden

[2]Leibnitz Institute DSMZ, Inhoffenstr. 7b, 38124 Braunschweig, Germany

[3]UFZ Centre for Environmental Research, Permoserstr. 15, 04318 Leipzig, Germany

[4]Karlsruhe Institute of Technology, Institute of Functional Interfaces Division, PO Box 3640, D-76021 Karlsruhe, Germany

A Gram-stain-negative, oxidase and phosphatase-positive and catalase-negative, short rod-shaped bacterium was isolated from sediment of a drinking water reservoir in Germany. Based on 16S rRNA gene sequence and phenotypic properties, the bacterium belongs to the genus *Rhodoferax* within the family *Comamonadaceae*. The new taxon differed from related species mainly with respect to its fatty acid composition, low growth temperature, lack of pigments in young cultures and ability to utilize glycerol and D-mannose but not urea. The major fatty acids were $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, $C_{16:0}$, and $C_{18:1}\omega7c$. The only ubiquinone detected was ubiquinone Q-8. The DNA G+C content was 60.3–61 mol%. Because of the phenotypic and genotypic differences from the most closely related taxa, the new strain represents a novel species for which the name *Rhodoferax saidenbachensis* sp. nov. is proposed. The type strain is ED16^T (=CCUG 57711^T=ATCC BAA-1852^T=DSM 22694^T). An emended description of the genus *Rhodoferax* is proposed. Based on the results of this study, strain T118^T (*Albidiferax ferrireducens*) is properly placed in the genus Rhodoferax as *Rhodoferax ferrireducens*.

The oligotrophic Saidenbach reservoir (50° 44′ 9″ N 13° 14′ 3″ E) with a retaining capacity of 22.3 million m^3 and a watershed of 60.7 km^2 is one of the largest drinking water reservoirs in Saxony, Germany. During microbial screening of the sediment and pelagial, with the aim of displaying temporal differences in the composition of the population during one year, an unknown organism related to the family *Comamonadaceae* was isolated and cultured. The environmental sampling parameters in the hypolimnion were 8.38 mg O_2 l^{-1}, a conductivity of 220 µS cm^{-1}, a temperature of 3.8 °C and pH 6.73 (Kaden, 2009).

Abbreviation: ANI, average nucleotide identity.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the whole genome sequence of strain ED16^T are FJ755906 and AWQR00000000, respectively.

The family *Comamonadaceae* within the class *Betaproteobacteria* (Garrity *et al.*, 2006) was first described by Willems *et al.* (1991). At the time of writing, besides the genera *Comamonas* (De Vos *et al.*, 1985), *Variovorax* (Willems *et al.*, 1991), *Rhodoferax* (Hiraishi *et al.*, 1991), *Curvibacter* (Ding & Yokota, 2004) and *Albidiferax* (Ramana & Sasikala, 2009), altogether more than 30 genera belong to this family. Species of the genus *Rhodoferax* are all vibroid or slightly curved Gram-negative rods. They are especially characterized by the occurrence of quinone type Q-8 or rhodoquinone RQ-8 and the presence of the major fatty acids $C_{16:1}$ and $C_{16:0}$ as well as $C_{18:1}$ (Hiraishi *et al.*, 1991). All bacteria of this genus described to date are able to grow in a phototrophic manner and produce a peach–brown pigment. The only species of the most closely related genus, *Albidiferax ferrireducens*, was isolated from brackish water and might, therefore, be tolerant of salinity.

A sample of water from the hypolimnion of the Saidenbach reservoir was autoclaved with pure agar added to a final concentration of 1 % (w/v). A 20 ml sample of the water was saved prior to this procedure and stored at 4 °C. Of this sample, 100 µl was plated out on the water/agar mixture. After 3 days, colonies were transferred into 1 ml physiological NaCl solution and 100 µl plated out on R2A agar (Difco). After a few passages to establish a pure culture with growth times of approximately 5 days, bacteria were cultivated in liquid R2A medium to increase biomass. All tests were performed at least in duplicate.

Motility was investigated using semi-solid R2A medium, and the presence of flagella was determined using an atomic force microscope with a cantilever with a spring constant of 0.9–1.75 N m^{-1}, a scan rate 0.8 Hz and scan points=scan lines=1024 (Binnig *et al.*, 1986). Cell dimensions were measured using light microscopy (Zeiss Axioskop) and atomic force microscopy on native cultures as described.

The Biolog system provides a microtitre plate with 95 different carbon sources. Active cells that are able to utilize the carbon sources transform 2,3,5-triphenyletetrazolium chloride to red formazane. First, 7.5 ml of the biomass from a culture in liquid R2A medium with an OD 590 of 0.4 was centrifuged for 5 min at 3000 ***g***. After decanting the supernatant, the pellet was transferred into physiological PBS with a final concentration of 10^7 to 10^8 bacteria ml^{-1}, estimated using the McFarland standard protocol (McFarland, 1907) and confirmed afterwards by a plate count after cultivation on R2A. Furthermore, 150 µl bacterial solution was pipetted into every well of a Biolog GN2 MicroPlate. During incubation for 20 days at 20 °C, OD was determined daily using a Tecan Scanphotometer Sunrise Basic at λ=590 nm and the software Magellan V.5.0.0 2005. API ZYM and API 20NE kits (BioMérieux) were used to determine the enzyme activity and other physiological properties of the strain. API 20NE was incubated for 7 days at 20 °C. API ZYM kits were evaluated after incubation for 1, 4 and 7 days at 20 °C. Long-term incubation of API 20NE, API ZYM and Biolog GN2 was necessary because of a cell division rate of about one per 24 h.

In addition to the carbon utilization and enzyme activity tests, bacteria were plated out on the solid media R2A, nutrient agar, tryptic soy agar (TSA), Kligler iron, urea, citrate, TTC-Chapman, MacConkey, Baird Parker, Endo, cetrimide, Oxidative-Fermentative (OF) agar with glucose (all Difco), Leifson and bile aesculin (Merck). Liquid media tested were lactose peptone bouillon (Difco) and DEV tryptophan bouillon (Merck).

The Voges–Proskauer reaction (Barritt, 1936) was also tested. Furthermore, the optimal temperature for growth was determined by incubating on R2A medium and increasing the temperature in 5 °C intervals between 4 and 35 °C. The optimal pH range for growth was investigated by measuring the OD at 595 nm of cultures in liquid R2A medium with pH 3–9 after incubation for 6 days at 20 °C. NaCl tolerance was tested in the same way, but at pH 6 and with a NaCl concentration of 0.9–3.9 % (w/v).

The presence of oxidase was detected using Bactident oxidase test strips (Merck). Catalase content was tested by using 3 % H_2O_2 on a colony placed on a microscope slide and observing whether O_2 bubbles emerged.

Fatty acid analyses were performed at German Culture Collection DSMZ in Braunschweig according to the methods of Miller (1982) and Kuykendall *et al.*, (1988). In preparation of fatty acid composition analysis the bacteria were cultured in liquid R2A medium for 14 days at 20°C until reaching the stationary growth phase. Analyses of ubiquinones were carried out by the Identification Service of the DSMZ using the method described by Tindall (1990a; 1990b).

Determination of mol% G+C content was done by HPLC method at the DSMZ according to the methods described by Cashion *et al.* (1977), Mesbah *et al.* (1989) and Tamaoka & Komagata (1984) in addition to the G+C content calculation of the complete genome.

DNA extraction was achieved by using the Q Biogene kit Fast DNA Spin kit for soil by following the manufacturer's protocol. For sequence analyses, the 16S rRNA gene was amplified using the primer combination 27F and 1492R (Lane, 1991). Commercially available primers 27F, 1492R (Lane, 1991), 519F, 907F, 1219F, 907R (Funke *et al.*, 2004), 519R (Götz *et al.*, 2002) and 1387R (Marchesi *et al.*, 1998) were used for DNA sequencing (Sigma).

After purification of the PCR products, they were sequenced using a Beckmann CEQ 2000XL sequencer. The sequence runs were assembled with the program Lasergene SeqMan Pro version 8.0.2 from DNASTAR. The 16S rRNA gene sequence was compared to all accessible sequences in databases using the BLAST (Altschul *et al.*, 1990) server at the National Centre for Biotechnology Information (NCBI). In addition, the sequences of the closest phylogenetic relatives were downloaded from NCBI. For alignment and tree calculation, the software Lasergene MegAlign version 8.0.2 (DNASTAR) applying CLUSTAL W and the neighbour-joining algorithm was used. Bootstrapping was performed based on 100 nucleotide substitutions, 1000 trails and a seed of 111.

In addition to analysis of the 16S rRNA gene fragment, the structural gene for the DNA gyrase β subunit, *gyrB*, was evaluated to determine phylogenetic distances. The *gyrB* sequences used for the analysis are available in the NCBI database: *A. ferrireducens* $T118^T$ (CP000267); *Rhodoferax fermentans* $FR2^T$ (AB020308); $ED16^T$ (AWQR00000000). Sequence analysis was performed as for the 16S rRNA gene.

For spectroscopic DNA–DNA hybridization, biomass of the most closely related species, *Curvibacter delicatus* (DSM 11558^T) and *Curvibacter fontanus* (CCUG 49444^T), was enriched in liquid media according to the culture conditions proposed by DSMZ and CCUG. Cells were disrupted by using a Constant Systems TS 0.75 kW (IUL Instruments) and

the DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977).

DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) under consideration of the modifications described by Huss *et al.* (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian). The similarity of the whole genomes of *A. ferrireducens* T118^T and ED16^T was determined using average nucleotide identity (ANI) because of known difficulties in culturing *A. ferrireducens* (Finneran *et al.*, 2003).

Libraries for whole genome sequencing were prepared with a Nextera XT sample preparation kit. Whole genome sequencing was performed using an Illumina MiSeq platform and a 2 × 250 paired-end run. Contigs were assembled with Mira 3.9.15. ANI was determined using the software Gegenees 2.0 with a threshold of 20 % (Ågren *et al.*, 2012). For visualization of phylogenetic relationships within the whole genome sequencing analysis, SplitsTree4 4.13.1 was used. The Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number AWQR00000000. The version described in this paper is version AWQR01000000 and belongs to NCBI Bioproject PRJNA215140.

On the basis of 16S rRNA gene sequence analysis, ED16^T is related to members of the genera *Curvibacter*, *Albidiferax*, *Rhodoferax* and *Variovorax* (Fig. 1). Within the genus *Curvibacter*, the most closely related taxa are *Curvibacter delicatus* (Leifson, 1962; Hylemon *et al.*, 1973; Ding & Yokota, 2004) and *Curvibacter fontanus* (Ding & Yokota, 2010) with sequence similarities of the 16S rRNA gene fragment of 97.9 and 97.8 %, respectively. The similarity between ED16^T and *A. ferrireducens*, based on 16S rRNA gene sequences, is 97.7 % and is almost comparable with the similarity of 97.1 % between *A. ferrireducens* and *R. fermentans*, which represents the type species of the genus *Rhodoferax* (Hiraishi *et al.*, 1991). All available sequences of uncultured bacteria with a 16S rRNA gene sequence similarity of more than 99 % with that of ED16^T were also included in the phylogenetic analysis (Fig. 1) to consider potential subspecies and the most closely related strains representing the same genus.

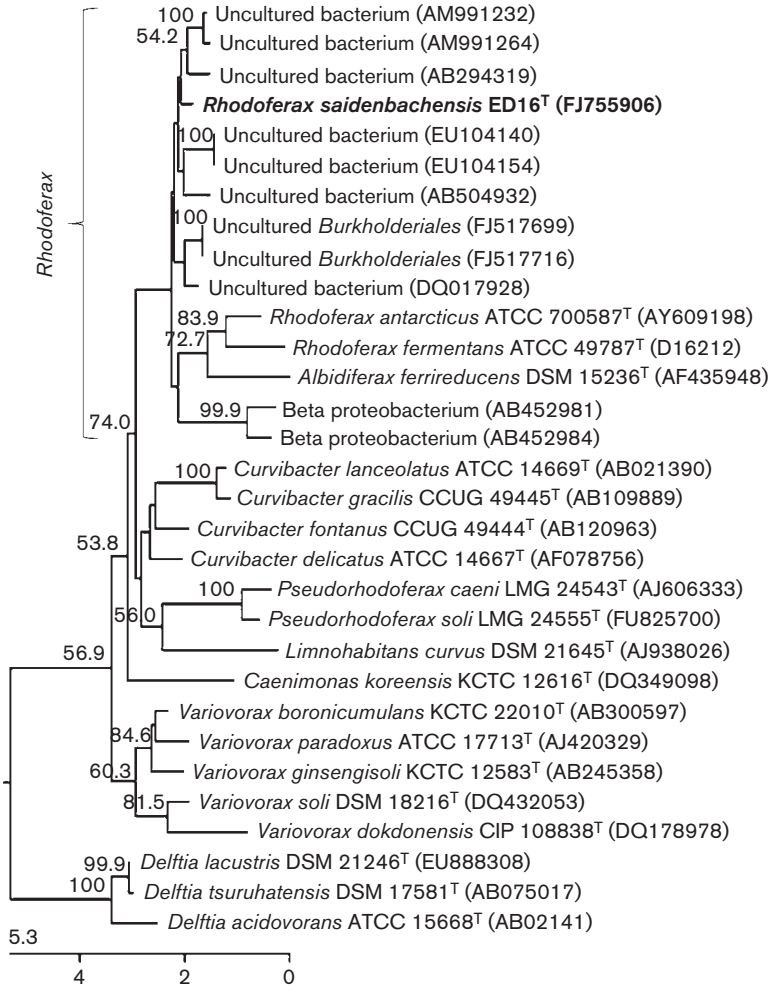

Fig. 1. Neighbour-joining tree of all described species within the genera *Curvibacter*, *Rhodoferax*, *Pseudorhodoferax*, *Delftia*, *Variovorax* and *Albidiferax* and all available sequences of uncultured bacteria with >99 % similarity to ED16^T, based on comparison of 1433 nt of the 16S rRNA gene (bootstrap values shown in % based on 1000 trials and a seed of 111). GenBank accession numbers are shown in parentheses. Bar, nucleotide substitutions (×100) per position.

The *gyrB* genes identity between and *Rhodoferax saidenbachensis* ED16^T and *Rhodoferax fermentans* FR2^T was 83.7 % and 80.9 % between *Rhodoferax saidenbachensis* ED16^T and *Albidiferax ferrireducens* T118^T. Between *Rhodoferax fermentans* FR2^T and *Albidiferax ferrireducens* T118^T an identity of 84.3 % was observed.

Based on comparisons of the 16S rRNA gene fragment and of the *gyrB* gene fragment it was shown that ED16^T and T118^T (*A. ferrireducens*) belong to the genus *Rhodoferax*. The genus *Albidiferax* was created due to the lack of photosynthetic properties of *A. ferrireducens* (Ramana & Sasikala, 2009). The species was previously assigned to the genus *Rhodoferax* (Finneran *et al.*, 2003).

DNA–DNA hybridization values between ED16^T and the most closely related species, *Curvibacter delicatus* and *Curvibacter fontanus*, were 17.6 % and 15.3 %, respectively. For determination of the genetic divergence, DNA–DNA-hybridization as well as ANI gave comparable results. The recommended 70 % cut off point for species delineation with DNA–DNA-hybridization corresponds to 94 % in ANI analysis (Richter & Rosselló-Móra, 2009).

The ANI of *A. ferrireducens* and ED16^T was 49.2 %. A phylogenetic analysis based on the whole genome sequences of ED16^T and all available sequences of the most closely related taxa is shown in Fig. 2.

The mol% G+C content of the DNA of ED16^T was 60.3 mol% when determined by the HPLC method and 60.99 mol% by analysing the sequence data of the complete genome. This value is comparable with those of the genera *Albidiferax* and *Rhodoferax* (~60 mol%) and is lower than those of species of the genus *Curvibacter* (63–66 mol%).

The most important phenotypic factors distinguishing strain ED16^T from the most closely related taxa are outlined in Table 1. Cells are Gram-stain-negative, short rods with a length of 1 to 1.8 μm and a diameter of 0.2 to 0.4 μm (Fig. 3) and polar flagella. Cells occurred singly or, in young cultures, in pairs. Circular, non-pigmented colonies with a diameter of 0.5 to 1 mm appeared on R2A agar after 5 days of incubation at 20 °C. Cultures more than 7 days old grown in liquid R2A rarely produced a brown pigment. The most closely related species, *Curvibacter fontanus*, and all species of the genus *Rhodoferax* are always pigmented.

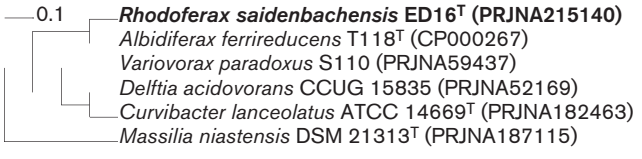

Fig. 2. Neighbour-joining tree of ED16^T and the most closely related taxa based on available whole genome sequences. *Massilia niastensis* DSM 21313^T was used as an outgroup. Generated in SplitsTree4 (4.13.1). Bar, 0.1 substitutions per nucleotide position.

The name *Albidiferax* is misleading since *A. ferrireducens* has a high cytochrome content (Risso *et al.*, 2009) that causes the reddish-brown colour of the colonies (Finneran *et al.*, 2003).

Strain ED16^T was strictly aerobic. While all described species of the genus *Rhodoferax* are able to photosynthesize, the corresponding genes were not detected in the whole genome of ED16^T. *A. ferrireducens* is a facultatively anaerobic species. In contrast to members of the genus *Albidiferax* (Ramana & Sasikala, 2009), ED16^T was not able to respire with Fe(III)-NTA or Mn(IV) oxide. All species of the genus *Curvibacter* are aerobic; only *Curvibacter fontanus* grows optimally with an oxygen concentration below 100 % of aerobiosis (Ding & Yokota, 2010).

The optimum temperature for growth of ED16^T was 20 °C but it was able to grow between 4 °C to 30 °C, which also distinguishes strain ED16^T from the most closely related taxa. ED16^T was oxidase-positive; however, the Voges–Proskauer reaction and the test for catalase were negative. In contrast, all species within the genus *Curvibacter* with the exception of *Curvibacter lanceolatus* are catalase-positive.

A. ferrireducens is saline tolerant (Ramana & Sasikala, 2009) while salinity and acid tolerance of strain ED16^T were not observed. ED16^T was able to grow at pH 6 to 9 and optimally at pH 6.

Physiological parameters as well as the DNA G+C content (mol%) that distinguish strain ED16^T from the type species of the most closely related genera are outlined in Table 1. While the species of the genus *Rhodoferax* and also *A. ferrireducens* require special growth factors such as biotin or thiamine, or special culture conditions (Brenner *et al.*, 2005; Finneran *et al.*, 2003; Madigan *et al.*, 2000), ED16^T grew on many standard media. Strain ED16^T grew on R2A-, Winkle- and blood-agar with β-haemolysis after incubation for 20 days. Strain ED16^T was not able to grow on the following media: Kligler, citrate, Leifson, aesculin, TTC-Chapman-agar, MacConkey, Baird Parker, Endo, cetrimide, OF-agar with glucose, lactose peptone bouillon or DEV tryptophan bouillon. Furthermore, it did not grow on nutrient agar or TSA.

As a result of the low frequency of cell division, Biolog GN2 analysis took 20 days to complete. Values above an OD of 0.5 were considered positive. Strain ED16^T was able to utilize DL-lactic acid, glycerol, methylsuccinate, α-D-glucose, D-mannose, succinamic acid, β-hydroxybutyric acid, L-fucose, monomethylpyruvate, γ-hydroxybutyric acid, L-alaninamide, α-hydroxybutyric acid, L-proline, *cis*-aconitic acid, L-asparagine, D-fructose, succinic acid, 2-aminoethanol and D-galactose. Adonitol, phenyethylamine and sucrose could not be utilized. All other substrates in the Biolog GN2 system resulted in a variable or low reaction. Of the most closely related taxa only *Curvibacter fontanus* and *R. fermentans* were able to degrade glycerol.

Strain ED16^T was positive for alkaline phosphatase, esterase C4, esterase lipase C8 and naphthol-AS-BI-phosphohydrolase activities but negative for the activities of arginine

Table 1. Characteristics that distinguish strain ED16^T from closely related species

Strains: 1, ED16^T; 2, *R. fermentans* JCM 7819^T (data from Brenner *et al.*, 2005; Hiraishi *et al.*, 1991); 3, *A. ferrireducens* DSM 15236^T (Finneran *et al.*, 2003; Ramana & Sasikala, 2009); 4, *Rhodoferax antarcticus* ATCC 700587^T (Madigan *et al.*, 2000); 5, *Curvibacter fontanus* CCUG 49444^T (Ding & Yokota, 2010); 6, *Curvibacter delicatus* IAM 14955^T (Brenner *et al.*, 2005; Ding & Yokota, 2004, 2010; Hylemon *et al.*, 1973; Leifson, 1962); 7, *Curvibacter gracilis* IAM 15033^T (Ding & Yokota, 2004, 2010); 8, *Curvibacter lanceolatus* IAM 14947^T (Ding & Yokota, 2004, 2010; Leifson, 1962); 9, *Variovorax paradoxus* CCUG 1777^T (Brenner *et al.*, 2005; Wen *et al.*, 1999; Willems *et al.*, 1991). +, Positive; − negative; W, weakly positive; NA, data not available.

Characteristic	1	2	3	4	5	6	7	8	9
Cell morphology	Short rods	Curved rods	Rods	Curved rods	Spirilla/curved rods	Curved rods	Curved rods	Short rods	Rods
Flagella	1	+	1	1	ND	1–2	ND	1	Peritrichious
Cell size (μm)	0.2–0.4 × 1.0–1.8	0.6–0.9	1 × 3–5	0.6–0.9 × 1.5–3	0.4–0.5 × 1.1–2.4	0.3–0.5 × 1.0–2.0	0.4–0.5 × 1.1–2.8	0.6–0.9 × 1.2–1.8	0.5–0.6 × 1.2–3.0
Oxygen demand	Aerobic; no photosynthesis	Aerobic respiration and fermentation; photosynthesis	Facultatively anaerobic; no photosynthesis	Phototrophic, chemo-organotrophic	Microaerobic	Aerobic	Aerobic	Aerobic	Facultatively autotrophic
Growth temperature (°C)	4–30	25–30	4–30	0–25	20–30*	9–40	9–40	20–37	30
Growth at 4 °C	+	NA	+	+	+	−	−	−	NA
Growth at 37 °C	−	−	−	−	−	+	+	+	−
pH range for growth	6–9	5–9	6.7–7.1	NA	7	5.5–8.5	5–8	Neutrophilic	Neutrophilic
Pigment	Old cultures brown	Peach–brown	Brown	Peach–brown	Yellow–brown	−	Yellow–brown	−	Yellow
DNA G+C (mol%)	60.3–61	59.8–60.3	59.5	61.5	66.6	63.0	66.0	66.2	66.8–69.4
Catalase	−	NA	−†	NA	+*	+	+	−	+
Utilization of:									
Glycerol	+	W	−	−	+*	−	−	ND	+
L-Arginine	−	W	+†	NA	−	−	+	−	+
Urea	−	NA	+†	NA	+	−	+	+	+
Aesculin	−	NA	NA	NA	−	−	−	−	+
Gelatin	−	W	−†	NA	−	−	−	−	+
D-Mannose	+	+	+†	−	−	−	−	W	+
Mannitol	+	+	+	−	+/−*	W	−	−	+
Maltose	−	W	−†	NA	+/−*	W	−	−	+
Gluconate	−	+	+†	NA	+/−*	−	+	−	+

*This study using strain CCUG 49444^T.

†Potentially; genes are present/absent (Risso *et al.* 2009; this study).

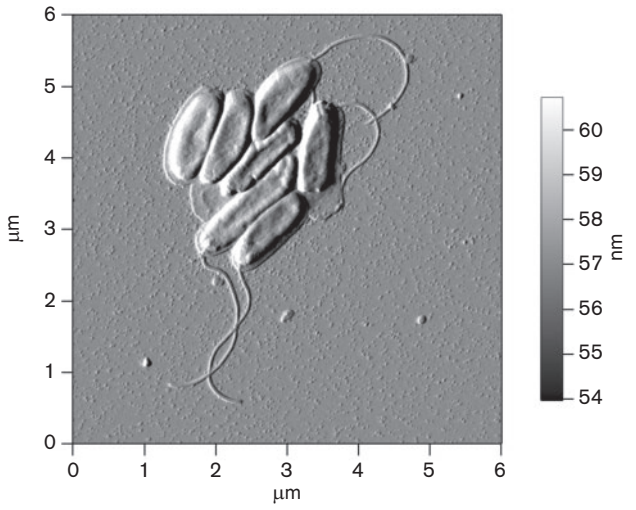

Fig. 3. Atomic force micrograph of cells of strain ED16^T.

dihydrolase, urease, β-glucosidase, gelatinase, cystine arylamidase, trypsin and α-chymotrypsin. Nitrite reduction was positive. No indole production was observed. In contrast to *Curvibacter fontanus*, urease activity was negative.

A. ferrireducens was not included in the comparison of cellular fatty acids (Table 2). As this species requires special culture conditions that are not suitable for ED16^T, the results would not be comparable.

Ubiquinone Q-8 was the only respiratory quinone found in strain ED16^T. Major fatty acids were $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, $C_{16:0}$, $C_{18:1}\omega7c$ and $C_{18:0}$. The occurrence of $C_{18:0}$ and iso-$C_{16:0}$ but the absence of $C_{17:0}$ cyclo is in contrast to the fatty acid profiles of the most closely related taxa and indicates that ED16^T represents a novel taxon within the genus *Rhodoferax*.

In the NCBI sequence database, there are seven 16S rRNA gene fragment sequences from uncultured strains, most of them PCR-amplified from fresh water sources, with greater than 99 % similarity to strain ED16^T, suggesting that close relatives to ED16^T have a high affinity for nutrient-poor habitats. These sequences (AB504932; FJ517699, FJ517716, FJ517721, FJ517679; DQ017928; AM991232, AM991264; AB294319; AB452984, AB452981; EU104140) have been isolated from iron-rich snow in Japan (Kojima *et al.*, 2009), the epithel of *Hydra magnipapillata*, the upland steam of the river Fulda (Beier *et al.*, 2008), karst spring water in Switzerland, a groundwater stream in Japan (Shimizu *et al.*, 2007), lake Hibara Fukushima in Japan and activated sludge in New Zealand. Psychrotolerance has also been reported for isolate AB504932 (Kojima *et al.*, 2009).

Emended description of the genus *Rhodoferax*

The description is based on earlier published data (Hiraishi *et al.* 1991) and own studies. Gram negative, vibroid or slightly curved rods, 0.2–1.0 × 1.0–5.0 µm; polar flagella occur. May grow brown pigmented or translucent aerobic, facultative anaerobic or with low oxygen concentrations at 4–30 °C and at pH 5.0–9.0. Some species show photosynthesis. Salinetolerance may occur. Oxidasepositive. Major fatty acids are $C_{16:1}$, $C_{16:0}$, and $C_{18:1}$, and 3-OH fatty acids $C_{8:0}$ 3-OH are present; ubiquinone type is Q-8; RQ-8 might occur. The DNA G+C content is 59.5–61.0 mol%. The type species is *Rhodoferax fermentans* (Hiraishi *et al.*, 1991).

Table 2. Fatty acid contents of ED16^T and closely related taxa

Strains: 1, ED16^T; 2, *R. fermentans* JCM 7819^T (data from Hiraishi *et al.*, 1991); 3, *Curvibacter gracilis* IAM 15033^T (Ding & Yokota, 2010); 4, *Curvibacter fontanus* IAM 15072^T (Ding & Yokota, 2010); 5, *Curvibacter delicatus* IAM 14955^T (Ding & Yokota, 2010); 6, *V. paradoxus* CCUG 1777^T (Urakami *et al.* 1995). Values are percentages of the total fatty acids. ND, Not detected.

Fatty acid	1	2	3	4	5	6
$C_{12:0}$	ND	ND	3.0	ND	1.0	ND
$C_{14:0}$	0.37	<1	0.9	4.1	ND	0.3
$C_{15:0}$	0.33	3.0	0.7	11.4	ND	0.9
$C_{16:0}$	39.42	35.0	19.7	21.7	32.0	33.9
iso-$C_{16:0}$	0.45	ND	ND	ND	ND	ND
$C_{17:0}$	0.39	2.0	ND	3.4	2.0	1.1
$C_{18:0}$	0.89	<1	NA	ND	ND	0.4
$C_{19:0}$	ND	ND	ND	ND	8.0	ND
$C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH	54.08	54.0	44.6	29.4	35	34.3
$C_{15:1}\omega6c$	0.20	ND	ND	3.1	ND	ND
$C_{17:1}\omega6c$	ND	ND	1.5	3.3	ND	ND
$C_{18:1}$ 1 w7c	3.17	5.0	25	9.2	23.0	17.2
$C_{8:0}$ 3-OH	0.45	Present	0.7	ND	Present	ND
$C_{10:0}$ 3-OH	ND	ND	ND	5.3	ND	2.1
$C_{17:0}$ cyclo	ND	ND	ND	5.7	ND	3.9

Taxonomic position of strain T118^T

Strain T118^T was originally designated as the type strain of *Rhodoferax ferrireducens* (Finneran *et al.* 2003). Ramana & Sasikala (2009) considered that this species should be transferred to the genus *Albidiferax* as *Albidiferax ferrireducens*. *Rhodoferax ferrireducens* (Finneran *et al.* 2003) and *Albidiferax ferrireducens* (Ramana & Sasikala 2009) share the same type strain, both names are validly published and are homotypic synonyms. Based on the data of this study, of Finneran *et al.* (2003), and Risso *et al.* (2009) (Fig. 1, Table 1) and despite the fact that strain T118^T does not grow phototrophically this type strain is more appropriately placed in the genus *Rhodoferax* as *Rhodoferax ferrireducens* (Finneran *et al.* 2003). Since *Rhodoferax ferrireducens* (Finneran *et al.* 2003) is validly published, the publication of this taxonomic opinion does neither create a new combination nor constitute a revived name. The description and type strain of *Rhodoferax*

ferrireducens (Finneran *et al.* 2003) are as given by Finneran *et al.* (2003).

The type strain is T118^T (=ATCC BAA-621^T=DSM 15236^T).

Description of *Rhodoferax saidenbachensis* sp. nov.

Rhodoferax saidenbachensis (sai.den.bach.en′sis N.L. masc. adj. *saidenbachensis* of Saidenbach reservoir in Germany, the geographical origin of isolation).

Cells are Gram-stain-negative rods 0.2–0.4 × 1.0–1.8 μm with polar flagella and occur individually or in pairs. Catalase-negative but oxidase-positive. Psychrotolerant with cell division between 4 and 30 °C (optimum 20 °C) but not at 37 °C. Has a very low rate of cell division. Colourless colonies on R2A but old cultures may be brown. Optimal growth at pH 6–9. No tolerance of NaCl above a physiological concentration of 0.9 %. No growth on nutrient agar, Kligler, TSA, citrate, Leifson, esculin, TTC-Chapman-agar, MacConkey, Baird Parker, Endo, Cetrimid and OF-agar with glucose, in lactose peptone bouillon and in DEV tryptophan bouillon. Shows β-haemolysis on blood agar in incubations longer than 20 days. Good utilization of most amides and mannose, D-glucose and glycerol but poor utilization of polymers. Utilizes DL-lactic acid, glycerol, methylsuccinate, succinamic acid, β-hydroxybutyric acid, L-fucose, monomethylpyruvate, γ-hydroxybutyric acid, L-alaninamide, α-hydroxybutyric acid, L-proline, *cis*-aconitic acid, L-asparagine, D-fructose, succinic acid, 2-aminoethanol and D-galactose. Urease-, indole-, gelatin- and aesculin-negative. Voges-Proskauer reaction negative. Activities of alkaline phosphatase, esterase C4, esterase lipase C8 and naphthol-AS-BI-phosphohydrolase are positive. Nitrite reduction positive. The major fatty acids are $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, $C_{16:0}$, $C_{18:1}\omega7c$. $C_{18:0}$ and $C_{8:0}$ 3-OH occur and the ubiquinone type is Q-8.

The type strain is *Rhodoferax saidenbachensis* ED16^T (=CCUG 57711^T=ATCC BAA-1852^T=DSM 22694^T). It was isolated from the sediment of the Saidenbach drinking water reservoir in Saxony, Germany. The G+C content of DNA of the type strain is 60.3 mol% (HPLC)–61 mol% (whole genome analysis).

Acknowledgements

We are grateful to Edward Moore, Peter Kämpfer and Enevold Falsen for the supportive correspondence. Furthermore we would like to thank Hans Georg Trüper for the assistance with Latin nomenclature. We thank Tatjana Ladnorg for providing the pictures taken by atomic force microscopy and Bettina Sträubler for excellent technical assistance.

References

Ågren, J., Sundström, A., Håfström, T. & Segerman, B. (2012). Gegenees: fragmented alignment of multiple genomes for determining phylogenomic distances and genetic signatures unique for specified target groups. *PLoS ONE* **7**, e39107.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.

Barritt, M. M. (1936). The intensification of the Voges-Proskauer reaction by the addition of α-naphthol. *J Pathol Bacteriol* **42**, 441–454.

Beier, S., Witzel, K.-P. & Marxsen, J. (2008). Bacterial community composition in Central European running waters examined by temperature gradient gel electrophoresis and sequence analysis of 16S rRNA genes. *Appl Environ Microbiol* **74**, 188–199.

Binnig, G., Quate, C. F. & Gerber, C. (1986). Atomic force microscope. *Phys Rev Lett* **56**, 930–933.

Brenner D. J., Krieg N. R., Staley J. T. & Garrity G. M. (editors) (2005). Bergey's Mannual of Systematic Bacteriololgy, 2nd edn, vol. 2 (*The Proteobacteria*). New York: Springer.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

De Vos, P., Kersters, K., Falsen, E., Pot, B., Gillis, M., Segers, P. & De Ley, J. (1985). *Comamonas* Davis and Park 1962 gen. nov., nom. rev. emend., and *Comamonas terrigena* Hugh 1962 sp. nov., nom. rev. *Int J Syst Bacteriol* **35**, 443–453.

Ding, L. & Yokota, A. (2004). Proposals of *Curvibacter gracilis* gen. nov., sp. nov. and *Herbaspirillum putei* sp. nov. for bacterial strains isolated from well water and reclassification of [*Pseudomonas*] *huttiensis*, [*Pseudomonas*] *lanceolata*, [*Aquaspirillum*] *delicatum* and [*Aquaspirillum*] *autotrophicum* as *Herbaspirillum huttiense* comb. nov., *Curvibacter lanceolatus* comb. nov., *Curvibacter delicatus* comb. nov. and *Herbaspirillum autotrophicum* comb. nov. *Int J Syst Evol Microbiol* **54**, 2223–2230.

Ding, L. & Yokota, A. (2010). *Curvibacter fontana* sp. nov., a microaerobic bacteria isolated from well water. *J Gen Appl Microbiol* **56**, 267–271.

Finneran, K. T., Johnsen, C. V. & Lovley, D. R. (2003). *Rhodoferax ferrireducens* sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe(III). *Int J Syst Evol Microbiol* **53**, 669–673.

Funke, G., Frodl, R. & Sommer, H. (2004). First comprehensively documented case of *Paracoccus yeei* infection in a human. *J Clin Microbiol* **42**, 3366–3368.

Garrtity, G. M., Bell, J., A. & Lilburn, T. (2006). Validation list No. 107. *Int J Syst Evol Microbiol* **56**, 1–6.

Götz, D., Banta, A., Beveridge, T. J., Rushdi, A. I., Simoneit, B. R. T. & Reysenbach, A. L. (2002). *Persephonella marina* gen. nov., sp. nov. and *Persephonella guaymasensis* sp. nov., two novel, thermophilic, hydrogen-oxidizing microaerophiles from deep-sea hydrothermal vents. *Int J Syst Evol Microbiol* **52**, 1349–1359.

Hiraishi, A., Hoshino, Y. & Satoh, T. (1991). *Rhodoferax fermentas* gen. nov., a phototrophic purple nonsulfur bacterium previously referred to as the "*Rhodocyclus gelatinosus*-like" group. *Arch Microbiol* **155**, 330–336.

Huss, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Hylemon, P. B., Wells, J. S., Jr, Krieg, N. R. & Jannasch, H. W. (1973). The genus *Spirillum*: a taxonomic study. *Int J Syst Bacteriol* **23**, 340–380.

Kaden, R. (2009). *Mikrobiologische Gewässeranalytik: am Beispiel der Untersuchung einer Trinkwassertalsperre*. Hamburg: Diplomica.

Kojima, H., Fukuhara, H. & Fukui, M. (2009). Community structure of microorganisms associated with reddish-brown iron-rich snow. *Syst Appl Microbiol* **32**, 429–437.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradorhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Leifson, E. (1962). The bacterial flora of distilled and stored water. III. New species of the genera *Corynebacterium*, *Flavobacterium*, *Spirillum* and *Pseudomonas*. *Int Bull Bacteriol Nomencl Taxon* **12**, 161–170.

Madigan, M. T., Jung, D. O., Woese, C. R. & Achenbach, L. A. (2000). *Rhodoferax antarcticus* sp. nov., a moderately psychrophilic purple nonsulfur bacterium isolated from an Antarctic microbial mat. *Arch Microbiol* **173**, 269–277.

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J., Dymock, D. & Wade, W. G. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl Environ Microbiol* **64**, 795–799.

McFarland, J. (1907). The nephelometer: an instrument for estimating the numbers of bacteria in suspensions used for calculating the opsonic index and for vaccines. *J Am Med Assoc* **49**, 1176–1178.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Miller, L. T. (1982). A single derivatization method for bacterial fatty acid methyl esters including hydroxy acids. *J Clin Microbiol* **16**, 584–586.

Ramana, C. V. & Sasikala, C. (2009). *Albidoferax*, a new genus of *Comamonadaceae* and reclassification of *Rhodoferax ferrireducens* (Finneran *et al.*, 2003) as *Albidoferax ferrireducens* comb. nov. *J Gen Appl Microbiol* **55**, 301–304.

Richter, M. & Rosselló-Móra, R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* **106**, 19126–19131.

Risso, C., Sun, J., Zhuang, K., Mahadevan, R., DeBoy, R., Ismail, W., Shrivastava, S., Huot, H., Kothari, S. & other authors (2009). Genome-scale comparison and constraint-based metabolic reconstruction of the facultative anaerobic Fe(III)-reducer *Rhodoferax ferrireducens*. *BMC Genomics* **10**, 447.

Shimizu, S., Akiyama, M., Naganuma, T., Fujioka, M., Nako, M. & Ishijima, Y. (2007). Molecular characterization of microbial communities in deep coal seam groundwater of northern Japan. *Geobiology* **5**, 423–433.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.

Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol. Lett* **66**, 199–202.

Urakami, T., Araki, H. & Komagata, K. (1995). Characteristics of newly isolated *Xanthobacter* strains and fatty acid compositions and quinone systems in yellow-pigmented hydrogen-oxidizing bacteria. *Int J Syst Bacteriol* **45**, 863–867.

Wen, A., Fegan, M., Hayward, C., Chakraborty, S. & Sly, L. I. (1999). Phylogenetic relationships among members of the *Comamonadaceae*, and description of *Delftia acidovorans* (den Dooren de Jong 1926 and Tamaoka *et al.* 1987) gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 567–576.

Willems, A., De Ley, J., Gillis, M. & Kersters, K. (1991). *Comamonadaceae*, a new family encompassing the acidovorans rRNA complex, including *Variovorax paradoxus* gen. nov., comb. nov., for *Alcaligenes paradoxus* (Davis 1969). *Int J Syst Bacteriol* **41**, 445–450.