***Rhodovulum iodosum* sp. nov. and *Rhodovulum robiginosum* sp. nov., two new marine phototrophic ferrous-iron-oxidizing purple bacteria**

Kristina L. Straub,¹ Frederick A. Rainey^{2,3} and Friedrich Widdel¹

Author for correspondence: Kristina L. Straub. Tel: +49 421 2028 736. Fax: +49 421 2028 580.
e-mail: kstraub@mpi-bremen.de

¹ Max-Planck-Institut für Marine Mikrobiologie, Celsiusstr. 1, D-28359 Bremen, Germany

² Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

³ Department of Microbiology, Louisiana State University, Baton Rouge, LA 70803, USA

Two new strains of marine purple bacteria, N1^T and N2^T, were isolated from coastal sediment of the North Sea (Germany) with ferrous iron as the only electron donor for anoxygenic photosynthesis. The isolates are the first salt-dependent, ferrous-iron-oxidizing purple bacteria characterized so far. Analysis of 16S rRNA gene sequences revealed an affiliation with the genus *Rhodovulum*, which until now comprises only marine species. The sequence similarity of both strains was 95.2 %, and their closest relative was *Rhodovulum adriaticum*. Like all known *Rhodovulum* species, the new strains had ovoid to rod-shaped cells, contained bacteriochlorophyll *a* and carotenoids of the spheroidene series, and were able to oxidize sulfide and thiosulfate. Like *Rhodovulum adriaticum*, both strains were unable to assimilate sulfate; for growth they needed a reduced sulfur source, e.g. thiosulfate. In contrast to the new strains, none of the known *Rhodovulum* species tested was able to oxidize ferrous iron or iron sulfide. In growth experiments, strains N1^T and N2^T oxidized 65 and 95 %, respectively, of the ferrous iron supplied. Electron diffraction analysis revealed ferrihydrite as the main product of ferrous iron oxidation. In addition, traces of magnetite were formed. Strains N1^T (= DSM 12328^T) and N2^T (= DSM 12329^T) are described as *Rhodovulum iodosum* sp. nov. and *Rhodovulum robiginosum* sp. nov., respectively.

Keywords: ferrous iron oxidation, purple bacteria, *Rhodovulum iodosum*, *Rhodovulum robiginosum*

INTRODUCTION

The utilization of ferrous iron as electron donor for anoxygenic photosynthesis was first recognized in crude enrichment cultures with iron-rich anaerobic freshwater and marine mud samples. Of subsequently isolated pure cultures, only those from freshwater habitats showed good growth on ferrous iron and were therefore characterized in detail (Ehrenreich & Widdel, 1994; Heising & Schink, 1998; Widdel *et al.*, 1993). Although marine enrichment cultures oxidized ferrous iron as efficiently as the freshwater enrichment cultures, marine isolates developed only poorly; these isolates were not preserved.

Coastal sediments often contain large pools of ferric iron, which may function as an important electron acceptor for the oxidation of organic carbon (Aller *et al.*, 1986; Canfield *et al.*, 1993a). It has been suggested that at high rates of ferric iron reduction, ferrous iron occurs in the upper millimetres of marine sediments (Canfield *et al.*, 1993b; Thamdrup *et al.*, 1994). Hence, the simultaneous occurrence of light and ferrous iron is likely so that the latter may serve as electron donor for anoxygenic phototrophs. Ferrous iron oxidation by anoxygenic phototrophs is understandable in terms of energetics. The Fe(III)/Fe(II) redox pair in bicarbonate-containing environments has an E'_0 around +0.2 V. Hence, electron transfer to the anoxygenic photosystem with a midpoint potential around +0.45 V is feasible (Widdel *et al.*, 1993). Another naturally widespread redox-active metal is manganese. Similar to ferrous iron, manganese(II) may also occur

The EMBL accession numbers for the 16S rDNA sequences determined in this study are Y15011 and Y15012 for strains N1^T and N2^T, respectively.

in the surface layers of sediments (Thamdrup *et al.*, 1994). However, according to thermodynamic data, manganese(II) is unlikely to serve as electron donor for anoxygenic phototrophs in environments with pH values below 9, since the redox potential of the Mn(IV)/Mn(II) couple is around +0.6 V and hence too positive (Brookins, 1988).

For comparative studies on ferrous-iron-oxidizing phototrophic bacteria we started enrichment cultures to obtain marine counterparts of the known freshwater isolates. Here we describe the isolation, physiological features and phylogenetic affiliation of two new marine species for which the names *Rhodovulum iodosum* and *Rhodovulum robiginosum* are proposed. Furthermore, the new isolates are compared to described *Rhodovulum* species.

METHODS

Source of bacteria. Sediment for enrichment cultures was collected from the mud flat of the Jadebusen (North Sea, Germany) at four different sites. *Rhodovulum adriaticum* DSM 2781^T, *Rhodovulum euryhalinum* DSM 4868^T and *Rhodovulum sulfidophilum* DSM 1374^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany.

Media and growth conditions. Techniques for preparation of media and cultivation of phototrophic bacteria under anoxic conditions have been described elsewhere (Widdel & Bak, 1992). For the present study, artificial seawater medium was used which contained (per litre distilled water): 0.4 g KH₂PO₄, 26.4 g NaCl, 0.25 g NH₄Cl, 6.8 g MgSO₄ · 7H₂O, 5.7 g MgCl₂ · 6H₂O, 1.5 g CaCl₂ · 2H₂O, 0.66 g KCl and 0.09 g KBr. After autoclaving and cooling under an atmosphere of N₂/CO₂ (90:10, v/v), 30 ml NaHCO₃ solution (84 g l⁻¹, autoclaved under CO₂), vitamins, EDTA-chelated mixture of trace elements, and selenite and tungstate solution (Widdel & Bak, 1992) were added. The pH was adjusted to 6.9. Unless indicated otherwise, 0.5 mM Na₂S₂O₃ was added as a source of reduced sulfur from a filter-sterilized stock solution (1 M).

FeSO₄ was added to culture medium from a 1.0 M anoxic stock solution; upon the addition of FeSO₄ to the medium (final concentration 10 mM), a white precipitate, most likely consisting of ferrous carbonate and ferrous phosphate, formed. An anoxic suspension of FeS was prepared as described previously (Ehrenreich & Widdel, 1994). For experiments with free sulfide, sodium sulfide was added to the media from an anoxic autoclaved stock solution (1 M) to the final concentrations indicated. Culture tubes and bottles were incubated horizontally on a Plexiglas plate and illuminated with two tungsten lamps (25 W) installed above and below the cultures at a distance of 30 cm. Enrichment cultures were incubated at 20 °C and gently shaken every other day to allow even distribution of bacteria and ferrous iron minerals.

Utilization of other compounds as electron donors for photosynthetic growth was tested in media without FeSO₄ (except for 7.5 µM FeSO₄ from trace element solution). Ferrous sulfide, free sulfide, propionate, butyrate, valerate, caproate, caprylate, methanol, ethanol, pyruvate, glucose and fructose were supplied at a final concentration of 2 mM. The final concentration of yeast extract and Casamino acids

was 0.5 g l⁻¹. All other compounds were added at a final concentration of 4 mM.

Isolation. Pure cultures were obtained via repeated agar dilution series (Widdel & Bak, 1992) prepared with a slight modification: to 3 ml 3% (w/v) agar molten in a test tube, 0.11 ml of a 1 M FeSO₄ solution and 8 ml prewarmed medium were subsequently added. FeSO₄ was supplied as the only electron donor. Purity was verified microscopically after incubation of isolates in medium with yeast extract (0.5 g l⁻¹), pyruvate (2 mM), glucose (1 mM) or acetate (5 mM). In addition, the homogeneity of colonies in agar dilutions was checked.

Analytical methods. Ferrous iron was quantified spectrophotometrically at 510 nm after chelation with 2 mM *o*-phenanthroline in 0.7 M sodium acetate buffer, pH 5, in a test volume of 1 ml (modified from Fachgruppe Wasserchemie, 1991). Immediately before withdrawing samples, cultures were agitated to disperse iron precipitates homogeneously. Samples were taken with anoxic syringes and were immediately acidified with HCl (final concentration 1 M). The concentration of ferric iron was determined in the same way after reduction with 0.28 M hydroxylammonium chloride; the ferrous iron concentration determined before reduction was subtracted.

The type of photosynthetically produced ferric iron was identified by electron diffraction analysis. Two millilitres of spent medium, with ferric iron produced during photoautotrophic growth on ferrous iron, was washed five times with 25 ml distilled water to remove medium components; then a drop of the ferric iron suspension was placed onto a Formvar-carbon-coated copper grid and air-dried. Selected area diffraction was carried out with a Zeiss EM10 electron microscope to identify the ferric iron and to determine its degree of crystallinity. The analysis was carried out by Marianne Hanzlik, Munich, Germany.

Sulfate was determined on a Sykam (Gilching) ion chromatograph according to Bak *et al.* (1991).

Carotenoids were extracted from cell pellets of acetate-grown cultures (10 ml) by using 100% acetone (3 ml) and sonication (Sun *et al.*, 1991). The carotenoid composition was determined by HPLC analysis according to Overmann *et al.* (1992); the column was Spherisorb ODS-2, 5 µm, and the eluent was acetonitrile/methanol/2-propanol (85:10:5). Carotenoids of *Rhodovulum sulfidophilum* were extracted for comparison.

16S rDNA sequence determination, phylogenetic analyses and DNA base composition. Genomic DNA extraction, PCR amplification of the 16S rDNA and sequencing of the PCR products were carried out as described previously (Rainey *et al.*, 1996). Sequence reaction products were electrophoretically separated using a model 373A automated sequencer (Applied Biosystems). The ae2 editor (Maidak *et al.*, 1994) was used to align the 16S rDNA sequences obtained in this study against the previously determined 16S rDNA sequences of *Rhodovulum* and *Rhodobacter* species available from the public databases. The method of Jukes & Cantor (1969) was used to calculate evolutionary distances. Phylogenetic dendrograms were generated using various treeing algorithms contained in the PHYLIP package (Felsenstein, 1993). The phylogenetic dendrogram shown in Fig. 1 was reconstructed from evolutionary distances using the algorithm of DeSoete (1983).

The G + C content of the DNA was determined by HPLC (DSMZ).

Strain accession numbers. Strains N1^T and N2^T are deposited at the DSMZ under accession numbers DSM 12328^T and DSM 12329^T, respectively. The strain designations and accession numbers of the reference strains used in the phylogenetic analyses are as follows: *Rhodobacter blasticus* ATCC 33485^T, D16429; *Rhodobacter capsulatus* ATCC 11166^T, D13474; *Rhodobacter sphaeroides* IFO 12203, D16425; *Rhodobacter veldkampii* ATCC 35703^T, D16421; *Rhodobacter* sp. strain SW2, X78717; *Rhodovulum adriaticum* DSM 2781^T, D16418; *Rhodovulum euryhalinum* DSM 4868^T, D13479; *Rhodovulum strictum* MB-G2, D16419; *Rhodovulum sulfidophilum* DSM 1374^T, D16423; *Roseobacter denitrificans* LMG 4218, X69159.

RESULTS AND DISCUSSION

Enrichment and isolation

Artificial seawater medium supplied with 10 mM FeSO₄ as the only electron donor was inoculated with marine sediment samples from four different sites of the Jadebusen. Enrichment cultures were incubated horizontally in the light at 20 °C. Rusty patches developed in particular on the sediment side which was illuminated from below, and after 3–4 weeks incubation the entire lower glass wall was covered by a rusty layer. After subsequent transfer of all four enrichment cultures to fresh FeSO₄-containing medium (inoculum size 5–10 %, v/v), the rate and extent of the ferrous iron oxidation decreased significantly. However, when transfers were made in the presence of a reduced sulfur source such as 0.5 mM FeS or 0.5 mM Na₂S₂O₃ the capacity for ferrous iron oxidation continued in all subcultures, and the precipitated ferrous iron minerals turned completely rusty within 2 weeks. Obviously, sulfate which was always present in the medium could not be used as sulfur source. Continued cultivation was therefore only possible when a reduced sulfur source was routinely added to the media. Because sample material was obtained from the same area as in the former study (Widdel *et al.*, 1993), one might speculate whether the poor growth of the formerly obtained isolates was due to the lack of a reduced sulfur source.

Agar dilutions with 10 mM FeSO₄ as the only electron donor were inoculated from the eighth subcultures. After 3 weeks incubation in the light, opaque rusty-orange colonies had formed amongst a variety of small red-coloured colonies. With the rusty-orange colonies subsequent agar dilution series were inoculated; from these 10 colonies were isolated and transferred to liquid media. All isolates were able to oxidize ferrous iron in the light; in controls in the dark no oxidation of ferrous iron occurred. Two isolates, strains N1^T and N2^T, which formed rusty deposits of distinct consistency were chosen for further characterization. When strain N1^T was grown without agitation, the ferric iron produced formed a solid layer sticking firmly to the glass wall. In contrast, strain N2^T formed a loose, powdery ferric iron mineral. The other isolates were not further studied.

Fig. 1. Dendrogram based on 16S rDNA sequence comparison indicating the position of strains N1^T and N2^T within the genus *Rhodovulum*. The genera *Rhodovulum* and *Rhodobacter* together form a major cluster within the α subclass of the *Proteobacteria*. The scale bar represents five nucleotide substitutions per 100 nucleotides.

Correction of *Rhodobacter blastica* (sic)

In the description of *Rhodobacter blastica* (sic) the name was incorrectly derived. The name is therefore corrected as follows: *Rhodobacter blasticus* corrig. [for *Rhodobacter blastica* (sic)]. The corrected name is used in this study.

Phylogenetic affiliation and G + C content of DNA

Almost complete 16S rDNA sequences of strains N1^T and N2^T comprising 1414 and 1415 nucleotides, respectively (>95% of the *Escherichia coli* sequence, positions 31–1519), were determined in this study. A total of 1034 nucleotides present in all of the strains between positions 47 and 1380 (*E. coli* positions) were used in the phylogenetic analyses presented in Fig. 1. The phylogenetic dendrogram (Fig. 1) shows strains N1^T and N2^T to fall within the radiation of the genus *Rhodovulum*, which, along with the species of the genus *Rhodobacter*, forms a distinct cluster within the α subclass of the *Proteobacteria* (Hiraishi & Ueda, 1994). The relationship of strains N1^T and N2^T to the species of the genus *Rhodovulum* is also seen from the 16S rDNA sequence similarity values. The 16S rDNA

Table 1. Compounds tested as substrates for photoautotrophic or photoheterotrophic growth

+, Utilized; –, not utilized; ND, no data.

Compound	Strain N1 ^T	Strain N2 ^T	<i>Rhodovulum adriaticum</i> *	<i>Rhodovulum euryhalinum</i> †	<i>Rhodovulum strictum</i> ‡	<i>Rhodovulum sulfidophilum</i> §
Inorganic						
Hydrogen	+	+	–	ND	ND	+
Sulfide	+	+	+	+	+	+
Sulfite	–	–	ND	ND	ND	–
Thiosulfate	+	+	+	+	+	+
Sulfur	+	+	ND	ND	ND	+
Ferrous iron	+	+	–	–	ND	–
Organic						
Formate	–	–	+	+	+	+
Acetate	+	+	+	+	+	+
Propionate	+	+	+	+	+	+
Butyrate	+	–	–	+	+	+
Valerate	+	–	+	+	+	+
Caproate	–	–	+	ND	+	+
Caprylate	–	–	ND	+	–	+
Methanol	–	–	–	–	–	–
Ethanol	–	–	+	+	–	–
Pyruvate	+	+	+	+	+	+
Lactate	+	+	+	+	+	+
Succinate	+	+	+	+	+	+
Fumarate	+	–	+	+	+	+
Malate	+	+	+	+	+	+
Citrate	–	–	–	–	+	–
Benzoate	–	–	–	–	–	–
Yeast extract	+	+	+	+	+	+
Casamino acids	+	+	ND	+	+	+
Aspartate	–	–	ND	+	–	–
Cysteine	+	–	ND	ND	ND	ND
Glutamate	+	+	ND	+	–	+
Glucose	–	–	+	+	+	+
Fructose	–	–	–	+	+	–
Glycerol	–	+	+	+	–	+
Mannitol	+	+	–	+	–	–

* Data from Neutzling *et al.* (1984).

† Data from Imhoff & Trüper (1992).

‡ Data from Hiraishi & Ueda (1995).

§ Data from Hansen & Veldkamp (1973); Imhoff & Trüper (1992).

sequences of strains N1^T and N2^T have similarity values in the range of 93.6–94.3% to species of the genus *Rhodovulum* while the sequence similarity to species of the genus *Rhodobacter* is lower and in the range of 90.9–92.9%. Pairwise comparison of the 16S rDNA sequences of strains N1^T and N2^T shows them to share 95.2% similarity. One of the previously characterized ferrous-iron-oxidizing phototrophic bacteria, strain SW2, isolated from a freshwater habitat is affiliated with the genus *Rhodobacter* (Ehrenreich & Widdel, 1994) and hence can be considered as a freshwater counterpart of strains N1^T and

N2^T within the α subclass. The G + C content of strains N1^T and N2^T was 66 and 69 mol%, respectively, as determined by HPLC.

Morphological features and photosynthetic pigments

The cell morphology of both strains was influenced by the type of growth substrate. In photoheterotrophically grown cultures, cells of both strains were ovoid to rod-shaped and non-motile. Cells of strain N1^T were approximately 0.8 × 2.4 µm in size; cells of strain N2^T were slightly smaller with a mean size of

0.8 × 1.6 µm. During growth on ferrous iron more elongated rod-shaped, non-motile cells (length up to 3.8 µm) prevailed. A similar influence of the substrate on cell morphology was reported for the ferrous-iron-oxidizing, *Rhodobacter*-like freshwater strain SW2 (Ehrenreich, 1994).

When grown on ferrous iron, the brownish-orange colour of the ferric iron produced masked the colour of the cells. When grown photoheterotrophically, cultures of both strains were yellow-brown under strictly anoxic conditions and pink when the head space of the culture contained air. The photosynthetic pigments of the strains were bacteriochlorophyll *a* and carotenoids of the spheroidene series (spheroidene, spheroidenone, hydroxyspheroidenone), as determined by HPLC. These are the characteristic photosynthetic pigments of *Rhodovulum* and *Rhodobacter* species (Imhoff & Trüper, 1992).

Physiological characterization

Like all known *Rhodovulum* species (Hiraishi & Ueda, 1994), the newly isolated strains also require NaCl for optimal growth. Strains N1^T and N2^T grew at NaCl concentrations of 20–70 and 10–70 g l⁻¹, respectively. An NaCl concentration of 25–50 g l⁻¹ was optimal for both strains.

Photoautotrophic growth of strains N1^T and N2^T was tested with electron donors other than ferrous iron. Both strains utilized sulfide and thiosulfate, like all other known *Rhodovulum* species, and in addition were able to oxidize hydrogen and sulfur, like *Rhodovulum sulfidophilum* (Hansen & Veldkamp, 1973; Hiraishi & Ueda, 1995; Imhoff & Trüper, 1992; Table 1). However, only strains N1^T and N2^T were able to oxidize ferrous iron (Table 1). Furthermore, both strains grew with iron sulfide, forming ferric iron and sulfate. The marine strains N1^T and N2^T share this capacity with the freshwater strains SW2 and L7, which also oxidized iron sulfide completely (Ehrenreich & Widdel, 1994). However, the freshwater strains SW2 and L7 were unable to oxidize free sulfide, thiosulfate and elemental sulfur (Ehrenreich, 1994). Strains N1^T and N2^T grew photoheterotrophically on a variety of organic compounds (Table 1). The range of substrates utilized by strains N1^T and N2^T differed slightly; only strain N1^T grew with butyrate, valerate, fumarate or cysteine, and only strain N2^T utilized glycerol. Like all other known *Rhodovulum* species, the new strains grew well on acetate, lactate, malate, pyruvate, succinate, yeast extract and Casamino acids (Hiraishi & Ueda, 1995; Imhoff & Trüper, 1992; Table 1). On acetate, malate or Casamino acids, a doubling time of 11 h was measured. The capacity for chemotrophic, respiratory growth was tested with acetate as organic substrate. Strains N1^T and N2^T grew in the dark with oxygen as electron acceptor. Under anaerobic conditions in the dark, growth was not observed with nitrate, nitrite, DMSO or with trimethylamine-*N*-oxide. Recently it was

Fig. 2. Ferric iron production during photoautotrophic growth with 10 mM ferrous iron. ▲, Ferric iron in a growing culture of strain N1^T; ●, ferric iron in a growing culture of strain N2^T; ○, ferric iron in a pasteurized control with strain N2^T.

reported that *Rhodobacter capsulatus* grew by dissimilatory reduction of ferric iron (Dobbin *et al.*, 1996). However, none of the marine or freshwater ferrous-iron-oxidizing strains obtained so far was able to use ferric iron for dissimilatory growth in the dark (Ehrenreich, 1994; Heising & Schink, 1998). Both strains did not grow fermentatively with yeast extract, Casamino acids, pyruvate or fumarate.

The pH range and optimum for growth depended on the substrate supplied. With acetate, both strains grew between pH 6 and 9, with an optimum between pH 7 and 7.3 for strain N1^T, and an optimum between pH 7.3 and 7.7 for strain N2^T. However, growth with ferrous iron was for both strains only observed between pH 6.3 and 6.8, with an optimum at pH 6.5. Such a striking, narrow pH range and pronounced optimum in ferrous-iron-containing media was also observed with anaerobic denitrifying ferrous-iron-oxidizing bacteria from freshwater sediments (Straub *et al.*, 1996; K. L. Straub, unpublished results) and with aerobic ferrous-iron-oxidizing bacteria (Pringsheim, 1949). The temperature range for growth of both strains was between 10 and 35 °C, with an optimum at 20–25 °C for strain N1^T and an optimum at 25–28 °C for strain N2^T. Both strains required biotin and nicotinate; strain N2^T also needed vitamin B₁₂.

Growth with ferrous iron

The time courses of ferrous iron oxidation were different in cultures of the two strains (Fig. 2). In repeated growth experiments, strain N1^T always oxidized 60–70 % of the ferrous iron within 15 d, after a lag phase of 8 d. During the same time, strain N2^T oxidized some 80 % of the ferrous iron, and then the oxidation of ferrous iron proceeded slowly and finally reached more than 90 %. Also the freshwater isolates, strains SW2 and L7, oxidized ferrous iron almost

completely (Ehrenreich & Widdel, 1994). A greyish-green intermediate oxidation state of iron, as observed in cultures of the freshwater strains (Ehrenreich & Widdel, 1994), did not appear in the presently described marine cultures; in cultures of the latter, the white ferrous iron precipitates turned gradually into rusty-orange ferric iron precipitates. In strains N1^T and N2^T, the ferric iron produced consisted of ferrihydrite (approx. 98%) and traces of magnetite, as determined by electron diffraction analysis.

Sulfur assimilation

Since the enrichment culture depended on the presence of a reduced sulfur source, the sulfur requirement was again tested with the isolates. Strains N1^T and N2^T were grown photoheterotrophically with 10 mM acetate and 0.5 mM of various sulfur compounds. Growth in subcultures continued only in the presence of thiosulfate, elemental sulfur, sulfide or cysteine, but not with sulfate or sulfite. The same demand for reduced sulfur sources has been reported for the closely related *Rhodovulum adriaticum* (Neutzling *et al.*, 1984). A possible toxic effect of sulfite was checked by growing the strains photoheterotrophically with 10 mM acetate in the presence of different concentrations of sulfite (0.1–5 mM). At a sulfite concentration of 1 mM and higher, growth of both strains was inhibited. Strain N1^T was not only able to use cysteine as sulfur source, but also utilized cysteine (4 mM) as the only substrate for photoheterotrophic growth (Table 1); sulfate was detected as the oxidation product. The capacity for photoheterotrophic growth on cysteine was for the first time reported for strain SiCys, classified as *Rhodovulum sulfidophilum* (Heising *et al.*, 1996). However, strain SiCys is unable to oxidize ferrous iron (B. Schink, personal communication).

Taxonomic conclusion

The results of the 16S rDNA sequence determination and analyses place strains N1^T and N2^T within the radiation of the α subclass of the *Proteobacteria* and more precisely within the radiation of the species of the genus *Rhodovulum*. Although sharing the capacity for ferrous iron oxidation with strain SW2, the two strains are distinct from the previously described strain SW2, which is a member of the genus *Rhodobacter* (Ehrenreich & Widdel, 1994).

On the basis of the low 16S rDNA sequence similarity values (< 95%) to other species of the genus *Rhodovulum* and their distinct physiological characteristics when compared to other species of this genus, strains N1^T and N2^T represent novel species. Strains N1^T and N2^T can be differentiated from each other on the basis of their low 16S rDNA sequence similarity (95.2%), the differing G + C content of their DNA and the differences in the range of substrates used for growth. On the basis of these characteristics and the differences observed between the two strains, they are

described as two new species of the genus *Rhodovulum*, *Rhodovulum iodosum* sp. nov., for strain N1^T, and *Rhodovulum robiginosum* sp. nov., for strain N2^T.

Description of *Rhodovulum iodosum* sp. nov.

Rhodovulum iodosum (i.o.do'sum. M.L. adj. from Gr. adj. *iodaēs* violet, and also rusty, indicating the formation of rusty, ferric iron deposits during photoautotrophic growth on ferrous iron).

Ovoid to rod-shaped, non-motile cells, 0.5–0.8 μ m wide and 2.4–3.8 μ m long. Multiplication by binary fission. Colour of strictly anaerobically grown cells is yellow–brown. Cells contain bacteriochlorophyll *a* and carotenoids of the spheroidene series. Electron donors for photoautotrophic growth are hydrogen, sulfide, thiosulfate, elemental sulfur and ferrous iron; the main product of ferrous iron oxidation is ferrihydrite. Photoheterotrophic growth occurs with acetate, propionate, butyrate, valerate, pyruvate, lactate, succinate, fumarate, malate, cysteine, glutamate and mannitol. Grows chemoheterotrophically in the dark with oxygen. Optimal growth on ferrous iron occurs at 20–25 °C, pH 6.5, and with 25–50 g NaCl l⁻¹. Growth is not possible with sulfate as sole sulfur source; thiosulfate, elemental sulfur, sulfide or cysteine is necessary as a source of reduced sulfur for cell synthesis. Biotin and nicotinate are required as growth factors. The G + C content of the DNA is 66 mol%. The type strain, N1^T (= DSM 12328^T), was isolated from sediment of the mud flat of the Jadebusen, North Sea coast, Germany.

Description of *Rhodovulum robiginosum* sp. nov.

Rhodovulum robiginosum (ro.bi.gi.no'sum. L. adj. *robiginosum*, rusty, indicating the formation of rusty, ferric iron deposits during photoautotrophic growth on ferrous iron).

Ovoid to rod-shaped, non-motile cells, 0.5–0.8 μ m wide and 1.6–3.2 μ m long. Multiplication by binary fission. Colour of strictly anaerobically grown cells is yellow–brown. Cells contain bacteriochlorophyll *a* and carotenoids of the spheroidene series. Electron donors for photoautotrophic growth are hydrogen, sulfide, thiosulfate, elemental sulfur and ferrous iron; the main product of ferrous iron oxidation is ferrihydrite. Photoheterotrophic growth occurs with acetate, propionate, pyruvate, lactate, succinate, malate, glutamate, glycerol and mannitol. Grows chemoheterotrophically in the dark with oxygen. Optimal growth on ferrous iron occurs at 25–28 °C, pH 6.5, and with 25–50 g NaCl l⁻¹. Growth is not possible with sulfate as sole sulfur source; thiosulfate, elemental sulfur, sulfide or cysteine is necessary as a source of reduced sulfur for cell synthesis. Biotin, vitamin B₁₂ and nicotinate are required as growth factors. The G + C content of the DNA is 69 mol%. The type strain, N2^T (= DSM 12329^T), was isolated from

ACKNOWLEDGEMENTS

We thank Marianne Hanzlik from the Institut für Allgemeine und Angewandte Geophysik, Munich, Germany, for electron diffraction analysis. This work was supported by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie and the Max-Planck-Gesellschaft.

REFERENCES

- Aller, R. C., Mackin, J. E. & Cox, R. T., Jr (1986). Diagenesis of Fe and S in Amazonian inner shelf muds: apparent dominance of Fe reduction and implications for the genesis of ironstones. *Continental Shelf Res* **6**, 263–289.
- Bak, F., Scheff, G. & Jansen, K.-H. (1991). A rapid and sensitive ion chromatographic technique for the determination of sulfate and sulfate reduction rates in freshwater lake sediments. *FEMS Microbiol Ecol* **85**, 23–30.
- Brookins, D. G. (1988). *Eh–pH Diagrams for Geochemistry*. Berlin, Heidelberg & New York: Springer.
- Canfield, D. E., Jørgensen, B. B., Fossing, H. & 7 other authors (1993a). Pathways of organic carbon oxidation in three continental margin sediments. *Mar Geol* **113**, 27–40.
- Canfield, D. E., Thamdrup, B. & Hansen, J. W. (1993b). The anaerobic degradation of organic matter in Danish coastal sediments: iron reduction, manganese reduction and sulfate reduction. *Geochim Cosmochim Acta* **57**, 3867–3883.
- DeSoete, G. (1983). A least square algorithm for fitting additive trees to proximity data. *Psychometrica* **48**, 621–626.
- Dobbin, P. S., Warren, L. H., Cook, N. J., McEwan, A. G., Powell, A. K. & Richardson, D. J. (1996). Dissimilatory iron(III) reduction by *Rhodobacter capsulatus*. *Microbiology* **142**, 765–774.
- Ehrenreich, A. (1994). *Die anaerobe Oxidation von Fe(II) durch Purpurbakterien*. PhD thesis, University of Bremen.
- Ehrenreich, A. & Widdel, F. (1994). Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. *Appl Environ Microbiol* **60**, 4517–4526.
- Fachgruppe Wasserchemie, G. D. C. (1991). *Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlamm-Untersuchung* (vol. Bd. II, E1). Weinheim: VCH.
- Felsenstein, J. (1993). PHYLIP (phylogenetic inference package) version 3.5.1. Department of Genetics, University of Washington, Seattle, USA.
- Hansen, T. A. & Veldkamp, H. (1973). *Rhodopseudomonas sulfidophila*, nov. spec., a new species of the purple nonsulfur bacteria. *Arch Microbiol* **92**, 45–58.
- Heising, S. & Schink, B. (1998). Phototrophic oxidation of ferrous iron by a *Rhodomicrobium vannielii* strain. *Microbiology* **144**, 2263–2269.
- Heising, S., Dilling, W., Schnell, S. & Schink, B. (1996). Complete assimilation of cysteine by a newly isolated non-sulfur purple bacterium resembling *Rhodovulum sulfidophilum* (*Rhodobacter sulfidophilus*). *Arch Microbiol* **165**, 397–401.
- Hiraishi, A. & Ueda, Y. (1994). Intrageneric structure of the genus *Rhodobacter*: transfer of *Rhodobacter sulfidophilus* and related marine species to the genus *Rhodovulum* gen. nov. *Int J Syst Bacteriol* **44**, 15–23.
- Hiraishi, A. & Ueda, Y. (1995). Isolation and characterization of *Rhodovulum strictum* sp. nov. and some other purple nonsulfur bacteria from colored blooms in tidal and seawater pools. *Int J Syst Bacteriol* **45**, 319–326.
- Imhoff, J. F. & Trüper, H. G. (1992). The genus *Rhodospirillum* and related genera. In *The Prokaryotes*, 2nd edn, pp. 2141–2155. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. Berlin, Heidelberg & New York: Springer.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Maidak, B. L., Larsen, N., McCaughey, M. J., Overbeek, R., Olsen, G. J., Fogel, K., Blandy, J. & Woese, C. R. (1994). The Ribosomal Database Project. *Nucleic Acids Res* **22**, 3485–3487.
- Neutzling, O., Imhoff, J. F. & Trüper, H. G. (1984). *Rhodopseudomonas adriatica* sp. nov., a new species of the Rhodospirillaceae, dependent on reduced sulfur compounds. *Arch Microbiol* **137**, 256–261.
- Overmann, J., Fischer, U. & Pfennig, N. (1992). A new purple sulfur bacterium from saline littoral sediments, *Thiorhodovibrio winogradskyi* gen. nov. and sp. nov. *Arch Microbiol* **157**, 329–335.
- Pringsheim, E. G. (1949). Iron bacteria. *Biol Rev* **24**, 200–245.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Straub, K. L., Benz, M., Schink, B. & Widdel, F. (1996). Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Appl Environ Microbiol* **62**, 1458–1460.
- Sun, M., Aller, R. C. & Lee, C. (1991). Early diagenesis of chlorophyll-a in Long Island Sound sediments: a measure of carbon flux and particle reworking. *J Mar Res* **49**, 379–401.
- Thamdrup, B., Fossing, H. & Jørgensen, B. B. (1994). Manganese, iron and sulfur cycling in a coastal marine sediment, Aarhus Bay, Denmark. *Geochim Cosmochim Acta* **58**, 5115–5129.
- Widdel, F. & Bak, F. (1992). Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes*, 2nd edn, pp. 3352–3378. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. Berlin, Heidelberg & New York: Springer.
- Widdel, F., Schnell, S., Heising, S., Ehrenreich, A., Assmus, B. & Schink, B. (1993). Ferrous iron oxidation by anoxygenic phototrophic bacteria. *Nature* **362**, 834–836.