Rhodobium pfennigii sp. nov., a phototrophic purple non-sulfur bacterium with unusual bacteriochlorophyll *a* antennae, isolated from a brackish microbial mat on Rangiroa atoll, French Polynesia

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A novel budding purple non-sulfur bacterium (strain AR2102^T) was isolated in pure culture from a microbial mat that had developed in brackish-water ponds on the coral rim of the atoll of Rangiroa (Tuamotu Islands, French Polynesia). Single cells of this strain were rod-shaped and motile by means of polar flagella and divided by budding. Their intracellular photosynthetic membranes were of the lamellar type. Bacteriochlorophyll *a* and carotenoids of the normal spirilloxanthin series, with spirilloxanthin as the main carotenoid, were present as photosynthetic pigments. Bacteriochlorophyll *a* absorption in the infrared portion of the light spectrum exhibited an unusual *in vivo* absorption peak at 909 nm. The strain grew optimally under photoheterotrophic conditions, but could grow photolithotrophically on thiosulfate or chemo-organotrophically under micro-oxic conditions. Optimal growth occurred in the presence of 1–2 % NaCl. Comparative sequence analysis of the 16S rRNA gene placed strain AR2102^T within the class *Alphaproteobacteria*, in a cluster with *Rhodobium* species. Representatives of this cluster form a closely related group of slightly to moderately halotolerant to halophilic, rod-shaped, purple non-sulfur bacteria that divide by budding. The new isolate exhibited some differences in physiology (no utilization of alcohols or carbohydrates) and genetic characteristics (low relatedness in DNA–DNA hybridization) as well as in its relation to light (differences in absorption wavelengths) from previously described *Rhodobium* species. Consequently, we propose that strain AR2102^T (= DSM 17143^T = ATCC BAA-1145^T) should be considered as the type strain of a novel species within the genus *Rhodobium*, *Rhodobium pfennigii* sp. nov.

For many years, the remarkable microbial mats found on Polynesian atolls in the central Pacific have been investigated in biogeochemical and microbiological analyses (Mao Che *et al.*, 2001; Guyoneaud *et al.*, 2002). These mats develop in shallow ponds encountered in the large motus (emerged parts) of several atolls in the Tuamotu archipelago of French Polynesia. These mats, locally called kopara, are very impressive in both their beautiful colours and their thickness, with depths of about 20–50 cm. They cover the entire sediment surface of the ponds above the coral reef. These mats consist of stratified layers of filamentous

cyanobacteria, mainly *Phormidium*, *Schizothrix* and *Scytonema*, that give the mats a gel-like structure. The cyanobacteria are active in the topmost dark-green layer, 2–3 mm in thickness, according to O₂ production (Mao Che *et al.*, 2001). Below, different bacterial groups are distributed along oxygen, sulfide and light gradients. Among these, purple and green anoxygenic phototrophic bacteria have been detected and isolated in large numbers in thin purple or brown layers just below the green cyanobacterial layer (Mao Che *et al.*, 2001). Among these isolates, strain AR2102^T was investigated, the only strain of purple non-sulfur bacteria that showed unusual light absorption above 900 nm.

Strain AR2102^T was isolated from a red layer in the first 2 cm of a microbial mat that had developed in a brackish

Abbreviations: BChl, bacteriochlorophyll; LHC, light-harvesting complex.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AR2102^T is AJ510235.

water pond (Pavete; station R2 of Mao Che *et al.*, 2001) on the rim of Rangiroa atoll. The salinity of the water ranged from 5 to 38 p.p.t. depending on the climatic conditions. At the time of sampling, the salinity was 7 p.p.t. and the temperature of the water above the mat was 34 °C. The oxic layer of the mat was 5 mm in depth; below this depth, the mat was anoxic, containing free sulfide ranging from 0.1 to 2.5 mM in the vertical gradient of the mat (Mao Che *et al.*, 2001).

The strain was isolated from deep-agar dilution series prepared from the red layer. The medium, prepared according to Pfennig & Trüper (1992), contained the following (per litre distilled water): KH_2PO_4 , 0.35 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.05 g; NH_4Cl , 0.5 g; NaCl, 10 g; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.7 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.35 g; NaHCO_3 , 1.5 g; vitamin solution V7 (Pfennig *et al.*, 1981), 1 ml; trace element solution SL12B (Overmann *et al.*, 1992), 1 ml; yeast extract, 0.5 g; disodium succinate, 1.35 g (5 mM); sodium acetate, 0.68 g (5 mM). The pH was adjusted to 6.8. For enrichments and to obtain better growth of pure cultures, the growth medium was supplemented with sodium ascorbate (0.5 g l^{-1}) in order to maintain reducing conditions.

The cultures were incubated at 30 °C with light intensity of $50 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ measured within the photosynthetically active radiation (16 h light, 8 h dark). Purity of cultures was checked by both microscopic observations and growth tests in oxygen gradients established in deep-agar AC medium (Difco), supplemented with thiosulfate (5 mM) and incubated in the dark. Pure cultures in liquid medium were stored in 60 ml screw-capped bottles at 4 °C in the dark.

Microscopic observations and photomicrographs were made with an Olympus BH-2 photomicroscope according to the method of Pfennig & Wagener (1986). Flagella were observed by transmission electron microscopy after negative staining with 1 % (w/v) tungstic acid neutralized to pH 7.2 (JEOL 1200 EX electron microscope). The fine structure of the cells was studied by transmission electron microscopy after fixation of a cell pellet with osmium tetroxide and ultrathin sectioning of the cells according to Reynolds (1963).

Under phase-contrast microscopy, individual cells of strain AR2102^T were short rods, 0.6 μm wide and 1–2 μm long, dividing by budding (Fig. 1a). The cells were motile by polar flagella, as revealed by electron microscopy with negative staining (not shown). Small individual cells were actively motile even after successive transfers in synthetic medium, whereas the large cells with buds were less motile or non-motile. Electron microscopy of thin sections revealed the presence of an intracellular system comprising stacks of lamellar membranes (Fig. 1b).

Absorption spectra of living cells were measured with a Perkin Elmer Lambda 12 spectrophotometer after suspension of a cell pellet in sucrose solution (Pfennig & Trüper,

Fig. 1. Phase-contrast photomicrograph (a) and electron transmission photomicrograph of an ultrathin section (b) of cells of strain AR2102^T. Bars, 5 μm (a) and 0.1 μm (b).

1992). Bacteriochlorophyll (BChl) was identified by its long-wavelength absorption band with the same spectrophotometer, after extraction of the pigments from cell pellets in acetone/methanol (7:2, v/v) in the dark and under N_2 flow for anaerobic conditions. Carotenoids were determined by HPLC analysis employing a Chromasil C18 separation column and acetonitrile/methanol/dichloromethane (70:15:15, by vol.) as the eluent at 1 ml min^{-1} . The percentage of each carotenoid was calculated from peak areas determined according to Buffan-Dubau *et al.* (1996).

The colour of the cell suspension of strain AR2102^T was pink. The absorption spectrum of living cells (Fig. 2) showed typical peaks of BChl *a*, at 378, 594 and 802 nm, and an unusual high peak at 909 nm. The peaks of carotenoids at 483, 512 and 548 nm, with the highest peak at 512 nm, are typical of carotenoids of the normal spirilloxanthin series. The pigment composition determined by HPLC confirmed this biosynthetic pathway, with spirilloxanthin (95.2 %) as the major carotenoid and small amounts of rhodovibrin and rhodopin (3.1 and 1.7 %, respectively).

This pigment composition, with an unusual long-wavelength absorption peak of BChl *a* at 909 nm, is one of the

Fig. 2. *In vivo* absorption spectra of strain AR2102^T (solid line) and *Rbi. marinum* DSM 2698^T (dotted line).

most interesting characteristics of strain AR2102^T. Several other phototrophic purple bacteria with unusual absorption peaks of BChl *a* between 911 and 920 nm, e.g. *Roseospira thiosulfatophila* (Guyoneaud *et al.*, 2002), *Roseospirillum parvum* (Glaeser & Overmann, 1999) and *Thermochromatium tepidum* (Garcia *et al.*, 1986), have been isolated previously. Generally, phototrophic bacteria containing BChl *a* have three major absorption bands in the near infrared region, at 800, 850–860 and 880–890 nm; the two first bands (800 and 850–860 nm) correspond to light-harvesting complex (LHC) II and the third (880 nm) to the low-energy LHC I. In strain AR2102^T, as in the three other species mentioned above, LHC I is different, with absorption peaks in the region 909–920 nm. Moreover, strain AR2102^T, together with *Rss. parvum*, possesses only LHC I, as demonstrated by an *in vivo* absorption spectrum with only two peaks, a small peak at 800 nm and a higher peak at 909–911 nm (LHC I). The absence of LHC II is also a characteristic of *Rhodobium marinum* (Fig. 2). The other two species mentioned above, *Ros. thiosulfatophila* and *Tch. tepidum*, possess both LHC I and LHC II, with three absorption bands in the near infrared region (Garcia *et al.*, 1986; Guyoneaud *et al.*, 2002). Another purple bacterium, containing BChl *b* (*Rhodospira trueperi*; Pfennig *et al.*, 1997), was found to absorb long wavelengths at 986 nm *in vivo* rather than 1025 nm, which is more typical of BChl *b*. Thus, a significant number of strains, all isolated from microbial mats, have the property of harvesting infrared light at wavelengths between 900 and 1000 nm, corresponding to a window in the light spectrum that is not used by most currently known phototrophic purple bacteria that contain typical BChl *a* or BChl *b* antennae. With the exception of *Tch. tepidum*, which was isolated from a mat in a thermal hot spring in Yellowstone National Park (USA), the other three species (*Rsa. trueperi*, *Rss. parvum*, *Ros. thiosulfatophila*) and strain AR2102^T originated from marine laminated microbial mats. They could be considered as characteristic micro-organisms in these mats, where infrared light penetrates deeper, down to the anoxic purple layers below

the cyanobacterial top layers. In these purple layers, such bacteria co-exist with purple bacteria containing typical BChl *a* or *b* antennae by using the intermediate wavelengths between 900 and 1000 nm, thus showing the capacity for growth without competition for light over a rather large part of the near-infrared spectrum, between 800 and 1025 nm.

DNA base composition and DNA–DNA hybridization tests were performed by the Identification Service of the DSMZ (Braunschweig, Germany). The G + C content of the DNA was determined by HPLC as described by Mesbah *et al.* (1989), using bacteriophage lambda DNA as the standard. Hybridization tests were performed according to the method of De Ley *et al.* (1970) as modified by Huß *et al.* (1983).

Genomic DNA isolation was performed as described by Precigou *et al.* (2001). The 16S rRNA gene was amplified from genomic DNA using universal primers (Lane, 1991; Weisburg *et al.*, 1991) for the bacterial domain. PCR amplification, 16S rRNA gene sequencing and sequence analysis were performed as described by Guyoneaud *et al.* (2002). Phylogenetic trees were constructed by using the PHYLIP computer package (Felsenstein, 1993). The confidence level of the phylogenetic tree topology was evaluated by performing 100 bootstrap replications with the programs SEQBOOT and CONSENSE.

The neighbour-joining phylogenetic tree is presented in Fig. 3. Strain AR2102^T is included in a cluster with all the *Rhodobium* strains identified so far and is closely related to *Rbi. marinum* DSM 2698^T, with 98.3% 16S rRNA gene sequence similarity. It is more distant from the type strain of the second species of the genus, *Rhodobium orientis* JCM 9397^T, with only 94.3% similarity. It conforms to the description of the genus *Rhodobium* (Hiraishi *et al.*, 1995). However, the DNA base composition of strain AR2102^T was 67.5 mol% G + C as determined by HPLC. In comparison, the DNA base composition of *Rbi. marinum* DSM 2698^T was determined as 64.2 mol% G + C by using the same method at the same time, thus showing that strain AR2102^T cannot be a member of the species *Rbi. marinum*. DNA–DNA hybridization between the most closely related type strain, *Rbi. marinum* DSM 2698^T, and strain AR2102^T was very low (10.4–17.2% DNA–DNA relatedness) and clearly confirmed the difference between the two strains.

The ability to utilize various substrates for growth and the optimal concentration of NaCl, optimal pH, light intensity and sulfide tolerance of strain AR2102^T were determined according to Guyoneaud *et al.* (2002).

Fermentative metabolism was tested under anaerobic conditions in the dark with pyruvate as the sole substrate, without addition of electron acceptors. Denitrification was tested under the same conditions with nitrate as the electron acceptor. Microaerophilic growth, vitamin requirements and utilization of nitrogen (ammonium chloride, 5 mM; sodium nitrate, 5 mM; organic nitrogen in sodium

Fig. 3. Dendrogram showing the relationships between the 16S rRNA gene sequences of strain AR2102^T and related phototrophic purple non-sulfur bacteria (strain numbers and EMBL accession numbers are indicated). The 16S rRNA gene sequence of *Allochromatium vinosum* ATCC 17899^T was included in the sequence analysis to root the tree. Bar, 2% difference in nucleotide sequence. Numbers on the dendrogram indicate the significance (percentage of outcomes) of the branches (bootstrap analysis).

glutamate, 5 mM) and sulfur (sulfate, 5 mM; sulfide, 2 mM; cysteine, 3 mM) compounds and hydrogen was tested according to Guyoneaud *et al.* (2002). The presence of catalase was determined by adding a few drops of 3% (v/v) H₂O₂ to 2 ml of a dense cell suspension.

Strain AR2102^T grew photo-organotrophically (50–80 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) on a wide number of organic substrates, mainly fatty acids and other organic acids (Table 1). The strain grew very well with the following substrates (mM, except where stated): butyrate (4), fumarate (4), succinate (5), pyruvate (5) and 2-oxoglutarate (4). The strain can also use acetate (5), propionate (4), valerate (4), crotonate (2), lactate (5), malate (4), glutamate (4), aspartate (3), cysteine (3), cyclohexanecarboxylate (2), Casamino acids (0.05%) and yeast extract (0.2%). It was not able to use the following substrates: sulfur, sulfite (2), formate (5), caprylate, pelargonate, palmitate (all at 2 mM), citrate, methanol, ethanol, propanol, butanol, glycerol, mannitol, glycolate, benzoate, sucrose, trehalose (all at 4 mM), glucose (5), fructose (5), gluconate, methionine, glycine betaine (all at 3 mM), thioacetamide, *N*-acetylglucosamine, tartrate, gallate, catechol and nicotinate (all at 2 mM). Poor photolithotrophic growth was observed only with thiosulfate (4 mM) as electron donor. Strain AR2102^T was not able to use sulfide (2 mM) but could tolerate sulfide concentrations up to 2–3 mM free sulfide. H₂ (2 bars) was not used as an electron donor. Strain AR2102^T utilized a rather similar selection of organic substrates, mainly fatty and organic acids, as the known species of the genus, *Rbi. marinum* and *Rbi. orientis* (Table 1). However, in contrast to the two known species, strain AR2102^T was not able to use alcohols or sugars, assimilation of which is an important characteristic of the genus *Rhodobium*. After three cultures, transferred at the same salinity, the strain could grow without NaCl in the synthetic medium ($\mu = 0.003 \text{ h}^{-1}$) and tolerated up to 5% NaCl ($\mu = 0.009 \text{ h}^{-1}$), with optimal growth at 1–2% (w/v) NaCl ($\mu = 0.05 \text{ h}^{-1}$). Like the two

described *Rhodobium* species, this new isolate is of marine origin, indicating halotolerant properties. Strain AR2102^T is thus well adapted to the brackish environment of the ponds from which it was isolated, the water salinity of which was found to vary between 5 and 38 p.p.t. (Mao Che *et al.*, 2001). It grew well between pH 6.3 and 8.5, with an optimum at pH 7.2–7.5. The strain showed good growth between 25 and 35 °C.

Growth of strain AR2102^T was tested at light intensities between 2 and 400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Incubated at 30 °C in the synthetic medium with optimal pH and salinity, best growth on succinate occurred at 400 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ($\mu = 0.100 \text{ h}^{-1}$; doubling time 6.9 h). Growth was still good at 80 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ($\mu = 0.08 \text{ h}^{-1}$) and slower at 50 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ($\mu = 0.05 \text{ h}^{-1}$). Very slow growth of strain AR2102^T still occurred at 2 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ($\mu = 0.005 \text{ h}^{-1}$). These data show the very wide adaptation to light intensity of strain AR2102^T.

Chemo-organotrophic growth of strain AR2102^T with acetate or succinate occurred under microaerophilic conditions in the dark at 2 mm depth in a tube of agar medium open to air. The strain was also able to grow in shaken liquid cultures under aerobic conditions, showing its capacity to develop in the presence of oxygen in the dark by using a respiratory metabolism. Catalase was present. The strain was unable to grow by fermentative metabolism in the dark, or with nitrate as an electron acceptor. Thiamine or yeast extract were required for growth. Strain AR2102^T was able to grow with sulfate as the sole sulfur source, indicating an assimilatory sulfate reduction pathway. The strain used ammonium chloride or glutamate as a nitrogen source for growth. It could fix dinitrogen but was not able to use nitrate as a nitrogen source.

Thus, according to phylogenetic differences, low relatedness in DNA–DNA hybridization and particularly such strong

Table 1. Major properties of strain AR2102^T in comparison with *Rbi. marinum*, *Rbi. orientis* and *Rss. parvum*

Taxa: 1, strain AR2102^T; 2, *Rbi. marinum* (data from Imhoff, 1983); 3, *Rbi. orientis* (Hiraishi *et al.*, 1995); 4, *Rss. parvum* 930I^T (Glaeser & Overmann, 1999). +, Utilized; (+), utilized poorly; –, not utilized; +/–, utilized by some strains; ND, no data available. The following substrates were utilized by all four taxa: acetate, butyrate, pyruvate, lactate, malate, fumarate and succinate. Sulfur and sulfite were not used.

Property	1	2	3	4
Cell size (µm)	0.6 × 1–2	0.7–0.9 × 1–2.5	0.7–0.9 × 2.5–5	0.4–0.6 × 1.8–2.6
Morphology	Rod	Rod	Rod	Vibrioid, spirilloid
Flagella	Polar	Polar	Polar	Bipolar
Cell division	Budding	Budding	Budding	Binary
BChl <i>a</i> absorption peak (nm)	909	883	870	911
Carotenoids*	Sp	Sp	Sp	Sp, la
Cytoplasmic membrane	Lamellar	Lamellar	Lamellar	Lamellar stacks
DNA G + C content (mol%)	67.5	61.5–64.1	65.2–65.7	71.2
Optimal pH	7.2–7.5	6.9–7.1	7–7.5	7.9
pH range	6.3–8.5	ND	ND	ND
Optimal salinity (%)	1–2	1–5	4–5	1–2
Salinity range (%)	0–5	1–5	2–8	1–6
Vitamin(s) required†	Thiamine	Complex	<i>p</i> -ABA, biotin	Complex
Sulfate assimilation	+	+	+	–
Substrates:				
Sulfide	–	+	(+)	+
Thiosulfate	(+)	–	+	+
Formate	–	+	+	–
Propionate	+	+	–	+
Citrate	–	+ / –	–	–
Glucose	–	+	+	–
Glycerol	–	+ / –	–	–
Mannitol	–	+	–	–
Ethanol	–	+ / –	–	–
Gluconate	–	ND	ND	–
Hydrogen	–	ND	ND	–

*la, Lycopenal; Sp, spirilloxanthin.

†*p*-ABA, *p*-Aminobenzoic acid

phenotypic differences in pigment composition and the use of substrates from the existing members of the genus, it is justified to consider strain AR2102^T as a representative of a novel species, with the name *Rhodobium pfennigii* sp. nov.

Description of *Rhodobium pfennigii* sp. nov.

Rhodobium pfennigii (pfen.ni'gi.i. N.L. gen. n. *pfennigii* of Pfennig, named after Norbert Pfennig, a German microbiologist).

Straight to ovoid rods, 0.6 µm wide and 1–2 µm long. Multiplication is by budding. Gram-negative cells are motile by polar flagella. Colour of cell suspension is pink. Stacks of lamellar photosynthetic membranes occur. Contains BChl *a* as photosynthetic pigment and spirilloxanthin as the major carotenoid, with small amounts of rhodovibrin and rhodopin. Absorption maxima of living cell suspensions at 395, 483, 512, 548 and 594 nm and infrared absorption peak

at 909 nm. Electron donors for photo-organotrophic growth are acetate, propionate, butyrate, valerate, crotonate, pyruvate, lactate, malate, fumarate, succinate, 2-oxoglutarate, glutamate, aspartate, yeast extract and Casamino acids. Photolithotrophic growth occurs with thiosulfate; chemo-organotrophic growth occurs under micro-oxic to oxic conditions in the dark with acetate and succinate. Capable of assimilatory sulfate reduction and nitrogen fixation. Thiamine or yeast extract is required for growth. Optimal pH is 7.2–7.5 (range pH 6.3–8.5). Optimal temperature is 30–35 °C. Salinity range is 0–5 % (w/v) NaCl (optimum 1–2 % NaCl). DNA base composition is 67.5 mol% G + C (HPLC). Habitat: microbial mats exposed to light in coastal marine environments or brackish ponds.

The type strain is strain AR2102^T (=ATCC BAA-1145^T = DSM 17143^T), isolated from a benthic microbial mat in a brackish pond (R2) located on the rim of the Rangiroa atoll (French Polynesia).

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References

- Buffan-Dubau, E., De Wit, R. & Castel, J. (1996). Feeding selectivity of the harpacticoid copepod *Canuella perplexa* in benthic muddy environments demonstrated by HPLC analyses of chlorin and carotenoid pigments. *Mar Ecol Prog Ser* **137**, 71–82.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Garcia, D., Parot, P., Vermeglio, A. & Madigan, M. T. (1986). The light-harvesting complexes of a thermophilic purple sulfur photo-synthetic bacterium *Chromatium tepidum*. *Biochim Biophys Acta* **850**, 390–395.
- Glaeser, J. & Overmann, J. (1999). Selective enrichment and characterization of *Roseospirillum parvum*, gen. nov. and sp. nov., a new purple nonsulfur bacterium with unusual light adsorption properties. *Arch Microbiol* **171**, 405–416.
- Guyoneaud, R., Moune, S., Eatok, C., Bothorel, V., Hirschler-Rea, A., Willison, J., Duran, R., Liesack, W., Herbert, R. & other authors (2002). Characterization of three spiral-shaped purple nonsulfur bacteria isolated from coastal lagoon sediments, saline springs, and microbial mats: emended description of the genus *Roseospira* and description of *Roseospira marina* sp. nov.; *Roseospira navarrensis* sp. nov., and *Roseospira thiosulfatophila* sp. nov. *Arch Microbiol* **178**, 315–324.
- Hiraishi, A., Urata, K. & Satoh, T. (1995). A new genus of marine budding phototrophic bacteria, *Rhodobium* gen. nov., which includes *Rhodobium orientis* sp. nov. and *Rhodobium marinum* comb. nov. *Int J Syst Bacteriol* **45**, 226–234.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Imhoff, J. F. (1983). *Rhodopseudomonas marina* sp. nov., a new marine phototrophic purple bacterium. *Syst Appl Microbiol* **4**, 512–521.
- 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.
- Mao Che, L., Andréfouët, S., Bothorel, V., Guezennec, M., Rougeaux, H., Guezennec, J., Deslandes, E., Trichet, J., Matheron, R. & other authors (2001). Physical, chemical, and microbiological characteristics of microbial mats (KOPARA) in the South Pacific atolls of French Polynesia. *Can J Microbiol* **47**, 994–1012.
- 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.
- 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.
- Pfennig, N. & Trüper, H. G. (1992). The family Chromatiaceae. In *The Prokaryotes*, 2nd edn, pp. 3200–3221. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Pfennig, N. & Wagener, S. (1986). An improved method of preparing wet mounts for photomicrographs of microorganisms. *J Microbiol Methods* **4**, 303–306.
- Pfennig, N., Widdel, F. & Trüper, H. G. (1981). The dissimilatory sulfate-reducing bacteria. In *The Prokaryotes*, vol. 1, pp. 926–940. Edited by M. P. Starr, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- Pfennig, N., Lünsdorf, H., Süling, J. & Imhoff, J. F. (1997). *Rhodospira trueperi* gen. nov., sp. nov., a new phototrophic proteobacterium of the alpha group. *Arch Microbiol* **168**, 39–45.
- Precigou, S., Goulas, P. & Duran, R. (2001). Rapid and specific identification of nitrile hydratase (NHase) encoding genes in soil samples by polymerase chain reaction. *FEMS Microbiol Lett* **204**, 155–161.
- Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J Cell Biol* **17**, 208–212.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.