Roseovarius mucosus sp. nov., a member of the *Roseobacter* clade with trace amounts of bacteriochlorophyll *a*

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Among a group of marine isolates that were found to be positive for genes of the bacterial photosynthetic reaction centre, a strain was selected for characterization that was phylogenetically close to the genus *Roseovarius*. The strain, designated DFL-24^T, originated from a culture of *Alexandrium ostenfeldii* (dinoflagellate) and contained small amounts of bacteriochlorophyll *a*, corresponding to about 1 % of the amount found in intensely pigmented aerobic phototrophs such as *Roseobacter litoralis*. Cells were rods of 0.5–0.7 × 1.3–3.0 µm often with uneven ends, suggesting a budding mode of division. True motility was not observed. Electron micrographs of ultrathin sections revealed a Gram-negative cell-wall structure. Cultures did not grow without addition of sea salts and tolerated up to 10 % (w/v) sea-water salts. Acetate, butyrate, tricarboxylic acid cycle intermediates, glutamate and glycerol were used as growth substrates, but not glucose or fructose. Biotin, thiamine and nicotinic acid were required as growth factors. The DNA G + C content was 63 mol%. 16S rRNA gene sequence analysis placed the strain within the *Roseobacter* lineage of the 'Alphaproteobacteria'. Its closest phylogenetic neighbour was *Roseovarius tolerans* showing 96.4 % 16S rRNA gene sequence similarity. Based also on its physiological and biochemical characteristics, the strain is considered to represent a novel species of the genus *Roseovarius*, *Roseovarius mucosus* sp. nov. Strain DFL-24^T (= DSM 17069^T = NCIMB 14077^T) is the type strain. The 16S rRNA gene sequence of DFL-24^T was found to have a similarity of 99.7 % with an unidentified strain, 253-13 (= DSM 17070), which was likewise characterized and shown to constitute another strain of the species.

A large number of pigmented marine isolates have been investigated recently for the presence of photosynthetic reaction-centre genes, *pufL* and *pufM*, using specific primers and sequencing (Allgaier *et al.*, 2003). The 16 strains that were found to be positive for these genes were characterized based on 16S rRNA gene sequence analysis and found to be phylogenetically diverse, forming five groups within the 'Alphaproteobacteria'. Only one group exhibited well-pigmented cultures under appropriate growth conditions and was shown to contain bacteriochlorophyll *a* (*bchl a*) (Allgaier *et al.*, 2003; Biebl *et al.*, 2005). The other groups appeared beige, slightly pink or yellow; *bchl a* was not detected initially, but was found later in small amounts or

under specific conditions. Two strains were related closely to *Roseovarius tolerans* (Labrenz *et al.*, 1999) according to their 16S rRNA gene sequence. One of these strains, DFL-24^T, is morphologically, physiologically and biochemically characterized here, and the results suggest that it should be classified as a novel species of the genus *Roseovarius*, a member of the *Roseobacter*–*Sulfitobacter*–*Silicibacter* group. In addition, a hitherto-unidentified marine strain, which was isolated by Hold *et al.* (2001), was also shown to be a member of this species.

All three strains of the novel species were obtained from dinoflagellates. DFL-24^T was isolated from a culture of *Alexandrium ostenfeldii* KO287, which was maintained in the collection of the Biological Institute, Helgoland. The algal cells were isolated and washed, and the attached bacteria were spread on an agar medium containing 0.25 g peptone, 0.25 g yeast extract, 0.25 g glucose and 10 g sea salts l⁻¹. After 2 weeks incubation at 18 °C, two faintly pink colonies were purified and maintained as strains DFL-24^T

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Abbreviation: *bchl a*, bacteriochlorophyll *a*.

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and DFL-35, which appeared to be very similar. DFL-24^T was further investigated.

Strain DFL-24^T grew well on marine agar 2216 (Difco). Colonies were whitish to faintly pink, circular, convex and had an entire margin and a glistening surface. A slimy consistency was often observed. By using a dissecting microscope and light from underneath, two stable colony morphotypes were discriminated: a rough, slightly slimy type and a glistening, somewhat darker type. The 16S rRNA gene sequences of the two types were found to be identical. For further characterization, cultures of the rough type were used. Cells were ovoid to rod-shaped and $0.5\text{--}0.7 \times 1.3\text{--}3.0\text{ }\mu\text{m}$ in size, often with unequal ends (Fig. 1a), suggesting a budding mode of division. The cells in the rough colony type were distributed evenly, whereas they formed star-shaped aggregates in the glistening colony type (Fig. 1b). Real motility was not observed, but occasionally cells showed a turning motion on the spot, which was therefore not caused by Brownian motion. In negatively stained preparations (Biebl *et al.*, 2005), the electron micrographs revealed extensions of the outer membrane at the cell poles (Fig. 2a), as has been described for

Fig. 1. Phase-contrast photomicrographs of cells of *Roseovarius mucosus* DFL-24^T grown in a medium containing 20 g sea salts, 3 g peptone and 0.5 g yeast extract l⁻¹, showing dispersed cells from the brighter colony type (a) and star-shaped aggregates from the darker, glistening colony type (b). Bar, 10 μm (applies to both images).

Fig. 2. Electron micrographs of cells of strain DFL-24^T. (a) Negatively stained preparation. Polarly situated extensions of the outer membrane are indicated (ome). Bar, 500 nm. (b) Oblique ultrathin section showing the Gram-negative cell-wall structure (om, outer membrane; cm, cytoplasmic membrane; asterisk in the inset, translucent periplasmic space) and the cytoplasm with the bacterial chromosome (Chr). Bars, 250 nm (main image); 50 nm (inset).

Roseovarius nubinihibens (González *et al.*, 2003). Flagella were not observed. In ultrathin sections, the periplasmic space appeared rather translucent (Fig. 2b, inset); the peptidoglycan sacculus was not visible. The outer membrane outlined the cell as an intensely stained layer with an undulating contour (Fig. 2b).

When grown in a medium containing 3 g peptone and 0.5 g yeast extract l⁻¹, the cultures developed readily at concentrations of 1–7 % sea salts (Sigma), but growth was somewhat delayed at 0.3, 0.6 and 10 %. No growth occurred without addition of sea salts. For the test cultures described below, a concentration of 2 % sea salts was used. Within the temperature range tested (15–43 °C at intervals of 3 °C; Biebl *et al.*, 2005), growth was observed throughout, with maximum growth observed at 31 °C. Growth was equally good at initial pH values between 6.5 and 8.8. Below

pH 6.5, growth was increasingly slower, and was lacking above pH 9.1.

Utilization of carbon sources was tested in a medium containing 20 g sea salts, 0.3 g (NH₄)₂SO₄, 0.1 g KH₂PO₄, 0.1 g yeast extract and 1 ml trace-element solution (Pfennig & Trüper, 1992) l⁻¹ at a pH of 7.5. The strain was able to use acetate and butyrate, intermediates of the tricarboxylic acid cycle, lactate, glutamate and glycerol, but not glucose, fructose, ethanol or methanol (see Table 1 and the species description for further details). Interestingly, the inability to grow on glucose was also found for *R. tolerans* (Labrenz *et al.*, 1999). DFL-24^T did not grow in a medium without yeast extract, but yeast extract could be replaced by a mixture of seven vitamins. Tests with mixtures in which one of these vitamins was omitted revealed that only biotin, thiamine and nicotinic acid were essential for growth. Strain DFL-24^T was able to degrade gelatin, but not starch, alginate or Tween 80. Dissimilatory nitrate reduction was not found; neither nitrite (sulfanilic acid/

N,N-dimethylnaphthylamine test) nor nitrogen (Durham tubes) was formed.

The ability to grow anaerobically or microaerobically in the light or in the dark was visualized in agar deeps using acetate as a carbon source. Molten agar medium (10 ml at 42 °C) in 22 ml test tubes was mixed with a drop of culture and incubated under air and nitrogen. There was no indication of growth under any of these conditions; cells developed only at the surface of the agar column in contact with air.

Allgaier *et al.* (2003) were not able to detect bchl *a* in the cells of strain DFL-24^T. However, when the pellet of the 25 ml culture grown in the above peptone medium was extracted with 3 ml acetone/methanol (7 : 2), a flat hump was seen in the absorption spectrum around 770 nm, the IR absorption range of bchl *a*. Faint IR absorption was also detected in a succinate-grown continuous culture of the strain, particularly during dark growth following an illumination period (H. Biebl, unpublished observations).

Table 1. Morphological and physiological properties of *Roseovarius mucosus* DFL-24^T in comparison with the other *Roseovarius* species

Species: 1, *Roseovarius mucosus*; 2, *Roseovarius tolerans*; 3, *Roseovarius nubinhibens*. +, Positive reaction; –, negative reaction; ±, variable from strain to strain; NT, not tested.

Characteristic	1	2*	3†
Cell shape	Short rods, uneven division	Short rods, uneven division	Short rods
Motility	–	– (tumbling)	–
Flagella	Not found	Not found	Not mentioned
Bacteriochlorophyll <i>a</i> :			
Presence	Traces	Variable	Not found
<i>In vivo</i> peaks (nm)		801, 878	
Sea salts requirement (%):			
Range	0.3–10	1–15	
Optimum	1–7	1–13	0.8–3.2‡
Substrates:			
Glucose	–	–	–
Fructose	–	NT	–
Ethanol	–	NT	–
Glycerol	–	NT	–
Hydrolysis of gelatin	±	–	–
Vitamin requirement:			
Biotin	–	–	–
Thiamine	–	–	–
Nicotinic acid	–	–	–
Cobalamin	–	±	–
Susceptible to:			
Penicillin G	±	–	NT
Tetracycline	–	–	NT
DNA G + C content (mol%)	60.9–62.9	62.3–63.8	66.0

*Data from Labrenz *et al.* (1999).

†Data from González *et al.* (2003).

‡NaCl only.

Very small levels of bchl *a* have previously been reported for aerobic anoxygenic phototrophs (Sato, 1978; Yurkov *et al.*, 1993; Hiraishi *et al.*, 2000; Hiraishi & Shimada, 2001). In visibly pigmented cultures, a specific bchl *a* content of 1–4 nmol (mg protein)^{−1} has been measured, whereas <0.1 nmol (mg protein)^{−1} was found generally in colourless or beige-coloured cultures. The lowest value, ≤0.004 nmol (mg protein)^{−1}, was reported for *Acidiphilium acidophilum* (Hiraishi & Shimada, 2001). For strain DFL-24^T, our estimated bchl *a* content was 0.05 nmol (mg protein)^{−1}. In *Blastomonas natatoria*, which was originally regarded as a pigment-free close relative of *Erythromonas ursincola* (Yurkov *et al.*, 1997), bchl *a* was identified by HPLC and photodiode-array detection and found to be present in very small amounts. Interestingly, in the genome of this species, the *pufL/M* genes were also shown to be present (Hiraishi *et al.*, 2000), as in strain DFL-24^T. For *R. tolerans*, no quantitative data on bchl *a* content are available, but it is noticeable that some strains belonging to this species formed bchl *a* and others did not, and two formed photosynthetic pigments only after several years of cultivation (Labrenz *et al.*, 1999). However, the *pufL/M* genes were present in all eight existing *Roseovarius* strains (Allgaier *et al.*, 2003). González *et al.* (2003) described a second species of *Roseovarius*, *R. nubinhibens*, which was free of photosynthetic pigments. Boettcher *et al.* (2005) showed that neither *R. nubinhibens* nor a newly proposed species, *Roseovarius crassostreae*, contains the *pufL/M* genes. The significance of very small amounts of bchl *a* for aerobic metabolism is unknown at present. Possibly, there are hitherto undetected conditions under which the bchl *a* content is increased to an

effective quantity, or the bchl *a* found is merely a remnant of a formerly expressed physiological activity.

Polar lipids were extracted with chloroform/methanol/0.3 % NaCl and were separated by TLC according to the method of Tindall (1990). Phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine were found, as previously shown in *R. tolerans*. By contrast, the lipid composition of other *Roseobacter* group members, such as *Antarctobacter heliothermus*, *Ruegeria algicola*, *Ruegeria denitrificans* and *Ruegeria litoralis*, differs considerably from this pattern (Labrenz *et al.*, 1999). The predominant lipoquinone was ubiquinone-10. The fatty acid profile of strain DFL-24^T was also similar to that of *R. tolerans* (Table 2). As in most members of the ‘*Alpha-proteobacteria*’, 18:1 ω 7 c is the predominant component, amounting to 70 % of the total fatty acid content (76 % as determined by González *et al.*, 2003), followed by 16:1 (about 10 %). Labrenz *et al.* (1999) also reported 10 % 18:2, which may have arisen from the low cultivation temperature used by these authors. The occurrence of the hydroxylated acids 12:0 2-OH, 12:0 3-OH and 12:1 3-OH might be more characteristic of the genus *Roseovarius*. 12:1 3-OH was present in both strain DFL-24^T and *R. tolerans*, whereas *R. nubinhibens* contained 12:0 3-OH instead. Detection of 12:0 2-OH requires acid hydrolysis, which was not applied by González *et al.* (2003).

The 16S rRNA gene sequence of DFL-24^T was determined as described by Allgaier *et al.* (2003) and Biebl *et al.* (2005) with the following amendments. The sequences were aligned manually and compared with published sequences from the

Table 2. Fatty acid composition of three species of the genus *Roseovarius*

Fatty acid	<i>Roseovarius mucosus</i> DFL-24 ^T	<i>Roseovarius tolerans</i> EL-172		<i>Roseovarius nubinhibens</i> ISM
	This study	Labrenz <i>et al.</i> (1999)	González <i>et al.</i> (2003)	González <i>et al.</i> (2003)
10:0 3-OH	0.7			
12:0	3.1			3.3
12:0 2-OH	2.5	2.4		
12:0 3-OH				1.5
12:1 3-OH	1.7	3.6	3.3	
16:1 ω 7 c				1.0
16:1 ω 9 c	0.8	0.8		
16:0	13.1	6.2	9.7	9.8
17:0			2.5	
18:0	1.8	0.8	1.7	0.6
Methyl 18:0	2.1			
18:1 ω 7 c	69.4	70.2	76.0	76.4
Methyl 18:1 ω 7	2.1		5.5	
Cyclo 19:1	3.7			5.3
18:2		10.6		
Unidentified (C ₁₈ ?)	1.1			

DSMZ 16S rRNA gene sequence database, including sequences available from the Ribosomal Data Project (Maidak *et al.*, 1999) and GenBank/EMBL. Alignment was achieved with the BioEdit program (Hall, 1999) and was used for calculating the distance matrix corrected by Kimura's two-parameter method (Kimura, 1980) and CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic dendrogram was inferred by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis was based on 1000 resamplings.

The strain was thus identified as a member of the *Roseobacter* group within the 'Alphaproteobacteria'. Its closest phylogenetic neighbour was found to be *R. tolerans* EL-172^T, showing a 16S rRNA gene sequence similarity value of 96.4 %, whilst *R. nubinihibens* showed only 93.9 % similarity. *R. tolerans*, *R. nubinihibens* and the novel species proposed herein, *Roseovarius mucosus* sp. nov., form a clearly separated group within the *Roseobacter* clade (Fig. 3). Furthermore, the 16S rRNA gene sequence from *R. crassostreae* (GenBank accession no. AF114484; Boettcher

Fig. 3. Neighbour-joining dendrogram based on 16S rRNA gene sequence comparisons showing the phylogenetic position of *Roseovarius mucosus* DFL-24^T within the 'Alphaproteobacteria'. Bootstrap confidence values > 70 % are shown at branching points (percentages of 1000 replicates). The tree was rooted with *Escherichia coli* as outgroup. GenBank accession numbers are given in parentheses. Bar, 0.1 % sequence divergence.

et al., 2005) is more closely related to *R. nubinhibens* and both species can clearly be differentiated from the *R. tolerans*/*R. mucosus* cluster by specific signature nucleotides at several positions of the secondary structure from the 16S rRNA gene. The pattern of 16S rRNA gene signatures consists of (A–U) for '*Roseovarius crassostreae*' and *Roseovarius nubinhibens*. The following helical regions are characterized by base-pair exchanges and the nucleotides detected for the *R. crassostreae*/*R. nubinhibens* group are given in parentheses: helical region 240–242/284–286 UGG/CCA (UAG/CUA), position 113–115/312–314 GUG/CAC (CAA/GUU), position 577–580/764–767 GCAC/GUGC (GCGC/GUGC). Additionally, sequences of the strains differ in the loop region of the fifth variable region of the 16S rRNA gene sequence. 16S rRNA gene sequence similarity of *R. mucosus* and *R. tolerans* was determined to be 96.4%, whereas that between *R. nubinhibens* and *R. tolerans* was 95.8% (González *et al.*, 2003), which is relatively low for species affiliation within a genus. However, if the pattern of the investigated characters is taken into consideration, the species designation is justified, whereby *R. nubinhibens* appears more distant from the other two species. The three species are morphologically very similar; they form short to medium-sized rods. For *R. mucosus* and *R. tolerans*, an uneven cell division was observed. Motility was almost absent in *R. mucosus*, tumbling in *R. tolerans* and present in *R. nubinhibens*, whilst flagella were not found in any of the strains. Substrate utilization agreed well in *R. mucosus* and *R. tolerans*, in particular the inability to use glucose, which is used readily by *R. nubinhibens*. The cellular fatty acid composition showed a good match for the three species; *R. nubinhibens* contained a different hydroxylated C₁₂ acid. Polar lipids were almost identical in *R. mucosus* and *R. tolerans*. The DNA G+C content of the novel species is again closer to that of *R. tolerans* than to that of *R. nubinhibens*. *Roseovarius* species differ strongly in bacteriochlorophyll content, but because formation of photosynthetic pigments in obligately aerobic bacteria is strongly dependent on cultivation conditions, it cannot be used as a taxonomically relevant criterion, given the pigmented and colourless strains reported for *R. tolerans* and the trace amounts reported for *R. mucosus*. However, both species have the *puf* genes of the photosynthetic reaction centre. It is remarkable that in *R. nubinhibens*, as well as in *R. crassostreae*, the *pufL/M* genes could not be detected (Boettcher *et al.*, 2005).

When aligning the 16S rRNA gene sequence of strain DFL-24^T with suitable database sequences, a hitherto-unidentified strain was found that was markedly closer to strains DFL-24^T and DFL-35 than to the *R. tolerans* strains (99 versus 95% similarity). This unidentified strain had been reported as an isolate from the dinoflagellate *Alexandrium lusitanicum* by Hold *et al.* (2001) and was listed as *Roseobacter* 253-13. We obtained this strain from C. Strömpl (GBF, Braunschweig) together with some identification data and additionally checked for pigment content, morphology, substrate utilization, fatty acids and polar

lipids. Surprisingly, we found *bchl a* to be present at about the same level as in DFL-24^T, i.e. 0.05 nmol (mg protein)⁻¹. Cells were similar under phase-contrast microscopy and were immotile, as were cells of strain DFL-24^T. Two types of colonies were observed, as with DFL-24^T. Substrate utilization was comparable, with the exception that lactate was not used. Strain 253-13 was unable to grow on glucose or fructose, as are DFL-24^T and *R. tolerans*. Fatty acid composition and polar lipid pattern were almost identical to those of DFL-24^T. Strain 253-13 differed from DFL-24^T in being unable to liquefy gelatin and in being resistant to penicillin G. The DNA G+C content was slightly lower (60.9 mol% for strain 253-13 versus 62.9 mol% for DFL-24^T). In view of the high 16S rRNA gene sequence similarity (99.7%), the *bchl a* content and a number of other characters, strain 253-13 can be unreservedly classified as belonging to *R. mucosus*.

Description of *Roseovarius mucosus* sp. nov.

Roseovarius mucosus (mu.co'sus. L. adj. *mucosus* slimy, a property of the colonies).

Cells are rods of 0.5–0.7 × 1.3–3.0 µm with pointed cell poles, frequently uneven before and after division (as described for the genus). Motility is rarely observed. Colonies on marine agar 2216 (Difco) appear whitish to faintly pink, circular, convex with an entire margin and a glistening surface, occasionally slimy. Two stable types of colony morphologies can be discriminated by using a dissecting microscope: a brighter, slightly slimy and a darker, somewhat smaller and more glistening type. Optimum growth occurs at a salinity between 1 and 7‰, a temperature range between 20 and 40 °C and a pH range between 6.0 and 8.8. Requires biotin, thiamine and nicotinic acid for growth. Growth substrates are acetate, butyrate, succinate, fumarate, malate, lactate (not for strain 253-13), glutamate, pyruvate and glycerol, but not glucose, fructose, citrate, ethanol or methanol. Gelatin is liquefied, but starch, alginate and Tween 80 are not degraded. Nitrate is not reduced to nitrite or to nitrogen. Cells are positive for catalase and oxidase. Indole is not formed from tryptophan. The DNA G+C content of strain DFL-24^T is 62.9 mol% and that of strain 253-13 is 60.9 mol%.

The type strain, DFL-24^T (=DSM 17069^T=NCIMB 14077^T), was isolated from a culture of *Alexandrium ostensfeldii* (dinoflagellate).

References

- Allgaier, M., Uphoff, H., Felske, A. & Wagner-Döbler, I. (2003). Aerobic anoxygenic photosynthesis in *Roseobacter* clade bacteria from diverse marine habitats. *Appl Environ Microbiol* **69**, 5051–5059.
- Biebl, H., Allgaier, M., Tindall, B. J., Koblizek, M., Lünsdorf, H., Pukall, R. & Wagner-Döbler, I. (2005). *Dinoroseobacter shibae* gen. nov., sp. nov., a new aerobic phototrophic bacterium isolated from dinoflagellates. *Int J Syst Evol Microbiol* **55**, 1089–1096.

- Boettcher, K. J., Geaghan, K. K., Maloy, A. P. & Barber, B. J. (2005).** *Roseovarius crassostreae* sp. nov., a member of the *Roseobacter* clade and the apparent cause of juvenile oyster disease (JOD) in cultured Eastern oysters. *Int J Syst Evol Microbiol* **55**, 1531–1537.
- González, J. M., Covert, J. S., Whitman, W. B. & 8 other authors (2003).** *Silicibacter pomeroyi* sp. nov. and *Roseovarius nubinhibens* sp. nov., dimethylsulfoniopropionate-demethylating bacteria from marine environments. *Int J Syst Evol Microbiol* **53**, 1261–1269.
- Hall, T. A. (1999).** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Hiraishi, A. & Shimada, H. (2001).** Aerobic anoxygenic photosynthetic bacteria with zinc-bacteriochlorophyll. *J Gen Appl Microbiol* **47**, 161–180.
- Hiraishi, A., Kuraishi, H. & Kawahara, K. (2000).** Emendation of the description of *Blastomonas natatoria* (Sly 1985) Sly and Cahill 1997 as an aerobic photosynthetic bacterium and reclassification of *Erythromonas ursincola* Yurkov et al. 1997 as *Blastomonas ursincola* comb. nov. *Int J Syst Evol Microbiol* **50**, 1113–1118.
- Hold, G. L., Smith, E. A., Rappé, M. S. & 7 other authors (2001).** Characterization of bacterial communities associated with toxic and non-toxic dinoflagellates: *Alexandrium* ssp. and *Scrippsiella trochoidea*. *FEMS Microbiol Ecol* **37**, 161–173.
- Kimura, M. (1980).** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Labrenz, M., Collins, M. D., Lawson, P. A., Tindall, B. J., Schumann, P. & Hirsch, P. (1999).** *Roseovarius tolerans* gen. nov., sp. nov., a budding bacterium with variable bacteriochlorophyll *a* production from hypersaline Ekho Lake. *Int J Syst Bacteriol* **49**, 137–147.
- Maidak, B. L., Cole, J. R., Parker, C. T., Jr & 11 other authors (1999).** A new version of the RDP (Ribosomal Database Project). *Nucleic Acids Res* **27**, 171–173.
- 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. Berlin: Springer.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sato, K. (1978).** Bacteriochlorophyll formation by facultative methylotrophs, *Protaminobacter ruber* and *Pseudomonas* AM 1. *FEBS Lett* **85**, 207–210.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tindall, B. J. (1990).** Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Yurkov, V. V., Krasilnikova, E. N. & Gorlenko, V. M. (1993).** Effect of light and oxygen on metabolism of the aerobic bacterium *Erythromicrobium sibiricum*. *Microbiology* (English translation of *Mikrobiologiya*) **62**, 35–38.
- Yurkov, V., Stackebrandt, E., Buss, O., Vermeglio, A., Gorlenko, V. M. & Beatty, J. T. (1997).** Reorganization of the genus *Erythromicrobium*: description of “*Erythromicrobium sibiricum*” as *Sandarcinobacter sibiricus* gen. nov., sp. nov., and of “*Erythromicrobium ursincola*” as *Erythromonas ursincola* gen. nov., sp. nov. *Int J Syst Bacteriol* **47**, 1172–1178.