Dinoroseobacter shibae gen. nov., sp. nov., a new aerobic phototrophic bacterium isolated from dinoflagellates

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A novel group of aerobic anoxygenic phototrophic bacteria was isolated from marine dinoflagellates, and two strains were characterized in detail. Cells were Gram-negative cocci or ovoid rods and were motile by means of a single, polarly inserted flagellum. They were obligate aerobes requiring 1–7 % salinity. The optimal pH range for growth was 6.5–9.0 and the temperature optimum was 33 °C. The bacteria contained bacteriochlorophyll *a* and spheroidenone as the only carotenoid. The *in vivo* absorption spectrum displayed two maxima in the infrared region at 804 and 868 nm. The distinct 804 nm band indicates the presence of light-harvesting system 2. Various organic carbon sources were assimilated, including many carboxylic acids, glucose and glycerol, but not butyrate, ethanol or methanol. Dissimilatory nitrate reduction was found for both strains. The physiological characteristics of the new strains resembled those of *Roseobacter denitrificans*, but there were differences in the lipid composition. Based on 16S rRNA gene sequence analysis the new strains are relatively distant from other recognized species, with the closest relatives *Jannaschia helgolandensis*, *Ruegeria atlantica* and *Rhodobacter veldkampii* showing 94.1–93.4 % similarity. Similarity to *Roseobacter denitrificans* was only 92.2 %, in line with numerous other species of the *Roseobacter* group. Therefore, it is proposed to classify the strains in a new genus and species within the *Roseobacter* clade, *Dinoroseobacter shibae* gen. nov., sp. nov. The type strain is DFL 12^T (= DSM 16493^T = NCIMB 14021^T).

INTRODUCTION

Aerobic anoxygenic phototrophs (AAPs) were discovered at the end of the 1970s in the Bay of Tokyo (Shiba *et al.*, 1979). These bacteria are related to the facultatively anaerobic purple non-sulfur bacteria, but during evolution they became adapted to an aerobic atmosphere and became

obligate aerobes with a heterotrophic metabolism (Yurkov & Beatty, 1998). They are capable of photosynthetic electron transfer under aerobic conditions without generation of oxygen (i.e. they are anoxygenic). Two of the first isolates were later classified as *Erythrobacter longus* and *Roseobacter denitrificans*, establishing two major genera of AAPs (Shiba & Simidu, 1982; Shiba, 1991). Significant anoxygenic photosynthesis was discovered in the open ocean as a result of kinetic measurements of bacteriochlorophyll *a* fluorescence emission (Kolber *et al.*, 2000). This activity was related to the presence of AAPs, which were estimated to comprise about 10 % of the total bacterial community in the North East Pacific (Kolber *et al.*, 2001). The ability of organisms to utilize light energy as a supplement to respiratory metabolism seems to be an effective strategy in nutrient-poor marine environments (Kolber *et al.*, 2001). Using cultivation-independent molecular methods it has been found that bacteria related to the genus *Roseobacter*

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This paper is dedicated to Professor Norbert Pfennig on the occasion of his 80th birthday.

Abbreviation: AAP, aerobic anoxygenic phototroph.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DFL 12^T is AJ534211.

A phase-contrast micrograph of cells of strain DFL 12^T, plus graphs of temperature and pH dependence for growth of the two strains are available as supplementary figures in IJSEM Online.

constitute a substantial fraction of the marine bacterioplankton (e.g. Zubkov *et al.*, 2001; Pinhassi & Berman, 2003; Selje *et al.*, 2004). However, high 16S rRNA gene sequence similarity among members of the group does not guarantee the presence of phototrophic metabolism because the *Roseobacter* clade contains both photoheterotrophic as well as purely heterotrophic bacteria. To overcome this uncertainty, Bja *et al.* (2002) selectively probed for the presence of *pufM* bacterial reaction centre genes and found that bacteria related to the *Roseobacter* clade probably form the major portion of the marine photoheterotrophic community.

Recently we surveyed a greater number of marine habitats for the presence of pigmented bacterial strains (Allgaier *et al.*, 2003). Putative photosynthetic activity was tested by probing for the presence of the *pufL* and *pufM* genes. Of 113 bacterial strains, 16 were shown to be positive for these genes. Using 16S rRNA gene sequence analyses these were classified into five phylogenetic groups. One group was yellow coloured, three showed only very weak pink pigmentation if at all, and one exhibited an intense red or pink pigment and contained fairly high levels of bacteriochlorophyll *a*. Cell morphology and culture properties of members of this last group resembled those of *Roseobacter* species, but their 16S rRNA gene sequences indicated only a distant relationship. Here we present a detailed characterization of this group. The results suggest classifying the group as a new genus and species within the *Roseobacter* clade.

METHODS

Isolation. The two strains investigated were obtained from washed single cells of cultivated marine dinoflagellates plated onto an agar surface (Allgaier *et al.*, 2003). Strain DFL 12^T was isolated from cells of *Prorocentrum lima* on a sea water medium containing 1 g tryptone and 0.5 g yeast extract per litre; strain DFL 27 was isolated from *Alexandrium ostenfeldii* on a sea water medium containing 40 mg peptone and 8 mg yeast extract per litre.

Cultivation. For general characterization the strains were grown in a complex medium containing 20 g sea salts (Sigma), 3 g Bacto peptone (Difco) and 0.5 g yeast extract (Difco) per litre (hereafter PY medium). In some tests a defined medium was used containing 20 g sea salts (Sigma), 0.3 g (NH₄)SO₄, 0.1 g KH₂PO₄, 1.37 g sodium acetate, 1 ml trace element solution SL12 (Pfennig & Trper, 1992), 5 ml vitamin solution and 0.8 ml 0.5 M H₂SO₄ per litre. Phosphate was added from a separately autoclaved 10% solution, pH 7.5. The filter-sterilized vitamin solution contained 2 mg biotin, 10 mg thiamine-HCl, 20 mg nicotinic acid, 5 mg pantothenic acid, 5 mg vitamin B₁₂, 8 mg pyridoxin and 8 mg 4-aminobenzoic acid per litre. Growth temperature was 30 C. If not otherwise indicated the cultures were incubated in the dark. Other growth conditions are given in the respective tests. Growth was measured turbidometrically at 600 nm, or at 650 nm if photosynthetic pigments were concerned. For long-term storage, 0.5 ml culture was mixed with 0.75 ml sterile 88% glycerol and maintained at -70 C.

Electron microscopy. Mid-exponentially growing cells were adsorbed onto carbon-Formvar foils for 1 min. Cells were washed once with water, blotted and air-dried. They were shadow-cast at

15 elevation with platinum-carbon and analysed with an energy-filtered transmission electron microscope (CEM902; Zeiss) as described by Golyshina *et al.* (2000). Embedding and thin-sectioning were performed as described by Yakimov *et al.* (1998).

Physiological and biochemical tests. All tests that required liquid cultures were performed in 22.5 ml metal-capped test tubes containing 5 ml medium. For determination of the salt requirement, Sigma sea salts in PY medium were used in concentrations up to 10.5%. For determination of temperature range and optimum for growth, a temperature gradient shaking incubator (Toyo Kogaku Sangyo Co. Ltd) was available that allowed us to create a gradient between 15 and 45 C at intervals of 3 C, if six strains were incubated simultaneously. At suitable time intervals the optical density was measured, and from the resulting growth curves the maximum growth rate at each temperature was calculated. The defined medium was used with addition of 0.5 g yeast extract l⁻¹. The pH range was tested at intervals of 0.5 units between pH 5.0 and 9.5 (heavy precipitation prevented preparation of media with higher pH values). The pH was readjusted after autoclaving, the medium was distributed to the tubes and the pH rechecked after inoculation. Growth was measured at an early stage of the growth phase before the pH was appreciably changed by growth. Usable carbon sources were tested in the synthetic medium, in which the vitamin solution was replaced by 0.1 g yeast extract l⁻¹. The carbon sources were used at a concentration of 1 g l⁻¹. General growth factor requirements, including amino acids, were determined by investigating growth in the above synthetic medium without vitamins, with vitamins, with 1 g yeast extract l⁻¹, and with vitamin-free and vitamin-containing Casamino acids (Difco). Requirement for single vitamins was determined in mixtures of seven vitamins from which one was omitted. Precultures were grown in a medium without any growth factors.

Degradation of polymers was tested on agar plates by using PY medium. Starch was added at 2 g l⁻¹, alginate at 7.5 g l⁻¹, and Tween 80 and gelatin at 4 g l⁻¹. Starch degradation was demonstrated with Lugol's solution, alginate degradation by clear zones around the colonies, reaction with Tween 80 by bubble formation (Labrenz, 1999) and gelatin liquefaction by precipitation of undigested gelatine with saturated ammonium sulfate solution. Nitrate reduction capacity was checked in 22.5 ml tubes with 10 ml synthetic medium supplemented by 0.4 g NaNO₃ and 0.5 g yeast extract l⁻¹. Nitrogen formation was demonstrated in Durham tubes, and formation of nitrite and consumption of nitrate with Merckoquant test sticks (Merck). The presence of catalase and oxidase and formation of indol was demonstrated according to Gerhardt *et al.* (1981).

Photosynthetic pigments, lipids and fatty acids. The *in vivo* absorption spectrum was recorded in a Shimadzu UV-3000 double beam spectrophotometer with a resolution of 0.5 nm, using a 2 nm slit width. Scattering was reduced by suspending centrifuged PY grown cells in 70% glycerol. Photosynthetic pigments were extracted in a mixture of acetone/methanol (7:2). A pellet of about 10 ml culture was resuspended in the remaining drop of medium, 1 ml of the solvent mixture was added and the suspension was incubated for 1 h at room temperature in the dark, vortexed and centrifuged at 9000 g. The bacteriochlorophyll *a* content was determined at 772 nm using an extinction coefficient of 75 mM⁻¹ cm⁻¹ (Clayton, 1963).

Pigment composition was analysed by HPLC using a Beckman system comprising a Beckman 420 controller, two Beckman 114M pumps and a Waters 990 photodiode array detector. Pigments were separated on a heated (40 C) VYDAC C18 column using a binary solvent system (A: 80% methanol + 0.5 M ammonium acetate; B: methanol/acetone, 80:20). The peak identity was confirmed by absorption spectra and retention times. Major carotenoid molecular mass was determined by

an offline injection into the Agilent 1100 Series LC/MSD Trap mass spectrometer. In parallel, the carotenoids were also characterized by TLC. One hundred micrograms of freeze-dried cell material was extracted in 9.5 ml of a chloroform/methanol/0.3 % NaCl mixture in a ratio of 1:2:0.8 under continuous stirring overnight. The mixture was extracted by hexane to record the overall absorption spectrum. The solution was then concentrated by evaporation and chromatographed in tertiary butyl ether on silica gel plates.

Lipoquinones and polar lipids were extracted and separated according to the methods described by Tindall (1990a, b). Fatty acid composition was determined by the method described by Labrenz *et al.* (1998). The G + C content of the DNA was determined by HPLC (Mesbah *et al.*, 1989).

16S rRNA gene sequences and phylogenetic inferences.

DNA extraction, amplification and sequencing of the 16S rRNA gene were as described by Allgaier *et al.* (2003). Sequences were manually aligned and compared to sequences published previously. These were stored in the DSMZ internal database, consisting of more than 6000 16S rRNA gene sequence entries, including those from the Ribosomal Database Project (Maidak *et al.*, 1999) and EMBL. Similarity values were transformed into phylogenetic distance values that compensate for multiple substitutions at any given site in the sequence (Jukes & Cantor, 1969). The algorithm of De Soete (1983) and the neighbour-joining method contained in the PHYLIP package (Felsenstein, 1993) were used in the construction of the phylogenetic dendrogram. All analyses were performed on a SUN SparcII workstation.

RESULTS AND DISCUSSION

Morphological features

A typical feature of strains DFL 12^T and DFL 27 is their relatively strong pigmentation. Liquid cultures grown in the dark appear distinctly pink or light red. When grown under strong illumination they are faintly beige. Streaks and colonies on complex agar media are deeply wine-red, with an intensity comparable to that of anaerobic phototrophs. Colonies are convex and smooth with entire margins. Cells are small cocci, occasionally oval rods, homogeneous in synthetic medium but somewhat variable in complex media (see Fig. A, available as supplementary material in IJSEM Online) and measure 0.3–0.7 µm in width and 0.3–1.0 (to 2.0) µm in length. Cell motility was observed in all the media used. Electron micrographs of whole cells revealed extracellular polymer substances of homogeneous or varying consistency, but capsule-free cells were also found in the same preparation (Fig. 1). Flagellation is monotrichous and shows a polar to subpolar insertion (Fig. 1a). Ultra-thin sections revealed a Gram-negative cell wall structure with a distinct outer membrane, an electron-translucent periplasmic space and a faint cytoplasmic membrane; the murein layer was not visible (Fig. 1b).

Physiological characteristics

Both strains grew between 15 and 38 °C, half maximum to maximum growth was observed between 25 and 37 °C, optimum growth at 33 °C and the upper limit at 38 °C (see Fig. B, available as supplementary material in IJSEM

Fig. 1. Electron micrographs of cells of strain DFL 12^T. Typically, short rod-shaped cells appear either free (white arrowheads) or encapsulated by extracellular polymer substances (arrows). The twin-arrowhead indicates the shadowing direction. (a) A single flagellum (black arrowheads) inserted at the polar region of the cell. (b) Gram-negative cell wall structure (ultra-thin section): OM, outer membrane; CM, cytoplasmic membrane; PS, periplasmic space.

Online). Strain DFL 27 grew at a lower rate than strain DFL 12^T (optimum at 0.17 h⁻¹ and 0.12 h⁻¹, respectively). The pH range for half maximum to maximum growth was found between 6.2 and 8.9 (DFL 12^T) and between 6.6 and 9.0 (DFL 27), the optimum range between 6.5 and 8.8 (DFL 12^T) and between 7.0 and 8.8 (DFL 27); no growth occurred above pH 9.2 (see Fig. C, available as supplementary material in IJSEM Online). The two strains were strictly salt-dependent and required at least 1 % salinity. Good growth still occurred at a salt concentration twice that of normal sea water (7 %).

The strains utilized a wide spectrum of organic substances (Table 1), but not ethanol or methanol. In contrast to the four other groups of aerobic phototrophs isolated from dinoflagellates, butyrate was not metabolized. Interestingly, *Roseobacter denitrificans* and *Roseobacter litoralis* showed an almost identical pattern of substrate utilization, including the inability to grow on butyrate. By contrast, *Jannaschia helgolandensis* (Wagner-Döbler *et al.*, 2003), which has the highest 16S rRNA gene sequence similarity to the two strains, displays a substantially different pattern of carbon source utilization (Table 1). Anaerobic growth was not observed, either photoheterotrophically on acetate in the light or by fermentation of glucose in the dark. There was

Table 1. Differences in utilization of organic carbon sources between strains DFL 12^T/DFL 27 and *Roseobacter denitrificans*, *Roseobacter litoralis* and *J. helgolandensis*

Taxa: 1, DFL 12^T/DFL 27; 2, *R. denitrificans* (Shioi, 1986); 3, *R. litoralis* (Shiba, 1991); 4, *J. helgolandensis* (Wagner-Döbler *et al.*, 2003). +, Utilized; –, not utilized; w, weakly utilized; NT, not tested.

Carbon source	1	2	3	4
Acetate	+	w	+	–
Succinate	+	w	+	+
Fumarate	+	+	NT	NT
Lactate	+	+	NT	+
Citrate	+	w	+	+
Glutamate	+	+	+	–
Pyruvate	+	+	+	–
Fructose	+	+	NT	+
Ethanol	w/–	–	NT	NT
Glycerol	+	+	NT	+

no indication for preferred growth under microaerobic conditions.

The new isolates were unable to grow in a pure mineral medium containing the carbon source only, as they required addition of yeast extract. The yeast extract could be entirely replaced by a mixture of biotin, nicotinic acid and 4-aminobenzoic acid (Table 2). Amino acids were not necessary for growth. According to Shiba (1991), biotin, nicotinic acid and thiamin are required by *Roseobacter* species.

Of the polymers tested, gelatine and Tween 80 (indicating lipase activity) were hydrolysed, but not starch or alginate (Table 2). The two strains were sensitive to penicillin G, tetracycline, chloramphenicol and polymyxin B, as are *Roseobacter* species. Nitrate reduction and nitrogen production were positive for both strains. Intermediary nitrite was not detected. Under aerobic conditions, nitrate was used as nitrogen source. Tests for catalase and oxidase were positive; indol was not formed.

Chemotaxonomic characteristics

The predominant respiratory lipoquinone was ubiquinone 10, which is characteristic for the α -*Proteobacteria*. The cellular polar lipids differed distinctly from those of *Roseobacter* species (Table 2). Phosphatidylglycerol and diphosphatidylglycerol were present, but not phosphatidylcholine as in *Roseobacter denitrificans*. An unidentified aminolipid was found in addition to an array of eight to ten unidentified substances. The polar lipid pattern of *J. helgolandensis* showed less similarity to DFL 12^T and DFL 27 (Table 2). The predominant cellular fatty acid was 18:1 ω 7c (75–82 % of the total), which is common among the α -*Proteobacteria* (Table 3). Five other fatty acids, two of them only tentatively identified, compose between 2 and 6 % of the total,

Table 2. Physiological and biochemical characteristics of strains DFL 12^T and DFL 27 in comparison to *Roseobacter denitrificans*, *Roseobacter litoralis* and *J. helgolandensis*

Taxa: 1, DFL 12^T/DFL 27; 2, *R. denitrificans* DSM 7001^T (Shiba, 1991; Labrenz *et al.*, 1999); 3, *R. litoralis* DSM 6996^T (Shiba, 1991; Labrenz *et al.*, 1999); 4, *J. helgolandensis* DSM 14858^T (Wagner-Döbler *et al.*, 2003). NT, Not tested.

Characteristic	1	2	3	4
Bacteriochlorophyll <i>a</i>	+	+	+	–
Nitrate reduction	+	+	–	–
Gelatin hydrolysed	+	+	+	–
Tween 80 hydrolysed	+	+	+	–
Vitamins required:	+	+	+	+
Biotin	+	+	+	NT
Nicotinic acid	+	+	+	NT
Thiamin	–	+	+	NT
4-Aminobenzoic acid	+	NT	NT	NT
Polar lipids:				
Phosphatidylethanolamine	–	–	–	+
Phosphatidylcholine	–	+	–	+
Unidentified aminolipid	+	–	–	+
Other polar lipids	8	–	–	1

and a sixth compound, tentatively identified as methyl 18:1, occurs only in strain DFL 27. The fatty acid pattern was similar to that of *Roseobacter* species but distinct from that of *J. helgolandensis*.

Photosynthetic pigments

Fig. 2 shows the *in vivo* absorption spectrum together with the acetone/methanol extract spectrum. Both display specific bacteriochlorophyll *a* peaks, at 373, 590, 804 and 868 nm *in vivo* and at 367, 590 and 775 nm in solvent. The infrared region of the *in vivo* spectrum, with a lower peak at 804 and a higher peak at 868 nm, corresponds to that of many other phototrophic bacteria (Yurkov & Beatty, 1998). The distinct absorption band at 804 nm is indicative of the presence of light-harvesting complex 2 as observed in the *Roseobacter* species (Shiba, 1991). Interestingly, recently isolated marine *Roseobacter* strains are entirely devoid of light-harvesting system 2, although they appear otherwise very similar to the strains described herein (M. Kobizek, P. G. Falkowski & Z. S. Kolber, unpublished results). The absolute amount of bacteriochlorophyll *a* reached values of 4 nmol (mg protein)^{–1}, which is among the highest yet published for aerobic phototrophs (Harashima *et al.*, 1980; Yurkov & van Gernerden, 1993). It was formed only in the dark and was increased under conditions of nutrient deficiency.

The absorption in the range 400–600 nm is due to a carotenoid. Its maximum of 482 nm in the solvent extract is almost identical to that of *Roseobacter denitrificans* and indicates spheroidenone as a major component (Harashima & Nakada, 1983). The identity of the pigments was

Table 3. Cellular fatty acid composition of strains DFL 12^T and DFL 27 in comparison to *Roseobacter denitrificans*, *Roseobacter litoralis* and *J. helgolandensis*

Fatty acid	DFL 12 ^T		DFL 27*		<i>R. denitrificans</i> DSM 7001 ^{T†}	<i>R. litoralis</i> DSM 6996 ^{T†}	<i>J. helgolandensis</i> DSM 14858 ^{T‡}	
	Ester linked	Ester and amide linked	Ester linked	Ester and amide linked	Ester linked§	Ester linked§	Ester linked	Ester and amide linked
3-OH 10:0	2.8	2.1	2.0	2.1	1.8	1.9	5.6	4.5
12:1	5.4	2.4	4.9	2.5			4.9	3.4
3-OH 14:0								1.4
3-OH 14:1	4.3	5.3	4.1	5.3	2.9	3.9		2.1
16:0				0.8	2.2	1.1		
17:0							1.0	1.0
18:1ω7c	77.8	81.8	77.5	76.4	87.7	88.8	45.1	45.0
18:2						1.4		
Methyl 18:1			2.6	3.1			5.7	7.6
18:0	4.3	6.3	3.4	4.7	2.3	1.3	10.8	11.7
Cyclo 19:0	5.4	2.1	5.6	2.5			25.1	22.4
20:0							0.9	1.0

*Strain DFL 27 contained three additional unidentified substances amounting to 0.8, 0.6 and 1.0 % of the total.

†Labrenz *et al.* (1999).

‡Wagner-Döbler *et al.* (2003).

§B. J. Tindall, personal communication.

||Not unambiguously identified for DFL 12^T and DFL 27.

confirmed by HPLC (Fig. 3). Based on its retention time, bacteriochlorophyll *a* contained a phetyl side chain as found in other AAPs. In the HPLC analysis, only one major (90 %) carotenoid was detected. This was identified as spheroidenone based on the absorption spectrum, retention time and mass spectrometry. Its identity was confirmed by

control extracts obtained from *Roseobacter denitrificans* and semi-aerobically grown *Rhodobacter sphaeroides*. TLC in tertiary butyl ether yielded two incompletely separated fractions with main absorption maxima in hexane at 479 and 482 nm, respectively; the raw extract peaked in between (481 nm). Exactly the same result was obtained with extracts of *Roseobacter denitrificans*. In the HPLC chromatogram, the presence of two fractions could also be inferred from

Fig. 2. Absorption spectra of strain DFL 12^T, obtained *in vivo* from isolated membrane particles, acetone/methanol extract (7:2). **Fig. 3.** Reversed-phase HPLC chromatogram of strain DFL 27 for bacteriochlorophyll *a* recorded at 780 nm and for spheroidenone recorded at 493 nm. Inset: on-line absorption of spheroidenone taken by diode array.

an asymmetric carotenoid peak. It is presumed that the two fractions represent *cis* and *trans* isomers of the same substance.

In experiments with washed cells, the effect of light on proton translocation and ATP formation is currently being studied by H. Cypionka (personal communication; for methods, see Sydow *et al.*, 2002). On illumination an increase in extracellular pH was observed, and the intracellular concentration of ATP increased considerably, indicating the presence of a functional photosynthetic apparatus. Moreover, oxygen uptake was impaired when the cells were exposed to light, as has been described previously by Harashima *et al.* (1982) for *Roseobacter* and *Erythrobacter* species.

Phylogenetic analysis

The phylogenetic position of strains DFL 12^T and DFL 27 was ascertained using about 1260 nt of the 16S rRNA gene sequence. About 100 bases at the beginning and at the end of the *Escherichia coli* sequence were not analysed. Comparison of the secondary structure of both sequences resulted in the detection of a few differences in some of the stem-loop structures, but no differences were observed in the variable regions. The sequences obtained showed

an overall similarity of 99.7% and allowed us to classify the strains as representing members of the *Roseobacter*–*Sulfitobacter*–*Silicibacter* group (Wagner-Döbler *et al.*, 2003) within the α -*Proteobacteria*. The closest phylogenetic neighbours of strain DFL 12^T were *J. helgolandensis* (Wagner-Döbler *et al.*, 2003), *Ruegeria atlantica* and *Rhodobacter veldkampii* with similarity values of 94.1, 93.7 and 93.4%, respectively. 16S rRNA gene sequence similarity values for the majority of the available relevant type strains ranged between 92 and 93%, making it difficult to mark a well-defined position in a possible lineage. The dendrogram obtained (Fig. 4) therefore has a relatively labile constitution and might change substantially if additional strains are included.

Regardless, the two presented strains are relatively distant neighbours from recognized species, which suggests that they should be classified within a separate genus. The apparent similarity to *Roseobacter* in pigmentation, morphology and physiological characters is not substantiated by 16S rRNA gene sequence analysis, suggesting that the strains are no more related to the type strains of *Roseobacter* species than they are to any other strain belonging to the *Roseobacter* clade. This conclusion is also supported by the rather specific polar lipid patterns of the two strains. In addition, the G+C content (64.8 mol%) falls outside

Fig. 4. Neighbour-joining dendrogram based on 16S rRNA gene sequences showing the phylogenetic position of *Dinoroseobacter shibae*, strains DFL 12^T and DFL 27, within the α -*Proteobacteria*. Bootstrap values with greater than 60% confidence are shown at branching points (percentages of 400 replicates). Bar, 5 nucleotide substitutions per 100 sequence positions. The tree was rooted with *Escherichia coli* as outgroup. Sequence accession numbers are given in parentheses.

the range of values reported for the genus *Roseobacter* (56.7–59.6 mol%). On the other hand, the strains showed only little similarity to *J. helgolandensis* with regard to phenotypic characters, including lack of phototrophy, and different physiological properties, fatty acids and polar lipids. However, with regard to phototrophy, it has been repeatedly shown in recent years that the presence of the photosynthetic apparatus is only of weak taxonomic relevance, and phototrophic and non-phototrophic strains have been grouped within the same genus or even species (Ruiz-Ponte *et al.*, 1998; Labrenz *et al.*, 1999; Hiraishi *et al.*, 2000). For *Rhodobacter*, a 16S rRNA gene sequence similarity equal to that of members of the *Roseobacter* group was unexpected, as *Rhodobacter* performs anaerobic photosynthesis. Relatedness between *Roseobacter* and *Rhodobacter* was known, however, and as inferred from 16S rRNA gene sequence analysis the new strains are somewhat closer to *Rhodobacter* than to *Roseobacter* species.

Description of *Dinoroseobacter* gen. nov.

Dinoroseobacter [Di'no.ro'se.o.bac.ter. Gr. n. *dinos* whirling, rotation, and the first compound of the Protozoan name Dinophyceae (dinoflagellates), the source from which the isolates were obtained; N.L. n. *Roseobacter* a bacterial genus; N.L. masc. n. *Dinoroseobacter* a *Roseobacter*-like organism originating from dinoflagellates].

Gram-negative cocci or ovoid rods. Motile by a single, polarly or subpolarly inserted flagellum. Liquid cultures pink to light red if grown in the dark or intermittent light; faint beige if grown in permanent light. Dark-grown colonies are wine-red. Strictly aerobic, non-fermentative heterotrophs requiring at least 1% sea salt. Contain photosynthetic pigments, bacteriochlorophyll *a* and spheroidenone. The predominant respiratory quinone is ubiquinone 10.

The type species is *Dinoroseobacter shibae*.

Description of *Dinoroseobacter shibae* sp. nov.

Dinoroseobacter shibae (shi'bae. N.L. gen. n. *shibae* of Shiba, named after Professor Tsuneo Shiba, who discovered the marine aerobic anoxygenic phototrophic bacteria and provided fundamental contributions to the description of this physiological group of bacteria).

Pseudococci or ovoid cells, 0.3–0.7 µm long and 0.3–1.0 µm (to 2.0) wide. Uses acetate, succinate, fumarate, malate, lactate, citrate, glutamate, pyruvate, glucose, fructose and glycerol as carbon source, but not methanol, ethanol or butyrate. Requires biotin, nicotinic acid and 4-aminobenzoic acid as growth factors. Nitrate is reduced to elemental nitrogen. Gelatin and Tween 80 are decomposed. The predominant fatty acid (75–82%) is 18:1 ω 7; 3-OH 10:1, 12:1, 3-OH 14:1, 18:0 and cyclo 19:0 are present in minor amounts. The polar lipids phosphatidylglycerol, diphosphatidylglycerol, an unidentified aminolipid and several other, as-yet-unidentified lipids are present.

G+C content of the type strain is 64.8 mol%. All other properties are as for the genus.

The type strain, DFL 12^T (=DSM 16493^T=NCIMB 14021^T), was isolated from cultured cells of the marine dinoflagellate *Prorocentrum lima*.

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