

***Albirhodobacter marinus* gen. nov., sp. nov., a member of the family *Rhodobacteriaceae* isolated from sea shore water of Visakhapatnam, India**

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Abstract

A novel marine, Gram-negative, rod-shaped bacterium, designated strain N9^T, was isolated from a water sample of the sea shore at Visakhapatnam, Andhra Pradesh (India). Strain N9^T was found to be positive for oxidase and catalase activities. The fatty acids were found to be dominated by C_{16:0}, C_{18:1} *ω*7*c* and summed in feature 3 (C_{16:1} *ω*7*c* and/or C_{16:1} *ω*6*c*). Strain N9^T was determined to contain Q-10 as the major respiratory quinone and phosphatidylethanolamine, phosphatidylglycerol, two aminophospholipids, two phospholipids and four unidentified lipids as polar lipids. The DNA G+C content of the strain N9^T was found to be 63 mol%. 16S rRNA gene sequence analysis indicated that *Rhodobacter sphaeroides*, *Rhodobacter johrii*, *Pseudorhodobacter ferrugineus*, *Rhodobacter azotoformans*, *Rhodobacter ovatus* and *Pseudorhodobacter aquimaris* were the nearest phylogenetic neighbours, with pair-wise sequence similarities of 95.43, 95.36, 94.24, 95.31, 95.60 and 94.74 % respectively. Phylogenetic analysis showed that strain N9^T formed a distinct branch within the family *Rhodobacteraceae* and clustered with the clade comprising species of the genus *Pseudorhodobacter*, together with species of the genera *Roseicitreum*, *Roseinatronobacter*, *Roseibaca* and *Rhodobaca*. Species of the genus *Pseudorhodobacter* are phylogenetically close with a 16S rRNA gene sequence dissimilarity of 5.9-7.3% (92.7-94.1% similarity). Based on the above-mentioned phenotypic characteristics and on phylogenetic inference, strain N9^T is proposed as a representative of a new genus and a novel species of the family *Rhodobacteraceae* as *Albirhodobacter marinus* gen. nov., sp. nov. The type strain of *Albirhodobacter marinus* is N9 (= MTCC 11277^T = JCM 17680^T)

Key words: *Albirhodobacter marinus*, 16S rRNA gene based phylogeny, chemotaxonomy.

Introduction

The family *Rhodobacteraceae* was established by Garrity et al. (2005) within the α -3 *Proteobacteria*. *Rhodobacteraceae* is an ecologically, metabolically and phenotypically diverse group, including both chemotrophic and phototrophic bacteria, with obligate aerobic to facultative anaerobic metabolism (Garrity et al. 2005). *Rhodobacter* (Imhoff et al. 1984; Srinivas et al. 2007) is the type genus, which contains fresh water bacteria closely related to the phototrophic species of the genera *Rhodovulum* (Hiraishi and Ueda, 1994), *Rhodobaca* (Milford et al. 2000), *Roseinatronobacter* (Sorokin et al. 2000), *Roseibaca* (Labrenz et al. 2009), *Rubrimonas* (Suzuki et al. 1999) and *Rubribacterium* (Boldareva et al. 2010) and the chemoheterotrophic genera *Albidovulum* (Albuquerque et al. 2002), *Albimonas* (Lim et al. 2008), *Catellibacterium* (Tanaka et al. 2004), *Haematobacter* (Helsel et al. 2007) and *Pseudorhodobacter* (Uchino et al. 2003). Presently the family comprises 95 genera (<http://www.bacterio.cict.fr/classifgenerafamilies.html#Rhodobacteraceae>). Recently we have

isolated a bacterial strain which is non-pigmented with chemoorganoheterotrophic, facultative anaerobic growth and phylogenetic analysis placed close to the species of the genus *Pseudorhodobacter*. In the present study, we focused on the characterization and classification of strain N9^T by using a polyphasic approach (Vandamme et al. 1996). From the results of phylogenetic and phenotypic analyses including chemotaxonomic characteristics, the strain was assigned to a new genus that belongs to the family *Rhodobacteraceae*.

Materials and Methods

Isolation and growth

Strain N9^T was isolated from marine water collected from the sea shore at Visakhapatnam (GPS position: 17°42' N 83°18' E), Andhra Pradesh, India. The sample (1 ml) was serially diluted (10 fold dilutions) in 2% NaCl (w/v) solution and 100 µl of each dilution was plated on Zobell marine agar (MA; HIMEDIA, Mumbai, India) medium and incubated at 30°C. A white to light cream colored colony observed after five days incubation was selected and characterized in the present study. Sub-cultivation of the isolate was carried out on MA at 30°C. Stock cultures of the isolate in marine broth with 10% glycerol were preserved at -80°C.

The type strain of *Pseudorhodobacter ferrugineus* LMG 22047^T was obtained from LMG Culture collection centre and cultured under the same conditions as strain N9^T throughout.

Morphological characteristics

Colony morphology was studied after 48 h growth of the strain on MA at 30 °C. Cell morphology was investigated by light microscopy (Olympus) at X 1000. Gram reaction was determined by using the HIMEDIA (Mumbai, India) Gram Staining Kit according to manufacturer's protocol. Endospore formation was determined after malachite-green staining of the isolate grown on R2A (HIMEDIA) plates supplemented with 2% NaCl for a week. Motility was also assessed on Motility-Indole-Lysine HiVegTM medium (cat. no. MV847; HIMEDIA) with agar 2 g l⁻¹ and also by phase contrast microscopy.

Physiological and biochemical characteristics

Growth was tested on MA, Nutrient Agar (NA; HIMEDIA) and Tryptone Soya Agar (TSA; HIMEDIA). Growth at 4, 10, 18, 25, 30, 35, 37, 40, 45, 47, 50, 55 and 60 °C was ascertained using marine broth medium and salt tolerance [0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18 and 20% (w/v) NaCl] were ascertained using nutrient broth without NaCl. Growth of strain N9^T at pH 5, 6, 7, 7.5, 8, 8.5, 9,

10, 11 and 12 was assessed in marine broth buffered with acetate buffer (pH 4-6), 100 mM NaH₂PO₄/Na₂HPO₄ buffer (pH 7-8), 100 mM NaHCO₃/Na₂CO₃ buffer (pH 9-10) or 100 mM Na₂CO₃/NaOH buffer (pH 11-12). Biochemical characteristics such as activity of oxidase, lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase; nitrate reduction; hydrolysis of aesculin, gelatin, ONPG, starch and Tween 20, 40, 60 and 80; carbon source assimilation; H₂S production; and the sensitivity to 18 different antibiotics using the disc diffusion method with commercially available discs (HIMEDIA) were determined by previously described methods (Lányi, 1987; Smibert and Krieg, 1994), except that media included 2.0 % (w/v) NaCl. Biochemical and enzymatic characterization of strain N9^T was also performed using Vitek 2 GN (bioMérieux), according to the manufacturer's protocol, except that sterile 2.0 % (w/v) NaCl was used to prepare the inoculum.

Chemotaxonomic analysis and DNA G+C content

Standardization of the physiological age of strain N9^T and the reference type strain *P. ferrugineus* LMG 22047^T was performed based on the protocol (http://www.microbialid.com/PDF/TechNote_101.pdf) provided by the Sherlock Microbial Identification System (MIDI, USA). For analysis of cellular fatty acids, strains N9^T and *P. ferrugineus* LMG 22047^T were grown on TSA plates with 2% NaCl (w/v) at 30 °C for two days and were of same physiological age. Cellular fatty acid methyl esters (FAMES) were obtained from cells by saponification, methylation and extraction following the protocol of the Sherlock Microbial Identification System (MIDI, USA). Cellular FAMES were separated using a Gas Chromatograph (6890), identified and quantified with Sherlock Microbial Identification System Software (version.6.0, using the aerobe TSBA6 method and TSBA6 database). Polar lipids and quinones were analysed by using freeze-dried cells following growth on marine agar 2216 (Difco) at 30 °C for 3 days under aerobic conditions. Cells were extracted for the polar lipid analysis (Bligh and Dyer 1959) and analyzed by two dimensional thin layer chromatograph followed by spraying with appropriate detection reagents (5% ethanolic molybdotophosphoric acid, molybdenum blue, ninhydrin and molisch reagents) (Komagata and Suzuki 1987). Isoprenoid quinones were extracted as described by Collins et al. (1977) and analyzed by high performance liquid chromatography (Groth et al. 1997). The DNA of strain N9^T was isolated according to the procedure of Marmur (1961) and the G+C content was determined from melting point (T_m) curves (Sly et al. 1986) obtained by using a Lambda 2 UV-Vis spectrophotometer (Perkin Elmer) equipped with the Templab 2.0 software package (Perkin Elmer). *Escherichia coli* strain DH5- α DNA was used as a standard in determining the DNA G+C content.

16S rRNA gene sequencing and phylogenetic analysis

For 16S rRNA gene sequencing, DNA was prepared using the Mo Bio microbial DNA isolation kit (Mo Bio Laboratories Inc.). PCR amplification of the 16S rRNA gene was performed with bacterial universal primers 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492r (5'-TAC GGY TAC CTT GTT ACG ACT T-3') (Brosius et al. 1978). The reaction mixture contained 100 ng of chromosomal DNA, 1 U of Deep Vent DNA polymerase, 1 X Thermopol reaction buffer, 200 μ M of each deoxynucleoside triphosphate (New England Biolabs) and 20 pmol of each primer (BioBasic Inc). PCR cycling parameters included an initial denaturation at 95 °C for 5 min, followed by 29 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 2 min and a final extension for 10 min at 72 °C. PCR products were separated and a 1.5 kb fragment detected and later eluted using a Qiaquick gel extraction kit (Qiagen). The 16S rRNA gene was sequenced using the forward and the reverse PCR primers (27f and 1492r) and in addition one forward and reverse primers, viz. 530f and 907r (Johnson, 1994), following the dideoxy chain-termination method as described earlier (Pandey et al. 2002). The 16S rRNA gene sequence of the isolate was subjected to BLAST sequence similarity search (Altschul et al. 1990) to identify the nearest taxa. The 16S rRNA gene sequences of closely related validly named taxa belonging to the family *Rhodobacteraceae* were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL_X program (Thompson et al. 1997). The alignment was then corrected manually by deleting gaps and missing data. There were a total of 1275 positions in the final dataset. Phylogenetic trees were reconstructed using the maximum likelihood method using the PhyML program (Guindon et al. 2005) and Neighbor-Joining method (Saitou and Nei 1987) using the PHYLIP package (transition/transversion ratio used was 2.0000)] (Felsenstein, 1989) and the tree topologies were evaluated by bootstrap analysis based on 100 and 1000 replicates respectively. The evolutionary distances were computed using the Kimura 2-parameter method Kimura (1980) and are in the units of the number of base substitutions per site.

Results and discussion

The cells of strain N9^T were observed to be rod-shaped (0.5-0.8 x 2-3 μ m) and to multiply by binary fission. The cells stained Gram-negative and were found to be non-motile. Colonies were observed to be circular, 2-4 mm in diameter, translucent, convex whitish (on one tenth diluted marine agar) to light cream colour (on marine agar). A chemoorganoheterotrophic mode of growth was observed. Anaerobic growth was observed in presence of hydrogen sulfide. The strain was found to grow between 30-37°C, the optimum being 30°C, and from pH 6-10, with optimum growth at pH

7.5. Growth was found to occur at salinities from 1% to 9% (NaCl, w/v), with an optimum around 2% NaCl (w/v). The strain was found to be positive for oxidase and catalase activities and negative for lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase activities. Strain N9^T was found to reduce nitrate, hydrolyze DNA and urea but did not hydrolyse aesculin, ONPG and Tween 60. It was observed to be negative for indole production. Strain N9^T can be differentiated from *Pseudorhodobacter ferrugineus* LMG 22047^T using different physiological characteristics, including the salinity, temperature and pH range for growth. Other differentiating characteristics between strain N9^T and the type strains representing related genera within the family *Rhodobacteraceae* and also with type strain *P. ferrugineus* LMG 22047^T are presented in Tables 1 and 2 respectively. Strain N9^T was found to be susceptible to (μg per disc unless indicated) chloramphenicol (30), kanamycin (30), neomycin (30), polymixin-B (300), ciprofloxacin (10), gentamycin (10), spectinomycin (100), novobiocin (30) and ceftazidime; and resistant to ampicillin (10), penicillin-G (10 units/disc), lincomycin (15), vancomycin (30), tetracycline (30), amoxicillin (20), cephadroxil (30), bacitracin (8) and chlortetracycline (30).

Analysis of the cellular fatty acid composition of strain N9^T identified 12 fatty acids with an abundance (>5 %) of C_{16:0}, C_{18:1} ω 7c and C_{16:1} ω 7c and/or C_{16:1} ω 6c (summed in feature 3 by the MIDI system) (Tables 1, 2, 3). The fatty acid profile was noted to be dominated by unsaturated fatty acids. The range of fatty acids detected in strain N9^T was comparable to that detected in *P. ferrugineus* LMG 22047^T (both in the present study and in Jung et al. 2012) but the proportions differed notably (Table 3). The respiratory quinone present in strain N9^T was determined to be ubiquinone 10 (Q-10). The polar lipids consisted of phosphatidylethanolamine, phosphatidylglycerol, two aminophospholipids, two phospholipids and four unidentified lipids as polar lipids (Fig. 2). The type strain *P. ferrugineus* LMG 22047^T was reported to contain phosphatidylcholine, phosphatidylglycerol, one unidentified aminophospholipid and two unidentified aminolipids as total polar lipids (Jung et al. 2012). Strain N9^T was determined to have a DNA G+C content of 63.2 \pm 0.6 mol%, which is higher than that of *P. ferrugineus* LMG 22047^T (58 mol%) and slightly lower than the G+C content of members of the genus *Rhodobacter* (64-73%) (Tables 1 and 2).

The phylogenetic relationships of strain N9^T was ascertained based on the 16S rRNA gene sequence similarity with other strains using a BLAST sequence similarity search (NCBI-BLAST). The 16S rRNA gene sequence analysis placed strain N9^T within the family *Rhodobacteraceae*. The results indicated that at the 16S rRNA gene sequence level, strain N9^T was closely related to the phylogenetic neighbours *Rhodobacter sphaeroides*, *Rhodobacter johrii*, *P. ferrugineus*, *Rhodobacter azotoformans*, *Rhodobacter ovatus* and *Pseudorhodobacter aquimaris* with pairwise sequence

similarity of 95.43, 95.36, 94.24, 95.31, 95.60 and 94.74 % respectively. Phylogenetic analysis based on maximum likelihood tree further revealed that the novel strain N9^T clearly formed a distinct branch within the family *Rhodobacteriaceae* and clustered with the clade comprising species of the genus *Pseudorhodobacter* and with species of the genera *Roseicitreum*, *Roseinatronobacter*, *Roseibaca* and *Rhodobaca*. Species of the genus *Pseudorhodobacter* are phylogenetically close with a distance of 5.9-7.3% (92.7-94.1% similarity). A similar topology was obtained for the neighbour joining tree (Supplementary Fig. S1).

The genus *Pseudorhodobacter* was first proposed by Uchino et al. (2002) when *Agrobacterium ferrugineum* was reclassified as *Pseudorhodobacter ferrugineum*. At the time of writing, only two validly named species have been described within this genus i.e *P. ferrugineum* by Uchino et al. (2002) and another strain isolated from seawater, Korea which was recently described by Jung et al. (2012) as *Pseudorhodobacter aquimaris*. The genus *Pseudorhodobacter* was differentiated from the genus *Rhodobacter* by the lack of photosynthetic abilities, bacteriochlorophyll a, lower DNA G+C content and an insertion in its 16S rRNA gene sequence (Uchino et al. 2002).

The characteristics that differentiate strain N9^T from the closely related genera in the *Rhodobacteraceae* family are given in Table 1. The characteristics that differentiate strain N9^T from the type species *P. ferrugineus* LMG 22047^T which is phylogenetically closely related are given in Tables 2 and 3. The 16S rRNA gene sequence analysis showed a clear dissimilarity between strain N9^T and its phylogenetic neighbour and other members of the family *Rhodobacteriaceae* (Fig. 1). Based on the 16S rRNA gene sequence phylogeny, the most closely related taxon in the family *Rhodobacteraceae* was *P. ferrugineus*. However, strain N9^T differed from *P. ferrugineus* LMG 22047^T with respect to colony colour, cell size, tolerance to salt, growth temperature, pH growth range, some biochemical characteristics, utilization of various carbon sources, fatty acid and polar lipid composition and DNA G+C content (Tables 2 and 3). The strain differed from the phototrophic members of the family *Rhodobacteriaceae* with respect to the lack of photosynthetic abilities, bacteriochlorophyll a, intracytoplasmic membrane systems and pigments. Thus, we conclude that the cumulative differences that strain N9^T exhibits from *P. ferrugineus* LMG 22047^T and other related genera in the family *Rhodobacteraceae* unambiguously support the creation of a new genus and species to accommodate strain N9^T, for which the name *Albirhodobacter marinus* gen. nov., sp. nov. is proposed.

Description of *Albirhodobacter* gen. nov.

Albirhodobacter (Al.bi.rho.do.bac'ter. L. adj. albus, white; N.L. masc. n. *Rhodobacter*, a bacterial genus name; N.L. masc. n. *Albirhodobacter*, a white colored bacterium related to *Rhodobacter* species.)

Cells are Gram stain negative, non-motile, facultative anaerobic, rod-shaped, oxidase and catalase positive. The major fatty acids are C_{16:0}, C_{18:1 ω7c} and C_{16:1 ω7c} and/or C_{16:1 ω6c} (summed in feature 3 as defined by the MIDI system). Q-10 is the predominant respiratory quinone. The predominant polar lipids are phosphatidylethanolamine and an unidentified phospholipid. The genomic DNA G+C content of the type strain of the type species is 63.2 ± 0.6 mol% (*T_m*). The genus is a member of the family *Rhodobacteraceae* of the class *Alphaproteobacteria* of the phylum *Proteobacteria*. The type species is *Albirhodobacter marinus*.

Description of *Albirhodobacter marinus* sp. nov.

Albirhodobacter marinus (ma.ri'nus. L. masc. adj. marinus, pertaining to marine, of sea water from Vishakhapatnam, Andhra Pradesh, India, referring to the isolation of the type strain from marine water).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.5-0.8 μm wide and 2-3 μm long. Colonies on marine agar are circular, 2-4 mm in diameter, smooth, whitish to light creamish in colour, translucent, convex. Anaerobic growth is observed in presence of hydrogen sulfide. Grows well in the temperature range 30-37°C, with an optimum temperature of 30 °C and optimum pH at 7.5. Optimal growth occurs in the presence of 2 % (w/v) NaCl and up to 9 % (w/v) NaCl is tolerated; growth does not occur in the absence of NaCl. Lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase activities are absent. Hydrogen sulfide gas and indole are not produced. DNA and urea are hydrolysed but not aesculin and Tween 60. Positive for L-pyrrolydonyl-arylamidase and α-glucosidase activities and negative for Ala-Phe-Pro-arylamidase, β-galactosidase, β-N-acetylglucosaminidase, glutamyl arylamidase, γ-glutamyl-transferase, β-glucosidase, β-xylosidase, β-alanine arylamidase, L-proline arylamidase, lipase, tyrosine arylamidase, β-N-acetylgalactosaminidase, α-galactosidase, phosphatase, glycine arylamidase, β-glucuronidase and glu-gly-arg-arylamidase activities. Positive for the ELLMAN reaction. Negative for L-lactate alkalisation, succinate alkalisation and 0/129 (vibriostatic agent) resistance. Utilises arabinose, D-mannitol, fructose, galactose, glucose, inulin, lactose, maltose, mannitol, mannose, melibiose and xylose but not adonitol, cellobiose, citrate, D-cellobiose,

courmarate, D-glucose, D-maltose, D-mannose, D-sorbitol, D-tagatose, D-trehalose, dulcitol, glucosamine, inositol, 5-keto-D-gluconate, L-arabitol, L-histidine, L-malate, malonate, palatinose, raffinose, rhamnose, salicin, sorbitol, sucrose and trehalose. The cellular fatty acid composition is given in Table 3. In addition to the major polar lipids listed above, significant amounts of phosphatidylglycerol, two aminophospholipids, one phospholipid and four unidentified lipids are also present.

The type strain, N9^T (= MTCC 11277^T = JCM 17680^T) was isolated from a marine water collected from sea shore of Visakhapatnam, Andhra Pradesh, India. The GenBank [EMBL/DDBJ] accession number for the 16S rRNA gene sequence of strain N9^T is FR827899.

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Legends to Figures

Fig. 1. Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between strain N9^T and representatives of the family *Rhodobacteraceae*. The filled circles indicate that a similar topology was obtained in both the maximum-likelihood and neighbor-joining phylogenetic trees. Numbers at the nodes are bootstrap values >70%. *Ancylobacter aquaticus* Ørskov^T (M62790) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Fig. 2. Two-dimensional thin-layer chromatogram of total polar lipids of strain N9^T. Detection of total polar lipids by 5% ethanolic molybdato-phosphoric acid. Abbreviations: PL1-2, phospholipids; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; L1-4, unidentified lipids; APL1-2, unidentified aminophospholipids; PL1-2, unidentified phospholipids.

Supplementary Fig. S1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between strain N9^T and representatives of the family *Rhodobacteraceae*. Numbers at the nodes are bootstrap values >70%. *Ancylobacter aquaticus* Ørskov^T (M62790) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 1. Distinguishing features of strain N9^T compared to those of members of the closely related genera of the family *Rhodobacteraceae*.

Strains: 1, strain N9^T (data from this study); 2, *Pseudorhodobacter ferrugineus* (data from this study); 3, *Rhodobacter*; 4, *Rhodovulum*; 5, *Paracoccus*; 6, *Amaricoccus* (data for taxa 3-6 taken from Imhoff 1984; Imhoff 1991; Uchino et al. 2002; Hiraishi and Ueda 1994; Ohara et al. 1990, Liu et al. 2008, Roh et al. 2009, Chen et al. 2011); 7, *Roseicitreum* (data taken from Yu et al. 2011); 8, *Roseinatronobacter* (data taken from Sorokin et al. 2000); 9, *Roseibaca* (data taken from Labrenz et al. 2009); 10, *Rhodobaca* (data taken from Milford et al,2000, Boldareva et al. 2008); 11, *Catellibacterium* (data taken from Tanaka et al, 2004); 12, *Haematobacter* (data taken from Helsel et al, 2007); 13, *Albimonas* (data taken from Lim et al. 2008); 14, *Rubribacterium* (data taken from Boldareva et al. 2009); 15, *Rubrimonas* (data taken from Suzuki et al. 1999)

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cell shape	R	R	O-R	O-R	Co-sh R	Co (Te)	L	O-L	R	S-R	O-R (Pa, Ch)	R	sh R	O-Po	sh R
Motility	Nm	Nm	Mo & Nm	Mo & Nm	Mo & Nm	Nm	Nm	Nm	Nm	M	Nm	Nm	Nm	M, Nm	M
Habitat	Ma	Ma	FW & Ter en	Ma or hy-s en	So, sw	Ac-s, sw	Ma	Ma	Ma	Ma	Ac-s	Bl	Ma	Ma	Ma
Requirement of NaCl	+	-	-	+	-/+	-	-	+	+	-	-	-	-	-	+
Aerobic dark growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic growth	+	-	+	+	-	-	-	-	-	+	-	-	-	+	-
Photosynthetic growth	-	-	+	+	-	-	-	-	-	+	-	-	-	+	-
BChl <i>a</i>	-	-	+	+	-	-	+	+	+	+	-	ND	-	+	+
Hydroxy fatty acids															
3-OH	10:0, 12:1	10:0	10:0, 14:1	ND	10:0	ND	10:0	ND	14:1	ND	None	10:0	None	None	ND
Polar lipids	PC, PE, PG, L	PC, PG, AL, APL	PC, SL	SL	DPG, PG, PC, AL, GL, L	ND	PG, PE, PC, AL	ND	DPG, PE, PC, AL	ND	ND	ND	PG, DPG, PE, PC	ND	ND
G + C content (mol%)	63.16	58-60.9*	64-73	62-69	62-71	51-63	63.3	59-61	61	58-60	64.5	65	72	69.9	74-74.8

Insertion in the 16S rRNA (190-204)	GATTGT GGTGAT TGG	CACTGT AGTGGT GGG	-	-	-	-	-	-	-	-	-	-	-	-
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*Data taken from Jung et al. (2012). ND, no data available; -, R, rod-shaped; O, ovoid; Co, coccus, L-lemon shaped, S-Spherical, Pa- arranged as Pairs, Po- Polymorphic; Ch- arranged as chain, Te, arranged as tetrads; sh, short; Nm, non-motile, Mo, motile, Ma, marine; FW, fresh water; Ter, terrestrial; en, environments; hy-s, hypersaline; So, soil; sw, sewage; Ac-s, Activated sludge; Bl-Blood (Specimen); -, negative/absent; +, positive/present; DPG, diphosphatidylglycerol; PE-phosphatidylethanolamine, PC, phosphatidylcholine; PG, phosphatidylglycerol; L, unidentified lipid; APL, unidentified aminophospholipid; AL, unidentified aminolipid; SL, sulfolipid; GL, unidentified glycolipid. Ubiquinone Q-10 is present in all taxa as far as tested.

Table 2. Features that distinguish strain N9^T from the closely related species *P. ferrugineus*

Data for both taxa is from the present study. Both strains were gram negative, rod-shaped, positive for catalase, oxidase, α -glucosidase and urease activities and ELLMAN reaction and negative for Ala-Phe-Pro-arylamidase, β -*N*-acetylglucosaminidase, glutamyl arylamidase pNA, γ -glutamyl-transferase, β -glucosidase, β -glucuronidase, β -xylosidase, β -alanine arylamidase pNA, L-proline arylamidase, lipase, tyrosine arylamidase, β -*N*-acetylgalactosaminidase, phosphatase, glycine arylamidase, lysine decarboxylase, ornithine decarboxylase and glu-gly-arg-arylamidase activities, H₂S production, fermentation of glucose, succinate alkalisation and 0/129 resistance. Both strains did not utilize adonitol, cellobiose, citrate, coumarate, malonate, sorbitol, sucrose, trehalose, L-arabitol, D-cellobiose, D-glucose, D-maltose, D-mannose, palatinose, D-sorbitol, D-tagatose, D-trehalose, 5-keto-D-gluconate, L-histidine and L-malate. Both strains resistant to penicillin-G (10 units/disc). Both strains contain Q-10 as the respiratory quinone. +, positive; -, negative; w, weak.

Characteristic	<i>Albirhodobacter marinus</i> N9 ^T	<i>Pseudorhodobacter ferrugineus</i> LMG 22047 ^T
Cell size (μ m)	0.5-0.8 x 2.0-3.0	0.6-1.6 x 1.0-4.0
Motility	-	+
Temperature growth range (°C)	30-37	25-37
Optimum growth temperature (°C)	30	25-27
NaCl range (% w/v)	1-9	0-3
Optimum NaCl concentration (% w/v)	2	1
pH growth range	6-10	6-9
Optimum pH	7.5	7
Biochemical characteristics		
Nitrate reduction	+	-
L-Pyrrolydonyl-arylamidase	+	-
β -galactosidase	-	+
α -galactosidase	-	+
L-Lactate Alkalisation	+	-
Utilization of:		
Glucose	+	-
Mannitol	+	-
Mannose	+	w
Melibiose	+	w
Xylose	+	w
D-Mannitol	+	-
Chemotaxonomic characteristics		
Major fatty acids	C _{16:0} , C _{18:1} ω 7c, SF 3 [#]	C _{16:0} , C _{18:0} , C _{18:1} ω 7c
Polar lipids	PE, PG, L1-L4, AL1, AL2, PL1, PL2	PC, PG, AL1, AL2, APL1
DNA G+C content (mol%)	63	58

[#]SF 3, summed feature 3. Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed Feature 3 contains C_{16:1} ω 7c and/or C_{16:1} ω 6c.

Table 3. Comparison of the fatty acid composition of strain N9^T with that of the closely related species *P. ferrugineus*

Results are presented as a percentage of the total fatty acids. Fatty acids amounting to 5% or more of the total fatty acids are in bold. Data for both taxa are from the present study. ND, not detected. The strains were grown on Trypticase Soy Agar plates with 2% (w/v) NaCl at 30°C for two days. Fatty acids amounting to <1% in either taxon are not presented.

Fatty acid	<i>Albirhodobacter marinus</i> N9 ^T	<i>Pseudorhodobacter ferrugineus</i> LMG 22047 ^T
C _{10:0} 3OH	2.3	2.3
C _{14:0}	0.2	1.4
C _{16:0}	7.2	13.2
C _{18:0}	0.6	10.4
C _{18:1} ω7c	77.5	63.7
C _{18:1} ω9c	ND	3.4
C _{18:2} ω6,9c	ND	1.3
[#] Summed feature 3	10.5	0.5

[#]Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed Feature 3 contains C_{16:1} ω7c and/or C_{16:1} ω6c.

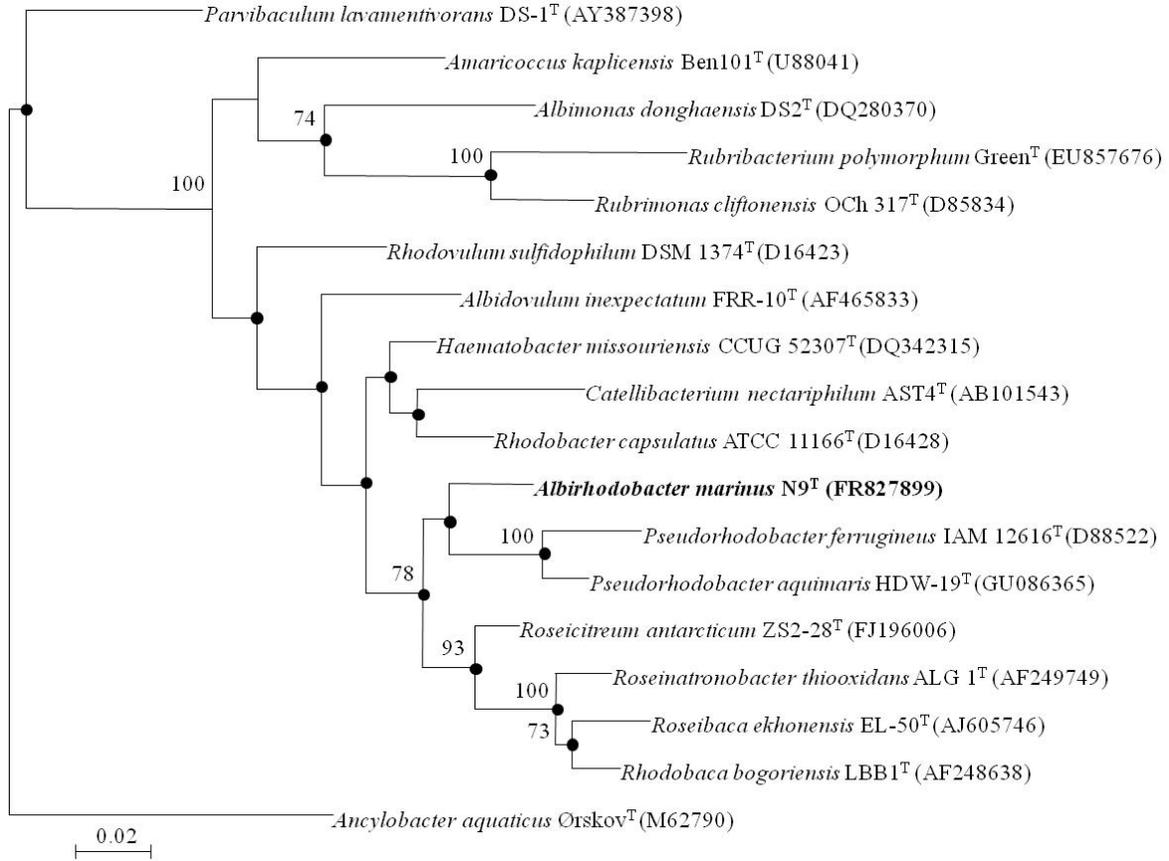


Fig. 1.

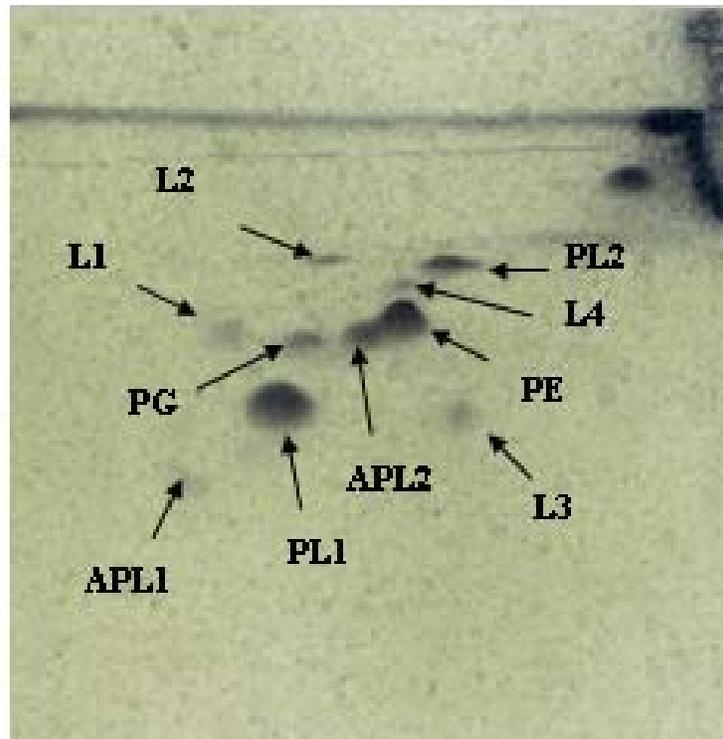
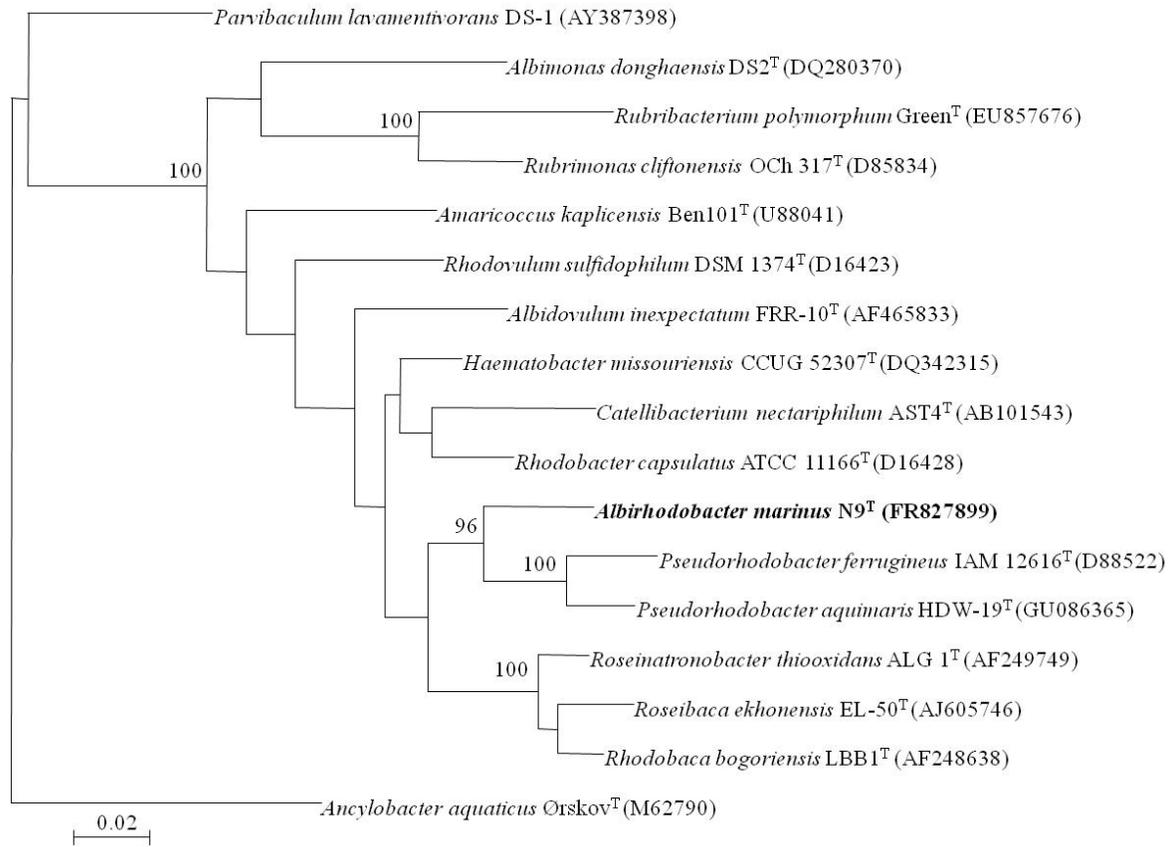


Fig. 2.



Supplementary Fig. S1.