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***Marinobacter nitratreducens* sp. nov., a halophilic and lipolytic bacterium of the family *Alteromonadaceae* isolated from coastal surface sea water**

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A novel Gram-stain-negative, rod shaped, motile bacterium, designated strain AK21^T, was isolated from coastal sea surface water, Visakhapatnam, India. The strain was positive for oxidase, catalase, lipase, L-proline arylamidase and tyrosine arylamidase activities. The predominant fatty acids were C_{12:0}, C_{12:0} 3OH, C_{16:0}, C_{16:1} ω⁹c, C_{18:1} ω⁹c and C_{16:1} ω⁷c and/or iso-C_{15:0} 2-OH (summed feature 3). The polar lipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), one unidentified aminophospholipids (APL), two unidentified phospholipids (PL) and one unidentified lipid (L). Q-10 is the predominant respiratory quinone. The DNA G + C content of the strain was 54.6 mol%. The 16S rRNA gene sequence analysis indicated that strain AK21^T was member of the genus *Marinobacter* and closely related to *Marinobacter xestospongiae* with pair-wise sequence similarity of 97.2% and with other members of the genus is between 94.0 to 96.8%. The average DNA-DNA relatedness of the strain AK21^T with *M. xestospongiae* JCM 17469^T was 34.5% and with *M. mobilis* JCM 15154^T was 40.5%. Phylogenetic analysis showed that strain AK21^T clustered with *Marinobacter xestospongiae* and *Marinobacter mobilis* with a distance of 2.9 and 2.8% (97.1 and 97.2% similarity), respectively. Based on the phenotypic characteristics and on phylogenetic inference, it appears that strain AK21^T represents a novel species of the genus *Marinobacter*, for which the name *Marinobacter nitratreducens* sp. nov. is proposed. The type strain of *Marinobacter nitratreducens* sp. nov. is AK21^T (= MTCC 11704^T = JCM 18428^T).

The genus *Marinobacter*, proposed by Gauthier et al. (1992) with the type species *Marinobacter hydrocarbonoclasticus*, isolated from sediment of the Gulf of Fos (French Mediterranean coast), belongs to the family *Alteromonadaceae* in the class *Gammaproteobacteria*. The genus currently comprises 36 species with validly published names, including the recently described *Marinobacter persicus* (Bagheri et al., 2013), *M. adhaerens* (Kaepfel et al., 2012), *M. antarcticus* (Liu et al., 2012), *M. xestospongiae* (Lee et al., 2012), *M. zhanjiangensis* (Zhuang et al. 2009), *M. daqiaonensis* (Qu et al., 2011), *M. oulmenensis* (Kharroub et al., 2011) and two validated species *M. salarius* and *M. similis* (Oren and Garrity, 2015). Members of the genus *Marinobacter* were isolated from diverse habitats like marine aggregates formed with the diatom *Thalassiosira weissflogii* (Kaepfel et al., 2012), paralytic shellfish toxin-producing dinoflagellates (Green et al., 2006), Antarctic marine habitats (Liu et al., 2012; Montes et al., 2008; Shivaji et al., 2005), Vietnamese oil-producing well (Nguyen et al. 1999), marine *Bryozoa* specimen (Romanenko et al., 2005), marine sediments (Gorshkova et al., 2003; Guo et al., 2007; Huo et al., 2008; Romanenko et al., 2005), sea water (Gauthier et al., 1992; Roh et al., 2008; Xu et al., 2008; Yoon et al., 2003, 2004; Zhuang et al., 2009), salt pond (Qu et al., 2011), oil-polluted saline soil (Gu et al., 2007), sea sand (Kim et al., 2006), hypersaline lake water (Aguilera et al., 2009; Bagheri et al., 2013), saline soil (Martín et al., 2003), coastal hot spring (Shieh et al., 2003), brine-seawater (Antunes et al., 2007), brine of a salt concentrator (Kharroub et al., 2011), marine solar saltern (Wang et al., 2009; Yoon et al., 2007), sea-ice of Arctic (Zhang et al., 2008), hydrothermal sediment (Handley et al., 2009), wine-barrel-decalcification wastewater (Liebgott et al., 2006) and marine sponge *Xestospongia testudinaria* (Lee et al., 2012). *Marinobacter aquaeolei* is a later heterotypic synonym of *Marinobacter hydrocarbonoclasticus* (Márquez and Ventosa 2005). In the present study we focused on the characterization and classification of a strain AK21^T, which was isolated from marine water sample, by polyphasic taxonomic approaches (Vandamme et al. 1996) and proposing new species of the genus *Marinobacter* for which the name *M. nitratireducens* sp. nov. is proposed.

Strain AK21^T was isolated from a coastal sea surface water sample collected from sea shore, Rushikonda beach (GPS positioning 17°46'54.08"N 83°23'10.29"E), Bay of Bengal, Visakhapatnam, India. The sample (1 ml) was serially diluted (10 fold dilutions) and 100 µl of each dilution was plated on marine agar medium and incubated at 30 °C. A shiny cream colored colony was observed upon five days incubation, which was purified and preserved as -70 °C glycerol stock for further characterization.

Type strains for comparative characterization were procured from Japan Collection of Microorganisms, Japan (*Marinobacter mobilis* JCM 15154^T, *Marinobacter xestospongiae* JCM

17469^T) and from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany (*Marinobacter hydrocarbonoclasticus* DSM 8798^T).

Strain AK21^T was characterized simultaneously with *M. mobilis* JCM 15154^T, *M. xestospongiae* JCM 17469^T and *M. hydrocarbonoclasticus* DSM 8798^T. Cell morphology studies of the strain AK21^T were done by phase contrast microscopy (Olympus). Physiological properties such as growth at different temperatures 5, 10, 15, 20, 25, 28, 30, 33, 35, 37, 40, 42 and 45 °C was tested by growing on Marine broth, and at different NaCl concentrations (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14% w/v) was tested by growing on Nutrient broth (Lányi, 1987). Growth of strain AK21^T at pH 5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10 and 11 was assessed on Marine broth buffered with citric acid/NaOH (for pH 5 and 6), NaHPO₄/Na₂HPO₄ (for pH 6.5, 7 and 8), glycine/NaOH (for pH 8.5, 9 and 10), Na₂HPO₄/NaOH (for pH 11). Different biochemical tests listed in description of species and as well as in Table 1 were carried out using the culture that was grown at 30 °C on MA medium as described by Lányi (1987) (catalase, oxidase activities, nitrate reduction, indole production and aesculin hydrolysis) and Smibert & Krieg (1994) (H₂S production, gelatin and urea hydrolysis). Extracellular enzymatic activities like amylase, lipase and protease, were studied as described by Srinivas et al. (2009). Biochemical and enzymatic characterizations, carbon substrate utilization, acid production and antibiotic susceptibility of the strains were performed using previously described methods (Anil Kumar et al., 2012).

Standardization of the physiological age of strains AK21^T, *M. mobilis* JCM 15154^T and *M. xestospongiae* JCM 17469^T was done based on the protocol (http://www.microbialid.com/PDF/TechNote_101.pdf) given by Sherlock Microbial Identification System (MIDI, USA). For cellular fatty acids analysis, strains AK21^T, *M. mobilis* JCM 15154^T and *M. xestospongiae* JCM 17469^T were grown on MA plates at 30 °C for 2-3 days (all three strains are at same phase of their growth). Cellular fatty acid methyl esters (FAMES) were obtained from cells by saponification, methylation and extraction following the protocol of MIDI. Cellular FAMES were separated by GC (6890) and identified and quantified using the Sherlock Microbial Identification System (MIDI-6890 with database TSBA6). Freeze-dried cells following growth were analysed for polar lipids and quinones. Isoprenoid quinones were extracted as described by Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1997). Cells of strain AK21^T and other three type strains were extracted for the polar lipid analysis (Bligh & Dyer, 1959) and analyzed by 2D thin-layer chromatography followed by spraying with appropriate detection reagents (5% ethanolic molybdato-phosphoric acid, molybdenum blue, ninhydrin and molisch reagents) (Komagata & Suzuki, 1987). Genomic DNA was isolated by using the procedure of Marmur (1961) and the mol%

G + C content was determined from melting point (T_m) curves (Sly *et al.*, 1986) obtained by using Lambda 35; Perkin Elmer spectrophotometer equipped with Templab 2.0 software package.

For 16S rRNA gene sequencing, DNA was prepared using a bacterial DNA isolation kit (Qiagen). The 16S rRNA gene was amplified by PCR using universal bacterial primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTTACGACTT -3'). The PCR product was purified using QIA quick PCR purification kit (Qiagen) and it was sequenced using an ABI PRISM model 3700 automatic DNA sequencer and Big Dye Terminator cycle sequencing kit (Applied Biosystems). The 16S rRNA gene sequence of strain AK21^T was subjected to BLAST sequence similarity search (Altschul *et al.*, 1990) and EzBioCloud (Kim *et al.*, 2012) to identify the nearest taxa. Based on BLAST results, all 16S rRNA gene sequences of the genus *Marinobacter* were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL_W program in MEGA5 (Tamura *et al.*, 2011). The evolutionary history was inferred by using the maximum-likelihood method (Tamura & Nei, 1993), neighbor-joining (NJ) method (Saitou & Nei, 1987) and maximum-parsimony method (Nei & Kumar, 2000) using the MEGA5 package (Tamura *et al.*, 2011). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 1355 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.*, 2011). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches (Felsenstein, 1985). DNA-DNA hybridization was performed by the membrane filter method (Tourova and Antonov, 1987) as described previously (Rakshak *et al.*, 2013).

Cells of the strain AK21^T were Gram-stain-negative, rod shaped, 0.8-1.0 μm wide x 2.0-3.0 μm long, multiply by binary fission and motile by single polar flagellum (Fig. 2). Chain formation is seen when grown on MA for 2-3 days at 30 °C. Colonies were circular, 2-3 mm in diameter, smooth, slimy, shiny, opaque, creamish and raised with entire margin on MA plates grown at 30 °C for two days. Grows chemoorganoheterotrophically. The strain grew between 15 to 42 °C temperature and optimum being 28 to 35 °C, from pH 6 to 10, with the optimum growth at pH 7 and from salinity 0.5 to 6% (NaCl, w/v), with optimum growth occurred at salinity 2% (NaCl, w/v). Other phenotypic characteristics of the strain AK21^T are listed in the species description and in Tables 1 and 2.

The fatty acid profile was dominated by saturated and unsaturated straight chain and hydroxy fatty acids, like C_{12:0} (9.5%), C_{12:0} 3OH (10.6%), C_{16:1} ω 9c (20.4%), C_{16:0} (24.7%), C_{18:1} ω 9c (19.9%) and C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH (summed feature 3) (5.3%) (Table 2). Over all fatty acid composition of strain AK21^T was similar to those of the two reference strains and however, the isolate AK21^T

could be clearly distinguished from the reference strains by the absence or low or high amount of C_{18:1} ω7c, summed feature 3, C_{18:1} ω9c and C_{16:1} ω9c (Table 2). The low and high amounts of fatty acids summed feature 3, C_{18:1} ω9c and C_{16:1} ω9c were comparable with *M. hydrocarbonoclasticus* DSM 8798^T (Table 2). Strain AK21^T contained diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), one unidentified aminophospholipids (APL), two unidentified phospholipids (PL) and one unidentified lipid (L) as total polar lipids (Fig. S1a). In type strains also nearly similar composition (DPG, PE, PG and APL) was observed but differed with strain AK21^T either in the absence or the presence of PL1-PL4 and L1-L7 (Fig. S1 b, c and d). Q-10 is the predominant respiratory quinone. The other type strains also comprise Q-10 as predominant respiratory quinone. DNA G + C content of the strain AK21^T was 54.6 mol% (T_m) (Table 1) which is different from the compared strains but within the genus.

Phylogenetic relationships of strain AK21^T based on the 16S rRNA gene sequence indicated that strain AK21^T was close to the *M. xestospongiae* with pair-wise sequence similarity of 97.2%. With the other members of the genus *Marinobacter* the strain AK21^T showed 94.0 to 96.8% pair-wise sequence similarity. The phylogenetic tree obtained using the neighbor-joining (Fig. 1) method revealed clustering of strain AK21^T with *M. xestospongiae*, *M. zhejiangensis* and *M. mobilis* with a distance of 2.9, 3.9 and 2.8% (97.1, 96.1 and 97.2% similarity), respectively. The other phylogenetic trees obtained using the maximum-likelihood (Supplementary Fig. S2) and maximum parsimony (Supplementary Fig. S3) methods revealed similar topology at this cluster. Based on the 16S rRNA gene sequence analysis strain AK21^T was falling within the cut off limit <97% similarity with two strains *M. xestospongiae* and *M. mobilis* and was above the cut off limit with *M. zhejiangensis* (96.1% similarity). DNA–DNA hybridization experiment was performed between the strain AK21^T and the type strains of two closely related phylogenetic neighbours, *M. xestospongiae* JCM 17469^T and *M. mobilis* JCM 15154^T. The average relatedness of the strain AK21^T with *M. xestospongiae* JCM 17469^T was 34.5% and with *M. mobilis* JCM 15154^T was 40.5%.

The main features of the strain AK21^T are in line with the original description of the genus *Marinobacter* but could be distinguished from published closely related species *M. xestospongiae*, *M. mobilis* and type species of the genus *M. hydrocarbonoclasticus* (Tables 1 and 2). Thus, the cumulative differences of strain AK21^T from the closely related type strains unambiguously classify the strain as a new species of the genus *Marinobacter* for which the name *Marinobacter nitratireducens* sp. nov. is proposed.

Description of *Marinobacter nitratireducens* sp. nov.

Marinobacter nitratireducens (ni.tra.ti.re.du'cens. N.L. n. *nitras* -atis nitrate; L. part. adj. *reducens* converting to a different state; N.L. adj. *nitratireducens* reducing nitrate).

Cells are Gram-stain-negative, rod shaped, 0.8-1.0 μm wide x 2.0-3.0 μm long and motile by single polar flagellum. Cells form chains, multiply by binary fission and are motile. Colonies grown for 2 days at 30 °C on MA plates are circular, 2-3 mm in diameter, smooth, slimy, shiny, opaque, cream and raised with entire margin. Grows at 15-42 °C (optimum, 28-35 °C), and at pH 6-10 (optimum, 7.0). Optimum growth occurred at salinity 2% (w/v) and tolerates up to 6% (w/v). Cells require NaCl for growth. Positive for catalase, oxidase and arginine dihydrolase activities. Methyl red and Voges Proskauer's reactions are negative. Nitrate is reduced but H₂S and indole are not produced. Hydrolyzed Tween 20/40/60/80 but not agar, aesculin, casein, gelatin, starch and urea. Produces acid from fructose, dextrose, sucrose, inulin, sodium gluconate, glycerol, inositol, sorbitol, mannitol, arabitol and α -methyl-D-glucoside in the Hi25™ *Enterobacteriaceae* Identification Kit, and is able to ferment the same sugars, using the HiCarbohydrate™ Kit and produce acid weakly from rhamnose but not from lactose, xylose, maltose, galactose, raffinose, trehalose, melibiose, D-arabinose, L-arabinose, mannose, salicin, dulcitol, adonitol, erythritol, cellobiose, melezitose, α -methyl-D-mannoside, xylitol, citrate, malonate and sorbose. Positive reactions (from VITEK 2 GN) in tests for L-proline arylamidase, lipase and tyrosine arylamidase; negative reactions were observed for Ala-Phe-Pro-arylamidase, L-pyrrolydonyl-arylamidase, β -galactosidase, β -N-acetylglucosaminidase, glutamyl arylamidase, γ -glutamyl-transferase, β -glucosidase, β -xylosidase, β -alanine arylamidase, α -glucosidase, β -N-acetylgalactosaminidase, α -galactosidase, phosphatase, glycine arylamidase, lysine decarboxylase, ornithine decarboxylase, β -glucuronidase and glu-gly-arg-arylamidase, adonitol, L-arabitol, D-cellobiose, D-glucose, D-maltose, D-mannitol, D-mannose, palatinose, D-sorbitol, sucrose, D-tagatose, D-trehalose, citrate, malonate, 5-keto-D-gluconate, L-histidine, L-lactate, L-malate, fermentation of glucose, L-lactate and succinate alkalisation, coumarate, ELLMAN and 0/129 resistance. Susceptible to antibiotics, gentamycin, chloramphenicol, amoxicillin and ampicillin; moderately resistant to N, ceftazidime and erythromycin; resistant to kanamycin, tetracycline, lincomycin, vancomycin, chlorotetracycline, cephadroxil, novobiocin, enoxacin and methicillin. The predominant fatty acids were C_{12:0}, C_{12:0} 3OH, C_{16:1} ω 9c, C_{16:0}, C_{18:1} ω 9c and C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH (summed feature 3). The polar lipids consisted of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), one unidentified aminophospholipids (APL), two unidentified phospholipids (PL) and one unidentified lipid (L) and Q-10 as predominant respiratory quinone. The G + C content of the genomic DNA is 54.6 mol%.

The type strain, AK21^T (= MTCC 11704^T = JCM 18428^T) was isolated from a coastal sea surface water sample collected from sea shore, Rushikonda beach, Bay Bengal, Visakhapatnam, India.

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Legends to Figures

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain AK21^T and closely related species of the genus *Marinobacter*. Numbers at the nodes are bootstrap values >70%. *Zooshikella ganghwensis* JC2044^T (AY130994) was taken as out group. Bar, 0.01 substitution per nucleotide position.

Fig. 2. Electron micrograph of negatively stained cells of strain AK21^T showing single polar flagellum. Bar, 1 μ m.

Supplementary Fig. S1. Two-dimensional thin-layer chromatogram of the total polar lipids of the strain AK21^T (a), *M. mobilis* JCM 15154^T (b) *M. xestospongiae* JCM 17469^T (c) and *M. hydrocarbonoclasticus* DSM 8798^T (d) after spraying the plates with molybdato-phosphoric acid. Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; PL, unidentified phospholipid. The spots identified as phospho-, amino- or glyco-lipids by spraying molybdenum blue, ninhydrin and α -naphthol reagents, respectively.

Supplementary Fig. S2. Maximum likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain AK21^T and closely related species of the genus *Marinobacter*. Numbers at the nodes are bootstrap values >60%. *Zooshikella ganghwensis* JC2044^T (AY130994) was taken as out group. Bar, 0.01 substitution per nucleotide position.

Supplementary Fig. S3. Maximum parsimony phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain AK21^T and closely related species of the genus *Marinobacter*. Numbers at the nodes are bootstrap values >60%. *Zooshikella ganghwensis* JC2044^T (AY130994) was taken as out group.

Table 1. Features that distinguish strain AK21^T from the related species *M. mobilis* JCM 15154^T, *M. xestospongiae* JCM 17469^T and *M. hydrocarbonoclasticus* DSM 8798^T

Data were from present study. All strains were rod-shaped, cream coloured and motile, positive for oxidase, catalase, L-proline arylamidase and tyrosine arylamidase activities, nitrate reduction and hydrolysis of Tween 20/40/60/80. All strains were negative for Voges Proskauer's reactions, aesculin, agar, casein, gelatin, ONPG, starch hydrolysis, Ala-Phe-Pro-arylamidase, L-Pyrrolydonyl-arylamidase, β -galactosidase, glutamyl arylamidase, β -glucosidase, β -xylosidase, β -alanine arylamidase, urease, α -glucosidase, α -galactosidase, β -N-acetylgalactosaminidase, α -galactosidase, phosphatase, glycine arylamidase, ornithine decarboxylase, lysine decarboxylase, β -glucuronidase and glu-gly-arg-arylamidase activities, fermentation of glucose, acid production from melibiose, D-arabinose, L-arabinose, salicin, adonitol, erythritol, cellobiose, melezitose, α -methyl-D-mannoside, sorbose and xylitol, utilization of citrate, malonate, adonitol, L-arabitol, D-cellobiose, D-glucose, D-maltose, D-mannitol, D-mannose, palatinose, D-sorbitol, sucrose, D-tagatose, D-trehalose, 5-keto-D-gluconate, L-histidine and L-malate, L-lactate and succinate alkalisation, 0/129 resistance and ELLMAN reaction and resistant to antibiotics (μ g/disc) tetracyclin (30) and vancomycin (30). +, positive; -, negative; (+), partially positive; S, sensitive; R, resistance; I, intermediate; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; PL, unidentified phospholipid.

Characteristic	<i>Marinobacter nitratireducens</i> AK21 ^T	<i>Marinobacter mobilis</i> JCM 15154 ^T	<i>Marinobacter xestospongiae</i> JCM 17469 ^T	<i>Marinobacter hydrocarbonoclasticus</i> DSM 8798 ^T
Cell size (μ m)	1 x 2.5	0.5-0.8 x 1.5-3.1	0.6-0.8 x 2-2.5	0.6-0.8 x 2-2.5
Temperature growth range ($^{\circ}$ C)	15-42	15-42	15-42	10-45
Optimum temperature ($^{\circ}$ C)	28-35	30-35	28-36	32
Salinity growth range (%)	0.5-6	0.5-10	0.5-6	0.5-20
Optimum NaCl concentration (% w/v)	2	3-5	2	3-6
pH growth range (Optimum)	6-10	6.5-9	5-10	6-12
Optimum pH	7	7-7.5	7-7.5	7-7.5
Indole production	-	-	+	-
Methyl red reaction	-	-	-	+
H ₂ S production	-	-	-	+
Arginine dihydrolase	+	+	+	-
Acid production from:				
Lactose	-	-	+	-
Citrate	-	-	+	+
Xylose	-	-	+	-
Maltose	-	-	+	-
Fructose	+	-	+	-
Dextrose	+	-	+	-
Galactose	-	-	+	-
Raffinose	-	-	+	-
Trehalose	-	-	+	-

Sucrose	+	-	-	-
Mannose	-	-	+	-
Inulin	+	-	-	-
Sodium gluconate	+	-	-	-
Glycerol	+	-	-	-
Dulcitol	-	-	+	-
Inositol	+	-	-	-
Characteristic	<i>Marinobacter nitratireducens</i> AK21 ^T	<i>Marinobacter mobilis</i> JCM 15154 ^T	<i>Marinobacter xestospongiae</i> JCM 17469 ^T	<i>Marinobacter hydrocarbonoclasticus</i> DSM 8798 ^T
Sorbitol	+	-	-	-
Mannitol	+	-	-	-
Arabitol	+	-	-	-
α -Methyl-D-glucoside	+	-	-	-
Rhamnose	(+)	-	-	-
Vitek GN information:				
β -N-Acetylglucosaminidase	-	-	+	-
β -N-Acetylgalactosaminidase	-	-	+	-
γ -Glutamyl-transferase	-	-	+	-
Lipase	+	-	+	+
Courmarate	-	-	+	-
Antibiotic susceptibility (μg/disc):				
Chloramphenicol (30)	S	S	R	S
Neomycin (30)	I	I	S	S
Ceftazidime (30)	I	S	R	I
Chlortetracyclin (30)	R	R	I	R
Amoxicillin (30)	S	I	R	S
Polymyxin B (300)	R	S	S	S
Cephahydroxil (30)	R	S	R	R
Ampicillin (25)	S	S	R	S
Novobiocin (30)	R	R	R	S
Polar lipids	DPG, PE, PG, APL, PL1, PL2, L1	DPG, PE, PG, APL, PL1, PL3, L2-L5	DPG, PE, PG, APL, PL1, PL2, PL4, L2	DPG, PE, PG, APL, PL1, PL3, L5-L7
DNA G+C content (mol%)	54.6	58.0-58.9	57.1	52.7

Table 2. Fatty acid composition of the strain AK21^T, *M. mobilis* JCM 15154^T, *M. xestospongiae* JCM 17469^T and *M. hydrocarbonoclasticus* DSM 8798^T

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 were from present study. ND, not detected. The strains were grown on MA plates at 30°C for two to three days. ND, not detected. Values of less than 1% for all strains are not shown. tr, traces (<1%).

Fatty acid	<i>Marinobacter nitratireducens</i> AK21 ^T	<i>Marinobacter mobilis</i> JCM 15154 ^T	<i>Marinobacter xestospongiae</i> JCM 17469 ^T	<i>Marinobacter hydrocarbonoclasticus</i> DSM 8798 ^T
C _{10:0}	ND	ND	ND	1.0
C _{12:0}	9.5	11.1	18.5	4.7
C _{12:0} 3OH	10.6	12.8	21.6	6.9
C _{14:0}	2.4	3.2	1.3	2.5
C _{16:0} N Alcohol	1.3	tr	1.9	1.7
C _{16:1} ω 9 <i>c</i>	20.4	1.1	3.9	9.2
C _{16:0}	24.7	21.6	19.1	24.4
C _{17:1} ω 8 <i>c</i>	1.1	4.6	1.8	0.8
C _{17:0}	1.0	3.5	1.6	0.5
C _{18:3} ω 6,9,12 <i>c</i>	1.4	ND	tr	1.0
C _{18:1} ω 9 <i>c</i>	19.9	3.8	4.1	35.9
C _{18:1} ω 7 <i>c</i>	ND	5.3	5.2	1.9
C _{18:0}	1.6	2.6	tr	2.0
Summed feature 3	5.3	28.1	18.8	6.3

Summed features are groups of two or three fatty acids that could not be separated by GC with the MIDI system. Summed feature 3 comprised C_{16:1} ω 7*c* and/or iso-C_{15:0} 2-OH.

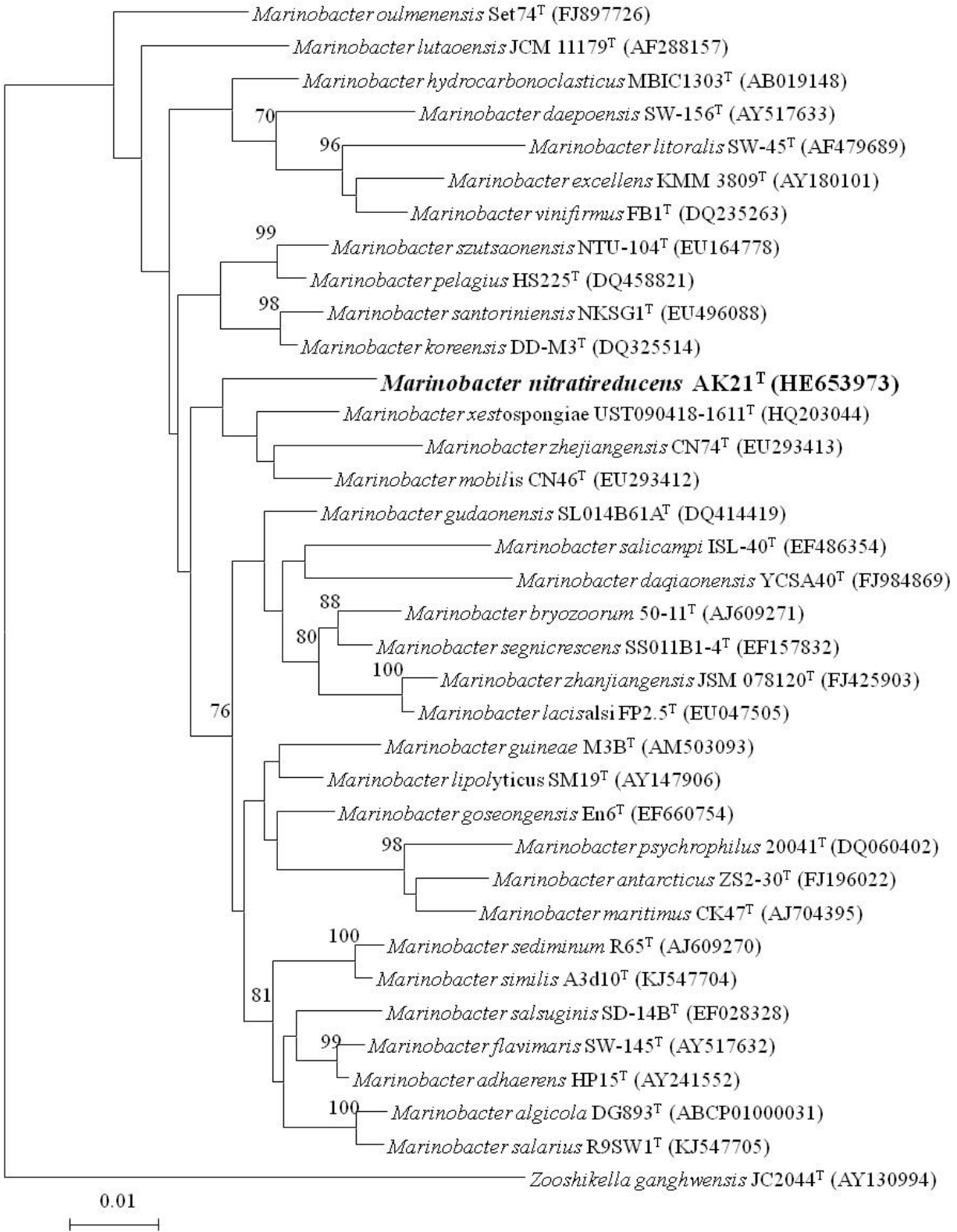
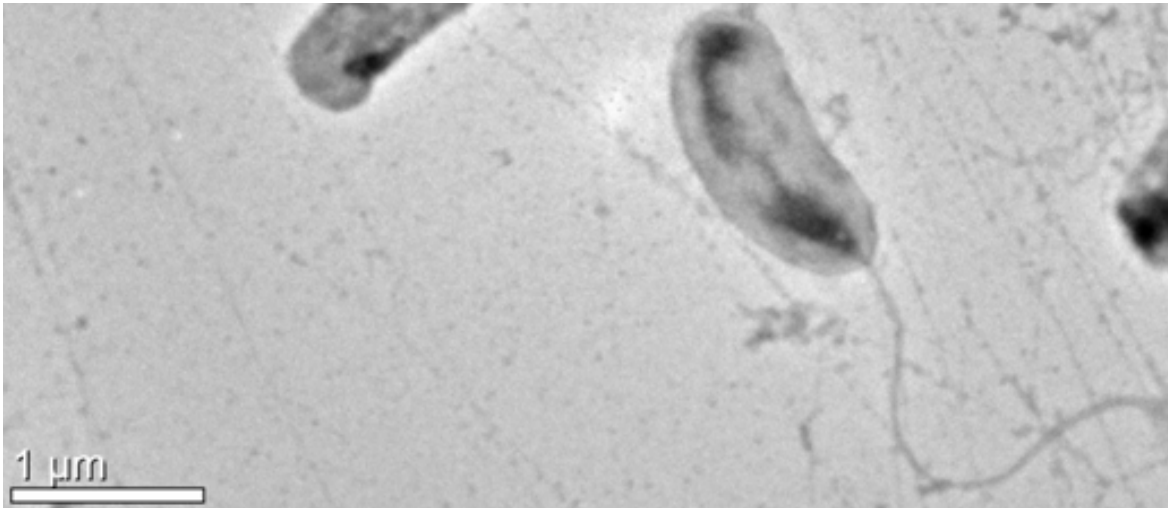
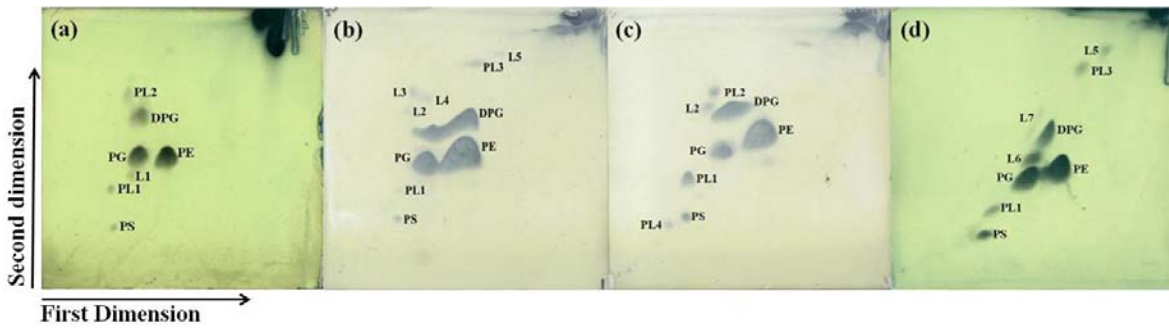
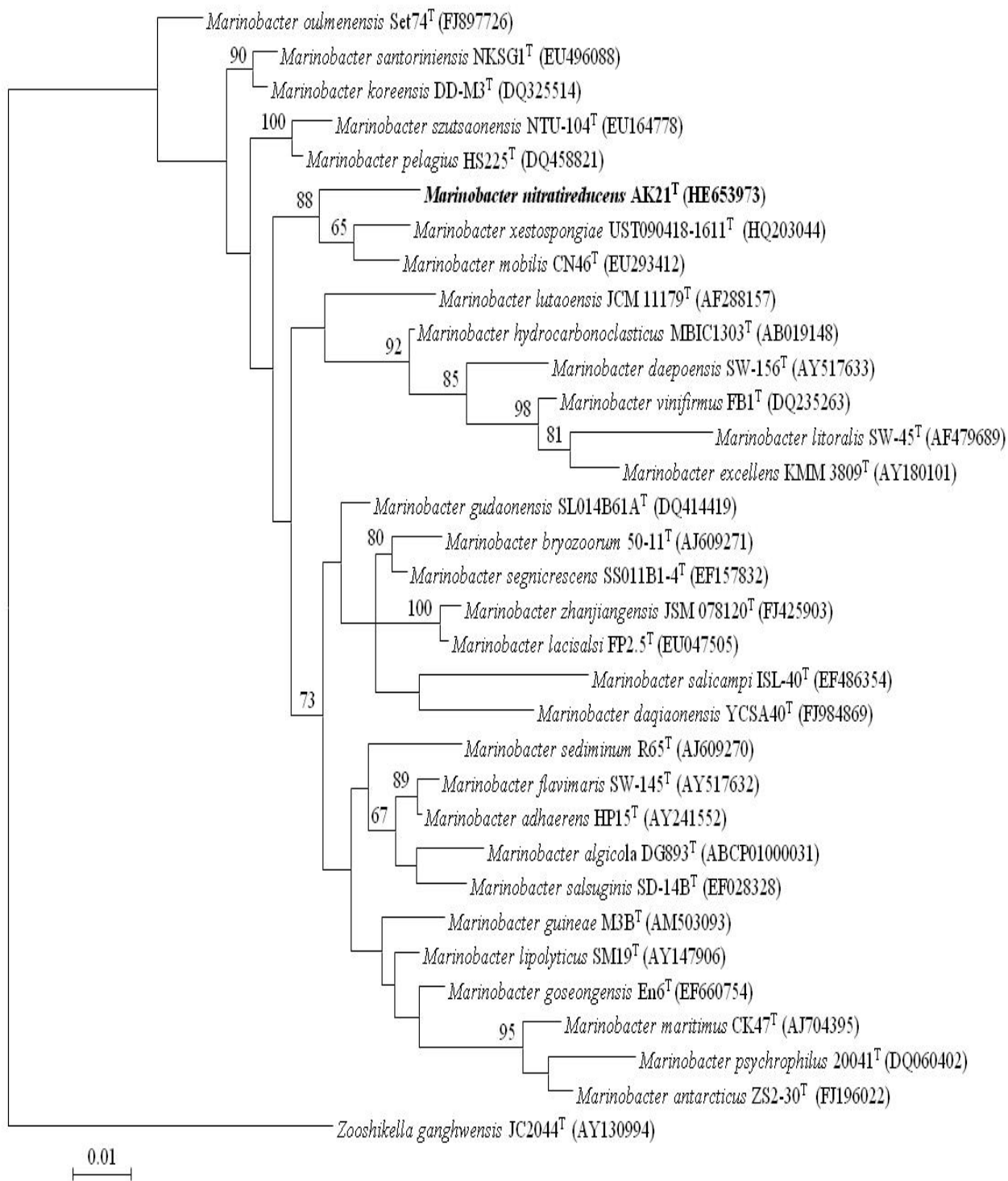
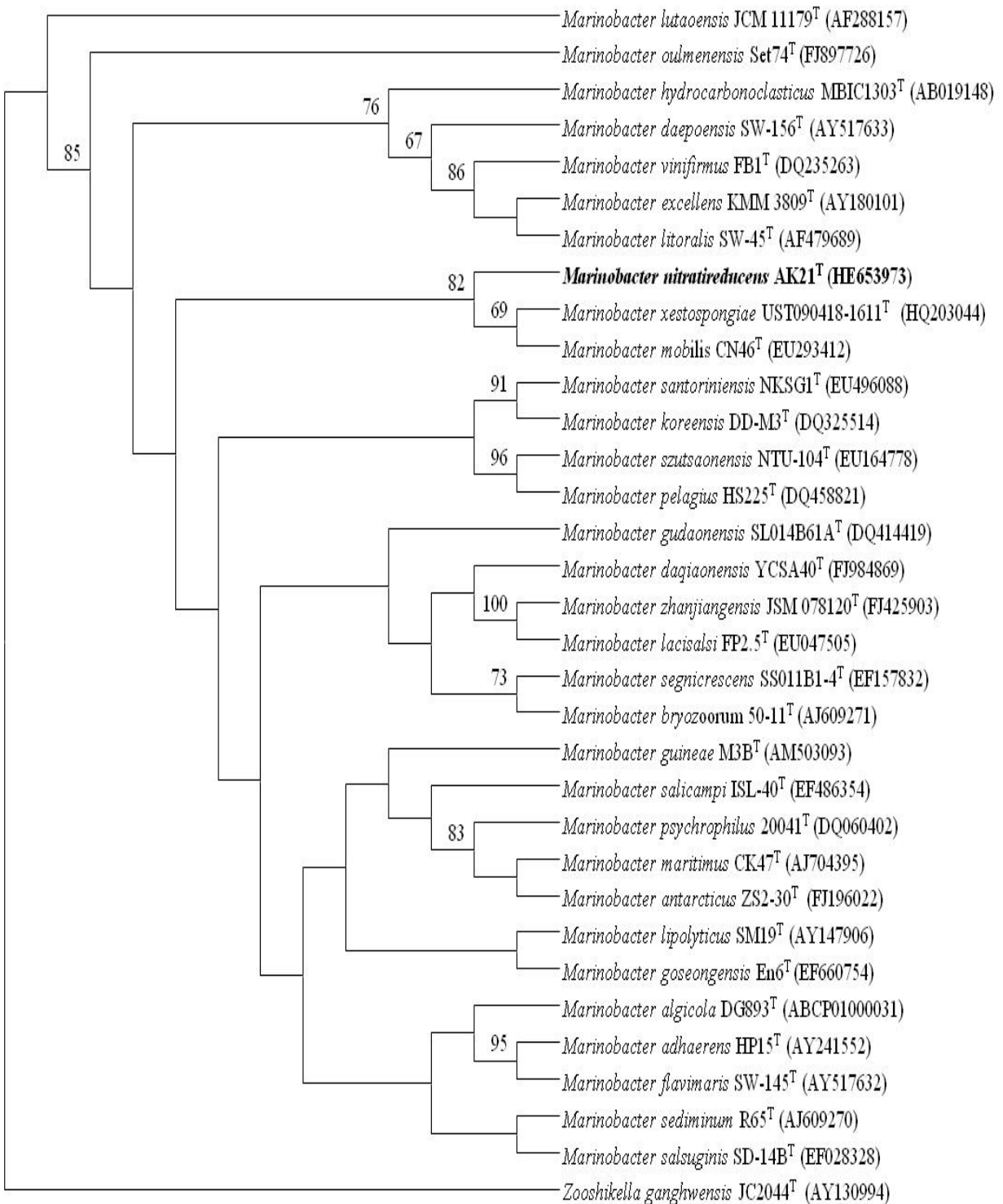


Fig. 1

**Fig. 2****Supplementary Fig. S1**



Supplementary Fig. S2



Supplementary Fig. S3