

Oceanospirillum sanctuarii sp. nov., isolated from a sediment sample collected from the Coringa wildlife sanctuary, India

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Abstract

A novel Gram-staining-negative, spiral-shaped, pale-yellow in color, non-sporulating, motile, aerobic bacterium designated strain AK56^T, was isolated from the sediment sample collected from Coringa Wildlife Sanctuary, India. Colonies on marine agar were circular, pale-yellow, shiny, translucent, 1–2 mm in diameter, convex, with entire margin. The major fatty acids included C_{16:1}, C_{16:1} ω7c/C_{16:1} ω6c and C_{18:1} ω7c fatty acids. Polar lipids included diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), two unidentified aminolipids (AL1, 2), one unidentified phospholipids (PL1) and five unidentified lipids (L1-5). DNA-DNA hybridization between strain AK56^T and with *Oceanospirillum linum* LMG 5214^T and *Oceanospirillum nioense* NIO-S6^T showed a relatedness of 39.91% and 23.62% respectively. The DNA G+C content of the strain AK56^T was found to be 50.3 mol%. Sequence similarity search for 16S rRNA gene sequence revealed that *Oceanospirillum linum* and *Oceanospirillum nioense* were the nearest phylogenetic neighbors, with a pair-wise sequence similarity of 98.9 and 98.2% respectively. Phylogenetic analysis also showed formation of a cluster of strain AK56^T with close relative *Oceanospirillum linum* and together clustered with *Oceanospirillum nioense*. Based on the observed phenotypic, chemotaxonomic characteristics and phylogenetic analysis, strain AK56^T is described in this study as a novel species in the genus *Oceanospirillum*, for which the name *Oceanospirillum sanctuarii* sp. nov. is proposed. The type strain of *Oceanospirillum sanctuarii* is AK56^T (= MTCC 12005^T = JCM 19193^T = KCTC 52973^T).

Keywords: *Oceanospirillum sanctuarii* sp. nov., 16S rRNA gene, phylogeny, polar lipids, fatty acids, DNA–DNA hybridization.

The genus *Oceanospirillum* is a member of the family *Oceanospirillaceae*, order *Oceanospirillales*, class *Gammaproteobacteria*, phylum *Proteobacteria* and was established by Williams and Rittenberg [1]. The family *Oceanospirillaceae* comprises of Gram-negative, aerobic spiral shaped motile bacteria [2] currently comprises of 18 genera (<http://www.bacterio.net/classifgenerafamilies.html#Oceanospirillaceae>). The different genera under the family *Oceanospirillaceae* have been described from diverse ecological niches and substrates such as: hydrothermal vent field at the Mid-Atlantic Ridge, Red sea, reef-building coral in Taiwan, cultured oysters and seawater, Mediterranean Sea, sediments adjacent to sperm whale carcasses in Japan, sediment contaminated with polycyclic aromatic hydrocarbon creosote, decomposing wood from the shore of Soap Lake in USA, haemolymph serum of the blacklip abalone *Haliotis rubra* and seawater from Indonesia [3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. Members of *Balneatrix alpica* have also been implicated in human disease symptoms such as pneumonia and meningitis [13]. The members of the genus *Oceanospirillum* are Gram-staining-negative, rigid, helical cells with clockwise helix, intracellular poly- β -hydroxybutyrate (PHB) granules, coccoid bodies, motile by bipolar tufts of flagella, chemo-organotrophic with strictly respiratory type of metabolism, oxidase positive, indole and nitrate reduction negative, cannot hydrolyze casein, starch, hippurate and aesculin, require sea water for growth, cannot ferment or oxidize carbohydrates, utilize amino acids or the salts of organic acids as carbon sources with DNA G+C content of 45-50 mol% (as determined by the thermal denaturation method). The type species is *Oceanospirillum linum* [14]. The genus *Oceanospirillum* presently comprises 3 species and two sub species (<http://www.bacterio.net/oceanospirillum.html>). In the present study, we focused on the characterization and classification of the strain AK56^T, which was isolated from mangrove sediment sample by a polyphasic taxonomic approach [15] and on the basis of the results assign the novel strain to the genus *Oceanospirillum*.

The strain AK56^T was isolated from the sediment sample (16° 48' N 82° 14' E) collected from Coringa Wildlife Sanctuary during an expedition to characterize microbial diversity of India's second largest stretch of mangrove forest located at Coringa Wildlife Sanctuary, Andhra Pradesh, India. For isolation of the strain AK56^T, the sediment sample was serially diluted (up to 10 fold dilutions) in 2% (w/v) NaCl solution and 100 μ l of each dilution was spread-plated on ZoBell's Marine Agar (ZMA; HIMEDIA, India) and incubated at 30 °C. Among the different colony morphotypes appeared a pale-yellowish coloured colony was selected and further characterized and preserved at -80 °C in marine broth with 10% glycerol.

The type strains of *Oceanospirillum linum* LMG 5214^T and *Oceanospirillum nioense* NIO-S6^T were also cultivated under same conditions as strain AK56^T. The colony morphology of strain

AK56^T was observed after 2 days of growth on ZMA at 30 °C. The cell morphology was studied by light microscopy (Zeiss, at 1000X). The cell motility was performed as described in Bernardet *et al.*, [16] and Gram staining was done using Gram staining kit (HIMEDIA, India) according to manufacturer's instructions. Endospore formation was determined by malachite-green staining of isolate grown on MA for a week.

Growth at 4, 10, 15, 20, 25, 30, 37, 42, 45 and 50 °C was ascertained using Marine Broth (MB; HIMEDIA, India) and salt tolerance [0, 1, 2, 3, 4, 5, 6, 8, 10 and 12% (w/v) NaCl] was ascertained using NB containing peptone (5 g l⁻¹) and beef extract (3 g l⁻¹). Growth of strain AK56^T at pH 4, 5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 11 and 12 was assessed on MB buffered either with acetate or citrate 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). Different biochemical tests listed in description of species and as well as in Table 1 were carried out for both strains under identical conditions using cultures grown at 37 °C on MA medium as described by Lányi [17]. Substrate hydrolysis (Aesculin, casein, gelatin, starch and Tweens), biochemical characteristics (Catalase and oxidase activity, production of H₂S and reduction of nitrate, Voges–Proskauer and methyl red test, oxidation–fermentation tests, oxidation of tetramethyl-p-phenylenediamine dihydrochloride) were determined as previously described [17, 18]. Utilization of different carbohydrate sources was determined by analyzing the acid production by the isolate using bromocresol purple as an acid–base indicator [19]. ONPG discs were used to determine the β -galactosidase activity (HIMEDIA). Biochemical and characterization of different enzyme activity of strain AK56^T was also performed using the Vitek 2 GN system (bioMérieux) according to the manufacturer's protocol. The inoculum for Vitek analysis was prepared using sterile 2.0% (w/v) NaCl and incubated at 30 °C. Antibiotic sensitivity was determined using commercially available antibiotic discs (HIMEDIA, India) as described by Baek *et al.* [20].

Standardization of the physiological age of strains AK56^T and *Oceanospirillum linum* LMG 5214^T was done based on the protocol [21] given by Sherlock Microbial Identification System (MIDI, USA). For cellular fatty acids analysis, strains AK56^T and *Oceanospirillum linum* LMG 5214^T were grown on MA plates at 37 °C for 2 days and were of same physiological age (at logarithmic phase of 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 analyzed using the Sherlock Microbial Identification System (MIDI-6890 with database TSBA6) according to the protocol described by Sherlock Microbial Identification System. Extraction of polar lipids was performed as described in Bligh & Dyer [22]

and analyzed using two-dimensional TLC followed by spraying with suitable detection reagents [23].

Isolation of genomic DNA, polymerase chain reaction (PCR) amplification and sequencing of 16S rRNA gene were performed as described in Surendra *et al.* [24]. The G+C content of strain AK56^T was determined from the melting point (T_m) curves obtained [25] using a Lambda 2 UV-Vis spectrophotometer with the Templab 2.0 software package (Perkin Elmer). For the determination of G+C content, *Escherichia coli* DH5 α DNA was used as a standard.

The 16S rRNA gene sequence obtained was 1488 bp long and subjected to EzBioCloud (<http://eztaxon-e.ezbiocloud.net/>) search [26] to find the nearest taxa. The 16S rRNA gene sequences of 21 closely related type strains were downloaded from NCBI (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL_W tool of MEGA version 5.2 [27]. Neighbor joining and maximum likelihood methods were used to construct the phylogenetic trees using MEGA. There were total 22 sequences and 1497 characters in the final dataset. Bootstrap analysis of 1000 replicates was performed to infer the robustness of the phylogenetic trees and the evolutionary distances were calculated using Kimura-2-parameter [28]. DNA-DNA hybridization was performed by the membrane filter method [29] as described previously [30].

Cells of strain AK56^T were Gram-negative, spiral-shaped, pale-yellow pigmented, non-sporulating and motile. Cells were 2.0–3.0 μm in length and 0.7–1.0 μm in width. Colonies on ZMA were observed as circular, 1–2 mm in diameter, pale-yellow, shiny, translucent, convex with entire margin after 48 h at 30 °C. Growth was observed at 10–37 °C with optimum growth at 30 °C, at 2–6 % (w/v) NaCl, with optimum growth at 2 % and at pH 6–10, with optimum growth at pH 7. The cellular morphology of strain AK56^T varied slightly as compared to the reference strain *Oceanospirillum linum* LMG 5214^T as the cells of strain AK56^T were slightly longer. Susceptible to (μg per disc unless indicated) amoxicillin (30), ampicillin (25), cephalexin (30), tetracycline (30) neomycin (30), novobiocin (30), vancomycin (30), streptomycin (10) and moderately susceptible to bacitracin (8), penicillin-G (2 units) and resistant to methicillin (5). Other characteristics are presented in Table 1.

The cellular fatty acids of strain AK56^T showed a spectrum of nine fatty acids (Table 2), and included C_{16:1}, C_{16:1} ω 7c/ C_{16:1} ω 6c (summed feature 3) and C_{18:1} ω 7c (summed feature 8) as the major cellular fatty acids. The overall fatty acid composition of strain AK56^T was similar to the *Oceanospirillum linum* LMG 5214^T and *Oceanospirillum nioense* NIO-S6^T. The polar lipid composition included diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), two unidentified aminolipids (AL1, 2), one unidentified phospholipids

(PL1) and five unidentified lipids (L1-5) (Supplementary Fig. S1a). In *O. linum* LMG 5214^T and *O. nioense* NIO-S6^T nearly similar composition (DPG, PE and PG) was observed but differed with strain AK56^T either in the absence of AL1-2, PL1 and L1-5 or the presence of APL1 and PL2-4 (Supplementary Fig. 1b and 1c). The G+C content of strain AK56^T was calculated as 50.3 mol%.

For phylogenetic assessment of strain AK56^T, 1488 bp of 16S rRNA gene sequence was compared with the database of with validly published type strains using EzBioCloud. The 16S rRNA gene sequence of strain AK56^T was found to be related to *Oceanospirillum* genus. Based on pair-wise sequence similarity, strain AK56^T was closest to *Oceanospirillum linum* with 98.9% and *Oceanospirillum nioense* NIO-S6^T, with 98.2% sequence similarity. Based on the NJ tree, strain AK56^T formed a distinct branch within the genus *Oceanospirillum* and clustered with *O. linum* and these two organisms clustered with *O. nioense* and *O. maris* (Fig. 1). Based on the ML and MP trees, strain AK56^T formed a distinct branch within the genus *Oceanospirillum* and clustered with *O. nioense* and these two organisms clustered with *O. linum* and *O. maris* (Supplementary Fig. S2 and S3). Results of DNA–DNA hybridization between the strain AK56^T and the type strains of two closely related phylogenetic neighbours, *O. linum* LMG 5214^T and *O. nioense* NIO-S6^T, revealed average relatedness of 39.91% (± 7.63) and 23.62% (± 1.51) respectively.

Based on the results of phylogenetic analysis, *Oceanospirillum linum* LMG 5214^T and *Oceanospirillum nioense* NIO-S6^T were selected for further comparative studies with the strain AK56^T and a number of differences were observed (Table 1). Strain AK56^T and strains *O. linum* LMG 5214^T and *O. nioense* NIO-S6^T, exhibited similar characteristics with respect to Gram-stain, cell shape and motility. However, the three strains differed in cell size, salt tolerance and optima for growth, temperature and pH growth range and optima, few biochemical characteristics, hydrolysis of few complex substrates, utilization or fermentation of few carbon substrates, few enzymatic activities, antibiotic susceptibility to few antibiotics, fatty acid and polar lipid composition (Tables 1 and 2). Based on the observed phenotypic, chemotaxonomic characteristics and phylogenetic analysis, strain AK56^T is described in this study as a novel species in the genus *Oceanospirillum*, for which the name *O. sanctuarii* sp. nov. is proposed.

Description of *Oceanospirillum sanctuarii* sp. nov.

Oceanospirillum sanctuarii (sanc.tu.a'ri.i. L. neut. gen. n. *sanctuarii* of a sanctuary, pertaining to Coringa Wildlife Sanctuary, the location from where the type strain was isolated)

Cells of strain AK56^T were Gram-stain-negative, spiral-shaped, 2.0–3.0 μm in long and 0.7–1.0 μm in wide, non-sporulating and motile. Colonies on Marine agar were observed as circular, 1–2 mm in

diameter, pale-yellow, shiny, translucent, convex with entire margin after 48 h at 30 °C. Growth at 10-37 °C with an optimum temperature of 30 °C and tolerates up to 6% (w/v) NaCl with optimum growth of 2% (w/v) NaCl. NaCl is required for growth. Growth at pH 6-10, with optimum growth at pH 7. Cells grow aerobically, with a respiratory, chemoorganotrophic mode of metabolism. Lysine decarboxylase and ornithine decarboxylase are negative. Nitrate is reduced; H₂S and indole are not produced. The methyl red and Voges-Proskauer reactions are negative. Gelatin and Tweens 20, Tween 40, Tween 60, Tween 80 are hydrolyzed but aesculin, casein and starch are not hydrolyzed. Strain AK56^T is positive for D-maltose, L-proline arylamidase, urease and coumarate but negative for Ala-Phe-Pro arylamidase, glutamyl arylamidase, lipase, tyrosine arylamidase, phosphatase, glycine arylamidase and glu-gly-arg-arylamidase, L-pyrrolidonyl arylamidase, β -galactosidase, β -N-acetylglucosaminidase, γ -glutamyl transferase, β -xylosidase, β -glucosidase, α -galactosidase, β -alanine arylamidase, β -N-acetylgalactosaminidase and β -glucuronidase. Does not utilize L-arabitol, D-mannitol, D-mannose, palatinose, D-glucose, D-sorbitol, D-tagatose, 5-keto-D-gluconate, L-histidine, L-malate and L-lactate. Negative for succinate alkalization, fermentation of glucose, and Ellman reaction and 0/129 resistance. Does not produce acid from cellobiose, glucose, fructose, L-arabinose, mannose, salicin, sucrose, adonitol, arabitol, dulcitol, erythritol, galactose, glycerol, inositol, inulin, lactose, maltose, mannitol, melezitose, raffinose, rhamnose, sodium gluconate, sorbitol, xylitol, xylose, α -methyl-D-glucoside and α -methyl-D-mannoside. The predominant polar lipids are diphosphatidylglycerol, phosphatidyl ethanolamine and phosphatidylglycerol. The G+C content of strain AK56^T was 50.3 mol%.

The type strain AK56^T (= MTCC 12005^T = JCM 19193^T = KCTC 52973^T) was isolated from sediment sample (16° 48' N 82° 14' E) collected from Coringa Wildlife Sanctuary, Andhra Pradesh, India.

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Author statements

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Conflict of Interest: There is no conflict of interest.

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Legends

Fig. 1. Neighbor joining tree showing phylogenetic relationships of strain AK56^T with representative members of the family *Oceanospirillaceae* based on 16S rRNA sequence dataset. Bootstrap support values more than 50% are shown at the nodes. *Alcanivorax balearicus* MACL04^T (AY686709) was used as out group taxon. Scale bar represents 0.01 substitutions per nucleotide position.

Supplementary Fig. S1. Two-dimensional thin-layer chromatogram of the total polar lipids of the strain AK56^T (a) *Oceanospirillum linum* LMG 5214^T (b) and *Oceanospirillum nioense* NIO-S6^T (c) after spraying the plates with molybdato-phosphoric acid. Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; AL, unidentified aminolipid; APL, unidentified aminophospholipid; PL, unidentified phospholipid; L, unidentified lipid. The the spots identified as phospho-, amino- or glyco-lipids by spraying molybdenum blue, ninhydrin and α -naphthol reagents, respectively.

Supplementary Fig. S2. Maximum likelihood tree showing phylogenetic relationships of strain AK56^T with representative members of the family *Oceanospirillaceae* based on 16S rRNA sequence dataset. Bootstrap support values more than 50% are shown at the nodes. *Alcanivorax balearicus* MACL04^T (AY686709) was used as out group taxon. Scale bar represents 0.02 substitutions per nucleotide position.

Supplementary Fig. S3. Maximum parsimony tree showing phylogenetic relationships of strain AK56^T with representative members of the family *Oceanospirillaceae* based on 16S rRNA sequence dataset. Bootstrap support values more than 50% are shown at the nodes. *Alcanivorax balearicus* MACL04^T (AY686709) was used as out group taxon.

Table 1. List of distinguishing features of strain AK56^T with the closely related type strains

Data were from present study. All strains were gram-stain-negative, spiral shaped, motile. All strains were negative for indole and H₂S production; hydrolysis of aesculin, casein and starch; utilization of lactose, xylose, fructose, galactose, raffinose, meliobiose, L-arabionse, sodium gluconate, salicin, dulcitol, inositol, sorbitol, D-sorbitol, palatinose, adonitol, arabitol, erythritol, α -methyl-D-glucoside, rhamnose, cellobiose, L-arabitol, D-cellobiose, D-glucose, 5-keto-D-gluconate, D-tagatose, melezitose, α -methyl D-mannoside, xylitol, ONPG, L-lactate, L-histidine, L-malate, D-arabinose and sorbose; Ala-Phe-Pro arylamidase, L-pyrrolidonyl arylamidase, β -N-acetyl glucosaminidase, glutamyl arylamidase, γ -glutamyl transferase, β -glucosidase, β -xylosidase, β -alanine arylamidase, lipase, tyrosine arylamidase, α -glucosidase, β -N-acetylgalactosaminidase, α -galactosidase, phosphatase, glycine arylamidase, β -glucoronidase and glu-gly-arg-arylamidase activities; L-lactate, succinate alkalisation; 0/129 resistance and ELLMAN reaction. All strains were susceptible to antibiotics (μ g/disc) cefalexin (30), amoxicillin (30), tetracycline (30), ampicillin (25), novobiocin (30) and resistant to methicillin (5). +, positive; -, negative; w, weak growth; S, sensitive; R, resistance; I, intermediate; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; AL, unidentified aminolipid, APL, unidentified amino phospholipid, PL, unidentified phospholipid, L, unidentified lipid.

Characteristic	<i>Oceanospirillum sanctuarii</i> AK56 ^T	<i>Oceanospirillum linum</i> LMG 5214 ^T	<i>Oceanospirillum nioense</i> NIO-S6 ^T
Cell size (μ m)	2.0-3.0 x 0.7-1.0	1.5-2.0 x 0.3-0.4	
Temperature growth range ($^{\circ}$ C)	10-37	10-40	20-40
Optimum temperature ($^{\circ}$ C)	30	32	30-37
pH growth range (Optimum)	6-10 (7)	6-9	6-10 (7-8)
Salinity growth range (%) (Optimum)	2-6 (2)	0.5-8 (2-3)	0-6 (2)
Biochemical characteristics			
Oxidase	+	-	+
Catalase	+	+	-
Arginine dihydrolase	+	-	-
Methyl red reaction	-	+	-
Voges Proskauer's reaction	-	+	-
Hydrolysis of			
Gelatin	+	+	-
Tween 20, 40, 60,80	+/+/+/+	+/+/-/-	-/-/-/-
Sugar Fermentation			
Maltose	-	+	-
Glucose	-	-	+
Trehalose	-	+	w
Sucrose	-	+	-
Mannose	-	+	-
Inulin	-	+	-
Glycerol	-	+	-
Mannitol	-	+	-
Vitek GN information			
β -galactosidase	-	+	-
Fermentation glucose	-	w	-

Characteristic	<i>Oceanospirillum sanctuarii</i> AK56^T	<i>Oceanospirillum linum</i> LMG 5214^T	<i>Oceanospirillum nioense</i> NIO-S6^T
D-Mannitol	-	+	-
D-Maltose	+	+	-
D-Mannose	-	+	-
L-Proline arylamidase	+	-	+
Urease	+	-	+
D-Trehalose	-	+	-
Citrate (Sodium)	-	+	-
Malonate	-	+	-
Ornithine decarboxylase	-	+	-
Lysine decarboxylase	-	+	+
Courmarate	+	-	-
Antibiotic susceptibility (µg/disc)			
Bacitracin (8)	I	S	S
Penicillin-G (2 units)	I	R	R
Neomycin (30)	S	S	R
Streptomycin (10)	S	S	R
Vancomycin (30)	S	S	R
Polar lipids	DPG, PE, PG, AL1-2, PL1, L1-5	DPG, PE, PG, APL1, PL2-4	DPG, PE, PG, AL3, L2-5
DNA G+C content (mol%)*	50.3	48-50	49.5

*Data for type strains was taken from Port & Gillis [31], Krishna *et al.* [32].

Table 2. Comparisons of fatty acid composition of strain AK56^T with the closely related type strains
Data were from present study. 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. ND, not detected. Values of less than 0.5% for all strains are not shown.

Fatty acid	<i>Oceanospirillum sanctuarii</i> AK56 ^T	<i>Oceanospirillum linum</i> LMG 5214 ^T	<i>Oceanospirillum nioense</i> NIO-S6 ^T
C _{10:0}	0.65	0.47	0.44
C _{10:0} 3OH	8.90	5.78	7.85
iso-C _{10:0}	ND	0.10	ND
C _{11:0}	ND	0.13	ND
C _{11:0} 2OH	0.12	ND	ND
C _{11:0} 3OH	0.06	0.14	ND
C _{12:0}	0.86	4.59	0.80
iso-C _{12:0}	0.07	ND	ND
C _{12:0} 3OH	0.06	ND	ND
C _{12:1} 3OH	0.12	ND	ND
C _{13:0}	ND	0.14	ND
anteiso-C _{13:0}	0.07	ND	ND
C _{14:0}	1.85	0.44	1.99
C _{14:0} 2OH	0.04	ND	ND
anteiso-C _{14:0}	0.09	ND	ND
C _{14:1} ω 5c	0.21	ND	ND
C _{15:1} ω 6c	ND	0.23	ND
iso-C _{15:1} F	0.05	ND	ND
anteiso-C _{15:0}	0.11	ND	ND
C _{16:0}	20.98	17.96	24.01
C _{16:0} 2OH	ND	ND	0.22
C _{16:0} 3OH	ND	ND	0.40
iso-C _{16:0}	0.08	ND	ND
iso-C _{16:0} 3OH	0.03	ND	ND
anteiso-C _{16:0}	0.06	ND	ND
C _{16:1} ω 5c	0.27	ND	0.14
C _{17:0}	0.16	1.21	ND
iso-C _{17:0} 3OH	0.16	ND	ND
C _{17:1} ω 5c	0.04	ND	ND
C _{17:1} ω 6c	0.08	0.51	ND
C _{17:1} ω 8c	0.06	0.37	ND
anteiso-C _{17:0}	0.11	0.44	ND
iso-C _{17:1} ω 5c	ND	0.10	ND
C _{18:0}	0.47	1.52	0.50
iso-C _{18:1} H	ND	0.36	ND

C _{18:1} 2OH	0.13	ND	0.68
C _{18:1} ω 5 <i>c</i>	0.20	0.25	ND
C _{18:1} ω 7 <i>c</i>	21.29	25.78	26.03
C _{18:1} ω 7 <i>c</i> 11-methyl	0.32	0.30	0.28
C _{18:3} ω 6,9,12 <i>c</i>	0.10	0.97	ND
C _{19:0} 10-methyl	ND	2.20	0.50
iso-C _{20:0}	0.05	ND	ND
C _{20:1} ω 7 <i>c</i>	0.35	ND	ND
Summed in feature 3*	41.66	33.48	36.01
Summed in feature 5*	ND	0.37	ND
Summed in feature 7*	0.02	2.09	0.10

*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 C_{16:1} ω 6*c*; Summed feature 5 comprised C_{18:2} ω 6,9*c* and/or anteiso-C_{18:0}; Summed feature 7 comprised C_{19:1} ω 7*c* and/or C_{19:1} ω 6*c* and/or C_{19:0} cyclo ω 10*c*.

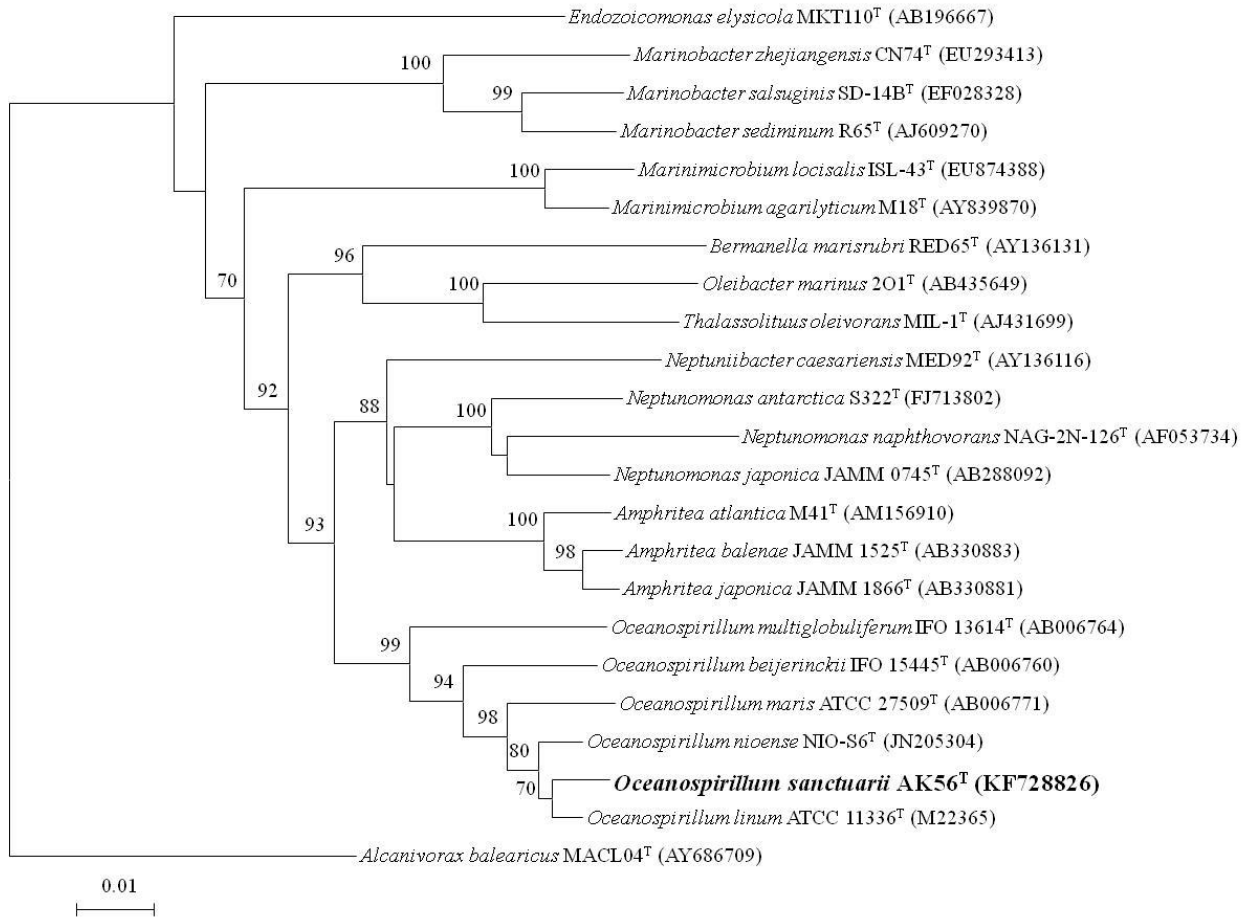
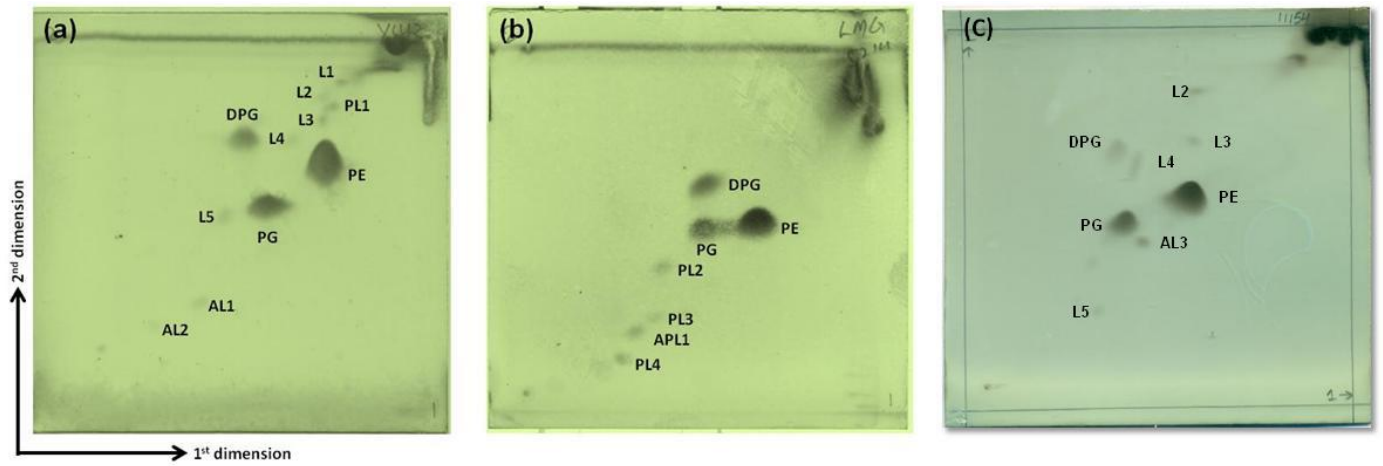
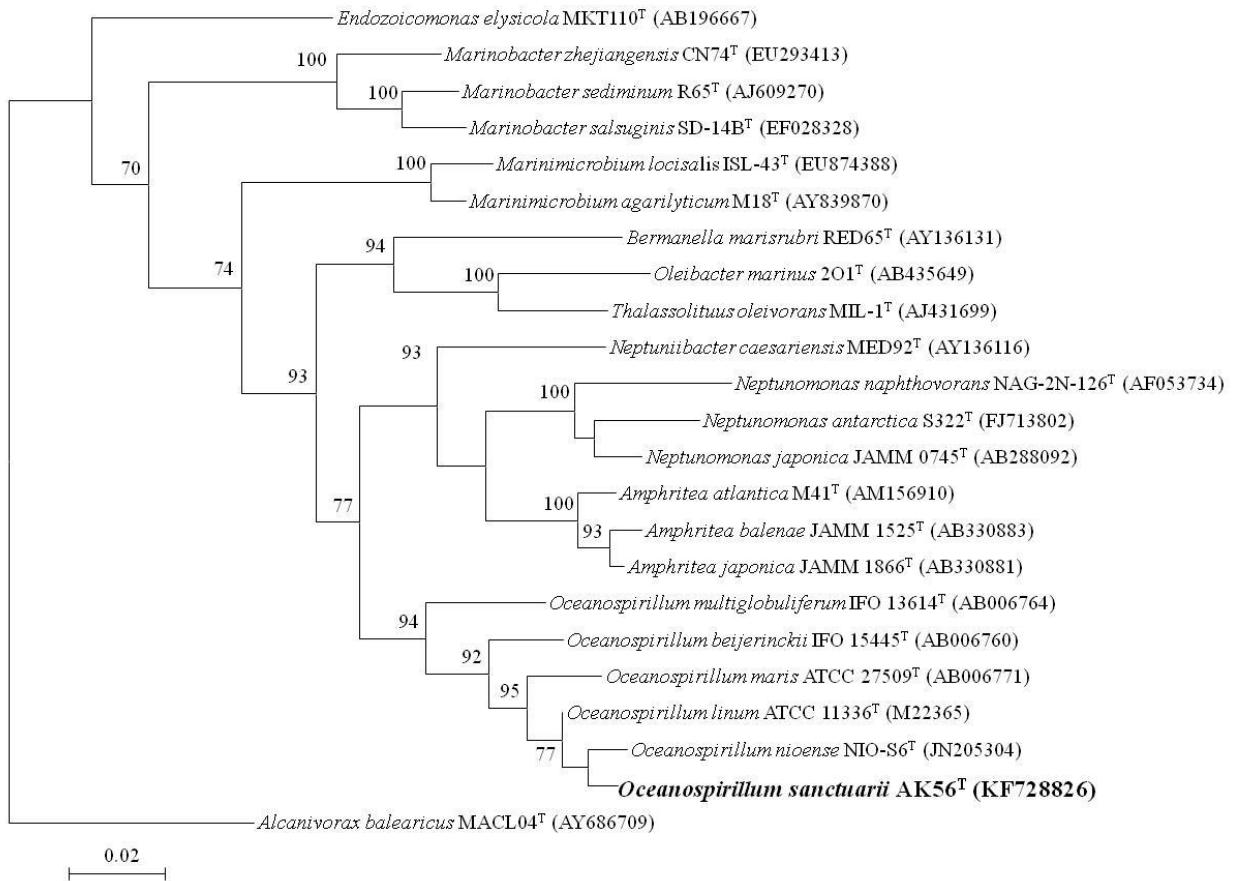


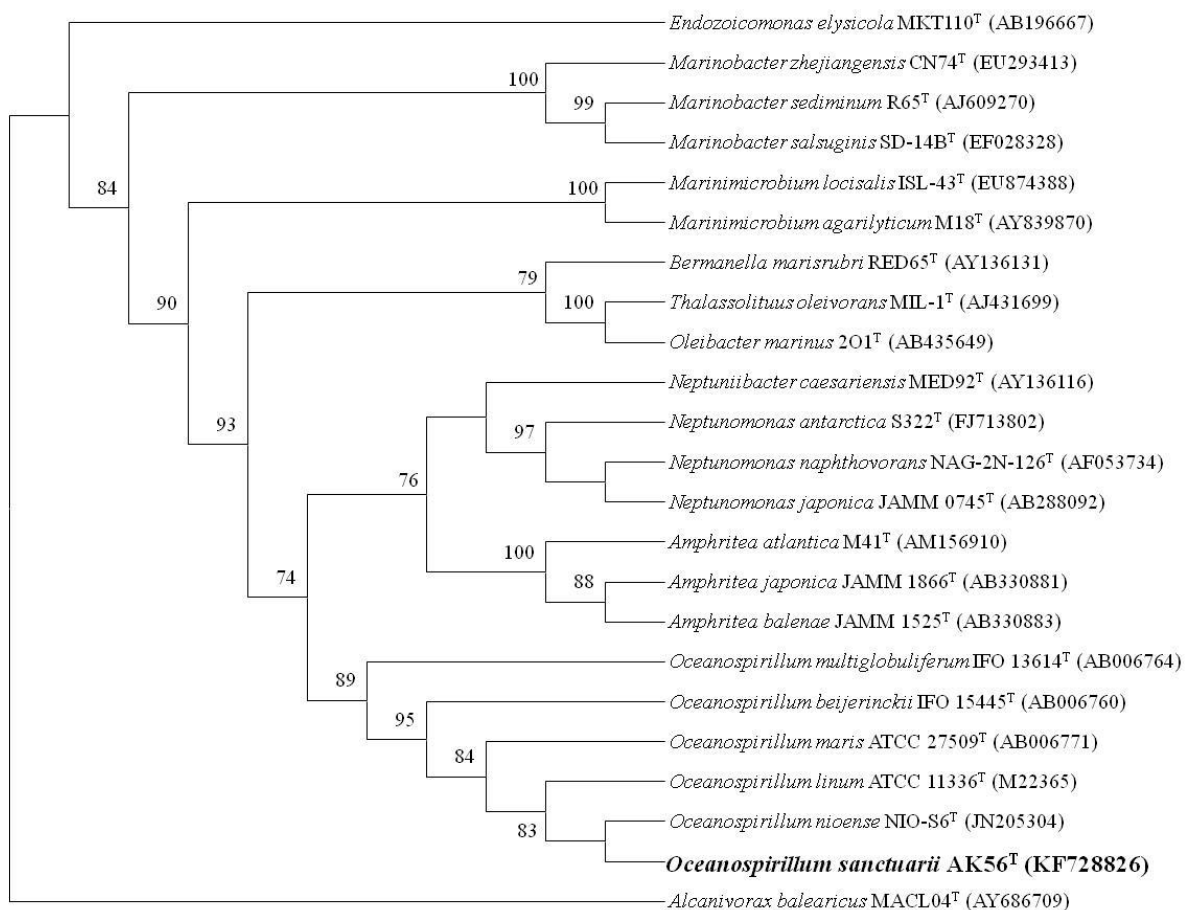
Fig. 1



Supplementary Fig. S1



Supplementary Fig. S2



Supplementary Fig. S3