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***Oceanospirillum nioense* sp. nov., a marine bacterium isolated from sediment sample of Palk bay, India**

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Running title

Oceanospirillum nioense sp. nov.

Subject category

New taxa (*Gammaproteobacteria*)

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All authors contributed equally to the work.

Abstract A novel Gram-negative, spiral shaped, motile bacterium, designated strain NIO-S6^T, was isolated from a sediment sample collected from Off-shore Rameswaram, Tamilnadu, India. Strain NIO-S6^T was positive for oxidase, DNase and lysine decarboxylase activities and negative for catalase, gelatinase, lipase, ornithine decarboxylase, nitrate reductase, aesculinase, amylase and urease activities. The fatty acids were dominated by C_{10:0} 3OH, C_{16:0}, C_{16:1} and C_{18:1}. Strain NIO-S6^T contained Q-8 as the major respiratory quinone. The DNA G + C content of the strain NIO-S6^T was 49.5 ± 0.6 mol%. Phylogenetic analysis based on 16S rRNA gene sequence of strain NIO-S6^T indicated *Oceanospirillum linum* and *Oceanospirillum maris* of the family *Oceanospirillaceae* (phylum *Proteobacteria*) are the closest related species with sequence similarities of 98.4 and 97.8% respectively. Other members of the family showed sequence similarities <96.4%. However, DNA-DNA hybridization with *Oceanospirillum linum* LMG 5214^T and *Oceanospirillum maris* LMG 5213^T showed a relatedness of 31.5 and 46.9% with respect to strain NIO-S6^T. Based on the phenotypic characteristics and on phylogenetic inference, strain NIO-S6^T is proposed as a novel species of the genus *Oceanospirillum* as *Oceanospirillum nioense* sp. nov. and the type strain is NIO-S6^T (= MTCC 11154^T = KCTC 32008^T).

Key words: *Oceanospirillum nioense*; 16S rRNA gene sequence based phylogeny; chemotaxonomy.

Introduction

The genus *Oceanospirillum* was proposed by Hylemon et al. (1973), with the type species *Oceanospirillum linum*. Phylogenetically, the genus belongs to the family *Oceanospirillaceae* within the class ‘*Gammaproteobacteria*’. The members of the genus *Oceanospirillum* are Gram-negative, rigid, helical cells with clockwise helix, motile, intracellular poly- β -hydroxybutyrate (PHB), form thin-walled coccoid bodies, which predominate in old cultures, chemoorganotrophic, having a strictly respiratory type of metabolism, optimum temperature for growth between 25 °C and 32 °C, positive for oxidase activity, utilize amino acids or salts of organic acids, negative for nitrate respiration, nitrate reduction, indole production, fermentation of carbohydrates, casein, starch, hippurate and aesculin hydrolysis, require sea water for growth and the G + C content of the genomic DNA ranges from 45 to 50 mol% (as determined by the thermal denaturation method). *Oceanospirillum* species have been isolated from coastal sea water, from decaying seaweed and from putrid infusions of marine mussels (Hylemon et al. 1973; Pot et al. 1989; Satomi et al. 2002). The type species is *Oceanospirillum linum* (Williams and Rittenberg 1957). At the time of writing, the genus *Oceanospirillum* accommodates *Oceanospirillum*

linum, *Oceanospirillum beijerinckii*, *Oceanospirillum maris* (Hylemon et al. 1973) and *Oceanospirillum multiglobuliferum* (Terasaki 1979) (Euzéby 2012, <http://www.bacterio.cict.fr/o/oceanospirillum.html>). In the present study, the focus was on the characterization and classification of strain NIO-S6^T by using a polyphasic approach (Vandamme et al. 1996). From the results of phylogenetic and phenotypic analyses, the strain is proposed as a representative of a novel species of the genus *Oceanospirillum*.

Materials and methods

Isolation and growth

Strain NIO-S6^T was isolated from a marine sediment sample collected from off-shore (GPS Positioning 21°45'39.35" N 88°13'48.05" E), Rameswaram, Tamilnadu, India. The sample that yielded strain NIO-S6^T had a pH and temperature of 8.0 and 32 respectively. For isolation of bacteria, 1 g of the sediment sample was serially diluted in 1 % sterile saline water and from each dilution 100 µl was plated on ZoBell Marine agar (MA) and Nutrient agar (NA)(HIMEDIA) plates, and incubated at 30 °C for 15 days. Out of different morphotypes, one cream-colored colony was selected and characterized. Subcultivation of the isolate was carried out on MA plates at 30 °C. Stock culture of the isolate in marine broth with 20% glycerol was preserved at -80 °C.

Type strains used for comparative study

Oceanospirillum maris LMG 5213^T and *Oceanospirillum linum* LMG 5214^T were obtained from the LMG bacteria collection, Belgium; strain NIO-S6^T was characterized simultaneously with *O. maris* LMG 5213^T and *O. linum* LMG 5214^T.

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 (Nikon) at X 1000. Gram reaction was determined by using the HIMEDIA Gram Staining Kit according to manufacturer's protocol. Endospore formation was determined after malachite-green staining of isolate grown on R2A (HIMEDIA) plates 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 under phase contrast microscope.

Physiological and biochemical characteristics

Growth was tested on MA, NA and Tryptone Soya Agar (TSA; HIMEDIA). Growth at 4, 10, 18, 25, 30, 35, 37, 40, 45, 47, 50, 55 and 60 °C was tested using nutrient broth medium and salt tolerance [0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18 and 20% (w/v) NaCl] were using nutrient broth without NaCl. Growth of strain NIO-S6^T at pH 5, 6, 7, 7.5, 8, 8.5, 9, 10, 11 and 12 was assessed on Tryptone Soya medium 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, nitrate reduction, hydrolysis of aesculin, gelatin, ortho-nitrophenyl- β -D-galactopyranoside (ONPG), starch, Tween 20, 40, 80, carbon source assimilation, H₂S production and the sensitivity to 22 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 using 2% (w/v) NaCl in medium and also for the preparation of the inoculum.

Chemotaxonomic analysis

Standardization of the physiological age of strain NIO-S6^T and the type strains 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 NIO-S6^T, *Oceanospirillum maris* LMG 5213^T and *Oceanospirillum linum* LMG 5214^T were grown on MA plates at 37 °C for 3, 2 and 2 days respectively 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 Sherlock Microbial Identification System (MIDI, USA). Cellular FAMES were separated by Gas Chromatograph (6890), identified and quantified with Sherlock Microbial Identification System Software (version.6.0, using the aerobe TSBA6 method and TSBA6 database). Freeze-dried cells were obtained from cultures grown on MA at 37 °C for 3 days under aerobic condition for the quinone

analysis. Isoprenoid quinones were extracted as described by Collins et al. (1977) and analyzed by high performance liquid chromatography (Groth et al. 1997).

Determination of DNA G + C content

The DNA of strain NIO-S6^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. DNA-DNA hybridization was performed by the membrane filter method (Tourova and Antonov 1987) as described previously (Shivaji et al. 1992; Reddy et al. 2000).

16S rRNA gene sequence determination 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 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') *Escherichia coli* 16S rRNA gene sequence numbering system (Brosius et al. 1978). 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 product was separated and 1.5 kb fragment detected and later eluted by 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. In EzBioCloud (Kim et al. 2012) a search is performed against a database of 16S rRNA gene sequences of type strains only using the algorithm of Myers and Miller (1988). The 16S rRNA gene sequences of closely related validly published taxa belonging to the family *Oceanospirillaceae* were

downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL_X program (Thompson et al. 1997) and the alignment was then corrected manually by deleting gaps and missing data. There were a total of 1341 positions in the final dataset. Phylogenetic tree was reconstructed using the maximum likelihood method using the PhyML program (Guindon et al. 2005) and Neighbor-Joining method (Saitou and Nei 1987) using the MEGA5 package (Tamura et al. 2011) 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.

Genomic fingerprinting analysis

REP, ERIC and BOX-PCR were performed according to the protocol described by Versalovic et al. (1991). RAPD-PCR was carried out with two random primers [5'-CTTGAGTGGA-3' and 5'-GAGATGACA-3'] of 10 pico mole concentration. Initial denaturation at 95 °C for 15 sec, and subsequent denaturation at 95 °C for 5 sec. Primer annealing was performed at 35-40 °C for 10 sec, and primer extension was performed at 72 °C for 30 sec. The final extension step was 5 min at 72 °C. Total 35 cycles were performed.

Results and discussion

Cells of strain NIO-S6^T were Gram-negative, motile spirals. Colonies were circular, 1-2 mm in diameter, smooth, translucent, creamish and raised with entire margins after 2 days growth on MA plates at 30 °C. The strain was positive for oxidase and lysine decarboxylase activities and negative for catalase and ornithine decarboxylase activities. Strain NIO-S6^T does not reduce nitrate and is negative for indole production. It hydrolyzed DNA, but did not hydrolyse aesculin, gelatin, starch, Tween 20, Tween 40, Tween 80 and urea. Growth was observed at 20-40 °C with optimum growth at 30-37 °C, at 0-6 % (w/v) NaCl, with optimum growth at 2 % (w/v) and at pH 6-10, with optimum growth at pH 7-8. Other phenotypic differentiating characteristics between the strain NIO-S6^T and related type strains are presented in Table 1 and Supplementary Table 2.

The cellular fatty acid composition of strain NIO-S6^T showed a spectrum of 12 fatty acids with a pronounced dominance of (>5 %) of C_{10:0} 3OH, C_{16:0}, C_{16:1} and C_{18:1} (Table 1 and Supplementary Table

1). Type strains also contain same dominant fatty acids but the composition varies (Supplementary Table 1). Compared with type strains the fatty acid composition detected in strain NIO-S6^T differed considerably (Supplementary Table 1). Other fatty acids (C_{11:0} 3OH, C_{12:0}, C_{16:0} 2OH, C_{16:0} 3OH, C_{17:0}, C_{18:1} 2OH, C_{18:0} 3OH and C_{20:2}) which are either dominant/absent in one or more strains (Supplementary Table 1). The respiratory quinone present in strain NIO-S6^T was ubiquinone 8 (Q-8) which is similar in other *Oceanospirillum* species (Pot and Gillis, 2005). Strain NIO-S6^T had a DNA G + C content of 49.5 ± 0.6 mol%, which is within the range of the genus *Oceanospirillum* (45-50 mol%) (Table 1).

The phylogenetic relationships of strain NIO-S6^T was ascertained based on the 16S rRNA gene sequence similarity with other strains using BLAST sequence similarity search (NCBI-BLAST). The 16S rRNA gene sequence analysis placed strain NIO-S6^T within the family *Oceanospirillaceae*. The results indicated that at the 16S rRNA gene sequence level, strain NIO-S6^T was close to the phylogenetic neighbors *Oceanospirillum linum* LMG 5214^T and *Oceanospirillum maris* LMG 5213^T with pair-wise sequence similarities of 98.4 and 97.8 % respectively. Other members of the family showed sequence similarities <96.4 %. Phylogenetic analyses based on maximum-likelihood and neighbour-joining trees further indicated that strain NIO-S6^T clustered with *Oceanospirillum linum* LMG 5214^T and together clustered with other species of the genus *Oceanospirillum* and distantly related to other genera *Neptuniibacter*, *Amphritea*, *Neptunomonas*, *Thalassolituus*, *Oleibacter*, *Bermanella*, *Marinimicrobium*, *Endozoicomonas* and *Marinobacter* (Fig. 1 and Supplementary Fig. S1). DNA-DNA hybridization with *Oceanospirillum linum* LMG 5214^T and *Oceanospirillum maris* LMG 5213^T showed a reassociation of 31.5±1.0 and 46.9±0.8 % respectively with respect to strain NIO-S6^T. This value was lower 70% confirming that the novel strain is not a member of the same species (Wayne et al. 1987). And a number of differences were observed between the strain NIO-S6^T and with type strains *Oceanospirillum linum* LMG 5214^T and *Oceanospirillum maris* LMG 5213^T in all three Rep-PCR genomic fingerprint patterns (REP-, ERIC-, BOX-PCR) (Supplementary Fig. S2). Very few bands were obtained in case of REP- and BOX-PCR for strain *Oceanospirillum linum* LMG 5214^T. In case of RAPD-PCR, amplicons were not obtained for any of the strains tested (Data not shown).

Strain NIO-S6^T was close to *Oceanospirillum linum* and *Oceanospirillum maris* based on phylogenetic analysis and physiological and biochemical characteristics and some major fatty acids. But strain NIO-S6^T also exhibits a number of differences with *O. maris* LMG 5213^T and *O. linum* LMG 5214^T at phenotypic and genotypic level (DNA-DNA hybridization and genomic fingerprinting analysis) (Table 1, Supplementary Tables 1 and 2, Supplementary Fig. S2). Thus, the cumulative

differences that strain NIO-S6^T exhibits from the closely related species of the genus *Oceanospirillum* unambiguously support the proposal of a new species to accommodate the strain NIO-S6^T, for which the name *Oceanospirillum nioense* sp. nov. is proposed.

Description of *Oceanospirillum nioense* sp. nov.

Oceanospirillum nioense (ni.o.en'se. N.L. gen. n. *nioense*, arbitrary name formed from the acronym of the National Institute of Oceanography, NIO, where taxonomic studies on this taxon were performed).

Cells are Gram-negative, motile, aerobic, spiral shaped. Colonies on MA plates were circular, 1-2 mm in diameter, smooth, translucent, creamish and raised with entire margins. Grows at 20 to 40 °C with an optimum growth at 30-37 °C and tolerates up to 6 % NaCl (w/v) with optimum growth at 2 % NaCl (w/v). Grows at pH 6-10, with optimum growth at pH 7-8. Oxidase, β -galactosidase and lysine decarboxylase activities are positive. Catalase, ornithine decarboxylase and phenylalanine deaminase activities are negative. Nitrate is not reduced and H₂S gas and indole are not produced. The methyl red and Voges-Proskauer's reactions are negative. DNA is hydrolyzed but, aesculin, agar, gelatin, starch, Tween 20, Tween 40, Tween 80 and urea are not hydrolysed. Does not produce acid from fructose, arabinose, cellobiose, glucose, xylose, rhamnose, mannose, salicin, mannitol, inulin, mannose, melibiose, sucrose, galactose, sorbitol, lactose, trehalose, raffinose, adonitol and meso-inositol. Utilize glucose and trehalose (weak) but not utilize following substrate used individually includes adonitol, arabinose, D-arabinose, L-arabinose, arabitol, cellobiose, citrate, dulcitol, erythritol, fructose, galactose, glycerol, inositol, inulin, lactose, melibiose, melizitose, melonate, maltose, mannitol, mannose, α -methyl-D-glucoside, α -methyl-D-mannoside, raffinose, rhamnose, salicin, sodium gluconate, sorbitol, sorbose, sucrose, xylitol and xylose. Susceptible to antibiotics (μ g per disc unless indicated) amoxycillin (10), ampicillin (10), azithromycin (15), bacitracin (8 units/disc), cephalixin (30), cephalothin (30), erythromycin (15), gentamicin (10), nalidixic acid (30), nitrofurantoin (300), norfloxacin (10), novobiocin (30), rifampicin (5), tetracycline (30) and trimthoprim (5) and resistant to kanamycin (30), methicillin (5), neomycin (30), penicillin-G (10 units/disc), streptomycin (10), tobramycin (10) and vancomycin (30). The major fatty acids are C_{10:0} 3OH, C_{16:0}, C_{16:1} and C_{18:1}. Q-8 is the predominant respiratory quinone. The DNA G + C content of the type strain is 49.5 \pm 0.6 mol%.

The type strain, NIO-S6^T (=MTCC 11154^T = KCTC 32008^T) was isolated from sediment sample collected from palk bay, India. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NIO-S6^T is JN205304.

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Legends to Figures

Fig. 1. Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strain NIO-S6^T and representatives of the family *Oceanospirillaceae*. Numbers at the nodes are bootstrap values >50%. *Alcanivorax balearicus* MACL04^T (AY686709) was used as an out group. Bar, 0.02 substitutions per nucleotide position.

Supplementary Fig. 1. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strain NIO-S6^T and representatives of the family *Oceanospirillaceae*. Numbers at the nodes are bootstrap values >50%. *Alcanivorax balearicus* MACL04^T (AY686709) was used as an out group. Bar, 0.01 substitutions per nucleotide position.

Supplementary Fig. S2. Characteristic Rep-PCR genomic fingerprint patterns of strains NIO-S6^T, *Oceanospirillum maris* LMG 5213^T and *Oceanospirillum linum* LMG 5214^T. Lanes 1 and 11 corresponds to 100 bp DNA marker. Lane 2, 5 and 8 corresponds to fingerprint patterns of strain *O. maris* LMG 5213^T, lanes 3, 6 and 9 corresponds to fingerprint patterns of strain *O. linum* LMG 5214^T and lanes 4, 7 and 10 corresponds to fingerprint patterns of strain NIO-S6^T. Lanes 2, 3 and 4 corresponds to REP (repetitive extragenic palindromic) -PCR genomic fingerprints; lanes 5, 6 and 7 corresponds to ERIC (enterobacterial repetitive intergenic consensus) -PCR genomic fingerprints and lanes 8, 9 and 10 corresponds to ERIC (enterobacterial repetitive intergenic consensus) -PCR genomic fingerprints.

Table 1. Features that distinguish strain NIO-S6^T from the closely related species of the genus *Oceanospirillum*

Characteristic	<i>Oceanospirillum nioense</i> NIO-S6 ^T	<i>Oceanospirillum maris</i> LMG 5213 ^T	<i>Oceanospirillum linum</i> LMG 5214 ^T
Salinity growth range (% w/v)	0-6	0-8	0.5-8
Optimum salinity (% w/v)	2	3	2-3
Temperature range for growth (°C)	20-40	4-37	10-40
Temperature optimum (°C)	30-37	25-30	30-37
Catalase	-	+	+
Voges Proskauer's reaction	-	+	+
Methyl red reaction	-	+	+
Hydrolysis of:			
Aesculin	-	-	W
Starch	-	-	+
Gelatin	-	-	+
Urea	-	+	+
Major fatty acids (> 5%)	C _{10:0} 3OH; C _{16:0} ; C _{16:1} ; C _{18:1}	C _{10:0} 3OH; C _{12:0} ; C _{16:0} ; C _{16:1} ; C _{18:1}	C _{10:0} 3OH; C _{16:0} ; C _{16:1} ; C _{18:1}
Major respiratory Quinone	Q8	Q8, Q6	Q8
DNA G + C content (mol%)	49.5	*46	*48-50

Data from the present study. All strains were Gram negative, motile, spiral-shaped, positive for oxidase activity and negative for indole production. +, positive; -, negative; w, weak.

*DNA G+C content of type strains was taken from Pot and Gillis 2005.

Supplementary Table 1. Comparison of the fatty acid composition of strain NIO-S6^T and the closely related species of the genus *Oceanospirillum*

Fatty acid	<i>Oceanospirillum</i> <i>nioense</i> NIO-S6 ^T	<i>Oceanospirillum</i> <i>maris</i> LMG 5213 ^T	<i>Oceanospirillum</i> <i>linum</i> LMG 5214 ^T
C _{10:0}	0.4	0.5	0.5
C _{10:0} 3OH	7.9	8.9	5.8
C _{11:0} 3OH	ND	0.2	0.1
C _{12:0}	0.8	5.9	4.6
C _{14:0}	2.0	0.5	0.4
C _{16:0}	24.0	23.8	18.0
C _{16:0} 2OH	0.2	ND	ND
C _{16:0} 3OH	0.4	ND	ND
C _{16:1}	36.0	36.6	33.5
C _{17:0}	ND	0.7	1.2
C _{18:0}	0.5	0.9	1.5
C _{18:0} 3OH	ND	2.8	ND
C _{18:1}	26.0	11.9	25.8
C _{18:1} 2OH	0.7	ND	ND
C _{19:0} 10-methyl	0.5	1.1	2.2
C _{20:2}	ND	2.0	ND

ND, not detected.

Supplementary Table 2. Phenotypic characteristics that distinguish the strain NIO-S6^T with the closely related species of the genus *Oceanospirillum*

All strains are negative for phenylalanine deamination, H₂S production and utilization of adonitol, arabinose, D-arabinose, L-arabinose, arabitol, cellobiose, dulcitol, erythritol, fructose, galactose, inositol, lactose, melibiose, melizitose, α -methyl-D-glucoside, α -methyl-D-mannoside, raffinose, rhamnose, salicin, sodium gluconate, sorbitol, sorbose, xylitol and xylose. All strains were susceptible to (μ g per disc) amoxycillin (10), ampicillin (10), azithromycin (15), bacitracin (8 units/disc), cephalixin (30), cephalothin (30), erythromycin (15), gentamicin (10), nitrofurantoin (300), novobiocin (30), rifampicin (5) and tetracycline (30) and resistant to kanamycin (30), methicillin (5) and penicillin-G (10 units/disc). +, positive; -, negative; w, weak; R, resistant; S, sensitive.

Characteristics	<i>Oceanospirillum</i> <i>nioense</i> NIO-S6 ^T	<i>Oceanospirillum</i> <i>maris</i> LMG 5213 ^T	<i>Oceanospirillum</i> <i>linum</i> LMG 5214 ^T
β -galactosidase	+	-	+
Lysine decarboxylase	+	-	+
Ornithine decarboxylase	-	+	+
Utilization of :			
Citrate	-	+	+
Glucose	+	W	w
Glycerol	-	-	+
Inulin	-	-	+
Malonate	-	-	+
Maltose	-	-	+
Mannitol	-	-	+
Mannose	-	-	+
Sucrose	-	W	+
Trehalose	w	W	+
Antibiotic Susceptibility (μg per disc):			
Nalidixic acid (30)	S	R	S
Neomycin (30)	R	S	S
Norfloxacin (10)	S	R	S
Streptomycin (10)	R	S	S
Tobramycin (10)	R	S	S
Trimthoprim (5)	S	R	S
Vancomycin (30)	R	S	S

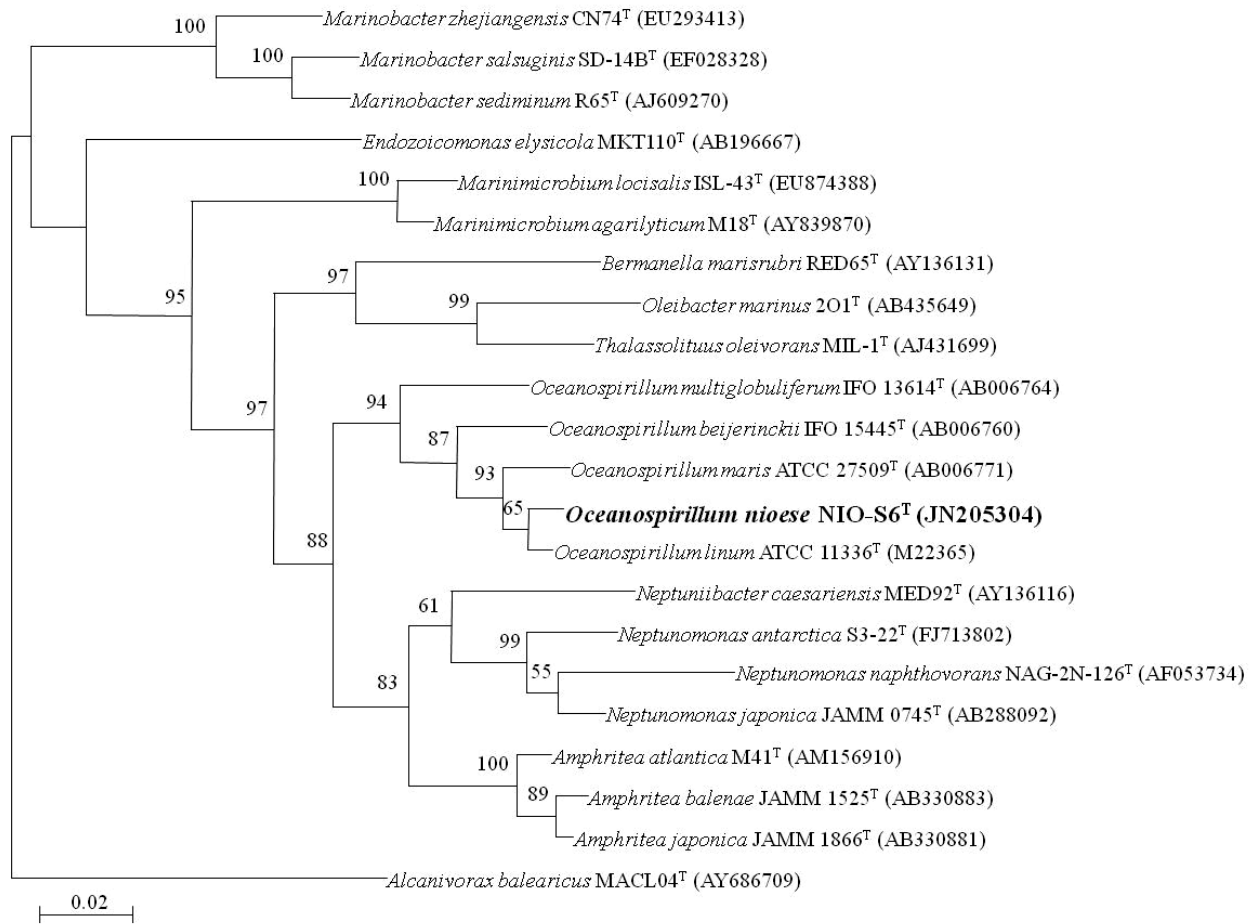
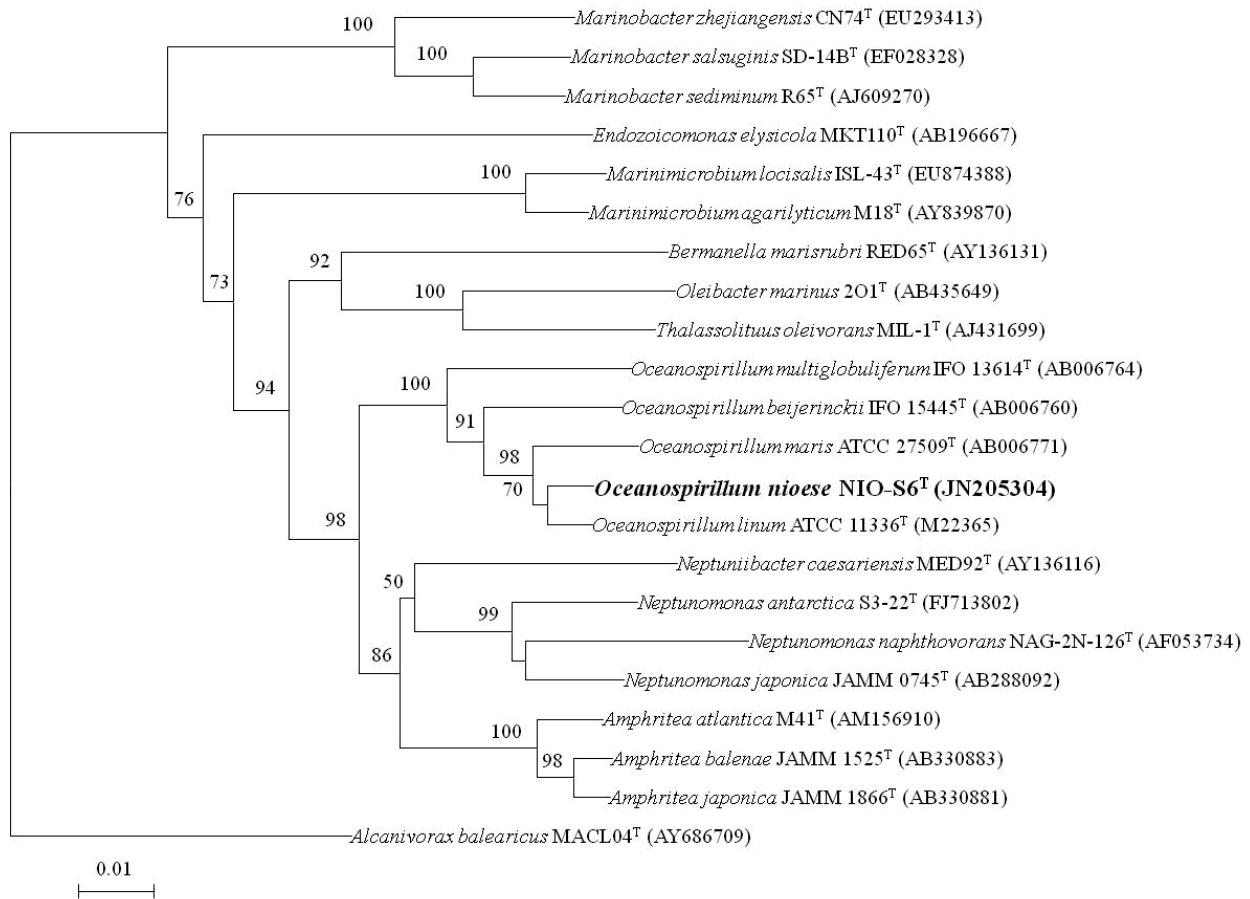
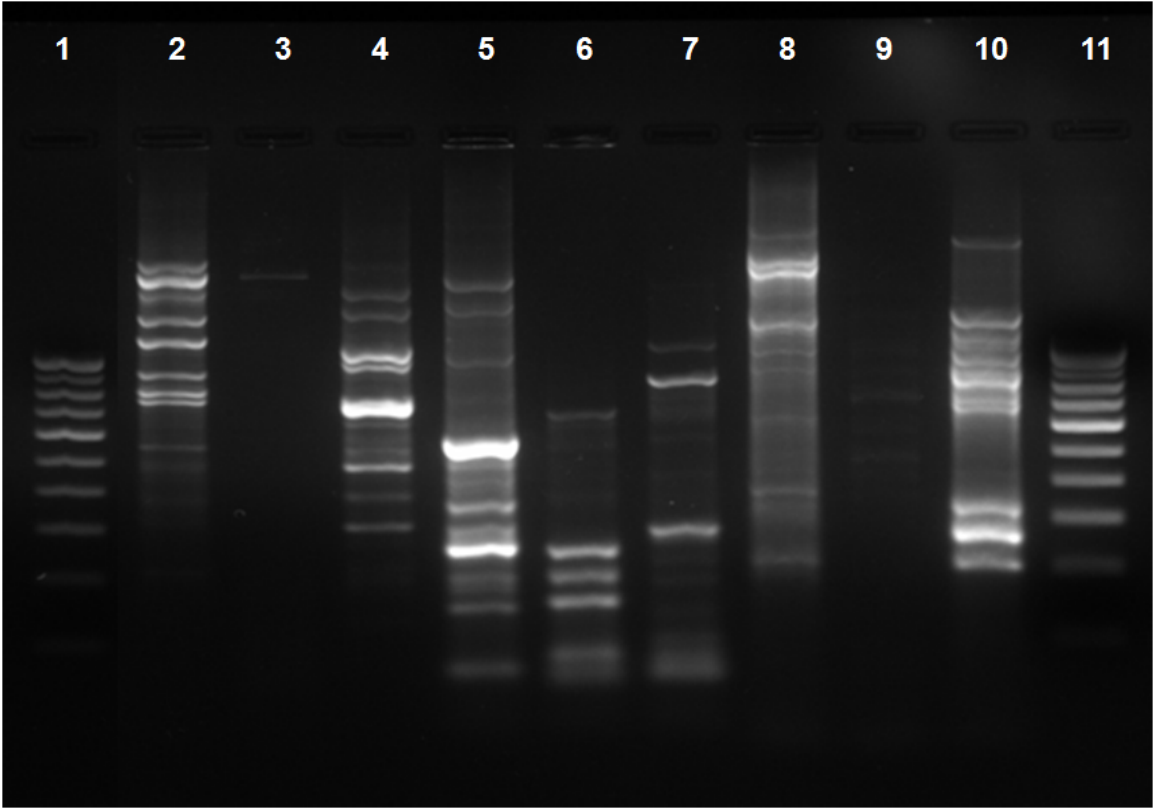


Fig. 1



Supplementary Fig. S1



Supplementary Fig. S2