

***Shewanella submarina* sp. nov., a gammaproteobacterium isolated from a marine
water, Visakhapatnam, India**

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Running title

Shewanella submarina sp. nov.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NIO-S14^T is KX645967.

Abstract

A curved rod shaped bacterium was isolated from a marine 100m depth water sample collected from Bay of Bengal, Visakhapatnam, India. Strain NIO-S14^T, was Gram-negative, motile, pale-yellow colored. Strain NIO-S14^T was able to grow aerobically and anaerobically and could utilize a number of organic substrates. Major fatty acids were C_{12:0}, iso-C_{13:0}, C_{14:0}, iso-C_{15:0}, C_{16:0} and C_{16:1 ω7c} and/or iso-C_{15:0} 2OH (Summed feature 3). Strain NIO-S14^T contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, two unidentified aminophospholipids and six unidentified lipids as polar lipids. The DNA G+C content of strain NIO-S14^T was 47.9 mol%. The 16S rRNA gene sequence comparisons indicated that the isolate represented a member of the family *Shewanellaceae* within the class *Gammaproteobacteria*. According to 16S rRNA gene sequence analysis, strain NIO-S14^T was closely related to *Shewanella corallii* with a pair-wise sequence similarity of 99.26%. Based on the sequence comparison, strain

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NIO-S14^T clustered with *Shewanella corallii* and together clustered with *Shewanella mangrovi* and 7 other *Shewanella* species but were distantly related. DNA–DNA hybridization between strains NIO-S14^T and *Shewanella corallii* DSM 21332^T showed a relatedness of 35%. Distinct morphological, physiological and genotypic differences from these previously described taxa supported the classification of strain NIO-S14^T as a representative of a novel species in the genus *Shewanella*, for which the name *Shewanella submarina* sp. nov. is proposed. The type strain of *Shewanella submarina* is NIO-S14^T (= MTCC 12524^T = KCTC 52277^T = LMG 30752^T).

Keywords: *Shewanella submarina*, 16S rRNA gene-based phylogeny, chemotaxonomy, Gammaproteobacteria

Introduction

The genus *Shewanella* (family *Shewanellaceae*, order *Alteromonadales*, class *Gammaproteobacteria*) was first described by MacDonell & Colwell [1] to accommodate Gram-negative, rod-shaped, facultatively anaerobic gammaproteobacteria. The different species of the genus *Shewanella* have been described from diverse ecological niches and substrates such as: deep-sea sediments, tidal flat, marine invertebrates, fish, shelf muds, marine water, Arctic marine sediment, black sand, lagoon, Red Sea coral, activated sludge of a waste-water treatment plant, coal mine, gut microflora of abalone, marine sponge *Ircinia dendroides*, Antarctic coastal areas, iron-rich microbial mats, mangrove sediment, sea-animal intestines, intestines of Pacific mackerel, marine sponge, bensasi goatfish *Upeneus bensasi* and sipuncula [2-23]. The members of the genus *Shewanella* are Gram-staining-negative, straight or curved rods, without endospores and microcysts, motile by single, unsheathed polar flagellum, oxidase and catalase positive, chemo-organoheterotrophic, facultatively anaerobic, fermentative, produce H₂S and may require Na⁺ ions for growth and DNA G+C content of 38-54 mol%. The type species is *Shewanella putrefaciens* [1]. The genus *Shewanella* presently comprises 63 species (<http://www.bacterio.net/shewanella.html>). In the present study, we focused on the characterization and classification of the strain NIO-S14^T, which was isolated from 100 m depth marine water sample by a polyphasic taxonomic approach [24] and on the basis of the results assign the novel strain to the genus *Shewanella*.

The strain NIO-S14^T was isolated from a marine water sample collected from 100m depth at off Visakhapatnam (GPS Positioning: 17°29'20.87" N 83°34'50.26" E), Andhra Pradesh, India. The temperature, salinity and pH of the water sample were 20.6 °C, 34.79 psu and 8.03 respectively. For isolation of the strain NIO-S14^T, the water sample was serially diluted (up to 10 fold dilutions) in 2% (w/v) NaCl solution and 100 µl of each dilution was spread-plated on ZoBell's Marine Agar (ZMA; HIMEDIA, India) and incubated at 25 °C. Among the different colony morphotypes appeared a pale-

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yellowish coloured colony was selected and further characterized and preserved at -80 °C in marine broth with 10% glycerol.

The type strain of *Shewanella corallii* DSM 21332^T was also cultivated under same conditions as strain NIO-S14^T. The colony morphology of strain NIO-S14^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.*, [25] 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, 45, 55 and 65 °C was ascertained using Marine Broth (MB; HIMEDIA, India) and salt tolerance [0, 0.5, 1, 1.5, 2, 3, 4, 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 NIO-S14^T at pH 4, 5, 6, 7, 8, 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 [26]. 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 [26, 27]. Utilization of different carbohydrate sources was determined by analyzing the acid production by the isolate using bromocresol purple as an acid–base indicator [28]. ONPG discs were used to determine the β -galactosidase activity (HIMEDIA). Biochemical and characterization of different enzyme activity of strain NIO-S14^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.* [29].

Standardization of the physiological age of strains NIO-S14^T and *Shewanella corallii* DSM 21332^T was done based on the protocol [30] given by Sherlock Microbial Identification System (MIDI, USA). For cellular fatty acids analysis, strains NIO-S14^T and *Shewanella corallii* DSM 21332^T were grown on MA plates at 30 °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 [31] and analyzed using two-dimensional TLC followed by spraying with suitable detection reagents [32].

Isolation of genomic DNA, polymerase chain reaction (PCR) amplification and sequencing of 16S rRNA gene were performed as described in Surendra *et al.* [33]. The G+C content of strain NIO-S14^T was determined from the melting point (T_m) curves obtained [34] 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 1359 bp long and subjected to EzBioCloud (<http://eztaxon-e.ezbiocloud.net/>) search [35] to find the nearest taxa. The 16S rRNA gene sequences of the species of the genus *Shewanella* were downloaded from NCBI (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL_W tool of MEGA version 6 [36]. Neighbour joining method was used to construct the phylogenetic tree using MEGA-6 [36]. There were total 64 sequences and 1184 positions in the final dataset. Bootstrap analysis of 10,000 replicates was performed to infer the robustness of the phylogenetic tree and the evolutionary distances were calculated using Kimura-2-parameter [37]. Phylogenetic trees were also reconstructed using the maximum-likelihood and maximum parsimony methods using MEGA-6 [36]. For DNA–DNA hybridization experiments, genomic DNA was isolated and purified according to the method of Marmur [38] with additional *RNase* treatment. The relative binding ratio experiment was done by fluorimetry method using a Step One Plus Real-Time PCR system (Applied Biosystems) fitted with 96 well thermal cycling block in 96 well plate as described by Loveland-Curtze *et al.* [39]. DNA suspended in the 2X SSC and 1:10000 SYBR Green was used for the analysis. DNA denaturation at 95.0 °C was carried out followed by the reassociation at optimum temperature 67.0 °C. Fluorescence readings were taken every 10 seconds throughout the protocol and relative binding ratio was calculated according to De Ley *et al.* [40] and Gillis *et al.* [41].

Cells of strain NIO-S14^T were Gram-negative, curved rod shaped, pale-yellow pigmented, non-sporulating and motile by means of polar flagella (Supplementary Fig. S1). Cells were 2.0-2.5 μ m in length and 0.6-0.7 μ m in width (Supplementary Fig. S1). 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 20-37 °C with optimum growth at 30 °C, at 0-6 % (w/v) NaCl, with

optimum growth at 2 % and at pH 6-9, with optimum growth at pH 7-8. In the Microlog, strain NIO-S14^T is positive for D-melibiose, growth at pH 6, growth with 4% NaCl (w/v), D-mannose, D-fructose, D-galactose, 3-methyl glucose, D-fucose, L-rhamnose, inosine, L-aspartic acid, niaproof 4, glucuronamide, vancomycin, tetrazolium blue, lithium chloride and aztreonam, weak positive for D-maltose, stachyose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, N-acetyl- β -D-mannosamine, growth with 1% NaCl (w/v), α -D-glucose, L-fucose, 1% sodium lactate, D-serine, D-mannitol, D-arabitol, D-glucose-6-PO₄, L-serine, lincomycin, mucic acid, quinic acid, D-saccharic acid, tetrazolium violet, methyl pyruvate, α -keto-glutaric acid and L-malic acid, but negative for dextrin, D-trehalose, sucrose, D-turanose, D-raffinose, α -D-lactose, β -methyl-D-glucoside, growth at pH 5, growth with 8% NaCl (w/v), D-sorbitol, myo-inositol, glycerol, D-fructose-6-PO₄, D-serine, troleandomycin, rifamycin SV, minocycline, gelatin, glycyl-L-proline, L-alanine, L-histidine, L-pyroglutamic acid, guanidine HCl, pectin, D-gluconic acid, p-hydroxy-phenylacetic acid, D-lactic acid methyl ester, L-lactic acid, citric acid, D-malic acid, bromo-succinic acid, nalidixic acid, potassium tellurite, tween 40, γ -amino-butyric acid, α -hydroxybutyric acid, β -hydroxy-D,L-butyric acid, α -keto-butyric acid, propionic acid, formic acid, sodium butyrate, sodium bromate. Susceptible to (μ g per disc unless indicated) ciprofloxacin (10) and chloramphenicol (30); moderately sensitive to gentamicin (10), streptomycin (25) and spectinomycin (100); resistant to cefmetazole (30), neomycin (30), kanamycin (30), ceftazidime (30), cephadroxil (30), polymyxin b (300), amoxycillin (30), cefazolin (30), cefalexin (30), novobiocin (30), sulphamethizole (300), cefprozil (30), chlortetracycline (30), bacitracin (8), ampicillin (25), vancomycin (30), lincomycin (2), tetracyclin (30) and penicillin-G (2 Units). Other characteristics are presented in Table 1.

The cellular fatty acid composition of strain AK61^T showed a spectrum of 17 fatty acids with a pronounced dominance of (>5 %) of C_{12:0}, iso-C_{13:0}, C_{14:0}, iso-C_{15:0}, C_{16:0}, C_{16:1} ω 7c/ C_{16:1} ω 6c (summed feature 3) (Supplementary Table S1). The fatty acids were dominated by saturated and branched fatty acids. The overall fatty acid composition of strain NIO-S14^T was similar to the *Shewanella corallii* DSM 21332^T except that in two major fatty acids (C_{17:1} ω 8c and C_{18:1} ω 7c) in type strain. The polar lipid composition included diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), two unidentified aminophospholipids (APL1, 2) and six unidentified lipids (L1-6) (Supplementary Fig. S2a). In *Shewanella corallii* DSM 21332^T nearly similar composition was observed but differed with strain NIO-S14^T in the absence of APL2 and L2 (Supplementary Fig. S2b). The G+C content of strain NIO-S14^T was calculated as 47.9 mol%.

For phylogenetic assessment of strain NIO-S14^T, 1491 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 NIO-S14^T was found to be related to *Shewanella* genus. Based on pair-wise sequence similarity, strain NIO-S14^T was closest to *Shewanella corallii* with 99.26% sequence similarity and rest of species of the genus *Shewanella* were between 93.16 to 96.02% sequence similarities. Based on the NJ tree, strain NIO-S14^T formed a distinct branch within the genus *Shewanella* and clustered with *Shewanella corallii* and these two organisms clustered with *Shewanella mangrovi* (Fig. 1). Similar topology was obtained in NJ, ML and MP trees (Supplementary Fig. S3, S4 and S5) considering all species of the genus *Shewanella*. Results of DNA–DNA hybridization between the strain NIO-S14^T and the type strain of closely related phylogenetic neighbour, *Shewanella corallii* DSM 21332^T, revealed average relatedness of 35% (± 1.64).

Based on the results of phylogenetic analysis, *Shewanella corallii* DSM 21332^T was selected for further comparative studies with the strain NIO-S14^T and a number of differences were observed (Table 1). Strain NIO-S14^T and *Shewanella corallii* DSM 21332^T, exhibited similar characteristics with respect to Gram-stain, motility, pH growth range and optimum and several other enzymatic activities and substrate utilization. However, the two strains differed in cell shape, size, salt tolerance and temperature range and optimum for growth, β -N-acetylgalactosaminidase and phosphatase activities, Ellman reaction and several biochemical characteristics in Microlog, antibiotic susceptibility, major fatty acids and polar lipid composition, DNA G+C content (mol%) (Table 1 and Supplementary Table S1) and DNA-DNA hybridization. Based on the observed phenotypic, chemotaxonomic characteristics and phylogenetic analysis, strain NIO-S14^T is described in this study as a novel species in the genus *Shewanella*, for which the name *Shewanella submarina* sp. nov. is proposed.

Description of *Shewanella submarina* sp. nov.

Shewanella submarina (sub.ma.ri'na. L. prep. *sub*, under; L. fem. adj. *marina* marine, of the sea; N.L. fem. adj. *submarina*, from under the sea).

Cells of strain NIO-S14^T were Gram-stain-negative, curved rod-shaped, 1.5–3.0 μ m in long and 0.5–0.8 μ m in wide, non-sporulating and motile by means of polar flagella. Colonies on Marine agar were observed as circular, 3–4 mm in diameter, pale-yellow, shiny, translucent, convex with entire margin after 48 h at 30 °C. Growth at 20–37 °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 not required for

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growth. Growth at pH 6-9, with optimum growth at pH 7-8. Cells grow aerobically, with a respiratory, chemoorganotrophic mode of metabolism and can grow facultative anaerobically. Nitrate is reduced; indole is not produced. The methyl red and Voges-Proskauer's reactions are negative. Aesculin and starch are not hydrolyzed. Utilize maltose, dextrose, galactose and trehalose but not mannose, melibiose, rhamnose, sucrose, fructose, xylose, raffinose, salicin, cellobiose, dulcitol, sorbitol, inulin, inositol, adonitol, mannitol and lactose in the Hi25™ *Enterobacteriaceae* Identification Kit, and is unable to ferment the same sugars, as well as citrate, using the HiCarbohydrate™ Kit. In the VITEK GN ID card, strain NIO-S14^T is positive for Ala-Phe-Pro arylamidase, L-pyrrolidonyl arylamidase, H₂S production, β-N-acetylglucosaminidase, glutamyl arylamidase, tyrosine arylamidase, α-glucosidase, β-N-acetylgalactosaminidase, phosphatase and glu-gly-arg-arylamidase, but negative for β-galactosidase, γ-glutamyl transferase, β-glucosidase, β-xylosidase, β-alanine arylamidase, L-proline arylamidase, lipase, urease, α-galactosidase, glycine arylamidase, lysine and ornithine decarboxylase, β-glucuronidase, coumarate, 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 alkalization, Ellman reaction and O/129 resistance. The cellular fatty acid composition is given in Supplementary Table S1. The major cellular fatty acids are C_{12:0}, iso-C_{13:0}, C_{14:0}, iso-C_{15:0}, C_{16:0}, C_{16:1 ω7c}/ C_{16:1 ω6c} (summed feature 3). The predominant polar lipids are diphosphatidylglycerol, phosphatidyl ethanolamine and phosphatidylglycerol. The G+C content of strain NIO-S14^T was calculated as 47.9 mol%.

The type strain NIO-S14^T (= MTCC 12524^T = KCTC 52277^T = LMG 30752^T) was isolated from a water sample collected from 100m depth at off Visakhapatnam, Andhra Pradesh, India.

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Author statements

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Figure Legend:

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences representing the phylogenetic relationships between the strain NIO-S14^T and the closely related species of the genus *Shewanella*. Numbers at the nodes are bootstrap values >60%. *Algicola bacteriolytica* IAM14595^T (D89929) was taken as the outgroup organism. Bar, 0.01 substitutions per nucleotide position.

Supplementary Fig. S1. Electron micrograph of negatively stained cells of strain NIO-S14^T. Bar, 0.5 μm. Strain NIO-S14^T was grown on MA plates at 30 °C for 2 days.

Supplementary Fig. S2. Two-dimensional thin-layer chromatogram of the total polar lipids of the strain NIO-S14^T (a) and *Shewanella corallii* DSM 21332^T (b) after spraying the plates with molybdato-phosphoric acid. Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidyl-ethanolamine; PG, phosphatidylglycerol; APL, unidentified aminophospholipid; L, unidentified lipid. The spots identified as phospho-, amino- or glycolipids by spraying molybdenum blue, ninhydrin and α-naphthol reagents, respectively.

Supplementary Fig. S3. Neighbour-joining tree based on 16S rRNA gene sequences representing the phylogenetic relationships between the strain NIO-S14^T and the species of the genus *Shewanella*. Numbers at the nodes are bootstrap values >60%. *Algicola bacteriolytica* IAM14595^T (D89929) was taken as the outgroup organism. Bar, 0.01 substitution per nucleotide position.

Supplementary Fig. S4. Maximum-likelihood tree based on 16S rRNA gene sequences representing the phylogenetic relationships between the strain NIO-S14^T and the species of the genus *Shewanella*. Numbers at the nodes are bootstrap values >60%. *Algicola bacteriolytica* IAM14595^T (D89929) was taken as the outgroup organism. Bar, 0.02 substitutions per nucleotide position.

Supplementary Fig. S5. Maximum-parsimony tree based on 16S rRNA gene sequences representing the phylogenetic relationships between the strain NIO-S14^T and the species of the genus *Shewanella*. Numbers at the nodes are bootstrap values >60%. *Algicola bacteriolytica* IAM14595^T (D89929) was taken as the outgroup organism.

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Table 1. Phenotypic features that distinguish strain NIO-S14^T from the most closely related strain *Shewanella corallii* DSM 21332^T

Data is from the present study. Both strains are Gram staining negative, motile, does not form spores, grew from pH 6 to 10 with optimum growth at pH 7-8 and weak growth at pH 5; positive for oxidase, catalase, Ala-Phe-Pro-arylamidase, L-pyrrolydonyl-arylamidase, β -N-acetylglucosaminidase, glutamyl arylamidase, tyrosine arylamidase, α -glucosidase, glu-gly-arg-arylamidase activities, nitrate reduction, anaerobic growth, H₂S production, utilization of maltose, dextrose, galactose, trehalose, D-fructose, D-galactose, D-fucose, L-rhamnose, inosine, L-aspartic acid, fusidic acid, L-arginine, L-glutamic acid, D-galacturonic acid, D-glucuronic acid, acetoacetic acid, acetic acid, lithium chloride and aztreonam; weak positive for N-acetyl- β -D-mannosamine, N-acetyl-D-galactosamine, α -D-glucose, L-fucose, 1% sodium lactate, D-serine and L-serine; negative for β -galactosidase, γ -glutamyl-transferase, β -glucosidase, β -xylosidase, β -alanine arylamidase, L-proline arylamidase, lipase, urease, α -galactosidase, glycine arylamidase, ornithine decarboxylase, lysine decarboxylase, β -glucuronidase activities, indole production, methyl red and Voges Proskauer's test, citrate utilization, esculine and starch hydrolysis, utilization of mannose, melibiose, rhamnose, fructose, xylose, raffinose, cellobiose, dulcitol, sorbitol, inulin, inositol, adonitol, mannitol, lactose, L-arabitol, D-cellobiose, D-glucose, D-maltose, D-mannitol, D-mannose, palatinose, D-sorbitol, D-tagatose, D-trehalose, malonate, 5-keto-D-gluconate, L-histidine, L-malate, L-lactate, β -methyl-D-glucoside, D-serine, troleandomycin, rifamycin SV, minocycline, guanidine HCl, pectin, gentiobiose, D-fructose-6-PO₄, citric acid, β -hydroxy-D,L-butyric acid, sodium butyrate, p-hydroxy-phenylacetic acid, bromo-succinic acid, α -hydroxybutyric acid, formic acid, sodium bromate, coumarate, fermentation glucose, 0/129 resistance, L-lactate and succinate alkalisation. Both strains were susceptible to antibiotics (μ g/disc) ciprofloxacin (10) and chloramphenicol (30); intermediate sensitive to streptomycin (25) and spectinomycin (100); resistant to ceftazidime (30), cefalexin (30), novobiocin (30), sulphamethizole (300), cefprozil (30), chlortetracycline (30), bacitracin (8), ampicillin (25), vancomycin (30), lincomycin (2), tetracyclin (30) and penicillin-G (2 Units).

Characteristic	<i>Shewanella submarina</i> NIO-S14 ^T	<i>Shewanella corallii</i> DSM 21332 ^T
Cell shape	Curved rod	Rod
Cell size (μ m)	0.6-0.7 x 2.0-2.5	0.8-0.9 x 2.0-2.2
Temperature growth range (°C)	20-37	10-37
Optimum temperature (°C)	25-30	25
Salinity range (%NaCl, w/v) (optimum)	0-6 (2)	0.5-8 (2-4)
Growth on MacConkey	+	-
Salicin utilization	-	+

Vitek (Biochemical test)		
<i>β-N</i> -acetylgalactosaminidase	w	+
Phosphatase	w	-
ELLMAN	-	+
Microlog (Biochemical test)		
Dextrin, D-trehalose, sucrose, glycerol, gelatin, glycy-L-proline, L-alanine, L-histidine, L-pyroglutamic acid, D-gluconic acid, D-lactic acid methyl ester, L-lactic acid, D-malic acid, nalidixic acid, potassium tellurite, <i>α</i> -keto-butyric acid	-	+
D-Maltose, stachyose, N-acetyl- D-glucosamine, D-mannitol, D-arabitol, D-glucose-6-PO ₄ , mucic acid, quinic acid, D-saccharic acid, <i>α</i> -keto-glutaric acid, L-malic acid	w	+
D-Turanose, D-raffinose, <i>α</i> -D-lactose, D-salicin, N-acetyl neuraminic acid, D-sorbitol, myo-inositol, Tween 40, <i>γ</i> -amino-butyric acid, propionic acid	-	w
Characteristic	<i>Shewanella submarina</i> NIO-S14^T	<i>Shewanella corallii</i> DSM 21332^T
D-Melibiose, D-mannose, 3-methyl glucose, glucuronamide	+	w
Lincomycin, tetrazolium violet, methyl pyruvate	w	-
Niaproof 4, L-galactonic acid lactone, vancomycin, tetrazolium blue	+	-
Antimicrobial susceptibility (μg/disc)		
Gentamicin (10)	I	S
Cefmetazole (30), neomycin (30), ceftazidime (30), cephadroxil (30), polymyxin B (300)	R	S
Kanamycin (30), amoxicillin (30)	R	I
Polar lipids	DPG, PE, PG, APL1-2, L1-6	DPG, PE, PG, APL1, L1, L3-6
Major fatty acids	C _{12:0} , iso-C _{13:0} , C _{14:0} , iso-C _{15:0} , C _{16:0} , summed feature 3	C _{12:0} , iso-C _{13:0} , C _{14:0} , iso-C _{15:0} , C _{16:0} , C _{17:1 ω8C} , C _{18:1 ω7C} , summed feature 3
DNA G+C content (mol%)	47.9	49.1*

*Data taken from [11].

+, substrate utilized or present; -, substrate not utilized or absent; w, weak growth; R, resistant; S, sensitive; I, intermediate; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; APL, aminophospholipid; L, lipid. Summed feature 3 is C_{16:1 ω7c} and/or C_{16:1 ω6c}.

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Supplementary Table S1. Comparison of the fatty acid composition of strain NIO-S14^T with the most closely related type strain DSM 21332^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. ND, not detected. Values of less than 1% for all strains are not shown. tr, traces (<1%).

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Fatty acid composition	<i>Shewanella submarina</i> NIO-S14 ^T	<i>Shewanella corallii</i> DSM 21332 ^T
C _{12:0}	5.4	5.5
C _{12:0} 3OH	3.8	3.7
C _{13:0}	1.5	1.4
iso-C _{13:0}	8.4	6.3
iso-C _{13:0} 3OH	3.4	2.6
C _{14:0}	5.8	5.3
iso-C _{15:0}	14.5	12.8
iso-C _{15:0} 3OH	1.2	1.0
C _{16:0}	12.7	14.4
C _{16:1} ω ⁹ C	2.4	2.8
C _{17:0}	tr	1.1
C _{17:1} ω ⁸ C	4.6	5.3
C _{18:1} ω ⁷ C	4.5	5.9
C _{18:1} ω ⁹ C	3.6	4.0
*Summed feature 1	1.4	1.3
*Summed feature 2	4.7	4.7
*Summed feature 3	17.3	17.4

*Summed features are groups of two or three fatty acids that could not be separated by GC with the MIDI system. Summed feature 4 comprised anteiso-C_{17:1} B and/or iso-C_{17:1} I.

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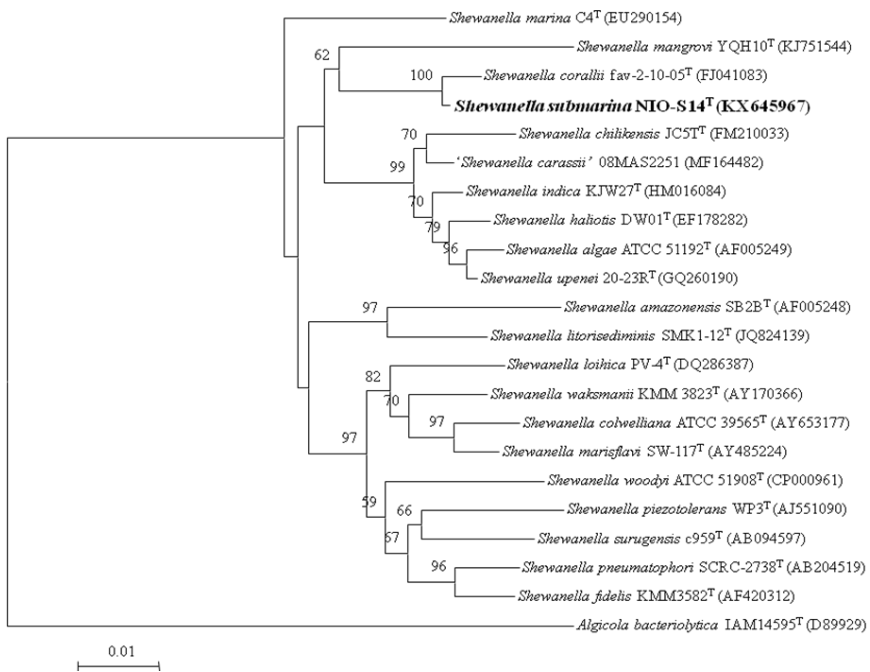
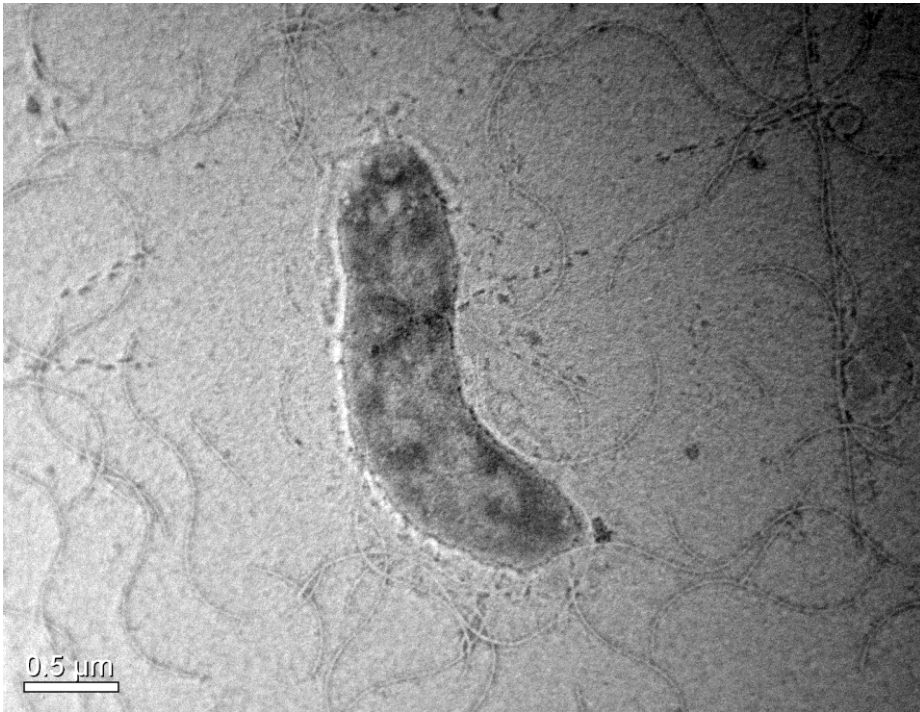
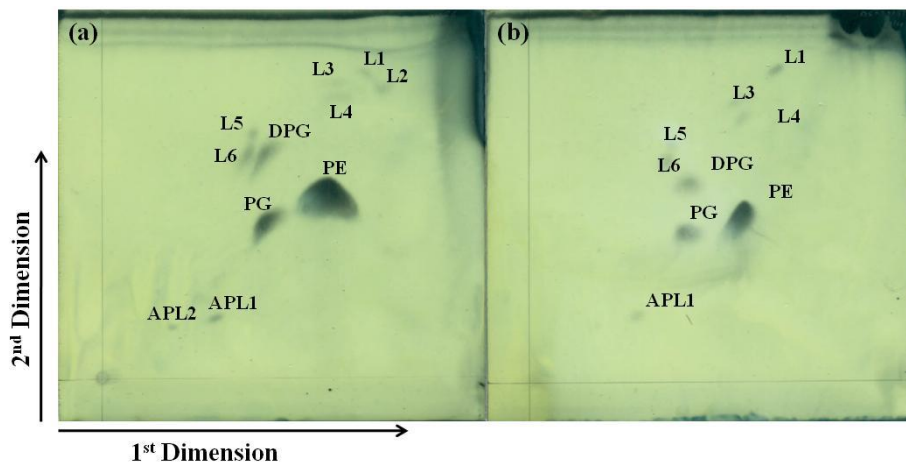


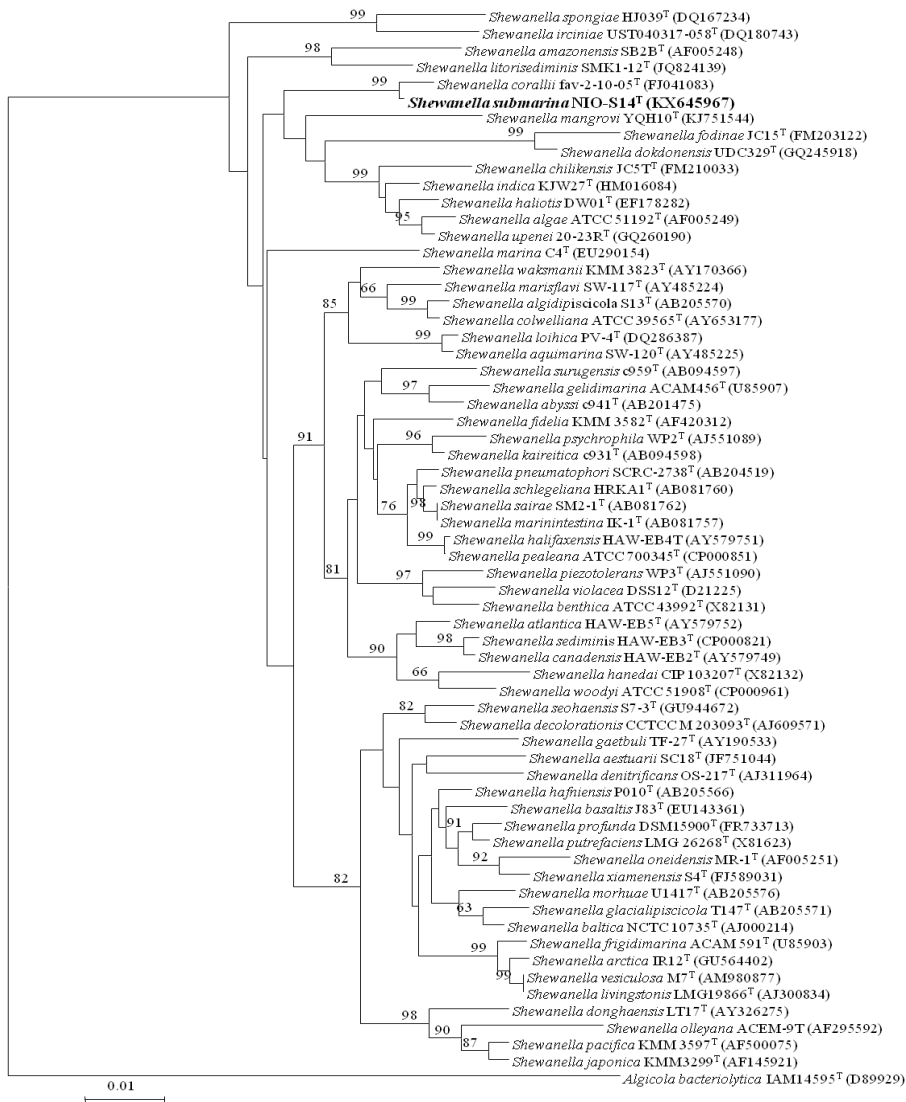
Fig. 1



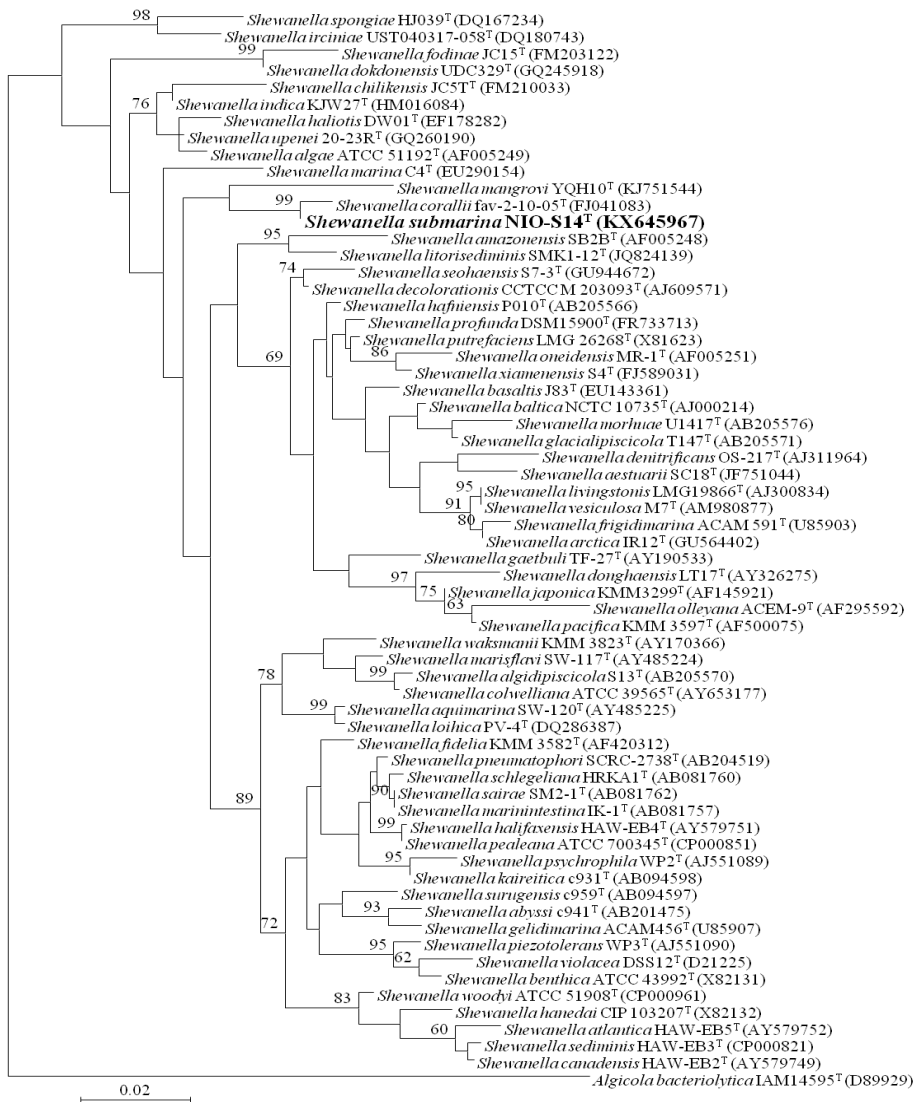
Supplementay Fig. S1



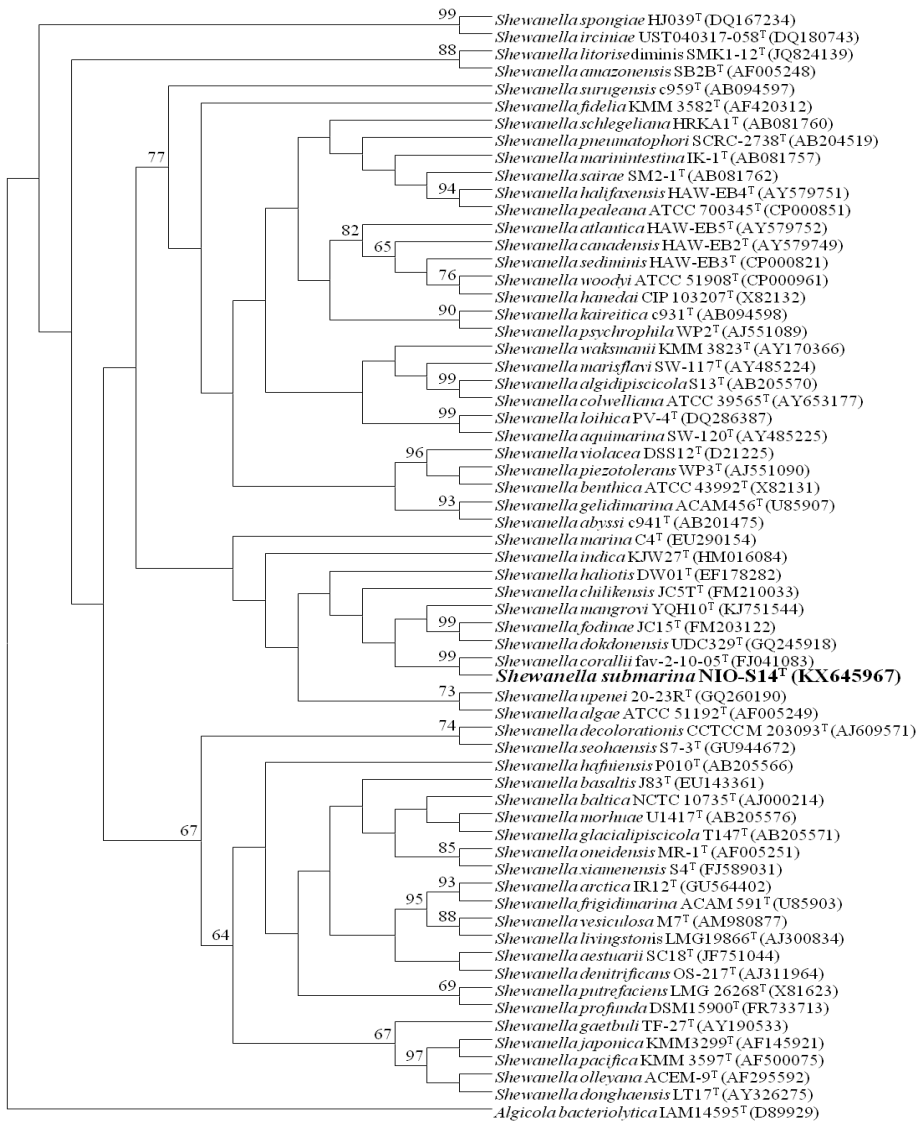
Supplementay Fig. S2



Supplementary Fig. S3



Supplementay Fig. S4



Supplementay Fig. S5