

***Nioella nitratireducens* gen. nov., sp. nov., a novel member of the family  
*Rhodobacteraceae* isolated from Azorean Island**

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**Running title:** *Nioella nitratireducens* gen. nov., sp. nov.

The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of the strain SSW136<sup>T</sup> is  
KC534331.

**Abstract**

A novel Gram-negative, non-spore forming, rod-shaped aerobic bacterium, designated SSW136<sup>T</sup>, was isolated from a surface seawater sample collected at Espalamaca (in Faial Island), Azores. Growth was found to occur from 10–37 °C, pH 6.0–8.0, and with 2–11 % of NaCl. 16S rRNA gene sequence indicated that the strain SSW136<sup>T</sup> belongs to the family *Rhodobacteraceae*. Strain SSW136<sup>T</sup> exhibited 96.3, 95.9, 95.7 and 95.5 sequence similarity to the type strains, *Oceanicola litoreus* M-M22<sup>T</sup>, *Roseovarius aestuarii* SMK-122<sup>T</sup>, *Marivita geojedonensis* DPG-138<sup>T</sup>, and *Pseudoruegeria aquimaris* SW-255<sup>T</sup> respectively. Neighbour-joining and maximum-parsimony phylogenetic trees based on 16S rRNA gene sequences revealed that strain SSW136<sup>T</sup> was affiliated to the family *Rhodobacteraceae* and formed a separate branch. The G+C content was 63.5 mol%. The major respiratory quinone was found to be Q-10. The polar lipids of strain SSW136<sup>T</sup> consisted of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, two unidentified aminolipids and three unidentified phospholipids. The major fatty acids were C<sub>18:1</sub> ω7c (46.5 %), Cyclo-C<sub>19:0</sub> ω8c (16.0 %) and C<sub>16:0</sub> (12.8 %). On the basis of the morphological, genotypic, chemotaxonomic characteristics and low DNA-DNA relatedness, strain SSW136<sup>T</sup> is proposed to represent a novel genus and novel species, *Nioella nitratireducens* gen. nov., sp. nov., in the family *Rhodobacteraceae*. The type strain is SSW136<sup>T</sup> (=KCTC 32417<sup>T</sup> = NCIM 5499<sup>T</sup>).

**Keywords** *Nioella nitratireducens* · Novel genus and species · Polyphasic taxonomy · Seawater · Espalamaca

## Introduction

The family *Rhodobacteraceae* which belongs to the phylogenetic class *Alphaproteobacteria* and comprises of more than 100 recognized genera (<http://www.bacterio.net/classifgenerafamilies.html#Rhodobacteraceae>) was proposed by Garrity et al. (2005) with *Rhodobacter* as the type genus of this family. Around 55 % of the genera from the family *Rhodobacteraceae* consist of only one species, on the other side *Paracoccus* being the largest, includes 40 species. During a comparative study on bacterial diversity from shallow water hydrothermal vent and non-vent regions of Espalamaca (Azorean Island), a strain designated SSW136<sup>T</sup>, was isolated from the surface seawater of the non-vent region at Espalamaca. The main aim of the present investigation was to establish the precise taxonomic position of the strain SSW136<sup>T</sup> using polyphasic approach.

## Materials and Methods

### Isolation and maintenance of isolate

Surface seawater was collected from the Espalamaca region, Azores (38°33'N; 28°39'W) through an Indo-Portugal bilateral program during September 2010. To isolate lead (Pb) resistant bacteria, serial dilutions of the samples were spread plated on diluted nutrient agar (Peptone 1.25 g/L, Beef extract 0.75 g/L and 1.5 % Agar) amended with 1mM Pb(NO<sub>3</sub>)<sub>2</sub> prepared in 50 % seawater. After 3 days of incubation at 30 °C, strain SSW136<sup>T</sup> was isolated and maintained on seawater nutrient agar (SWNA: Peptone 5.0 g/L, Beef extract 3.0 g/L, 1.5% Agar prepared in 50 % seawater) at 4 °C for short-term maintenance and as a 15 % glycerol suspension at -80 °C for long-term maintenance.

### Morphological, physiological and biochemical characterization

Gram staining was carried out using the standard Gram staining method, and counter checked by KOH test (Cerny 1978). Motility was determined by hanging drop method (Collee et al. 2008) under 1000 × magnification and confirmed by motility agar test. Spore staining was performed using Schaeffer and Fulton's spore stain kit (K006, Himedia, Mumbai). Morphological characteristics of strain SSW136<sup>T</sup> were observed by light microscopy (Olympus BX-60) and scanning electron microscopy (Hitachi TM3000). Growth at temperature range of 4, 10, 20, 25, 30, 37, 40 and 50 °C was tested on sea water nutrient broth (SWNB, the compositions are same as SWNA excluding agar). The pH range for growth was determined in SWNB adjusted to pH 4–10 (1 unit increment) by using acetate (pH 4–5), carbonate (pH 9–10) (Lee et al. 2012) and phosphate (pH 6–8) buffer systems (Wang et al. 2012). For pH and temperature experiments, cell densities for growth were measured at 600 nm using a spectrophotometer (Cary 300) after 2 days incubation. NaCl tolerance

tests were examined with different NaCl concentrations from 0 to 15 % (1 % increment) on nutrient agar (NA) prepared with distilled water. Catalase activity was detected by the production of bubbles after the addition of a drop of 3 % H<sub>2</sub>O<sub>2</sub> (Smibert and Krieg 1994). Oxidase activity was determined by the oxidation of tetramethyl-*p*-phenylenediamine. Nitrate reduction, Methyl red, Voges-Proskauer tests, determination of indole and H<sub>2</sub>S production, were assessed using Hi25<sup>TM</sup> Enterobacteriaceae identification kit (Himedia). Utilization of various carbon sources was determined using API50CH strips (bioMérieux) according to the manufacturer's instructions with inoculation medium API CHB/E amended with marine cations supplement (MCS, Farmer and Hickman-Brenner 2006). Hydrolysis of urea and DNA were determined on Urea agar base (M112, Himedia) and DNase test agar (M1041, Himedia) respectively. Xylan, starch and carboxymethyl cellulose hydrolysis were determined as per the methods provided by Khandeparker et al. (2011). Hydrolysis of casein (1 %), gelatin (1 %), Tweens 20 and 80 (1 %), Tributyrin (1 %), xanthine (0.4 %) and alginate (1 %) (Smibert and Krieg 1994) were tested on SWNA medium. API ZYM kit (bioMérieux) was used to analyse the various substrate utilization according to manufacturer's protocol.

Antibiotic susceptibility tests were performed on SWNA media using antibiotic discs (HiMedia) containing the following concentrations (µg per disc unless indicated): ampicillin (25), kanamycin (30), streptomycin (300), chloramphenicol (25), tetracycline (10), ciprofloxacin (30), ceftazidime (30), lincomycin (10), novobiocin (30), neomycin (30), vancomycin (5), amoxicillin (30), cefadroxyl (30), tobramycin (10), chlortetracycline (30), rifampicin (15), amikacin (30), norfloxacin (10), penicillin-G (2 U), polymyxin-B (50 U) and bacitracin (8 U).

#### Phylogenetic analyses

Genomic DNA of strain SSW136<sup>T</sup> was isolated using DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions. The 16S rRNA gene was PCR amplified using universal eubacterial primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') (Lane 1991). The PCR products were purified using PCR cleanup kit (Sigma) as per the method provided by the manufacturer. Sequencing of the amplified 16S rRNA gene was performed by using the automated 3130xl DNA analyzer (Applied Biosystems). The sequence alignment was carried out using Clustal W sequence alignment program (Thompson et al. 1994). The 16S rRNA gene sequences of the neighbouring taxa were obtained from the GenBank database. Phylogenetic trees were established with neighbour-joining (Saitou and Nei 1987) and maximum-parsimony (Fitch 1971) algorithms using MEGA 5 software (Tamura et al. 2011) with bootstrap values based on 1,000 replications (Felsenstein 1985).

DNA-DNA hybridization was performed between strain SSW136<sup>T</sup> and *Oceanicola litoreus* M-M22<sup>T</sup> by the method proposed by Ezaki et al. (1989). Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded and the means of the remaining three values are quoted as DNA-DNA relatedness values.

#### Chemotaxonomy

Cell biomass of the strain SSW136<sup>T</sup> for the analysis of polar lipids, isoprenoid quinones and DNA extraction was obtained from the cultures grown in LB broth (M1245, Himedia) prepared in 50 % seawater for 2 days at 30 °C. For the analysis of whole cell fatty acids, strain SSW136<sup>T</sup> and *Oceanicola litoreus* M-M22<sup>T</sup> were harvested from MA plates after cultivation for 5 days at 30 °C. Fatty acids were saponified, methylated and extracted using the standard procedure of Sherlock microbial identification system (MIDI 6.2B). The fatty acids were analysed by Gas Chromatograph (Hewlett Packard 6890) and identified using TSBA 6.0 database (Sasser 1990). Polar lipids were extracted and examined according to the procedures described by Collins et al. (1980). Briefly, polar lipids were extracted from freeze-dried cells and separated by two dimensional silica gel thin layer chromatography (Merck). The first direction was developed in chloroform/methanol/water (65:25:3.8, by vol.) and the second direction was developed in chloroform/methanol/acetic acid/water (40:7.5:6:1.8, by vol.). Total lipid material and specific functional groups were detected using molybdophosphoric acid (total lipids), molybdenum blue spray reagent (phosphate), ninhydrin (free amino groups), periodate-Schiff ( $\alpha$ -glycols), and  $\alpha$ -naphthol reagent (sugars). Isoprenoid quinones were determined as depicted by Minnikin et al. (1984). The DNA G+C content of strain SSW136<sup>T</sup> was determined by reverse-phase HPLC of nucleosides according to Mesbah et al. (1989).

#### Results and Discussion

Morphological observations of 2 days old colonies grown in SWNA media were observed to be punctiform, white, convex and circular in shape. Strain SSW136<sup>T</sup> was found to be a Gram negative, non-motile, rod shaped (Supplementary Fig. S1) and non-spore forming bacterium. The temperature range for growth was determined to be 10–37 °C, with optimal growth at 30 °C. The pH range for growth was determined to be 6–8, with optimal pH 6. Growth of strain SSW136<sup>T</sup> was observed at NaCl concentrations between 2 and 11 %, with an optimum of 8 %. Strain SSW136<sup>T</sup> was found to be susceptible to all the tested antibiotics. Strain SSW136<sup>T</sup> was distinguishable from the four recognized relative species (different genera) by differences in several phenotypic characteristics represented in Table 1.

The major isoprenoid quinone was determined to be Q-10. Polar lipids of the strain SSW136<sup>T</sup> consisted of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, two unidentified aminolipids and three unidentified phospholipids (Supplementary Fig. S2). Cellular fatty acid analysis revealed that C<sub>18:1</sub> ω7c (46.5 %), Cyclo-C<sub>19:0</sub> ω8c (16.0 %) and C<sub>16:0</sub> (12.8 %) are the major components (Table 2) observed in 5 days old culture. Whereas, in 2 days old culture C<sub>18:1</sub> ω7c (62.6 %) and C<sub>16:0</sub> (20.0 %) are dominant (Supplementary Table 1). The proportion of Cyclo-C<sub>19:0</sub> ω8c was found to be lower (2.9 %) in 2 days and tried to be higher with longer incubation of 5 days (16.0 %). At the same time, proportion of major fatty acid C<sub>18:1</sub> ω7c was reduced nearly 16 % as the cultures got older. It may be estimated that there is difference between the growth phases of strain SSW136<sup>T</sup> and *Oceanicola litoreus* M-M22<sup>T</sup>. Comparative analysis with *Oceanicola litoreus* M-M22<sup>T</sup> revealed that Methyl-C<sub>19:0</sub> ω7c, C<sub>12:0</sub> 3-OH and C<sub>16:0</sub> 2-OH were detected only in strain SSW136<sup>T</sup>. Further, Strain SSW136<sup>T</sup> could be distinguished from its closest relative *Oceanicola litoreus* M-M22<sup>T</sup> by the differences in proportions of fatty acids (Table 2). The chemotaxonomic properties of strain SSW136<sup>T</sup> i.e., ubiquinone Q-10, the presence of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and the large proportion of unsaturated fatty acid C<sub>18:1</sub> ω7c are similar to those described for phylogenetically related genera of the order *Rhodobacterales*, class *Alphaproteobacteria* (Romanenko et al. 2011). However, strain SSW136<sup>T</sup> differed from their closest relatives by some unidentified phospholipids and aminolipids, and the percentage variations of fatty acids (Table 2). The DNA G+C content of the strain SSW136<sup>T</sup> was determined to be 63.5 mol% which matched the values reported for related type strains (58.6 – 67.6 mol%).

The nearly complete 16S rRNA gene sequence of the strain SSW136<sup>T</sup> (1,411 nt) has highest similarity values with the type strains of the family *Rhodobacteraceae*. EzTaxon-e (Kim et al. 2012) showed closest similarities to *Oceanicola litoreus* M-M22<sup>T</sup> (96.3 %) *Roseovarius aestuarii* SMK-122<sup>T</sup> (95.9 %), *Marivita geojedonensis* DPG-138<sup>T</sup> (95.7 %), *Pseudoruegeria aquimaris* SW-255<sup>T</sup> (95.5 %), *Marivita cryptomonadis* CL-SK44<sup>T</sup> (95.4 %), *Tropicimonas sediminicola* M97<sup>T</sup> (95.4 %), *Roseibacterium elongatum* DSM 19469<sup>T</sup> (95.4 %), *Celeribacter neptunius* H 14<sup>T</sup> (95.3 %), *Jannaschia cystaugens* CFPB-A9<sup>T</sup> (95.2 %), *Roseovarius tolerans* EL-172<sup>T</sup> (95.2 %), *Roseisalinus antarcticus* EL-88<sup>T</sup> (95.2 %) *Roseovarius nubinhibens* ISM<sup>T</sup> (95.2 %), *Jannaschia donghaensis* DSW-17<sup>T</sup> (95.1 %), *Roseovarius lutimaris* 112<sup>T</sup> (95.1 %) and *Thioclava pacifica* DSM 10166<sup>T</sup> (95.0 %). The remaining type strains of the *Rhodobacteraceae* members had similarity of < 95 %.

Various phylogenetic tree algorithms (neighbour-joining and maximum-parsimony) based on 16S rRNA gene sequences (Fig. 1 and Supplementary Fig. S3) revealed a close phylogenetic relationship between strain SSW136<sup>T</sup> and members of the family *Rhodobacteraceae* by forming a separate

branch within the type species of closely related genera. In addition, Strain SSW136<sup>T</sup> exhibited DNA-DNA relatedness value of 14.3±4.5 % to *Oceanicola litoreus* M-M22<sup>T</sup>. This information confirms that the strain SSW136<sup>T</sup> belongs to a novel genus within the *Roseobacter* lineage of the *Alphaproteobacteria*.

#### **Description of *Nioella* gen. nov.**

*Nioella* (N.L. fem. dim. n. *Nioella*, arbitrary name after NIO, the National Institute of Oceanography, where the taxonomic study of this taxon was conducted).

Cells are Gram-negative, aerobic, non-spore forming, rod shaped bacteria that are positive for catalase and oxidase. Sodium ions are necessary for growth. Predominant quinone is Q-10. The polar lipids comprised of phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl choline, two unidentified aminolipids and three unidentified phospholipids. Major fatty acids are C<sub>18:1</sub> ω7c, Cyclo-C<sub>19:0</sub> ω8c and C<sub>16:0</sub>. The DNA G+C content is 63.5 mol%. Based on 16S rRNA gene sequence, the genus represents a separate branch within the class *Alphaproteobacteria*, closely related to the genera, *Oceanicola*, *Roseovarius*, *Marivita* and *Pseudoruegeria*. The type species is *Nioella nitratreducens*.

#### **Description of *Nioella nitratreducens* sp. nov.**

*Nitratreducens* (N.L. n. *nitratum*, nitrate; L. v. *reducens*, bringing back to a state or condition; N.L. part. adj. *nitratreducens*, reducing nitrate).

Cells are aerobic, Gram-staining negative, non-motile and small rods- long rod shaped (0.6–0.9 × 2.4–13.5 μm). Colonies on SWNA are punctiform, white, convex, circular and opaque after incubation at 30 °C for 48 h. Optimal growth temperature is 30 °C; growth occurs between 10 and 37 °C but not at 40 °C. Optimal pH for growth is 6.0; growth occurs at pH 6.0–8.0. Optimum NaCl concentration for growth is 8 % (w/v); growth occurs in the presence of 2–11 % of NaCl (w/v). Mg<sup>2+</sup> ions are not required for growth. Catalase and oxidase are positive. Nitrate is reduced to nitrite. Gelatin and aesculin are hydrolyzed but Tweens 20, and 80, casein, starch, agar, alginate, xylan, CMC, DNA, urea and xanthine are not. Acid is produced from erythritol, L-arabinose, D-xylose, D-adonitol, D-galactose, D-glucose, D-fructose, D-mannose (weak), D-mannitol, D-sorbitol, D-cellobiose, D-maltose, D-lactose (weak), D-turanose, D-fucose and D-arabitol. In assays with the API ZYM system, alkaline phosphatase, esterase (C 4), esterase lipase (C 8), leucine arylamidase, acid phosphatase, α-galactosidase, β-galactosidase (weak) and α-glucosidase (weak) activities are present, but lipase (C 14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-

mannosidase and  $\alpha$ -fucosidase activities are absent. The main respiratory quinone is Q-10. The major fatty acids (>10 %) are C<sub>18:1</sub>  $\omega$ 7c (46.5 %), Cyclo-C<sub>19:0</sub>  $\omega$ 8c (16.0 %) and C<sub>16:0</sub> (12.8 %). The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, two unidentified aminolipids and three unidentified phospholipids. The DNA G+C content of the type strain is 63.5 mol%.

The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of strain SSW136<sup>T</sup> is KC534331. The type strain, SSW136<sup>T</sup> (= KCTC 32417<sup>T</sup> = NCIM 5499<sup>T</sup>), was isolated from coastal surface seawater at the Espalamaca, Azores, Portugal.

### Acknowledgments

The authors are grateful to Dr. N. Ramaiah and RM. Meena for providing DNA sequence facility. Author Rajasabapathy acknowledges CSIR for providing senior research fellowship. We acknowledge Department of Science & Technology, Govt. of India for the support through an Indo-Portugal bilateral program. We acknowledge MMRF-COMAPS under ICMAM, MoES for FAME facility supported by Dr. Shanta Nair, Dr. Anas Abdulaziz and V. Vijitha. We thank Dr. VK. Banakar and Mr. Sarath for SEM analysis. IMAR-DOP/Uaz is Research and Development Unit #531 and LARSyS – Associated Laboratory # 9 funded by the Portuguese Foundation for Science and Technology (FCT) through PEst project (Pest/OE/EEI/LA0009/2011–2014), and by DRCTC – Regional Government of the Azores through a Pluriannual Funding scheme. This is NIO contribution ----.

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**Table 1** Characteristics that differentiate strain SSW136<sup>T</sup> and closest type strains in the family *Rhodobacteraceae*

Characteristic	1	2	3	4	5
Colony colour <sup>†</sup>	Wh	GY	C	GY	GY
Cell size (>10 µm)	+	-	-	-	-
Motility	-	-	+	-	-
Growth at 4°C	-	+	-	-	-
Growth at 40°C	-	+	-	+	+
Growth at 10 % NaCl	+	-	-	-	-
Nitrate reduction	+	-	+	+	-
Hydrolysis of:					
Casein	-	-	+	+	-
Gelatin	+	+	-	-	-
Tween 20	-	+	-	+	-
Tween 80	-	+	-	+	-
Aesculin	+	+	-	+	+
Urea	-	+	-	ND	-
API ZYM results*					
Lucine arylamidase	+	+	-	+	+
Acid phosphatase	+	-	-	+	+
Naphthol-AS-BI-phosphohydrolase	-	-	-	+	W
α-galactosidase	+	-	-	-	-
β-galactosidase	W	-	-	-	+
α-glucosidase	W	-	-	-	-
N-Acetyl-β-glucosaminidase	-	-	-	-	+
Major polar lipids	PC, PG, PE, PL, AL	PC, PG, PE, AL, L	ND	PC, PG, PE, AL	PG, DPG, PE, PL, GL
DNA G+C content (mol%)	63.5	67.6	58.6	59.9	67.0

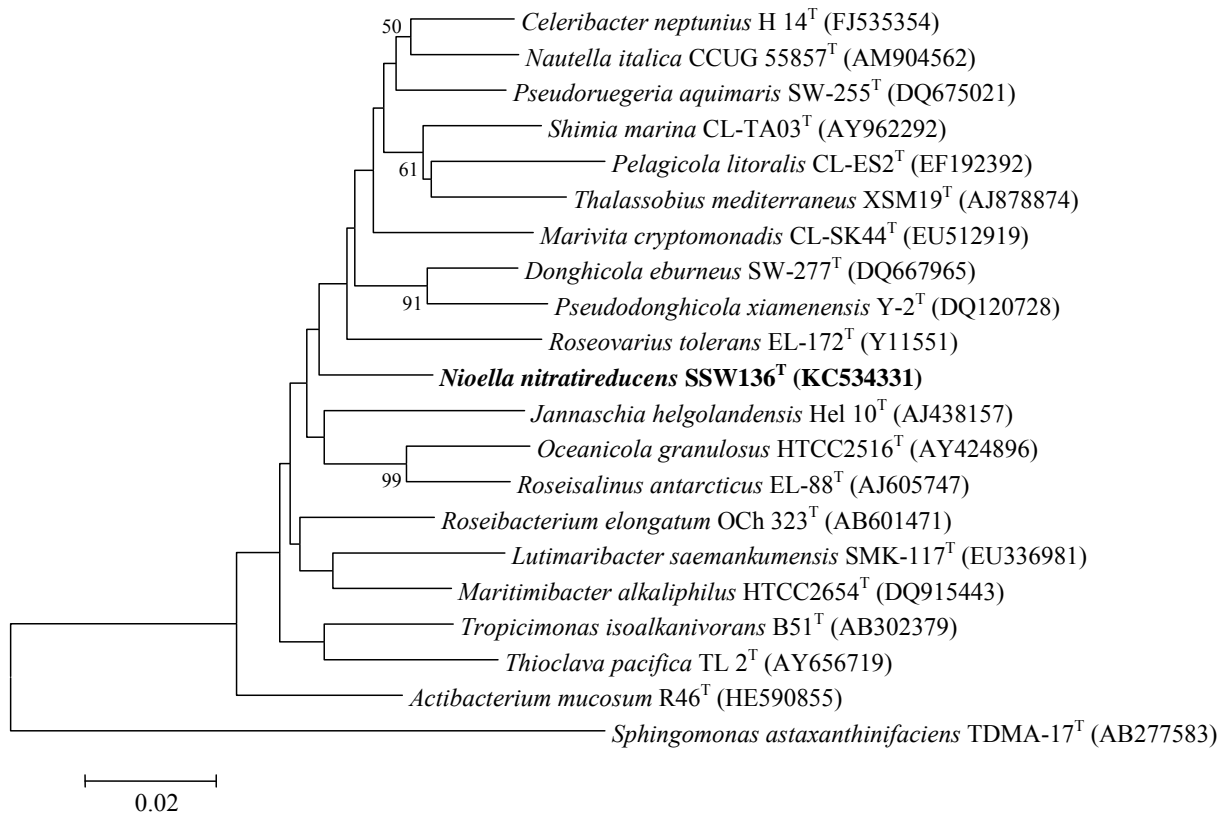
Strains: 1, SSW136<sup>T</sup>; 2, *Oceanicola litoreus* M-M22<sup>T</sup>; 3, *Roseovarius aestuarii* SMK-122<sup>T</sup>; 4, *Marivita geojedonensis* DPG-138<sup>T</sup>; 5, *Pseudoruegeria aquimaris* SW-255<sup>T</sup>; 6, *Jannaschia cystaugens* CFPB-A9<sup>T</sup>. Data for column 2 (except \*asterisk) were taken from Park *et al.* 2013; Data for columns 3, 4, 5 were taken from Yoon *et al.* (2007, 2008 and 2013). Data for column 1 and asterisk (\*) shown in column 2 were obtained from this study. +, positive; -, negative; W, weak; ND, No data available; <sup>†</sup>Wh, white; C, cream; GY, greyish yellow. All five taxa are positive for catalase, oxidase, alkaline phosphatase, esterase (C4) and esterase lipase (C8). All are negative for Gram-staining, starch hydrolysis, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase. Polar lipids: AL, unidentified aminolipid; PL, unidentified phospholipid; GL, unidentified glycolipid; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; L, unidentified lipid.

**Table 2** Cellular fatty acid compositions of strain SSW136<sup>T</sup> and closest type strain *Oceanicola litoreus* M-M22<sup>T</sup>.

Strains: 1, SSW136<sup>T</sup>; 2, *Oceanicola litoreus* M-M22<sup>T</sup>. Data were obtained from this study. Fatty acids that represented  $\geq 1.0$  % are given. Summed feature 3 contained C<sub>16:1</sub>  $\omega$ 7c and/or iso-C<sub>15:0</sub> 2-OH.

Fatty acids	1	2
C <sub>16:0</sub>	12.8	7.1
C <sub>17:0</sub>	-	1.7
C <sub>18:0</sub>	2.1	11.5
C <sub>10:0</sub> 3-OH	3.3	4.3
C <sub>12:0</sub> 3-OH	3.5	-
C <sub>16:0</sub> 2-OH	3.7	-
C <sub>18:1</sub> $\omega$ 7c	46.5	65.4
Methyl-C <sub>18:1</sub> $\omega$ 7c	8.4	-
Cyclo-C <sub>19:0</sub> $\omega$ 8c	16.0	5.0
Summed feature 3	-	1.1

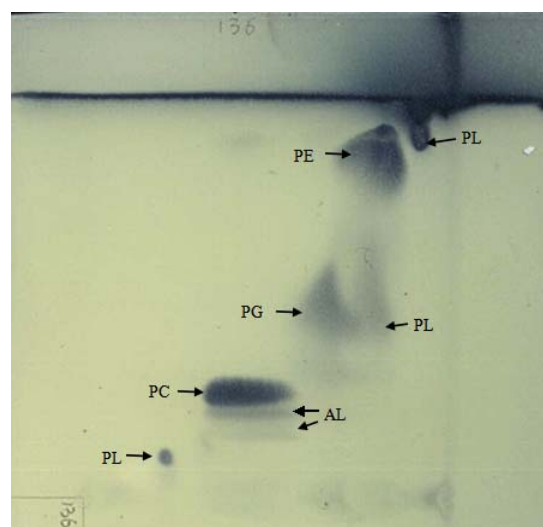
**Fig. 1** Neighbour-joining phylogenetic tree showing the relationship of strain SSW136<sup>T</sup> and phylogenetically related species of the family *Rhodobacteraceae* based on 16S rRNA gene sequences. Numbers at the nodes indicate percentage bootstrap values above 50 (1000 replicates). Bar, 0.02 substitutions per nucleotide position. *Sphingomonas astaxanthinifaciens* (AB277583) was used as an out group.



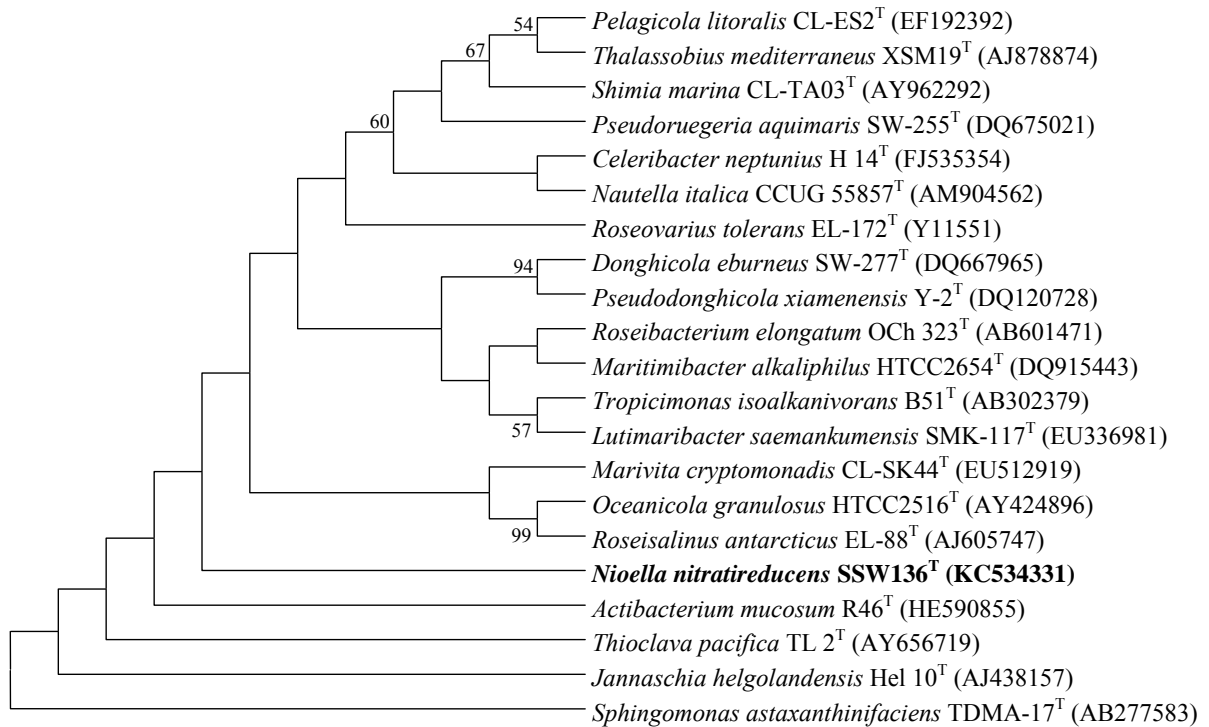
**Supplementary Fig. S1** Scanning electron microscopic image of the strain SSW136<sup>T</sup>



**Supplementary Fig. S2** Two-dimensional TLC of polar lipids of the strain SSW136<sup>T</sup>  
PL, unidentified phospholipid; AL, unidentified aminolipid; PE, phosphatidylethanolamine;  
PG, phosphatidylglycerol; PC, phosphatidylcholine.



**Supplementary Fig. S3** 16S rRNA gene sequence based Maximum-parsimony phylogenetic tree showing the relationship of strains SSW136<sup>T</sup> and its closest neighbours of the class *Alphaproteobacteria* from the GenBank database.



**Supplementary Table 1** Cellular fatty acid compositions of strain SSW136<sup>T</sup> with different time interval

Fatty acids extracted from two days old culture (A) and five days old culture (B) of strain SSW136<sup>T</sup>. Fatty acids that constituted <1.0 % were omitted.

Fatty acids	A	B
C <sub>12:0</sub>	-	-
C <sub>14:0</sub>	1.0	-
C <sub>16:0</sub>	20.0	12.8
C <sub>17:0</sub>	-	-
C <sub>18:0</sub>	4.0	2.1
C <sub>10:0</sub> 3-OH	2.3	3.3
C <sub>12:0</sub> 3-OH	2.0	3.5
C <sub>16:0</sub> 2-OH	-	3.7
C <sub>18:1</sub> ω7c	62.5	46.5
Methyl-C <sub>18:1</sub> ω7c	1.7	8.4
Cyclo-C <sub>19:0</sub> ω8c	2.9	16.0