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ORIGINAL PAPER

Pseudoroseovarius zhejiangensis gen. nov., sp. nov., a novel alpha-proteobacterium isolated from the chemical wastewater, and reclassification of *Roseovarius crassostreae* as *Pseudoroseovarius crassostreae* comb. nov., *Roseovarius sediminilitoris* as *Pseudoroseovarius sediminilitoris* comb. nov. and *Roseovarius halocynthiae* as *Pseudoroseovarius halocynthiae* comb. nov.

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Abstract A Gram-stain negative, aerobic, non-motile and ovoid- to rod-shaped bacterial strain, designated JB3^T, was isolated from a wastewater sample collected from the biochemical reaction basin of Haiyan fine chemical factory in Zhejiang, China. Strain JB3^T was found to grow optimally at pH 7.0–8.0, at 28 °C and in the presence of 1.0–2.0 % (w/v) NaCl. Chemotaxonomic analysis showed that strain JB3^T contains ubiquinone-10 (>99 %) as the predominant respiratory quinone and $C_{18:1}$ $\omega 7c$ (70.9 %) as the most abundant fatty acid. The polar lipids of strain JB3^T were identified as phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an aminophospholipid, an unidentified aminolipid, four unidentified phospholipids and three unidentified lipids. The DNA G+C content of strain JB3^T was determined to be 68.1 mol%. The 16S rRNA gene sequence similarities between the isolate and *Roseovarius crassostreae* DSM 16950^T, *Roseovarius sediminilitoris* KCTC 23959^T and *Roseovarius halocynthiae* MA1-10^T were found to be 97.1, 96.8 and 96.2 %, respectively. Morevoer, the similarity between strain JB3^T and the type strain of the genus *Roseovarius* (*Roseovarius tolerans* DSM 11457^T) was found to be 93.8 %. The phylogenetic trees reconstructed with all three treeing methods showed that strain JB3^T constituted a different taxon, which was separate from other taxa with validly published names, and formed a cluster with *R. crassostreae* DSM 16950^T, *R. sediminilitoris* KCTC 23959^T and *R. halocynthiae* MA1-10^T. These three species were not placed within the phylogenetic cluster formed by *R. tolerans* DSM 11457^T. Differential phenotypic properties, together with the phylogenetic distinctiveness, demonstrated that strain JB3^T is clearly distinct from species of the genus *Roseovarius*. On the basis of these features, we propose strain JB3^T represents a novel species of a novel genus with the

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name *Pseudoroseovarius zhejiangensis* gen. nov., sp. nov. The type strain is JB3^T (=MCCC 1K00457^T = KCTC 42443^T). We also propose that *R. crassostreae*, *R. sediminilitoris* and *R. halocynthiae* should be transferred to this new genus as *Pseudoroseovarius crassostreae* comb. nov., *Pseudoroseovarius sediminilitoris* comb. nov. and *Pseudoroseovarius halocynthiae* comb. nov., respectively.

Keywords *Pseudoroseovarius zhejiangensis* · *Alpha-proteobacteria* · Polyphasic taxonomy · Chemical wastewater · Fine chemical factory

Introduction

The genus *Roseovarius* was first established by Labrenz et al. (1999) with the type species *Roseovarius tolerans*, isolated from water samples form Ekho Lake, Antarctica. Members of this genus are Gram-negative bacteria belonging to the *alpha-proteobacteria*. At the time of writing, according to LPSN (Parte 2014; http://www.bacterio.net/index.html), the genus *Roseovarius* contains 18 species with validly published names. Members of the genus *Roseovarius* have been isolated from seawater, oysters, marine sediments, a sea squirt, a hypersaline lake, an amphioxus breeding zone and a dinoflagellate culture (Biebl et al. 2005; Boettcher et al. 2005; Kim et al. 2012b; Labrenz et al. 1999; Li et al. 2013; Park and Yoon 2013; Yoon et al. 2008). However, based on this and former studies, it is clear that the genus *Roseovarius* is polyphyetic and in need of revision (Lucena et al. 2014; Park et al. 2014; Rajasabapathy et al. 2014).

In this study, strain JB3^T was isolated from a wastewater sample collected from the biochemical reaction basin of Haiyan fine chemical factory in Zhejiang, China. On the basis of polyphasic taxonomic characterisation, we propose that strain JB3^T should be assigned to a novel species of a novel genus, *Pseudoroseovarius zhejiangensis* gen. nov., sp. nov., within the family *Rhodobacteraceae* of *Alpha-proteobacteria*. Additionally, this study shows that three species, *Roseovarius crassostreae* DSM 16950^T, *Roseovarius sediminilitoris* KCTC 23959^T and *Roseovarius halocynthiae* MA1-10^T which do not fall within the cluster of the genus *Roseovarius*, are very closely related to strain JB3^T. Therefore, we propose the transfer of these three species to the new genus *Pseudoroseovarius*, as *Pseudoroseovarius crassostreae* comb. nov., *Pseudoroseovarius sediminilitoris* comb. nov. and *Pseudoroseovarius halocynthiae* comb. nov., respectively.

Materials and methods

Strains and culture conditions

In March 2014, a study of microbial diversity in wastewater led to the isolation of a novel *alpha-proteobacterium*. The wastewater sample was collected from the biochemical reaction basin of Haiyan fine chemical factory (Zhejiang, China). The wastewater sample was diluted, using a tenfold dilution series method, spread on modified ZoBell 2216E agar medium (Oppenheimer and Zobell 1952) and incubated at 28 °C. The modified ZoBell 2216E agar medium contained (per liter distilled water): yeast extract 0.5 g, peptone 0.1 g, ferric citrate 0.1 g, NaCl 19.45 g, $MgCl_2{\cdot}6H_2O$ 8.8 g, $CaCl_2{\cdot}2H_2O$ 1.8 g, KCl 0.55 g, $NaHCO_3$ 0.16 g, Na_2SO_4 3.24 g, KBr 0.08 g, $SrCl_2$ 34 mg, H_3BO_4 22 mg, $NaSiO_4$ 4 mg, NaF 2.4 mg, NH_4NO_3 1.6 mg, Na_2HPO_4 8 mg, agar 20 g, pH 7.4 adjusted with NaOH. After 72 h of incubation, a pink-coloured colony was collected and named as JB3^T. After repeated purifying, the strain was routinely cultured on marine agar (MA; BD).

Strain JB3^T can be maintained for short-term storage on marine agar (MA, BD) slants or plates at 4 °C for 4 weeks. For long-term preservation, cultures can be preserved in liquid nitrogen, by lyophilization or frozen at −80 °C in glycerol. Strain JB3^T has been deposited at the MCCC (Marine Culture Collection of China) and the KCTC (Korean Collection for Type Cultures).

Reference strains

Reference strains used in this study (*R. crassostreae* DSM 16950^T, *R. sediminilitoris* KCTC 23959^T and *R. tolerans* DSM 11457^T) were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) or the KCTC. Data for the reference strain *Roseovarius halocynthiae* MA1-10^T are cited from Kim et al. (2012b).

Phenotypic characterisation

Gram reaction was tested by using the Gram staining method as described (Dong and Cai 2001). Cell morphology was examined by optical microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL) using exponentially growing cells which were incubated on MA for 24 h.

Growth at various NaCl concentrations (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10 and 12 %, w/v) was determined in modified marine broth with different NaCl concentrations. The pH range for growth was determined with an interval of 0.5 by adding 40 mM 2-(*N*-morpholino) ethanesulfonic acid (MES; BBI, pH 5.0–6.0), 3-(*N*-morpholino) propanesulfonic acid (MOPS; BBI, pH 6.5–7.5), Tricine buffer (BBI, pH 8.0–8.5) and 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic (CAPSO; BBI, pH 9.0–10.5) to marine broth (MB, BD), respectively. The temperature range for growth was determined in MB at 4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 42 and 45 °C. Growth was monitored by measuring OD590 in a UV/Visible Spectrophotometer (Ultrospec 6300 pro, Amersham Biosciences).

Antibiotic sensitivity was detected on MA with discs containing the following antibiotics (μg per disc unless stated otherwise): ampicillin (10), bacitracin (0.04 IU), carbenicillin (100), chloramphenicol (30), erythromycin (10), gentamycin (10), kanamycin (30), rifampicin (5), streptomycin (10) and tetracycline (30).

Oxidation of 1 % p-aminodimethylaniline oxalate was used to detect oxidase activity. Catalase activity was determined by observing bubble production in 3 % (v/v) H_2O_2 solution with optical microscopy (BX40, Olympus). Hydrolysis of hypoxanthine and xanthine were tested as described (Gordon and Mihm 1957). Degradation of starch, L-tyrosine and hydrolysis of Tweens 20, 40, 60, 80 were tested as described (Sun et al. 2014). Nitrate reduction, urease activity and the ability to hydrolyse aesculin, casein, chitin, carboxymethyl cellulose (CMC), filter paper and gelatin were determined according to Dong and Cai (2001). Production of bacteriochlorophyll a (Bchl a) was analysed as described (Shiba and Simidu 1982). The H_2S production, methyl red and Voges–Proskauer reactions were determined as described (Wu et al. 2010). Anaerobic growth was determined in modified MB, to which 20 mM sodium thiosulfate, 5 mM sodium sulfite, 20 mM sodium sulfate, 5 mM sodium nitrite, 20 mM sodium nitrate, 5 g l^{-1} L-arginine and 0.5 g l^{-1} cysteine were added as electron acceptors, respectively. Hungate tubes filled with N_2 were used for incubation.

GN2 MicroPlates (Biolog) were used to detect the utilisation of organic substrates according to the manufacturers' instructions. The inoculating fluid for GN2 MicroPlates was replaced by artificial seawater, which contained (per liter distilled water) NaCl 24 g, $MgCl_2$ 5.1 g, Na_2SO_4 4 g, $CaCl_2$ 1.1 g, KCl 0.7 g, $NaHCO_3$ 0.2 g, KBr 0.1 g, H_3BO_3 0.027 g, $SrCl_2$ 0.024 g and NaF 0.003 g (Lyman and Fleming 1940). After incubation at 30 °C for 96 h, the data were read using the Biolog Microbial ID System. Acid production was tested by using API 50CH (bioMérieux) strips. Leifson modified O/F medium (MOF; Leifson 1963) was used to suspend the cells for the inoculation of API 50CH tests. API 50CH strips were read after 24 and 48 h. Additional physiological characteristics and enzyme activities were tested by API 20NE and API ZYM strips (bioMérieux), and they were read after 24 and 4 h, respectively.

Determination of fatty acids, polar lipids and isoprenoid quinones

The cells for fatty acid methyl ester analysis were incubated on MA at 28 °C for 24 h and were analysed according to the instructions of the Microbial Identification System (MIDI; Microbial ID) with the standard MIS Library Generation Software version 4.5. The polar lipids were extracted and separated on silica gel plate (10 × 10 cm, Merck 5554) and further analysed as described (Fang et al. 2012; Minnikin et al. 1984). Isoprenoid quinones were analysed using reversed-phase HPLC (Komagata and Suzuki 1987).

Determination of 16S rRNA gene sequence and phylogenetic analysis

The 16S rRNA gene was amplified by PCR with the bacterial universal 16S rRNA primer pair 27F (5′-GAGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGAC-3′). PCR products were cloned into the pMD 19-T vector (TaKaRa) for sequencing (Xu et al. 2007). The complete 16S rRNA sequence of strain JB3^T (1393 bp) was identified using the EzTaxon-e service (Kim et al. 2012a) by

using EzTaxon-e tool. Phylogenetic trees were constructed by the neighbour-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1987) and maximum-likelihood (Felsenstein 1981) methods with the MEGA 5 program package (Tamura et al. 2011). According to the algorithm of the Kimura two-parameter model (Kimura 1980) for the neighbour-joining method, evolutionary distances were calculated with the MEGA 5 program package.

Determination of chromosomal DNA G+C content

Genomic DNA was collected using the method described by Marmur and Doty (1962) and hydrolysed with P1 nuclease (Sigma). The nucleotides were dephosphorylated with calf intestine alkaline phosphatase (TaKaRa). The G+C content of these deoxyribonucleosides was determined by reversed-phase HPLC and calculated from the ratio of deoxyguanosine (dG) and thymidine (dT) (Mesbah and Whitman 1989).

Results and discussion

Phenotypic characterisation

Cells of strain JB3^T were observed to be Gram-stain negative and ovoid- to rod-shaped measuring 0.6–0.8 μm wide by 1.0–1.5 μm long (Supplementary Fig. S1). Strain JB3^T was found to grow optimally at pH 7.0–8.0, at 28 °C and in the presence of 1.0–2.0 % (w/v) NaCl. Strain JB3^T was found to be sensitive to ampicillin, carbenicillin, chloramphenicol, erythromycin, gentamycin, rifampicin, streptomycin and tetracycline but not to bacitracin and kanamycin. Other physiological and biochemical characteristics of strain JB3^T are included in the species description. A comparison of the physiological and biochemical characteristics of strain JB3^T, *R. crassostreae* DSM 16950^T, *R. sediminilitoris* KCTC 23959^T, *R. halocynthiae* MA1-10^T and *R. tolerans* DSM 11457^T are shown in Table 1 and Supplementary Table S1. Several characteristics were found to discriminate the proposed genus *Pseudoroseovarius* from *Roseovarius*, and also strain JB3^T from *R. crassostreae* DSM 16950^T, *R. sediminilitoris* KCTC 23959^T, *R. halocynthiae* MA1-10^T and *R. tolerans* DSM 11457^T. As shown in Table 1, species in the genus *Pseudoroseovarius* (strain JB3^T, *R. crassostreae* DSM 16950^T, *R. sediminilitoris* KCTC 23959^T and *R. halocynthiae* MA1-10^T) can hydrolyse hypoxanthine and L-tyrosine but not Tween 20, while *R. tolerans* DSM 11457^T (the type strain of the genus *Roseovarius*) showed results contrary to these strains.

Additionally, strain JB3^T was found to be able to hydrolyse gelatin and Tween 40, produce N-acetyl-β-glucosaminidase and produce acid from sucrose, while, other strains showed contrary results.

Fatty acid, polar lipids and isoprenoid quinones characterisation

The most abundant fatty acid of strain JB3^T was found to be $C_{18:1}$ $\omega 7c$ (70.9 %), as for other strains in the genus *Pseudoroseovarius*. The fatty acid profiles of strain JB3^T, *R. crassostreae* DSM 16950^T, *R. sediminilitoris* KCTC 23959^T, *R. halocynthiae* MA1-10^T and *R. tolerans* DSM 11457^T are shown in Table 2. There were some differences in the proportions of some fatty acids. For examples, many fatty acids such as $C_{15:0}$, $C_{17:0}$, $C_{12:1}$ 3-OH and $C_{16:0}$ 2-OH were found in *R. tolerans* DSM 11457^T but were not detected or only found in trace amounts (<1.0 %) in the other strains, which could discriminate the genus *Pseudoroseovarius* from *Roseovarius*. The main polar lipids of strain JB3^T were found to consist of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an aminophospholipid, an unidentified aminolipid, four unidentified phospholipids and three unidentified lipids (Supplementary Fig. S2). The predominant respiratory quinone of the isolate was determined to be ubiquinone-10 (>99 %), as in other species in the genus *Pseudoroseovarius*.

Genotypic characterisation and taxonomic conclusion

The G+C content of strain JB3^T was found to be 68.1 mol% (as determined by HPLC), which could discriminate the isolate from *R. crassostreae* DSM 16950^T (59.0 mol%, Boettcher et al. 2005), *R. sediminilitoris* KCTC 23959^T (63.0 mol%, Park and Yoon 2013), *R. halocynthiae* MA1-10^T (55.4 mol%, Kim et al. 2012b) and *R. tolerans* DSM 11457^T (63.3–63.4 mol%, Labrenz et al. 1999).

Table 1 Characteristics that differentiate strain JB3^T from related species of the genus *Pseudoroseovarius* and the genus *Roseovarius*

Characteristic	1	2	3	4[‡]	5
Flagella	Not found	Subpolar to lateral[†]	Polar[§]	Polar	Not found[*]
Motility	−	+	+	+	−
Bchl *a*	+	+	−	−	+
VP test	+	−	+	ND	+
Growth at					
4 °C	+	-[†]	w[§]	−	+[*]
40 °C	+	+[†]	-[§]	−	+[*]
NO_3^- reduced to NO_2^-	−	−	−	+	+
Hydrolysis of					
Esculin	+	−	+	+	−
Gelatin	+	−	−	−	−
Hypoxanthine	+	+	+	+	−
Tween 20	−	−	−	−	+
Tween 40	+	−	−	−	−
Tween 60	−	+	−	−	−
L-Tyrosine	+	+	+	+	−
Xanthine	−	+	−	−	−
Production of					
N-Acetyl-*β*-glucosaminidase	+	−	−	−	−
Acid phosphatase	w	+	+	w	w
Cysteine arylamidase	−	+	−	−	−
α-Glucosidase	+	−	+	−	−
β-Glucosidase	+	−	+	−	−
Valine arylamidase	−	+	−	−	+
Acid production from					
Amygdalin	+	−	−	ND	−
L-Arabinose	+	−	+	−	+
Arbutin	+	−	+	ND	−
D-Cellobiose	+	−	+	−	−
D-Fructose	+	−	+	−	−
D-Glucose	+	+	+	−	−
D-Mannose	+	−	+	−	−
Potassium 2-ketogluconate	+	+	−	ND	+
Potassium 5-ketogluconate	−	+	+	ND	+
D-Saccharose (sucrose)	+	−	−	−	−
Salicin	+	−	+	ND	−
D-Trehalose	+	−	−	−	+
DNA G+C content (mol%)	68.1	59.0[†]	63.0[§]	55.4	63.3-63.4[*]

Strains 1 JB3^T, *2 R. crassostreae* DSM 16950^T, *3 R. sediminilitoris* KCTC 23959^T, *4 R. halocynthiae* MA1-10^T, *5 R. tolerans* DSM 11457^T

* Data from Labrenz et al. (1999)

† Data from Boettcher et al. (2005)

§ Data from Park and Yoon (2013)

‡ Data from Kim et al. (2012a)

+ positive, − negative, *w* weakly positive, *ND* no data available. Unless stated otherwise, data were obtained from this study under identical growth conditions

As indicated by 16S rRNA gene sequence analysis, strain JB3^T was found to represent a novel species of a novel genus within the family *Rhodobacteraceae* of the *alpha-proteobacteria*. 16S rRNA gene sequence similarities between the isolate and *R. crassostreae* DSM 16950^T, *R. sediminilitoris* KCTC 23959^T and *R. halocynthiae* MA1-10^T were found to be 97.1, 96.8 and 96.2 %, respectively. Moreover, the similarity between strain JB3^T and *R. tolerans* DSM 11457^T was found to be 93.8 %. The phylogenetic trees reconstructed with all three treeing methods showed that strain JB3^T constituted a different taxon, which was

Table 2 Values of total fatty acid contents in strain JB3^T, *Roseovarius crassostreae* DSM 16950^T, *Roseovarius sediminilitoris* KCTC 23959^T, *Roseovarius halocynthiae* MA1-10^T and *Roseovarius tolerans* DSM 11457^T

Fatty acid	1	2	3	4†	5
Straight-chain fatty acids					
$C_{12:0}$	3.8	–	3.6	4.4	0.1
$C_{14:0}$	0.7	–	1.1	–	0.2
$C_{15:0}$	–	–	–	0.2	1.3
$C_{16:0}$	10.4	5.8	11.1	9.7	9.0
$C_{17:0}$	–	–	–	0.6	2.5
$C_{18:0}$	1.6	1.3	0.7	1.6	1.0
10-methyl $C_{19:0}$	0.8	0.6	1.9	–	0.2
Unsaturated fatty acids					
$C_{18:1}$ $\omega 7c$	70.9	79.0	68.4	65.5	70.5
11-methyl $C_{18:1}$ $\omega 7c$	0.5	–	1.3	9.7	5.4
Hydroxy fatty acids					
$C_{10:0}$ 3-OH	3.0	3.8	2.8	2.0	0.1
$C_{12:1}$ 3-OH	–	–	–	–	3.8
$C_{16:0}$ 2-OH	–	–	–	–	1.2
Summed features[a]					
Summed Feature 2	6.8	7.3	5.6	–	–
Summed Feature 3	1.4	1.6	2.4	1.6	0.6

Summed feature 2 contains one or more of iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH

Summed feature 3 contains one or more of $C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$ 2-OH

Strains 1 JB3^T, *2 R. crassostreae* DSM 16950^T, *3 R. sediminilitoris* KCTC 23959^T, *4 R. halocynthiae* MA1-10^T, *5 R. tolerans* DSM 11457^T. Unless stated otherwise, data were obtained from this study under identical growth conditions. Fatty acids that represented <1 % in all strains were omitted

† Data from Kim et al. (2012b)

[a] Summed features represent groups could not be separated by GLC with MIDI system

– Not detected

separate from other taxa with validly published names, and formed a cluster with *R. crassostreae* DSM 16950^T, *R. sediminilitoris* KCTC 23959^T and *R. halocynthiae* MA1-10^T. These three species were not placed within the phylogenetic cluster formed by *R. tolerans* DSM 11457^T (Fig. 1).

On the basis of the physiological, chemotaxonomic and genotypic characteristics, we propose that strain JB3^T represents a novel species of a novel genus, for which the name *Pseudoroseovarius zhejiangensis* gen. nov., sp. nov. is proposed. We also propose that *R. crassostreae*, *R. sediminilitoris* and *R. halocynthiae* should be transferred to this new genus *Pseudoroseovarius* as *Pseudoroseovarius crassostreae* comb. nov., *Pseudoroseovarius sediminilitoris* comb. nov. and *Pseudoroseovarius halocynthiae* comb. nov., respectively.

Description of *Pseudoroseovarius* gen. nov.

Pseudoroseovarius (Pseu.do.ro.se.o.va'ri.us. Gr. adj. pseudes false; M.L. masc. n. *Roseovarius* the varying rose-coloured one and also a bacterial genus name; N.L. masc. n. *Pseudoroseovarius* false *Roseovarius*).

Cells are Gram-stain negative, aerobic, ovoid- to rod-shaped and non-motile or motile by flagellum. Colonies on MA are smooth, convex and cream, pink, pinkish-beige, greenish-yellow or greyish-yellow. Bchl *a* can be produced. Growth occurs in a wide range of NaCl concentrations (0.5–7.0 %). Some strains have weak growth in the absence of NaCl. Mesophilic, neutrophilic and chemotrophic. Catalase and oxidase positive. The major isoprenoid quinone is ubiquinone-10 (Q-10). The most abundant fatty acid is $C_{18:1}$ $\omega 7c$. The main polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and one unidentified aminolipid. The DNA G+C content is 55.4–68.1 mol%. The type species of the genus is *Pseudoroseovarius zhejiangensis*.

Description of *Pseudoroseovarius zhejiangensis* sp. nov.

Pseudoroseovarius zhejiangensis (zhe.ji.ang.en'sis. N.L. masc. adj. *zhejiangensis* pertaining to Zhejiang province in China, where the type strain was isolated).

Cells are Gram-stain negative, aerobic, non-motile and ovoid- to rod-shaped, 0.6–0.8 μm wide by 1.0–1.5 μm long. Colonies are 1–2 mm in diameter, circular, smooth, elevated and pink-coloured on MA after 24 h incubation at 28 °C. Growth occurs at pH 5.5–10, but not pH 5.0 and 10.5; optimal pH for growth is pH 7.0–8.0. Growth occurs at 4 and 45 °C; optimal temperature for growth is 28 °C. Growth occurs in presence of 0.5–12.0 % (w/v) NaCl; optimal growth occurs in presence of 1.0–2.0 % (w/v) NaCl. Oxidase and catalase- positive. Nitrate is not reduced to nitrite. Aesculin, gelatin, hypoxanthine, L-tyrosine and Tween 40 are hydrolysed but carboxymethyl

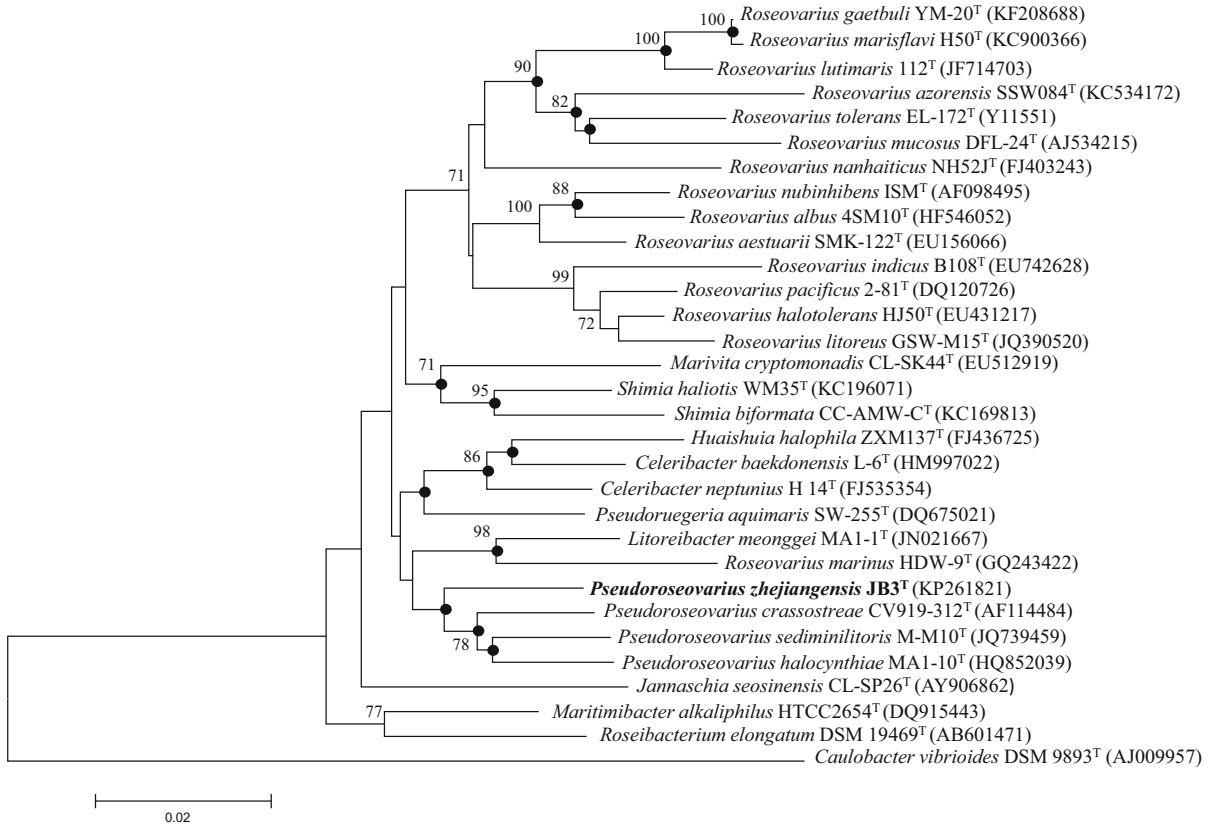

Fig. 1 Neighbour-joining tree using the Kimura two-parameter model based on the 16S rRNA gene sequences, showing the phylogenetic relationships of the novel isolate and related members of the genus *Pseudoroseovarius* and other related genera. Bootstrap values are based on 1000 replicates; values >70 % are shown. *Filled circles* indicate nodes also obtained in both maximum-likelihood and maximum-parsimony trees. *Bar*, 0.02 substitutions per nucleotide position

cellulose (CMC), casein, chitin, filter paper, starch, Tween 20, Tween 60, Tween 80, urea and xanthine are not. Positive for the Voges–Proskauer test. Negative for the methyl red test. Produces H_2S and bacteriochlorophyll a (Bchl a). In GN2 MicroPlates, utilisation of 2,3-butanediol, 2-aminoethanol, D,L-α-glycerol phosphate, D,L-lactic acid, D-cellobiose, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucose-6-phosphate, D-glucuronic acid, D-mannose, D-psicose, formic acid, glucuronamide, glycyl-L-glutamic acid, itaconic acid, L-alanyl-glycine, L-arabinose, L-asparagine, L-aspartic acid, L-histidine, L-pyroglutamic acid, L-rhamnose, L-threonine, *N*-acetyl-D-glucosamine, quinic acid, succinic acid, succinic acid mono-methyl ester, urocanic acid, xylitol, α-keto valeric acid, β-hydroxybutyric acid and γ-hydroxybutyric acid are positive; utilisation of D-alanine, D-glucosaminic acid, D-raffinose, D-serine, i-erythritol, inosine, L-alanine, L-glutamic acid, *N*-acetyl-D-galactosamine, turanose, α-cyclodextrin, α-D-lactose, α-keto glutaric acid, β-methyl-D-glucoside and γ-aminobutyric acid are negative. In API 20NE tests, fermentation of glucose, hydrolysis of esculin and gelatin are positive; β-galactosidase is weak; arginine dihydrolase, hydrolysis of urea, indole production and reduction of nitrate to nitrite are negative. In API ZYM tests, production of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, *N*-acetyl-β-glucosaminidase, α-glucosidase and β-glucosidase are positive; production of acid phosphatase and napthol-AS-BI-phosphohydrase are weak; production of chymotrypsin, cysteine arylamidase, lipase (C14), trypsin, valine arylamidase, α-fucosidase, α-galactosidase, α-mannosidase, β-galactosidase and β-glucuronidase are negative. In API 50CH tests, acid is produced from amygdalin, arbutin, D-cellobiose, D-fructose, D-glucose, D-mannose, D-ribose, D-saccharose (sucrose), D-trehalose, esculin

ferric citrate, L-arabinose, potassium 2-ketogluconate and salicin, but not amidon (starch), D-adonitol, D-arabinose, D-arabitol, D-fucose, D-galactose, D-lactose (bovine origin), D-lyxose, D-maltose, D-mannitol, D-melezitose, D-melibiose, D-raffinose, D-sorbitol, D-tagatose, D-turanose, dulcitol, D-xylose, erythritol, gentiobiose, glycerol, glycogen, inositol, inulin, L-arabitol, L-fucose, L-rhamnose, L-sorbose, L-xylose, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, Methyl-β-D-xylopyranoside, *N*-acetylglucosamine, potassium 5-ketogluconate, potassium gluconate and xylitol. The predominant respiratory quinone is ubiquinone-10 (>99 %). The main polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an aminophospholipid, an unidentified aminolipid, four unidentified phospholipids and three unidentified lipids. The major fatty acids are $C_{18:1}$ $\omega 7c$, $C_{16:0}$ and Summed Feature 2 (iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH). The DNA G+C content of the type strain is 68.1 mol% (as determined by HPLC).

The type strain is JB3^T (=MCCC 1K00457^T = KCTC 42443^T), isolated from the biochemical reaction basin of Haiyan fine chemical factory (Zhejiang, China). The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JB3^T is KP261821.

Description of *Pseudoroseovarius crassostreae* comb. nov.

Pseudoroseovarius crassostreae (crass.o.stre'ae. N.L. n. Crassostrea systematic name of an oyster genus; N.L. gen. n. *crassostreae* of Crassostrea).

Basonym: *Roseovarius crassostreae* Boettcher et al. (2005).

The description is identical to that given for *Roseovarius crassostreae* by Boettcher et al. (2005).

The type strain is CV919-312^T (=ATCC BAA-1102^T = DSM 16950^T).

Description of *Pseudoroseovarius sediminilitoris* comb. nov.

Pseudoroseovarius sediminilitoris (se.di.mi.ni.li'to.ris. L. n. sedimen -inis sediment; L. n. litus -oris the seashore, beach; N.L. gen. n. *sediminilitoris* of sediment, of seashore, from which the type strain was isolated).

Basonym: *Roseovarius sediminilitoris* Park and Yoon (2013).

The description is identical to that given for *Roseovarius sediminilitoris* by Park and Yoon (2013).

The type strain is M-M10^T (=KCTC 23959^T - = CCUG 62413^T).

Description of *Pseudoroseovarius halocynthiae* comb. nov.

Pseudoroseovarius halocynthiae (ha.lo.cyn'thi.ae. N.L. gen. n. *halocynthiae* of *Halocynthia*, named after the generic name of the sea squirt *Halocynthia roretzi*, from which the type strain was isolated).

Basonym: *Roseovarius halocynthiae* Kim et al. (2012b).

The description is identical to that given for *Roseovarius halocynthiae* by Kim et al. (2012b).

The type strain is MA1-10^T (=KCTC 23462^T - = CCUG 60745^T).

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