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Roseovarius marisflavi sp. nov., isolated from an amphioxus breeding zone in the coastal region of the Yellow Sea, China

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Abstract A novel Gram stain-negative, catalase- and oxidase-positive, strictly aerobic bacterium, designated strain H50^T, was isolated from an amphioxus breeding zone in the coastal region of the Yellow Sea, China. Cells were observed to be ovoid or short rods, lacked flagella and were found to contain bacteriochlorophyll *a*. Poly-beta-hydroxybutyrate was found to be accumulated. The temperature range for growth was determined to be 0–37 °C (optimum 28–37 °C). The halotolerance range for growth is 1–15 % NaCl (optimum 2–7 %). The pH range for growth is 6.0–8.0 (optimum 7.0). The major fatty acids were identified as $C_{18:1}\omega7c$ and $C_{16:0}$. The following polar lipids were found to be present: diphosphatidylglycerol, phosphatidylglycerol and a lipid. The predominant respiratory quinone was determined to be Q-10. DNA G+C content was determined to be 57.7 mol%. Strain H50^T exhibited the highest 16S rRNA gene sequence similarity to *Pelagicola litoralis* DSM 18290^T (96.1 %), *Roseovarius mucosus* DSM 17069^T (95.8 %) and *Roseovarius tolerans* DSM 11457^T (95.7 %). In the phylogenetic trees, strain H50^T was clustered with the genus *Roseovarius* but not *Pelagicola*. On the basis of phenotypic, chemotaxonomic and genotypic data, strain H50^T is considered to represent a novel species in the genus *Roseovarius*, for which the name *Roseovarius marisflavi* sp. nov. is proposed. The type strain is H50^T (=CGMCC 1.10799^T=JCM 17553^T).

Keywords *Roseovarius marisflavi* sp. nov. · Amphioxus breeding zone · 16S rRNA · Taxonomy

Introduction

The family *Rhodobacteraceae* is a major group in the class *Alphaproteobacteria* (Garrity et al. 2005) and currently it comprises 93 genera according to the NCBI database. The genus *Roseovarius*, a member of the family *Rhodobacteraceae*, was proposed by Labrenz et al. (1999) with the description of a single species, *Roseovarius tolerans*, which was isolated from a hypersaline lake. Ten further *Roseovarius* species, *R. nubinhibens* (González et al. 2003), *R. crassostreae* (Boettcher et al. 2005), *R. mucosus* (Biebl et al. 2005), *R. aestuarii* (Yoon et al. 2008), *R. pacificus* (Wang et al. 2009), *R. halotolerans* (Oh et al. 2009), *R. nanhaiticus* (Wang et al. 2010), *R. marinus* (Jung et al. 2011), *R. indicus* (Lai et al. 2011), *R. halocynthiae* (Kim et al. 2012b) and *R. litoreus* (Jung et al. 2012) have since been described; all of them were isolated from marine environments. The genus *Pelagicola*, another member of the family

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Rhodobacteraceae, was proposed by Kim et al. (2008). Presently, there is only one species in this genus, *Pelagicola litoralis*, which was isolated from coastal seawater (Kim et al. 2008).

A novel bacterial strain, designated H50^T, was isolated from a bottom seawater sample collected from an amphioxus breeding zone in the coastal region of the Yellow Sea, China in November 2009. In the present study, strain H50^T has been characterized using a polyphasic taxonomic approach and was found to represent a novel species belonging to the genus *Roseovarius*, for which we propose the name *R. marisflavi* sp. nov.

Materials and methods

Organism and culture conditions

A bottom seawater sample (about 10-m deep) was collected from an amphioxus breeding zone in the coastal region of the Yellow Sea (36°5′N, 120°32′E), China, two miles off shore in November 2009. The cultivation of microorganisms from the sample was carried out using a modified gel microbead (GMD) cultivation method (Zengler et al. 2002; Ji et al. 2012). The individual microcolony-forming GMD was picked out by flow cytometry through Fluorescence Activated Cell Sorting and then sorted into 96-well microtiter plates filled with marine R2A (0.5 g/L yeast extract, 0.5 g/L proteose peptone, 0.5 g/L casamino acids, 0.5 g/L dextrose, 0.5 g/L soluble starch, 0.3 g/L sodium pyruvate, 20 g Agar No. 1, 75 % sea water) medium (Suzuki et al. 1997). After 8–12 days cultivation at 16 °C, the turbid wells ($OD_{600} \geq 0.1$) were chosen for bacterial purification on marine R2A agar plates. Individual colonies showing different traits were picked off and purified by successive streaking on fresh marine agar 2216 (MA; BD) plates at 28 °C. Stocks were kept at −80 °C in sterile 0.9 % (w/v) NaCl supplemented with 15 % (v/v) glycerol. One of the isolates, designated H50^T, has been further characterized.

The type strains of phylogenetically related species, *R. tolerans* DSM 11457^T, *R. mucosus* DSM 17069^T and *P. litoralis* DSM 18290^T, obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany), were used as reference strains and were cultured under the same conditions as H50^T [MA or marine broth 2216 (MB) at 28 °C], unless otherwise specified.

Determination of 16S rRNA gene sequence and phylogenetic analysis

Genomic DNA was extracted from 48 h old cultures on MA by standard methods (Ausubel et al. 1995). The 16S rRNA gene was amplified by PCR using universal primers B8f (5′-AGAGTTTGATCCTGGCTCAG-3′) and B1510 (5′-GGTTACCTTGTTACGACTT-3′). Purified PCR products were ligated to the pMD 18-T (TaKaRa) and cloned to *Escherichia coli* JM109 according to the manufacturer's instructions. Sequencing reactions were carried out using ABI BigDye 3.1 Sequencing kits (Applied BioSystems) and an automated DNA sequencer (model ABI3730; Applied BioSystems). The near-complete 16S rRNA gene sequence of strain H50^T was submitted to GenBank/EMBL to search for similar sequences using the BLAST algorithm. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al. 2012a). Sequences were aligned using CLUSTAL X1.8 (Thompson et al. 1997). Phylogenetic trees were constructed using the neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) methods with Kimura 2-state parameter model analyses implemented in the program MEGA version 5 (Tamura et al. 2007). In each case, bootstrap values were calculated based on 1,000 replicates.

Phenotypic characterization

Cell morphology was determined by transmission electron microscopy (JEOL; JEM-1200EX) after cells had been negatively stained with 1 % (w/v) phosphotungstic acid. Gram and endospore staining was carried out using the standard method (Beveridge et al. 2007). Poly-beta-hydroxybutyrate (PHB) accumulation was tested by fluorescence microscope (BH2; OLYMPUS) using Nile Blue A as a fluorescent stain (Tindall et al. 2007). Growth under anaerobic conditions was determined on MA after incubation in an anaerobic jar which was filled with nitrogen and with a packet of Anero-Pack-Anaero (Mitsubishi Gas Chemical Co.) at 28 °C for 30 days. Halotolerance was investigated using synthetic marine ZoBell broth [5 g Bacto peptone,

1 g yeast extract and 0.1 g $FePO_4$ in 1 L modified artificial seawater (Lyman and Fleming 1940), in which starting Na^+ was replaced by the appropriate K^+] supplied with various concentrations of NaCl (0–15 %, w/v, in increments of 1 %). Tolerance of sea salts was determined using synthetic ZoBell broth (5 g Bacto peptone, 1 g yeast extract and 0.1 g FePO4 in 1 L distilled water) supplied with different concentrations (0–15 %, w/v, in increments of 1 %) of sea salts (Sigma). The temperature range for growth was determined on MA plates incubated at 0–45 °C (0, 4, 16, 22, 28, 37 and 45 °C) for 30 days. The pH range for growth was tested at pH 2–10 (in steps of 1 pH unit) in MB with citrate/phosphate buffer (pH 2.0–7.0), Tris/HCl (pH 8.0–9.0) (Breznak and Costilow 1994) or sodium carbonate/sodium bicarbonate (pH 10.0). Susceptibility to antibiotics was investigated on MA plates by using discs (Hangzhou Microbiology Reagent) containing different antibiotics.

Standard protocols (Tindall et al. 2007) were used to assess catalase and oxidase activities, and degradation of casein, starch, gelatin, lecithin and Tween 20, 40, 80. The ability to hydrolyse alginate was tested as described by Smibert and Krieg (1994). Degradation of chitin was examined on chitin agar with sterile seawater (Hsu and Lockwood 1975). DNase activity was examined by using DNase agar (Qingdao Hope Bio-technology Co., Ltd) with sterile seawater, according to the manufacturer's instructions.

Bacteriochlorophyll (Bchl) *a* content of cells was determined in acetone/methanol (7:2, v/v) extracts using a TU-1810 spectrophotometer (Pgeneral) as described previously (Martens et al. 2006). Activities of constitutive enzymes, substrate oxidation, carbon source utilization and other physiological properties were determined by using the API 20NE, API 20E and Gram-negative MicroPlates (Biolog GN2), according to the manufacturer's instructions except that sterile seawater was used to prepare the inocula.

Determination of chromosomal DNA G+C content

Genomic DNA was extracted from 48 h old cultures on MA by standard method (Ausubel et al. 1995). The G+C content of the chromosomal DNA was determined as described by Mesbah et al. (1989) using a reverse-phase HPLC.

Determination of fatty acid, polar lipids compositions and isoprenoid ubiquinones

Cell masses of H50^T, *R. mucosus* DSM 17069^T, *R. tolerans* DSM 11457^T and *P. litoralis* DSM 18290^T were obtained from MB after cultivation at 28 °C for 2–7 days when the bacterial communities reached the late exponential stage. Cellular fatty acids of H50^T, *R. tolerans* DSM 11457^T and *P. litoralis* DSM 18290^T were prepared and analysed according to the standard protocol described in the Sherlock System (MIDI) (Sasser 1990) with the Sherlock version 6.0 and the included database. Polar lipids of H50^T, *R. tolerans* DSM 11457^T and *P. litoralis* DSM 18290^T were analysed by using standard procedures, in which extracted lipids were separated by two-dimensional TLC and identified by spraying with appropriate detection reagents (Minnikin et al. 1984). Respiratory quinones of H50^T were analysed by Dr B. J. Tindall of the Identification Service of the DSMZ (Braunschweig, Germany).

Results

Determination of 16S rRNA gene sequence and phylogenetic analysis

A BLASTN search using the almost-complete 16S rRNA gene sequence (1,438 bp; GenBank accession number KC900366) of strain H50^T placed it among the members of family *Rhodobacteraceae* in the *Alphaproteobacteria*. Strain H50^T exhibited the highest 16S rRNA gene sequence similarity to *P. litoralis* DSM 18290^T (96.1 %), *R. mucosus* DSM 17069^T (95.8 %) and *R. tolerans* DSM 11457^T (95.7 %), and lower than 95.7 % to all the other related strains. In the phylogenetic tree based on the NJ algorithm (Fig. 1), Strain H50^T formed a coherent cluster with two species of the genus *Roseovarius*, i.e. *R. mucosus* DSM 17069^T and *R. tolerans* DSM 11457^T (the type species of genus *Roseovarius*) with a high bootstrap resampling value of 77 % but occupied a separate phylogenetic branch in this cluster. However, strain H50^T did not form a monophyletic cluster with either *P. litoralis* DSM 18290^T (the only species in the genus) or other genera. Topologies of phylogenetic trees constructed using the ML (Supplementary Fig. S1) and MP (Supplementary Fig. S2) algorithms were

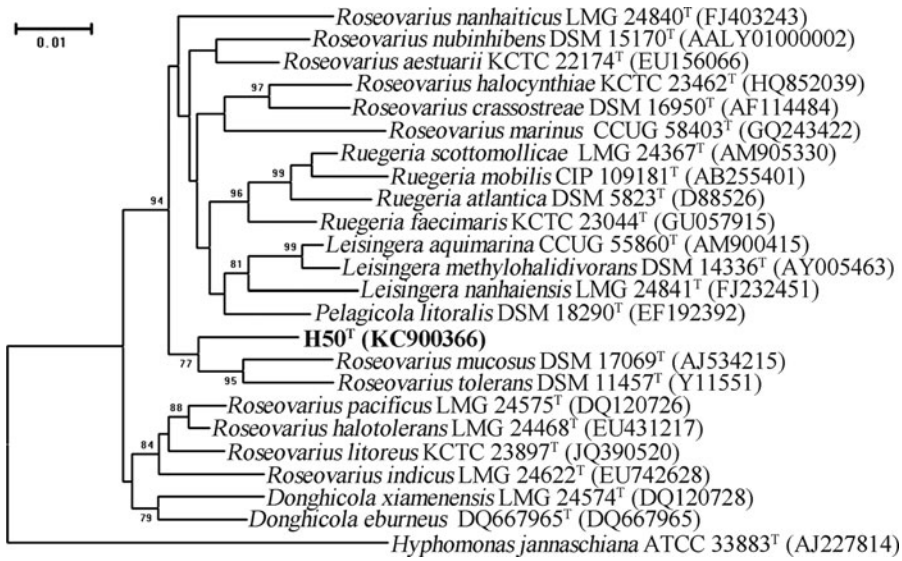

Fig. 1 Phylogenetic dendrogram of *Roseovarius marisflavi* H50^T and related species based on 16S rRNA gene homology. The tree was constructed using the neighbor-joining method with Kimura 2-state parameter model analyses implemented in the program MEGA version 5. *Bar* 0.01 nucleotide substitutions per site

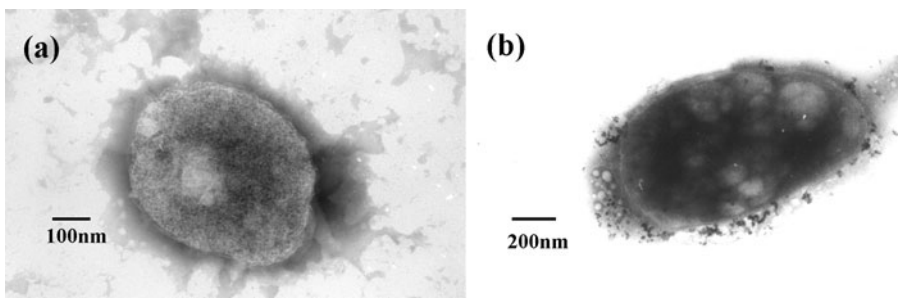

Fig. 2 Transmission electron micrographs of negatively stained cells of *Roseovarius marisflavi* H50^T. **a** In stationary phase, **b** in log phase. *Bars* **a** 100 nm, **b** 200 nm

similar to the NJ phylogenetic tree but the bootstrap resampling values were lower than 70 %. On the basis of the phylogenetic evidence, strain H50^T should be classified as a novel species in the genus *Roseovarius* instead of genus *Pelagicola*.

Phenotypic characterization

Cells of strain H50^T were observed to be Gram stain-negative, strictly aerobic, ovoid or short rods, approximately 0.5–1.3 μm long by 0.4–0.8 μm wide, with no flagella (Fig. 2). Cells did not form endospores. PHB was found to be accumulated. Bacteriochlorophyll *a* (Bchl *a*) was found to be produced. The halotolerance range for growth was determined to be 1–15 % NaCl (optimum 2–7 %). The sea salts range for growth is 1–11 %. The pH range for growth was determined to be 6.0–8.0 (optimum 7.0). The temperature range for growth was determined to be 0–37 °C (optimum 28–37 °C).

The oxidase and catalase tests were positive. Tween 20, DNA and esculin ferric citrate were degraded by strain H50^T but starch, Tween 80, gelatin, casein, alginate, lecithin or chitin was not degraded. Tween 40 was weakly degraded. Arginine dihydrolase and urease tests were positive but β-galactosidase activity was negative. Acid was not observed to be produced from D-glucose. Antibiotic susceptibilities were determined as follows: susceptible to streptomycin (10 μg, 17 mm), rifampicin (5 μg, 24 mm), tobramycin (10 μg, 16 mm), trimethoprim (5 μg, 16 mm), ofloxacin (5 μg, 23 mm), amoxicillin (10 μg, 32 mm), minocycline (30 μg, 24 mm), tetracycline (30 μg, 22 mm), cefobid (75 μg, 41 mm), gentamicin (10 μg, 20 mm), ceftriaxone (30 μg, 48 mm), chloromycetin (30 μg, 22 mm) and oxacillin (1 μg, 16 mm); slightly susceptible to

neomycin (30 μg, 22 mm), lincomycin (2 μg, 20 mm) and erythromycin (15 μg, 18 mm); resistant to midecamycin (30 μg, 12 mm), doxycycline (30 μg, 12 mm), amikacin (30 μg, 14 mm), ciprofloxacin (5 μg, 9 mm), clindamycin (2 μg, 11 mm), vancomycin (30 μg, 9 mm), polymyxin B (300 IU, 8 mm), penicillin (10 IU, 0 mm), ampicillin (10 μg, 0 mm), carbenicillin (100 μg, 0 mm).

The chromosomal DNA G+C content

The DNA G+C content of strain H50^T was determined to be 57.7 mol%.

Determination of fatty acid, polar lipids compositions and isoprenoid ubiquinones

There were eight kinds of fatty acid detected in strain H50^T (Table 1), with $C_{18:1}\omega7c$ and $C_{16:0}$ as the major components of cellular lipid acids; and other cellular fatty acids present were identified as $C_{14:0}$, $C_{18:0}$, $C_{16:1}\omega7c/C_{16:1}\omega6c$, $C_{19:0}$ cyclo$\omega8c$, $C_{18:1}$ 2-OH and $C_{18:1}\omega7c$ 11-methyl. The following polar lipids were found to present in H50^T: phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and a lipid (L) (Supplementary Fig. S3; the polar lipid profiles for *R. tolerans* DSM 11457^T and *P. litoralis* DSM 18290^T are available in Fig. S4 and S5, respectively). The predominant isoprenoid quinone of H50^T was determined to be Q-10, consistent with members of the genus *Roseovarius*.

Taxonomic conclusion

The cultural, physiological and biochemical characteristics of strain are given in Table 2 and the species description (see below). Strain H50^T exhibited the highest 16S rRNA gene sequence similarity to members of the genera *Roseovarius* and the *Pelagicola*, but the phylogenetic evidence and chemotaxonomic data support the view that H50^T represents a novel species in the genus *Roseovarius* instead of the genus *Pelagicola*. The reasons are as following: (1) In the

Table 1 Cellular fatty acid content (% of total) of H50^T and the related species

Fatty acid	1	2	3	4
Straight-chain fatty acids				
$C_{12:0}$	–	–	6.8	–
$C_{14:0}$	0.5	0.1	0.3	–
$C_{16:0}$	17.0	12.2	13.4	3.5
$C_{17:0}$		1.5		
$C_{18:0}$	1.5	1.3	3.1	1.5
Unsaturated fatty acids				
$C_{16:1}\omega7c/C_{16:1}\omega6c$	2.4	0.6	0.7	–
$C_{17:1}\omega8c$		0.6		–
$C_{18:1}\omega7c$	70.8	70.3	58.6	86.5
$C_{18:1}\omega9c$			0.6	–
$C_{19:0}$ cyclo$\omega8c$	1.8	0.5	2.6	–
Unknow $C_{18.846}/_{19:1}\omega6c$	–	0. 9	1.8	–
Hydroxy fatty acids				
$C_{10:0}$ 3-OH	–	0.3		5.8
$C_{12:0}$ 2-OH	–	1.2	1.5	
$C_{12:0}$ 3-OH	–		0.5	
$C_{12:1}$ 3-OH	–	4.9	3.9	
$C_{15:0}$ 2-OH	–	0.3		
$C_{16:0}$ 2-OH	–	1.6	4.0	1.7
$C_{18:1}$ 2-OH	2.1		1.4	
$C_{18:1}\omega7c$ 11-methyl	3.9	3.7	0.8	1.0

Strains 1. H50^T; 2. *Roseovarius tolerans* DSM 11457^T; 3. *Roseovarius mucosus* DSM 17070^T; 4. *Pelagicola litoralis* DSM 18290^T. All data are from this study. –, not detected. Cell masses of the four strains were obtained from MB after cultivation at 28 °C for 4, 3, 2, and 7 days, respectively, when the bacterial communities reached the late exponential phase

Table 2 Characteristics that differentiate H50^T from related strains

Characteristics	1	2	3	4
Temperature range for growth (°C)	0–37	0-37	0–37	16–28
Sea salts requirement (%)	1–11	0–15	1–10	2–6
Optimum temperature (°C)	28–37	28–37	28–37	28
Optimum NaCl (%)	2–7	1–8^A	1–7^B	ND^C
Optimum pH	7.0	6–9^A	6–9^B	7^C
Fermentation D-glucose	–	–	–	+
Arginine dihydrolase	+	–	–	–
Urease	+	–	–	–
β-Galactosidase	–	–	–	+
Hydrolyse of				
Tween 80	–	+	–	+
Tween 40	W	–	+	–
Tween 20	+	+	+	W
Starch	–	–	–	+
DNA	+	–	–	–
Esculin ferric citrate	+	–	–	+
Oxidation of				
D-Arabitol	+	–	–	–
D-Glucuronic Acid	+	–	–	–
α-Ketovaleric Acid	+	–	–	–
D, L-Lactic Acid	+	–	+	–
Polar lipids				
Diphosphatidylglycero (DPG)	+	+	+^{B}	–
Phosphatidylglycerol (PG)	+	+	+^{B}	+
Phosphatidylcholine (PC)	–	+	+^{B}	+
Phosphatidylethanolamine (PE)	–	+	+^{B}	+
Lipid	+	–	–^{B}	+
Unknown phospholipid	–	+	–^{B}	–
Aminolipid	–	+	–^{B}	+
DNA G+C content (mol%)	57.7	62.2–63.8^A	62.9^B	47.0^C

Strains 1. H50^T; 2. *Roseovarius tolerans* DSM 11457^T; 3. *Roseovarius mucosus* DSM 17070^T; 4. *Pelagicola litoralis* DSM 18290^T. All data are from this study, except when otherwise stated. +, positive; −, negative; W, weak reaction; ND, not detect. All organisms have no flagella, negative for Gram staining, nitrate reduction and H_2S production, but positive for oxidase and catalase

[A] Data from Labrenz et al. (1999)

[B] Data from Biebl et al. (2005)

[C] Data from Kim et al. (2008)

phylogenetic trees based on the NJ, ML and MP methods, strain H50^T clearly formed a coherent cluster with the type species of *Roseovarius*, but not the sole representative of the genus *Pelagicola*; (2) The presence of Bchl *a* and PHB of strain H50^T are consistent with the description of the genus *Roseovarius* (Labrenz et al. 1999), whereas no Bchl *a* or PHB are present in *P. litoralis* DSM 18290^T (Kim et al. 2008); (3) The temperature range (0–37 °C) and sea salts range (1–11 %) for growth of strain H50^T is similar to that of *R. tolerans* DSM 11457^T and *R. mucosus* DSM 17069^T but obviously wider than that of *P. litoralis* DSM 18290^T (16–28 °C and 2–6 %, respectively); (4) The DNA G+C content of strain H50^T (57.7 mol%) is within the range for members of the genus *Roseovarius* (55.4–66 mol%) (Lai et al.

2011; Kim et al. 2012b; Jung et al. 2012) but much higher than that of *P. litoralis* DSM 18290^T (47.0 mol%; Kim et al. 2008); (5) $C_{18:1}\omega7c$ and $C_{16:0}$ were the major fatty acid components of H50^T, which is consistent with the profile of *R. tolerans* DSM 11457^T and *R. mucosus* DSM 17069^T. However, in *P. litoralis* DSM 18290^T, $C_{18:1}\omega7c$ and $C_{10:0}$ 3-OH were major fatty acid components, but there was no detectable $C_{10:0}$ 3-OH in strain H50^T; (6) The two identified polar lipids (PG and DPG) in strain H50^T were also found in *R. tolerans* DSM 11457^T and *R. mucosus* DSM 17069^T (Biebl et al. 2005) but DPG could not be found in *P. litoralis* DSM 18290^T.

Phylogenetic analysis, phenotypic characterization, DNA G+C content and chemotaxonomic characteristics support the assignment of strain H50^T to the genus *Roseovarius* instead of the genus *Pelagicola*. However, the new strain H50^T also showed significantly different physiological and biochemical characteristics to the phylogenetically closest species in the genus *Roseovarius*: (1) Hydrolytic activities. DNases, arginine dihydrolase and urease were found to be positive in strain H50^T, which is in contrast to either *R. tolerans* DSM 11457^T or *R. mucosus* DSM 17069^T; (2) Sole carbon sources. Strain H50^T can utilize D-arabitol, D-glucuronic acid and α-ketovaleric acid as sole carbon sources, but *R. tolerans* DSM 11457^T or *R. mucosus* DSM 17069^T could not; (3) Polar lipids. Phosphatidylcholine (PC) and Phosphatidylethanolamine (PE) were detected in both *R. tolerans* DSM 11457^T (Labrenz et al. 1999) and *R. mucosus* DSM 17069^T (Biebl et al. 2005) but were not present in strain H50^T. Other differential properties between H50^T and the phylogenetically closely related species are given in Table 2. The different physiological and biochemical characteristics imply that strain H50^T should not be allocated to the closely related species *R. mucosus* DSM 17069^T or the type species *R. tolerans* DSM 11457^T within the genus *Roseovarius*. In summary, genotypic, phenotypic, and chemotaxonomic data support the conclusion that strain H50^T from an amphioxus breeding zone in the coastal region of the Yellow Sea represents a novel species of the genus *Roseovarius,* for which the name *R. marisflavi* sp. nov. is proposed.

Description of *Roseovarius marisflavi* sp. nov.

Roseovarius marisflavi (ma.ris.fla'vi. L. gen. neut. n. *maris* of the sea; L. masc. adj. *flavus* yellow; N.L. gen. masc. n. *marisflavi* of the Yellow Sea).

Cells are Gram stain-negative, strictly aerobic, ovoid or short rods, approximately 0.5–1.3 μm long by 0.4–0.8 μm wide, with no flagella. Cells do not form endospores. PHB is accumulated. Colonies are cream-coloured, uniformly round, 0.2–0.8 mm in diameter, regular, convex and smooth. The halotolerance range for growth is 1–15 % NaCl (optimum 2–7 %). The sea salts range for growth of strain H50^T is 1–11 %. The temperature range for growth is 0–37 °C (optimum 28–37 °C). The pH range for growth is 6.0–8.0 (optimum 7.0). Cells contain Bchl *a*. Oxidase and catalase are positive. Tween 20 and DNA are degraded, but starch, Tween 80, gelatin, casein, alginate, lecithin or chitin is not degraded. Tween 40 is weakly degraded.

According to API 20NE tests: nitrate cannot be reduced to nitrite; indole is not produced; arginine dihydrogenase and urease are positive; aesculin ferric citrate is degraded; β-galactosidase is negative; D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not utilized. According to API 20E tests: lysine decarboxylase, ornithine decarboxylase, tryptophane desaminase and VP reaction are negative; H_2S is not produced; acid is not produced from D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-saccharose, D-melibiose, amygdalin or L-arabinose. According to Biolog GN2 tests, D-arabitol, D-glucuronic acid, α-ketovaleric acid and D, L-lactic acid are oxidized.

$C_{18:1}\omega7c$ and $C_{16:0}$ are the major components of the cellular lipids and other fatty acids present include $C_{14:0}$, $C_{18:0}$, $C_{16:1}\omega7c/C_{16:1}\omega6c$, $C_{19:0}$ cyclo$\omega8c$, $C_{18:1}$ 2-OH, $C_{18:1}\omega7c$ 11-methyl. The following polar lipids are present: diphosphatidylglycerol, phosphatidylglycerol and a lipid. The predominant respiratory quinone is Q-10. The DNA G+C content of the type strain is 57.7 mol%.

The type strain, strain H50^T (=CGMCC 1.10799^T=JCM 17553^T) was isolated from seawater collected from an amphioxus breeding zone in the coastal region of the Yellow Sea, China. The GenBank accession number for the 16S rRNA gene sequence of *R. marisflavi* H50^T is KC900366.

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References

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1995) Short protocols in molecular biology: a compendium of methods from current protocols in molecular biology, 3rd edn. Wiley, New York

Beveridge TJ, Lawrence JR, Murray RGE (2007) Sampling and staining for light microscopy. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf G, Schmidt TM, Snyder LR (eds) Methods for general and molecular microbiology. American Society for Microbiology, Washington, DC, pp 19–33

Biebl H, Allgaier M, Lünsdorf H, Pukall R, Tindall BJ, Wagner-Döbler I (2005) *Roseovarius mucosus* sp. nov., a member of the *Roseobacter* clade with trace amounts of bacteriochlorophyll *a*. Int J Syst Evol Microbiol 55:2377–2383

Boettcher KJ, Geaghan KK, Maloy AP, Barber BJ (2005) *Roseovarius crassostreae* sp. nov., a member of the *Roseobacter* clade and the apparent cause of juvenile oyster disease (JOD) in cultured Eastern oysters. Int J Syst Evol Microbiol 55:1531–1537

Breznak JA, Costilow RN (1994) Physicochemical factors in growth. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC, pp 137–154

Garrity GM, Bell JA, Lilburn T (2005) Family I. *Rhodobacteraceae* fam. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds). Bergey's manual of systematic bacteriology, 2nd edn, vol. 2, part C, Springer, New York, pp 161–229

González JM, Covert JS, Whitman WB, Henriksen JR, Mayer F, Scharf B, Schmitt R, Buchan A, Fuhrman JA et al (2003) *Silicibacter pomeroyi* sp. nov. and *Roseovarius nubinhibens* sp. nov., dimethylsulfoniopropionate-demethylating bacteria from marine environments. Int J Syst Evol Microbiol 53:1261–1269

Hsu SC, Lockwood JL (1975) Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. Appl Microbiol 29:422–426

Ji S, Zhao R, Yin Q, Zhao Y, Liu C, Xiao T, Zhang X (2012) Gel microbead cultivation with a subenrichment procedure can yield better bacterial cultivability from a seawater sample than standard plating method. J Ocean Univ China 11(1): 45–51

Jung Y-T, Lee JS, Oh K-H, Oh T-K, Yoon J-H (2011) *Roseovarius marinus* sp. nov., isolated from seawater. Int J Syst Evol Microbiol 61:427–432

Jung YT, Park S, Yoon JH (2012) *Roseovarius litoreus* sp. nov., isolated from seawater of southern coast of Korean peninsula. Antonie Van Leeuwenhoek 102(1):141–148

Kim Y-G, Hwang CY, Cho BC (2008) *Pelagicola litoralis* gen. nov., sp. nov., isolated from coastal water in Korea. Int J Syst Bacteriol 58:2102–2106

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012a) Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Kim Y-O, Kong HJ, Park S, Kang S-J, Kim W-J, Kim K-K, Oh T-K, Yoon J-H (2012b) *Roseovarius halocynthiae* sp. nov., isolated from the sea squirt Halocynthia roretzi. Int J Syst Evol Microbiol 62:931–936

Labrenz M, Collins MD, Lawson PA, Tindall BJ, Schumann P, Hirsch P (1999) *Roseovarius tolerans* gen. nov., sp. nov., a budding bacterium with variable bacteriochlorophyll a production from hypersaline Ekho Lake. Int J Syst Bacteriol 49:137–147

Lai Q, Zhong H, Wang J, Yuan J, Sun F, Wang L, Zheng T, Shao Z (2011) *Roseovarius indicus* sp. nov., isolated from deep-sea water of the Indian Ocean. Int J Syst Evol Microbiol 61:2040–2044

Lyman J, Fleming RH (1940) Composition of seawater. J Mar Res 3:134–146

Martens T, Heidorn T, Pukall R, Simon M, Tindall BJ, Brinkhoff T (2006) Reclassification of *Roseobacter gallaeciensis* Ruiz- Ponte et al. 1998 as *Phaeobacter gallaeciensis* gen. nov., comb. nov., description of *Phaeobacter inhibens* sp. nov., reclassification of *Ruegeria algicola* (Lafay et al. 1995) Uchino et al. 1999 as *Marinovum algicola* gen. nov., comb. nov., and emended descriptions of the genera *Roseobacter*, *Ruegeria* and *Leisingera*. Int J Syst Evol Microbiol 56:1293–1304

Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic acid by highperformance liquid chromatography. Int J Syst Bacteriol 39:159–167

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athayle M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Oh Y-S, Lim H-J, Cha I-T, Im W-T, Yoo J-S, Kang UG, Rhee S-K, Roh D-H (2009) *Roseovarius halotolerans* sp. nov., isolated from deep seawater. Int J Syst Evol Microbiol 59:2718–2723

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101. MIDI Inc., Newark, DE

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC, pp 607–654

Suzuki MT, Rappé MS, Haimberger ZW, Winfield H, Adair N, Ströbel J, Giovannoni SJ (1997) Bacterial diversity among small-subunit rRNA gene clones and cellular isolates from the same seawater sample. Appl Environ Microbiol 63:983–989

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Tindall BJ, Sikorski J, Smibert RM, Krieg NR (2007) Phenotypic characterization and the principles of comparative

systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf G, Schmidt TM, Snyder LR (eds) Methods for General and Molecular Microbiology. American Society for Microbiology, Washington, DC, pp 330–393

Wang B, Tan T, Shao Z (2009) *Roseovarius pacificus* sp. nov., isolated from deep-sea sediment. Int J Syst Evol Microbiol 59:1116–1121

Wang B, Sun F, Lai Q, Du Y, Liu X, Li G, Luo J, Shao Z (2010) *Roseovarius nanhaiticus* sp. nov., a member of the *Roseobacter* clade isolated from marine sediment. Int J Syst Evol Microbiol 60:1289–1295

Yoon J-H, Kang SJ, Oh TK (2008) *Roseovarius aestuarii* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. Int J Syst Evol Microbiol 58:1198–1202

Zengler K, Toledo G, Rappé M, Elkins J, Mathur EJ, Short JM, Keller M (2002) Cultivating the uncultured. Proc Natl Acad Sci USA 26:15681–15686