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ORIGINAL PAPER

Roseovarius litoreus sp. nov., isolated from seawater of southern coast of Korean peninsula

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Abstract A gram-negative, non-flagellated and ovoid- to rod-shaped bacterial strain, designated GSW-M15^T, was isolated from seawater on the southern coast of South Korea. Strain GSW-M15^T grew optimally at 30 °C, at pH 7.0–7.5 and in the presence of 2 % (w/v) NaCl. The phylogenetic trees based on 16S rRNA gene sequences revealed that strain GSW-M15^T belonged to the genus *Roseovarius*. Strain GSW-M15^T exhibited highest 16S rRNA gene sequence similarity values (98.3 and 97.5 %) to *Roseovarius halotolerans* HJ50^T and *Roseovarius pacificus* 81-2^T and 92.8-96.2 % sequence similarity values to the type strains of the other *Roseovarius* species. Strain GSW-M15^T contained Q-10 as the predominant ubiquinone and $C_{18:1}$ $\omega 7c$ and 11-methyl-$C_{18:1}$ $\omega 7c$ as the major fatty acids. The major polar lipids detected in strain GSW-M15^T were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, one unidentified aminolipid and two unidentified lipids. The DNA G+C content of strain GSW-M15^T was 62.9 mol% and its mean DNA–DNA relatedness values with *R. halotolerans* KCTC 22224^T and *R. pacificus* LMG 24575^T were 33 and 18 %, respectively. Differential phenotypic properties of strain GSW-M15^T, together with the phylogenetic and genetic distinctiveness, demonstrated that this strain is distinguishable from other *Roseovarius* species. On the basis of the data presented here, strain GSW-M15^T (=KCTC 23897^T = CCUG 62218^T) represents a novel species of the genus *Roseovarius*, for which the name *Roseovarius litoreus* sp. nov. is proposed.

Keywords *Roseovarius litoreus* · Novel species · *Alphaproteobacteria* · Seawater

Introduction

The genus *Roseovarius,* a member of the *Alphaproteobacteria*, was established by Labrenz et al. (1999) with the description of *Roseovarius tolerans*, isolated from water samples from Ekho Lake, Antarctica, as the sole recognized species. Subsequently, nine further

The GenBank accession number of the 16S rRNA gene sequence of *Roseovarius litoreus* GSW-M15^{T}is JQ390520.

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Roseovarius species with validly published names, *Roseovarius nubinhibens* (González et al. 2003), *Roseovarius crassostreae* (Boettcher et al. 2005), *Roseovarius mucosus* (Biebl et al. 2005), *Roseovarius aestuarii* (Yoon et al. 2008), *Roseovarius pacificus* (Wang et al. 2009, 2010), *Roseovarius halotolerans* (Oh et al. 2009), *Roseovarius nanhaiticus* (Wang et al. 2010), *Roseovarius marinus* (Jung et al. 2011) and *Roseovarius indicus* (Lai et al. 2011), have been described from a hypersaline lake, seawater, a dinoflagellate culture, oysters and marine sediments (Labrenz et al. 1999; González et al. 2003; Biebl et al. 2005; Boettcher et al. 2005; Yoon et al. 2008; Wang et al. 2009, 2010). In this study, we describe a bacterial strain, designated GSW-M15^T, which was isolated from seawater on the southern coast of South Korea and found to be phylogenetically affiliated to the genera *Roseovarius* and *Donghicola* on the basis of comparative 16S rRNA gene sequence analysis. This study describes a polyphasic characterization, which included determination of the chemotaxonomic and other phenotypic properties, a detailed phylogenetic investigation based on 16S rRNA gene sequences and genetic analysis, to determine the exact taxonomic position of strain GSW-M15^T.

Materials and methods

Bacterial strains and culture conditions

Seawater was collected from the coast of Geojedo, South Korea, and used as the source for the isolation of bacterial strains. Strain GSW-M15^T was isolated by means of the standard dilution plating technique at 25 °C on marine agar 2216 (MA; Becton–Dickinson). Strain GSW-M15^T was maintained on MA at 4 °C for short-term preservation and as a glycerol suspension (20 %, w/v in distilled water) at −80 °C for long-term preservation. Strain GSW-M15^T has been deposited in the Korean Collection for Type Cultures (KCTC; South Korea) and the Culture Collection, University of Göteborg (CCUG; Sweden) as KCTC 23897^T and CCUG 62218^T, respectively. *R. halotolerans* KCTC 22224^T and *R. pacificus* LMG 24575^T, which were used as reference strains for fatty acid analysis, phenotypic characterization and DNA–DNA hybridization, were obtained from the Korean Collection for Type Cultures (KCTC), Daejeon, South Korea, and Laboratorium voor Microbiologie Universiteit Gent (LMG), Gent, Belgium, respectively.

Cell biomass of strain GSW-M15^T for DNA extraction and for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown for 3 days in marine broth 2216 (MB; Becton–Dickinson) at 30 °C. For fatty acid methyl ester analysis, cell mass of strain GSW-M15^T, *R. halotolerans* KCTC 22224^T and *R. pacificus* LMG 24575^T was harvested from MA plates after incubation for 3 days at 30 °C.

Morphological, physiological and biochemical characterization

The morphological, physiological and biochemical characteristics of strain GSW-M15^T were investigated using routine cultivation on MA at 30 °C. The cell morphology was examined by light microscopy (Olympus BX51) and transmission electron microscopy (Philips CM-20). The latter technique was also used to assess the presence of flagella on cells from exponentially growing cultures. For this purpose, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Strain GSW-M15^T was cultured at various temperatures (4, 10, 20, 25, 28, 30, 35, 37, 40 and 45 °C) to measure optimal temperature and temperature range for growth. The pH range for growth was determined in MB adjusted to pH 4.5–9.5 (using increments of 0.5 pH unit) by using sodium acetate/acetic acid and Na_2CO_3 buffers. The pH values were verified after autoclaving. Growth in the presence of 0–20.0 % (w/v) NaCl was investigated by using marine broth prepared according to the formula of the Becton–Dickinson medium except that NaCl was excluded. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber (1029; Forma, N_2:CO_2:H_2, 86:7:7 %) on MA and on MA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under nitrogen atmosphere. Catalase and oxidase activities were investigated as described by Cowan and Steel (1965). Hydrolysis of casein, starch, hypoxanthine, L-tyrosine and xanthine was tested on MA by using the substrate concentrations described by Cowan and Steel (1965). Hydrolysis of aesculin and Tweens 20, 40, 60 and 80 and nitrate reduction were

investigated as described previously (Lányí 1987) with the modification that artificial seawater was used for preparation of media. Hydrolysis of gelatin and urea was investigated by using Nutrient gelatin and Urea agar base media (BD), respectively, with the modification that artificial seawater was used for the preparation of media. The artificial seawater contained (l^{-1} distilled water): 23.6 g NaCl, 0.64 g KCl, 4.53 g $MgCl_2 \cdot 6H_2O$, 5.94 g $MgSO_4 \cdot 7H_2O$ and 1.3 g $CaCl_2 \cdot 2H_2O$ (Bruns et al. 2001). For analysis of pigments, the absorption spectrum was determined for strain GSW-M15^T that was cultivated aerobically in the dark at 30 °C in MB. The culture was centrifuged, washed twice using a MOPS buffer (MOPS/NaOH 0.01 M; KCl 0.1 M; $MgCl_2$ 0.001 M; pH 7.5) and disrupted by sonication with a Branson Sonifier 450. After removal of cell debris by centrifugation, the absorption spectrum of the supernatant was examined in 200–950 nm on a Beckman Coulter DU800 spectrophotometer. Utilization of substrates as sole carbon and energy sources was tested as described by Baumann and Baumann (1981) using supplementation with 2 % (v/v) Hutner's mineral base (Cohen-Bazire et al. 1957) and 1 % (v/v) vitamin solution (Staley 1968). Susceptibility to antibiotics was tested on MA plates using antibiotic discs (Advantec) containing the following (μg per disc unless otherwise stated): ampicillin (10), carbenicillin (100), cephalothin (30), chloramphenicol (100), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), novobiocin (5), oleandomycin (15), penicillin G (20 U), polymyxin B (100 U), streptomycin (50) and tetracycline (30). Enzyme activities were determined, after incubation for 8 h at 30 °C, by using the API ZYM system (bioMérieux).

16S rRNA gene sequencing and phylogenetic analysis

Chromosomal DNA was extracted and purified according to the method described previously (Yoon et al. 1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene amplification was performed using two universal primers (5′-GAGTTTGATCCTGGCTCAG-3′ and 5′-ACGGTTACCTTGTTACGACTT-3′) as described previously (Yoon et al. 1998) and the PCR products were purified by using a QIAquick PCR purification kit (Qiagen). Sequencing of the amplified 16S rRNA gene was performed as described by Yoon et al. (2003). Alignment of sequences was carried out with CLUSTAL W software (Thompson et al. 1994). Gaps at the 5′ and 3′ ends of the alignment were omitted from further analysis. Phylogenetic trees were inferred by using three tree-making algorithms, the neighbour-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Kluge and Farris 1969) methods implemented within the PHYLIP package (Felsenstein 1993). Evolutionary distance matrices for the neighbour-joining method were calculated by the algorithm of Jukes and Cantor (1969) using the program DNADIST. The stability of relationships was assessed by a bootstrap analysis based on 1,000 resampling of the neighbour-joining dataset by using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package.

DNA–DNA hybridization

DNA–DNA hybridization was performed fluorometrically by the method of Ezaki et al. (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values are quoted as DNA–DNA relatedness values. The DNAs of strain GSW-M15^T, *R. halotolerans* KCTC 22224^T and *R. pacificus* LMG 24575^T were used individually as labelled DNA probes for reciprocal hybridization.

Chemotaxonomic characterization

Isoprenoid quinones were extracted according to the method of Komagata and Suzuki (1987) and analyzed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6-mm) column. The isoprenoid quinones were eluted by a mixture of methanol/isopropanol (2:1, v/v), using a flow rate of 1 ml min^{-1} at room temperature and detected by UV absorbance at 275 nm. Fatty acids were saponified, methylated and extracted using the standard protocol of the MIDI (Sherlock Microbial Identification System, version 4.0). The fatty acids were analysed by GC (Hewlett Packard 6890) and identified by using the TSBA40 database of the Microbial Identification System

(Sasser 1990). Polar lipids were extracted according to the procedures described by Minnikin et al. (1984) and separated by two-dimensional TLC using chloroform/methanol/water (65:25:3.8, v/v) for the first dimension and chloroform/methanol/acetic acid/water (40:7.5:6:1.8, v/v) for the second dimension as described by Minnikin et al. (1977). Individual polar lipids were identified by spraying with the ethanolic molybdophosphoric acid, molybdenum blue, ninhydrin and α-naphthol reagents (Minnikin et al. 1984; Komagata and Suzuki 1987) and the Dragendorff's reagent (Sigma). The DNA G+C content was determined by the method of Tamaoka and Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC equipped with a YMC ODS-A (250 × 4.6-mm) column. The nucleotides were eluted by a mixture of 0.55 M $NH_4H_2PO_4$ (pH 4.0) and acetonitrile (40:1, v/v), using flow rate of 1 ml min^{-1} at room temperature and detected by UV absorbance at 270 nm.

Results and discussion

Morphological, cultural, physiological and biochemical characteristics

Strain GSW-M15^T was found to be aerobic, gram-negative, non-spore-forming and ovoid- to rod-shaped. Strain GSW-M15^T was non-motile, whereas its two closest phylogenetic neighbours, *R. halotolerans* KCTC 22224^T and *R. pacificus* LMG 24575^T, are non-motile and motile, respectively (Oh et al., 2009; Wang et al., 2009). Strain GSW-M15^T grew optimally at 30 °C and pH 7.0–7.5. Strain GSW-M15^T is a moderate halophile as it grew optimally in the presence of 2 % (w/v) NaCl; it grew in the presence of 0.5–15 % NaCl. Strain GSW-M15^T showed catalase and oxidase activities but no urease activity. Strain GSW-M15^T did not produce bacteriochlorophyll *a* and reduced nitrate to nitrite. Morphological, cultural, physiological and biochemical characteristics of strain GSW-M15^T are given in the species description (see below) or in Table 1.

Phylogenetic analysis

The almost complete 16S rRNA gene sequence of strain GSW-M15^T determined in this study comprised

Table 1 Phenotypic characteristics of *Roseovarius litoreus* GSW-M15^T and the type strains of *Roseovarius halotolerans* and *Roseovarius pacificus*

Characteristic	1	2	3
Motility	−	−	+
Nitrate reduction[a]	+	−	−
Hydrolysis of[b]			
Tween 80	−	−	+
Utilization of[a]			
Citrate	−	+	+
L-Malate	−	+	+
Pyruvate	−	+	+
Succinate	−	+	+
Susceptibility to[b]			
Ampicillin	+	+	−
Gentamicin	+	−	w
Neomycin	+	−	w
Novobiocin	+	−	−
Oleandomycin	w	−	−
Penicillin G	+	+	−
Streptomycin	+	−	−
Enzyme activity (API ZYM)[b]			
Alkaline phosphatase	w	−	+
Lipase (C 14)	w	−	−
Naphthol-AS-BI-phosphohydrolase	−	+	+
DNA G + C content (mol%)	62.9	58.9–59.1	62.3

Symbol: (+) positive reaction, (−) negative reaction, *w* weakly positive reaction

[a] Data of reference strains taken from this study

[b] Data of reference strains taken from Kim et al. (2011)

Strains: *1. R. litoreus* GSW-M15^T; *2. R. halotolerans* KCTC 22224^T; *3. R. pacificus* LMG 24575^T. Data for reference strains were taken from Oh et al. (2009) and Wang et al. (2009) unless indicated otherwise. All strains are positive for catalase and oxidase activities; growth at 10 and 40 °C[a]; hydrolysis of[b] Tweens 20, 40 and 60; utilization of[a] acetate; susceptibility to[b] carbenicillin, cephalothin, chloramphenicol and kanamycin; and activity of[b] esterase (C 4), esterase lipase (C 8) and leucine arylamidase. All strains are negative for Gram staining; growth at 4 °C[a]; production of bacteriochlorophyll *a*; hydrolysis of[b] aesculin, casein, gelatin, hypoxanthine, xanthine, starch and L-tyrosine; utilization of[a] L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, sucrose, D-trehalose, D-xylose, benzoate, formate, salicin and L-glutamate; susceptibility to[b] lincomycin, polymyxin B and tetracycline; and activity of[b] valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase

1,389 nucleotides (approximately 96 % of the *Escherichia coli* 16S rRNA sequence). In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain GSW-M15^T clustered with the type strains of *R. halotolerans* and *R. pacificus* (Fig. 1). The relationships among strain GSW-M15^T, *R. halotolerans* KCTC 22224^T and *R. pacificus* LMG 24575^T were also maintained in the trees constructed using the maximum-likelihood and maximum-parsimony algorithms (Supplementary Figs. 1, 2). The maximum-likelihood and maximum-parsimony phylogenetic trees revealed that strain GSW-M15^T fell also within the clade comprising *Roseovarius* species (Supplementary Figs. 1, 2). Strain GSW-M15^T exhibited highest 16S rRNA gene sequence similarity values of 98.3 and 97.5 % to the type strains of *R. halotolerans*

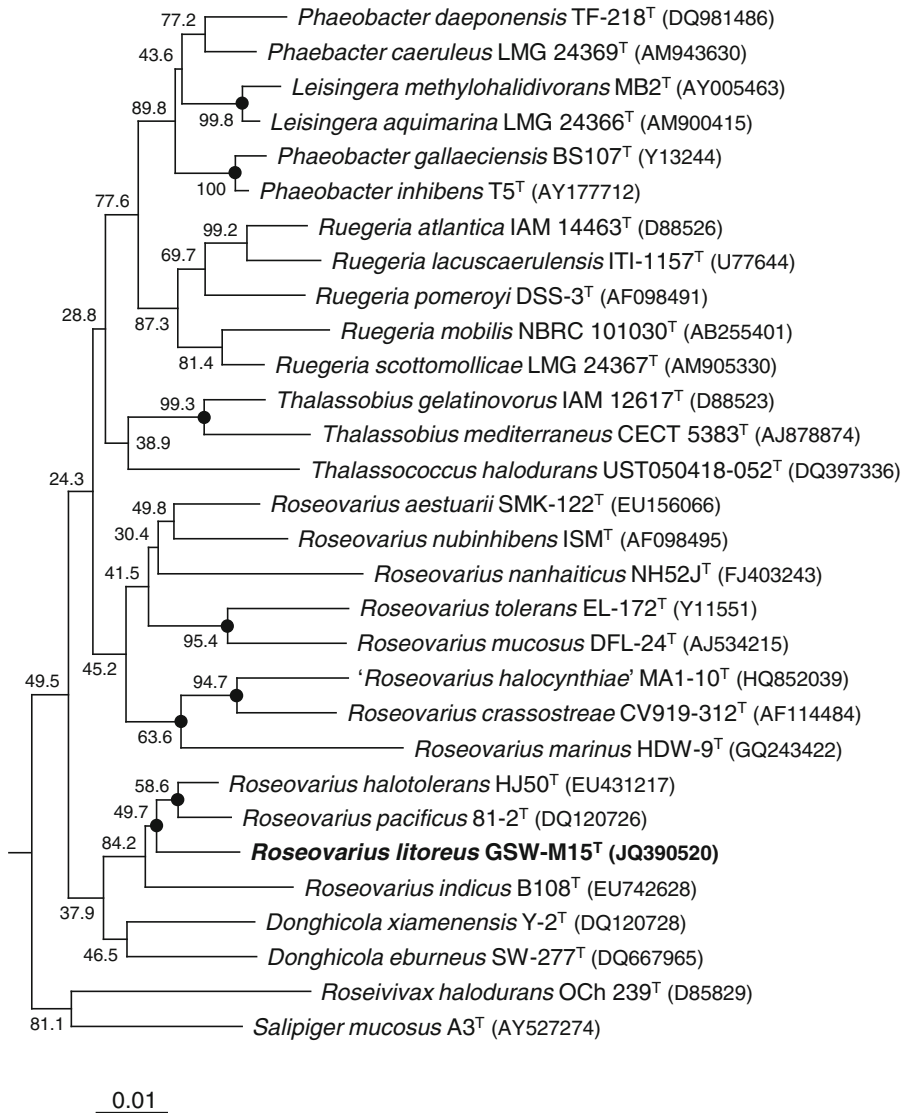

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Roseovarius litoreus* GSW-M15^T, other *Roseovarius* species and representatives of some other related taxa. Bootstrap values (expressed as percentages of 1,000 replications) are shown at branching points. *Filled circles* indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Altermonas marina* SW-47^T (GenBank Accession No: AF529060) was used as an outgroup (not shown). *Scale bar* 0.01 substitutions per nucleotide position

and *R. pacificus*, respectively. Strain GSW-M15^T exhibited slightly higher 16S rRNA gene sequence similarity values (96.6 and 96.2 %) to the type strains of *Donghicola xiamenensis* and *Donghicola eburneus* than to the type strains of the other *Roseovarius* species, with which it exhibited 92.8–96.2 % sequence similarity. In view of this and the close relationship of strain GSW-M15^T to the type strains of *R. halotolerans* and *R. pacificus* (Fig. 1; Supplementary Figs. 1, 2), these three species may represent a novel genus but further studies with additional strains would be needed to confirm this.

DNA–DNA relatedness

Strain GSW-M15^T exhibited mean DNA–DNA relatedness values of 33 ± 7 and 18 ± 5 % to *R. halotolerans* KCTC 22224^T and *R. pacificus* LMG 24575^T, respectively.

Chemotaxonomic characteristics

The predominant isoprenoid quinone detected in strain GSW-M15^T was ubiquinone-10 (Q-10) which is compatible with other *Roseovarius* species (Labrenz et al. 1999; Yoon et al. 2008, Jung et al. 2011). The cellular fatty acid profiles of strain GSW-M15^T, *R. halotolerans* KCTC 22224^T and *R. pacificus* LMG 24575^T, which were analyzed in this study, are compared in Table 2. The major fatty acids (>10 % of the total fatty acids) found in strain GSW-M15^T were $C_{18:1}$ *ω7c* and 11-methyl-$C_{18:1}$ *ω7c* (Table 2). The fatty acid profile of strain GSW-M15^T was similar with those of the two reference strains and of the type strain of *R. tolerans* (type species of the genus *Roseovarius*) analyzed previously under the same conditions (Yoon et al. 2008), even though there were differences in the proportions of some fatty acids. The major polar lipids detected in strain GSW-M15^T were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, one unidentified aminolipid and two unidentified lipids (Supplementary Fig. 3). This profile was similar with that of the type strain of *R. tolerans* shown by Kim et al. (2011) in that phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, one unidentified aminolipid and two unidentified lipids exist as major polar lipids, although diphosphatidylglycerol, which is major polar lipid in the type strain of *R. tolerans*, was only detected as a minor component in strain GSW-M15^T. The DNA G+C content of strain GSW-M15^T was 62.9 mol%, a value in the range reported for *Roseovarius* species (Wang et al. 2009, 2010; Jung et al. 2011; Lai et al. 2011).

Table 2 Cellular fatty acid compositions (%) of *Roseovarius litoreus* GSW-M15^T and the type strains of *Roseovarius halotolerans* and *Roseovarius pacificus*

Fatty acid	1	2	3
Straight-chain fatty acid			
$C_{10:0}$	–	0.6	–
$C_{12:0}$	0.7	4.3	4.1
$C_{16:0}$	8.4	10.1	5.3
$C_{18:0}$	5.8	3.5	1.0
Unsaturated fatty acid			
$C_{18:1}$ *ω7c*	61.2	60.3	60.8
Hydroxy fatty acid			
$C_{10:0}$ 3-OH	tr	tr	0.6
$C_{12:0}$ 3-OH	0.6	3.9	3.1
$C_{12:1}$ 3-OH	–	2.0	1.6
$C_{16:0}$ 2-OH	1.4	1.9	4.4
Cyclo-$C_{19:0}$ *ω8c*	6.3	4.7	9.4
11-methyl-$C_{18:1}$ *ω7c*	11.1	6.3	8.0
Summed features			
3	tr	tr	0.8
7	0.8	–	–
Unknown fatty acid			
ECL 11.799	2.4	0.6	tr

tr Traces (<0.5 %), *ECL* equivalent chain length, (–) not detected

Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 3 contained iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ *ω7c*. Summed feature 7 contained unknown fatty acid (ECL 18.846), $C_{19:1}$ *ω6c* and/or cyclo $C_{19:0}$ *ω10c*

Strains: 1, *R. litoreus* GSW-M15^T; 2, *R. halotolerans* KCTC 22224^T; 3, *R. pacificus* LMG 24575^T. All data from this study; cells of all strains were grown for 3 days at 30 °C on MA. Fatty acids that represented <0.5 % in all strains were omitted

Conclusion

It is appropriate to classify strain GSW-M15^T as a member of the genus *Roseovarius* as shown by the phylogenetic inference and the absence of differentiating chemotaxonomic properties from *Roseovarius* species, including *R. halotolerans* and *R. pacificus*

(Labrenz et al. 1999; González et al. 2003; Wang et al. 2009, 2010; Jung et al. 2011; Lai et al. 2011). Phenotypic characteristics of strain GSW-M15^T were compared with *R. halotolerans* KCTC 22224^T and *R. pacificus* LMG 24575^T i.e. related species that showed 16S rRNA gene sequence similarity of >97 % (Table 2). Strain GSW-M15^T was distinguishable from *R. halotolerans* KCTC 22224^T and *R. pacificus* LMG 24575^T by differences in several phenotypic characteristics, including motility, nitrate reduction, utilization of some substrates, susceptibility to some antibiotics and activity of some enzymes, most of which were determined under the same conditions and methods (Table 1; Kim et al. 2011). The phylogenetic and genetic distinctiveness of strain GSW-M15^T, together with the differential phenotypic properties, is sufficient to show that this strain is separate from other *Roseovarius* species (Wayne et al. 1987; Stackebrandt and Goebel 1994). Therefore, on the basis of the phenotypic, chemotaxonomic, phylogenetic and genetic data, strain GSW-M15^T is considered to represent a novel species of the genus *Roseovarius*, for which the name *Roseovarius litoreus* sp. nov. is proposed.

Description of *Roseovarius litoreus* sp. nov

Roseovarius litoreus (li.to.re'us. L. masc. adj. *litoreus* of or belonging to the seashore).

Cells are gram-negative, non-spore-forming, non-flagellated and ovoid- to rod-shaped, 0.4–0.9 μm in diameter and 0.7–7.0 μm in length. Colonies on MA are circular, flat to raised, smooth, glistening, grayish yellow in colour and 0.5–1.0 mm in diameter after incubation for 3 days at 30 °C. Optimal growth occurs at 30 °C; growth occurs at 10 and 40 °C, but not at 4 and 45 °C. Optimal pH for growth is between 7.0 and 7.5; growth occurs at pH 5.5, but not at pH 5.0. Optimal growth occurs in the presence of 2 % (w/v) NaCl; growth occurs at 0.5–15.0 % (w/v) NaCl. Mg^{2+} ions are required for growth. Growth does not occur under anaerobic conditions on MA and on MA supplemented with nitrate. Bacteriochlorophyll *a* is not produced. Catalase- and oxidase-positive. Nitrate is reduced to nitrite. Tweens 20, 40 and 60 are hydrolysed, but aesculin, casein, gelatin, hypoxanthine, xanthine, starch, Tween 80 and L-tyrosine are not. Acetate is utilized but L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, sucrose, D-trehalose, D-xylose, citrate, benzoate, formate, L-malate, pyruvate, salicin, succinate and L-glutamate are not. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, novobiocin, penicillin G and streptomycin, but not to lincomycin, polymyxin B and tetracycline. In assays with the API ZYM system, activity of alkaline phosphatase (weak), esterase (C 4), esterase lipase (C 8), lipase (C 14) (weak) and leucine arylamidase are present, but activity of valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. The predominant ubiquinone is Q-10. The major fatty acids (>10 % of the total fatty acids) are $C_{18:1}$ $\omega7c$ and 11-methyl-$C_{18:1}$ $\omega7c$. The major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine, one unidentified aminolipid and two unidentified lipids. The DNA G+C content is 62.9 mol% (determined by HPLC).

The type strain, GSW-M15^T (=KCTC 23897^T = CCUG 62218^T), was isolated from seawater of Geojedo on the South Sea, South Korea.

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