**Roseivivax roseus** sp. nov., an alphaproteobacterium isolated from a solar saltern soil sample

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A pink, Gram-stain-negative, motile, halotolerant bacterium with subpolar flagellum, designated strain BH87090T, was isolated from a saline soil sample collected from the south-west coastal area of South Korea (125° 58′ 58.08″ E 34° 45′ 37.32″ N). The isolate formed opaque pink to red colonies on marine agar plates at 30 °C. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, sulfoquinovosyl diacylglycerol, phosphatidylcholine and one unidentified phospholipid. The sole respiratory quinone was ubiquinone-10 (Q-10). The major cellular fatty acids were C18:1ω7c, C19:0 cyclo ω8c, C16:0 and 11-methyl C18:1ω7c. The genomic DNA G+C content was 61.8 mol%. These chemotaxonomic characteristics were all consistent with specific properties of the genus *Roseivivax*. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate affiliated to the cluster with members of the genus *Roseivivax* in the *Roseobacter* clade, which suggested that the strain belonged to the genus *Roseivivax*. However, the low 16S rRNA gene similarities (93.5–95.3 %) of strain BH87090T with all the members of the genus *Roseivivax* indicated that it represented a novel species of the genus *Roseivivax*. On the basis of phenotypic and genotypic data, strain BH87090T should be classified as a novel species of the genus *Roseivivax*. The name *Roseivivax roseus* sp. nov. is proposed, with strain BH87090T (=DSM 23042T=KCTC 22650T) as the type strain.

The genus *Roseivivax* is a small group of the *Roseobacter* clade of the family *Rhodobacteraceae* in the phylum *Proteobacteria*. At the time of writing, there are six species with validly published names, which were all isolated from saline environments (Suzuki et al., 1999; Park et al., 2010; Chen et al., 2012; Xiao et al., 2012; Wu et al., 2013). Here, we describe another *Roseivivax* strain, BH87090T, discovered from a saline soil sample collected from the southwest coastal area of South Korea (125° 58′ 58.08″ E 34° 45′ 37.32″ N). Based on polyphasic taxonomic studies, a novel species, *Roseivivax roseus* sp. nov., is proposed.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BH87090T is FJ897782.

Two supplementary figures and one supplementary table are available with the online version of this paper.

Strain BH87090T was obtained by the dilution plating method on R2A (DSMZ 830 medium) plates supplemented with 5 % (w/v) NaCl. After seven days of incubation at 28 °C, the single pink colony was picked up and transferred to marine broth 2216 with 1.5 % (w/v) agar (MA; BD Difco) by a series of streaking purifications. The purified culture was maintained on R2A and trypticase soy agar (TSA; DSMZ 545 medium) supplemented with 5 % (w/v) NaCl, and MA slants at 4 °C, and also as suspensions of cells in 20 % (v/v) glycerol at −80 °C and in liquid nitrogen.

The temperature range for growth and the temperature for optimum growth were determined at 0, 4, 10, 28 to 37 (at intervals of 1.0 °C), 40, 45 and 55 °C using marine broth 2216 (MB) and trypticase soy broth (TSB). The pH range for growth was tested at pH 5.0–11.5 (at intervals of 0.5 pH...
unit), using the buffer systems described by Xu et al. (2005). Tolerance to sodium chloride, potassium chloride, magnesium chloride and calcium chloride (1, 3, 5, 7, 10, 13, 15, 20 and 25%, w/v) was tested. Metabolic characteristics were determined using Biolog GEN III MicroPlates, and API 50CH and API ZYM test kits (bioMérieux) according to the manufacturers’ instructions, except that the cell suspension was supplemented with NaCl at a final concentration of 5% (w/v). Catalase and oxidase activities were determined following the procedure described previously (Zhang et al., 2008). The ability of the strain to hydrolyse gelatin and starch and to produce H₂S was tested as described by Smibert & Krieg (1994). Other physiological tests on the strain were carried out according to previously described procedures (Yuan et al. 2008). Strains Roseivivax pacificus 22DY03T, R. halotolerans DSM 15490T, R. halodurans DSM 15395T, R. sediminis YIM D21T, R. isoporae sw-2T and R. lentus KCTC 22708T were included in parallel physiological and biochemical tests.

Strain BH87090T grew well at 28–32 °C, with poor growth at 10 and 37 °C, and no growth at 4 or 40 °C. Growth was observed at initial pH values of between pH 5.5 and 9.0 on R2A or TSA medium, with 3–15% NaCl (w/v), and NaCl could also be substituted with KCl or MgCl₂, 6H₂O. Optimal growth occurred at pH 7.0–7.5 on R2A or TSA with 5–7% NaCl (w/v) at 30 °C. Strain BH87090T was resistant (μg ml⁻¹) to bacitracin (10), erythromycin (10), nystatin (10), penicillin G (10), spectinomycin (10), streptomycin (30) and tetracycline (30). Detailed physiological and biochemical characteristics are given in Table S1 (available in the online Supplementary Material) and in the species description.

Colonial morphology was determined at 30 °C on MA, R2A and TSA media containing 5% (w/v) NaCl. Gram staining was carried out by the standard Gram reaction and observed by light microscopy (Olympus microscope BH-2). Motility of cells was examined using 0.3% (w/v) MB swarming agar and confirmed using Olympus phase-contrast microscopy BX63 with the hanging drop method. Cellular morphology was observed using a JEOL JEM-1010 electron microscope (transmission electron microscopy mode) with cells from exponentially growing cultures on MA. Beforehand, cells were mounted on Formvar-coated copper grids (Electron Microscopy Science), and negatively stained with 2% (w/v) uranyl acetate for 15 s.

Pink colonies with a maximum diameter of 2.1 mm were formed on MA, R2A and TSA [containing 5% (w/v) NaCl] plates after 72 h of growth. Colonies were opaque with a moist surface. No diffusible pigment was produced on any of the media tested. Cells of BH87090T were Gram-stain-negative, rod-shaped and 0.6–1.0 μm x 1.1–2.1 μm, with subpolar flagellum (Fig. S1).

Biomass for chemical and molecular studies was obtained by cultivation in shaken flasks (about 150 r.p.m.) using TSB medium containing 5% (w/v) NaCl, with incubation at 30 °C for 4 days, during the late exponential growth phase of the cells. Polar lipids were extracted and examined by two-dimensional TLC and identified using previously described procedures (Minnikin et al., 1984). The respiratory quinone was isolated, purified and analysed as described by Lee et al. (2001). Analysis of the whole-cell fatty acid pattern followed the methods described using the MIDI system (Microbial ID) (Kroppenstedt, 1985; Meier et al. 1993). Pigment characterization examination was carried out as described by Reddy et al. (2006).

The polar lipids detected were diphasphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, sulfoquinovosyl diacylglycerol, phosphatidylcholine and one unidentified phospholipid (Fig. S2). The sole respiratory quinone was ubiquinone-10 (Q-10). The cellular fatty acid profile showed the major fatty acids present to be C₁₈ : 1ω₇c (64.9%), C₁₉ : 0 cyclo ω₈c (13.6%), C₁₆ : 0 (6.9%) and 11-methyl C₁₈ : 1ω₇c (6.5%), with trace amounts of hydroxyl fatty acids. Methanolic extracts of the pigments of strain BH87090T exhibited no obvious absorption peak at 800 nm—850 nm and 875 nm, which indicated that strain BH87090T did not contain bacteriochlorophyll a.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene were conducted as described by Li et al. (2007). Purified PCR products were sequenced with an ABI PRISM automated sequencer. The sequence obtained was compared with available 16S rRNA gene sequences from GenBank using the BLAST program and the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012) to determine an approximate phylogenetic affiliation. Multiple alignments with sequences of the most closely related taxa and calculations of levels of sequence similarity were carried out using MEGA version 5.0 (Tamura et al., 2011). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987) from K$_{\text{vac}}$ values (Kimura, 1980, 1983), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) methods with MEGA version 5.0 (Tamura et al., 2011; Kumar et al., 2008). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The DNA base composition was determined by reverse-phase HPLC of nucleosides according to Mesbah et al. (1989).

The genomic DNA G+C content was 61.8 mol%. Sequence similarities of strain BH87090T with type strains of recognized species of the genus Roseivivax were in the range of 93.5–95.3%, and all of the values were below 97.0%. In the phylogenetic tree (Fig. 1), based on the 16S rRNA gene sequences, the new isolate fell within the Roseivivax clade, forming a clade with R. pacificus 22DY03T, R. sediminis ACCC 10710T, R. halotolerans DSM 15490T, R. halodurans DSM 15395T and R. isoporae BCRC 17966T, which suggests the classification of this strain as a different species of the genus Roseivivax (Stackebrandt & Goebel, 1994). This proposal was also supported by the chemotaxonomic, morphological and some biochemical properties. C₁₈ : 1ω₇c, C₁₉ : 0 cyclo ω₈c, C₁₆ : 0 and 11-methyl...
C<sub>18:1ω7c</sub> were present as the main components of the cellular fatty acid profile, with C<sub>18:1ω7c</sub> the predominant one, Q-10 was the respiratory quinone, and phosphatidylglycerol and phosphatidylethanolamine the diagnostic polar lipids, also suggesting that strain BH87090<sup>T</sup> should be a member of the genus _Roseivivax_. However, strain BH87090<sup>T</sup> could not utilize formate, L-arginine, L-serine, N-acetylglucosamine or succinate as the sole carbon and energy source, nor could it hydrolyse urea, which served to distinguish it from its nearest phylogenetic neighbour, _Roseivivax pacificus_ 22DY03<sup>T</sup>.

Based on the data above, we propose that strain BH87090<sup>T</sup> represents a novel species of the genus _Roseivivax_, with the name _Roseivivax roseus_ sp. nov.

**Description of _Roseivivax roseus_ sp. nov.**

_Roseivivax roseus_ (ro’se.u.s. L. masc. adj. roseus rose-coloured, pink).

Colonies are smooth, slightly convex, opaque and pink, with a maximum diameter of 2.1 mm. No diffusible pigment is produced on any media tested. Cells are Gram-strain-negative, rod-shaped, 0.6–1.0 μm × 1.1–2.1 μm, and motile with a subpolar flagellum. Optimum growth occurs in R2A or TSA at pH 7.0–7.5, with 5–7 % NaCl (w/v) and at 30 °C. Positive for reduction of nitrate and catalase activity, but negative for oxidase, methyl red and Voges–Proskauer tests, gelatin hydrolysis, casein and starch degradation, and H<sub>2</sub>S production. Resistant (μg ml<sup>–1</sup>) to bacitracin (10), erythronin (10), nystatin (10), penicillin G (10), spectinomycine (10), streptomycin (30) and tetracycline (30). Acetate, citrate, D-arabitol, cellobiose, dextrin, D-fructose, D-fructose, D-galactose, D-glucosamine, D-mannose, melibiose, raffinose, D-salicin, D-sorbitol, trehalose, glycerol, L-alanine, maltose, _myo_-inositol, propionate, succrose, α-D-glucose and α-lactose can be utilized as the sole carbon sources, but formate, L-arabinose, L-serine, N-acetylglucosamine and succinate cannot be assimilated. Acid is produced from arbutin, cellobiose, D-glucose, fucose, gentiobiose, salicin and potassium 5-ketogluconate. In API ZYM strips, acid and alkaline phosphatases, esterase (C4), esterase lipase (C8), α- and β-D-glucosidases, α- and β-glucosaminidase, α-D-glucuronidase, β-naphthol-AS-BI-phosphohydrolase and valine arylamidase are positive, while cystein arylamidase, leucine aminopeptidase, naphthol AS-BI-phosphohydrolase and valine arylamidase are positive, with cysteine arylamidase, lipase (C14), N-acetyl-β-glucosaminidase, α-chymotrypsin, α-mannosidase, β-fucosidase, β-glucuronidase and trypsin activities are negative. The polar lipid profile consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, sulfoquinovosyl diacylglycerol, phosphatidylcholine and one unidentified phospholipid. The sole respiratory quinone is ubiquinone-10 (Q-10). The major cellular fatty acids are C<sub>18:1ω7c</sub>, C<sub>19:0 cyclo ω8c</sub>, C<sub>16:0</sub> and 11-methyl C<sub>18:1ω7c</sub>.
The type strain, BH87090T (＝DSM 23042T＝KCTC 22650T), was isolated from a saline soil sample collected from the south-west coastal area of South Korea. The genomic DNA G+C content of the type strain is 61.8 mol%.

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References


