**Roseomonas rosulenta** sp. nov., isolated from rice paddy soil

Hyo-Jin Lee1,2 · Kyung-Sook Whang1,2

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**Abstract**

Three bacterial isolates, Gram-stain-negative, non-motile, cocccobacilli-shaped bacteria, strains OP-27\(^T\), OP-5 and OP-30, were isolated from rice paddy soil. Phylogenetic analyses based on 16S rRNA gene sequences revealed that three isolates belonged to the genus *Roseomonas*, showing the highest sequence similarities to *Roseomonas sediminicola* FW-3\(^T\) (98.1%) and *Roseomonas lacus* TH-G33\(^T\) (98.0%). The genome size of strain OP-27\(^T\) was 5.2 Mb in a single contig with DNA G+C content of 71.2%. The genome included 5164 predicted protein-coding genes, as well as 48 tRNA, 4 rRNA and 4 mRNA genes. The average nucleotide identity value between strain OP-27\(^T\) and type strains of related species of the genus *Roseomonas* were 81.1–83.1%, and the digital DNA–DNA hybridization values of strain OP-27\(^T\) and the related strains were 24.6–26.8%, respectively. The DNA–DNA hybridization values between strains OP-27\(^T\), OP-5 and OP-30 were 84–100% and its closest relative, *Roseomonas sediminicola* KACC 16616\(^T\) was 21.1%. The major fatty acids were C\(_{18:1}\) \(\omega_7\) c, C\(_{18:1}\) 2-OH and C\(_{16:0}\) and predominant quinone was Q-10. Based on its distinctive phenotypic, phylogenetic, and chemotaxonomic characteristics, the three strains are considered to represent novel species of the genus *Roseomonas*, for which the name *Roseomonas rosulenta* sp. nov. is proposed. The type strain is OP-27\(^T\) (=KACC 21501\(^T\)= NBRC 114497\(^T\)).

**Keywords** PPB · *Roseomonas* · Novel species · Rice paddy soil

**Introduction**

The genus *Roseomonas* was first classified by Rihs et al. (1993). Currently, 50 species of the genus *Roseomonas* have been reported (https://lpsn.dsmz.de/genus/Roseomonas) and isolated from various environmental habitats (air, freshwater sediment, plants, soil, surfaces and water etc.). Most members of the genus *Roseomonas* are Gram stain negative, red, orange, pink, yellow, white, brown pigmented, cocccobacilli shaped and occasionally rod shaped (Yoon et al. 2007; Ramana et al. 2010; Subhash et al. 2016, 2017; Wang et al. 2016). Among them, 42 strains are pink pigmented bacteria. In the previous study, we isolated purple phototrophic bacteria (PPB) from paddy soil samples managed long-term for over 20 years with different chemical fertilizer. Researchers have tried to improve cultivation methods to isolate diverse and novel PPB in rice paddy soil. We succeeded in isolating purple phototrophic bacteria belonging to diversity of genera. Phylogenetic analysis indicated that some isolates belonged to be a member of novel genera and species. Currently, there are published data on four novel species (Lee and Whang 2020, 2022; Cho and Whang 2021). In this study, we propose that strains OP-27\(^T\), OP-5 and OP-30 represent a novel species of the genus *Roseomonas* based on polyphasic taxonomic approach.

**Materials and methods**

**Isolation and culture conditions**

Strains OP-27\(^T\), OP-5 and OP-30 were isolated from rice paddy soil collected from Cheongju, Chungbuk in the Republic of Korea (36° 43’ 38.1” N 127° 27’ 47.6” E). These strains were isolated from the PSBA medium [sodium lactate 10 ml\(^{-1}\), sodium glutamate 1.1 g\(^{-1}\), K\(_2\)HPO\(_4\) 1.0 g, ...
KH₂PO₄ 0.5 g l⁻¹, yeast extract 2.0 g l⁻¹, MgSO₄·7H₂O 0.2 g l⁻¹, CaCl₂·2H₂O 0.075 g l⁻¹, sodium-EDTA 0.02 g l⁻¹, FeSO₄·7H₂O 0.012 g l⁻¹ and agar 0.8% (w/v, pH 6.8), and the isolation method as described in the Lee and Whang 2020. The deep rose or pink pigmented strains, designated OP-27ᵀ, OP-5 and OP-30, were picked up and purified by repeated streaking on Reasoner’s 2A (R2A) agar (BD Difco). The strains were stored at −80 °C on this medium without agar and supplemented with 20% (v/v) glycerol solution. Strains OP-27ᵀ, OP-5 and OP-30 phenotypically, the isolates were routinely grown aerobically on R2A agar for 5 days at 30 °C. The reference type strains, Roseomonas sediminicola KACC 16616ᵀ, Roseomonas lacus KACC 11678ᵀ, Roseomonas soli KACC 16376ᵀ, Roseomonas eburnea KACC 17166ᵀ, Roseomonas alkaliterra KACC 18530ᵀ, Roseomonas oryzicola KCTC 22478ᵀ and Roseomonas terrae KACC 12677ᵀ were obtained from the KACC and the KCTC. These strains were cultured under the same conditions for comparative phenotypic testing.

Phylogenetic analysis and genome information

The genomic DNA of strains OP-27ᵀ, OP-5 and OP-30 were extracted using the Genomic DNA Prep Kit for Bacterium, Cultured Cell (BIOFACT). The 16S rRNA gene sequence was amplified by PCR using the universal primers 27F, and 1492R (Lee et al. 2012). The identification of phylogenetic neighbors and calculations of pairwise 16S rRNA gene sequence similarity was determined using the EzBioCloud server (www.ezbiocloud.net/identify) (Yoon et al. 2017). The 16S rRNA gene sequence was aligned with the published sequences of closely related bacteria with ClustalW 2.1 software (Larkin et al. 2007). Phylogenetic trees were reconstructed by the neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971), algorithms within the MEGA 7.0 program (Kumar et al. 2016). Evolutionary distance matrices for the neighbor-joining method were calculated using the algorithm of Kimura’s two-parameter model (Kimura 1980). To evaluate the stability of the phylogenetic tree, a bootstrap analysis was performed based on 1000 replications (Felsenstein 1985). To determine genomic relatedness, DNA–DNA hybridization (DDH) was performed using the modified method of Ezaki et al. (1989). Probe labeling for DDH was conducted using the non-radioactive DIG-High prime system (Roche); hybridized DNA was visualized using the DIG luminescent detection kit (Roche) and the level of DNA–DNA relatedness was quantified using a densitometer (Bio-Rad). The genome sequence of strain OP-27ᵀ was determined using the Illumina Miseq 300 platform at ChunLab. Illumina reads was assembled by SPAdes 3.13.0 (Bankevich 2012) to obtain the draft genomes. The Rapid Annotation using Subsystem Technology (RAST) (Aziz et al. 2008) server was used for the genome annotation. The average nucleotide identity (ANI) score was by calculated using the ANI calculator (www.ezbiocloud.net/tools/ani) (Lee et al. 2016). In silico digital DNA-DNA hybridization (dDDH) were calculated using Genome-to-Genome Distance Calculator 3.0 method, with the recommended formula 2, available at the TYGS web service, respectively (Meier-Kolthoff and Göker 2019).

Morphological, physiological and biochemical characteristics

Morphological, physiological and biochemical characteristics of strains OP-27ᵀ, OP-5 and OP-27 were determined after 5 days at 30 °C using R2A agar. Cell morphology was observed with stereoscopy (Leica EZ4) and transmission electron microscopy (FEI Tecnai G2 spirit TWIN) of cultures on R2A agar incubated at 30 °C for 5 days. Growth at different temperatures (4, 10, 15, 20, 25, 30, 37, 40 and 45 °C) was examined using R2A agar for 10 days. The pH ranges for growth at pH 4.0–11.0 (in increments of 0.5 pH units, adjusted with HCl or NaOH, 1 M, respectively) was assessed in R2A broth after 10 days. The tolerance range for salts was tested at 0, 0.5, 1.0, 2.0, 3.0, 5.0 and 7.0% NaCl (w/v) in R2A broth at pH 7.0. Gram staining was performed by the Hucker method (Gerhardt et al. 1994). Motility was determined by the hanging-drop method. Catalase activity was determined by assessing bubble production in 3.0% (w/v) H₂O₂. Oxidase activity was tested using oxidase reagent (bioMérieux) according to the instructions of the manufacturer. Enzyme activity and other biochemical characteristics were tested using API 20NE and API ZYM test kits (bioMérieux) at 30 °C for 5 days.

Chemotaxonomic analysis

Cellular fatty acids were extracted from cells grown on R2A agar for 5 days at 30 °C. The cellular fatty acid pattern analysis was performed as described by Sasser (1990), using the Microbial Identification System (Sherlock Version 4.5; MIDI database: TSBA 40). For chemotaxonomic analysis, freeze-dried cells were obtained from a culture grown in R2A agar for 10 days at 30 °C. The isoprenoid quinones were extracted as described by Collins (1985) and analyzed by high-performance liquid chromatography (SPD-10AV; Shimadzu) (Kroppenstedt 1982). Polar lipids were examined by two-dimensional thin-layer chromatography and identified according to the method of Minnikin et al. (1984).
Results and discussion

Phylogenetic analysis and genome information

Phylogenetic analyses based on 16S rRNA gene sequences showed that the strains OP-27T, OP-5, OP-30 belongs to the genus *Roseomonas* and it was closely related to *Roseomonas sediminicola* FW-3T (98.1%) and *Roseomonas lacus* TH-G33T (98.0%). The 16S rRNA gene sequences of strain OP-27 were the same as those of strains OP-5 and OP-30. In the maximum-likelihood tree, strains OP-27T, OP-5 and OP-30 clustered with members of the genus *Roseomonas* and were most closely related to *R. lacus* TH-G33T and *R. terrae* DS-48T (Fig. 1). The phylogenetic position in the neighbor-joining (Fig. S1) and maximum-parsimony (Fig. S2) trees showed that strains OP-27, OP-5 and OP-30 monophyletic cluster with recognized members of the genus *Roseomonas*. The complete genome of strain OP-27T obtained 671 contigs, with a total length of 5,277,075 bp and an N50 length of 15,298 bp (GenBank accession no. JACADR000000000). A total of 5438 genes were predicted, which included 5164 protein-coding genes and 56 RNA genes (Table S1). As the result of categorizing functional genes using the RAST subsystem, 5735 genes were predicted as coding sequence by the RAST server; 1198 CDSs (21%) were in the subsystem and 4537 CDSs (79%) were not in the subsystem. According to the functional subsystem distribution, a total of 1147 genes were counted as subsystem features. Strain OP-27T had the photosynthetic genes for chlorophyllide synthase subunit encoding genes (bchF, bchJ, bchX, bchY and bchZ) in the chromosome. The DNA G+C content of strain OP-27T was 71.2 mol% obtained from the genomic sequence. The ANI values between strain OP-27T and *R. lacus* CGMCC 1.3617T, *R. soil* LMG 31231T, *R. eburnea* LMG 31228T, *R. alkaliterra* DSM 25895T, *R. oryzae* KCTC 22478T, *R. rubra* MO17T and *R. terrae* LMG 31159T were 81.1–83.1%, which are lower than the 95–96% cut-off values previously proposed for species

![Phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of strains OP-27T, OP-5 and OP-30 among closely related members of the genus *Roseomonas*. The tree was reconstructed using the maximum-likelihood method. Numbers at the branch nodes are bootstrap values (> 50%), expressed as a percent-age of 1000 replicates. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-parsimony and neighbor-joining algorithms. Bar, 0.01 substitutions per nucleotide position](image-url)
delimitation (Richter and Rosselló-Móra 2009). In addition, digital DNA–DNA relatedness values between strain OP-27T and the type strains of *Roseomonas* species were 24.6–26.8%, respectively (Table S2). The DNA–DNA relatedness values for strains OP-27T, OP-5 and OP-30 were within the range 84–100% and were identified as the same strain. The DNA–DNA hybridization values between strain OP-27T and its closest relative, *Roseomonas sediminicola* KACC 16616T was 21.1% and related species of the genus *Roseomonas* were 19.2–31.2%, which are lower than the 70% proposed threshold for species designation (Wayne et al. 1987). These results further suggest also that strain OP-27T represents a novel species of the genus *Roseomonas*.

**Morphological, physiological and biochemical characteristics**

The morphological characteristics of strains OP-27T, OP-5 and OP-30 by stereoscopy and transmission electron microscopy of 5-day cultures on R2A agar. Colonies are circular with smooth, convex, with entire margins and deep pink or rose on R2A agar. Cells are Gram stain negative, aerobic, non-motile without flagella and coccobacilli shaped (1.4–1.6 × 2.5–2.9 μm in size) (Fig. S4). The results of the physiological and biochemical analyses are presented in Table 1. Negative results in API 20NE and API ZYM tests are listed in Table S3.

**Chemotaxonomic analysis**

The cellular fatty acids (> 10%) of strains OP-27T, OP-5 and OP-30 were C<sub>18:1</sub>ω7c (50.0, 47.7, 51.9%), C<sub>18:1</sub>2OH (23.4, 23.2, 21.6%) and C<sub>16:0</sub> (9.9, 10.6, 9.5%). The cellular fatty acid profiles of the three novel strains and their most closely related type strains of *Roseomonas* species are presented in Table S4. The predominant menaquinone in all strains was Q-10. The polar lipids of strain OP-27 T were identified as diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, one unidentified aminolipid, one unidentified phospholipid and two unknown lipids (Fig. S5).

**Taxonomic conclusion**

In this study, phylogenetic analysis, morphological, physiological and biochemical characteristics, chemotaxonomic analysis, strains OP-27T, OP-5 and OP-30 should be affiliated to the genus *Roseomonas* (Table 1). Moreover, the low ANI values (81.1–83.1%) and DDH values (24.6–26.8%) and the phenotypic and chemotaxonomic properties presented above, we suggest that strains OP-27T, OP-5 and OP-30 is a novel species of the genus *Roseomonas*, and the name *Roseomonas rosulenta* sp. nov. is proposed.

**Description of Roseomonas rosulenta sp. nov**

*Roseomonas rosulenta* (ro.su.len’ta. L. fem. adj. *rosulenta* pink, rose colored, referring to the color of the colonies).

Cells are aerobic, Gram stain negative, aerobic, non-motile and coccobacilli shaped (1.4–1.6 wide and 2.5–2.9 μm long). Colonies on R2A agar are deep rose, smooth, circular, entire edges and convex after 5 days of incubation. Growth occurs at 4–37 °C (optimally at 30 °C) and at pH 4.0–11.0 (optimally at pH 6.0–8.0). Cells tolerate up to 1.0% NaCl in R2A broth. Catalase and oxidase are positive. Nitrate is reduced to nitrite. Negative for indole production, urea, gelatine, aesculin hydrolysis and arginine dihydrolase. In the API 20NE system, positive for adipic acid but negative for D-glucose, L-arabiose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malate, trisodium citrate and phenylacetic acid. In the API ZYM strip, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase and naphthol-AS-BI-phosphohydrolase, weakly positive for α-chymotrypsin but negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-galactosidase, β-glucuronidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. The major fatty acids are C<sub>18:1</sub>ω7c, C<sub>18:1</sub>2OH and C<sub>16:0</sub>. The predominant isoprenoid quinone is Q-10. The polar lipids profile consists of DPG, PG, PE, PC, one unidentified aminolipid, one unidentified phospholipid and two unknown lipids. The genome size is 5.2 Mb and DNA G+C content is 71.2 mol%.

The type strain, OP-27T (= KACC 21501T = NBRC 114497T) which was isolated from rice paddy soil. The GenBank accession numbers of 16S rRNA gene sequence and the whole-genome sequence of strain OP-27T are LC505025 and JACADR0000000000, respectively.
Table 1  Different characteristics between the strains OP-27\textsuperscript{T}, OP-5 and OP-30 and the type strains of related species of the genus *Roseomonas*

| Characteristics | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|-----------------|---|---|---|---|---|---|---|---|---|----|----|
| Habitat         | Paddy soil | Paddy soil | Paddy soil | Freshwater sediment | Freshwater lake sediment | Cabbage rhizosphere | Sludge | Alkaline geothermal soil | Cabbage rhizosphere | Paddy soil | Soil |
| Colony color    | Deep pink | Deep pink | Deep pink | Pale red | Pale red | White | White | White | Pale pink | Pale pink | Pale red | Pink |
| Catalase activity | + | + | + | – | – | + | + | – | – | – | + | + |
| Oxidase activity | + | + | + | – | – | + | + | – | + | + | + | + |
| Nitrate reduction | + | + | + | + | + | + | + | + | – | – | – | – |
| Growth at/with  |   |   |   |   |   |   |   |   |   |   |   |   |
| Temperature (°C) | 4–37 | 4–37 | 4–37 | 10–37 | 15–40 | 15–40 | 15–35 | 40–50 | 10–40 | 4–37 | 10–36 |
| pH              | 4.0–11.0 | 4.0–11.0 | 4.0–11.0 | 5.5–10.0 | 6.0–9.0 | 5.0–8.5 | 3.0–10.0 | 6.0–11.0 | 5.0–10.0 | 4.0–11.0 | 5.0–10.0 |
| Salinity (%, w/v) | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | <6.5 | <1.0 | <8.0 | <2.0 | <2.0 | <1.0 | <2.0 |
| Hydrolysis of   |   |   |   |   |   |   |   |   |   |   |   |   |
| Urea            | – | – | – | – | – | + | – | – | – | + | – | + |
| Utilization of (API 20NE) |   |   |   |   |   |   |   |   |   |   |   |   |
| Potassium gluconate | – | – | – | – | + | – | – | – | – | + | – | – |
| Adipic acid     | + | + | + | + | + | + | + | + | + | + | + | + |
| Malate          | – | – | – | – | – | + | + | + | + | + | + | + |
| Citric acid     | – | – | – | – | – | – | + | + | + | + | + | + |
| Enzyme activity (API ZYM) |   |   |   |   |   |   |   |   |   |   |   |   |
| Leucine arylamidase | – | – | – | – | – | – | – | – | – | – | – | w |
| Acid phosphatase | + | w | w | – | + | w | – | + | + | – | + | w |
| Alkaline phosphate | + | + | + | + | + | w | – | – | – | – | + | + |
| α-chymotrypsin  | w | w | w | – | – | – | – | – | – | – | + | – |
| Naphthol-AS-BI-phosphohydrolase | + | + | + | + | + | + | + | + | + | + | + | + |
| DNA G+C mol %   | 71.2 | ND | ND | 68.0 | 71.9 | 68.3 | 67.6 | 63.0 | 70.5 | 71.2 | 69.3 |   |

Strain: 1 OP-27\textsuperscript{T}, 2 OP-5, 3 OP-30, 4 *R. sediminicola* KACC 16616\textsuperscript{T}, 5 *R. lacus* KACC 11678\textsuperscript{T}, 6 *R. soli* KACC 16376\textsuperscript{T}, 7 *R. eburnea* KACC 17166\textsuperscript{T}, 8 *R. alkaliterrae* KACC 18530\textsuperscript{T}, 9 *R. oryzicola* KCTC 22478\textsuperscript{T}, 10 *R. rubea* MO17\textsuperscript{T}, 11 *R. terrae* KACC 12677\textsuperscript{T}. All data are this study except for growth and DNA G+C contents of strains 4–9, 11 which are from He et al. (2014), Jiang et al. (2006), Kim and Ka (2014), Wang et al. (2016), Dong et al. (2014), Chung et al. (2015), Lee and Whang (2022), Yoon et al. (2007). All strains are positive for activities of esterase (C4) and esterase lipase (C8). All strains are negative for indole production, hydrolysis of gelatin and valine arylamidase.

+ positive activity and/or growth occurs, – no activity and/or no growth, ND not determined/no data available, w weakly positive
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Author contributions HJL: performed experiments, data analysis and wrote the manuscript, HJL and KSW: designed research and revised the manuscript. All authors read and approved the final manuscript.

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Data availability The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains OP-27T, OP-5 and OP-30 are LC505025, LC505024 and LC505026. The GenBank/EMBL/DDBJ accession number for the draft genome sequence of strain OP-27T is JACADR0000000000.

Declarations Conflict of interest The authors declare that there are no conflicts of interest.

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