

Roseomonas vinacea sp. nov., a Gram-negative coccobacillus isolated from a soil sample

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Strain CPCC 100056^T, which was isolated from a soil sample collected from the Qinghai–Tibet plateau, China, was subjected to a polyphasic taxonomic study. The organism was coccobacillus-shaped, non-motile and formed vinaceous colonies on ISP2 agar medium. The respiratory quinone was ubiquinone-10. The major fatty acids were C_{18:1ω7c} and C_{16:1ω7c} and/or C_{16:1ω6c}. The G + C content of the genomic DNA was 67.3 mol%. A comparison of sequences in GenBank revealed that strain CPCC 100056^T exhibited highest 16S rRNA gene sequence similarity (84.5–95.5%) with *Roseomonas* species. Strain CPCC 100056^T could be distinguished from all *Roseomonas* species with validly published names by differences in phenotypic and genotypic properties. In view of the combined phenotypic, chemotaxonomic and phylogenetic data, strain CPCC 100056^T should be classified as a representative of a novel species in the genus *Roseomonas*, *Roseomonas vinacea* sp. nov.; the type strain is CPCC 100056^T (=KCTC 22045^T =CCM 7468^T).

Members of the genus *Roseomonas*, which belong to the class *Alphaproteobacteria*, are Gram-negative coccobacilli with oxidative metabolism that are widely distributed in nature and their colonies have a pink pigmentation (Jiang *et al.*, 2006). Currently, the genus *Roseomonas* comprises the following species: *Roseomonas cervicalis* (Rihs *et al.*, 1993), *Roseomonas gilardii* (Han *et al.*, 2003), divided into *R. gilardii* subsp. *gilardii* and *R. gilardii* subsp. *rosea*, *Roseomonas mucosa* (Han *et al.*, 2003), *Roseomonas lacus* (Jiang *et al.*, 2006), *Roseomonas aquatica* (Gallego *et al.*, 2006) and *Roseomonas terrae* (Yoon *et al.*, 2007). In this study, a vinaceous *Roseomonas*-like coccobacillus, designated CPCC 100056^T, was isolated from a soil sample collected from the Qinghai–Tibet plateau, China. Phenotypic characteristics and phylogenetic analyses

demonstrated that the isolate should be assigned to a novel species of the genus *Roseomonas*.

With the study of extremophiles gaining increasing attention, more and more psychrophiles have been isolated from both permanently and temporarily cold habitats. The Qinghai–Tibet plateau provides a cold environment for such microbes: the altitude is above 4000 m and the temperature seldom or never reaches 10 °C. However, during the course of a screening programme for new antibiotics, rather more non-psychrophiles were discovered from such environments. Strain CPCC 100056^T was isolated by using the dilution plating method on ISP2 agar medium (Shirling & Gottlieb, 1966) at 20 °C from a soil sample collected from the Qinghai–Tibet plateau, where there is very little rain throughout the year and the mean monthly air temperature ranges from 0 °C during January to 7 °C during July. The pH of the tested soil sample was 7.8, with 7.6% NaCl and 5.9% KCl as the main salts. The isolation medium contained [(1 distilled water)⁻¹]: yeast extract (Difco), 4.0 g; malt extract (Difco), 10.0 g; glucose (Difco), 4.0 g; and agar, 15.0 g; pH 7.2. The strain was maintained on ISP2 agar slants at

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CPCC 100056^T is EF368368.

Cellular fatty acid profiles of strain CPCC 100056^T and type strains of related species are available as supplementary material with the online version of this paper.

4 °C and as 20% (w/v) glycerol suspensions at -20 °C. Biomass for chemical and molecular studies was obtained by cultivation in shake flasks (about 150 r.p.m.) using ISP2 broth at 32 °C for 5 days.

Morphology and motility were examined by light microscopy (model BH2; Olympus) and electron microscopy (JEM-1010) using cells from exponentially growing cultures. For transmission electron microscopic observation, cells were negatively stained with 1% (w/v) phosphotungstic acid after air-drying. Colony morphology was observed on ISP2 and ISP5 media (Shirling & Gottlieb, 1966) and R2A after incubation at 32 °C for 5 days. Colony colour was determined with the ISCC-NBS colour charts (Kelly, 1964). Gram staining and the KOH lysis test were carried out according to Gram (1884) and Cerny (1978), respectively. Growth was tested at 0, 4, 10, 20, 28–37 (at intervals of 0.5 °C), 40, 45 and 55 °C on ISP2, trypticase soy agar (TSA) and R2A media. The ability of the strain to grow at different pH (pH 5.0–11.0, at intervals of 0.5 pH units) and NaCl concentrations (0–20%, w/v, at intervals of 0.5%) was examined using ISP2 or R2A (Difco) as basal medium. The following buffer solutions were used to adjust the pH: pH 5.0–8.5, NaOH/KH₂PO₄; pH 9.0–9.5, borax/boric acid; pH 10.0–10.5, borax/NaOH; pH 11.0–11.5, Na₂HPO₄/NaOH; pH 12.0–13.0, KCl/NaOH. Simmons' citrate test was carried out in Simmons' citrate agar (Sigma). Metabolic properties were determined using API 50CH, API 20E and API ZYM test kits (bioMérieux) according to the manufacturer's instructions. The utilization of sugars and other compounds listed in API 50CH and API 20E as carbon sources and acid production from these sugars were reassessed on basal medium (Shirling & Gottlieb, 1966) supplemented with 1% (w/v) of the tested substrate and using the basal medium as a control. Growth was monitored by measuring OD₆₀₀ using an Ultrospec 2000 photometer (Pharmacia Biotech); a decrease in the pH to less than 6.0 was considered as evidence of acid production. Catalase activity was determined by the production of bubbles after the addition of a drop of 3% H₂O₂. The ability of the strain to produce H₂S, to hydrolyse casein, starch and Tweens 20 and 80 and the methyl red and Voges–Proskauer tests were assessed as described by Smibert & Krieg (1981). Susceptibility to antibiotics was determined by using the disc-diffusion plate method. Discs containing the following amounts of antibiotic were tested on R2A medium: amikacin (30 µg), aureomycin (30 µg), ciprofloxacin (10 µg), chloramphenicol (30 µg), erythromycin (15 and 30 µg), gentamicin sulfate (10 µg), kanamycin (15 µg), netilmicin (10 µg), novobiocin (5 and 30 µg), oleandomycin (10 µg), penicillin G (10 U), polymyxin B (10 and 300 U), streptomycin sulfate (10 and 25 µg), terramycin (2.5 and 30 µg), tetracycline (10 and 30 µg), tobramycin sulfate (10 µg) and vancomycin (10 µg).

Cocci bacillus cells were observed for strain CPCC 100056^T, but neither flagella nor spores were found. Vinaceous colonies with smooth surfaces and a maximum

diameter of 1.0 mm were formed on ISP2 or R2A medium after incubation for 3 days at 32 °C. Strain CPCC 100056^T was aerobic, stained Gram-negative and grew well at 30–32 °C on ISP2, TSA, R2A, 5% defibrinated sheep blood agar and nutrient agar, but very slowly at 4 and 40 °C. Growth was observed at initial pH values between 6.5 and 8.0 and on ISP2 and R2A containing 0–2% (w/v) NaCl. The strain grew optimally at pH 7.0–7.5 and in the presence of 0–0.5% (w/v) NaCl. No growth occurred on MacConkey agar. Other physiological and biochemical characteristics of the strain are given in detail in Table 1 and in the species description.

Respiratory quinones were isolated, purified and analysed as described by Lee *et al.* (2001). Analysis of the whole-cell fatty acid pattern was carried out according to the instructions of the MIDI system (Microbial ID) (Kroppenstedt, 1985) using exponential phase cultures on R2A medium. The respiratory quinone was ubiquinone-10 and the major fatty acids were C_{18:1}ω7c (33.6%), C_{16:1}ω7c and/or C_{16:1}ω6c (31.3%) and C_{16:0} (13.0%), all of which support classification of strain CPCC 100056^T as a member of the genus *Roseomonas*. The cellular fatty acid profile of strain CPCC 100056^T was determined on R2A medium using the same cultivation conditions used for the closely related *Roseomonas* species identified in this study (see Supplementary Table S1, available in IJSEM Online). The significant differences in the amounts of the straight-chain fatty acid C_{18:0} and the hydroxyl fatty acid C_{18:1} 2-OH clearly separated strain CPCC 100056^T from all tested strains of *Roseomonas* species.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Li *et al.* (2007). The G+C content of the genomic DNA was determined using the thermal denaturation method (Marmur & Doty, 1962). The 16S rRNA gene sequence of the tested strain was compared with sequences in GenBank to find the best matches; the BLAST result displayed the highest level of similarity to those of *Roseomonas* species. Multiple alignments with sequences of members of the most closely related genera of the *Alphaproteobacteria* (such as *Roseomonas*, *Muricoccus*, *Paracraurococcus*, *Teichococcus* and *Gluconobacter*) and calculations of levels of sequence similarity were carried out using CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) from *K_{nuc}* values (Kimura, 1980) using MEGA version 2.1 (Kumar *et al.*, 2001). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The results of 16S rRNA gene sequence (1421 bp) comparison indicated that strain CPCC 100056^T showed a high similarity to *Roseomonas*, *Muricoccus*, *Teichococcus*, *Paracraurococcus*, *Acetobacter* and *Azospirillum* species within the *Alphaproteobacteria*. In the phylogenetic tree based on 16S rRNA gene sequences, isolate CPCC 100056^T formed a separate clade with *R. aquatica*

Table 1. Differential characteristics of strain CPCC 100056^T and other members of the genus *Roseomonas*

Strains: 1, CPCC 100056^T; 2, *R. aquatica* CCM 7538^T; 3, *R. lacus* CGMCC 1.3617^T; 4, *R. mucosa* CCM 7177^T; 5, *R. gilardii* subsp. *gilardii* CCM 4846^T; 6, *R. gilardii* subsp. *rosea* CCM 7178^T; 7, *R. cervicalis* CCM 4881^T. Data are from this study; sources of isolation of reference strains are from Jiang *et al.* (2006), Han *et al.* (2003), Rihs *et al.* (1993) and Gallego *et al.* (2006).

Characteristic	1	2	3	4	5	6	7
Motility	–	–	–	+	–	–	+
Optimum growth temperature (°C)	30–32	28	30	35	35	35	35
Growth on NaCl (%)	≤2.0	≤2.0	≤7.0	≤7.0	≤7.0	≤7.0	≤7.0
Hydrolysis of urea	–	+	+	+	+	+	+
Reduction of nitrate	+	+	+	–	–	–	–
Utilization of:							
D-Glucose	+	–	–	+	+	+	–
D-Mannose	+	–	–	–	–	–	–
D-Mannitol	+	–	–	+	–	+	–
L-Arabinose	+	–	+	+	+	+	+
Trisodium citrate	–	–	–	+	+	+	+
Fructose	–	–	–	+	+	+	+
Ribose	+	–	+	–	+	–	–
D-Galactose	–	–	+	–	–	+	–
D-Xylose	–	–	+	–	–	+	+
Glycerol	–	–	–	–	+	–	–
Isolation source	Soil sample	Potable water	Fresh water	Lake sediment	Blood	Potable water	Blood

TR53^T (95.5% 16S rRNA gene sequence similarity) within the genus *Roseomonas* (Fig. 1). In the tree shown in Fig. 1, *Azospirillum brasilense* ATCC 49958 (Helsel *et al.*, 2006) (the type strain of *Roseomonas fauriae*) and *Roseomonas* genomospecies 6 ATCC 49961 formed a stable clade with the genus *Azospirillum* rather than with *Roseomonas*, which supports a previous proposal to remove *Roseomonas* genomospecies 6 from the genus *Roseomonas* (Cohen *et al.*, 2004; Han *et al.*, 2003; Jiang *et al.*, 2006). *Paracraurococcus ruber* NS89^T and *Muricoccus roseus* 173/96^T clustered together with members of the genus *Roseomonas*. The former two genera have coccus morphology (Kämpfer *et al.*, 2003), whereas all members of the genus *Roseomonas* have coccobacillus cells; isolate CPCC 100056^T also had coccobacillus

morphology. In addition, the respiratory quinone (ubiquinone-10), DNA G + C content (67.3 mol%) and major fatty acid composition of the strain CPCC 100056^T corresponded with those of *Roseomonas* species, thus supporting the classification of strain CPCC 100056^T as a member of the genus *Roseomonas*. However, the 16S rRNA gene sequence similarity between strain CPCC 100056^T and the other *Roseomonas* species (84.5–95.5%) was significantly below 97%, which demonstrated that the strain represents a different genomic species (Stackebrandt & Goebel, 1994). In addition, strain CPCC 100056^T could be distinguished from its closest phylogenetic neighbour, *R. aquatica* CCM 7538^T, in some phenotypic characteristics (Table 1) and by its fatty acid profile (Supplementary Table S1).

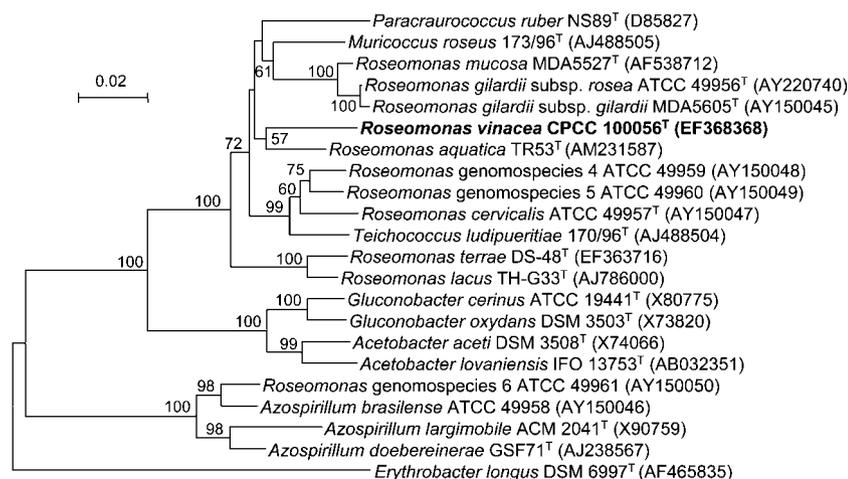


Fig. 1. Phylogenetic tree obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain CPCC 100056^T among phylogenetic neighbours. Numbers on branch nodes are bootstrap values (1000 resamplings). The sequence of *Erythrobacter longus* DSM 6997^T was used as an outgroup. Bar, 2% sequence divergence.

Therefore, based on the above phenotypic characteristics and 16S rRNA gene sequence analysis, it is proposed that isolate CPCC 100056^T be classified as a representative of a novel species of genus *Roseomonas*, for which the name *Roseomonas vinacea* sp. nov. is proposed.

Description of *Roseomonas vinacea* sp. nov.

Roseomonas vinacea (vi.na'ce.a. L. fem. adj. *vinacea* of or belonging to wine or to the grape, referring to the colony colour).

Cells are Gram-negative, coccoid rods, 0.9–1.0 × 0.9–2.2 μm, non-motile and non-spore-forming. The vinaceous colonies are circular, opaque and approximately 0.7–1.0 mm in diameter after 3 days at 32 °C. Grows at 4–40 °C and pH 6.5–8.0, with optimal growth at 30–32 °C and pH 7.0–7.5. Oxidase- and catalase-positive. Grows in NaCl at concentrations up to 2%. Nitrate is reduced to nitrite, but nitrite is not reduced. Casein, gelatin, starch and aesculin are not hydrolysed, but Tweens 80 and 20 are hydrolysed. The Voges–Proskauer test and methyl red reaction and tests for milk peptonization and coagulation and H₂S, indole and melanin production are negative. Alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase and naphthol-AS-BI-phosphohydrolase are present, but tests for urease, DNase, acid phosphatase, leucine arylamidase, arginine dihydrolyase, valine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are negative. D-Glucose, D-ribose, L-arabinose, L-rhamnose and melibiose can be utilized as sole carbon sources and no acid is produced from any carbon sources listed in the API 20E and API 50CH strips. Resistant to amikacin, erythromycin, gentamicin sulfate, penicillin G, streptomycin sulfate, vancomycin, polymyxin B and ciprofloxacin, but sensitive to terramycin, aureomycin, tetracycline, tobramycin sulfate, netilmicin, oleandomycin, novobiocin, kanamycin, nalidixic acid and chloramphenicol. The respiratory quinone is ubiquinone-10. The cellular fatty acid profile consists of C_{18:1}ω7c (33.6%), C_{16:1}ω7c and/or C_{16:1}ω6c (31.3%), C_{16:0} (13.0%), C_{18:0} (10.2%), C_{18:1}ω5c (1.3%), C_{19:0} cyclo ω8c (1.3%), C_{16:1}ω5c (0.6%), C_{18:1} 2-OH (0.6%), C_{14:0} (0.4%), C_{17:1}ω7c (0.4%) and C_{12:0} (0.1%).

The type strain is CPCC 100056^T (=KCTC 22045^T =CCM 7468^T), isolated from a soil sample collected from the Qinghai–Tibet plateau, north-west China. The DNA G + C content of the type strain is 67.3 mol%.

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