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

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Strain CPCC 100056T, 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 C18:1ω7c and C16:1ω7c and/or C16:1ω6c. The G+C content of the genomic DNA was 67.3 mol%. A comparison of sequences in GenBank revealed that strain CPCC 100056T exhibited highest 16S rRNA gene sequence similarity (84.5–95.5%) with Roseomonas species. Strain CPCC 100056T 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 100056T should be classified as a representative of a novel species in the genus Roseomonas, Roseomonas vinacea sp. nov.; the type strain is CPCC 100056T (=KCTC 22045T =CCM 7468T).

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 100056T, 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 ºC. However, during the course of a screening programme for new antibiotics, rather more non-psychrophiles were discovered from such environments. Strain CPCC 100056T was isolated by using the dilution plating method on ISP2 agar medium (Shirling & Gottlieb, 1966) at 20 ºC 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 ºC during January to 7 ºC 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 [(l 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 100056T is EF368368.

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

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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. Simmon’s 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 sugars were reassessed on basal medium (Shirling & Gottlieb, 1966) supplemented 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. Simmon’s 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).

Coccobacillus cells were observed for strain CPCC 100056T, 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 100056T 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 C18 : 0 and C16 : 1ω7c (33.6 %), C16 : 1ω7c and/or C16 : 1ω6c (31.3 %) and C16 : 0 (13.0 %), all of which support classification of strain CPCC 100056T as a member of the genus Roseomonas. The cellular fatty acid profile of strain CPCC 100056T 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 C18 : 0 and the hydroxyl fatty acid C18 : 1ω2-OH clearly separated strain CPCC 100056T 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 100056T 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 100056T formed a separate clade with R. aquatica
Table 1. Differential characteristics of strain CPCC 100056T and other members of the genus Roseomonas

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<th>Characteristic</th>
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<td>Motility</td>
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<td>–</td>
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<td>Optimum growth temperature (°C)</td>
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<td>35</td>
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<td>Growth on NaCl (%)</td>
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<td>Reduction of nitrate</td>
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<td>Fresh water</td>
<td>Lake sediment</td>
<td>Blood</td>
<td>Potable water</td>
<td>Blood</td>
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TR53T (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 NS89T and Muricoccus roseus 173/96T 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 100056T 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 100056T corresponded with those of Roseomonas species, thus supporting the classification of strain CPCC 100056T as a member of the genus Roseomonas. However, the 16S rRNA gene sequence similarity between strain CPCC 100056T 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 100056T could be distinguished from its closest phylogenetic neighbour, R. aquatica CCM 7538T, in some phenotypic characteristics (Table 1) and by its fatty acid profile (Supplementary Table S1).
Therefore, based on the above phenotypic characteristics and 16S rRNA gene sequence analysis, it is proposed that isolate CPCC 100056T 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, coccolid 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 melainin 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 dihydrodrolase, valine arylamidase, trypsin, x-chymotrypsin, x-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, vancomycin, polymyxin B and ciprofloxacin, but sensitive to terramycin, aureomycin, tetracycline, tobramycin sulfate, netilmicin, oleanodonic, novobiocin, kanamycin, nalidixic acid and chloramphenicol. The respiratory quinone is ubiquinone-10.

The type strain is CPCC 100056T (=KCTC 22043T =CCM 7468T), 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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References


