Roseomonas lacus sp. nov., isolated from freshwater lake sediment

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An aerobic, Gram-negative bacterial strain, TH-G33^T, was isolated from freshwater sediment of Taihu Lake in China. The taxonomy of strain TH-G33^T was studied by using phenotypic and phylogenetic methods. Cells of strain TH-G33^T were coccoid rods or rods and formed colourless to pale-pink colonies on nutrient agar. Phylogenetic analysis based on nearly complete 16S rRNA gene sequences showed that strain TH-G33^T was related to *Roseomonas mucosa* (94.4%), *Roseomonas gilardii* subsp. *gilardii* (94.1%), *Roseomonas gilardii* subsp. *rosea* (94.8%) and *Roseomonas cervicalis* (93.9%). Cells contained ubiquinone 10 (Q-10) as the major quinone and the G+C content was 71.9 mol%. Thus, strain TH-G33^T represents a novel species of the genus *Roseomonas*, for which the name *Roseomonas lacus* sp. nov. is proposed. The type strain is TH-G33^T (=CGMCC 1.3617^T=JCM 13283^T).

The genus *Roseomonas* (Rihs *et al.*, 1993) was created for pink coccoid isolates that caused bacteraemia and other human infections. *Roseomonas* species have been isolated frequently from blood, wounds, exudates, abscesses and genitourinary specimens (Rihs *et al.*, 1993; Sandoe *et al.*, 1997; Bibashi *et al.*, 2000; Subudhi *et al.*, 2001). Recently, bacterial strains that are phylogenetically related to members of the genus *Roseomonas* have also been isolated frequently from environmental samples such as drinking water distribution systems (September *et al.*, 2004), oil wells (*Roseomonas* strain BON 1; GenBank accession no. AY219712), biological soil crust (*Roseomonas* strain CP4B2; AJ871456), agriculture drainage water (*Roseomonas* strain SA-2; AY730026), as well as from water (Rihs *et al.*, 1993). *Roseomonas*-related 16S rRNA gene sequences have also been detected frequently during molecular surveys of microbial diversity in various environments, for example, in the freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes (Eiler & Bertilsson, 2004). All these facts indicate that members of the genus *Roseomonas* could occur widely in nature. However, no names of environmental isolates (non-medical source) of *Roseomonas* have been validly published. Currently, all members of the genus *Roseomonas* with validly published names, including *Roseomonas cervicalis*,

Roseomonas fauriae, *Roseomonas gilardii* subsp. *gilardii* (Rihs *et al.*, 1993), *Roseomonas gilardii* subsp. *rosea* and *Roseomonas mucosa* (Han *et al.*, 2003), were isolated from medical samples.

Strain TH-G33^T was isolated from sediment of the freshwater Taihu Lake (also called lake Tai; 120° 02' 16.8" E 31° 27' 10.7" N, Jiangsu Province, China), by plating 10-fold dilutions of samples on dilute nutrient medium (DNM) containing (l⁻¹): 0.3 g beef extract, 1.0 g fish peptone, 0.1 g yeast extract, 0.5 g NaCl and 15 g agar. Sediment samples were obtained from 10 cm beneath the sediment surface. LB or nutrient medium (NM) agar or broth at 30 °C were used for routine cultivation.

Gram reactions were determined by staining cells grown on NM agar at 30 °C for 24 h according to the method described by Gerhardt *et al.* (1994). Endospore formation was determined after malachite green staining of cells grown on LB agar. Cell flagellation and morphology were examined by using transmission and scanning electron microscopy. For assimilation of carbon sources, the standard mineral base solution of Stanier *et al.* (1966) was used. Each compound was added at a concentration of 0.2% (w/v) after the mineral base solution had been autoclaved. Growth was examined after incubation at 30 °C for 1, 3, 7 and 14 days. Aerobic and anaerobic production of acid (O–F reaction) from carbohydrates was determined using O–F basal medium (Hugh & Leifson, 1953). Carbohydrate solutions sterilized by filtration were added at a final concentration of 1% (w/v) and acid production was recorded after 7 and 14 days of incubation. For cellular fatty acid analysis, strains were grown for 72 h at 30 °C on NM agar. Cells were

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Roseomonas lacus* TH-G33^T is AJ786000.

A table showing the cellular fatty acid profile of *Roseomonas lacus* TH-G33^T is available as supplementary material in IJSEM Online.

harvested from the plates and saponified, and the liberated fatty acids were methylated and analysed by using the Sherlock system (Microbial ID Inc.), following the manufacturer's instructions. Isoprenoid quinones were extracted from freeze-dried cells (200 mg) with methyl chloride/methanol (2:1) and analysed by reversed-phase HPLC. The sensitivity to various antibiotics was tested by using the disc diffusion method (Kirby–Bauer method).

The DNA base composition was determined by thermal denaturation (Marmur & Doty, 1962) using DNA from *Escherichia coli* DH-5 α as a control. The 16S rRNA gene was amplified as described previously (Zhang *et al.*, 2003) and 16S rRNA gene sequence alignments were performed with the CLUSTAL_X program (version 1.64b; Thompson *et al.*, 1997). The phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) with Kimura's two-parameter calculation model in TREECON W version 1.3b.

Cells of strain TH-G33^T were Gram-negative, aerobic, non-spore-forming coccoid rods or rods (Fig. 1). Flagella were not observed. Growth occurred at 15–40 °C and pH 6.0–9.0, with optima at 30 °C and pH 7.0. Cultures grown in NM broth were colourless. Colonies on NM agar were colourless to pale-pink. Colonies were pinpoint, raised, entire, runny and mucoid, with a diameter of 0.5–1.0 mm after incubation for 3–4 days. Catalase and oxidase reactions were positive. 16S rRNA gene sequence analysis showed that strain TH-G33^T was phylogenetically related to members of the genus *Roseomonas* (similarities 93.2–94.8 %), with the greatest similarity to *R. gilardii* subsp. *rosea* (94.8 %) followed by *R. mucosa* (94.4 %). A neighbour-joining tree based on these 16S rRNA gene sequences was constructed (Fig. 2), which showed that strain TH-G33^T and other species of the genus *Roseomonas* except *R. fauriae*, which has been suggested not to be a member of the genus *Roseomonas* (Cohen *et al.*, 2004; Han *et al.*, 2003), were grouped together. Thus, strain TH-G33^T represents a novel species of the genus *Roseomonas*.

Phenotypic properties, such as assimilation of sugars and other carbon sources, hydrolysis of complex organic compounds and reduction of nitrate, were determined. The results are included in the species description. In conclusion, strain TH-G33^T was shown to be a unique isolate from freshwater sediment and could be differentiated from other species of *Roseomonas* by a range of features. Strain TH-G33^T did not have flagella, grew in NaCl at concentrations up to 6.5 % and had an optimum growth temperature of 30 °C. Unlike *R. mucosa*, strain TH-G33^T reduced nitrate, but did not assimilate D-glucose or citrate. Some properties that differentiate species of *Roseomonas* are given in Table 1. The major cellular fatty acids of strain TH-G33^T were iso-C_{15:0} (19.9 %), iso-C_{16:0} (38 %) and iso-C_{17:1 ω 9c} (12.1 %). The cellular fatty acid profile of strain TH-G33^T (Supplementary Table in IJSEM Online) was also significantly different from those reported for *R. mucosa*, *R. gilardii*, *R. cervicalis* and *R. fauriae*. Strain TH-G33^T was susceptible to ampicillin, piperacillin, amoxicillin, piperacillin/tazobactam, cefazolin, cephalothin, cefuroxime, cefotaxime, cefaclor, cefoperazone, ceftazidime, amikacin, gentamicin, tobramycin, kanamycin, streptomycin, tetracycline, doxycycline, minocycline, ciprofloxacin, levofloxacin, norfloxacin, lomefloxacin, fleroxacin, erythromycin, nitrofurantoin, polymyxin and chloramphenicol, but not to trimethoprim/sulfamethoxazole or enoxacin.

These results suggest that strain TH-G33^T represents a novel aquatic species, for which we propose the name *Roseomonas lacus* sp. nov.

Description of *Roseomonas lacus* sp. nov.

Roseomonas lacus (la'cus. L. masc. gen. n. *lacus* of a lake, indicating the site of isolation of this organism).

Cells are coccoid rods or rods, 0.5–0.8 $\times$ 0.8–1.5 μ m. Non-motile, without flagella. Gram-negative. Colonies on NM or DNM agar are circular, raised, entire, runny and mucoid, colourless to pale-pink and 0.5–1.0 mm in

Fig. 1. Transmission (a) and scanning (b) electron micrographs showing the morphology of *Roseomonas lacus* sp. nov. strain TH-G33^T. The dark ring (arrow) in (a) indicates the position at which the cell may divide. Bars, 1 μ m (a) and 5 μ m (b).

Fig. 2. Phylogenetic tree constructed using the neighbour-joining method based on 16S rRNA gene sequences of strain TH-G33^T and related bacteria. Numbers at nodes indicate bootstrap support based on 1000 resampled datasets. *Methylobacterium hispanicum* was used as the outgroup. Bar, evolutionary distance (K_{nuc}) of 0.05. *R. fauriae* is out of the *Roseomonas* group and most probably represents a member of a different bacterial genus, as also proposed by others (Cohen *et al.*, 2004; Han *et al.*, 2003).

diameter after incubation for 3–4 days. Grows at 15–40 °C and pH 6.0–9.0, with optima at 30 °C and pH 7.0. Oxidase- and catalase-positive. Grows in NaCl at concentrations up to 6.5 %. Nitrate is reduced. Casein, gelatin, starch, Tween 80 and aesculin are not hydrolysed. Voges–Proskauer test and methyl red reaction are negative. Oxidizes ribose, L-rhamnose, D-galactose, L-arabinose, DL-sorbose, fucose,

sucrose, D-cellobiose, D-melibiose and D-xylose. Does not utilize D-glucose, fructose, DL-sorbitol, DL-raffinose, DL-lactose, L-lysine, D-mannitol, acetate, propionate, glycerol or citrate. The major quinone is ubiquinone 10 (Q-10). The major cellular fatty acids (>1 %) are iso-C_{14:0} (5.8 %), iso-C_{15:0} (19.9 %), iso-C_{15:1} (1.6 %), anteiso-C_{15:0} (4.3 %), C_{15:0} (1.5 %), iso-C_{16:0} (38 %), iso-C_{16:1} (5.0 %), C_{16:0} (1.9 %), iso-C_{17:0} (4 %), anteiso-C_{17:0} (2.1 %) and iso-C_{17:1ω9c} (12.1 %). Sensitive to a range of antibiotics but not to trimethoprim/sulfamethoxazole or enoxacin.

The type strain, TH-G33^T (=CGMCC 1.3617^T=JCM 13283^T), was isolated from freshwater lake sediment. The G+C content of the type strain is 71.9 mol%.

Table 1. Characteristics that differentiate between members of the genus *Roseomonas*

Taxa: 1, strain TH-G33^T; 2, *R. mucosa*; 3, *R. gilardii*; 4, *R. cervicalis*; 5, *R. fauriae*. Data for *R. mucosa*, *R. gilardii*, *R. cervicalis* and *R. fauriae* from Han *et al.* (2003), Rihs *et al.* (1993) and Wallace *et al.* (1990). +, Positive; –, negative; V, variable; ND, not determined.

Characteristic	1	2	3	4	5
Flagellum	–	+	+	+	+
Motility	–	+	–	+	+
Reduction of NO ₃ ⁺	+	–	V	–	+
H ₂ S production	ND	ND	–	–	–
Indole production	ND	–	–	–	–
Hydrolysis of aesculin	–	–	–	–	+
Gelatin liquefaction	–	ND	–	–	–
Utilization of carbon compounds:					
D-Glucose	–	+	V	–	V
Fructose	–	–	+	+	+
Ribose	+	ND	ND	ND	ND
D-Galactose	+	ND	V	–	+
L-Arabinose	+	+	V	V	+
D-Xylose	+	ND	V	V	+
D-Mannitol	–	ND	V	–	–
Glycerol	–	ND	+	–	+
Citrate	–	+	+	V	V
Optimum growth temperature (°C)	30	35	35	35	35

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