Paracoccus sulfuroxidans sp. nov., a sulfur oxidizer from activated sludge

Xing-Yu Liu,^{1,2} Bao-Jun Wang,¹ Cheng-Ying Jiang¹
and Shuang-Jiang Liu¹

Correspondence
Shuang-Jiang Liu
liusj@sun.im.ac.cn

¹State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Zhong-Guan-Cun, Haidian, Beijing 100080, P. R. China

²Graduate University of Chinese Academy of Sciences, Beijing 100049, P. R. China

Bacterial strain LW36^T was isolated from activated sludge of a wastewater-treatment bioreactor. Cells of strain LW36^T were Gram-negative coccoids to short rods, 1.0–1.2 × 0.5 µm in size. Colonies were cream-coloured, smooth and circular. Strain LW36^T was hetero-organotrophic and chemolithotrophic and was able to use reduced sulfur as an energy resource. Growth was observed at 25–36 °C and pH 5–10. The most abundant cellular fatty acid of strain LW36^T was C_{18:1}ω7c (64.2 %). The sole respiratory quinone was ubiquinone-10. The G + C content of the genomic DNA was 61.3 mol% (T_m). 16S rRNA gene sequence analysis indicated that strain LW36^T was phylogenetically related to members of the genus *Paracoccus*, with similarities ranging from 92.4 to 94.9 %. Based on these results, it is concluded that strain LW36^T represents a novel species of the genus *Paracoccus*, for which the name *Paracoccus sulfuroxidans* is proposed. The type strain is strain LW36^T (= CGMCC 1.5364^T = JCM 14013^T).

The genus *Paracoccus* was first erected by Davis *et al.* (1969) and the description was emended by Katayama *et al.* (1995). At the time of writing, the genus comprises 19 recognized species (see Fig. 1). Members of this genus are metabolically versatile and are widely distributed in soil, sediment and activated sludge environments. They take part in bioconversion of organic and inorganic compounds. For example, *Paracoccus denitrificans* utilizes organic compounds for growth and can use nitrate for respiration (Rainey *et al.*, 1999, and references therein). Some other *Paracoccus* species, such as *Paracoccus pantotrophus* (Robertson & Kuenen, 1983; Rainey *et al.*, 1999), *Paracoccus thiocyanatus* and *Paracoccus versutus*, can use reduced sulfur as an energy source (Harrison, 1983; Katayama *et al.*, 1995). In this study, a sulfur-oxidizing, heterotrophic bacterial strain, designated LW36^T, was isolated from activated sludge. Based on phenotypic and genotypic studies it is concluded that this isolate represents a novel species of the genus *Paracoccus*.

Bacterial strain LW36^T was isolated from activated sludge of a sequential batch reactor treating mixed wastewater containing various nitroaromatic compounds (nitrobenzene, nitrophenol, 2,4-dinitrophenol) and aniline. The sludge sample was suspended in sterile saline solution by using vigorous

vortexing, and a portion of the suspension was spread directly on LB agar plates. The plates were incubated at 30 °C for about a week. Single colonies on the plates were picked up and bacterial strain LW36^T was obtained by repeatedly streaking cultures on new plates from a single colony.

Routine cultivation was conducted at 30 °C with LB media. Gram reactions were determined according to the method described by Gerhardt *et al.* (1994). Presence of cell flagella and cell morphology were examined by transmission electron microscopy (H600; Hitachi) and scanning electron microscopy (FEI QUANTA 200). Growth temperature range was determined with a TN3F temperature-gradient incubator (Advantec). Catalase and oxidase activities, the Voges–Proskauer reaction, carbon source utilization and other biochemical characterization methods were performed according to Dong & Cai (2001). For testing growth on reduced sulfur and elemental sulfur, strain LW36^T was inoculated into Allen's medium (Allen, 1959), the pH of which was adjusted to 6.5, supplemented with elemental sulfur (1 %), sulfide (1 %), thiosulfate (1 %) or sulfite (1 %). Cell growth was estimated either by monitoring the increase in optical density at 600 nm or by determining the increase in protein content of cultures.

Cells of strain LW36^T were Gram-negative, coccoid to short rods with a size range of 1.0–1.2 × 0.5 µm (see Supplementary Fig. S1 in IJSEM Online). Colonies were cream-coloured, smooth, circular and 0.4–2.0 mm in diameter. Growth was observed over a temperature range

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LW36^T is DQ512861.

A scanning electron micrograph of cells of strain LW36^T and a table showing its cellular fatty acid profile are available as supplementary material in IJSEM Online.

Fig. 1. Phylogenetic tree constructed with the neighbour-joining method according to 16S rRNA gene sequence evolutionary distances among strain LW36^T and recognized members of the genus *Paracoccus*. *Rhodobacter capsulatus* ATCC 11166^T was used as the outgroup. Bar, evolutionary distance (K_{nuc}) of 0.01.

of 25–36 °C and a pH range of 5–10. Strain LW36^T grew chemolithotrophically on thiosulfate, sulfide and elemental sulfur but growth was not observed with sulfite. Other physiological and biochemical characteristics are given in Table 1 and in the species description below.

Biomass for chemotaxonomic analyses was harvested from LB cultures at 30 °C after incubation for 24 h. Cellular fatty acids were extracted and analysed as described by Hu *et al.* (2004). Quinones were extracted and purified according to the method of Collins (1985) and were analysed by HPLC (Wu *et al.*, 1989), with a commercial ubiquinone-10 (Q-10)

sample (China Pharmacy Corp.) as reference. Results showed that the major cellular fatty acids (>1% of the total) of strain LW36^T were C_{18:1ω7c} (64.2%), C_{16:0} (16.4%), C_{18:0} (3.7%), C_{14:0} 3-OH (3.0%), C_{16:1ω7c} (2.3%), C_{14:0} (1.9%), iso-C_{15:1} (1.8%) and C_{19cyc} (1.4%). Detailed information on the cellular fatty acid composition of strain LW36^T is provided in Supplementary Table S1 in IJSEM Online. Strain LW36^T had Q-10 as the sole respiratory quinone. DNA base composition was determined by thermal denaturation (Marmur & Doty, 1962), with *Escherichia coli* K-12 as reference. The DNA G+C content of strain LW36^T was 61.3 mol%.

Table 1. Phenotypic characteristics that differentiate strain LW36^T from related species of the genus *Paracoccus*

Taxa: 1, strain LW36^T; 2, *P. denitrificans* (data from Doronina *et al.*, 1998; Dworkin, 2001); 3, *P. methylutens* (Doronina *et al.*, 1998; Dworkin, 2001); 4, *P. versutus* (Katayama *et al.*, 1995; Dworkin, 2001); 5, *P. pantotrophus* (Robertson & Kuenen, 1983; Dworkin, 2001). +, Positive; –, negative; NA, no data available.

Characteristic	1	2	3	4	5
Optimal pH	6.5–7.5	6.5–7.5	7.0–8.0	7.5–8.0	NA
Nitrate reduction	–	+	–	–	+
Utilization of:					
Sucrose	–	+	+	+	NA
D-Fructose	–	+	+	+	+
Inositol	–	+	+	NA	NA
D-Mannitol	–	+	+	+	NA
Mannose	–	+	+	NA	+
Lactose	–	–	NA	–	–
Arabinose	+	+	+	+	–
Glutamic acid	–	NA	+	+	+
Sulfur compounds used for energy					
Thiosulfate	+	+	–	+	+
Sulfide	+	+	NA	+	+
DNA G+C content (mol%)	61.3	65–68	67.0	67–68	66

The nearly complete 16S rRNA gene of strain LW36^T (1343 bp) was amplified and sequenced as described by Zhang *et al.* (2003). Alignments of 16S rRNA gene sequences were performed with the CLUSTAL_X program, version 1.64b (Thompson *et al.*, 1997). A neighbour-joining (Saitou & Nei, 1987) phylogenetic tree (Fig. 1) was constructed based on evolutionary distances calculated with the Kimura two-parameter model. When aligned, positions with insertions or deletions were excluded from the calculations. The 16S rRNA gene sequence analysis indicated that strain LW36^T was phylogenetically related to members of the genus *Paracoccus*, with similarities ranging from 92.4 to 94.9%.

Based on our phenotypic and phylogenetic studies, it is clear that strain LW36^T represents a member of the genus *Paracoccus*. Strain LW36^T showed a range of phenotypic characteristics that differentiated it from recognized species of the genus (as listed in Table 1). Its phylogenetic divergence (Fig. 1) also differentiates strain LW36^T from recognized *Paracoccus* species. Therefore, we conclude that strain LW36^T represents a novel species of the genus *Paracoccus*, for which the name *Paracoccus sulfuroxidans* sp. nov. is proposed.

Description of *Paracoccus sulfuroxidans* sp. nov.

Paracoccus sulfuroxidans (sul.fur.ox'i.dans. L. n. *sulfur* sulfur; N.L. part. adj. *oxidans* oxidizing; N.L. part. adj. *sulfuroxidans* pertaining to the ability to oxidize sulfur).

Cells are Gram-negative, coccoid to short rods, 1.0–1.2 × 0.5 µm in size. Colonies are cream-coloured, smooth, circular and 0.4–2.0 mm in diameter. Growth occurs over a temperature range of 25–36 °C (optimum growth at 31.5–33.5 °C) and a pH range of 5–10. Positive for catalase and oxidase activities. Negative for Voges–Proskauer reaction and lipase activity. Does not hydrolyse gelatin, starch or casein. Utilizes citric acid, nitriloxides, L-arabinose, D-glucose and maltose, but not D-arabitol, fructose, mannitol, rhamnose, melibiose, xylitol, malic acid, L-glutamic acid, L-lactic acid, cellobiose, D-lactose, inositol, D-mannose, D-melibiose, raffinose, D-ribose, salicin, sorbitol, glycerol, trehalose or dextrin. Grows chemolithotrophically on thiosulfate, sulfide and elemental sulfur but not on sulfite. The sole respiratory quinone is Q-10. The predominant cellular fatty acid is C_{18:1ω7c} (64.2%). The G + C content of the DNA is 61.3 mol% (T_m).

The type strain, LW36^T (=CGMCC 1.5364^T=JCM 14013^T), was isolated from activated sludge of a wastewater-treatment bioreactor.

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