NOTE

Emended description of *Paracoccus kondratievae*

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An aerobic, facultatively chemolithotrophic and methylotrophic strain, GB, was isolated from a maize rhizosphere. On the basis of comparative analysis of its phenotypic and genotypic properties, it is proposed that strain GB^T (= VKM B-2222^T = NCIMB 13773^T) be assigned to the genus *Paracoccus* as *Paracoccus kondratievae* sp. nov.

Keywords: methylotroph, chemolithotroph, thermotolerant, alkaliphilic, *Paracoccus*

Until recently, the genus *Paracoccus* contained only two species, namely *Paracoccus denitrificans* and *Paracoccus halodenitrificans* (Van Verseveld & Stouthamer, 1992). At present, the genus *Paracoccus* includes 13 validated species and has become the focus of many studies on its physiological versatility, and on the ability to degrade unusual and potentially polluting compounds, as extensively reviewed by Kelly *et al.* (2000). Some members of this genus are able to oxidize reduced sulfur compounds such as thiosulfate, thiocyanate, carbonyl sulfide and carbon disulfide to gain energy for autotrophic growth (Katayama *et al.*, 1995; Siller *et al.*, 1996; Lipski *et al.*, 1998; Rainey *et al.*, 1999). Most *Paracoccus* species are able to grow on carbon dioxide by using hydrogen oxidation as the source of metabolic energy; some species can also grow methylotrophically with methanol, methylamine (Urakami *et al.*, 1990; Ohara *et al.*, 1990; Van Verseveld & Stouthamer, 1992), dichloromethane (Doronina *et al.*, 1998), or with more complex substances such as *N,N*-dimethylformamide (Urakami *et al.*, 1990) or methylated sulfides (Rainey *et al.*, 1999). Phylogenetic analyses have demonstrated that all of the described species of the genus *Paracoccus* form a coherent cluster within the α -3 subclass of the *Proteobacteria* (Kelly *et al.*, 2000).

Very recently, we isolated (and made a preliminary description of) a colourless, methanol-utilizing *Paracoccus*-like strain – strain GB^T – that possessed the ribulose biphosphate pathway. Based on analysis of cytophysiological and metabolic properties, cellular

ubiquinones, phospholipids and fatty acids, the DNA base composition and DNA-DNA hybridization, isolate GB was preliminarily classified as *Paracoccus kondratievae* (Doronina & Trotsenko, 2000, 2001). This paper reports on the phylogenetic analysis and further taxonomic characterization of *Paracoccus kondratievae* sp. nov. The 16S rRNA gene was amplified and sequenced according to Lane (1991). The almost complete 16S rRNA gene sequence from strain GB^T was aligned, using the CLUSTAL program for sequence alignment to the representative paracocci. The positions of sequence uncertainties were omitted; in total, 1290 nucleotides were used in the analysis. Their phylogenetic relationships were determined by the neighbour-joining method and the programs of the TREECON package (Van de Peer & De Wachter, 1994), by maximum-likelihood by using the PUZZLE program (Strimmer & von Haeseler, 1996), and by maximum-parsimony by using the program DNAPARS from the PHYLIP package (Felsenstein, 1989) with bootstrap analysis of 100 trees.

Some differential phenetic characteristics of *Paracoccus kondratievae* GB and representatives of the genus *Paracoccus* are summarized in Table 1.

Approximately 95% of the 16S rRNA gene sequence of strain GB was determined by direct sequencing of PCR-amplified 16S rDNA. As follows from the phylogenetic tree (Fig. 1), strain GB belongs to the genus *Paracoccus* within the *Rhodobacter* group of the α -*Proteobacteria*. The highest 16S rRNA gene similarity values exist between *Paracoccus kondratievae* GB and *P. versutus* IAM 12814^T (97.6%), *P. pantotrophus* ATCC 35512^T (97.5%) and a new sequence of '*P. thiophilus*' AS 001 (97.5%). *Paracoccus kondratievae*

The GenBank/EMBL/DDJB accession number for the 16S rRNA sequence of strain GB^T is AF250332.

Table 1. Some differential phenetic characteristics of *Paracoccus* species

1, Strain GB^T; 2, *P. denitrificans*; 3, *P. thiocyanatus*; 4, *P. versutus*; 5, *P. kocurii*; 6, *P. alcaliphilus*; 7, *P. aminophilus*; 8, *P. aminovorans*; 9, *P. solventivorans*; 10, *P. marcusii*; 11, *P. carotinifaciens*; 12, *P. alkenifer*; 13, *P. pantotrophus*; 14, *P. methylutens*. Data are from Doronina *et al.* (1998), Doronina & Trotsenko (2000) and Kelly *et al.* (2000). –, Absent; +, present, NR, not reported.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Motility	–	–	–	+	–	–	–	–	–	–	+	–	–	–
Pigmentation	–	–	–	–	–	–	–	–	–	+	+	NR	–	–
Denitrification	+	+	+	+	+	–	–	–	+	–	–	+	+	–
Urease	+	+	–	–	–	+	–	–	NR	NR	–	NR	–	+
Growth on:														
H ₂ + CO ₂	+	+	+	+	NR	–	–	–	–	NR	NR	NR	+	+
Thiosulfate + CO ₂	+	+	+	+	–	NR	NR	NR	NR	NR	NR	NR	+	+
Thiocyanate + CO ₂	–	–	+	–	NR	NR	NR	NR	NR	NR	NR	NR	NR	–
Formate	+	+	+	+	+	–	–	–	–	+	NR	–	–	+
Methanol	+	+	–	+	–	+	–	–	–	–	NR	+	–	+
Methylamine	+	+	–	+	+	+	+	+	+	–	NR	–	–	+
Trimethylamine	–	+	–	–	+	–	+	+	–	–	NR	–	–	–
Glycerol	+	+	–	+	+	+	+	+	–	+	NR	–	+	+
Xylose	–	–	–	–	–	+	+	–	–	NR	NR	–	NR	–
Sucrose	–	+	–	+	–	–	–	–	–	+	NR	–	+	+
Fructose	+	+	+	+	–	+	–	+	–	+	NR	–	+	+
Mannitol	+	+	+	+	–	+	–	+	–	+	+	–	+	+

GB forms a single cluster with these species and *P. methylutens* (96.2% of sequence similarity) in all the trees constructed by different methods, but the highest bootstrap probability (100) of this branching point was only for a neighbour-joining method. An emended formal taxonomic description of *Paracoccus kondratievae* GB follows.

Emended description of *Paracoccus kondratievae* (Doronina and Trotsenko 2001)

Paracoccus kondratievae (kon.dra.tie'vae. N.L. gen. fem. n. *kondratievae* of Kondratieva, named after the prominent Russian microbiologist Elena N. Kondratieva).

Cells are coccoid (0.5–0.6 µm in diameter) or short rods (0.5–0.6 to 1.0–1.3 µm long). Cells occur singly, in pairs, chains or clusters. Gram-negative, non-motile, colourless, asporogenous. Multiplies by binary fission. Alkaliphilic and thermotolerant: grows at 30–50 °C and at pH 7.5–10.5, with optima at 38–42 °C and at pH 8.0–9.0. Does not grow in the presence of 3% NaCl. Aerobic, facultatively chemolithotrophic and methylotrophic, assimilating C₁ compounds via the ribulose biphosphate pathway. Utilizes the following carbon and energy sources: L-arabinose, D-galactose, D-glucose, D-ribose, D-fructose, adonitol, dulcitol, glycerol, inositol, mannitol, sorbitol, ethanol, acetate, malate, 2-oxoglutarate, succinate, fumarate, L-alanine, L-aspartate, L-glutamate, sarcosine, N,N-dimethylglycine, betaine, serine, methanol, methylamine, formalde-

hyde, CO₂ + H₂ + O₂ and thiosulfate + NaHCO₃. Growth factors are not required. Utilizable nitrogen sources are ammonium salts, nitrates, urea, methylamine and amino acids. Indole is produced from L-tryptophan in the medium with nitrate as nitrogen source. Urease-, oxidase- and catalase-positive. Glucose is not fermented. Grows anaerobically in the presence of nitrate. Denitrification occurs under heterotrophic growth conditions. The major cellular phospholipids are phosphatidylethanolamine, phosphatidylglycerol, cardiolipin and phosphatidylcholine. The predominant cellular fatty acids are cyclopropane C_{19cyc} (29%), palmitic acid (C_{16:0}; 18%) and *cis*-vaccenic acid (C_{18:1}; 49%). The main ubiquinone is Q-10. The DNA G + C content is 62.5 mol% (T_m). The level of DNA–DNA similarity with type cultures *P. denitrificans* ATCC 17441^T and *P. methylutens* VKM B-2164^T is approximately 40%. The GenBank accession number for the 16S rRNA gene sequence of strain GB^T is AF250332. The type strain, GB^T (= VKM B-2222^T = NCIMB 13773^T), was isolated from maize rhizosphere.

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Fig. 1. Phylogenetic trees showing the positions of strain GB^T among members of the genus *Paracoccus*. (a) Neighbour-joining method; (b) maximum-likelihood method; (c) maximum-parsimony method. Bootstrap values (each expressed as a percentage of 100 replications) and puzzling steps are shown at branch points; values greater than 95 % were considered as significant. Bars indicate the Jukes & Cantor (1969) distance and maximum-parsimony distances (2 nucleotide substitutions per 100 nucleotides).

the *Paracoccus* reference strains. Special thanks are due to the referees for their useful comments.

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