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# *Paracoccus bengalensis* sp. nov., a novel sulfur-oxidizing chemolithoautotroph from the rhizospheric soil of an Indian tropical leguminous plant

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## Abstract

*Paracoccus versutus*-like isolates from the rhizosphere of *Clitoria ternatea*, a slender leguminous herb (family—Papilionaceae), found ubiquitously in waste places and village forests of the Lower Gangetic plains of India, presented a case of graduated infraspecific variation that was capped by the identification of a new species *Paracoccus bengalensis* (type strain JJJ$^T$ = LMG 22700$^T$ = MTCC 7003$^T$). The diverged phenetic and genetic structure of these sulfur-oxidizing chemolithoautotrophs presented a case of apparent nonconformity of 16S rRNA gene sequence similarities with results of DNA–DNA hybridization. Despite high 16S rRNA gene sequence similarity with *P. versutus* one of the newly isolated strains, viz., JJJ$^T$ was identified as a new species of *Paracoccus* by virtue of its explicitly low DNA–DNA hybridization (42–45%) with the type strain of the closest species *P. versutus* (ATCC 25364$^T$), distinct G + C content (65.3 mol%), physiological and biochemical differences amounting to <60% phenetic similarity with strains of *P. versutus* as well as new isolates akin to the species. The newly described species also had a unique fatty acid profile that was distinguished by the absence of 18:1 $\omega$9*c*, unique possession of Summed feature 3 (16:1$\omega$7*c* & 15:0 iso 2-OH), 19:0 10 methyl, and a much higher concentration of 19:0 cyclo$\omega$8*c*.


***Keywords:*** Sulfur oxidation; Chemolithoautotrophic paracocci; *Paracoccus bengalensis* sp. nov.; Leguminous rhizosphere

## Introduction

The genus *Paracoccus* is constituted by a group of catalase and oxidase positive, nitrate reducing, aerobic, nonmotile, Gram-negative bacteria that are spherical in shape and also occur in the form of short rods. The three closely related species, viz., *Paracoccus denitrificans*, *Paracoccus pantotrophus* and *Paracoccus versutus* have attracted the attention of microbiologists because of their exclusive aerobic respiratory system, which has several components in common with those of the mitochondria. Members of the genus exhibit a wide range of metabolic flexibility, particularly with respect to processes involving respiration and energy transduction and are generally found in the soil as well as natural and artificial brines [2]. The chemolithotrophic species of *Paracoccus* have so far mostly been isolated from activated sludge or anaerobic digesters and sometimes from soils [8,10,16,21] but never from plant rhizospheres.

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[1]*This work is only a small part of the wide perspectives and vision of Dr. Pradosh Roy whose untimely demise begets his unfortunate student WG to see the publication through on his behalf.

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We have elsewhere described the isolation of 16 neutrophilic, mesophilic and facultatively chemolithotrophic sulfur-oxidizing bacterial strains from the soils immediately adjacent to the roots of the slender leguminous herb *Clitoria ternatea* (family—Papilionaceae) found ubiquitously in waste places and village forests of the Lower Gangetic plains of India and delineated their 16S rRNA gene sequence-based taxonomic affinities (W. Ghosh & P. Roy, Systematic and Applied Microbiology, manuscript no. SAM 2624, submitted for publication). FASTA analysis had ascribed 13 out of the 16 isolates to the α-3 subclass (order *Rhodobacterales*) of '*Proteobacteria*' with species of *Paracoccus* as their closest relatives. While two leguminous plant rhizospheric (LPR) chemolithotrophic isolates, phylogenetically closest to *P. pantotrophus* and *Paracoccus thiocyanatus*, respectively, have been described earlier polyphasic characterization of the rest of the 11 isolates related to *P. versutus* have been described in the present treatise. These novel strains presented a case of graduated infraspecific divergence where high 16S rRNA gene sequence similarity of the new isolates was not reflected in their discrete physiological and biochemical properties as well as whole cell protein and cellular fatty acid profiles. Moreover, 16S rRNA gene sequence-based phylogeny of the new isolates did not corroborate with their genomic relatedness with members of the closest species. The diverged taxonomic structure of the novel *P. versutus*-like isolates was found to be capped by the speciation of *Paracoccus bengalensis*.

## Materials and methods

### Phenotypic characterization

Chemolithoautotrophic as well as chemoorganoheterotrophic growth experiments were performed at 30 °C in basal MS solution supplemented at a time with a single sulfur compound or a single carbon source (5 g $l^{-1}$), respectively. The modified basal and mineral salts (MS) solution contained the following (per liter of distilled water): 1 g, $NH_4Cl$; 4 g, $K_2HPO_4$; 1.5 g, $KH_2PO_4$; 0.5 g, $MgSO_4.7H_2O$ and 5.0 ml trace metals solution [25]. Bacteria were tested for their ability to use thiosulfate (12–20 mM), sulfide (2 mM), sulfite (3 mM), thiocyanate (3 or 5 mM), elemental sulfur (0.5% and 1.0%, wt./vol.) or tetrathionate (10 mM) as substrates for chemolithotrophic growth. The level of thiosulfate or tetrathionate in the media was estimated by cyanolytic method described earlier [11]. For strains having growth factor requirements all the aforesaid synthetic media were supplemented with yeast extract (50 mg $l^{-1}$) or a vitamin mixture (10 mg $l^{-1}$ each of nicotinic acid, pantothenic acid pyridoxine, thiamin, *para*-aminobenzoic acid, riboflavin and biotin). All other phenotypic tests were performed using standard techniques described elsewhere [7].

Comparison of the phenotypes was made with previously published data and/or experimentally verified using the type as well as other strains of the nearest phylogenetic relatives of the new isolates. A numerical analysis of all the comparative data was performed using the Simple matching coefficient ($S_{SM}$) or the Jaccard coefficient ($S_J$) [22] followed by generation of phenograms using the unweighted pair group with mathematical mean (UPGMA) algorithm. Similar phenograms were yielded by both the coefficients.

### Genomic relatedness studies

High molecular mass DNA was prepared as described by Ezaki et al. [4] with minor modifications. To determine genomic relatedness of the new isolates dot-blot hybridization experiments were carried out with DIG-labeled genomic DNA as described earlier [13] using the Detection kit from Roche Applied Sciences following manufacturer's instructions. Colorimetric quantification of dot intensities was done using the Molecular Analyst software (BioRad Inc.) by determining mean pixel densities in equal sized circles.

Since comparisons were between closely related organisms all hybridization experiments were executed at temperatures 10–15 °C below the melting temperatures ($T_m$) of the tested genomic DNA samples. The membranes were washed under highly stringent conditions, i.e., 2 × 5 min in 2 × SSC, 0.1% SDS at 15–25 °C; and finally once for 15 min in 0.1 × SSC, 0.1% SDS at 10 °C below the melting temperatures ($T_m$) of the genomic DNA samples.

### Amplification, restriction analysis, sequencing and phylogeny of 16S rRNA genes

16S rRNA genes were amplified by PCR with bacteria-specific primers f27 (nucleotide position 8–27 of the 16S rRNA gene of *E. coli*) and r1492 (reverse complementary of nucleotide position 1492–1510 of the 16S rRNA gene of *E. coli*) [7] from DNA samples or boiled cell extracts of the isolates using High fidelity PCR Master kit (Roche Applied Science) according to the manufacturer's instructions. 16S rRNA gene sequences from the PCR products were determined using 'universal primers' [7], according to the manufacturer's specifications for *Taq* DNA polymerase-initiated cycle sequencing reactions using fluorescently labeled dideoxynucleotide terminators with an ABI PRISM 377 automated DNA sequencer (Perkin-Elmer Applied Biosystems, Inc.).