Paracoccus koreensis sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket (UASB) reactor

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A Gram-negative, short rod- to coccus-shaped, non-spore-forming bacterium (Ch05^T) was isolated from granules in an upflow anaerobic sludge blanket (UASB) reactor. On the basis of 16S rRNA gene sequence similarity, strain Ch05^T was shown to belong to the subclass α -Proteobacteria, being related to *Paracoccus solventivorans* (97.5%), *Paracoccus alkenifer* (96.9%) and *Paracoccus kocurii* (96.4%). The phylogenetic distance from *Paracoccus* species with validly published names was always less than 96%. Physiological and chemotaxonomic data (major ubiquinone, Q-10; major fatty acids, C_{18:1} and C_{18:0}) supported the affiliation of strain Ch05^T to the genus *Paracoccus*. The results of DNA–DNA hybridization and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain Ch05^T from the 17 *Paracoccus* species with validly published names. Ch05^T therefore represents a novel species, for which the name *Paracoccus koreensis* sp. nov. is proposed. The type strain is Ch05^T (=KCTC 12238^T =IAM 15216^T).

During the course of studies on the culturable aerobic bacterial community in granules that were used to treat wastewaters of brewing factories in Korea, a large number of novel bacterial strains were isolated (Im *et al.*, 2003). One of these isolates (strain Ch05^T) was found to be a member of the genus *Paracoccus* in the α -Proteobacteria lineage and was the subject of a taxonomic investigation.

The genus *Paracoccus* was first described by Davis *et al.* (1969); the genus currently comprises 17 species with validly published names. The members of the genus *Paracoccus* consist of Gram-negative cocci or short rods that show substantial metabolic versatility. Representatives are able to grow aerobically on a wide range of organic compounds. A number of species can also grow anaerobically, using nitrate as electron acceptor, and some representatives are able to use hydrogen as electron donor for chemoautotrophic growth. Phylogenetically, the genus belongs to the α -3 subgroup of the

Proteobacteria. Recently, *Paracoccus seriniphilus* (Pukall *et al.*, 2003), *Paracoccus yeei* (Daneshvar *et al.*, 2003) and *Paracoccus zeaxanthinifaciens* (Berry *et al.*, 2003) were introduced.

Strain Ch05^T was isolated from granules involved in the treatment of wastewater from a beer-brewing factory in Chung-Ju, Republic of Korea. Anaerobic granules are bacterial aggregates that result from the flocculation of sludge in an upflow anaerobic sludge blanket (UASB) reactor (de Zeeuw & Lettinga, 1980; Lettinga, 1995). They are composed of micro-organisms, inorganic nuclei and extracellular polymers (Fukuzaki *et al.*, 1991; Shen *et al.*, 1993). Great attention has been paid to the internal structure and catalytic activities of such granules (MacLeod *et al.*, 1990; Schmidt & Ahring, 1996). In our laboratory, the relationship between the structure and the resistance to toxic chemicals in anaerobic granules from a brewery-wastewater-treatment UASB reactor was studied (Bae & Lee, 1999; Bae *et al.*, 2000). In a series of studies, we attempted to isolate micro-organisms from the anaerobic granules in order to investigate the community structure by using a culture system. Interestingly, the granules contained aerobic bacteria even though they had been kept under anaerobic conditions for 2 years. Strain Ch05^T is one of the dominant bacterial isolates grown under aerobic conditions.

In the present study, we conducted a phylogenetic analysis on the basis of 16S rRNA gene sequences, DNA–DNA

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Abbreviation: UASB, upflow anaerobic sludge blanket.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Ch05^T is AB187584.

relatedness and some important phenotypic characteristics to determine the precise taxonomic position of this strain. On the basis of the results obtained in this study, we propose that strain Ch05^T should be placed in the genus *Paracoccus* as the type strain of a novel species.

For the isolation of aerobic bacteria, brownish black granules (around 2 mm in diameter) from a brewery-wastewater-treating UASB reactor, which had been operated anaerobically for 2 years, were homogenized by using an Ace homogenizer (Nihonseiki). The suspension was spread on R2A agar plates (Scharlau) after being serially diluted with 50 mM phosphate buffer (pH 7.0). The plates were incubated at 30 °C for 2 weeks. Single colonies on the plates were purified by transferring them onto new plates and incubating them once again under the same conditions. The purified colonies were tentatively identified by means of partial sequences of 16S rRNA. Ch05^T was one of the dominant isolates appearing on the plates under aerobic conditions.

A Gram-reaction was performed by using the non-staining method as described by Buck (1982). Cell morphology was observed under a Nikon E600 light microscope at × 1000, using cells grown for 2 days at 30 °C on R2A agar. Catalase and oxidase tests were performed by using the procedures outlined by Cappuccino & Sherman (2002). The utilization of various substrates as sole carbon sources, and some physiological characteristics, were determined with API 32GN and API 20NE galleries according to the instructions of the manufacturer (bioMérieux). Nitrate and nitrite reduction were later confirmed by inoculating cells, in each case, into three serum bottles (25 ml) containing 13 ml R2A medium, while nitrate and nitrite were added as KNO₃ and NaNO₂ at concentrations of 10 mM. The reduction of nitrate and nitrite was monitored by ion chromatograph (model 790 personal IC; Metrohm) equipped with a conductivity detector and anion exchange column (Metrosep Anion Supp 4; Metrohm). The physiological characteristics of strain Ch05^T and related type strains are summarized in Table 1.

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene sequences and sequencing of purified PCR products were carried out according to Im *et al.* (2004). The 16S rRNA gene sequences of related taxa were obtained from GenBank. The multiple alignments were performed using the CLUSTAL X program (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). A phylogenetic tree was constructed by using the neighbour-joining methods (Saitou & Nei, 1987) in the MEGA2 program (Kumar *et al.*, 2001), with bootstrap values based on 1000 replications (Felsenstein, 1985) (Fig. 1). The 16S rRNA gene sequence of strain Ch05^T was a continuous stretch of 1386 bp. Sequence-similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain

Table 1. Comparison of selected characteristics of *P. koreensis* sp. nov. Ch05^T with those of its nearest phylogenetic neighbours within the genus *Paracoccus*

Strains: 1, *P. koreensis* Ch05^T; 2, *P. kocurii* KCTC 12189^T; 3, *P. alkenifer* KCTC 12188^T; 4, *P. solventivorans* KCTC 12190^T. All strains are Gram-negative and short rod- to coccus-shaped and are positive for oxidase and catalase activities and assimilation of acetate, 3-hydroxybutyrate, DL-lactate and propionate. All strains are negative for assimilation of adipate, L-arabinose, caprate, citrate, L-fucose, D-glucose, 3-hydroxybenzoate, inositol, itaconate, 2-ketogluconate, malate, maltose, mannitol, mannose, D-melibiose, N-acetylglucosamine, rhamnose, salicin, suberate and D-sucrose, indole production, acid production from glucose and for the production of arginine dihydrolase, urease, β-glucosidase, β-galactosidase and protease (casein hydrolysis).

Characteristic	1	2	3	4
Denitrification	—	+	+	+
Carbon utilization				
L-Alanine	+	—	—	—
Gluconate	—	—	—	+
Glycogen	+	—	—	—
Histidine	+	—	+	+
4-Hydroxybenzoate	—	—	—	+
5-Ketogluconate	—	—	+	—
Malonate	—	—	+	+
Phenylacetate	—	—	+	—
L-Proline	+	—	+	+
D-Ribose	+	—	—	—
L-Serine	—	—	+	+
D-Sorbitol	+	—	—	—
Valerate	+	—	+	+

Ch05^T were *Paracoccus solventivorans* (97.5 %), *Paracoccus alkenifer* (96.9 %) and *Paracoccus kocurii* (96.4 %), and the phylogenetic distance from other *Paracoccus* species with validly published names was more than 4 %.

Fig. 1. Phylogenetic tree, constructed from a comparative neighbour-joining analysis of 16S rRNA gene sequences, showing the relationships between *P. koreensis* Ch05^T and other related species. Bootstrap values (expressed as percentages of 1000 replications) greater than 70 % are shown at branch points. Bar, 1 substitution per 100 nt positions.

The G + C content of the chromosomal DNA was determined as described by Mesbah *et al.* (1989), using reverse-phase HPLC. Quinones were extracted from cells grown on a nutrient broth (Scharlau) and then analysed as described by Komagata & Suzuki (1987), using reverse-phase HPLC. Cellular fatty acids were analysed in micro-organisms grown on trypticase soy agar (Scharlau) for 2 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed using gas chromatography (6890; Hewlett Packard) and identified by using the Microbial Identification software package (Sasser, 1990).

The G + C content of genomic DNA of strain Ch05^T was 69 mol%, while those of *Paracoccus* species were in the range 63–71 mol% (Harker *et al.*, 1998). The respiratory quinone system of Ch05^T supports the affiliation of this strain to the α -Proteobacteria, as the majority of *Paracoccus* species contain Q-10 as the major quinone. The fatty acid profile of strain Ch05^T was composed mainly of C_{18:1} (79.6%), C_{18:0} (13.4%), C_{10:0} 3-OH (2.4%) and C_{16:0} (2.3%). A comparison of the fatty acid profile of Ch05^T with those of phylogenetically related strains is presented in Table 2. The fatty acid profile of strain Ch05^T is relatively simple compared with the others.

DNA–DNA hybridization experiments were performed between Ch05^T and type strains of the phylogenetically closest *Paracoccus* species, using the method described by Ezaki *et al.* (1989) with photobiotin-labelled DNA probes and microdilution wells. The DNA–DNA relatedness values for strain Ch05^T with respect to the type strains of the phylogenetically closest *Paracoccus* species were in the range 14–36%, which is sufficiently low to allow the assignment of strain Ch05^T to the genus *Paracoccus* as a novel species. On the basis of the data and observations described above, strain Ch05^T should be assigned to the genus *Paracoccus* as the type strain of a novel species, for which the name *Paracoccus koreensis* sp. nov. is proposed.

Description of *Paracoccus koreensis* sp. nov.

Paracoccus koreensis (ko.re.en'sis. N.L. masc. adj. *koreensis* pertaining to Korea, the location of the granule samples from which the type strain was isolated).

Cells are Gram-negative, aerobic, non-motile, short rod- to coccus-shaped and 0.5–1.0 µm by 1.0–1.5 µm in size after 2 days culture on R2A agar. Colonies grown on R2A agar for 1–2 days are smooth, circular, non-glossy, creamy white and convex. Grows well at 15–37 °C and at pH 6–8, but does not grow at 4 or 45 °C. Catalase- and oxidase-positive; shows oxidative metabolism. Unable to grow anaerobically using nitrate as electron acceptor. Unable to reduce nitrate but able to reduce nitrite. Substrate utilization, enzyme production, acid production and other physiological characteristics are indicated in Table 1. Q-10 is the predominant ubiquinone. C_{18:1} (79.6%) and C_{18:0} (13.4%) are the major

Table 2. Cellular fatty acid profile of *P. koreensis* sp. nov. Ch05^T and related type strains of *Paracoccus* species

Strains: 1, *P. koreensis* Ch05^T; 2, *P. kocurii* KCTC 12189^T; 3, *P. alkenifer* KCTC 12188^T; 4, *P. solventivorans* KCTC 12190^T. tr, Trace amounts (<1%).

Fatty acid	1	2	3	4
Saturated				
C _{12:0}	–	1.1	–	–
C _{14:0}	–	tr	–	–
C _{16:0}	2.3	4.0	1.6	–
C _{17:0}	–	3.2	2.3	2.2
C _{18:0}	13.4	14.8	4.5	7.1
C _{19:0}	–	1.0	0.9	–
Unsaturated				
C _{20:1} ω9t	–	2.6	1.7	1.4
Hydroxy fatty acids				
C _{16:0} 2-OH	–	1.1	–	–
C _{10:0} 3-OH	2.4	–	–	–
C _{19:0} cyclo ω8c	–	34.5	–	–
Summed features*				
3	–	tr	2.3	3.2
7	79.6	36.5	86.7	86.1
Unknown†				
ECL 11.798	2.3	–	–	–

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains C_{14:0} 3-OH and/or C_{16:1} iso I; summed feature 7 contains C_{18:1}ω7c/ω9t/ω12t.

†Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified. Equivalent chain lengths (ECL) are given.

cellular fatty acids. The G + C content of the genomic DNA is 69 mol% (as determined by HPLC).

The type strain, Ch05^T (= KCTC 12238^T = IAM 15216^T), was isolated from granules used in the wastewater-treatment plant of a beer-brewing factory in Chung-Won, Republic of Korea.

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