Cellulomonas terrae sp. nov., a cellulolytic and xylanolytic bacterium isolated from soil

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A bacterial strain (DB5^T), with polysaccharide-degrading activities, was isolated from garden soil in Daejeon, Republic of Korea. The cells were Gram-positive, aerobic or facultatively anaerobic, non-motile straight rods. Phylogenetic analysis based on 16S rRNA gene sequences showed that this strain belongs to the genus *Cellulomonas* and that it is most closely related to *Cellulomonas xylanilytica* LMG 21723^T and *Cellulomonas humilata* ATCC 25174^T (98.0 and 97.9 % similarity, respectively). Chemotaxonomic data also supported the classification of strain DB5^T in the genus *Cellulomonas*, i.e. L-ornithine as the cell-wall diamino acid, anteiso-C_{15:0} and iso-C_{15:0} as the major fatty acids, MK-9(H₄) as the predominant menaquinone and the presence of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol mannosides in the polar lipid profile. The results of DNA–DNA hybridization in combination with chemotaxonomic and physiological data demonstrated that strain DB5^T (=KCTC 19081^T=NBRC 100819^T) should be classified as the type strain of a novel species within the genus *Cellulomonas*, for which the name *Cellulomonas terrae* sp. nov. is proposed.

Polysaccharide-degrading enzymes such as amylase, cellulase and xylanase are widespread in nature. They can be found in every type of organism, including mammals, plants, algae, moulds, bacteria and phages (Oshima *et al.*, 2002; Sutherland, 1999; Terra & Ferreira, 1994). For the production of polysaccharases, micro-organisms are usually the most convenient sources and they can be obtained from various natural environments.

During a study of the bacterial community that produces polysaccharases, a large number of novel bacterial strains were isolated (Kang *et al.*, 2003) from soil near the Korean city of Daejeon. On the basis of 16S rRNA sequence data, one of these isolates, designated strain DB5^T, was found to be a member of the genus *Cellulomonas* in the suborder *Micrococcineae* and was subjected to taxonomic investigation.

The aim of this study was to determine the taxonomic position of strain DB5^T by using chemotaxonomic, physiological and DNA–DNA hybridization analyses. The results provided evidence that DB5^T is a representative of a novel bacterial species.

Strain DB5^T was isolated using the plate technique together with an insoluble chromogenic substrate (Ten *et al.*, 2004). After isolation, strain DB5^T was cultivated by being transferred on R2A agar (Difco) every month. Cell morphology and motility were observed under a Nikon E600 light microscope (1000 × magnification) using cells grown on R2A agar for 3 days at 30 °C. Growth at different temperatures and pH values was assessed after 5 days incubation. Salt tolerance was tested in R2A broth (Difco) supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth was estimated by monitoring the OD₆₀₀ value. Anaerobic growth was observed in serum bottles by adding thioglycolate (1 g l⁻¹) to R2A broth and substituting the upper air layer with nitrogen gas. Carbon-source utilization and some enzyme activities were tested by using API 20NE, API ID 32 and API 50 CH test kits (bioMérieux). Catalase activity was determined by using 3 % (v/v) H₂O₂, while oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine (Acros). Degradation of DNA (using DNA agar from Difco, supplemented with 0.01 % toluidine blue from Merck), degradation of casein, cellulose and starch (Atlas, 1993), degradation of lipid (Kouker & Jaeger, 1987) and degradation of xylan (Ten *et al.*, 2004) were also investigated; reactions were read after 5 days. Duplicate antibiotic-sensitivity tests were done using filter-paper discs containing the following: streptomycin (5, 10 and 15 µg), tetracycline (5, 10 and 15 µg), kanamycin (1.0, 1.5 and 2.0 mg) and ampicillin (20, 25 and 30 µg) (all from Sigma). Discs were placed on R2A plates

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DB5^T is AY884570.

Results of two-dimensional TLC analysis of polar lipids of strain DB5^T are available as supplementary material in IJSEM Online.

Table 1. Comparison of selected characteristics of *C. terrae* sp. nov. DB5^T with those of its nearest phylogenetic neighbours in the genus *Cellulomonas*

Taxa: 1, *C. terrae* sp. nov. DB5^T; 2, *C. xylanilytica*; 3, *C. humilata*; 4, *Cellulomonas cellasea*; 5, *Cellulomonas biazotea*; 6, *Cellulomonas fimi*; 7, *Cellulomonas hominis*. Data for taxa 1–3 are from this study; data for taxa 4–7 are from Rivas *et al.* (2004). Symbols: +, positive; (+), positive, weak or delayed response; –, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Motility	–	–	–	–	+	+	+
Catalase	–	+	–	+	+	+	+
Growth on:							
Acetate	–	–	–	+	+	–	ND
Gluconate	–	–	+	–	–	–	+
Lactose	+	+	+	–	+	+	+
Lactate	–	–	–	+	+	+	ND
Mannose	+	+	+	+	+	+	ND
Rhamnose	(+)	+	+	–	+	+	+
Mannitol	–	–	+	+	–	–	–
Hydrolysis of:							
Cellulose	+	+	+	+	+	+	ND
Xylan	+	+	+	ND	ND	ND	ND
Lipid	–	–	–	ND	ND	ND	ND
Casein	–	–	–	ND	ND	ND	ND
Starch	+	+	–	ND	ND	ND	ND
Peptidoglycan composition	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn
Cell-wall sugars*	Rha, Gal, Glc	Rha, Man, Fuc	Rha, Fuc, Glc	Rha, Man, 6dTal	Rha, Man, Gal, 6dTal	Rha, Fuc, Glc	ND
Predominant cellular fatty acids	anteiso-C _{15:0} , iso-C _{15:0} , C _{16:0}	anteiso-C _{15:0} , iso-C _{15:0} , C _{18:0}	anteiso-C _{15:0} , anteiso-C _{17:0} , C _{16:0}	anteiso-C _{15:0} , C _{16:0} , anteiso-C _{17:0}	anteiso-C _{15:0} , iso-C _{15:0} , C _{16:0}	anteiso-C _{15:0} , C _{16:0} , anteiso-C _{17:0}	anteiso-C _{15:0} , C _{16:0} , anteiso-C _{17:0}
DNA G + C content (mol%)	73.9	73	ND	75	71.5–75.6	71.3–72.0	76

*6dTal, 6-Deoxytalose; Fuc, fucose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose.

spread with DB5^T culture and were then incubated at 30 °C for 5 days. The physiological and biochemical characteristics of strain DB5^T and related type strains are summarized in Table 1.

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Im *et al.* (2004). The 16S rRNA gene partial sequences were compiled using SeqMan software; the 16S rRNA gene sequences of the test strain and related taxa (obtained from GenBank) were edited using the BioEdit program (Hall, 1999). Multiple alignments were performed by using the CLUSTAL_X program (Thompson *et al.*, 1997). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). A phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) in the MEGA2 program (Kumar *et al.*, 2001). Bootstrap analysis with 1000 replicates was performed to obtain the confidence level of the branches (Felsenstein, 1985). All 12 species in the genus *Cellulomonas* were included in the phylogenetic tree.

Purified cell-wall preparations were obtained as described by Schleifer & Kandler (1972). Amino acids and peptides in cell-wall hydrolysates were analysed with two-dimensional TLC on cellulose plates using solvent systems described by Schleifer & Kandler (1972) and cell-wall sugar analysis as described by Stanek & Roberts (1974). Menaquinone was extracted from cells grown on R2A broth and analysed as described by Komagata & Suzuki (1987), using reverse-phase HPLC. Cellular fatty acids were analysed in organisms grown on trypticase soy agar (Difco) for 2 days. The cellular fatty acids were saponified, methylated, extracted and identified by the Microbial Identification software package (Sasser, 1990). Polar lipids were extracted and examined by using two-dimensional TLC (Minnikin *et al.*, 1984).

The G + C content of the chromosomal DNA was determined as described by Mesbah *et al.* (1989), using reverse-phase HPLC. DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes (Sigma) and microdilution wells (Greiner). Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values are quoted as DNA hybridization values.

The cells of strain DB5^T were found to be Gram-positive, non-motile, straight rods that were 0.3–0.6 µm in diameter and 1.6–2.3 µm in length. Colonies on R2A agar were yellow and smooth with clear edges. The strain showed negative results for catalase and oxidase activity, it was aerobic or facultatively anaerobic and it could reduce nitrate and hydrolyse aesculin, DNA, xylan and starch. The strain grew at 10–37 °C but not at 4 or 45 °C; the optimum temperature was 30 °C. Growth was observed at pH values in the range 6.5–9.0, the optimum pH being from 7.0 to 8.0. Strain DB5^T showed salt tolerance of 4 % (w/v) NaCl.

Details of various differentiating characteristics of strain DB5^T and of phylogenetically related species are shown in Table 1; other characteristics are given in the species description.

The peptidoglycan composition of strain DB5^T corresponded to type A4β; it contained L-ornithine–D-glutamic acid, which is reported for most members of the genus *Cellulomonas* and has been emphasized as an important feature for delineation, at genus level, in the *Actinobacteria* (Stackebrandt & Schumann, 2000). In the case of *Cellulomonas humilata* ATCC 25174^T, Gledhill & Casida (1969) found that the peptidoglycan of this strain contained lysine and ornithine. However, in our study, the determination of peptidoglycan for *C. humilata* ATCC 25174^T showed the presence of L-ornithine–D-glutamic acid, which corresponds with the results of Stackebrandt *et al.* (2002); furthermore, the latter composition is found in most *Cellulomonas* species, including *Cellulomonas xylanilytica* LMG 21723^T. The cell-wall sugars of strain DB5^T were galactose, glucose and rhamnose. Fatty acid profiles of the strain and its closest neighbours are shown in Table 2. The major fatty acids were anteiso-C_{15:0} (51.9 %), iso-C_{15:0} (13.3 %) and C_{16:0} (10.9 %). No significant differences in the fatty acid profiles were found with respect to the other *Cellulomonas* species. HPLC analysis of the menaquinones revealed two peaks: the main peak corresponded to MK-9(H₄) and the smaller one to MK-8(H₄). In addition, the major menaquinone found in members of the family *Cellulomonadaceae* is MK-9(H₄). The polar lipids detected were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol mannosides and other unidentified phosphoglycolipids. The results of

Table 2. Cellular fatty acid composition of strain DB5^T and related type strains

Taxa: 1, *C. terrae* DB5^T; 2, *C. xylanilytica*; 3, *C. humilata*. Fatty acids that account for less than 0.5 % of the total fatty acid content in all strains are not shown; –, not detected.

Fatty acid	1	2	3
C _{14:0}	3.6–3.8	4.6–4.8	1.0–1.8
C _{16:0}	8.0–9.5	9.5–10.6	11.2–12.8
C _{17:0}	1.8–2.3	1.8–2.7	1.2–2.1
C _{18:0}	7.1–8.2	10.7–13.0	0.8–1.8
iso-C _{14:0}	5.1–5.9	7.7–9.6	7.8–8.9
iso-C _{15:0}	15.2–18.5	18.6–22.1	10.9–12.2
anteiso-C _{15:0}	44.5–45.6	40.1–45.5	32.8–44.9
anteiso-C _{15:1} A	–	1.9–2.6	2.4–3.3
iso-C _{16:0}	–	1.8–2.3	1.7–7.0
iso-C _{17:0}	–	–	0.8–1.6
anteiso-C _{17:0}	7.4–7.6	5.5–6.6	11.6–13.7
Unknown 14:258*	2.9–3.4	3.0–3.3	1.2–2.7

*Unknown 14:258 represents an unknown peak that appeared at 14.258 min.

Fig. 1. Rooted phylogenetic tree based on the 16S rRNA gene sequences of the proposed species *C. terrae* sp. nov. DB5^T and its closest phylogenetic neighbours. This tree was made using the neighbour-joining method (Saitou & Nei, 1987) with a Kimura two-parameter distance matrix and pairwise deletion. Bootstrap values (expressed as percentages of 1000 replications) greater than 70 % are shown at branch points. Bar, 10 substitutions per 1000 nt.

polar lipid analysis of strain DB5^T are available as supplementary data in IJSEM Online.

The almost-complete 16S rRNA gene sequence (1475 bp) of strain DB5^T was compared with 16S rRNA gene sequences from the GenBank database, with the aid of the BLASTN program (Altschul *et al.*, 1990) and the Ribosomal Database Project (Maidak *et al.*, 1997). The two species most closely related to strain DB5^T were found to be *C. xylanilytica* LMG 21723^T (98.0 %) and *C. humilata* ATCC 25174^T (97.9 %). A phylogenetic tree obtained by using the neighbour-joining method shows this close relationship (Fig. 1), which was supported by a high bootstrap value based on 1000 resamplings. Furthermore, the results of DNA–DNA hybridization showed that strain DB5^T has 41 % relatedness with *C. xylanilytica* LMG 21723^T and 20 % relatedness with *C. humilata* ATCC 25174^T. These results suggest that strain DB5^T should be classified as a novel species, particularly in view of the recommendations of Wayne *et al.* (1987) and Stackebrandt & Goebel (1994) for species delineation. The DNA G + C content of the strain is 73.9 mol%, a value that falls within the range reported for members of the genus *Cellulomonas*.

In summary, the characteristics of strain DB5^T are consistent with descriptions of the genus *Cellulomonas* with regard to morphological, biochemical and chemotaxonomic properties. The characteristics also supported the conclusion inferred from the 16S rRNA gene sequence analysis, i.e. that strain DB5^T belongs to the genus *Cellulomonas*. However, some biochemical reactions and physiological characteristics differentiate strain DB5^T from any *Cellulomonas* species with validly published names, and DNA–DNA hybridization confirmed that this strain represents a taxonomically distinct species. On the basis of these results, DB5^T (=KCTC 19081^T=NBRC 100819^T) is proposed as the type strain of a novel species of the genus *Cellulomonas*, namely *Cellulomonas terrae* sp. nov.

Description of *Cellulomonas terrae* sp. nov.

Cellulomonas terrae (ter'rae. L. gen. n. *terrae* of the earth).

Grows well under aerobic or facultatively anaerobic conditions at 30 °C on R2A agar. Cells are Gram-positive,

straight rods and produce creamy yellow, circular, smooth colonies. No growth at 4 or 45 °C or at 4 % NaCl. Shows negative results for oxidase and catalase activity and production of urease, indole and acylamidase, and positive results for β -galactosidase, β -glucosidase, reduction of nitrate and hydrolysis of aesculin, DNA, starch and xylan. Can also use the following as sole carbon sources: arabinose, cellulose, fructose, galactose, gentiobiose, glycogen, glucose, inositol, 5-ketogluconate, maltose, mannose, D-melibiose, N-acetylglucosamine, rhamnose, D-sucrose, starch and xylan. Shows no growth with the following: acetate, adipate, L-alanine, caprate, citrate, L-fucose, gluconate, 3-hydroxybenzoate, 3-hydroxybutyrate, 4-hydroxybutyrate, histidine, itaconate, 2-ketogluconate, lactate, mannitol, malate, malonate, propionate, L-proline, phenylacetate, D-ribose, salicin, L-serine, D-sorbitol, suberate and valerate. Can produce acid from L-arabinose, cellobiose, fructose, galactose, gentiobiose, glycerol, glycogen, glucose, D-lyxose, maltose, mannose, melezitose, melibiose, N-acetylglucosamine, raffinose, sucrose, trehalose, D-turanose and D-xylose. It is also resistant to discs containing 30 μ g ampicillin, 15 μ g tetracycline, 15 μ g streptomycin and 2 mg kanamycin. The predominant isoprenoid quinones are MK-9(H₄) and MK-8(H₄). The most abundant cellular fatty acids are anteiso-C_{15:0} (51.9 %), iso-C_{15:0} (13.3 %) and C_{16:0} (10.9 %). The G + C content of the genomic DNA of the type strain is 73.9 mol%. The peptidoglycan contains L-Orn–D-Glu (type A4 β). The cell-wall sugars are galactose, glucose and rhamnose.

The type strain, DB5^T (=KCTC 19081^T=NBRC 100819^T), was isolated from soil near Yusong in Daejeon City, Republic of Korea.

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