Cellulomonas composti sp. nov., a cellulolytic bacterium isolated from cattle farm compost

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A bacterial strain, TR7-06^T, which has cellulase and β -glucosidase activities, was isolated from compost at a cattle farm near Daejeon, Republic of Korea. It was a Gram-positive, aerobic or facultatively anaerobic, non-motile, rod-shaped bacterium. Phylogenetic analysis based on 16S rRNA gene sequences showed that this strain belongs to the genus *Cellulomonas*, with highest sequence similarity to *Cellulomonas uda* DSM 20107^T (98.5%). Cell wall analysis revealed the presence of type A4 β , L-Orn-D-Glu peptidoglycan. The cell-wall sugars detected were mannose and glucose. The predominant menaquinone was MK-9(H₄); MK-8(H₄) was detected in smaller quantities. The major fatty acids were anteiso-C_{15:0}, C_{16:0}, C_{14:0} and C_{18:0}. The polar lipids detected were diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. The results of DNA–DNA hybridization and physiological and biochemical tests clearly demonstrated that TR7-06^T represents a novel species. The combined genotypic and phenotypic data show that strain TR7-06^T (=KCTC 19030^T=NBRC 100758^T) merits description as the type strain of a novel *Cellulomonas* species, *Cellulomonas composti* sp. nov.

The genus *Cellulomonas* has a long taxonomic history and comprises species of clinical (Funke *et al.*, 1995) and environmental (Bagnara *et al.*, 1985; Elberson *et al.*, 2000) significance. The genus was proposed by Bergey *et al.* (1923) for several organisms which were previously classified by Kellerman & McBeth (1912) as *Bacillus* species. Applications of chemotaxonomic, molecular systematic and numerical phenetic methods were significant in the establishment of the genus, and these methods form the basis for its differentiation from closely related genera by a combination of morphological and biochemical properties; in particular, *Cellulomonas* species can be differentiated from members of related genera on the basis of chemotaxonomic and phenotypic properties (Stackebrandt & Kandler, 1979).

Along with *Oerskovia*, *Cellulomonas* currently belongs to the family *Cellulomonadaceae* (Stackebrandt & Schumann, 2000) in the suborder *Micrococccineae* (Prévot, 1961; Stackebrandt *et al.*, 1997). At the time of writing, the genus contains 14 species with validly published names.

Strain TR7-06^T was isolated along with a large member of other strains during a study of the cultivable aerobic bacterial community in compost from a cattle farm near Daejeon, Republic of Korea. Strain TR7-06^T was shown to be a member of the genus *Cellulomonas* based on partial 16S rRNA gene sequence data and it was subjected to a taxonomic investigation by using chemotaxonomic, physiological and DNA–DNA hybridization analyses. The results provided evidence that TR7-06^T is a representative of a novel bacterial species.

TR7-06^T was one of the isolates from the compost, which was composed of cow dung and rice straw, that grew on R2A agar (Difco) plates under aerobic conditions. The strain was maintained on R2A agar; stock cultures were preserved as a

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TR7-06^T is AB166887.

API ID32 test results for strain TR7-06^T and related *Cellulomonas* strains are available with the online version of this paper.

suspension in glycerol (20 %, v/v) at -70°C . Biomass for chemotaxonomic studies was prepared by cultivating the strain in shake flasks of R2A broth (Difco) at 150 r.p.m. for 3 days at 30°C . Cultures were checked for purity, harvested by centrifugation and freeze-dried.

Cell morphology and motility were observed under a Nikon light microscope ($1000\times$ magnification) with cells grown on R2A agar for 3 days at 30°C . The Gram reaction was conducted according to the non-staining method described by Buck (1982). Growth at different temperatures and pH was assessed after 5 days incubation on R2A agar. Salt tolerance was tested in R2A broth supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth was estimated by monitoring OD₆₀₀. Anaerobic growth was observed in serum bottles by adding thioglycolate (1 g l^{-1}) to R2A broth and replacing the upper air layer with nitrogen gas. Carbon source utilization and some enzyme activities were tested by using the API 20NE, API ID32 and API 50CH test kits (bioMérieux). Catalase activity was determined using 3 % (v/v) H_2O_2 and oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine. Tests for the degradation of DNA [flooding DNase agar plates (Scharlau) with 1 M HCl], casein, chitin, starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987), xylan and cellulose (Ten *et al.*, 2004) were performed and evaluated after 5 days. Results regarding the physiological characteristics of strain TR7-06^T are summarized in the species description; the data enable the novel strain to be differentiated from other *Cellulomonas* species, as shown in Table 1.

Extraction of genomic DNA was performed with a commercial genomic DNA extraction kit (Solgent) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR products were carried out according to Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled using SEQMAN software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed using the program CLUSTAL_X (Thompson *et al.*, 1997). Gaps were edited in the program BioEdit (Hall, 1999). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using a neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) using the program MEGA3 (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

Purified cell-wall preparations were obtained as described by Schleifer & Kandler (1972). Amino acids and peptides in cell-wall hydrolysates were analysed by two-dimensional TLC on cellulose plates using solvent systems described by Schleifer & Kandler (1972). Cell-wall sugars were analysed as described by Stanek & Roberts (1974). Menaquinones were extracted from cells grown in R2A broth (Difco) and analysed as described by Komagata & Suzuki (1987) using reverse-phase HPLC. Cellular fatty acid profiles were determined for strains grown on TSA (Difco) for 2 days. The cellular fatty acids were saponified, methylated and

extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). Fatty acids analysed by GC (Hewlett Packard 6890) were identified by the Microbial Identification software package (Sasser, 1990). Polar lipids were extracted and examined by 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 by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. 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–DNA hybridization values.

Phenotypic properties of strain TR7-06^T were consistent with its classification in the genus *Cellulomonas* (Stackebrandt & Kandler, 1979). It was an aerobic or facultatively anaerobic, Gram-positive, rod-shaped and non-motile actinomycete that did not form mycelium. It contained L-Orn–D-Glu as diamino acid in the peptidoglycan (peptidoglycan type A4β) and mannose and glucose in the cell wall (cell-wall type VIII). The predominant menaquinone was MK-9(H₄) and the DNA G + C content was 73.7 mol%.

The almost complete 16S rRNA gene sequence (1453 bp) obtained for strain TR7-06^T was compared with sequences of representatives of the suborder *Micrococineae*. High 16S rRNA gene sequence similarity was found between the tested strain and representatives of the genus *Cellulomonas* (94.4–98.3 %), which supports its inclusion in the genus *Cellulomonas*. It is evident from Fig. 1 that strain TR7-06^T is most closely related to *Cellulomonas uda* DSM 20107^T, *Cellulomonas gelida* DSM 20111^T and *Cellulomonas iranensis* ATCC 700643^T, supported by a moderately high bootstrap value; 16S rRNA gene sequence similarities between strain TR7-06^T and these three type strains were 97.7–98.3 % (*C. uda* DSM 20107^T showed the highest sequence similarity). However, it is evident from DNA–DNA hybridization data that strain TR7-06^T and *C. uda* DSM 20107^T should not be classified within the same species as they shared a DNA–DNA hybridization value of 10.7 %, well below the 70 % cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987). The organism also has a profile of phenotypic properties that distinguish it from representatives of all species of *Cellulomonas* with validly published names, including *C. uda*, the most closely related species (Table 1). Moreover, strain TR7-06^T could be differentiated easily from the three species of the genus *Cellulomonas* that are phylogenetically most closely related using tests for the utilization of substrates as sole carbon sources (see Supplementary Table S1 available in IJSEM Online). Unlike *C. uda* DSM 20107^T and *C. gelida* DSM 20111^T, strain TR7-06^T could not utilize salicin or acetate.

Table 1. Phenotypic properties that distinguish strain TR7-06^T from the type strains of *Cellulomonas* species

Strains: 1, strain TR7-06^T; 2, *C. uda* DSM 20107^T; 3, *C. gelida* DSM 20111^T; 4, *C. iranensis* DSM 14785^T; 5, *C. persica* ATCC 700642^T; 6, *C. flavigena* DSM 20109^T; 7, *C. cellasea* DSM 20118^T; 8, *C. biazotea* DSM 20112^T; 9, *C. fimi* DSM 20113^T; 10, *C. humilata* ATCC 25174^T; 11, *C. xylanilytica* LMG 21723^T; 12, *C. fermentans* DSM 3133^T; 13, *C. hominis* DSM 9581^T. +, Positive; –, negative; W, weakly positive; ND, not determined/no data available. Data for reference strains were taken from Bagnara *et al.* (1985), Collins & Pascual (2000), Elberson *et al.* (2000), Funke *et al.* (1995), Rivas *et al.* (2004) and Stackebrandt & Kandler (1979).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Motility	–	–	+	+	+	–	–	+	+	–	–	–	+
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Diphtheroid, coccoid	Rod, coccoid	Polymorphic	Rod
Mycelium	–	–	–	–	–	–	–	–	–	+	–	–	–
Colony colour*	Y	WH	YW	YW	YW	Y	Y	YW	YW	WH	Y	WH	Y
Nitrate reduction	+	+	+	+	+	+	+	+	+	–	+	+	+
Peptidoglycan	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn–D-Asp	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn–D-Glu	L-Orn–D-Glu, Lys	L-Orn–D-Glu	L-Orn	L-Orn
Cell-wall sugars†	Glc, Man	GlcN, Glc	GlcN, Glc	GlcN, Rha, Glc, Man	GlcN, Rha, Glc, Man	GlcN, Rha, Rib, Man	Rha, Man, 6dTal	GlcN, Rha, 6dTal, Gal	GlcN, Rha, Fuc	Rha, Glc, Fuc	Man, Rha, Fuc	Glc, Rha, Rib	ND
Fermentation of:													
D-Glucose	+	+	+	ND	ND	+	+	+	+	+	+	+	+
Maltose	+	+	+	ND	ND	+	+	+	+	+	+	+	+
L-Rhamnose	–	–	–	ND	ND	–	–	+	+	+	+	–	+
Sucrose	+	+	+	ND	ND	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	ND	ND	+	+	+	+	+	+	+	+
Methyl β-xyloside	–	–	–	ND	ND	–	–	–	W	ND	ND	+	+
Gluconate	–	–	–	ND	ND	W	–	–	–	ND	ND	–	+
Dextrin	ND	+	+	+	+	+	–	–	+	+	ND	+	+
Mannitol	–	–	–	ND	ND	–	+	–	–	+	ND	+	–
Enzyme activities													
Catalase	–	+	+	ND	ND	+	+	+	+	–	+	–	+
Cellulase	+	+	+	+	+	+	+	+	+	ND	+	+	–
Urease	–	–	–	+	+	–	–	–	–	–	–	–	–
Hydrolysis of:													
Aesculin	+	+	+	ND	ND	+	+	+	+	+	+	+	+
Gelatin	W	+	+	W	W	+	–	+	+	W	W	+	+
Major fatty acids‡	ai-C _{15:0} , C _{16:0} , C _{14:0}	ai-C _{15:0} , C _{16:0} , C _{15:0}	ai-C _{15:0} , C _{16:0} , C _{15:0}	ND	ND	ai-C _{15:0} , C _{16:0} , C _{14:0}	ai-C _{15:0} , C _{16:0} , ai-C _{17:0}	ai-C _{15:0} , i-C _{15:0} , C _{16:0}	ai-C _{15:0} , C _{16:0} , ai-C _{17:0}	ND	ai-C _{15:0} , C _{16:0} , C _{18:0}	ai-C _{15:0} , C _{16:0} , C _{14:0}	ai-C _{15:0} , C _{16:0} , ai-C _{17:0}
DNA G+C content (mol%)	73.7	72.0–76.3	72.4–74.4	ND	ND	72.7–74.8	75	71.5–75.6	71.3–72	73	73	75.8	73.0–76.0

*WH, White; Y, yellow; YW, yellow–white.

†6dTal, 6-Deoxytalose; Fuc, fucose; Gal, galactose; Glc, glucose; GlcN, glucosamine; Man, mannose; Rha, rhamnose; Rib, ribose.

‡ai, Anteiso; i, iso.

Fig. 1. Phylogenetic tree based on almost-complete 16S rRNA gene sequences showing relationships between strain TR7-06^T and representatives of *Cellulomonas* species (neighbour-joining method). Dots indicate generic branches of the tree that were also found using the maximum-parsimony treeing algorithm. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; values greater than 60% are shown. Bar, 0.005 substitutions per nucleotide site.

The genotypic and phenotypic data indicate that strain TR7-06^T merits recognition as a representative of a novel species of *Cellulomonas*. It is proposed that the organism should be classified in the genus *Cellulomonas* as *Cellulomonas composti* sp. nov.

Description of *Cellulomonas composti* sp. nov.

Cellulomonas composti (N.L. n. *compostum* compost; N.L. gen. n. *composti* of compost).

Gram-positive, aerobic or facultatively anaerobic, non-motile, rod-shaped cells. Colonies on R2A agar plates are circular, smooth, yellow circles within 3 days at 25 °C. Optimal growth occurs at 30 °C and pH 6.8–7.5. Utilizes D-glucose, maltose and glycogen as sole carbon sources. Acid is produced aerobically and anaerobically from L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-cellobiose, D-maltose, sucrose, D-trehalose, starch, glycogen and gentiobiose. Acid is produced weakly under anaerobic conditions from D-ribose, D-lactose, inulin and D-lyxose. Hydrolyses gelatin weakly. Positive for nitrate reduction. β -Glucosidase, amylase, cellulase and DNase are produced. Negative for catalase and urease. MK-9(H₄) is the predominant menaquinone. The major cellular fatty acids are anteiso-C_{15:0} (44.0%), C_{16:0} (14.6%), C_{14:0} (9.0%) and C_{18:0} (7.9%). Cell wall sugars are mannose and glucose.

The type strain is TR7-06^T (=KCTC 19030^T=NBRC 100758^T), isolated from compost from a cattle farm near Daejeon, Republic of Korea. The G+C content of the genomic DNA of the type strain is 73.7 mol% (determined by HPLC).

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