

Cellulomonas xylanilytica sp. nov., a cellulolytic and xylanolytic bacterium isolated from a decayed elm tree

Raúl Rivas, Martha E. Trujillo, P. F. Mateos, E. Martínez-Molina and Encarna Velázquez

Departamento de Microbiología y Genética, Edificio Departamental, Campus Miguel de Unamuno, Universidad de Salamanca, Spain

Correspondence
Martha E. Trujillo
mett@usal.es

A Gram-positive, aerobic, non-motile bacterium was isolated from a decayed elm tree. Phylogenetic analysis based on 16S rDNA sequences revealed 99.0% similarity to *Cellulomonas humilata*. Chemotaxonomic data that were determined for this isolate included cell-wall composition, fatty acid profiles and polar lipids; the results supported the placement of strain XIL11^T in the genus *Cellulomonas*. The DNA G+C content was 73 mol%. The results of DNA–DNA hybridization with *C. humilata* ATCC 25174^T, in combination with chemotaxonomic and physiological data, demonstrated that isolate XIL11^T should be classified as a novel *Cellulomonas* species. The name *Cellulomonas xylanilytica* sp. nov. is proposed, with strain XIL11^T (= LMG 21723^T = CECT 5729^T) as the type strain.

During a study of bacterial populations that degrade plant polymers, we isolated several bacteria that produced hydrolytic enzymes; isolate XIL11^T was able to break down several polymers, namely cellulose, starch and xylan. On the basis of 16S rDNA sequence data, this strain was characterized primarily as a member of the genus *Cellulomonas*, which currently contains 14 species with validly published names (Stackebrandt & Keddie, 1986; Funke *et al.*, 1995; Collins & Pascual, 2000; Elberson *et al.*, 2000; Stackebrandt *et al.*, 2002). Further study of this strain, based on a polyphasic approach that included chemotaxonomic, physiological and DNA–DNA hybridization analyses, confirmed its position as a representative of a novel species within the genus *Cellulomonas*.

Strain XIL11^T was isolated on XED medium at 28 °C from decayed wood of an elm tree, as described previously (Rivas *et al.*, 2003). Extraction and amplification of genomic DNA for 16S rDNA sequence analysis were carried out as described previously (Rivas *et al.*, 2001, 2003). An almost-complete 16S rDNA sequence was obtained and aligned against 16S rDNA sequences that were available from public databases. Pairwise evolutionary distances were computed by using the correction algorithm of Jukes & Cantor (1969). The least-squares distance method of DeSoete (1983) was

used in the construction of the phylogenetic dendrogram from distance matrices. The MEGA2 package (Kumar *et al.*, 2001) was used for all analyses.

Isolate XIL11^T was observed through a phase-contrast microscope after 48 h growth in YED medium (0.4% yeast extract, 0.7% dextrose, 1.8% agar) to check for cell shape and motility. Cells were also stained according to the classical Gram procedure that was described by Doetsch (1981).

Physiological and biochemical tests were determined by using API 20NE, API 20E and API 50 CH strips (bio-Mérieux) according to the manufacturer's instructions. Enzyme activities for amylase, casein, catalase, cellulase and oxidase were determined as described previously (Rivas *et al.*, 2003). A temperature range for growth on YED medium of 4–45 °C was determined.

Amino acid and sugar analyses of whole-cell hydrolysates were performed according to procedures described by Stanek & Roberts (1974). Peptidoglycan type was determined as described by Schleifer & Kandler (1972) and Schleifer (1985). Menaquinone and cellular fatty acid compositions were determined as described by Zimmermann *et al.* (1998). Polar lipids were extracted and examined by two-dimensional TLC (Minnikin *et al.*, 1984).

The G+C content of genomic DNA was determined by using the thermal denaturation method (Mandel & Marmur, 1968).

DNA–DNA hybridization was performed [in 2 × SSC + 10%

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The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain XIL11^T is AY303668.

A fuller phylogenetic tree is available as supplementary material in IJSEM Online.

DMSO (v/v) at 68 °C] between strain XIL11^T and *Cellulomonas humilata* ATCC 25174^T. DNA was isolated as described by Cashion *et al.* (1977). Renaturation rates were calculated by using the TRANSFER.BAS program (Jahnke, 1992).

Strain XIL11^T was characterized morphologically as a Gram-positive, non-filamentous actinomycete that grew as lemon-yellow colonies on XED and YED media. Cells were non-motile and coccoid in shape (2.4 × 1.4 µm). Details of various differentiating characteristics of strain XIL11^T and phylogenetically related species are shown in Table 1. Other characteristics determined are given in the species description. Isolate XIL11^T grew at 4–37 °C. No growth was recorded at 45 °C.

The 16S rDNA sequence of strain XIL11^T was obtained and compared with those of members of the genus *Cellulomonas*. The sequence of strain XIL11^T showed 99.0% similarity to the 16S rDNA sequence of *C. humilata* ATCC 25174^T. A phylogenetic tree that was obtained by using the least-squares method shows this close relationship (Fig. 1), which was supported by a high bootstrap value (100%) based on 1000 resamplings. A fuller phylogenetic tree is available as supplementary material in IJSEM Online.

The 16S rDNA sequence of strain XIL11^T contained most of the signature nucleotides that have been defined for

members of the genus *Cellulomonas* (Stackebrandt *et al.*, 2002), except for the nucleotides at positions 614–626 (A–U) and 1438–1463 (A–U), which were identical to those found in the sequence of *C. humilata*, but differed from those of the rest of the species in the genus *Cellulomonas*.

The peptidoglycan composition of strain XIL11^T corresponded to type A4β; it contained D-ornithine–D-glutamic acid. This composition is reported for most members of the genus *Cellulomonas* and has been emphasized as an important feature for delineation at genus level in actinobacteria (Stackebrandt & Schumann, 2000). In the case of *C. humilata* ATCC 25174^T, Gledhill & Casida (1969) found that the peptidoglycan of this strain contained lysine and ornithine, whereas Stackebrandt *et al.* (2002) reported L-ornithine–D-glutamic acid; the latter composition is found in most *Cellulomonas* species, including strain XIL11^T. If the peptidoglycan results obtained by Stackebrandt *et al.* (2002) are confirmed, the genus *Cellulomonas* would then embrace a homogeneous group with respect to peptidoglycan structure, with L-ornithine in the third position of the peptide side-chain and D-aspartic acid or D-glutamic acid in the interpeptide bridge.

The cell-wall sugars in strain XIL11^T were rhamnose, mannose and traces of fucose. The major fatty acids were anteiso-C_{15:0} (41.66%), iso-C_{16:0} (13.37%), iso-C_{18:0} (12.83%) and anteiso-C_{17:0} (6.87%). The main difference

Table 1. Characteristics that differentiate *C. xylanilytica* from phylogenetically related *Cellulomonas* species

Species: 1, *C. xylanilytica*; 2, *C. humilata*; 3, *C. biazotea*; 4, *C. cellasea*; 5, *C. fimi*; 6, *C. hominis*. +, Positive; –, negative; W, weak; ND, not determined. All strains were positive for fermentation and negative for urea hydrolysis. Data are from Collins & Pascual (2000), Elberson *et al.* (2000), Schumann *et al.* (2001) and this study.

Characteristic	1	2	3	4	5	6
Shape	Curved rods or coccoid	Diphtheroid or coccoid	Straight or curved rods	Straight or curved rods	Straight or curved rods	Regular short rods
Mycelium	–	+	–	–	–	–
Motility	–	–	+	–	+	+
Catalase	+	–	+	+	+	+
Growth in:						
Acetate	–	–	+	+	–	ND
Gluconate	–	+	–	–	–	+
Lactose	+	+	+	–	+	+
Mannitol	–	+	–	+	–	–
Rhamnose	+	+	+	–	+	+
Hydrolysis of gelatin	W	W	+	–	+	+
Peptidoglycan type*	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, Man, Fuc	Rha, Glc, Fuc	Rha, Gal, Man, 6-deoxy-Tal	Rha, Man, 6-deoxy-Tal	Rha, Fuc, Glc	ND
Principal fatty acids	ai-C _{15:0} , C _{16:0} , C _{18:0}	ND	ai-C _{15:0} , i-C _{15:0} , C _{16:0}	ai-C _{15:0} , C _{16:0} , ai-C _{17:0}	ai-C _{15:0} , C _{16:0} , ai-C _{17:0}	ai-C _{15:0} , C _{16:0} , ai-C _{17:0}

*Glu, Glutamic acid; Orn, Ornithine.

†Composition according to Stackebrandt *et al.* (2002). Lysine and ornithine have been reported by Gledhill & Casida (1969).

‡Fuc, Fucose; Glc, glucose; Man, mannose; Rha, rhamnose; Tal, talose.

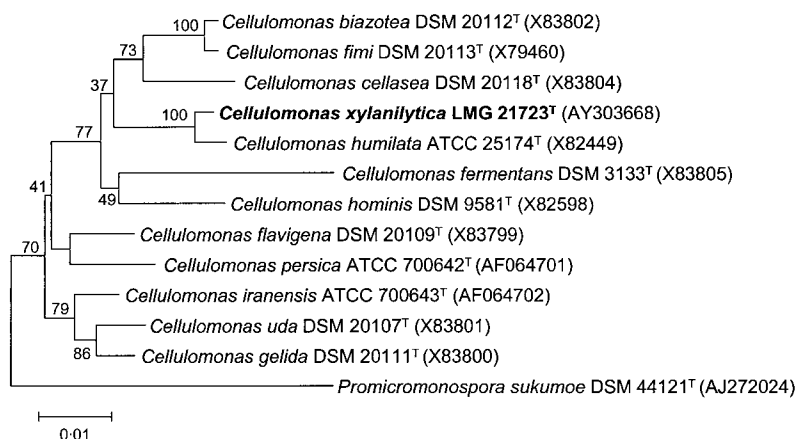


Fig. 1. Dendrogram based on comparison of 16S rRNA gene sequences of the proposed species *C. xylanilytica* XIL11^T and its closest phylogenetic neighbours. Bootstrap values are expressed as percentages of 1000 replications. Bar, 1 substitution in 100 nt.

was the fairly large amount of C_{18:0} in the new isolate with respect to other *Cellulomonas* species (Schumann *et al.*, 2001).

HPLC analysis of menaquinones revealed two peaks: the main peak corresponded to MK-9(H₄) and the smaller one to MK-8(H₄). MK-9(H₄) is the major menaquinone found in members of the family *Cellulomonadaceae*. Polar lipids detected were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides.

The results of DNA–DNA hybridization showed 36.6% relatedness between strain XIL11^T and *C. humilata* ATCC 25174^T, suggesting that isolate XIL11^T should be classified as a novel species, in view of the recommendations of Wayne *et al.* (1987) for species delineation. The G+C content of the DNA of this strain is 73 mol%, a value that falls within the range reported for other members of the genus. Therefore, on the basis of chemotaxonomic, phylogenetic and physiological data, we propose that isolate XIL11^T should be classified as the type strain of a novel species, *Cellulomonas xylanilytica* sp. nov.

Description of *Cellulomonas xylanilytica* sp. nov.

Cellulomonas xylanilytica (xy.la.ni.ly'ti.ca. N.L. n. *xylan* xylan, a plant polymer; N.L. adj. *lyticus* from Gr. adj. *lytos* dissolving; N.L. fem. adj. *xylanilytica* xylan-dissolving).

Gram-positive, non-flagellated, non-spore-forming, coccoid or rod-shaped cells (2.4 × 1.4 μm). Aerobic or facultatively anaerobic and chemo-organotrophic. Colonies on YED medium are smooth, yellow and usually 1–3 mm in diameter within 7 days at 28 °C. Aerial mycelium is not formed. Optimal growth temperature is 30 °C. Optimal growth pH is 7. Oxidase- and catalase-positive. Phylogenetically related to members of the family *Cellulomonadaceae*. Utilizes arabinose, cellulose, gentiobiose, maltose, mannose, *N*-acetylglucosamine, starch and xylan as sole carbon sources. By contrast, no growth occurs in acetate, adipate, caprate, citrate, malate, mannitol, phenylacetate or ribose.

Acid is produced from amygdalin, arbutin, cellobiose, D-fructose, galactose, glucose, glycerol, glycogen, inulin, lactose, D-lyxose, maltose, D-mannose, melezitose, melibiose, D-raffinose, rhamnose, salicin, L-sorbose, sucrose, trehalose, xylitol, D-xylose, methyl α-D-glucoside, methyl α-D-mannoside, *N*-acetylglucosamine and β-gentiobiose. Amylases, cellulases, β-galactosidase and xylanases are actively produced. Aesculin, casein and gelatin are hydrolysed. Nitrate is reduced to nitrite. Arginine dehydrolase, indole, tryptophan deaminase and urease are not produced. Major fatty acids are ai-C_{15:0}, C_{16:0} and C_{18:0}. Major isoprenoid quinones are MK-9(H₄) and MK-8(H₄). Peptidoglycan contains L-ornithine–L-glutamic acid (type A4β). Cell-wall sugars are rhamnose, mannose and fucose.

The type strain is XIL11^T (= LMG 21723^T = CECT 5729^T).

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