Xylanibacterium ulmi gen. nov., sp. nov., a novel xylanolytic member of the family *Promicromonosporaceae*

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A bacterial strain designated XIL08^T was isolated from an elm tree affected by Dutch elm disease. Strain XIL08^T is Gram-positive, aerobic, rod-shaped and non-motile. The complete 16S rDNA sequence of this micro-organism was obtained and phylogenetic analysis based on the neighbour-joining method indicated that the closest related organism belongs to the genus *Xylanimonas* of the family *Promicromonosporaceae*, suborder *Micrococcineae*. Cell-wall analyses revealed the presence of type A4_x, L-Lys–L-Ala–D-Glu peptidoglycan. The cell-wall sugars found were rhamnose in large amounts, fucose, mannose and galactose and traces of arabinose and glucose. HPLC analysis of menaquinones revealed two peaks, the main peak corresponding to MK-9(H₄) and the smaller one to MK-8(H₄). The major fatty acid found was anteiso-C_{15:0}. Mycolic acids were absent. The polar lipids detected were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. The G + C content of the DNA was 72 mol%. Isolate XIL08^T hydrolysed xylan but not cellulose. Growth was observed with many carbohydrates including acetate and xylan as the only carbon source. Catalase activity was not detected. The data from this polyphasic study suggest that this bacterium belongs to a novel genus of the family *Promicromonosporaceae*. It is proposed that isolate XIL08^T (=LMG 21721^T=CECT 5731^T) be classified in a new genus, *Xylanibacterium* gen. nov., as the type strain of *Xylanibacterium ulmi* sp. nov.

During the last decade, *Ulmus nigra* trees in Spain have been extensively affected by Dutch elm disease. Consequently, elm trees have been eliminated from parks and gardens and the remaining stumps were reduced to a powdery material by the action of humidity. An actinomycete strain, XIL08^T, was isolated from one of these stumps. The phylogenetic data obtained suggest that this micro-organism belongs to a novel genus within the family *Promicromonosporaceae* (Stackebrandt *et al.*, 1997), which includes Gram-positive bacteria with high G + C content, within the order *Actinomycetales*. In the present paper, we describe the chemotaxonomic, morphological, phylogenetic and physiological characteristics of this organism. On the basis of the present data, isolate XIL08^T should be

placed in a novel genus, *Xylanibacterium*, as *Xylanibacterium ulmi* gen. nov., sp. nov.

A sample of wood decay from a stump of an elm tree was collected under aseptic conditions and 1 g was suspended in sterile water and stirred for 60 min. From this suspension, 100 µl was spread on XED medium (0.7% xylan, 0.3% yeast extract, 2.5% agar) and incubated at 28 °C. A bacterial strain, XIL08^T, was isolated after 10 days incubation and a pure culture was maintained as a glycerol suspension (25%, v/v) at –80 °C.

Strain XIL08^T was grown in YED medium (0.5% yeast extract, 0.7% glucose, 1.5% agar) for 48 h to check for motility by phase-contrast microscopy. Cells were also stained according to the classical Gram procedure described by Doetsch (1981).

Isolate XIL08^T was cultivated in TSB (Becton Dickinson) for 4 days at 28 °C in a rotary shaker (90 r.p.m.) for cell-wall and menaquinone analyses using freeze-dried cells.

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The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain XIL08^T is AY273185.

The same medium amended with 15 g agar l⁻¹ was used to cultivate the strain for examination of the fatty acid composition and presence of mycolic acids. Cell-wall sugar analysis was performed according to described procedures (Staneck & Roberts, 1974). Menaquinone and cellular fatty acid compositions were analysed as described by Zimmermann *et al.* (1998). Peptidoglycan and cell-wall amino acids were determined as described by Schleifer (1985) and Schleifer & Kandler (1972). GC/MS analysis of amino acid derivatives was performed as described by Mackenzie (1987).

Amylase, catalase and oxidase activities were detected as described previously (Rivas *et al.*, 2003). Cellulases were detected after 7 days incubation on plates containing 0.5 % carboxymethylcellulose as the carbon source, 0.3 % yeast extract and 1.5 % agar. Plates were stained with a 1 % aqueous Congo red solution. Different growth temperatures were determined on XED medium incubated at 4, 10, 20, 25, 30 and 37 °C. Other physiological and biochemical tests were done using API 20NE and API 50CH strips (bioMérieux) following the manufacturer's instructions.

DNA for base composition analysis was prepared according to Chun & Goodfellow (1995). The G + C content of DNA was determined using the thermal denaturation method (Mandel & Marmur, 1968).

DNA extraction, PCR amplification of the 16S rRNA gene and sequencing of PCR products were performed as described previously (Rivas *et al.*, 2003). An almost complete 16S rRNA gene sequence was obtained and compared with those deposited in the databases. Sequences were aligned using the CLUSTAL X software (Thompson *et al.*, 1997). Distances were calculated according to Kimura's two-parameter method (Kimura, 1980). Phylogenetic trees were inferred using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis was based on 1000 resamplings. The MEGA2 package (Kumar *et al.*, 2001) was used for all analyses. Trees were rooted using *Bifidobacterium bifidum* DSM 20456^T as the outgroup.

Based on 16S rDNA sequence analysis, the organism under study formed a novel branch within the family *Promicromonosporaceae* and was closest to the recently described *Xylanimonas cellulosilytica*; this relationship was supported by a high bootstrap value (Fig. 1). Furthermore, the 16S rRNA gene sequence of strain XIL08^T contained many of the nucleotide signatures that define the family *Promicromonosporaceae* according to Stackebrandt *et al.* (1997) and Stackebrandt & Schumann (2000). Five differences were found, at nucleotide positions 384 (A for G), 648 (G for A), 615 (A for U), 624 (C for U) and 1263–1272 (G–C for A–U). The 16S rRNA gene sequence of strain XIL08^T showed 95.8 % similarity to that of *X. cellulosilytica*, 94.02 % to *Promicromonospora citrea*, 94.4 % to *Promicromonospora sukumoe* and 94.4 % to *Cellulosimicrobium cellulans*.

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences of *Xylanibacterium ulmi* XIL08^T and other related organisms of the family *Promicromonosporaceae*. The significance of each branch is indicated by a bootstrap percentage calculated for 1000 subsets. Bar, 2 substitutions per 100 nt.

The peptidoglycan composition of strain XIL08^T corresponded to type A4 α , L-Lys–L-Ala–D-Glu; this composition differs from that reported among the genera encompassed by the family *Promicromonosporaceae* (Table 1), with the exception of the genus *Promicromonospora* (Evtushenko *et al.*, 1984); the genus *Cellulosimicrobium* (Schumann *et al.*, 2001) has L-Lys, D-Ser and D-Asp while, in the genus *Xylanimonas* (Rivas *et al.*, 2003), L-Lys and D-Asp have been detected. The cell-wall sugars detected for strain XIL08^T were rhamnose (in large amounts), fucose, mannose, galactose and traces of arabinose and glucose. In many strains of the genus *Promicromonospora*, only galactose has been detected (Kalakoutsii *et al.*, 1989). In the case of *Cellulosimicrobium* species, fucose, galactose, glucose, mannose and rhamnose have been found (Bakalidou *et al.*, 2002; Schumann *et al.*, 2001). In the genus *Xylanimonas*, galactose and rhamnose were detected (Rivas *et al.*, 2003).

As expected for members of the family *Promicromonosporaceae*, mycolic acids were not found.

The cellular fatty acid pattern of strain XIL08^T was composed of iso- and anteiso-branched fatty acids. The main fatty acid detected was anteiso-C_{15:0}, followed by iso-C_{15:0} and C_{14:0} in smaller amounts. According to published data, the cellular fatty acid patterns of members of the genus *Promicromonospora* are composed of iso- and anteiso-C_{15:0} (Kalakoutsii *et al.*, 1989), while, for members of the genus *Cellulosimicrobium*, the fatty acid pattern reported was anteiso-C_{15:0}, iso-C_{15:0}, C_{16:0} and iso-C_{16:0} (Schumann *et al.*, 2001). Members of the recently described genus *Xylanimonas* (Rivas *et al.*, 2003) present a fatty acid profile similar to that of strain XIL08^T, although some differences were found (Table 1).

HPLC analysis of the menaquinones revealed two peaks; the

Table 1. Characteristics that differentiate the genus *Xylanibacterium* gen. nov. from other genera of the family *Promicromonosporaceae*

Data for reference genera were taken from Bakalidou *et al.* (2003), Evtushenko *et al.* (1984), Goodfellow (1989), Rivas *et al.* (2003) and Schumann *et al.* (2001). +, Positive; –, negative; V, variable; W, weak; ND, not determined. Abbreviations: Gal, galactose; Rha, rhamnose; Fuc, fucose; Man, mannose; Glu, glucose; Ara, arabinose; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PET, phosphatidyl ethanolamine.

Characteristic	<i>Xylanibacterium</i>	<i>Xylanimonas</i>	<i>Promicromonospora</i>	<i>Cellulosimicrobium</i>
Shape	Small rods	Coccoid	Rod-like, coccoid, Y- or V-shaped	Short rods
Aerial hyphae	–	–	+	–
Catalase	–	W	+	+
Fermentation	+	W	Rarely	+
Chemotaxonomic characteristics:				
Peptidoglycan composition	L-Lys–L-Ala–D-Glu	L-Lys–D-Asp	L-Lys–L-Ala–D-Glu	L-Lys–D-Ser–D-Asp
Cell-wall sugars	Rha, Fuc, Man, Gal, Ara, Glu	Gal, Rha	Gal	Rha, Fuc, Gal, Man, Glu
Major menaquinones	MK-9(H ₄), MK-8(H ₄)	MK-9(H ₄), MK-8(H ₄)	MK-9(H ₄)	MK-9(H ₄)
Predominant cellular fatty acids	ai-C _{15:0} , i-C _{15:0} , C _{16:0} , C _{14:0}	ai-C _{15:0} , i-C _{15:0} , C _{14:0}	ai-C _{15:0} , i-C _{15:0}	ai-C _{15:0} , i-C _{15:0} , C _{16:0} , i-C _{16:0}
Phospholipid composition	PG, DPG, PI, PIM*	PG, DPG, PI, PIM	PG, PI	PG, PI, PIM, PET
Growth (as only carbon source) in:				
Acetate	+	–	+	+
Malate	–	–	+	–
Gluconate	+	–	ND	+
Citrate	–	–	+	ND
D-Raffinose	–	–	+	–
Mannitol	–	–	+	+
Inositol	–	–	V	+
Hydrolysis of:				
Cellulose	–	+	+	+
Casein	–	–	+	ND
Urea	–	–	+	ND

*Other unidentified phosphoglycolipids have been detected.

main peak corresponded to MK-9(H₄) and the smaller one to MK-8(H₄). MK-9(H₄) is the major menaquinone in both *Promicromonospora* and *Cellulosimicrobium* (Kalakoutskii *et al.*, 1989; Schumann *et al.*, 2001).

The major polar lipids of strain XIL08^T were phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylinositol (PI), phosphatidylinositol mannosides (PIM) and other unidentified phosphoglycolipids. This profile is similar to that obtained for members of the genus *Xylanimonas* (Rivas *et al.*, 2003). The main difference between *Promicromonospora* and XIL08^T is the presence of PIM (Goodfellow, 1989). With respect to *Cellulosimicrobium*, strain XIL08^T contains DPG, but not phosphatidylethanolamine (Bakalidou *et al.*, 2002).

The strain isolated was a Gram-positive, non-motile, rod-shaped organism (0.9 × 0.3 µm). Cells grew as white- to cream-coloured colonies on XED medium. They were smooth and mostly flat. Morphologically, strain XIL08^T was different from *Xylanimonas cellulosilytica* XIL07^T.

Strain XIL08^T showed high xylanase and β-xylosidase activities; however, no cellulase production was observed. Differentiating physiological characteristics of strain XIL08^T and those of the remaining genera of the family *Promicromonosporaceae* are presented in Table 1. According to these data, this strain is different from members of *Promicromonospora* in aerial hyphae formation, caseinase, catalase and urease production as well as in growth with citrate, malate, mannitol and D-raffinose. Strain XIL08^T is different from members of *Cellulosimicrobium* in catalase production and in growth with inositol and mannitol as the only carbon source. Finally, isolate XIL08^T differed from *Xylanimonas cellulosilytica* in the assimilation of acetate and gluconate and the production of cellulase. The optimum growth temperature was 25–28 °C; no growth was detected at 4 or 10 °C.

The DNA G + C content of strain XIL08^T was 72 mol%. This value is similar to those obtained for members of the genera *Cellulosimicrobium*, *Promicromonospora* and *Xylanimonas*.

In conclusion, the results of the present study, which include a low similarity value for the 16S rDNA sequence together with differences found in chemotaxonomic, morphological and physiological properties, indicate that isolate XIL08^T should be classified in the new genus *Xylanibacterium* as *Xylanibacterium ulmi* gen. nov., sp. nov.

Description of *Xylanibacterium* gen. nov.

Xylanibacterium (Xy.la.ni.bac.te'ri.um. N.L. neut. n. *xylanum* xylan, a polysaccharide; N.L. neut. n. *bacterium* from Gr. neut. n. *baktron* small rod; N.L. neut. n. *Xylanibacterium* xylan-hydrolysing small rod).

Gram-positive, non-spore-forming, small rod-shaped bacterium. Aerobic or facultatively anaerobic, chemo-organotrophic and xylanolytic. Aerial mycelium is not formed. Optimal pH for growth is 7. Oxidase-positive and catalase-negative. Phylogenetically related to members of the family *Promicromonosporaceae*. Cell-wall sugars are galactose and rhamnose. Major menaquinones are MK-9(H₄) and MK-8(H₄); predominant fatty acid is anteiso-C_{15:0} (12-methyltetradecanoic acid). Mycolic acids are absent. Contains PG, DPG, PI, PIM and other unidentified phosphoglycolipids. The DNA G+C content of the type strain of the type species is 72.3 mol%. The type species of the genus is *Xylanibacterium ulmi*.

Description of *Xylanibacterium ulmi* sp. nov.

Xylanibacterium ulmi (ul' mi. L. gen. n. *ulmi* of the elm tree, referring to the isolation source of the type strain).

In addition to the properties listed in the genus description, the following properties are reported. Colonies on YED are circular convex, white, opaque and usually 1–3 mm in diameter within 7 days at 28 °C. Strain XIL08^T utilizes acetate, *N*-acetylglucosamine, L-arabinose, cellobiose, D-fructose, galactose, gentiobiose, gluconate, 2- and 5-ketogluconate, glycerol, L-xylose, D-mannose, ribose, L-sorbose, sucrose, trehalose, D-turanose, xylitol, D-xylose and xylan as sole carbon sources. Does not utilize adipate, adonitol, amygdalin, D-arabinose, D- and L-arabitol, arbutin, caprate, citrate, dulcitol, erythritol, D- and L-fucose, gluconate, glycogen, inositol, inulin, lactose, malate, maltose, mannitol, melibiose, melezitose, phenylacetate, raffinose, rhamnose, salicin, sorbitol, L-sorbose, starch, D-tagatose, L-xylose, methyl α -D-glucoside, methyl α -D-mannoside, *N*-acetylglucosamine or methyl β -xyloside. Actively produces xylanases, β -xylosidase and β -galactosidase. Gelatinase production is weak. Aesculin is hydrolysed. Nitrate reduction is negative. Does not produce arginine dihydrolase, caseinase, tryptophan deaminase or urease.

The type strain, XIL08^T (=LMG 21721^T=CECT 5731^T), was isolated from decayed wood of the rotten stump of an elm tree, *Ulmus nigra*.

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