**INTRODUCTION**

The families *Cellulomonadaceae* and *Promicromonosporaceae* are closely related and are Gram-positive bacteria with high G+C content within the order *Actinomycetales*. The taxonomy of these two families has been the subject of important changes in recent years: species from the genus *Actinomyces* have been reclassified into the genus *Cellulomonas* (Stackebrandt *et al.*, 1980, 1982); the genus *Rarobacter* has been included in the new family *Rarobacteraceae* (Stackebrandt & Schumann, 2000); and *Cellulomonas cellsulans* has been reclassified in the new genus *Cellulosimicrobium* (Schumann *et al.*, 2001). This latter genus has been separated from the family *Cellulomonadaceae* (Schumann *et al.*, 2001) and included in the family *Promicromonosporaceae* (Stackebrandt *et al.*, 1997), which also includes the genus *Promicromonospora*. One of the most common characteristics of species included in the families *Cellulomonadaceae* and *Promicromonosporaceae* is their ability to hydrolyse cellulose, often linked to the hydrolysis of xylan and other polysaccharides. Cellulolytic bacteria are commonly isolated from soil, but taking into account that these micro-organisms can destroy wood, cellulolytic and xylanolytic species may be found in decayed trees. From these sources, a strain has been isolated, strain XIL07\textsuperscript{T}, that hydrolyses xylan. The present investigation was designed to establish the taxonomic position of isolate XIL07\textsuperscript{T}; phylogenetic data obtained suggest that this micro-organism belongs to a new genus closely related to members of the family *Promicromonosporaceae*. The chemotaxonomic and phenotypic data support these results. The name *Xylanimonas cellulosilytica* gen. nov., sp. nov. is proposed; the type strain is strain XIL07\textsuperscript{T} (=LMG 20990\textsuperscript{T} = CECT 5975\textsuperscript{T}).

**METHODS**

**Isolation of strains and culture conditions.** A sample of sawdust from a decayed tree, *Ulmus nigra*, was collected under aseptic conditions and 1 g was suspended in sterile water and stirred for 30 min. From this suspension, 100 \(\mu\)l was spread onto XED medium (xylan, 0.7 %; yeast extract, 0.3 %; agar, 2.5 %) and incubated at 28 °C. A bacterial strain, strain XIL07\textsuperscript{T}, was isolated that hydrolysed xylan. This strain was maintained as a glycerol suspension (25 %, v/v) at \(-80\) °C.
**Morphology.** Strain XIL07<sup>T</sup> was grown in YED medium (yeast extract, 0·5 %; glucose, 0·7 %; agar, 1·5 %) for 48 h to check for motility by phase-contrast microscopy. The cells were also stained according to the classical Gram procedure described by Doetsch (1981). For EM, the cells were grown in liquid YED for 3 days at 180 r.p.m. and 28 °C. The samples were fixed overnight in 2 % glutaraldehyde. Sections were mounted according to Reynolds (1963) and were examined at 80 kV with a Zeiss EM 209 TEM.

**Chemotaxonomic characterization.** Strain XIL07<sup>T</sup> was cultivated in TSB (Becton Dickinson, BBL) for 4 days at 28 °C in a rotary shaker (90 r.p.m.) for cell wall and menaquinone analyses. The same medium amended with 1·5 % agar was used to cultivate the strain for fatty acid composition and the presence of mycolic acids. Amino acid and cell-wall sugar analyses were performed according to described procedures (Staneck & Roberts, 1974). Menaquinone and cellular fatty acid composition were determined as described by Zimmermann et al. (1998).

**Phenotypic tests and determination of DNA base composition.** Isolate XIL07<sup>T</sup> was grown in YED plates for 48 h. Catalase production was assayed using 0·3 % hydrogen peroxide with one colony taken from YED agar plates. Oxidase activity was detected using N,N,N′,N′-tetramethyl-1,4-phenylenediamine dihydrochloride. Cellulases were detected after 7 days incubation in plates containing 0·5 % carboxymethylcellulose as the carbon source, 0·3 % yeast extract and 1·5 % agar. Plates were stained with 1 % Congo Red water solution. Casein activity was detected on skimmed milk agar after 7 days incubation. Other physiological and biochemical tests were done using the API 20NE, API 20E and API 50CH strips (bioMérieux) according to the manufacture’s instructions.

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

**16S rDNA analysis.** DNA extraction was carried out as described previously (Rivas et al., 2001). PCR amplification of 16S rDNA was carried out using primers 5′-AGAGTTTGATCCTGCTCAG-3′ and 5′-AAGGAGGTAGTCCGCCCCA-3′ under conditions described previously (Velázquez et al., 2001). The sequence reaction was performed on an ABI377 sequencer (Applied Biosystems) using a BigDye terminator v3.0 cycle sequencing kit as supplied by the manufacturer. The following primers were used (corresponding positions in the *Escherichia coli* small-subunit rDNA sequence are given in parentheses): 5′-AGATTTGATCCTGCTCAG-3′ (8–27); 5′-CTCTACGAGGAGCAGCT-3′ (339–358); 5′-CACGACGGCCG GGTGATTAC-3′ (519–537); 5′-GCCTGGGAGTAGCACCGCA-3′ (849–869); 5′-ACTGCTGGCTCCGCTAGGAG-3′ (1093–1112); and 5′-AAGGAGGTAGTCCGCCCCA-3′ (1498–1522). The sequence obtained was compared with those from GenBank using the FASTA program (Pearson & Lipman, 1988). Sequences were aligned using CLUSTAL W software (Thompson et al., 1997). The distances were calculated according to Kimura’s 2-parameter method (Kimura, 1980). Phylogenetic trees were inferred using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis was based on 1000 resamplings. The PHYLIP package (Felsenstein, 1993) was used for all analyses. The trees were rooted using *Rarobacter faecitabidus* DSM 4813<sup>T</sup> as the outgroup.

**RESULTS AND DISCUSSION**

**Morphology**

The strain isolated from sawdust was a Gram-positive, non-motile, coccoid organism (1·1 × 0·8 μm). Fig. 1 shows the cell morphology of strain XIL07<sup>T</sup> as observed by TEM. Colonies were white- to cream-coloured on XED medium. They were smooth and mostly flat.

**Phenotypic tests**

Strain XIL07<sup>T</sup> showed high cellulase and xylanase activity. Phenotypic characteristics of strain XIL07<sup>T</sup> and species of closely related genera are shown in Table 1. According to the data, this strain differs from *Promicromonospora* in mycelium formation, caseinase, catalase and urease production, and growth on acetate, citrate, malate, mannitol and D-raffinose as sole carbon source. Strain XIL07<sup>T</sup> differs from *Cellulosimicrobium* in mycelium formation, catalase production and growth with acetate, gluconate, inositol and mannitol as sole carbon source.
Chemotaxonomic characteristics and DNA base composition

The results of the chemotaxonomic analyses are shown in Table 1. The peptidoglycan of strain XII07^T contained the amino acids L-lysine and D-aspartic acid. This composition differs from that reported for peptidoglycan of the genus Promicromonospora (Kalakoutskii et al., 1989), which contains L-lysine and alanine, and from peptidoglycan of the genus Cellulosimicrobium (Schumann et al., 2001), where L-lysine, D-serine and D-aspartic acid have been detected.

The cell-wall sugars detected for strain XII07^T were galactose and rhamnose. In many strains of the genus Promicromonospora, only galactose has been detected (Kalakoutskii 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).

As expected for members of the family Promicromonosporaceae (Kalakoutskii et al., 1989; Bakalidou et al., 2002), mycolic acids were not detected.

The cellular fatty acid pattern of strain XII07^T was composed of iso- and anteiso-branched fatty acids. The main fatty acid detected was anteiso-C15:0 (12-methyl tetradecanoic acid). According to the published data, the cellular fatty acid pattern of the genus Promicromonospora is made up of iso- and anteiso-C15:0 (Kalakoutskii et al., 1989), whereas for the genus Cellulosimicrobium the fatty acid pattern reported contains anteiso-C15:0, iso-C15:0, C16:0 and iso-C16:0 (Schumann et al., 2001).

HPLC analysis of the 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 in both Promicromonospora and Cellulosimicrobium (Kalakoutskii et al., 1989; Schumann et al., 2001).

The major polar lipids detected for strain XII07^T were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and other unidentified phosphoglycolipids. The main difference between Promicromonospora and XII07^T is the presence of phosphatidylinositol mannosides (Goodfellow, 1989). With respect to Cellulosimicrobium, strain XII07^T contains diphosphatidylglycerol, but not phosphatidylethanolamine (Bakalidou et al., 2002).

The DNA G+C content was 73 mol%. This value is

<table>
<thead>
<tr>
<th>Character</th>
<th>Xylanimonas</th>
<th>Promicromonospora</th>
<th>Cellulosimicrobium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Coccoid</td>
<td>Rod-like, coccoid, Y- or V-shaped</td>
<td>Short rods</td>
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<td>Aerial hyphae</td>
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<td>+</td>
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<tr>
<td>Catalase</td>
<td>W</td>
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<td>Fermentation</td>
<td>W</td>
<td>Rare</td>
<td>+</td>
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<td>Cell-wall sugars*</td>
<td>Gal, Rha</td>
<td>Gal</td>
<td>Rha, Fuc, Gal, Man, Glu</td>
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<td>Major menaquinones</td>
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<td>MK-9(H4)</td>
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<td>Predominant cellular fatty acids</td>
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<td>ai-C&lt;sub&gt;15:0&lt;/sub&gt;, i-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>ai-C&lt;sub&gt;15:0&lt;/sub&gt;, i-C&lt;sub&gt;15:0&lt;/sub&gt;, C&lt;sub&gt;16:0&lt;/sub&gt;, i-C&lt;sub&gt;16:0&lt;/sub&gt;</td>
</tr>
<tr>
<td>Phospholipid composition†</td>
<td>PG, DPG, PI, PIM‡</td>
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<td>PG, PI, PIM, PET</td>
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<td>Growth on sole carbon source:</td>
<td></td>
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<tr>
<td>Acetate</td>
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<td>ND</td>
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</table>

*Gal, Galactose; Rha, rhamnose; Fuc, fucose; Man, mannose; Glu, glucose.
†PG, Phosphatidylglycerol; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PET, phosphatidylethanolamine.
‡Other unidentified phosphoglycolipids were detected.

Table 1. Characters that differentiate the genus Xylanimonas from other genera of the family Promicromonosporaceae

Data from Bakalidou et al. (2002), Goodfellow (1989), Kalakoutskii et al. (1989) and Schumann et al. (2001). +, Positive; −, negative; V, variable; ND, not determined; w, weak.

http://ijs.sgmjournals.org 101
similar to those obtained for species of the genera *Cellulosimicrobium* and *Promicromonospora*.

**16S rDNA analysis**

The complete 16S rDNA sequence for isolate XIL07<sup>T</sup> was obtained. A comparison with 16S rDNA sequences held in GenBank indicated that the organism is phylogenetically related to members of the family *Promicromonosporaceae*. The 16S rDNA sequence of strain XIL07<sup>T</sup> contained the nucleotide signatures of this family according to Stackebrandt & Schumann (2000). Fig. 2 shows the phylogenetic tree obtained by the neighbour-joining method. Strain XIL07<sup>T</sup> formed a separate group from the *Cellulomonas* species. This group included strain XIL07<sup>T</sup>, *Promicromonospora citrea* DSM 43110<sup>T</sup>, *Promicromonospora sukumoe* DSM 44121<sup>T</sup> and *Cellulosimicrobium cellulans* DSM 43879<sup>T</sup>. The 16S rDNA sequence of strain XIL07<sup>T</sup> showed 95·02% similarity with that of *P. citrea*, 95·03% with that of *P. sukumoe* and 94·67% with that of *C. cellulans*. These results indicate that the isolate merits genus status.

Recovery of *Promicromonospora enterophila* DSM 43852<sup>T</sup> in the branch corresponding to *Cellulomonas* species indicates that this strain probably belongs to the genus *Cellulomonas* (Bakalidou et al., 2002; M. Takeuchi and others, unpublished results).

Therefore, on the basis of phylogenetic, chemotaxonomic and phenotypic data, it is proposed that isolate XIL07<sup>T</sup> should be classified in a new genus, *Xylanimonas*, as *Xylanimonas cellulosilytica* gen. nov., sp. nov.

**Description of Xylanimonas gen. nov.**

*Xylanimonas* (Xy.lan.i.mo.nas. N.L. n. *xylanum* xylan, a polysaccharide; Gr. n. *monas* a unit; N.L. n. *Xylanimonas* a monad from xylan).

Gram-positive, non-spore-forming, coccolid cells. Aerobic or facultatively anaerobic, chemo-organotrophic and xylanolytic. Aerial mycelium is not formed. Optimal growth temperature is 30 °C; pH 7 is optimal for growth. Oxidase is produced, but catalase production is weak. Phylogenetically related to members of the family *Promicromonosporaceae*. Peptidoglycan type is A4<sub>α</sub>, L-Lys–D-Asp. Cell-wall sugars are galactose and rhamnose. Major menaquinones are MK-9(H<sub>4</sub>) and MK-8(H<sub>4</sub>); predominant fatty acid is anteiso-C<sub>15</sub>:0 (12-methyl tetradecanoic acid). Mycolic acids are absent. Contains phosphatidyglycerol, diposphatidyglycerol, phosphatidylinositol, phosphatidylinositol mannosides and other unidentified phosphoglycolipids. The type species of the genus is *Xylanimonas cellulosilytica*.

**Description of Xylanimonas cellulosilytica sp. nov.**


![Fig. 2. Comparative sequence analysis of 16S rDNA from Xylanimonas cellulosilytica XIL07<sup>T</sup> and representative strains from GenBank. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Bar, 5 nt substitutions per 100 nt.](image-url)
Gram-positive, aerobic or facultatively anaerobic, non-motile, non-spore-forming coccoid cells of 1.1 × 0.8 µm. Colonies on YED are circular, convex, white, opaque and usually 1–3 mm in diameter within 7 days at 28°C. Utilizes L-arabinose, carboxymethylcellulose, mannose, maltose, rhamnose, starch and xylan as sole carbon sources. Does not grow in acetate, adonitol, D-arabinose, citrate, dulcitol, erythritol, gluconate, inositol, inulin, malate, mannitol, raffinose, sorbitol, L-sorbose, L-xylene, methyl α-D-glucoside, methyl β-D-mannoside, N-acetylglucosamine or methyl β-xylidine. Produces acid from amygdalin, L-arabinose, arbutin, cellobiose, fructose, galactose, gentobiose, glucose, glycerol, glycogen, lactose, lyxose, maltose, mannose, melezitose, rhamnose, salicin, sucrose, trehalose, turanose and D-xylene. Actively produces amylases, cellulases, gelatinase, xylanases and β-galactosidase. Aesculin is hydrolysed. Reduces nitrate and produces acetoin (Voges–Proskauer reaction). Does not produce arginine dihydrolase, caseinase, indole, lysine decarboxylase, ornithine decarboxylase, ornithine, p-xylose. Actively produces amylases, cellulases, gelatinase, xylanases and β-galactosidase. Aesculin is hydrolysed. Reduces nitrate and produces acetoin (Voges–Proskauer reaction). Does not produce arginine dihydrolase, caseinase, indole, lysine decarboxylase, ornithine decarboxylase, ornithine, p-xylose.

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REFERENCES


