Xylan is a heterogeneous polymer composed of (1,4)-linked β-D-xylosyl residues. It is the major hemicellulose component in plant cell walls and is the most abundant polysaccharide after cellulose. Several bacterial and fungal species produce the full complement of enzymes necessary to utilize xylan as a carbon source (Uffen, 1997). Some of these bacterial species belong to the genus Paenibacillus described by Ash et al. (1994). Some members of the genus Paenibacillus excrete a diverse range of extracellular polysaccharide-hydrolysing enzymes, including xylanases (Zamost et al., 1991; Morales et al., 1995; Hespell, 1996; Ay et al., 1998; Nielsen & Sorensen, 1997; Lee et al., 2000; Velázquez et al., 2004).

Here we describe the isolation and classification of a novel xylan-degrading bacterium.

The bacterial strain examined was isolated from a leaf of the palm tree Phoenix dactylifera. The sample was collected aseptically, and 1 g was chopped, placed in 9 ml sterile water and stirred for 60 min. Aqueous portions (100 ml of the mixture) were spread on XED medium (0.7% xylan, 0.3% yeast extract, 2.5% agar) in triplicate and incubated at 28°C. A bacterial strain, designated PALXIL04T, was isolated after 7 days incubation and a pure culture was maintained in a glycerol suspension (25%, v/v) at −80°C.

Strain PALXIL04T was grown in YED medium (0.5% yeast extract, 0.7% glucose, 1.5% agar) for 48 h to check for motility by using phase-contrast microscopy. Gram staining was carried out by using the procedure described by Doetsch (1981). Cells were gently suspended in sterile water, stained with 0.2% uranyl acetate and examined at 80 kV with a Zeiss EM 209 transmission electron microscope (Peix et al., 2003).

Strain PALXIL04T was cultivated in TSB (Becton Dickinson) for 24 h at 28°C in a rotary shaker (90 r.p.m.) for menaquinone analyses using freeze-dried cells. The same medium but amended with 15 g agar l−1 was used to cultivate the strain for examination of its fatty acid composition. Menaquinone and cellular fatty acids were analysed as described by Zimmermann et al. (1998).

Physiological and biochemical tests were determined using API 20NE, API 20E and API 50CH strips (bioMérieux) according to the manufacturer’s instructions. Amylase, caseinase, catalase, cellulase and oxidase were analysed as described by Rivas et al. (2003). Growth was determined at temperatures of 4–45°C in YED medium.
DNA for base composition analysis was prepared according to the method of Chun & Goodfellow (1995). The G+C content of the DNA was determined using the thermal denaturation method of Mandel & Marmur (1968).

DNA–DNA hybridization was carried out as described by De Ley et al. (1970) with the modifications described by Huss et al. (1983) and Escara & Hutton (1980). DNA was isolated as described by Cashion et al. (1977).

For 16S rRNA gene sequencing, DNA extraction was carried out as described by Rivas et al. (2001). Amplification and sequencing of the 16S rRNA gene were performed according to the method described by Rivas et al. (2003). An almost-complete 16S rRNA gene sequence was obtained and compared with those deposited in public databases. Sequences were aligned using CLUSTAL X software (Thompson et al., 1997). Evolutionary 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.

A comparison of the 16S rRNA gene sequence of strain PALXIL04T and sequences held in GenBank indicated that the organism is phylogenetically related to members of the genus Paenibacillus. Fig. 1 shows the phylogenetic tree obtained with the neighbour-joining method (an expanded tree is available as Supplementary Fig. A in IJSEM Online). The closest related species are Paenibacillus kobensis DSM 10249T (95·7 % similarity) and Paenibacillus curdlanolyticus DSM 10247T (95·0 %).

Details of characteristics that differentiate strain PALXIL04T and phylogenetically related species are given in Table 1. Other characteristics determined are given under the species description below.

Unsaturated menaquinone with seven isoprene units (MK-7) was the predominant isoprenoid quinone found in strain PALXIL04T. The major cellular fatty acid of strain PALXIL04T, Paenibacillus curdlanolyticus and Paenibacillus kobensis was anteiso-C15:0 (Table 2), which is the predominant cellular fatty acid found in all recognized members of the genus Paenibacillus (Shida et al., 1997). The fatty acid profile of PALXIL04T was similar to those of the type strains of Paenibacillus curdlanolyticus and Paenibacillus kobensis, but differed in the proportions of some fatty acids (Table 2). The main differences were the amounts of C16:0, C17:0 and iso-C17:0 in the novel isolate compared to Paenibacillus curdlanolyticus and those of anteiso-C15:0 and anteiso-C17:0 compared to Paenibacillus kobensis.

DNA–DNA relatedness values for strain PALXIL04T against Paenibacillus curdlanolyticus DSM 10247T and Paenibacillus kobensis DSM 10249T were 15 and 20 %, respectively, suggesting that the novel isolate was not closely related to species with validly published names according to current species concepts. The results confirm that strains with less than 97 % 16S rRNA gene sequence similarity have DNA–DNA relatedness values lower than 70 % (Stackebrandt & Goebel, 1994).

The DNA G+C content of strain PALXIL04T was 74.4 mol%. This value was similar to that obtained for Paenibacillus curdlanolyticus and Paenibacillus kobensis (Shida et al., 1997).

On the basis of phylogenetic, chemotaxonomic and phenotypic data, we propose that strain PALXIL04T should be described as a novel species of the genus Paenibacillus, for which the name Paenibacillus phyllosphaerae sp. nov. is proposed.
Table 1. Characteristics that differentiate *Paenibacillus phyllosphaerae* sp. nov. from phylogenetically related *Paenibacillus* species


<table>
<thead>
<tr>
<th>Test</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>Anaerobic growth</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Urease</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Degradation of:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Carboxymethyl cellulose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methyl β-D-xyloside</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>w</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Methyl α-D-mannoside</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methyl α-D-glucoside</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arbutin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salicin</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Melezitose</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Turanose</td>
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<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Gluconate</td>
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<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2-Ketogluconate</td>
<td>+</td>
<td>−</td>
<td>−</td>
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</table>

classified as a novel species, with the name *Paenibacillus phyllosphaerae* sp. nov.

**Description of Paenibacillus phyllosphaerae sp. nov.**

*Paenibacillus phyllosphaerae* (phyllo.spha’rae. Gr. neut. n. phyllon leaf; L. fem. n. sphaera ball, sphere; N.L. gen. fem. n. phyllosphaerae of the phyllosphere).

Spore-forming rods, 0.9–1.6 µm wide, 3.3–4.2 µm long. Gram-variable. Motile by means of peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia and they are in the terminal position in cells. Aerobic or facultatively anaerobic, chemo-organotrophic and xylanolytic bacterium. Colonies on YED medium are circular, flat, whitish cream, opaque and usually 1–3 mm in diameter within 48 h at 28 °C. Growth occurs at 10–37 °C (optimal growth occurs at 28 °C) and optimal pH for growth is 7. Oxidase- and catalase-positive. The pH in Voges–Proskauer broth is 5–3. Could not grow in the presence of 5% NaCl. The major quinone is MK-7. The main fatty acid is anteiso-C15:0. Gas is not produced from D-glucose. D-Glucose, glycerol, L-arabinose, D-xylose, galactose, D-fructose, mannitol, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, D-raffinose, starch, glycogen, β-gentobiose, gluconate, 2-ketogluconate, xylan, carboxymethyl cellulose and gentiobiose are utilized as carbon sources. Assimilation of D-mannose, rhamnose and D-turanose is weakly positive. In contrast, erythritol, D-arabino, ribose, L-xylose, adonitol, methyl β-D-xyloside, L-sorbose, dulcitol, inositol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, inulin, melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 5-ketogluconate, adipecate, caproate, citrate, phenylacetate and malate do not serve as carbon sources for growth. Xylanase, cellulase, amyrase and β-galactosidase are produced actively, but caseinase, arginine dihydrolase, indole, lysine decarboxylase, ornithine decarboxylase, urease, phenylalanine deaminase, tryptophan deaminase, hydrogen sulfide and acetoin (Voges–Proskauer medium) are not produced. Nitrate is reduced to nitrite.

The type strain, PALXIL04T (=LMG 22192T = CECT 5862T), was isolated from the phyllosphere of *Phoenix dactylifera* in Palma de Mallorca (Spain). The DNA G+C content of the type strain is 50-7 mol%.

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