Paenibacillus phyllosphaerae sp. nov., a xylanolytic bacterium isolated from the phyllosphere of *Phoenix dactylifera*

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A bacterial strain, designated PALXIL04^T, was isolated from the phyllosphere of *Phoenix dactylifera*. Phylogenetic analysis placed the isolate within the genus *Paenibacillus* with the closest relatives being *Paenibacillus curdianolyticus* and *Paenibacillus kobensis*. DNA–DNA hybridization measurements showed low DNA relatedness (15–20%) between the isolate and its closest relatives. Cells were Gram-variable, facultatively anaerobic, motile, sporulating rods. Catalase and oxidase were produced by the organism. Cellulose, starch, aesculin and xylan were hydrolysed. Growth was supported by many carbohydrates as the carbon source. MK-7 was the predominant menaquinone and anteiso-C_{15:0} the major fatty acid. The G+C content of the DNA was 50.7 mol%. Phylogenetic, DNA–DNA relatedness and phenotypic analyses indicated that strain PALXIL04^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus phyllosphaerae* sp. nov. is proposed. The type strain is PALXIL04^T (=LMG 22192^T = CECT 5862^T).

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; Aÿ *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 μ l 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 PALXIL04^T, was isolated after 7 days incubation and a pure culture was maintained in a glycerol suspension (25%, v/v) at –80 °C.

Strain PALXIL04^T 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 PALXIL04^T 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.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PALXIL04^T is AY598818.

An expanded neighbour-joining tree based on the 16S rRNA gene sequence of strain PALXIL04^T and representative *Paenibacillus* strains is available as supplementary material in IJSEM Online.

Fig. 1. Comparative analysis of the 16S rRNA gene sequence of *Paenibacillus phyllosphaerae* PALXIL04^T and those of representative strains from GenBank using the neighbour-joining method. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. Bar, 2 nt substitutions per 100 nt.

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 PALXIL04^T 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 10249^T (95.7 % similarity) and *Paenibacillus curdlanolyticus* DSM 10247^T (95.0 %).

Details of characteristics that differentiate strain PALXIL04^T 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 PALXIL04^T. The major cellular fatty acid of strain PALXIL04^T, *Paenibacillus curdlanolyticus* and *Paenibacillus kobensis* was anteiso-C_{15:0} (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 PALXIL04^T 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 C_{16:0}, C_{17:0} and iso-C_{17:0} in the novel isolate compared to *Paenibacillus curdlanolyticus* and those of anteiso-C_{15:0} and anteiso-C_{17:0} compared to *Paenibacillus kobensis*.

DNA–DNA relatedness values for strain PALXIL04^T against *Paenibacillus curdlanolyticus* DSM 10247^T and *Paenibacillus kobensis* DSM 10249^T 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 PALXIL04^T was 50.7 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 PALXIL04^T should be

Table 1. Characteristics that differentiate *Paenibacillus phyllosphaerae* sp. nov. from phylogenetically related *Paenibacillus* species

Species: 1, *P. phyllosphaerae*; 2, *P. curdlanolyticus*; 3, *P. kobensis*. Data are from Kanzawa *et al.* (1995), Shida *et al.* (1997), Dasman *et al.* (2002) and this study. +, Positive; –, negative; w, weak.

Test	1	2	3
Anaerobic growth	+	–	–
Oxidase	+	–	–
Urease	–	+	+
Degradation of:			
Carboxymethyl cellulose	+	–	–
Utilization of:			
Glycerol	+	+	–
Methyl β -D-xyloside	–	+	+
D-Fructose	+	+	–
D-Mannose	w	–	–
Rhamnose	w	–	–
Mannitol	+	–	–
Methyl α -D-mannoside	–	+	–
Methyl α -D-glucoside	–	+	+
N-Acetylglucosamine	–	+	+
Arbutin	+	+	–
Salicin	+	+	–
Melezitose	–	+	+
D-Turanose	w	+	–
Gluconate	+	–	–
2-Ketogluconate	+	–	–

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

Description of *Paenibacillus phyllosphaerae* sp. nov.

Paenibacillus phyllosphaerae (phyl.lo.sphae'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 μ m wide, 3.3–4.2 μ m long. Gram-variable. Motile by means of peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia and they are in subterminal 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-C_{15:0}. 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,

Table 2. Cellular fatty acid composition of *Paenibacillus phyllosphaerae* sp. nov. and phylogenetically related species of the genus *Paenibacillus*

Species: 1, *P. phyllosphaerae*; 2, *P. curdlanolyticus*; 3, *P. kobensis*. Data are from Dasman *et al.* (2002) and this study. ND, Not detected.

Fatty acid	1	2	3
Saturated			
Straight-chain:			
C _{14:0}	1.8	0.8	1.2
C _{15:0}	ND	0.4	0.9
C _{16:0}	11.8	0.6	16.1
C _{17:0}	0.6	9.9	0.2
iso-Branched:			
C _{14:0}	2.2	1.8	2.3
C _{15:0}	9.9	5.4	6.1
C _{16:0}	11.1	15.4	14.5
C _{17:0}	3.3	16.4	5.2
anteiso-Branched:			
C _{15:0}	48.6	44.6	38.5
C _{17:0}	6.4	4.7	14.0
Unsaturated			
C _{16:1ω7c}	1.9	ND	ND
C _{16:1ω11c}	1.4	ND	ND

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-arabinose, ribose, L-xylose, adonitol, methyl β -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, adipate, caproate, citrate, phenylacetate and malate do not serve as carbon sources for growth. Xylanase, cellulase, amylase 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, PALXIL04^T (=LMG 22192^T=CECT 5862^T), 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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