Paenibacillus xylanilyticus sp. nov., an airborne xylanolytic bacterium

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During a search for xylan-degrading micro-organisms, a sporulating bacterium was recovered from xylan-containing agar plates exposed to air in a research laboratory (Salamanca University, Spain). The airborne isolate (designated strain XIL14^T) was identified by 16S rRNA gene sequencing as representing a *Paenibacillus* species most closely related to *Paenibacillus illinoisensis* JCM 9907^T (99.3% sequence similarity) and *Paenibacillus pabuli* DSM 3036^T (98% sequence similarity). Phenotypic, chemotaxonomic and DNA–DNA hybridization data indicated that the isolate belongs to a novel species of the genus *Paenibacillus*. Cells of strain XIL14^T were motile, sporulating, rod-shaped, Gram-positive and facultatively anaerobic. The predominant cellular fatty acids were anteiso-C_{15:0} and C_{16:0}. The DNA G + C content of strain XIL14^T was 50.5 mol%. Growth was observed with many carbohydrates, including xylan, as the only carbon source and gas production was not observed from glucose. Catalase was positive and oxidase was negative. The airborne isolate produced a variety of hydrolytic enzymes, including xylanases, amylases, gelatinase and β -galactosidase. DNA–DNA hybridization levels between strain XIL14^T and *P. illinoisensis* DSM 11733^T and *P. pabuli* DSM 3036^T were 43.3 and 36.3%, respectively. According to the data obtained, strain XIL14^T is considered to represent a novel species for which the name *Paenibacillus xylanilyticus* sp. nov. is proposed (=LMG 21957^T = CECT 5839^T).

Xylan is a heterogeneous polymer composed of (1,4)-linked β -D-xylosyl residues. It is very abundant in nature, being the major component of hemicelluloses of monocotyledon cell walls. Xylanases (1,4- β -D-xylanohydrolase) are a group of xylanolytic enzymes that hydrolyse xylan to xylan-oligosaccharides and xylose. The genus *Paenibacillus*, which was originally proposed by Ash *et al.* (1993) (valid publication by Ash *et al.*, 1994), contains some species that are able to hydrolyse xylan (Ay *et al.*, 1998; Hespell, 1996; Lee *et al.*, 2000; Morales *et al.*, 1995; Nielsen & Sorensen, 1997; Zamost *et al.*, 1991). Recently, a novel xylanolytic *Paenibacillus* species, *Paenibacillus favisporus*, was described (Velázquez *et al.*, 2004).

Within the framework of a screening programme to search for xylan-degrading micro-organisms, we isolated a bacterial strain (XIL14^T) that actively hydrolysed xylan. Isolation was made in YNBX medium containing xylan as single source of carbon, as previously described (Velázquez *et al.*,

2004). Colonies of the airborne bacterium were cream coloured, opaque, rounded and convex.

Strain XIL14^T was grown for 48 h and motility was checked under a scanning electron microscope. Gram behaviour of the cells was ascertained by staining (Doetsch, 1981). Cells of strain XIL14^T were Gram-positive, rod-shaped, sporulating and motile (peritrichous flagellation).

Amplification and sequencing of the 16S rRNA gene were performed as described by Rivas *et al.* (2003a). The sequence obtained was compared with those from GenBank using the FASTA program (Pearson & Lipman, 1988). Sequences were aligned using the CLUSTAL W software (Thompson *et al.*, 1997). Distances were calculated by using the method of Kimura (1980). Phylogenetic trees were inferred using the neighbour-joining method (Saitou & Nei, 1987). The MEGA 2.1.0 package (Kumar *et al.*, 2001) was used for all analyses.

The complete (1546 bp) 16S rRNA gene sequence of strain XIL14^T was determined. A comparison against the 16S rRNA gene sequences held in the GenBank database indicated that strain XIL14^T is phylogenetically related to strains and species of the genus *Paenibacillus*. 16S rRNA

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of XIL14^T is AY427832.

An extended phylogenetic tree of representative *Paenibacillus* strains is available as a supplementary figure in IJSEM Online.

Fig. 1. Comparative analysis of the 16S rRNA gene sequences of *Paenibacillus xylanilyticus* sp. nov. and representative strains from GenBank using the neighbour-joining method. The analysis was based on 1411 nt. The significance of each branch is indicated by a bootstrap percentage calculated for 1000 subsets. Bar, 1 substitution per 100 nt. An extended tree containing a wider selection of reference sequences is available in IJSEM Online.

gene sequence similarities to all other recognized *Paenibacillus* species ranged from 89.8% with *Paenibacillus alginolyticus* to 99.3% with *Paenibacillus illinoisensis*. Fig. 1 shows the phylogenetic tree obtained with the neighbour-joining method (a more complete phylogenetic tree is available as a supplementary figure in IJSEM Online). The closest related recognized species are *P. illinoisensis* JCM 9907^T (=DSM 11733^T) with 99.3% similarity and *Paenibacillus pabuli* DSM 3036^T with 98.4% similarity.

For base composition analysis, DNA was prepared according to the method of Chun & Goodfellow (1995), and the G+C content was determined using the thermal denaturation method (Mandel & Marmur, 1968). The DNA G+C content of strain XIL14^T was 50.5 mol%.

For DNA–DNA hybridization analyses, DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion *et al.* (1977), which was carried out as described by De Ley *et al.* (1970) but using the modifications described by Huß *et al.* (1983) and Escara & Hutton (1980). Renaturation rates were determined with the TRANSFER.BAS program (Jahnke, 1992). DNA–DNA relatedness was tested [in 2 × SSC + 10% (v/v) DMSO at 68 °C] among strains XIL14^T, *P. illinoisensis* DSM 11733^T and *P. pabuli* DSM 3036^T. Levels of DNA–DNA relatedness between strain XIL14^T and *P. illinoisensis* DSM 11733^T and *P. pabuli* DSM 3036^T were 43.3 and 36.3%, respectively. These results indicate that strain XIL14^T is not a representative of either of these species, using the recommended threshold value of 70% DNA–DNA relatedness for the definition of genomic species (Wayne *et al.*, 1987).

The predominant fatty acids analysed by GLC as described by Rivas *et al.* (2003b) were anteiso-C_{15:0} and C_{16:0}, respectively comprising 47.9 and 16.6% of the total. These values coincide with those reported for *P. illinoisensis* and *P. pabuli* (Shida *et al.*, 1997a, b) (Table 2). Other fatty acids

Table 1. Distinguishing phenotypic characteristics of the species phylogenetically related to *Paenibacillus xylanilyticus* sp. nov.

Strains: 1, *P. xylanilyticus* sp. nov. XIL14^T; 2, *P. illinoisensis* NRRL NRS-1356^T; 3, *P. pabuli* NRRL NRS-924^T; 4, *P. amylolyticus* NRRL NRS-290^T. Data for reference strains are from Meehan *et al.* (2001) and Velázquez *et al.* (2004). +, Positive; –, negative; v, variable; ND, no data. All species grow anaerobically and are positive for catalase, production of amylases and acid production from L-arabinose, mannitol and D-xylose. All species are negative for oxidase and production of tyrosinase and citrate.

Test	1	2	3	4
Optimum growth temperature (°C)	37	37	28–30	28–30
Nitrate reduction	–	–	–	+
Production of:				
Acetylmethylcarbinol	+	–	–	–
Indole	–	ND	–	–
Dihydroxyacetone	–	ND	ND	ND
pH in MR-VP broth	<4	5.0–5.2	<5.5	<5.5
Caseinase	–	+	v	–
Growth at pH 5.6	–	+	+	+
Growth at 50 °C	–	+	–	–
Growth in the presence of 5% NaCl	+	–	v	–

detected were C_{16:1ω11c} (8.1%), iso-C_{15:0} (7.1%), anteiso-C_{17:0} (4.9%), iso-C_{16:0} (4.7%), iso-C_{17:0} (4.5%), C_{14:0} (3.9%) and iso-C_{14:0} (2.3%).

Phenotypic characterization was performed according to the standard methods described by Claus & Berkeley (1986) and Logan & Berkeley (1984) using strains *P. illinoisensis* DSM 11733^T and *P. pabuli* DSM 3036^T as references. The API 20E and API 20NE systems were also used to characterize the isolate according to the manufacturer's instructions (bioMérieux). Table 1 lists the phenotypic properties of strain XIL14^T as well as those of closely related *Paenibacillus* species. Strain XIL14^T differs from *P. illinoisensis* in terms of acetoin production, final pH in Voges–Proskauer medium, caseinase, growth at pH 5.6 and growth at 50 °C. It differs with respect to *P. pabuli* in terms of optimum growth temperature, acetoin production and growth at pH 5.6.

Therefore, on the basis of the polyphasic taxonomic data we propose that isolate XIL14^T should be classified as representing a novel *Paenibacillus* species, for which the name *Paenibacillus xylanilyticus* sp. nov. is proposed.

Description of *Paenibacillus xylanilyticus* sp. nov.

Paenibacillus xylanilyticus (xy.la.ni.ly'ti.cus. N.L. n. *xylanum* xylan; Gr. adj. *lytikos* able to loosen; N.L. adj. *lyticus* -a -um dissolving; N.L. masc. adj. *xylanilyticus* xylan-dissolving).

Cells are rod-shaped, measuring 3.9–4 × 1.5–1.55 µm and motile by means of peritrichous flagella. Oval subterminal

Table 2. Percentage of total cellular fatty acids from strain XIL14^T and phylogenetically related species of the genus *Paenibacillus*

Species: 1, *P. xylanilyticus* sp. nov. XIL14^T; 2, *P. illinoisensis* (data from Shida *et al.*, 1997b); 3, *P. pabuli* (Shida *et al.*, 1997a); 4, *P. amylolyticus* (Shida *et al.*, 1997b). ND, Not detected.

Fatty acid	1	2	3	4
C _{14:0}	3.9	2.0	0.7	2.9
C _{15:0}	ND	1.0	0.1	0.5
C _{16:0}	16.6	24.7	10.1	12.8
iso-C _{14:0}	2.3	1.8	0.7	2.3
iso-C _{15:0}	7.1	1.5	2.2	2.0
iso-C _{16:0}	4.7	6.3	4.8	9.0
iso-C _{17:0}	4.5	1.4	1.3	3.5
anteiso-C _{15:0}	47.9	56.6	73.7	46.2
anteiso-C _{17:0}	4.9	4.9	4.1	2.2
C _{16:1} ω11	8.1	ND	ND	0.1

spores are formed that slightly swell the sporangia. Colonies grown (48 h, 37 °C) on YNBX agar are circular, convex, cream-coloured, opaque and usually 1–3 mm in diameter. Cells are facultatively anaerobic and stain Gram-positive. Optimum growth temperature is 37 °C and optimum pH is 7. Oxidase-negative and catalase-positive. The DNA G+C content of the type strain is 50.5 mol%. The predominant fatty acids are anteiso-C_{15:0} and C_{16:0}. Does not produce gas from glucose. Acid is produced from D-glucose, L-arabinose, N-acetylglucosamine, sucrose, D-mannose, rhamnose, melibiose, maltose, xylose and mannitol. Able to use xylan as a carbon source. Unable to use inositol, sorbitol, citrate, propionate, caprate, adipate, malate or phenylacetate as sole sources of carbon. Produces xylanases, gelatinase, amylase and β-galactosidase but not urease, caseinase, phenylalanine deaminase, lysine decarboxylase, arginine dehydrolase, ornithine decarboxylase, tryptophan deaminase, tyrosinase, indole, dihydroxyacetone, hydrogen sulphide or acetoin (Voges–Proskauer medium). Nitrate reduction to nitrite is positive.

The type strain, XIL14^T (=LMG 21957^T=CECT 5839^T), was isolated from air in a research laboratory from Salamanca University, Spain.

Acknowledgements

This work was supported by Spanish Government Grants to E. M.-M. and E. V. We are grateful to Professor Dr R. M. Kroppenstedt and Dr P. Schumann (DSMZ, Braunschweig, Germany) for their help with analysis of fatty acid methyl esters and DNA–DNA hybridization, respectively.

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