Paenibacillus xylanisolvens sp. nov., a xylan-degrading bacterium from soil

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A xylan-degrading bacterium, strain X11-1^T, was isolated from soil collected in Nan province, Thailand. The strain was characterized based on its phenotypic and genotypic characteristics. Strain X11-1^T was a Gram-stain-positive, facultatively anaerobic, spore-forming, rod-shaped bacterium. It contained meso-diaminopimelic acid in the cell-wall peptidoglycan. The major menaquinone was MK-7, anteiso-C_{15:0} (56.6%) and C_{16:0} (14.0%) were the predominant cellular fatty acids and diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine and phosphatidylglycerol were the major phospholipids. The DNA G + C content was 51.6 mol%. Phylogenetic analysis using 16S rRNA gene sequences showed that strain X11-1^T was affiliated to the genus *Paenibacillus* and was closely related to *Paenibacillus naphthalenovorans* KACC 11505^T and *Paenibacillus validus* CCM 3894^T, with 96.5% sequence similarity. Therefore, the strain represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus xylanisolvens* sp. nov. is proposed. The type strain is X11-1^T (=KCTC 13042^T =PCU 311^T =TISTR 1829^T).

The genus *Paenibacillus*, proposed by Ash *et al.* (1993, 1994), has undergone considerable changes, with a consequent increase in the number of species. At the time of writing, there are 112 recognized species (Euzéby, 2010). *Paenibacillus* strains have been isolated from diverse environmental samples such as soil, cow faeces, dead honeybees and honeybee larvae, plant roots, food, a warm spring, raw and heat-treated milk and blood cultures (Shida *et al.*, 1997; Berge *et al.*, 2002; Roux & Raoult, 2004; Scheldeman *et al.*, 2004; Velázquez *et al.*, 2004; Chou *et al.*, 2007). Some of these bacteria excrete a diverse assortment of extracellular enzymes that hydrolyse polysaccharides (Zamost *et al.*, 1991; Morales *et al.*, 1995; Hespell, 1996; Nielsen & Sørensen, 1997; Aÿ *et al.*, 1998; Lee *et al.*, 2000; Dasman *et al.*, 2002; Kim *et al.*, 2004). Among them, strains of *Paenibacillus barcinonensis*, *P. cellulosilyticus*, *P. favisporus*, *P. montaniterrae*, *P. nanensis*, *P. panacisoli*, *P. phyllosphaerae*, *P. septentrionalis*, *P. siamensis*, *P. soli*,

P. thailandensis, *P. tundrae*, *P. woosongensis*, *P. xylanexedens* and *P. xylanilyticus* have been reported to have xylan-degrading activity (Velázquez *et al.*, 2004; Rivas *et al.*, 2005a, b, 2006; Sánchez *et al.*, 2005; Ten *et al.*, 2006; Park *et al.*, 2007; Lee & Yoon, 2008; Khiangnam *et al.*, 2009a, b; Nelson *et al.*, 2009). In this paper, we describe a novel xylan-degrading bacterium belonging to the genus *Paenibacillus* by means of a polyphasic taxonomic study.

Strain X11-1^T was isolated from a soil sample collected in Viengsa district, Nan province, in the northern part of Thailand, by the spread-plate method on XC agar medium (1⁻¹: 10 g oat spelt xylan, 5 g peptone, 1 g yeast extract, 4 g K₂HPO₄, 1 g MgSO₄·7H₂O, 0.2 g KCl, 0.02 g FeSO₄·7H₂O, 15 g agar; pH 7.0). In this screening step, the agar plates were incubated at 40 °C for 2 days. Xylan-degrading capacity of the cultures was detected by using a Congo red overlay method, as reported previously (Teather & Wood, 1982; Ruijsenaars & Hartsmans, 2001). Isolates that showed xylanase production were transferred to C agar medium. This medium had the same composition as XC medium apart from the omission of the oat spelt xylan. Cells grown on C agar medium were examined for their morphological and cultural characteristics, including cell

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain X11-1^T is AB495094.

Three supplementary figures are available with the online version of this paper.

shape, colony appearance, endospore formation and pigmentation, after incubation at 37 °C for 2 days. Motility was observed under a phase-contrast microscope (Nikon ECLIPSE 80i) by the hanging-drop method. Physiological and biochemical characterization was performed using the API 20NE and API 50CH (combined with API 50CHB/E medium) strips (bioMérieux), in accordance with the manufacturer's directions. Tests for Gram staining, catalase and oxidase, hydrolysis of casein, DNA, starch, Tween 80 and L-tyrosine, the methyl red/Voges–Proskauer reactions, indole production, citrate utilization and H₂S production were determined as described by Barrow & Feltham (1993). Growth under anaerobic conditions on agar plates was investigated using a Gaspak (BBL) anaerobic jar. Growth at pH 5, 6, 7, 8 and 9, in 3 and 5 % (w/v) NaCl and at 10, 15, 20, 25, 30, 40, 45, 50, 55 and 60 °C was tested by using C agar medium. All tests were carried out by incubating the cultures at 37 °C, except for the investigation of the effect of temperature on growth.

The type of diaminopimelic acid in the cell wall and menaquinones were determined as described by Komagata & Suzuki (1987). Total cellular fatty acid analysis was performed with cells grown on tryptic soy agar (TSA; Difco) for 48 h at 30 °C, by using the standard Microbial Identification System (MIDI Inc.) for automated GC analysis (Sasser, 1990). Polar lipids were extracted, examined by two-dimensional TLC and identified by using published procedures (Minnikin *et al.*, 1977).

DNA was prepared by the method of Saito & Miura (1963). DNA base composition was determined by reversed-phase HPLC (Tamaoka & Komagata, 1984). The 16S rRNA gene of the novel strain was amplified and the PCR product was purified and sequenced as described previously (Tanasupawat *et al.*, 2004). The sequence of strain X11-1^T (1472 nt) was aligned with selected sequences obtained from GenBank by using CLUSTAL_X version 1.83 (Thompson *et al.*, 1997). The alignment was edited manually to remove gaps and ambiguous nucleotides prior to the construction of phylogenetic trees. Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in the MEGA4 software (Tamura *et al.*, 2007). Confidence values of branches of the trees were determined using bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings.

Strain X11-1^T was a Gram-stain-positive, facultatively anaerobic, motile, rod-shaped bacterium. Central or subterminal ellipsoidal endospores were observed in swollen sporangia (Supplementary Fig. S1, available in IJSEM Online). Colonies were 1–5 mm in diameter, circular, flat, white and opaque after incubation on C medium for 2 days. Phenotypic characteristics are listed in the species description and in Table 1. Strain X11-1^T contained anteiso-C_{15:0} (56.6 %) and C_{16:0} (14.0 %) as the predominant cellular fatty acids. Strain X11-1^T, *Paenibacillus naphthalenovorans* KACC 11505^T and *Paenibacillus validus* CCM 3894^T showed similar cellular fatty acid profiles, but significant quantitative

Table 1. Differential characteristics of strain X11-1^T and closely related *Paenibacillus* type strains

Strains: 1, X11-1^T; 2, *P. naphthalenovorans* KACC 11505^T; 3, *P. validus* CCM 3894^T. Data were obtained in this study unless otherwise indicated. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3
Growth in 3 % NaCl	w	w	–
Growth at:			
15 °C	+	–	w
50 °C	+	+	–
55 °C	–	+	–
Voges–Proskauer reaction	–	+	–
Nitrate reduction	+	–	+
Hydrolysis of:			
Aesculin	w	–	+
Casein	+	–	–
Starch	–	–	+
Assimilation of potassium gluconate	–	+	+
Acid production from:			
Amygdalin	–	–	+
D-Arabinose	+	+	–
D-Arabitol	+	–	–
Cellobiose	–	–	+
Glycerol	+	–	+
Glycogen	–	–	+
Inositol	–	–	+
D-Lyxose	+	+	–
Melibiose	–	+	+
Raffinose	–	+	+
Starch	–	+	+
Xylitol	–	+	–
DNA G+C content (mol%)	51.6	46.6 ^a *	50.9 ^b

*Data from: a, Daane *et al.* (2002); b, Heyndrickx *et al.* (1995).

differences were also found, as shown in Table 2. In the 16S rRNA gene sequence-based phylogenetic tree reconstructed according to the neighbour-joining method, strain X11-1^T was placed in a monophyletic cluster of closely related *Paenibacillus* species (Fig. 1). Trees constructed by the neighbour-joining (Supplementary Fig. S2) and maximum-parsimony (not shown) methods including strain X11-1^T and representatives of all *Paenibacillus* species were compared and strain X11-1^T was closely related to *P. naphthalenovorans* PR-N1^T, *P. validus* JCM 9077^T, *P. ehimensis* KCTC 3748^T, *P. elgii* SD17^T, *P. koreensis* YC300^T, *P. soli* DCY03^T and *P. filicis* S4^T based on 16S rRNA gene sequence similarity (less than 97 %). The DNA G+C content was 51.6 mol%, which was in the range observed for members of the genus *Paenibacillus* (Shida *et al.*, 1997). It contained meso-diaminopimelic acid in the cell-wall peptidoglycan, the major menaquinone was MK-7 and the major phospholipids were diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine and phosphatidylglycerol (Supplementary Fig. S3); glycolipids were not detected in this study. Based on its phenotypic and chemotaxonomic

Table 2. Cellular fatty acid compositions of strain X11-1^T and closely related *Paenibacillus* type strains

Strains: 1, X11-1^T; 2, *P. naphthalenovorans* KACC 11505^T; 3, *P. validus* CCM 3894^T. Data were obtained in this study. Values are percentages of total fatty acids. tr, Trace (<1 %); ND, not detected.

Fatty acid	1	2	3
Straight-chain saturated			
C _{12:0}	ND	ND	tr
C _{14:0}	2.4	2.2	2.0
C _{15:0}	3.1	tr	tr
C _{16:0}	14.0	17.2	15.2
C _{17:0}	tr	ND	tr
Branched saturated			
iso-C _{14:0}	2.4	1.2	1.3
iso-C _{15:0}	5.1	6.5	9.9
iso-C _{16:0}	7.1	8.8	6.9
iso-C _{17:0}	1.7	4.5	7.7
anteiso-C _{15:0}	56.6	47.2	44.6
anteiso-C _{17:0}	5.2	9.3	9.7
Monounsaturated			
C _{16:1} ω7c alcohol	ND	ND	tr
C _{16:1} ω11c	1.1	2.5	tr
iso-C _{17:1} ω10c	ND	ND	tr

characteristics and phylogenetic analysis using 16S rRNA gene sequences, we conclude that strain X11-1^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus xylanisolvens* sp. nov. is proposed.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain X11-1^T and the type strains of closely related *Paenibacillus* species. *Thermobacillus xylanilyticus* XE^T was used as the outgroup. Based on 1000 resamplings, bootstrap percentages ≥56 % are shown. Bar, 0.01 substitutions per nucleotide position. An extended neighbour-joining tree is available as Supplementary Fig. S2.

Description of *Paenibacillus xylanisolvens* sp. nov.

Paenibacillus xylanisolvens (xy.la.ni.sol'vens. N.L. n. xylanum xylan; L. part. adj. solvens dissolving; N.L. part. adj. xylanisolvens xylan-dissolving).

Cells are Gram-stain-positive, facultatively anaerobic, motile rods (0.4–0.8 × 1.5–3.0 μm). Central or subterminal ellipsoidal endospores are observed in swollen sporangia. Colonies are 1–5 mm in diameter, circular, flat, white and opaque after 2 days of incubation on C agar medium. Growth is observed at pH 6–9 (optimally at pH 7–8), at 15–50 °C (optimally at 30–37 °C), in 3 % NaCl (weakly) and under anaerobic conditions, but not at pH 5, at 10, 55 or 60 °C or in 5 % NaCl. Positive for catalase, oxidase, nitrate reduction, hydrolysis of aesculin (weak), casein, Tween 80, urea and xylan and assimilation of D-glucose, maltose, D-mannitol and D-mannose, but negative for methyl red and Voges–Proskauer reactions, citrate utilization, H₂S production, indole production, fermentation of glucose, hydrolysis of L-arginine, DNA, gelatin, *p*-nitrophenyl β-D-galactopyranoside, starch and L-tyrosine and assimilation of *N*-acetylglucosamine, L-arabinose, adipic acid, capric acid, phenylacetic acid and potassium gluconate. Able to produce acid from aesculin, D-arabinose, D-arabitol, D-fructose, α-glucopyranoside, D-galactose, D-glucose, glycerol, D-lyxose, maltose, D-mannitol, D-mannose, D-ribose, sucrose, trehalose, turanose and D-xylose, but not from *N*-acetylglucosamine, D-adonitol, amygdalin, L-arabinose, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D- or L-fucose, gentiobiose, gluconate, glycogen, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, α-mannopyranoside, melezitose, melibiose, raffinose, L-rhamnose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, β-xylopyranoside, xylitol or L-xylose. MK-7 is the predominant menaquinone. The predominant fatty acids are anteiso-C_{15:0} and C_{16:0}. The cell-wall diamino acid is *meso*-diaminopimelic acid. Major phospholipids are diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine and phosphatidylglycerol. The DNA G + C content of the type strain is 51.6 mol%.

The type strain is X11-1^T (=KCTC 13042^T =PCU 311^T =TISTR 1829^T), isolated from soil.

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