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Paenibacillus xylaniclasticus sp. nov., a Xylanolytic-Cellulolytic Bacterium Isolated from Sludge in an Anaerobic Digester[§]

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A mesophilic, facultative, anaerobic, xylanolytic-cellulolytic bacterium, TW1^T, was isolated from sludge in an anaerobic digester fed with pineapple waste. Cells stained Gram-positive, were spore-forming, and had the morphology of straight to slightly curved rods. Growth was observed in the temperature range of 30 to 50°C (optimum 37°C) and the pH range of 6.0 to 7.5 (optimum pH 7.0) under aerobic and anaerobic conditions. The strain contained *meso*-diaminopimelic acid in the cell-wall peptidoglycan. The predominant isoprenoid quinone was menaquinone with seven isoprene units (MK-7). Anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, and $C_{16:0}$ were the predominant cellular fatty acids. The G+C content of the DNA was 49.5 mol%. A phylogenetic analysis based on 16S rRNA showed that strain TW1^T belonged within the genus *Paenibacillus* and was closely related to *Paenibacillus cellulosilyticus* LMG 22232^T, *P. curdlanolyticus* KCTC 3759^T, and *P. kobensis* KCTC 3761^T with 97.7, 97.5, and 97.3% sequence similarity, respectively. The DNA-DNA hybridization values between the isolate and type strains of *P. cellulosilyticus* LMG 22232^T, *P. curdlanolyticus* KCTC 3759^T, and *P. kobensis* KCTC 3761^T were found to be 18.6, 18.3, and 18.0%, respectively. The protein and xylanase patterns of strain TW1^T were quite different from those of the type strains of closely related *Paenibacillus* species. On the basis of DNA-DNA relatedness and phenotypic analyses, phylogenetic data and the enzymatic pattern presented in this study, strain TW1^T should be classified as a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus xylaniclasticus* sp. nov. is proposed. The type strain is TW1^T (=NBRC 106381^T =KCTC 13719^T =TISTR 1914^T).

Keywords: anaerobic digester, facultative anaerobic bacterium, *Paenibacillus xylaniclasticus*, xylanase, xylanolytic-cellulolytic bacterium

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Introduction

Plant cells represent a renewable, abundant biopolymer source and consist of a mixture of polysaccharides, cellulose, and hemicellulose (Ljungdahl and Eriksson, 1985). Cellulose is primarily found in the plant cell wall, where it is embedded with xylan, other hemicelluloses, and lignin. Microorganisms that are involved in the degradation of cellulose and xylan play an important role in the carbon cycle. The conversion of plant cell-wall polysaccharides through hydrolysis into soluble sugars is potentially an important source of building blocks for multiple value-added products (Patrick *et al.*, 2010). It has been reported that many microorganisms produce extracellular polysaccharide-degrading enzymes including xylanase and cellulase (Bhat, 2000; Subaramaniyan and Prema, 2002; Zhang *et al.*, 2006). Among these, members of the genus *Paenibacillus* in the family *Paenibacillaceae* have also been reported to produce polysaccharide-degrading enzymes (Sánchez *et al.*, 2005; Rivas *et al.*, 2006; Park *et al.*, 2007; Lee and Yoon, 2008; Oh *et al.*, 2008; Khianngam *et al.*, 2009; Kim *et al.*, 2009). In this study, we describe a novel xylanolytic-cellulolytic enzyme-producing bacterium, TW1^T, isolated from sludge in an anaerobic digester, based on a polyphasic taxonomic approach.

Materials and Methods

Isolation of bacterial strain and culture conditions

Sludge samples were collected from several waste treatment reactors. These samples were suspended in Berg's mineral salt medium (MS), pH 7.0 (Berg *et al.*, 1972) containing 0.2% $NaNO_3$, 0.05% K_2HPO_4, 0.02% $MgSO_4{\cdot}7H_2O$, 0.002% $MnSO_4{\cdot}H_2O$, 0.002% $FeSO_4{\cdot}7H_2O$, 0.002% $CaCl_2{\cdot}2H_2O$, and 0.5% oat spelt xylan as the sole carbon source and incubated aerobically at 37°C in a rotary shaker at 200 rpm. Aliquots of grown cultures were transferred to fresh medium and incubated under the same conditions. This sub-culture step was repeated several times to obtain good candidates. Isolation and purification procedures were carried out on MS agar plates by conventional spread plate techniques. Xylanase-producing bacteria were detected by a halo around the colony. The isolated strain was designed as TW1^T. This bacterium was then submitted to the National Institute of Technology and Evaluation Biological Resource Centre, Japan (=NBRC 106381^T), Korean Collection for Type Cultures (=KCTC

13719^T) and the MIRCEN Culture Collection, Thailand Institute of Scientific and Technological Research (=TISTR 1914^T). *P. cellulosilyticus* LMG 22232^T, *P. curdlanolyticus* KCTC 3759^T, and *P. kobensis* KCTC 3761^T were obtained from the Belgian Coordinated Collections of Microorganisms (BCCM/LMG) and the Korean Collection for Type Cultures (KCTC).

Phenotypic and biochemical characteristics

To examine cell shape and spore formation, strain TW1^T was grown in the same medium without agar (MS broth) at 37°C for 3 days. Traditional Gram staining was performed as described previously (Singleton, 2004), and spore formation was examined using the Schaeffer-Fulton staining method (Schaeffer and Fulton, 1933). Microscopic analysis was performed using an Olympus (CH20BIMF200) light microscope. Scanning electron microscopy (SEM) was used to study the morphology of the isolate (Bozzola and Russel, 1999). SEM was performed using a JEOL JSM-35 scanning electron microscope. Acid production from carbohydrates was evaluated with API 50CH (combined with API 50CHB/E medium) strips (bioMérieux, France), and biochemical characteristics were assessed using the API 20NE and API 20E systems (bioMérieux) according to the manufacturer's instructions. Activities for catalase and oxidase, hydrolysis of casein, starch, tyrosine, Tween 80 and DNA, and the methyl red/Voges-Proskauer (MR-VP) reaction were assessed as described by Barrow and Feltham (1993). Growth under anaerobic conditions was determined on MS agar incubated in an anaerobic chamber (Bactron, USA) at 37°C. Growth was monitored by measuring culture turbidity at 600 nm. Detection of motility was performed using semi-solid agar (Tittsler and Sandholzer, 1936). To determine the optimum growth temperature, the isolate was cultured in MS broth containing glucose as the carbon source and incubated at different temperatures (25, 28, 30, 37, 45, 50, and 55°C) in incubators. To determine the optimum pH range for growth, the pH was adjusted with 2 M HCl or 1% (w/v) Na_2CO_3 to an acidic or alkaline pH, respectively (pH 5.0, 6.0, 7.0, 7.5, 8.0, and 9.0). The effect of salt concentration on growth was examined by adding NaCl to the MS broth to give a final concentration of 0–5% (w/v).

Chemotaxonomic characterisations

The whole-cell fatty acid compositions of strain TW1^T and the type strains were analysed by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) GmbH, Braunschweig, Germany. Quantitative analysis of cellular fatty acids was done with the Microbial Identification System (MIDI) (Sasser, 1990) using the TSBA40 method, after cultivation in tryptic soy broth until the early stationary growth phase at the optimum temperature for each strain under aerobic conditions. Fatty acids were determined by gas chromatography using a flame ionisation detector. An Ultra 2 column (phenyl methyl silicone fused silica capillary column) was used for analysis. The temperature programme ramped from 170°C to 270°C at 5°C per min. Following analysis, a ballistic increase to 300°C allowed cleaning of the column during a hold of 2 min. Hydrogen was the carrier gas, nitrogen was the "make up" gas, and air was used to support the flame. Diaminopimelic acid (DAP) in the peptidoglycan and isoprenoid quinones were analysed according to the procedure of Komagata and Suzuki (1987). Polar lipids were extracted from freeze-dried cells, separated by two-dimensional silica gel TLC and detected with appropriate detection reagents (Minnikin *et al.*, 1977).

Molecular characterisations

To determine the DNA G+C content of strain TW1^T, the genomic DNA was prepared and purified according to the method of Saito and Miura (1963). The G+C content of DNA was determined by reverse-phase high-performance liquid chromatography (Tamaoka and Komagata, 1984). DNA-DNA hybridization was carried out by the photobiotin-labeling method in microdilution wells at 43°C for 5 h (Ezaki *et al.*, 1989). DNA-DNA relatedness values are the mean of three values. The 16S rRNA gene was amplified by PCR using the following primers: 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Weisburg *et al.*, 1991). PCR products were purified using the QIAquick PCR purification kit (QIAGEN, Germany). The purified PCR product was ligated into the *p*GEM-T Easy vector using the cloning kit (Promega, USA) and transformed into competent cells (*E. coli* DH5α). The nucleotide sequence of the 16S rRNA gene was determined using T7 and SP6 primers according to the manufacturer's instructions. The sequence data were applied to the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007) to obtain highly similar sequences. The nearly complete sequence (1,513 bp) was aligned with other sequences by CLUSTAL_X version 1.83 (Thompson *et al.*, 1997). Gaps and ambiguous bases were eliminated from the calculations. The distance matrices for the aligned sequences were calculated by Kimura's two-parameter model (Kimura, 1980). A phylogenetic tree was constructed by the neighbour-joining method (Saitou and Nei, 1987) with MEGA 4.0 software (Tamura *et al.*, 2007). The confidence values of individual branches of the phylogenetic tree were estimated by bootstrap analysis (Felsenstein, 1985) based on 1,000 resamplings.

Nucleotide sequence accession number

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TW1^T is FJ532373.

Enzyme production

Strain TW1^T was grown in MS broth containing 0.5% (w/v) birchwood xylan as the sole carbon source. The culture was incubated in a rotary shaker at 200 rpm and 37°C until the late exponential growth phase (2 days) and harvested by centrifugation (8,000×g for 5 min) at 4°C. The supernatant was concentrated by ultrafiltration with a 10-kDa molecular weight cut-off membrane (QuixStand benchtop system; Amersham, USA). The concentrated supernatant was used as an extracellular crude enzyme. Three type strains were cultivated in the above medium until the late exponential growth phase at the optimum temperatures of each strain, then harvested as described above.

Table 1. Differential characteristics of strain TW1^T and related *Paenibacillus* species
Strains: 1, TW1^T; 2, *P. cellulosilyticus* LMG 22232^T; 3, *P. curdlanolyticus* KCTC 3759^T; 4, *P. kobensis* KCTC 3761^T. Data were obtained from this study under the same conditions, unless otherwise indicated. All strains produced acid from aesculin, amygdaline, L-arabinose, D-cellobiose, D-galactose, gentiobiose, D-glucose, D-lactose, D-maltose, D-melibiose, starch, methyl-β-D-xylopyranoside, and D-xylose. All strains did not produce acid from D-arabinose, D-arabitol, L-arabitol, dulcitol, D-fucose, inulin, D-lyxose, D-mannitol, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, D-sorbitol, L-sorbose, D-tagatosexylitol, and L-xylose. All four strains assimilated D-glucose and D-maltose. The following substrates were not assimilated by any of the four strains: D-mannitol, adipate, caprate, malate, phenyl-acetate, and trisodium citrate. +, Positive; W, weakly positive; -, negative.

Characteristic	1	2	3	4
Colony color	White/brown	White/cream	White	White
Brown-black pigment production	+	-	-	-
Anaerobic growth	W	-	-	-
Catalase	-	+	+	+
Optimum temperature (°C) for growth	37	28	30	30
Growth at 50°C	+	-	-	-
Nitrate reduction	-	-	+	+
Production of:				
Acetoin	-	+	-	-
Cellulase on CM-cellulose	+	+	-	-
Assimilation of:				
N-Acetylglucosamine	-	-	+	+
L-Arabinose	+	+	-	-
D-Mannose	+	+	-	-
Potassium gluconate	-	+	+	+
Acid from:				
N-Acetylglucosamine	-	-	+	+
D-Adonitol	+	-	-	-
Arbutin	-	-	+	-
Erythritol	+	-	-	-
D-Fructose	-	-	+	-
L-Fucose	+	-	-	-
Glycerol	-	-	+	-
Glycogen	-	+	+	+
Inositol	+	-	-	-
D-Mannose	+	+	-	-
D-Melezitose	-	+	+	+
Methyl-α-D-glucopyranoside	-	+	+	+
Methyl-α-D-mannopyranoside	-	-	+	-
D-Raffinose	-	+	+	+
L-Rhamnose	-	+	-	-
D-Ribose	+	-	-	-
Salicin	+	-	+	-
D-Saccharose	-	+	+	+
D-Trehalose	-	+	+	+
D-Turanose	-	-	+	-
DNA G+C content (mol%)	49.5	51.0[a]	50.1–51.8[b]	50.1–51.8[b]

[a] Data from Rivas *et al.* (2006)
[b] Data from Kanzawa *et al.* (1995)

Enzyme assays

Xylanase, β-xylosidase, α-L-arabinofuranosidase, β-glucosidase, carboxymethyl cellulase (CMCase), and avicelase activities were determined as previously described (Pason *et al.*, 2006). The increase in reducing sugars was determined by the Somogyi-Nelson method (1944). One unit of enzyme activity was defined as the amount of enzyme that liberated 1 µmol of reducing sugars per min. Cellobiohydrolase activity was determined by the method of Kohring *et al.* (1990) using *p*-nitrophenyl β-D-cellobioside as the substrate. β-Xylosidase, α-L-arabinofuranosidase, β-glucosidase, and cellobiohydrolase activities were expressed as µmol of *p*-nitrophenol released per min per ml of enzyme solution.

Protein determination

Protein concentrations were measured by the Lowry method (1951) using bovine serum albumin as the standard.

Gel electrophoresis and zymograms

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a 10% polyacrylamide gel by the Laemmli method (1970). After electrophoresis, the proteins were stained with Coomassie brilliant blue R-250, whereas SDS-zymograms for xylanase activity were prepared from SDS-10% (w/v) polyacrylamide gels containing 0.1% (w/v) soluble xylan, as previously described (Phitsuwan *et al.*, 2010). Gels were incubated with 50 mM sodium phosphate buffer at 50°C for 1 h. After incubation, the gels were stained with 0.1% (w/v) Congo red solution for 30 min at room temperature. Destaining was performed with 1 M NaCl until clear bands were seen. Afterward, the gels were fixed with 5% (v/v) acetic acid.

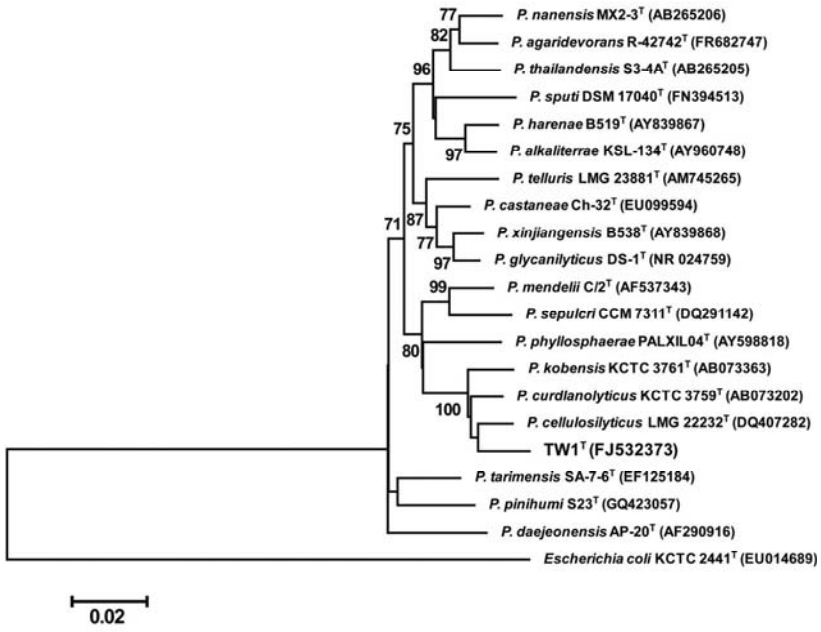

Fig. 1. Phylogenetic relationship of *Paenibacillus xylaniclasticus* sp. nov. with members of the genus *Paenibacillus* and *E. coli* as an out-group. The phylogenetic tree is based on the comparison of nearly complete 16S rRNA gene sequences (1,513 bp) and was constructed using the neighbour-joining method. Bar represents 0.02 substitutions per nucleotide position.

Results and Discussion

Phenotypic and biochemical characteristics

Strain TW1^T was a facultatively anaerobic, Gram-positive, non-motile, rod-shaped (0.5–0.8×1.4–2.3 μm) bacterium, and endospores were observed in swollen sporangia. Colonies on MS agar plates were translucent white to slightly brown, 1.0–2.0 mm in diameter, with irregular undulate margins, and produced a brown-black pigment after 3 days at 37°C. Growth was inhibited in the presence of greater than 2% (w/v) NaCl. Optimal growth was obtained at 37°C, and no growth occurred at 55°C. Strain TW1^T could be differentiated from the related *Paenibacillus* species by means of some phenotypic characteristics (Table 1). For example, strain TW1^T was able to grow at 50°C and under anaerobic conditions, while related type strains could not. Moreover, the assimilation test and acid production from several carbon sources were also different among strains. The physiological and biochemical properties of strain TW1^T are summarised in the species description below.

Phylogenetic analysis

Phylogenetic analysis of the nucleotide sequence of 16S rRNA gene revealed that strain TW1^T belonged to the genus *Paenibacillus* (Fig. 1). Trees constructed by the neighbor-joining and maximum-parsimony methods, including strain TW1^T and closely related species, are shown in Supplementary data Figs. S1 and S2. Strain TW1^T showed high phylogenetic relatedness with *P. cellulosilyticus* LMG 22232^T (97.7% similarity), *P. curdlanolyticus* KCTC 3759^T (97.5% similarity), and *P. kobensis* KCTC 3761^T (97.3% similarity).

Cellular fatty acids, G+C content, isoprenoid quinones, and polar lipids

Strain TW1^T contained *meso*-diaminopimelic acid in the cell-wall peptidoglycan and MK-7 as the major isoprenoid quinone. The G+C content of the DNA was 49.5 mol%, which is similar to related type strains (Shida *et al.*, 1997; Rivas *et al.*, 2006). The major cellular fatty acid components were anteiso-$C_{15:0}$ (36.6%), iso-$C_{16:0}$ (24.2%), anteiso-$C_{17:0}$ (17.9%), and $C_{16:0}$ (9.6%). The isolated strain showed a similar major fatty acid composition to the related type strains of the genus *Paenibacillus*, but there were significant quantitative differences when cultivated under the same conditions (Table 2). Thus, the results indicate that strain TW1^T might

Table 2. Cellular fatty acid composition of strain TW1^T and related *Paenibacillus* species[a]

Strains: 1, TW1^T; 2, *P. cellulosilyticus* LMG 22232^T; 3, *P. curdlanolyticus* KCTC 3759^T; 4, *P. kobensis* KCTC 3761^T. Data were obtained in this study according to the Analytical Service Microbial Identification System (DSMZ, Braunschweig, Germany).

Fatty acid	1	2	3	4
iso-$C_{10:0}$	1.0	ND	ND	ND
anteiso-$C_{11:0}$	2.1	ND	ND	ND
anteiso-$C_{13:0}$	ND	1.3	tr	tr
iso-$C_{14:0}$	tr	2.4	1.3	1.7
$C_{14:0}$	tr	1.1	1.0	1.4
iso-$C_{15:0}$	1.3	2.4	7.7	6.0
anteiso-$C_{15:0}$	36.6	36.9	49.7	43.3
$C_{15:0}$	3.1	1.0	tr	tr
iso-$C_{16:0}$	24.2	22.7	8.4	11.5
$C_{16:0}$	9.6	12.9	10.3	13.4
iso-$C_{17:0}$	1.2	2.7	6.9	5.6
anteiso-$C_{17:0}$	17.9	11.2	9.8	15.1
$C_{18:1}\omega 9c$	ND	1.1	1.1	ND

[a] Values are percentages of total fatty acids; fatty acids detected in amounts of less than 1% in four taxa are not shown.
ND, not detected.
tr, trace

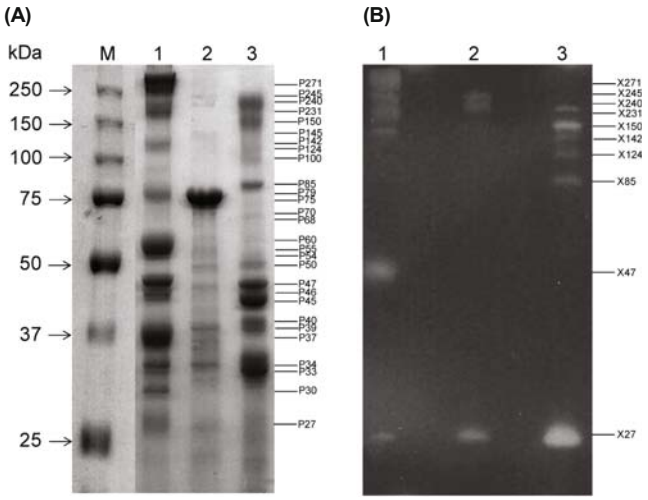

Fig. 2. SDS-PAGE (A) and xylanase zymogram (B) of the extracellular crude enzymes from *Paenibacillus* species. All samples contained approximately 80 µg of protein. Lanes: M, molecular weight markers; 1, strain TW1^T; 2, *P. cellulosilyticus* LMG 22232^T; 3, *P. curdlanolyticus* KCTC 3759^T.

be a new species in the genus *Paenibacillus*. Furthermore, the polar lipid composition of strain TW1^T was analysed. Diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), an unknown aminophospholipid, and an unknown phospholipid were found in strain TW1^T (Supplementary data Fig. S3). These polar lipids have been reported in several members of the genus *Paenibacillus*; however, the components were different from those found in other *Paenibacillus* species (Sánchez *et al.*, 2005; Baik *et al.*, 2011; Khianngam *et al.*, 2011).

DNA-DNA hybridization

DNA-DNA hybridization revealed that strain TW1^T showed low levels of DNA-DNA relatedness to *P. cellulosilyticus* LMG 22232^T (18.6%), *P. curdlanolyticus* KCTC 3759^T (18.3%), and *P. kobensis* KCTC 3761^T (18.0%). Thus, according to the recommendations of the *ad hoc* committee (Wayne *et al.*, 1987), who defined a threshold value of over 70% for DNA-DNA relatedness as a definition of bacterial species, strain TW1^T is not a member of these *Paenibacillus* species.

Protein and zymogram analysis

The component patterns of the crude enzymes from four strains were investigated using gel electrophoresis and xylanase zymograms. However, *P. kobensis* KCTC 3761^T grew poorly in MS broth containing 0.5% (w/v) birchwood xylan as the sole carbon source. Thus, *P. kobensis* KCTC 3761^T was dropped from this analysis. Three strains were cultivated and harvested under the same conditions as described in the 'Materials and Methods' section. The crude enzymes of strain TW1^T, *P. cellulosilyticus* LMG 22232^T, and *P. curdlanolyticus* KCTC 3759^T exhibited sixteen, fourteen, and eighteen proteins, respectively (Fig. 2A, lanes 1-3) with a molecular mass range of 27 to 271 kDa on SDS-PAGE. The zymogram of strain TW1^T revealed six proteins (27, 47, 142, 231, 245, and 271 kDa) with xylanase activity (Fig. 2B, lane 1), whereas *P. cellulosilyticus* LMG 22232^T and *P. curdlanolyticus* KCTC 3759^T revealed three (27, 240, and 245 kDa) and six proteins (27, 85, 124, 142, 150, and 231 kDa) with xylanase activity (Fig. 2B, lanes 2 and 3, respectively). These results strongly indicate that newly isolated strain TW1^T produced different xylanases when compared with closely related type strains. Strain TW1^T produced high molecular mass proteins containing xylanase activity. A similar pattern was reported in the multienzyme complex of *P. curdlanolyticus* B-6 (Pason *et al.*, 2006). Thus, the newly isolated strain TW1^T probably produced multienzymes in a complex form.

Taxonomic conclusion

On the basis of these results, including phenotypic and chemotaxonomic characteristics, 16S rRNA gene analysis, DNA-DNA relatedness, as well as protein and xylanase zymogram patterns, strain TW1^T represents a member of a novel species, for which the name *Paenibacillus xylaniclasticus* sp. nov. is proposed.

Description of *Paenibacillus xylaniclasticus* sp. nov.

Paenibacillus xylaniclasticus (xy.la.ni.clas'ti.cus. N.L. neut. n. *xylanum*, xylan; N.L. adj. *clasticus -a –um*; Gr. adj. *klastos* -ē -on, broken in pieces, breaking; N.L. masc. adj. *xylaniclasticus*, xylan-breaking).

Cells are rod-shaped (0.5–0.8×1.4–2.3 µm), usually single or in pairs. Endospores are formed in the terminal position. Cells are Gram-positive, non-motile, and facultatively anaerobic. The isolated strain produces a brownish-black pigment when cultivated in mineral salt broth or on agar medium. Colonies are white to slightly brown, irregular with undulate margins and are about 1.0–2.0 mm in diameter after 3 days of cultivation on MS agar plates. The strain does not produce catalase or oxidase. The DNase, methyl red test, Voges-Proskauer reaction, and hydrolysis of tyrosine, casein, and Tween 80 are negative, but starch hydrolysis is positive. Nitrate is not reduced to nitrite. Using API 20E strips, indole production, acetoin production, and citrate utilisation are negative. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, gelatinase, and hydrogen sulphide are not produced. Growth occurs between 30 and 50°C, with optimum growth at 37°C and no growth at 55°C. The pH range for growth is 6.0 to 7.5,

with optimum growth at pH 7.0. There is no growth in the presence of 2% (w/v) NaCl. Using API 50CH strips, after an incubation time of 48 h, acid is produced from D-adonitol, aesculin, amygdaline, L-arabinose, D-cellobiose, erythritol, L-fucose, D-galactose, gentiobiose, D-glucose, inositol, D-lactose (bovine origin), D-maltose, D-mannose, D-melibiose, L-rhamnose, D-ribose, salicin, starch, methyl-β-D-xylopyranoside, and D-xylose. Acid is not produced from *N*-acetylglucosamine, D-arabinose, arbutin, D-arabitol, L-arabitol, dulcitol, D-fructose, D-fucose, glycerol, glycogen, inulin, D-lyxose, D-mannitol, D-melezitose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, D-raffinose, D-saccharose, D-sorbitol, L-sorbose, D-tagatose, D-trehalose, D-turanose, xylitol, or L-xylose. Xylanolytic-cellulolytic enzymes are actively produced, including xylanase, α-L-arabinofuranosidase, β-xylosidase, carboxymethyl cellulase, avicelase, β-glucosidase, β-galactosidase, and cellobiohydrolase. Using API 20NE strips, hydrolysis of aesculin is positive. Strain TW1^{T} assimilates D-glucose, D-maltose, L-arabinose, and D-mannose. However, the following are negative for assimilation: D-mannitol, *N*-acetylglucosamine, potassium gluconate, caprate, adipate, malate, trisodium citrate, and phenylacetate. The major cellular fatty acids (>10% of the total fatty acids) are anteiso-$C_{15:0}$, iso-$C_{16:0}$, and anteiso-$C_{17:0}$. The polar lipids identified are DPG, PE, PG, an unknown aminophospholipid and an unknown phospholipid. The cell wall peptidoglycan is *meso*-diaminopimelic acid. The major menaquinone is MK-7. The G+C content of DNA is 49.5 mol%.

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