Paenibacillus sacheonensis sp. nov., a xylanolytic and cellulolytic bacterium isolated from tidal flat sediment

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A Gram-negative-staining, rod-shaped bacterium, designated strain SY01^T, was isolated from tidal flat sediment from Sacheon Bay, South Korea. Strain SY01^T was characterized with respect to its phenotypic and phylogenetic characteristics. The novel strain was spore-forming, motile, catalase-negative and oxidase-positive. Optimal growth of the strain occurred at 30 °C and pH 7.0. The DNA G + C content was 56.1 mol%. The predominant menaquinone was MK-7. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and several unknown lipids were detected in the polar lipid profile. Anteiso-C_{15:0} (47.2%), iso-C_{15:0} (18.9%) and iso-C_{16:0} (10.5%) were the major cellular fatty acids of strain SY01^T. The highest 16S rRNA gene sequence similarities were found with *Paenibacillus phyllosphaerae* PALXIL04^T (95.9%), *Paenibacillus tarimensis* SA-7-6^T (94.6%) and *Paenibacillus mendelii* C/2^T (94.4%). Based on the phylogenetic, chemotaxonomic and physiological characteristics presented in this study, strain SY01^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus sacheonensis* sp. nov. is proposed. The type strain is SY01^T (=DSM 23054^T=KACC 14895^T).

Xylan is a heterogeneous and abundant polymer composed of (1,4)-linked β -D-xylosyl residues. It is the major component of the hemicelluloses of monocotyledon cell walls (Timell, 1967). Xylanases (1,4- β -D-xylanohydrolases) are a group of xylanolytic enzymes that hydrolyse xylan to xylan oligosaccharides and xylose. Several bacterial and fungal species produce the full complement of enzymes necessary to utilize xylan as a carbon source (Uffen, 1997). The genus *Paenibacillus* contains several species that are able to hydrolyse xylan (Akaracharanya *et al.*, 2009; Aý *et al.*, 1998; Beneduzi *et al.*, 2010; Hespell, 1996; Khianngam *et al.*, 2009a, b; Lee *et al.*, 2000; Lee & Yoon, 2008; Morales *et al.*, 1995; Nelson *et al.*, 2009; Nielsen & Sorensen, 1997; Park *et al.*, 2007, 2009; Rivas *et al.*, 2005a, b, 2006; Sánchez *et al.*, 2005; Ten *et al.*, 2006; Velázquez *et al.*, 2004; Valverde *et al.*, 2010; Yoon *et al.*, 2002; Zamost *et al.*, 1991). At the time of writing, the genus *Paenibacillus*, which was originally

proposed by Ash *et al.* (1993), contained 118 species with validly published names. Generally, members of this genus are facultatively anaerobic or strictly aerobic, rod-shaped bacteria that produce spores in swollen sporangia and have DNA G + C contents of 45–57 mol% (Ash *et al.*, 1993). In this paper, the isolation and classification of a novel species of the genus *Paenibacillus* is described.

A novel bacterium, designated strain SY01^T, was isolated from tidal flat sediment from Sacheon Bay, South Korea. The sediment sample was serially diluted with sterile water, spread on half-strength tryptic soy broth (TSB) agar (Difco) and incubated at 30 °C. Strain SY01^T was isolated after 7 days of incubation and cryopreserved at –80 °C in 10-fold-diluted TSB supplemented with 20% (v/v) glycerol. Cultures were maintained in 10-fold-diluted TSB agar.

Strain SY01^T was cultivated for 2 days at 30 °C in 10-fold-diluted TSB medium to determine the cell morphology by using a Zeiss light microscope at $\times 1000$ magnification. The presence of flagella was investigated using a transmission electron microscope (Tecnai G2 Spirit Twin; FEI). TEM images with scale bars were used to determine the cell size. Growth at 4, 10, 20, 28, 37, 40 and 50 °C and pH 5, 6, 7, 8, 9, 10 and 11 was tested on 10-fold-diluted TSB medium after

Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SY01^T is GU124597.

Supplementary figures are available with the online version of this paper.

7 days of incubation. The pH of the TSB was adjusted by adding 1 M NaOH or 1 M HCl. Salt tolerance was tested in 10-fold-diluted TSB supplemented with 0–5.0 % (w/v) NaCl at 1 % intervals after 7 days of incubation at 30 °C. For antibiotic susceptibility testing of the strain, the following antibiotic discs (Oxoid) were placed on 10-fold-diluted TSB agar plates spread with strain SY01^T: kanamycin (30 µg), streptomycin (10 µg), vancomycin (30 µg), gentamicin (10 µg), penicillin G (10 U), erythromycin (15 µg), ampicillin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg) and piperacillin/tazobactam 10:1 (110 µg).

Gram staining was carried out by using bioMérieux Gram staining kit according to the manufacturer's instructions. Spore formation was determined by staining with malachite green. Strain SY01^T was grown in 10-fold-diluted TSB for 48 h at 30 °C for menaquinone analyses and genomic DNA isolation. Isoprenoid quinones and the G + C content of the genomic DNA were analysed using reversed-phase HPLC according to the methods described by Komagata & Suzuki (1987) and Mesbah *et al.* (1989), respectively. For examination of the fatty acid composition, strain SY01^T was grown on 10-fold-diluted TSB agar at 30 °C for 48 h until the late exponential phase of growth. The analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI; Microbial ID). Polar lipid analyses were carried out by the Identification Service of the DSMZ and Dr B. J. Tindall (DSMZ, Braunschweig, Germany). Hydrolysis of casein, xylan, aesculin, gelatin, carboxymethylcellulose (CMC), starch, tyrosine, urea, Tween 20 and Tween 80 was tested as described by Lányi (1987) and Smibert & Krieg (1994).

Physiological and biochemical properties and enzyme activities were analysed using API 20NE, API 20E and API 50CH strips combined with API 50CHB/E medium, according to the manufacturer's instructions (bioMérieux). Oxidase activity was determined using 1 % (w/v) *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride (T3134; Sigma-Aldrich) and catalase activity was tested by the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution. The phenotypic characteristics of *Paenibacillus phyllosphaerae* PALXIL04^T, the closest neighbour of strain SY01^T according to 16S rRNA gene sequences, differed from those of strain SY01^T as regards nitrate reduction, catalase activity and carbon source utilization (Table 1). Strain SY01^T was Gram-negative-staining, catalase-negative and oxidase-positive. Strain SY01^T did not reduce nitrate to nitrite. Differential characteristics between strain SY01^T and closely related species of the genus *Paenibacillus* are shown in Table 1.

For 16S rRNA gene sequencing, extraction of genomic DNA was carried out using a commercial genomic DNA extraction kit (GeneAll). Amplification of the 16S rRNA gene was performed using the universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTACGACTT-3') (Lane, 1991). The PCR products were sequenced by Invitrogen Corporation,

Table 1. Distinctive characteristics of strain SY01^T and closely related species of the genus *Paenibacillus*

Strains: 1, SY01^T; 2, *P. phyllosphaerae* PALXIL04^T; 3, *P. tarimensis* SA-7-6^T; 4, *P. mendelii* C/2^T; 5, *P. kobensis* DSM 10249^T; 6, *P. humicus* PC-147^T. All data are from this study unless indicated otherwise. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4	5	6
Gram stain	–	–*	–*	–*	+	–
Oxidase	+	+	+	+	–	+
Catalase	–	+	+	+	+	+
Nitrate reduction	–	+	–	–	–*	–
DNA G + C content (mol%)†	56.1	50.7 ^a	53.7 ^b	50.8 ^c	50–52 ^d	58 ^e
Hydrolysis of:						
Xylan	+	+	+	–	–	+
Casein	–	w	+	–	–	+
CMC	+	+	+	–	–	+
Utilization of:						
Glycerol	w	w	–	+	w	–
D-Ribose	w	–	–	+	+	–
D-Xylose	w	+	+	+	–	–
Methyl β-D-xylopyranoside	–	+	w	+	–	–
D-Glucose	+	+	+	+	+	+
D-Fructose	+	+	–	+	+	–
D-Mannose	–	–*	+	–	+	–
L-Rhamnose	–	+	–	+	–	–
D-Mannitol	+	+	–*	–	–	+
N-Acetylglucosamine	w	+	–	–	+	w
Amygdalin	–	+	w	–	w	+
Arbutin	–	+	–	–	+	–
Salicin	–	+	+	+	+	–
Melezitose	+	–	–	+	–	+
Gluconate	–	–	–	–	+	–
5-Ketogluconate	–	–	–	+	–	–

*Results different from those in the studies by Rivas *et al.* (2005a), Wang *et al.* (2008), Šmerda *et al.* (2005), Kanzawa *et al.* (1995) and Vaz-Moreira *et al.* (2007).

†Data from other studies indicated as follows: a, Rivas *et al.* (2005a); b, Wang *et al.* (2008); c, Šmerda *et al.* (2005); d, Kanzawa *et al.* (1995); e, Vaz-Moreira *et al.* (2007).

where a 1405 bp fragment was obtained. Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were carried out by using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Phylogenetic trees were constructed by using three different methods, the neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms (Thompson *et al.*, 1994). The ML and MP programs were available in PHYLIP software, version 3.6 (Felsenstein, 2002), while the NJ algorithm was taken from the MEGA4 package (Tamura *et al.*, 2007). Evolutionary distance matrices were calculated using Kimura's two-parameter model (Kimura, 1980) for the NJ method. Phylogenetic

analysis based on the 16S rRNA gene sequences indicated that the strain SY01^T formed a distinct phyletic lineage within the genus *Paenibacillus* (Fig. 1). The topologies of phylogenetic trees based on the ML and MP algorithms also supported the result of the NJ tree (Saiton & Nei, 1987) and confirmed that strain SY01^T represented a novel member of the genus *Paenibacillus* (see Supplementary Figs S1 and S2 in IJSEM Online).

16S rRNA gene sequence comparisons showed that strain SY01^T was most closely related to *P. phyllosphaerae* PALXIL04^T (95.9%), *Paenibacillus tarimensis* SA-7-6^T (94.6%) and *Paenibacillus mendelii* C/2^T (94.4%). Sequence similarities to the type strains of other members of the genus *Paenibacillus* were below 94.0%. The predominant isoprenoid quinone detected in strain SY01^T was MK-7. The major fatty acid of strain SY01^T was anteiso-C_{15:0}, which is the predominant cellular fatty acid found in the genus *Paenibacillus* (Table 2) (Shida *et al.*, 1997a, b). The level of fatty acid anteiso-C_{15:0} in strain SY01^T was higher than that found for other type strains of the genus. Strain SY01^T showed higher levels of iso-C_{15:0} but lower levels of C_{16:0} when compared with *P. phyllosphaerae* PALXIL04^T. The major polar lipids present were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, four unknown aminophospholipids, five unknown phospholipids and an unknown phosphoglycolipid. The DNA G+C content of strain SY01^T was 56.1 mol%. This value was similar to that obtained from closely related species of the genus *Paenibacillus*.

On the basis of the phylogenetic, chemotaxonomic and physiological characteristics, strain SY01^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus sacheonensis* sp. nov. is proposed.

Description of *Paenibacillus sacheonensis* sp. nov.

Paenibacillus sacheonensis (sa.che.o.nen'sis. N.L. masc. adj. *sacheonensis* of or belonging to Sacheon, the city in Korea where the type strain was isolated).

Cells are Gram-negative-staining, motile and rod-shaped (0.8–1.0 µm wide and 2.8–3.8 µm long) (see Supplementary Fig. S2 in IJSEM Online). A spherical endospore is formed at a central to subterminal position. After 48 h incubation at 30 °C on 10-fold-diluted TSB agar plates, colonies are 1–2 mm in diameter, smooth, round, flat and translucent/white. The temperature range for growth is 10–40 °C and the optimum temperature for growth is 30 °C. The pH growth range is between 6.0 and 9.0, with an optimum at pH 7.0. Growth occurs in the absence of NaCl and no growth occurs in the presence of >1.0% (w/v) NaCl in 10-fold-diluted TSB medium. Xylan, aesculin, starch, gelatin and CMC are hydrolysed. Gives a positive result in tests for oxidase, but catalase is negative. Positive result in tests for β-galactosidase and acetoin production (weak), but negative result for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H₂S production, urease, tryptophan deaminase, indole production, glucose fermentation and nitrate reduction. No assimilation of caproate, adipate, citrate, phenylacetate or malate. Acid is produced from glycerol, L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannitol, N-acetylglucosamine, aesculin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, glycogen, gentiobiose and turanose, but not from erythritol, D-arabinose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside,

Fig. 1. The phylogenetic relationships of strain SY01^T within the genus *Paenibacillus* determined using 16S rRNA gene sequence analysis. The phylogenetic tree was constructed by using the NJ method and Kimura's two-parameter model as the substitution model. GenBank accession numbers are given in parentheses. Bootstrap values (expressed as percentage of 1000 replications) >50% are shown at the branch points. Bar, 1 substitution per 100 nt.

Table 2. Cellular fatty acid composition of strain SY01^T and phylogenetically related species of the genus *Paenibacillus*

Strains: 1, SY01^T; 2, *P. phyllosphaerae* PALXIL04^T; 3, *P. tarimensis* SA-7-6^T; 4, *P. mendelii* C/2^T; 5, *P. kobensis* DSM 10249^T; 6, *P. humicus* PC-147^T. All data are from this study. ND, Not detected.

Fatty acid	1	2	3	4	5	6
Saturated fatty acids						
C _{13:0}	ND	ND	ND	2.0	ND	ND
C _{14:0}	ND	2.9	1.1	ND	4.3	3.8
C _{15:0}	6.0	2.4	5.2	8.2	ND	4.7
C _{16:0}	3.1	22.3	7.8	8.6	26.2	4.7
C _{18:0}	ND	2.0	7.5	ND	ND	3.2
Branched fatty acids						
iso-C _{14:0}	3.6	4.6	ND	5.0	ND	1.9
iso-C _{15:0}	18.9	5.1	8.1	6.2	3.5	7.1
anteiso-C _{15:0}	47.2	34.6	43.0	42.8	38.2	40.3
iso-C _{16:0}	10.5	10.9	3.7	16.1	8.2	11.5
iso-C _{16:1} H	ND	ND	0.2	ND	ND	3.2
iso-C _{17:0}	4.0	1.9	2.1	ND	ND	ND
iso-C _{17:0} 3-OH	ND	ND	ND	ND	ND	1.7
anteiso-C _{17:0}	4.0	6.7	14.5	5.2	5.6	9.7
iso-C _{18:0}	ND	ND	2.1	ND	ND	ND
iso-C _{20:0}	ND	ND	ND	ND	ND	0.7
Unsaturated						
C _{14:1} ω5c	2.7	5.2	0.9	5.9	5.2	7.5
C _{16:1} ω7c alcohol	ND	1.4	ND	ND	ND	ND
C _{16:1} ω9c	ND	ND	ND	ND	3.6	ND
C _{16:1} ω11c	ND	ND	1.5	ND	ND	ND
C _{18:1} ω9c	ND	ND	ND	ND	5.3	ND
Summed feature 4*	ND	ND	2.3	ND	ND	ND

*Summed feature 4 represents iso-C_{17:1} I and/or anteiso-C_{17:1} B, which could not be separated by GLC using the MIDI system.

amygdalin, arbutin, salicin, inulin, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, gluconate, 2-keto-gluconate or 5-ketogluconate. Sensitive to kanamycin, streptomycin, vancomycin, gentamicin, penicillin G, erythromycin, ampicillin, chloramphenicol, tetracycline and piperacillin/tazobactam 10:1. The main cellular fatty acids are anteiso-C_{15:0} (47.2%), iso-C_{15:0} (18.9%), iso-C_{16:0} (10.5%). The major polar lipids present are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, four unknown aminophospholipids, five unknown phospholipids and an unknown phosphoglycolipid. The predominant menaquinone is MK-7.

The type strain, SY01^T (=DSM 23054^T=KACC 14895^T), was isolated from tidal flat sediment from Sacheon Bay in South Korea. The G+C content of the genomic DNA of the type strain is 56.1 mol%.

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