Paenibacillus thailandensis sp. nov. and *Paenibacillus nanensis* sp. nov., xylanase-producing bacteria isolated from soil

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Two strains of xylanase-producing bacteria, S3-4A^T and MX2-3^T, isolated from soils in Thailand, were characterized on the basis of their phenotypic and chemotaxonomic characteristics, DNA–DNA relatedness and 16S rRNA gene sequences. The novel strains were Gram-positive, facultatively anaerobic, spore-forming, rod-shaped bacteria. They contained *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The DNA G+C contents of strains S3-4A^T and MX2-3^T were 52.7 and 52.9 mol%, respectively. The major isoprenoid quinone was MK-7. The dominant cellular fatty acids were anteiso-C_{15:0} and iso-C_{16:0}. Phylogenetic analyses using 16S rRNA gene sequences showed that both novel strains were affiliated to the genus *Paenibacillus*. Strains S3-4A^T and MX2-3^T were closely related to *Paenibacillus agaridevorans* DSM 1355^T with 97% and 97.3% gene sequence similarities, respectively. The DNA–DNA relatedness between strains S3-4A^T, MX2-3^T and *P. agaridevorans* DSM 1355^T was low (6.0–30.3%). The novel strains could be clearly distinguished from *P. agaridevorans* DSM 1355^T by physiological and biochemical characteristics. Therefore, these two strains represent novel species of the genus *Paenibacillus*, for which the names *Paenibacillus thailandensis* sp. nov. (type strain S3-4A^T=KCTC 13043^T=PCU 275^T=TISTR 1827^T) and *Paenibacillus nanensis* sp. nov. (type strain MX2-3^T=KCTC 13044^T=PCU 276^T=TISTR 1828^T) are proposed.

The genus *Paenibacillus* proposed by Ash *et al.* (1994), was deemed to be a member of the family ‘*Paenibacillaceae*’. Species of the genus *Paenibacillus* have been found in such diverse environments as soils, cattle faeces, dead honeybees, honeybee larvae, plant roots, food, a warm spring, raw and heat-treated milk and in blood cultures (Berge *et al.*, 2002; Chou *et al.*, 2007; Roux & Raoult, 2004; Scheldeman *et al.*, 2004; Shida *et al.*, 1997; Velázquez *et al.*, 2004). Some of these bacteria have been found to excrete diverse assortments 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). Strains of *Paenibacillus barcinonensis*, *Paenibacillus favisporus*, *Paenibacillus phyllosphaerae*, *Paenibacillus xylanilyticus* and *Paenibacillus panacisoli* have been reported as displaying xylan-degrading properties (Velázquez *et al.*, 2004; Rivas *et al.*, 2005a, b; Sánchez *et al.*, 2005; Ten *et al.*, 2006). In this paper, we describe the novel xylanase-producing bacteria, isolated from soils in Thailand, based on their phenotypic, and chemotaxonomic characteristics, DNA–DNA relatedness and 16S rRNA gene sequence analyses.

Two novel strains of xylanase-producing bacteria were isolated from soil samples collected from Muang district, Nan province, Thailand, by the spread plate method on XC agar medium [containing (l⁻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; at pH 7.0]. In this screening step, the agar plates were incubated at 40 °C

Abbreviation: NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains S3-4A^T and MX2-3^T are AB265205 and AB265206, respectively.

Supplementary figures showing colonies of the novel strains on XC medium, scanning electron micrographs of cells showing endospores and two extended phylogenetic trees based on 16S rRNA gene sequences and constructed by the neighbour-joining and maximum-parsimony methods are available with the online version of this paper.

for 2 days. The xylanase-producing capacity of the cultures was detected by using a Congo red overlay method, as reported previously (Teather & Wood, 1982; Ruijsenaars & Hartmans, 2001). Isolates showing xylanase-producing capacity were transferred to C agar medium. This medium had the same composition of XC medium apart from the omission of the oat spelt xylan. Colonies grown on C agar medium were examined for their morphological and cultural characteristics, including cell shape, colony appearance, endospore formation and pigmentation, after incubation at 37 °C for 2 days. Flagella were stained by the method described by Forbes (1981). Tests for catalase, oxidase, hydrolysis of L-arginine, aesculin, casein, gelatin, starch and L-tyrosine, DNase, urease activity, nitrate reduction, citrate utilization, hydrogen disulphide production, dihydroxyacetone from glycerol and acid from carbohydrates and the methyl red/Voges-Proskauer (VP) reaction and indole test were determined as described by Barrow & Feltham (1993). Additional biochemical characteristics were recorded after 2 days incubation in API 50 CH strips (bioMérieux). Growth under anaerobic conditions on agar plates was investigated using a Gaspak (BBL) anaerobic jar. Growth at different pH values (5, 6, 6.5, 7, 7.5, 8, 8.5 and 9), at different temperatures (10, 15, 20, 30, 37, 45, 50, 55 and 60 °C) and in 3 and 5 % (w/v) NaCl were tested by using C medium. All tests were carried out by incubating the cultures at 37 °C, except for investigations into the effect of temperature on growth. The detection of the diaminopimelic acid in the cell wall and menaquinone were determined as described by Komagata & Suzuki (1987). For total cellular fatty acid analysis, cells were cultivated on TSA for 48 h at 30 °C using the standard Microbial Identification System (MIDI Inc.) for automated GC analysis (Sasser, 1990).

DNA was isolated from cells grown in C medium agar for 18 to 48 h and purified by the method of Saito & Miura (1963). DNA base composition was determined by reversed-phase HPLC (Tamaoka & Komagata, 1984). Photobiotin labelled DNA–DNA hybridization was determined in 2 × SSC and 50 % formamide solution at 45 °C for 15 h (Ezaki *et al.*, 1989). The 16S rRNA gene of the novel strains was amplified, purified and sequenced as described previously (Tanasupawat *et al.*, 2004). The sequences of strain S3-4A^T (1485 bp) and MX2-3^T (1460 bp) were 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. The phylogenetic tree was constructed by using the neighbour-joining (NJ) method (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) in MEGA4 software (Tamura *et al.*, 2007). The confidence values of branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Strains S3-4A^T and MX2-3^T were Gram-positive, spore-forming, rod-shaped bacteria. Colonies were yellowish white or white, circular, convex and had entire margins (1–3 mm in diameter). They exhibited a clear zone of xylanase

activity on XC medium (see Supplementary Fig. S1 in IJSEM Online). Central or subterminal ellipsoidal endospores were observed in swollen sporangia (see Supplementary Fig. S2 in IJSEM Online). Cells of both novel strains were motile by means of peritrichous flagella. Both strains were facultatively anaerobic. The phenotypic and chemotaxonomic characteristics are listed in the relevant species descriptions and in Tables 1 and 2.

In the 16S rRNA gene-based phylogenetic tree constructed according to the NJ method, strains S3-4A^T and MX2-3^T were placed in a monophyletic cluster consisting of the genus *Paenibacillus* as shown in Fig. 1. Extended trees constructed by the NJ method and the maximum-parsimony method showing the positions of strains S3-4A^T and MX2-3^T in relation to all recognized species of the genus *Paenibacillus* are available as Supplementary Figs S3 and S4, respectively, in IJSEM Online. Strains S3-4A^T and MX2-3^T showed 16S rRNA gene sequence similarities of 97 % to each other and were closely related to *Paenibacillus agaridevorans* DSM 1355^T with 97 % and 97.3 % similarities, respectively. The novel strains were related to *Paenibacillus granivorans* A30^T, *Paenibacillus glycanilyticus* DS-1^T, *Paenibacillus agarexedens* DSM 1327^T, *Paenibacillus alkaliterrae* DSM 17040^T and *Paenibacillus naphthalenovorans* DSM 14203^T with 92.8–96.4 % gene sequence similarities (Van der Maarel *et al.*, 2000; Dasman *et al.*, 2002; Uetanabaro *et al.*, 2003; Yoon *et al.*, 2005).

Strains S3-4A^T and MX2-3^T showed low DNA–DNA relatedness to *P. agaridevorans* KCTC 3849^T (6.0–30.3 %) and strain S3-4A^T showed low DNA–DNA relatedness to MX2-3^T (20.5 %). The DNA G + C contents of strains S3-4A^T and MX2-3^T were 52.7 and 52.9 mol%, respectively. Strains S3-4A^T and MX2-3^T contained anteiso-C_{15:0} (34.9–40.5 %) and iso-C_{16:0} (18.6–19.6 %) as the dominant cellular fatty acids. The two novel strains showed the same cellular fatty acid profiles as *P. agaridevorans* KCTC 3849^T, but significant quantitative differences were found between the strains (Table 2). In addition, the novel strains could be differentiated from the most closely related species, *P. agaridevorans* KCTC 3849^T, and other related *Paenibacillus* species by pigmentation, nitrate reduction, the VP reaction, growth at 50 °C, acid production from carbohydrates and DNA G + C content (mol%) (Table 1).

On the basis of their phenotypic and chemotaxonomic characteristics, DNA–DNA relatedness and phylogenetic analysis using 16S rRNA gene sequences, it is concluded that the two new isolates represent two novel species of the genus *Paenibacillus* (Ash *et al.*, 1994), for which the names *Paenibacillus thailandensis* sp. nov. and *Paenibacillus nanensis* sp. nov. are proposed.

Description of *Paenibacillus thailandensis* sp. nov.

Paenibacillus thailandensis (thai.lan.den'sis. N.L. masc. adj. *thailandensis* pertaining to Thailand, where the strain was isolated).

Table 1. Differential characteristics of the novel strains and related *Paenibacillus* species

Strains: 1, S3-4A^T; 2, MX2-3^T; 3, *P. agaridevorans* KCTC 3849^T; 4, *P. agarexedens* KCTC 3848^T; 5, *P. alkaliterrae* KCTC 3956^T; 6, *P. glycanilyticus* KCTC 3808^T. +, Positive; w, weakly positive; –, negative; yw, yellowish white; wh, white; py, pinkish yellow; i, ivory.

Characteristic	1	2	3	4	5	6
Colony pigment	YW	WH	WH	WH	I	PY
Nitrate reduction	–	–	+	–	–	–
VP reaction	+	+	–	–	–	–
Growth at 50 °C	+	–	–	–	–	–
Acid from:						
<i>N</i> -Acetylglucosamine	+	+	–	–	–	–
D-Adonitol	+	+	–	–	–	–
D-Arabinose	+	+	–	–	+	+
L-Arabinose	+	+	–	w	+	+
D-Arabitol	+	+	–	–	–	–
L-Arabitol	+	+	–	–	–	–
Arbutin	+	+	–	+	+	+
Dulcitol	+	+	–	–	–	–
Erythritol	+	+	–	–	–	–
D-Fructose	+	+	–	+	+	–
D-Fucose	+	+	–	–	–	–
L-Fucose	+	+	–	–	–	+
Gluconate	+	+	–	–	+	–
5-Ketogluconate	+	+	–	–	–	–
Glycerol	+	+	–	w	–	+
Glycogen	+	+	–	–	+	+
Inositol	+	+	–	–	–	+
Inulin	+	+	–	–	–	–
D-Lyxose	+	+	–	–	–	–
Maltose	+	–	+	–	+	+
D-Mannitol	+	–	–	+	–	+
D-Mannose	+	–	–	w	+	+
Methyl α -D-mannoside	+	+	–	–	–	–
L-Rhamnose	+	–	–	+	w	–
D-Ribose	+	+	–	w	–	+
D-Sorbitol	+	+	–	–	–	–
L-Sorbose	+	+	–	–	–	–
Starch	+	+	–	–	+	+
Sucrose	–	–	+	+	+	+
D-Tagatose	+	+	–	+	–	–
Trehalose	+	–	+	w	+	+
Xylitol	+	+	–	–	–	–
D-Xylose	+	+	–	w	+	+
L-Xylose	+	+	–	–	–	–
DNA G + C (mol%)	52.7	52.9	51*	47*	49.4†	50.5‡

*Data from Uetanabaro *et al.* (2003).

†Data from Yoon *et al.* (2005).

‡Data from Dasman *et al.* (2002).

Gram-positive, spore-forming rods, $0.8\text{--}1.2 \times 4\text{--}12\ \mu\text{m}$ that are motile by means of peritrichous flagella. Central or subterminal ellipsoidal endospores are observed in

Table 2. Cellular fatty acid content of strains

Values are percentages of total fatty acids. ND, Not detected; tr, <0.5.

Fatty acids	S3-4A ^T	MX2-3 ^T	KCTC 3849 ^T
Saturated fatty acids			
C _{12:0}	ND	tr	tr
C _{14:0}	0.5	0.5	0.9
C _{15:0}	tr	0.7	1.2
C _{16:0}	5.6	14.3	13.1
C _{16:0} N alcohol	tr	tr	ND
C _{17:0}	ND	0.5	0.5
C _{18:0}	tr	tr	1.7
Unsaturated fatty acids			
C _{14:1ω5c}	tr	tr	tr
C _{16:1ω7c} alcohol	ND	0.6	tr
C _{16:1ω11c}	ND	1.3	tr
C _{18:1ω7c}	ND	ND	tr
C _{18:1ω9c}	ND	ND	0.6
C _{18:3ω6c} (6, 9, 12)	ND	tr	ND
Branched fatty acids			
Anteiso-C _{11:0}	ND	tr	ND
iso-C _{14:0}	1.3	1.3	2.7
iso-C _{15:0}	7.4	4.1	5.4
anteiso-C _{15:0}	40.5	34.9	42.4
iso-C _{16:0}	18.6	19.6	17.7
iso-C _{17:1ω10c}	ND	0.5	ND
iso-C _{17:0}	6.1	7.8	5.6
iso-C _{17:0} 3-OH	ND	tr	ND
anteiso-C _{17:0}	18.3	12.2	6.5
iso-C _{18:0}	tr	tr	tr
iso-C _{19:0}	ND	ND	tr
Summed feature 4*	ND	0.5	ND
Summed feature 5†	tr	ND	ND

*Summed feature 4 contained iso-C_{17:1} I/anteiso-B.

†Summed feature 5 contained C_{18:2 ω 6}, 9c/anteiso-C_{18:0}.

swollen sporangia. Colonies are yellowish white, circular, convex and have entire margins (1–3 mm in diameter). Facultatively anaerobic. Growth over a pH range of 7–9 (optimal growth at pH 7.5), at 20–55 °C (optimal at 37 °C) and in 3% (w/v) NaCl. No growth at pH 5–6.5, at 10–15 °C or in 5% (w/v) NaCl. Positive in tests for catalase and oxidase activities, for hydrolysis of aesculin, starch and Tween 80, DNase activity and the VP reaction, but negative for the hydrolysis of L-arginine, casein, gelatin, for activities of L-tyrosine and urease, and nitrate reduction, the methyl red test, indole production, citrate utilization, hydrogen sulphide production and production of dihydroxyacetone from glycerol. Acid production from adonitol, aesculin, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fructose, D-fucose, L-fucose, D-galactose, gentiobiose, gluconate, 5-ketogluconate, D-glucose, *N*-acetylglucosamine, methyl α -D-glucoside, glycerol, glycogen, inositol, inulin, D-lactose, D-lyxose, maltose, D-mannitol, D-mannose, methyl α -D-mannoside, melezitose, melibiose, raffinose, L-rhamnose,

Fig. 1. Neighbour-joining tree of 16S rRNA gene sequences showing the phylogenetic relationships between strains S3-4A^T, MX2-3^T and selected members of the genus *Paenibacillus*. Bootstrap percentages (based on 1000 replications) above 54 % are shown at nodes. Bar, 0.01 substitutions per nucleotide position.

D-ribose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, trehalose, turanose, xylitol, D-xylose, L-xylose and methyl β -D-xyloside. No acid production from 2-ketogluconate or sucrose. MK-7 is the major isoprenoid quinone. The major cellular fatty acids are anteiso-C_{15:0} and iso-C_{16:0}. The diagnostic diamino acid in the cell-wall hydrolysates is *meso*-diaminopimelic acid.

The type strain, S3-4A^T (=KCTC 13043^T=PCU 275^T=TISTR 1827^T), was isolated from soil in Thailand. The DNA G + C content of the type strain is 52.7 mol%.

Description of *Paenibacillus nanensis* sp. nov.

Paenibacillus nanensis (nan.en'sis. N.L. masc. adj. *nanensis* pertaining to Nan, a province in Thailand, from where the strain was isolated).

Gram-positive, spore-forming rods, 1–1.2 × 3–8 µm that are motile by means of peritrichous flagella. Central or subterminal ellipsoidal endospores are observed in swollen sporangia. Colonies are white, circular, convex and have entire margins (1–3 mm in diameter). Facultatively anaerobic. Grows over a pH range of 6.5–9 (optimal growth at pH 7.5), at 20–45 °C (optimal at 37 °C) and in 3 % (w/v) NaCl. No growth at pH 5–6, at 10, 15 or 50–60 °C, or at 5 % (w/v) NaCl. Positive in tests for catalase, oxidase and urease activities, for hydrolysis of aesculin, starch and Tween 80, and for the VP reaction and nitrate reduction. Negative in tests for hydrolysis of L-arginine, casein, gelatin and L-tyrosine, for DNase activity, for the methyl red test, for production of hydrogen disulphide and indole, for citrate utilization and production of dihydroxyacetone from glycerol. Acid production from adonitol, aesculin, amygdalin, D-arabinose, L-arabinose, D-arabitol,

L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D-fructose, D-fucose, L-fucose, D-galactose, gentiobiose, gluconate, 5-ketogluconate, D-glucose, N-acetylglucosamine, methyl α -D-glucoside, glycerol, glycogen, inositol, inulin, D-lactose, D-lyxose, methyl α -D-mannoside, melezitose, melibiose, raffinose, D-ribose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, turanose, xylitol, D-xylose, L-xylose, and methyl β -D-xyloside. No acid production from 2-ketogluconate, maltose, D-mannitol, D-mannose, L-rhamnose, trehalose or sucrose. MK-7 is the major isoprenoid quinone. The major cellular fatty acids are anteiso-C_{15:0} and iso-C_{16:0}. The diagnostic diamino acid in cell-wall hydrolysates is *meso*-diaminopimelic acid.

The type strain, MX2-3^T (=KCTC 13044^T=PCU 276^T=TISTR 1828^T), was isolated from soil in Thailand. The DNA G + C content of the type strain is 52.9 mol%.

Acknowledgements

The Government Research Grant, Chulalongkorn University (2005), the scholarship from the Royal Golden Jubilee Ph. D. Program (2007) to S.K., and a grant from KRIBB Research Initiative Program are gratefully acknowledged.

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