Paenibacillus taiwanensis sp. nov., isolated from soil in Taiwan

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Among a large collection of Taiwanese soil isolates, a novel Gram-variable, rod-shaped, motile and endospore-forming bacterial strain, designated G-soil-2-3^T, was isolated from farmland soil in Wu-Feng, Taiwan. The isolate was subjected to a polyphasic study including 16S rRNA gene sequence analysis, DNA–DNA hybridization experiments, fatty acid analysis and comparative phenotypic characterization. 16S rRNA gene sequence analysis indicated that the organism belongs within the genus *Paenibacillus*. It contained menaquinone MK-7 as the predominant isoprenoid quinone and anteiso-C_{15:0} (40.5%), iso-C_{15:0} (13.1%), iso-C_{16:0} (10.8%) and anteiso-C_{17:0} (7.3%) as the major fatty acids. Phylogenetically, the closest relatives of strain G-soil-2-3^T were the type strains of *Paenibacillus assamensis*, *Paenibacillus alvei* and *Paenibacillus apiarius*, with 16S rRNA gene sequence similarity of 95.7, 95 and 95.2%, respectively. DNA–DNA hybridization experiments showed levels of relatedness of 2.8–9.0% of strain G-soil-2-3^T with these strains. The G + C content of the DNA was 44.6 mol%. Strain G-soil-2-3^T was clearly distinguishable from *P. assamensis*, *P. alvei* and *P. apiarius* and thus represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus taiwanensis* sp. nov. is proposed. The type strain is G-soil-2-3^T (=BCRC 17411^T = IAM 15414^T = LMG 23799^T = DSM 18679^T).

In a comparative analysis of 16S rRNA gene sequences of 51 species of the genus *Bacillus*, Ash *et al.* (1991) described five principal groups of strains, of which rRNA group 3 and group 4 bacilli of Ash, Farrow, Wallbanks and Collins have since been afforded generic status as *Paenibacillus* (Ash *et al.*, 1993) and *Brevibacillus* (Shida *et al.*, 1996), respectively. The genus *Paenibacillus* was considered to be a member of the family 'Paenibacillaceae', proposed to group several bacterial taxa previously attributed to the genus *Bacillus* (Garrrity & Holt, 2001). At the time of writing, the genus *Paenibacillus* comprised more than 70 recognized species, with *Paenibacillus polymyxa* as the type species. The description of the genus was emended by Shida *et al.* (1997a, b).

In the present study, the taxonomic position of a Taiwanese soil isolate, designated strain G-soil-2-3^T, was studied by using a polyphasic approach. Based on levels of 16S rRNA gene sequence similarity, DNA–DNA relatedness values,

fatty acid composition, phenotypic characterization and the generally accepted standards for describing novel species (Kämpfer *et al.*, 2003; Stackebrandt & Goebel, 1994; Vandamme *et al.*, 1996; Wayne *et al.*, 1987), the results of DNA–DNA hybridization and physiological and biochemical tests allowed the genotypic and phenotypic differentiation of strain G-soil-2-3^T from the phylogenetically most closely related *Paenibacillus* species.

Strain G-soil-2-3^T was isolated on Luria–Bertani agar (USB Corporation) from a sample of farmland soil from Taiwan. *Paenibacillus assamensis* JCM 13186^T, *Paenibacillus alvei* IAM 1258^T and *Paenibacillus apiarius* NRRL B-23460^T were received from culture collections. All strains were reactivated on trypticase soy agar (Difco) and checked for purity on nutrient agar (NA; Difco) after cultivation at 30 °C for 24–48 h. Strains were preserved at –80 °C in trypticase soy broth or by lyophilization.

Genomic DNA was extracted and purified by using the Qiagen Blood & Cell Culture DNA kit. 16S rRNA genes were amplified by PCR and sequenced by using the MicroSeq Full

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain G-soil-2-3^T (=BCRC 17411^T) is DQ890521.

Gene 16S rDNA Bacterial Sequence kit (PE Applied Biosystems). Sequencing was performed with an Applied Biosystems 310 DNA sequencer. Sequence assembly was performed by using the ABI PRISM DNA Sequencing Analysis software (PE Applied Biosystems) and phylogenetic analyses were performed via CLUSTAL X (Thompson *et al.*, 1997) for alignment and MEGA version 3.1 (Kumar *et al.*, 2004) for the neighbour-joining analysis. The 16S rRNA gene sequence of strain G-soil-2-3^T was aligned with those of *Paenibacillus* species retrieved from the NCBI database. Sequences of *Thermobacillus xylanilyticus* CNCM I-1017^T and *Bacillus subtilis* subsp. *subtilis* DSM 10^T were also included in the phylogenetic analysis for comparative purposes. Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Confidence values of branches on the neighbour-joining tree were determined by performing a bootstrap analysis with 1000 replicates.

DNA G + C content and levels of DNA–DNA relatedness were determined with genomic DNA prepared by using a commercial kit (Genomic-tips; Qiagen). The G + C content was determined via reversed-phase HPLC according to Tamaoka & Komagata (1984) with slight modifications. Nucleotides were separated by using a Cosmosil 5C₁₈ column (4.0 × 150 mm) (Waters) in a mobile phase composed of 0.2 M NH₄H₂PO₄/acetonitrile (20 : 1, v/v) at a flow rate of 1 ml min⁻¹ at room temperature. Nucleotides were detected and quantified by absorption at 260 nm. DNA–DNA relatedness values between the novel strain and the type strains of recognized *Paenibacillus* species were determined by using the fluorometric hybridization method in microdilution wells (Ezaki *et al.*, 1989; Tai *et al.*, 2006). The fluorescence intensity of each well was measured with a Fluoroskan II microplate fluorometer (LabSystems) at a wavelength of 360 nm for excitation and at 450 nm for emission. Hybridization temperature was 45 °C.

The fatty acid composition was determined with the Sherlock Microbial Identification System (MIDI Inc.) as described by Chern *et al.* (2004). Extracts of the methylated fatty acids were prepared according to the protocol provided by the manufacturer and analysed with an Agilent 6890N gas chromatograph equipped with a flame-ionization detector and 7683 Automatic Liquid Sampler. Identification of the peaks was made by comparing the results with the built-in TSBA 50 database (MIDI Inc.).

Respiratory quinones of the novel bacterium were identified according to Collins & Jones (1981) and Komagata & Suzuki (1987). The TLC-purified quinones were analysed with a Nova-Pak C₁₈ (15 × 3.9 cm) column (Waters). Peaks were detected at 270 nm after elution with methanol/2-propanol (2 : 1) at a flow rate of 1 ml min⁻¹.

All phenotypic tests were performed on bacteria grown on NA at 30 °C unless otherwise specified. General cell morphology was studied by using phase-contrast microscopy (Eclipse E600; Nikon). The Gram reaction was performed with a four-step Gram stain kit (Becton Dickinson) according to the manufacturer's instructions. Catalase activity, oxidase activity and oxidation–fermentation reactions were determined by using standard methods (Barrow & Feltham, 1993). Hydrolysis of starch and casein were determined as described by Cowan & Steel (1974). The silver-plating method was applied to stain the flagella (West *et al.*, 1977). Additional physiological and biochemical tests were performed with the API 50CHB and API 20E systems (bioMérieux) according to the manufacturer's instructions.

16S rRNA gene sequence analysis indicated that strain G-soil-2-3^T belongs within the genus *Paenibacillus* (Fig. 1). The levels of sequence similarity of 95.0–95.7 % between strain G-soil-2-3^T and the type strains of the phylogenetically most closely related species, *P. assamensis*, *P. alvei* and *P. apiarius* seem to exclude a possible relationship at the

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships among *Paenibacillus* species. Database accession numbers for the sequences used in the analysis are given in parentheses. Bootstrap percentages (based on 1000 replicates) are given at branching points. *Thermobacillus xylanilyticus* XE^T and *Bacillus subtilis* subsp. *subtilis* DSM 10^T were used as the outgroup to root the tree. Bar, 2 % nucleotide substitution rate.

species level with one of these taxa (Stackebrandt & Goebel, 1994; Vandamme *et al.*, 1996; Hagström *et al.*, 2000), and DNA–DNA relatedness values between strain G-soil-2-3^T and strains *P. assamensis* BCRC 17411^T, *P. alvei* DSM 29^T and *P. apiarius* DSM 5581^T were only 2.8–9.0 %. Therefore, the new isolate is considered to represent a novel species of the genus *Paenibacillus*.

The fatty acid profiles of strain G-soil-2-3^T and related taxa are presented in Table 1. Only minor differences were noted between strain G-soil-2-3^T and other *Paenibacillus* species. The dominant fatty acids for all strains investigated (mean level above 10 %) were anteiso-C_{15:0} (40.5 %), iso-C_{15:0} (13.1 %) and iso-C_{16:0} (10.8 %) (Table 1). Strain G-soil-2-3^T contained menaquinone MK-7 as the predominant isoprenoid quinone (97.1 %); this is in accordance with all other members of the genus *Paenibacillus*.

Phenotypic features that distinguish the novel species from phylogenetically related *Paenibacillus* species are listed in Table 2 and in the species description below.

Description of *Paenibacillus taiwanensis* sp. nov.

Paenibacillus taiwanensis (tai.wan.en'sis. N.L. masc. adj. *taiwanensis* of Taiwan, where the type strain was isolated).

Table 1. Cellular fatty acid compositions of strain G-soil-2-3^T and type strains of selected *Paenibacillus* species

Strains: 1, strain G-soil-2-3^T; 2, *P. assamensis* GPTSA 11^T (data from Saha *et al.*, 2005); 3, *P. apiarius* MTCC 1497^T (Saha *et al.*, 2005); 4, *P. alvei* IFO 3343^T (Shida *et al.*, 1997a). Values are percentages of total fatty acids. ND, Not detected; NM, not mentioned; tr, trace (<1 %).

Fatty acid	1	2	3	4
iso-C _{14:0}	2.47	4.74	tr	tr
C _{14:0}	2.42	1.80	1.15	2.3
iso-C _{15:0}	13.08	6.03	13.55	12.3
C _{15:0}	ND	ND	ND	1.6
anteiso-C _{15:0}	40.47	48.42	49.96	53.9
C _{16:1} ω7c alcohol	1.59	5.01	ND	NM
iso-C _{16:0}	10.78	11.59	6.52	2.3
C _{16:1} ω11c	4.25	6.16	2.18	1.3*
C _{16:0}	6.16	3.49	5.9	15.3
iso-C _{17:1} ω10c	2.75	1.18	1.35	tr*
iso-C _{17:0}	6.13	1.59	6.39	3.3
anteiso-C _{17:0}	7.30	5.68	8.80	3.3
C _{17:0}	tr	1.56	1.25	NM
Summed feature 4†	1.42	1.64	tr	NM

*The *cis/trans* isomeric nature of the fatty acid was not specified.

†Fatty acids that could not be separated by GC with the Microbial Identification System (Microbial ID) software were considered summed features. Summed feature 4 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B.

Cells are rod-shaped (2.5–6.8 × 0.7–0.9 μm), Gram-variable, facultatively anaerobic, mesophilic, capable of forming endospores and motile by means of peritrichous flagella. Colonies are flat with undulate margins and greyish white on NA. Grows at 10–45 °C (optimum 30 °C) on NA. Unable to grow below 5 °C or above 50 °C. The pH range for growth is 4.5–12 (optimum pH 6–8). Grows on NA containing 4 % NaCl (w/v). Positive for catalase and β-galactosidase but negative for oxidase, urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and gelatinase (API 20E). Indole, H₂S and acetoin are not produced. Hydrolyses starch and casein. Produces acid from ribose, D-glucose, glycerol, methyl α-D-glucoside, N-acetylglucosamine, amygdalin, aesculin, D-cellobiose, D-maltose, sucrose, D-trehalose, D-melezitose, gentiobiose, D-turanose and gluconate. No acid is produced from any other substrate in the API 50CH system. The major fatty acids are anteiso-C_{15:0} (40.5 %), iso-C_{15:0} (13.1 %), iso-C_{16:0} (10.8 %), anteiso-C_{17:0} (7.3 %), C_{16:0} (6.2 %) and iso-C_{17:0} (6.2 %). The predominant menaquinone is MK-7

Table 2. Differential phenotypic characteristics of strain G-soil-2-3^T and type strains of phylogenetically related *Paenibacillus* species

Strains: 1, strain G-soil-2-3^T; 2, *P. assamensis* GPTSA 11^T; 3, *P. apiarius* BCRC 17384^T; 4, *P. alvei* BCRC 11220^T. Data for *P. assamensis* GPTSA 11^T were taken from Saha *et al.* (2005); the remaining data were from this study. +, Positive; –, negative.

Characteristic	1	2	3	4
Growth at:				
10 °C	+	–	–	–
45 °C	+	–	–	+
pH 4.5	+	–	+	–
Production of indole	–	–	–	+
Acid production from:				
Glycerol	+	+	+	–
Adonitol	–	–	–	+
D-Galactose	–	–	+	–
D-Mannose	–	+	–	–
Inositol	–	–	+	–
Methyl α-D-glucoside	–	+	+	–
Amygdalin	+	–	+	–
Arbutin	–	–	+	+
Salicin	+	–	+	–
D-Cellobiose	+	–	+	–
D-Melibiose	–	+	+	+
Sucrose	+	–	+	–
D-Trehalose	+	–	+	–
D-Melezitose	+	–	+	–
D-Raffinose	–	–	+	+
Starch	–	+	+	+
Glycogen	–	+	+	–
Gluconate	+	–	+	–

(97.1 %). The DNA G + C content is 44.6 mol%. The species is phylogenetically most closely related to *P. assamensis*.

The type strain, G-soil-2-3^T (=BCRC 17411^T=IAM 15414^T=LMG 23799^T=DSM 18679^T), was isolated in 2000 from farmland soil in Wu-Feng, Taiwan.

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