Paenibacillus terrigena sp. nov., isolated from soil

Cheng-Hui Xie and Akira Yokota

Correspondence
Cheng-Hui Xie
cxie@uams.edu

Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi,
Bunkyo-Ku, Tokyo 113-0032, Japan

A novel Gram-positive bacterium, strain A35^T, was isolated from coastal soil at Chiba, Japan, and was identified as a member of the genus *Paenibacillus* on the basis of phenotypic and phylogenetic analyses. The bacterium was found to be a facultatively anaerobic and endospore-forming rod. The predominant menaquinone was MK-7, the major cellular fatty acid was anteiso-C_{15:0} and the DNA G + C content was 48.1 mol%. The levels of 16S rRNA gene sequence similarity between strain A35^T and *Paenibacillus* species with validly published names were less than 94 %. Strain A35^T was clearly distinguishable from reference species for the genus *Paenibacillus*. Therefore, on the basis of these data, a novel species of the genus *Paenibacillus*, *Paenibacillus terrigena* sp. nov., is proposed. The type strain is A35^T (= IAM 15291^T = CCTCC AB206026^T).

The genus *Paenibacillus* (Ash *et al.*, 1993), which comprises aerobic or facultatively anaerobic endospore-forming bacilli, is phenotypically related to other genera belonging to the family *Bacillaceae*. Phylogenetic analysis based on 16S rRNA gene sequences is the most useful method for identifying *Paenibacillus* species and differentiating them from other members of the family *Bacillaceae*. The genus currently contains 69 recognized species and the type species is *Paenibacillus polymyxa*. During the screening of nitrogen-fixing bacteria in a coastal soil sample from Chiba, Japan, a novel Gram-positive bacterium, designated strain A35^T, was isolated. Strain A35^T neither fixes nitrogen nor possesses the *nifH* gene (Xie & Yokota, 2006). On the basis of 16S rRNA gene sequence analysis, strain A35^T showed a very low level of similarity (92 %) to the type species of the genus *Paenibacillus*, *P. polymyxa*. The next most closely related species were *Paenibacillus koreensis* (GenBank accession no. AF130254), *Paenibacillus larvae* (X60619) and *Paenibacillus validus* (AB073203), which showed approximately 94 % 16S rRNA gene sequence similarity to strain A35^T. In comparisons with other recognized species of the genus *Paenibacillus*, the novel strain showed less than 94 % similarity. To elucidate the taxonomic position of strain A35^T, we used a polyphasic taxonomic approach that included physiological tests and analyses of the fatty acid content, quinone system, DNA G + C content and 16S rRNA gene sequence. On the basis of the phenotypic and phylogenetic analyses, it is proposed that strain A35^T represents a novel species of the genus *Paenibacillus*.

Strain A35^T was incubated in nutrient broth (NB; Difco) at 29 °C. Cell morphology and motility were examined by phase-contrast microscopy (BX60; Olympus). Growth

under anaerobic growth conditions was determined after 2 weeks incubation in an AnaeroPack (Mitsubishi Gas Chemical Co.). Gram staining was performed using 3 % KOH. Catalase activity was determined using 3 % H₂O₂ with the production of bubbles indicating a positive result. Oxidase was determined using cytochrome oxidase paper (Nissui Pharmaceutical Co.). API 20E and API 50 CH microtest galleries (bioMérieux) were used to determine physiological and biochemical characteristics. API strips were incubated for 2 days at 30 °C. Tolerance of salinity was determined by inoculating cells into NB supplemented with 0–4.0 % NaCl (w/v). For fatty acid analysis, cells were incubated on trypticase soy agar medium (Becton Dickinson) for 3 days at 29 °C. Cellular fatty acid methyl esters were prepared, separated and identified by using the Microbial Identification System (Microbial ID). Cells used to determine the respiratory quinone system and the DNA G + C content were incubated in NB for 5 days at 29 °C and then characterized by HPLC (Shimadzu) as described by Xie & Yokota (2003). Genomic DNA was isolated according to the method of Marmur (1961) using cells cultured for 2 days in NB. An acetylene-reduction assay and growth in nitrogen-free medium were used to determine nitrogenase activity (Xie & Yokota, 2006).

A fragment (approx. 1500 bp) of the 16S rRNA gene sequence was amplified by a PCR and sequenced as described by Xie & Yokota (2003). The 16S rRNA gene sequences obtained in this study and from GenBank were aligned using the CLUSTAL W software package (Thompson *et al.*, 1994), and the evolutionary distances and the *K*_{nuc} value (Kimura, 1980) were generated. Alignment gaps and ambiguous bases were excluded from the calculation. A phylogenetic tree based on a comparison of 1189 bases was constructed using the neighbour-joining method (Saitou & Nei, 1987). The topology of the tree was evaluated by using

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain A35^T is AB248087.

the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. Similarity values were calculated using PAUP, version 4.0b1 (Swofford, 1998).

The 16S rRNA gene sequence search using BLAST revealed that strain A35^T exhibited very low levels of similarity (not more than 94 %) with respect to the recognized species of *Paenibacillus*, which is sufficient to indicate that strain A35^T represents a novel member of the genus (Stackebrandt & Goebel, 1994). The phylogenetic tree (Fig. 1) showed that strain A35^T was affiliated to the genus *Paenibacillus* but was not closely related to any other species as poor bootstrap support (less than 60 %) was found at the branch node.

Chemotaxonomic analysis also supported the view that strain A35^T was a member of the genus *Paenibacillus* (Ash *et al.*, 1993). The presence of MK-7 as the major quinone system, the G+C DNA content (48.1 mol%) and the presence of anteiso-C_{15:0} as the major fatty acid (56.4–57.1 %) reflected the similarity of strain A35^T to all members of the genus *Paenibacillus* (Shida *et al.*, 1997). The fatty acid contents of the novel strain and the type strains of selected *Paenibacillus* species are shown in Table 1. Phenotypic differences between strain A35^T and the reference strains are shown in Table 2.

In summary, on the basis of the phylogenetic and phenotypic analyses, strain A35^T can be classified as representing a novel species of the genus *Paenibacillus*, for which we propose the name *Paenibacillus terrigena*.

Description of *Paenibacillus terrigena*

Paenibacillus terrigena [ter.ri.ge'na. L. n. *terra* soil; L. v. *gignere* to bear; L. n. *terrigena* (nominative in apposition) born of, or from, the earth, earth-born].

Cells are Gram-positive. Vegetative cells are motile rods; terminal, oval spores can be observed within swollen sporangia. Facultatively anaerobic. Grows at 4–32 °C, pH 4.0–10.0 and 3.5 % NaCl. Shows poor growth in carbohydrate-containing media (determined on O/F basal medium; Xie & Yokota, 2006), but grows well in Luria–Bertani and trypticase soy agar media, producing irregular, smooth, cream-coloured colonies. Catalase-, oxidase- and β -galactosidase-positive. Gives a positive result for nitrate reduction, aesculin hydrolysis and in the Voges–Proskauer test. Acid is produced from glucose, sucrose, melibiose and amygdalin. Glucose, glycerol, ribose, D-xylose, amygdalin, arbutin, methyl β -D-xyloside, galactose, methyl α -D-mannoside, methyl α -D-glucoside, aesculin, salicin, cellobiose,

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain A35^T within the genus *Paenibacillus*. *Bacillus subtilis* NCDO 1769^T was used as the outgroup. Numbers at the nodes indicate the percentages of occurrence in 1000 bootstrapped trees; only values greater than 50 % are shown. Bar, 0.02 substitutions per nucleotide position.

Table 1. Cellular fatty acids (%) of strain A35^T and some type strains in the genus *Paenibacillus*

Strains: 1, A35^T; 2, *P. polymyxa* DSM 36^T; 3, *P. larvae* NBRC 15408^T; 4, *P. validus* DSM 3037^T; 5, *Paenibacillus chondroitinus* DSM 5051^T. All of the data (except those for strain A35^T) are from Shida *et al.* (1997).

Fatty acid	1	2	3	4	5
C _{14:0}	0.8	0.4	0.6	0.7	0.9
C _{15:0}	3.8–4.1	0.3	1.3	1.4	2.0
C _{16:0}	4.0–4.4	9.3	6.4	10.8	6.4
C _{16:1ω11c}	1.0	–	0.8	0.4	–
anteiso-C _{13:0}	0.3	–	–	–	0
iso-C _{14:0}	2.6	0.5	0.2	1.4	1.7
iso-C _{15:0}	5.7–5.8	1.0	9.5	4.1	2.4
anteiso-C _{15:0}	56.4–57.1	62.9	48.8	57.3	69.7
iso-C _{16:0}	13.0	5.6	5.3	11.7	10.4
iso-C _{17:0}	1.9–2.1	1.6	4.5	3.4	0.7
anteiso-C _{17:0}	7.9–8.1	16.9	0.7	7.5	3.2

Table 2. Distinctive phenotypic properties of strain A35^T and reference strains of *Paenibacillus*

Strains: 1, *P. koreensis* YC300^T; 2, *P. polymyxa* ATCC 842^T; 3, *P. validus* DSM 3037^T; 4, *P. larvae* DSM 7030^T; 5, *Paenibacillus alginolyticus* DSM 5050^T; 6, A35^T. Data for reference species are from Chung *et al.* (2000) and Yoon *et al.* (2002). +, Positive; –, negative; V, variable reaction; W, weak reaction; ND, not determined.

Characteristic	1	2	3	4	5	6
Anaerobic growth	+	+	–	+	–	+
Catalase	+	–	+	–	+	+
Oxidase	+	+	+	–	–	+
Nitrate reduction	+	+	–	V	–	–
Hydrolysis of:						
Gelatin	ND	+	–	+	ND	–
Starch	+	+	ND	–	+	+
Urea	–	–	–	–	+	–
Acid produced from:						
L-Arabinose	+	+	+	–	+	–
D-Cellobiose	ND	+	V	–	+	–
D-Fructose	ND	+	+	V	+	–
Inulin	–	+	V	–	ND	–
Lactose	+	+	V	–	+	+
Maltose	+	+	+	–	+	W
D-Mannitol	+	+	+	+	+	–
D-Raffinose	+	–	–	–	ND	–
L-Rhamnose	–	+	+	–	+	–
Salicin	+	–	–	–	+	+
D-Sorbitol	+	–	–	–	–	+
D-Xylose	–	–	–	–	+	W
DNA G+C content (mol%)	54.0	43–46	50–52	42–43	47–49	48.1

lactose, melibiose, sucrose, raffinose, starch, gentiobiose, gluconate and D-turanose are assimilated. *N*-acetylglucosamine and maltose are poorly assimilated. Erythritol, arabinose, L-xylose, adonitol, fructose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, trehalose, inulin, melezitose, glycogen, xylitol, D-lyxose, tagatose, fucose, arabitol, caprate, adipate, malate, citrate, phenylacetate and 2-ketogluconate are not utilized. The major quinone system is MK-7. The DNA G+C content is 48.1 mol%. The fatty acids comprise anteiso-C_{15:0} (56.4–57.1 %), iso-C_{16:0} (13.0 %), anteiso-C_{17:0} (7.9–8.1 %), iso-C_{15:0} (5.7–5.8 %), C_{16:0} (4.0–4.4 %), C_{15:0} (3.8–4.1 %), iso-C_{14:0} (2.6 %), iso-C_{17:0} (1.9–2.1 %) and C_{14:0} (0.8 %).

The type strain, A35^T (= IAM 15291^T = CCTCC AB206026^T), was isolated from coastal soil from Chiba, Japan.

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