Paenibacillus aestuarii sp. nov., isolated from an estuarine wetland

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A novel bacterial strain designated CJ25^T was isolated from the estuarine wetland of the Han river in Korea. Identification of this strain was carried out on the basis of polyphasic taxonomy. The isolate was Gram-staining-positive, rod-shaped, non-pigmented and motile. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that the isolate was closely related to *Paenibacillus chondroitinus* DSM 5051^T with 96.1 % similarity. The predominant fatty acids were anteiso-$C_{15:0}$ (50.25 %), iso-$C_{16:0}$ (18.54 %) and iso-$C_{15:0}$ (10.00 %). The major isoprenoid quinone was MK-7. The G+C content of genomic DNA was 50 mol%. According to physiological data and 16S rRNA gene sequence analysis, the isolate was discriminated from related members of the genus *Paenibacillus*. Therefore, strain CJ25^T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus aestuarii* sp. nov. is proposed. The type strain is CJ25^T (=KACC 13125^T =JCM 15521^T).

The genus *Paenibacillus* is a diverse group of bacteria that are commonly found in soil and estuarine wetlands and comprises 99 species with validly published names at the time of writing (DSMZ, http://www.dsmz.de/). The estuarine wetlands of the Han river have been regarded as contaminated environments where large amounts of industrial wastewater and sewage accumulate in the river (Li *et al.*, 2004). As microbial diversity in this environment is expected to be exceptionally high, we tried to isolate bacteria capable of degrading hazardous pollutants such as polycyclic aromatic hydrocarbons. During the enrichment culture, a *Paenibacillus*-like bacterium was isolated and characterized. In this study, we report the description of a novel species of the genus *Paenibacillus*, which showed low 16S rRNA gene sequence similarity to all recognized species of this genus.

A bacterial strain designated CJ25^T was isolated from the estuarine wetland of the Han river, South Korea. Enrichment culture was performed using pyrene as a sole carbon source in a minimal medium that contained: 0.5 g $NaNO_3$, 1.0 g $(NH_4)_2SO_4$, 2.5 g KH_2PO_4, 1.0 g Na_2HPO_4, 0.03 g $CaCl_2$, 0.02 g $MgCl_2$ and 1 ml trace element solution (containing 1.94 g $Al_2(SO_4)_3.18H_2O$, 1.0 g $CoSO_4.7H_2O$, 1.0 g $CuSO_4.5H_2O$, 54 g $FeSO_4.7H_2O$, 11.0 g H_3BO_3, 0.5 g KBr, 0.5 g LiCl, 1.0 g KI, 7.0 g $MnCl_2.4H_2O$, 0.5 g $Na_2MoO_4.2H_2O$, 0.5 g $Na_2WO_4.2H_2O$, 1.0 g $NiCl_2.6H_2O$, 0.5 g $SnCl_2.2H_2O$ and 0.62 g $ZnSO_4.H_2O$ in a total volume of 18 l) per litre distilled water. During cultivation, spreading and selection of colonies were carried out on R2A agar (Difco). The isolate was routinely cultured in R2A broth at 30 °C and maintained as a glycerol suspension (30 %, w/v) at −80 °C. Anaerobic atmosphere was prepared by using a BD GasPak pouch (Becton Dickinson) for anaerobic growth. Growth was tested at different temperatures (4–50 °C) and pH (2.0–10.0 at 1 pH unit intervals, adjusted by adding HCl or NaOH; Takeda *et al.*, 2005). Growth was generally monitored by measuring optical density at 600 nm. Motility was tested on semisolid R2A media containing 0.4 % agar (Smibert & Krieg, 1994). Growth occurred at 20–37 °C and at pH 5–10. Optimal growth temperature and pH ranges of the strain were 30–37 °C and pH 6–8, respectively. Growth occurred in the presence of 0–10 % NaCl with an optimum of 0–1 %. Although the strain was isolated during enrichment culture for pyrene-degrading bacteria, degradation of pyrene was not observed. Additional growth properties of strain CJ25^T are summarized in the species description.

The 16S rRNA gene was amplified by PCR using AccuPower PCR Premix (Bioneer) that contained DNA polymerase, dNTPs, Tris/HCl (pH 9.0), KCl, $MgCl_2$, tracking dye and primers pBact27F (5′-AGAGTTTGA-TCMTGGCTCAG-3′) and pUniv 1492R (5′-GGYTA-

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CJ25^T is EU570250.

An extended phylogenetic tree is available with the online version of this paper.

CCTTGTTACGACTT-3′) (Lane, 1991). Purification of the PCR product from agarose gel was performed using a Rapid Gel Extraction kit (TAKARA). Sequencing of the 16S rRNA gene was performed at Solgent corp. (Daejeon, South Korea). The results of 16S rRNA gene sequencing were analysed using the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007). Phylogenetic analyses were performed by using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. Evolutionary distance matrices for the neighbour-joining method were generated according to the model of Jukes & Cantor (1969). The neighbour-joining tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. The phylogenetic tree was reconstructed using the MEGA4 (Tamura *et al.*, 2007) and PHYLIP (Felsenstein, 2005) programs. According to 16S rRNA gene sequence analyses, isolate CJ25^T showed the highest 16S rRNA gene sequence similarity with *Paenibacillus chondroitinus* DSM 5051^T (96.2 %), followed by *Paenibacillus larvae* subsp. *larvae* DSM 7030^T (95.6 %), *Paenibacillus chitinolyticus* IFO 15660^T (95.5 %) and *Paenibacillus alginolyticus* DSM 5050^T (95.5 %). In the phylogenetic tree, strain CJ25^T represented a distinct phyletic line corresponding to a novel species in the genus *Paenibacillus* (Fig. 1). For an extended phylogenetic tree, see Supplementary Fig. S1 in IJSEM Online.

Catalase activity was evaluated by assessing the production of O_2 bubbles in a 3 % (v/v) aqueous H_2O_2 solution (Lim *et al.*, 2006). Oxidase activity was tested by assessing the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck). Citrate utilization was tested by using Simmons' citrate agar. Hydrolysis of casein was tested as described by Smibert & Krieg (1994). Starch hydrolysis was tested using R2A with iodine. Carbon source utilization was determined by using the API ZYM, API 50CHB and API 20NE galleries according to the instructions of the manufacturer (bioMérieux). Biochemical and physiological properties are presented in Table 1 and in the species description.

Chemotaxonomic characteristics were determined from cells grown on R2A agar at 30 °C for 2 days. Isoprenoid quinones were extracted and purified as described by Minnikin *et al.* (1984) and preparations dried under nitrogen gas were dissolved in 200 µl acetone; samples were analysed by HPLC (Varian) as described by Collins (1985) using a SYNERGI 4µ Hydro-RP column (150 × 4.60 mm). Strain CJ25^T contained menaquinone 7 as the major quinone. Genomic DNA for G+C content analysis was isolated using a Wizard genomic DNA purification kit (Promega). DNA G+C content was determined by the method of Mesbah *et al.* (1989) and analysed by HPLC (Varian) using a Supelcosil LC-18-S column. Experiments were performed in triplicate and the calculated G+C content was 50 mol%. Fatty acid methyl ester analysis was carried out by GLC according to the instructions of the Microbial Identification system (MIDI; Microbial ID). The fatty acid profiles of strain CJ25^T and related taxa are presented in Table 2. The major fatty acids for strain CJ25^T were anteiso-$C_{15:0}$ (50.25 %) and iso-$C_{16:0}$ (18.54 %), which was consistent with type strains of closely related species. Unlike the reference strains compared in Table 2, iso-$C_{15:0}$ (10.00 %) was relatively high and unsaturated fatty acids were not detected in strain CJ25^T.

As shown by the 16S rRNA gene sequence analysis, strain CJ25^T formed an independent phyletic line among recognized species of the genus *Paenibacillus*. Data from the polyphasic taxonomy study also differentiated the strain from related members of this genus. Therefore, we propose that strain CJ25^T be classified as the type strain of a novel species in the genus *Paenibacillus*, with the name *Paenibacillus aestuarii* sp. nov.

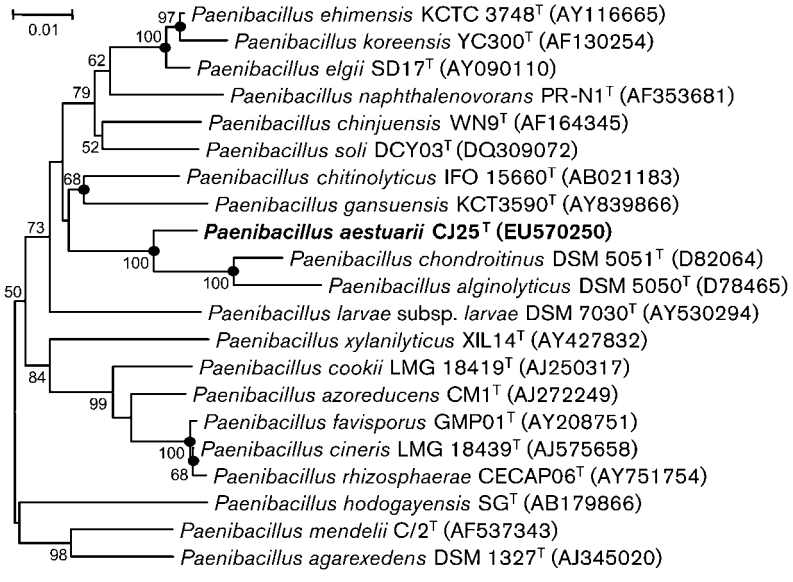

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain CJ25^T and other related species of the genus *Paenibacillus*. Bootstrap values greater than 50 % are shown at branch points based on neighbour-joining analyses of 1000 resampled datasets. Solid circles indicate that the corresponding nodes are also recovered in trees generated by the maximum-likelihood and maximum-parsimony algorithms. The tree is rooted using *Bacillus subtilis* as an outgroup. Bar, 0.01 nucleotide substitutions per position.

Table 1. Differential characteristics of strain CJ25^T and related members of the genus *Paenibacillus*.

Strains: 1, *Paenibacillus aestuarii* sp. nov. CJ25^T; 2, *P. chondroitinus* DSM 5051^T; 3, *P. chitinolyticus* IFO 15660^T; 4, *P. gansuensis* KCTC 3950^T; 5, *P. alginolyticus* CIP 103122^T. DNA G+C content data are from Nakamura (1987), Kuroshima *et al.* (1996) and Lim *et al.* (2006). All strains are motile with swollen sporangia and are negative for oxidase, nitrate reduction, indole production and hydrolysis of gelatin. +, Positive; −, negative.

Characteristic	1	2	3	4	5
Anaerobic growth	−	+	+	−	+
Optimum growth temp. (°C)	30–37	30	37	30–37	30
Catalase	−	+	+	−	+
Urease	−	+	−	−	−
Citrate utilization	−	−	−	−	+
Acid production from:					
L-Arabinose	+	+	−	+	+
D-Mannitol	+	+	−	−	+
D-Xylose	+	+	−	+	+
Lactose	+	+	−	+	−
Maltose	+	+	+	+	−
D-Mannose	−	+	+	+	−
Hydrolysis of:					
Starch	+	+	−	+	+
Casein	−	−	+	+	−
DNA G+C content (mol%)	50	47–48	51–53	50	47–49

Description of *Paenibacillus aestuarii* sp. nov.

Paenibacillus aestuarii (aes.tu.a′ri.i. L. gen. n. *aestuarii* of a wetland, from where the type strain was isolated)

Cells are Gram-staining-positive, rod-shaped and motile. Growth occurs only under aerobic conditions. Colonies on R2A agar are circular and non-pigmented. Ellipsoidal spores are produced in swollen sporangia. Optimal growth at 30–37 °C and at pH 6–8. Catalase- and oxidase-negative. Nitrate is not reduced to nitrite. Citrate is not utilized. Indole is not produced. Starch is hydrolysed but gelatin and casein are not hydrolysed. β-Galactosidase, esterase, esterase lipase, leucine arylamidase, cystine arylamidase and naphthol-AS-BI-phosphohydrolase activities are present but urease, arginine dihydrolase, alkaline phosphatase, lipase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Acid is produced from the following substrates: L-arabinose, D-xylose, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-mannitol, methyl α-D-glucopyranoside, amygdalin, aesculin, salicin, cellobiose, D-maltose, lactose, melibiose, D-sucrose, D-trehalose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose and 5-keto-gluconate. Acid is not produced from the following substrates: glycerol, erythritol, D-arabinose, D-ribose, L-xylose, D-adonitol, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, *N*-acetylglucosamine, arbutin, inulin, D-melezitose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate or 2-keto-gluconate. The major quinone is MK-7. The predominant fatty acids are anteiso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{15:0}$. The DNA G+C content of the type strain is 50 mol%.

The type strain is CJ25^T (=KACC 13125^T =JCM 15521^T), isolated from the sediment of the estuarine wetland of the Han river, South Korea.

Table 2. Cellular fatty acid profiles of strain CJ25^T and type strains of related species of the genus *Paenibacillus*

Strains: 1, *Paenibacillus aestuarii* sp. nov. CJ25^T; 2, *P. chondroitinus* DSM 5051^T; 3, *P. chitinolyticus* IFO 15660^T; 4, *P. gansuensis* KCTC 3950^T; 5, *P. alginolyticus* CIP 103122^T. Values are percentages of total fatty acids. −, Not detected.

Fatty acid	1	2	3	4	5
Saturated fatty acids					
$C_{14:0}$	1.05	1.09	2.15	0.95	0.75
$C_{15:0}$	0.56	2.70	−	−	−
$C_{16:0}$	6.29	5.22	9.18	5.32	4.39
$C_{17:0}$	−	−	−	0.44	0.10
$C_{18:0}$	0.24	0.22	0.33	0.30	−
Unsaturated fatty acids					
$C_{16:1}\omega7c$ alcohol	−	−	0.39	0.49	0.19
$C_{16:1}\omega11c$	−	−	2.68	1.49	0.22
$C_{18:1}\omega9c$	−	0.32	0.27	0.34	0.21
Branched fatty acids					
anteiso-$C_{13:0}$	−	−	0.26	−	−
iso-$C_{14:0}$	7.77	4.94	1.56	2.80	2.25
iso-$C_{15:0}$	10.00	2.44	3.50	2.90	3.11
anteiso-$C_{15:0}$	50.25	66.22	65.42	67.62	69.65
iso-$C_{16:0}$	18.54	14.98	5.09	10.13	12.2
iso-$C_{17:0}$	1.65	0.48	1.33	0.76	0.73
anteiso-$C_{17:0}$	3.19	3.53	6.67	5.63	5.06
iso-$C_{17:1}\omega10c$	−	−	0.15	−	−

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