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Cohnella damensis sp. nov., a Motile Xylanolytic Bacteria Isolated from a Low Altitude Area in Tibet

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A bacterial strain, 13-25^T with xylanolytic activity isolated from a single soil sample, was characterized with respect to its phenetic and phylogenetic characteristics. The cells of the isolate are Gram-staining variable rods, but spore formation was not observed. This strain is catalase- and oxidase-positive, and able to degrade starch and xylan. The predominant fatty acids are anteiso-$C_{15:0}$, $C_{16:0}$, and iso-$C_{16:0}$. The major respiratory quinone is menaquinone 7(MK-7), with a polar lipid profile consistent with the genus *Cohnella*. The DNA G+C content is 54.3 mol%. The 16S rRNA gene sequence analysis indicates that this organism belongs to the genus *Cohnella*, with *Cohnella panacarvi* as the closest phylogenetic neighbor. Low levels of 16S rRNA gene sequence similarity (<97.0%) with respect to other taxa with published names and the identification of distinctive phenetic features in the isolate indicate that the strain 13-25^T represents a novel species of the genus *Cohnella*, for which the name *Cohnella damensis* sp. nov. is proposed. The type strain is 13-25^T (=CCTCC AB 208103^T=KCTC 13422^T).

Keywords: *Cohnella damensis* sp. nov., motility, xylanolytic

The genus *Cohnella* was first proposed by Kämpfer [5] and species in this genus can be easily separated from the genus *Paenibacillus* by phylogenic analysis, forming a distinct group outside the genus *Paenibacillus*. In addition, the presence of several unknown aminophospholipids can well differentiate them from the type species *Paenibacillus polymyxa* of the genus *Paenibacillus*. At the time of writing, five species have been described previously, namely *Cohnella thermotolerans* (type species), *Cohnella hongkongensis* isolated from a patient with neutropenic fever [5, 12], *Cohnella laeviribosi* with ability to produce a novel thermophilic D-lyxose isomerase [3], the xylanolytic bacterium *Cohnella panacarvi* that has been effectively published [14], and *Cohnella phaseoli* isolated from root nodules of *Phaseolus coccineus* [4], which indicated species in this genus can be retrieved from various habitats. In the present study, a single strain with xylanolytic activity from Tibet was isolated from a soil sample collected from an 800-m altitude area.

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Materials and Methods

Bacteria Strains

Strain 13-25^T was isolated on 0.2-fold marine agar. Subcultivation was done on tryptone soybean broth (TSB; Difco) at 30°C for 24–72 h. The nearest two type strains DSM 17683^T and KCTC 13060^T were obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, German Collection of Microorganisms and Cell Cultures) and KCTC (Korean Collection for Type Cultures).

Morphological, Physiological, and Biochemical Characteristics

The cell morphology of 13-25^T was examined by light microscopy and the motility was observed with an optical microscope (Olympus; BX51) using the hanging-drop technique and silver staining method [1, 10]. To examine flagellum type, the cells were gently suspended in sterile water and then stained with 0.2% uranyl acetate before being examined by transmission electron microscopy.

Growth at different temperatures was observed in tryptic soybean agar (TSA; Difco) at 10, 20, 30, 37, 40, 43, and 45°C. Growth was assessed by monitoring the optical density at 600 nm of bacteria in 5 ml of tryptic soy broth, adjusted to pH 4.0–10.0 using 100 mM citric acid/200 mM Na_2HPO_4 buffer at pH 4.0–5.0, 100 mM Na_2HPO_4/NaH_2PO_4 buffer at pH 6.0–8.0, or 100 mM glycine/NaOH buffer at pH 9.0–10.0, based on a previously described method [15]. The ability of the isolate to grow in different NaCl concentrations (0, 0.5, 1, 2, 5, 10%) was also tested with TSB (Difco) as the basal medium at pH 6.5 and 30°C.

Table 1. Differential characteristics of *Cohnella damensis* sp. nov. and the other members of the genus *Cohnella.*

Characteristic	1	2	3.	4	5	6
Oxidase	+	+	+	-	+	w
Voges–Proskauer test	+	-	+	ND	-	+
Temperature range (°C)	16–40	18–45	20–55	37–52	37–65	10–45
Flagella	Peritrichous	-	-	-	-	Peritrichous
Acid from						
Glycerol	w	-	-	+	-	(-)
D-Arabinose	-	-	w	+	-	+
L-Arabinose	+	+	+	+	-	+
D-Ribose	-	-	(-)	+	-	+
D-Xylose	+	+	+	+	-	+
Adonitol	-	-	(-)	+	-	-
Methyl-β-D-xyloside	+	-	-	+	-	+
Galactose	w	+	+	+	-	-
D-Glucose	+	+	+	+	-	+
Fructose	-	+	+	+	-	+
D-Mannose	+	+	+	+	-	+
Sorbose	-	-	w	-	-	-
Rhamnose	w	+	w	+	-	-
Methyl-α-D-glucoside	+	+	w	+	-	+
Amygdalin	+	+	w	+	-	+
Arbutin	+	-	(-)	-	-	+
D-Cellobiose	+	+	+	+	-	+
Maltose	+	+	+	+	-	+
Lactose	+	+	+	+	-	+
Melibiose	+	+	+	+	-	+
Sucrose	(-)	+	+	+	-	+
Trehalose	+	+	+	+	-	+
Raffinose	+	w	+	+	-	+
Starch	+	-	+	+	-	+
Glycogen	+	-	+	+	-	+
Xylitol	-	-	-	+	-	-
Gentiobiose	+	-	+	-	-	+
Turanose	+	+	+	+	-	+
D-Lyxose	+	-	(-)	+	-	-
L-Fucose	+	-	(-)	+	-	+
D-Arabitol	-	-	(-)	+	-	-
L-Arabitol	-	-	-	+	-	-
Salicin	+	-	+	-	-	+
N-Acetyl-glucosamine	(-)	-	w	-	-	+
Assimilation						
D-Mannitol	-	-	+	+	+	-
N-Acetyl-glucosamine	-	-	-	-	w	+
Gluconate	-	-	-	-	+	+
Malate	-	-	-	-	-	+
DNA G+C content (mol%)	54.3	53.4	59	51	47.6	60.3

Strains: 1, strain 13-25^T; 2, *Cohnella panacarvi* KCTC 13060^T ; 3, *C. thermotolerans* DSM 17683^T this study; 4, *C. laeviribosi* RI-39^T [3]; 5, *C. hongkongensis* HKU3^T [5, 12]; 6, *C. phaseoli* GSPC1^T [4]. All strains were negative for anaerobic growth, assimilation of caprate, adipitate, malate, and phenylacetate, and acid production from L-xylose, sorbose, methyl-α-D-mannoside, erythritol, inositol, 2-ketogluconate, D-tagatose, gluconate, D-fucose, and glucitol; and positive for catalase assimilation of glucose, L-arabinose, D-maltose, and D-mannose. +, Positive; -, negative; (-), no clear negative in repetitive experiment; w, positive or weakly positive in repetitive experiment; ND, not determined.

Other physiological and biochemical characterizations were performed using the API 20NE and API 20E strips and 50CH strips combined with API 50CHB/E medium (bioMérieux), in accordance with the manufacturer's directions. Hydrolysis of gelatin, casein, starch, aesculin, and xylan, production of catalase, oxidase, and urease, and the methyl red test were examined according to the standardized methods [10].

Determination of Cellular Fatty Acids, DNA G+C Content, and Polar Lipids

Fatty acids were extracted and analyzed according to the instructions of the Microbial Identification System (MIDI; Microbial ID) after the strains were cultivated on R2A (Difico) agar for 48 h. The DNA G+C content of this strain was determined by HPLC (UltiMate 3000, Dionex) [9], and the respiratory quinone was extracted and determined by HPLC as described by Xie and Yokota [13]. The polar lipids were extracted from dried cells using methanol/chloroform [2:1 (v/v)] and the total polar lipid fraction was collected by evaporation. Total polar lipids were subjected to TLC (10×10 cm, 0.5 mm silica gel 60 glass plates; Merck). Solvent systems were used as previously described [6].

PCR Amplification, 16S rRNA Gene Sequencing, and Phylogenetic Analysis

Genomic DNA was isolated by a bacteria genomic kit (CASarray Co., Ltd). The fragments comprising 16S rRNA gene were amplified by PCR using universal primers described by Lane [8]. The PCR products were sequenced by Invitrogen Corporation. The identification of phylogenetic neighbors and calculation of 16S rRNA gene sequence similarity were achieved using NCBI BLAST and the EzTaxon server [2]. Phylogenetic trees were constructed using the neighbor-joining method, and maximum likelihood method of the Kimura-2 parameter model of MEGA version 3.1 [7]. The topology of the phylogenetic tree was evaluated by using the bootstrap resampling method with 1,000 replicates.

Results and Discussion

Morphological, Physiological, and Biochemical Characteristics

The strain was found to be Gram-staining-variable, rod-shaped, and motile by means of peritrichous flagella (Supplementary Fig. S1). No spore formation was observed at 30°C. The colonies were white, mucoid, translucent, and convex on this medium. Phenotypic characterizations were listed in the species description and shown in Table 1.

Chemotaxonomy analysis indicated this strain contained MK-7 as the predominant menaquinone. Anteiso-$C_{15:0}$, iso-$C_{16:0}$, and $C_{16:0}$ were detected as major fatty acids. Limited quantitative differences of fatty acids composition can be observed among its closely related species (Table 2).

The polar lipids profile of the novel strain showed that it possesses diphosphatidylglycerol, phosphatidylglycerol, and diphosphatidylethanolamine as the predominant phospholipids that are typical in bacilli, as well as a number of phospholipids and aminophospholipids that are not present in the type species of the genera *Paenibacillus*, and *Bacillus*. In addition, a few unknown phospholipids and aminophospholipids can differentiate between the novel strain and other species in *Cohnella* (Supplementary Fig. S1).

Phylogenetic Analysis Based on 16S rRNA Gene Sequences

16S rRNA gene analysis showed that the sequence of this strain shared the highest sequence similarity with *C. panacarvi* (96.9%). The phylogenetic tree indicated that strain 13-25^T with *C. panacarvi* as its closest species belonged to the genus *Cohnella* (Fig. 1). The topologies of the phylogenetic tree generated using maximum-parsimony algorithms (data not shown) was similar to that of the tree constructed by neighbor-joining analysis.

Table 2. Cellular fatty acid composition of *Cohnella damensis* and all type strains of the genus *Cohnella*.

Fatty acid	1	2	3	4	5	6
Saturated acids:						
Straight-chain:						
$C_{14:0}$	-	4.7	-	1.9	5.0	1.8
$C_{15:0}$	3.3	-	-	1.3	8.0	5.3
$C_{16:0}$	12.5	13.9	5.1	9.2	25.3	8.9
$C_{18:0}$	6.6	6.8	-	-	-	-
Iso-branched:						
$C_{14:0}$	8.2	1.4	3.0	3.9	2.3	2.6
$C_{15:0}$	2.9	4.3	10.4	11.7	8.1	14.3
$C_{16:0}$	18.9	9.6	38.2	40.5	11.9	14.1
$C_{17:0}$	0.8	1.4	0.6	2.8	1.9	3.1
Anteiso-branched:						
$C_{15:0}$	30.1	31.8	35.4	22.4	31.2	44.5
$C_{17:0}$	2.0	4.9	5.1	5.8	2.6	2.3
Unsaturated acids						
$C_{16:1}\omega7c$ alcohol	-	0.9	1.2	-	-	-
$C_{16:1}\omega11c$	-	2.3	-	-	-	1.2
$C_{17:1}\omega6c$	-	-	1.0	-	-	-
$C_{18:1}\omega9c$	1.8	2.2	-	-	-	-
$C_{18:1}\omega5c$	1.3	-	-	-	-	-
$C_{18:1}\omega7c$	1.9	1.8	-	-	-	-
$C_{16:1}\omega7c$	-	4.7	-	-	-	1.9

Data from this study. Strains: 1, strain 13-25^T; 2, *C. panacarvi* KCTC 13060^T; 3, *C.thermotolerans* DSM 17683^T. 4, *Cohnella laeviribosi* RI^T [3]; 5 *Cohnella hongkongensis* HKU3^T [5]; 6, *Cohnella phaseoli* GSPC1^T [4]. -, not detected or <1 %.

Taxonomic Conclusions

Chemotaxonomic markers support the affiliation of strain 13-25^T to the genus *Cohnella*, such as predominant isoprenoid quinone MK-7, major fatty acids anteiso-$C_{15:0}$ and iso-$C_{16:0}$, and the similar polar lipids profile. Sequence similarity between strain 13-25^T and *C. panacarvi* is just below the threshold value of the species (97.0%) [11]. These two strains can hydrolyze xylan and starch. Strain 13-25^T, however, is positive for Voges–Proskauer test and tryptophane deaminase, contains peritrichous flagella, and has a lower temperature range for growth. Furthermore, this isolate can be differentiated from published *Cohnella* species by several phenotypic characteristics listed in Table 1, including tests of acid from carbohydrates and carbon sources assimilation.

Based on these polyphasic evidences, we propose a novel species in the genus *Cohnella*, for which the name

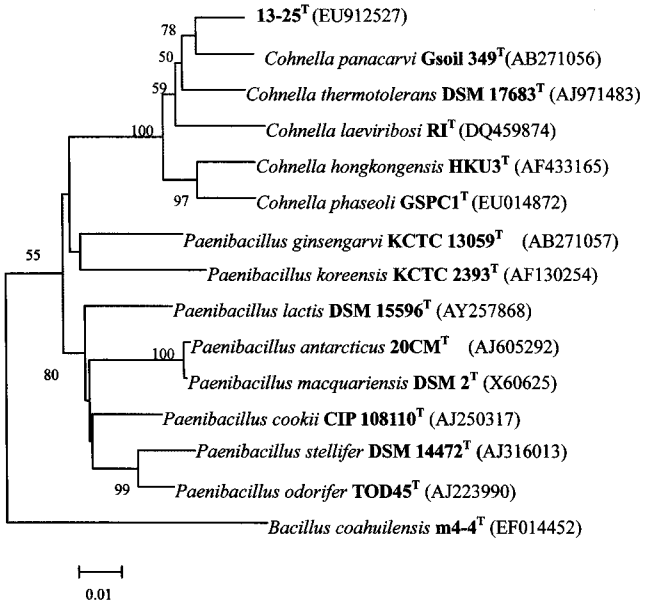

Fig. 1. Comparative sequence analysis of 16S rDNA from *Cohnella damensis* 13-25^T and representative strains from genera *Cohnella* and *Paenibacillus* using the neighbour-joining method.
Bacillus coahuilensis m4-4^T was used as the outgroup. The significance of each branch is indicated by a bootstrap value calculated for 1,000 replicates. Bar, 0.01 (bootstrap value less than 50 was not shown).

Cohnella damensis is proposed with the type strain 13-25^T (= CCTCC AB 208103^T= KCTC 13422^T)

Description of *Cohnella damensis* sp. nov.

(dam'ensis. N.L. masc. adj. dam'ensis. pertaining to Damu one of the villages in Tibet, China, where the type strain was isolated.)

Cells are Gram-staining-variable, rod-shaped of 0.5–0.7 μm×1.5–2.5 μm, and motile by means of peritrichous flagella. Colonies on tryptone soybean agar (TSA; Difco) are circular, flat, white cream, opaque, and usually 2 to 3 mm in diameter within 48 h at 28°C. Growth occurs from 10 to 40°C (optimal 28°C) and from pH 5.5 to 7.5 (optimal 7.0). Strain can grow in the presence of 1% NaCl. Nitrate is weakly reduced to nitrite. Oxidase and catalase are positive. Aesculin is hydrolyzed. The type strain is positive for Voges–Proskauer test, and xylan and starch hydrolysis; shows activities of alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), leucine and valine arylamidases, naphthol-AS-BI-phosphohydrolase, galactosidases (both α and β) and glucosidases (both α and β), and assimilation of glucose, arabinose, mannose and dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophane deaminase, gelatin hydrolysis, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, and gel liquificience. Results of acid from sugars are listed in Table 2. The major quinone is MK-7. The main fatty acids are anteiso-$C_{15:0,}$ iso-$C_{16:0}$, and $C_{16:0.}$ The polar lipids contain diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, lysyl-phosphatidylglycerol, several unknown phospholipids, unknown aminophospholipids, and unknown glycolipids. DNA G+C content is 54.3 mol%.

The type strain is 13-25^T (=CCTCC AB 208103^T= KCTC 13422^T), isolated from Damu village in Tibet, China.

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