Cohnella thailandensis sp. nov., a xylanolytic bacterium from Thai soil

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A xylanolytic bacterium, strain S1-3^T, was isolated from soil collected in Nan province, Thailand. It was characterized taxonomically based on phenotypic characteristics and 16S rRNA gene sequence comparison. The strain was a Gram-stain-positive, facultatively anaerobic, spore-forming, rod-shaped bacterium. It contained *meso*-diaminopimelic acid in the cell-wall peptidoglycan. The major menaquinone was MK-7. Iso-C_{16:0} (39.5%) and anteiso-C_{15:0} (26.8%) were predominant cellular fatty acids. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysyl-phosphatidylglycerol were the major polar lipids. The DNA G + C content was 53.3 mol%. Phylogenetic analysis using 16S rRNA gene sequences showed that strain S1-3^T was affiliated to the genus *Cohnella*, and was closely related to *Cohnella ginsengisoli* GR21-5^T and *Cohnella thermotolerans* CCUG 47242^T with 95.7 and 95.3% sequence similarity, respectively. Strain S1-3^T could be clearly distinguished from related species of the genus *Cohnella* by its physiological and biochemical characteristics as well as by its phylogenetic position. Therefore, the strain represents a novel species of the genus *Cohnella*, for which the name *Cohnella thailandensis* sp. nov. is proposed. The type strain is S1-3^T (=KCTC 22296^T =TISTR 1890^T =PCU 306^T).

The genus *Cohnella* was proposed by Kämpfer *et al.* (2006) and the type species is *Cohnella thermotolerans*. Members of the genus *Cohnella* are Gram-positive, endospore-forming, aerobic, rod-shaped organisms. At the time of writing, the genus *Cohnella* contains eight species, *Cohnella thermotolerans* isolated from a sample of industrial starch production in Sweden, *Cohnella hongkongensis* from a Chinese patient with neutropenic fever, *Cohnella laeviribosi* from a volcanic area in Likupang, Indonesia, *Cohnella phaseoli* from root nodules of *Phaseolus coccineus* in Spain, *Cohnella fontinalis* from fresh water in Japan, *Cohnella luojiensis* from soil of a Euphrates poplar forest in Xinjiang, China, and *Cohnella yongneupensis* and *Cohnella ginsengisoli* from wetland and ginseng soils in Korea, respectively (Teng *et al.*, 2003; Kämpfer *et al.*, 2006; Cho *et al.*, 2007; García-Fraile *et al.*,

2008; Shiratori *et al.*, 2010; Cai *et al.*, 2010; Kim *et al.*, 2010). In this paper, we describe the characterization of a novel xylanase-producing bacterium belonging to the genus *Cohnella* by means of a polyphasic taxonomic study.

Strain S1-3^T was isolated from a soil sample collected in 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; pH 7.0]. In this screening step, agar plates were incubated at 40 °C for 2 days. The xylanase-producing capacity of the cultures was detected by using a Congo red overlay method, as reported by Teather & Wood (1982) and Ruijsenaars & Hartsmans (2001). Isolates showing xylanase-producing capacity were transferred to C agar medium. This medium had the same composition as XC medium without 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

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S1-3^T is FJ001840.

Neighbour-joining and maximum-parsimony trees based on 16S rRNA gene sequences, and the polar lipid profile of strain S1-3^T, are available with the online version of this paper.

Table 1. Differential characteristics of strain S1-3^T and related species of the genus *Cohnella*

Strains: 1, S1-3^T; 2, *C. ginsengisoli* GR21-5^T; 3, *C. thermotolerans* CCUG 47242^T; 4, *C. yongneupensis* 5YN10-14^T; 5, *C. hongkongensis* HKU3^T. Data were obtained from this study unless otherwise indicated. All strains are positive for catalase, oxidase, growth at pH 7–9 and 30–40 °C, hydrolysis of aesculin, and acid production from amygdalin, aesculin and glycogen, but negative for indole production, growth in 5% NaCl, hydrolysis of L-arginine, casein, DNA, Tween 80 and L-tyrosine, and acid production from D-adonitol, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, gluconate, inositol, inulin, 2-ketogluconate, 5-ketogluconate, D-sorbitol, L-sorbose and D-tagatose. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5
Growth at/in:					
3 % NaCl	+	–	w	–	–
pH 5	w	+	w	+	–
pH 6	+	+	+	+	–
10 and 15 °C	–	+	–	+	–
20 and 25 °C	+	+	+	+	–
45 and 50 °C	+	–	+	–	+
55 °C	–	–	+	–	+
60 °C	–	–	–	–	+
Citrate utilization	–	w	–	–	–
Nitrate reduction	–	+	–	–	+
Urease activity	–	+	–	+	–
Hydrolysis of:					
Gelatin	+	–	+	–	–
Starch	+	+	w	+	–
Acid from:					
N-Acetylglucosamine	–	–	–	–	+
D-Arabinose	w	–	+	–	–
L-Arabinose	+	–	–	–	+
Arbutin	+	+	–	–	+
Cellobiose	+	+	–	–	+
D-Fructose	+	+	+	–	+
L-Fucose	+	–	–	–	–
D-Galactose	+	–	+	–	+
Gentiobiose	+	+	–	–	+
Glucose	+	+	–	–	+
α-Glucopyranoside	+	–	–	–	+
Glycerol	–	+	–	+	–
Lactose	+	+	–	+	+
D-Lyxose	–	–	+	–	–
Maltose	+	+	–	–	+
D-Mannitol	+	+	–	–	+
D-Mannose	+	+	–	–	+
α-Mannopyranoside	–	–	+	–	–
Melibiose	+	+	–	–	+
Melezitose	+	–	–	–	–
Raffinose	+	+	–	–	+
L-Rhamnose	+	–	–	–	–
D-Ribose	+	+	+	–	–
Salicin	+	+	–	–	+
Sucrose	+	+	–	–	–
Starch	+	+	+	–	+
Trehalose	+	+	–	–	+

Table 1. cont.

Characteristic	1	2	3	4	5
Turanose	+	+	+	–	–
Xylitol	–	–	+	–	–
D-Xylose	+	+	–	–	+
L-Xylose	–	–	+	–	–
β-Xylopyranoside	+	+	+	–	w
DNA G+C content (mol%)	53.3	61.3*	59.0†	58.8*	60.9†

*Data from Kim *et al.* (2010).

†Data from Kämpfer *et al.* (2006).

incubation at 37 °C for 2 days. Motility was observed under a phase-contrast microscope (Eclipse 80i; Nikon). Tests for catalase, oxidase and urease activity, hydrolysis of aesculin, L-arginine, casein, DNA, gelatin, starch, Tween 80 and L-tyrosine, the methyl red/Voges–Proskauer reactions, indole production, nitrate reduction, citrate utilization and hydrogen sulfide (H₂S) production were performed as described by Barrow & Feltham (1993). Additional biochemical characteristics were recorded after 2 days of incubation in API 50 CH strips. Growth under anaerobic conditions on agar plates was investigated by using a Gaspak (BBL) anaerobic jar. Growth at different pH (5, 6, 7, 8 and 9), in 3 and 5% (w/v) NaCl and at different temperatures (10, 15, 20, 25, 30, 40, 45, 50, 55 and 60 °C) was tested by using C agar medium. All tests were carried out by incubating the cultures at 37 °C, except for investigations into the effect of temperature on growth.

Detection of menaquinones and the cell-wall diamino acid was performed as described by Komagata & Suzuki (1987). For total cellular fatty acid analysis, cells were cultivated on TSA for 2 days at 30 °C, and fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Polar lipids were extracted, examined by two-dimensional TLC and identified by using published procedures (Minnikin *et al.*, 1977).

DNA was prepared by the method of Saito & Miura (1963). DNA base composition was determined by reversed-phase HPLC (Tamaoka & Komagata, 1984). The 16S rRNA gene of the novel strain was amplified, and the PCR product was purified and sequenced as described previously (Tanasupawat *et al.*, 2004). The sequence of strain S1-3^T (1531 bp) was 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 reconstruction of phylogenetic trees. Phylogenetic trees were reconstructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with MEGA4 software (Tamura *et al.*, 2007). Confidence values of branches of the phylogenetic trees were determined by using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Strain S1-3^T was a Gram-stain-positive, facultatively anaerobic, motile, rod-shaped bacterium. Subterminal ellipsoidal endospores were observed in swollen sporangia. After 2 days of incubation on C medium, colonies were 0.5–1.0 mm in diameter, circular, flat and white. The phenotypic characteristics are listed in the species description and in Tables 1 and 2. In the 16S rRNA gene-based phylogenetic tree reconstructed according to the neighbour-joining method, S1-3^T was placed in a monophyletic cluster consisting of all known species of the genus *Cohnella*, as shown in Fig. 1. Trees reconstructed by the neighbour-joining and maximum-parsimony methods, including strain S1-3^T and all species of the genera *Cohnella* and *Paenibacillus*, are shown as Supplementary Figs S1 and S2 (available in IJSEM Online). Strain S1-3^T was closely related to *C. ginsengisoli* GR21-5^T (95.7%), *C. thermotolerans* CCUG 47242^T (95.3%), *C. yongneupensis* 5YN10-14^T (94.6%), *C. hongkongensis* HKU3^T (94.2%), *C. laeviribosi* RI-39^T (94.0%), *C. luojiensis* HY-22R^T (93.8%), *C. phaseoli* GSPC1^T (93.5%) and *C. fontinalis* YT-1101^T (93.5%) based on 16S rRNA gene sequence similarity. The low 16S rRNA gene sequence similarity (93.5–95.7%) of strain S1-3^T with respect to related type strains was below the recommended range of 98.7–99.0% where DNA–DNA reassociation experiments should be mandatory (Stackebrandt & Ebers, 2006). The DNA G+C content was 53.3 mol%, which was close to the values

Table 2. Cellular fatty acid composition (%) of strain S1-3^T and related species of the genus *Cohnella*

Strains: 1, S1-3^T; 2, *C. ginsengisoli* GR21-5^T; 3, *C. thermotolerans* CCUG 47242^T; 4, *C. yongneupensis* 5YN10-14^T; 5, *C. hongkongensis* HKU3^T. Data were obtained in this study. ND, Not detected; tr, <1.0.

Fatty acid	1	2	3	4	5
Straight-chain saturated					
C _{10:0}	ND	ND	tr	ND	tr
C _{12:0}	ND	ND	tr	ND	1.6
C _{14:0}	1.8	5.5	1.5	2.3	8.4
C _{15:0}	5.2	1.5	1.1	1.7	tr
C _{16:0}	7.7	16.2	6.8	10.2	29.6
C _{17:0}	tr	ND	ND	ND	ND
Branched saturated					
iso-C _{11:0}	tr	ND	ND	ND	ND
iso-C _{12:0}	ND	ND	ND	tr	ND
iso-C _{14:0}	4.4	3.8	3.4	2.1	1.2
iso-C _{15:0}	7.5	9.2	4.4	1.3	5.1
iso-C _{16:0}	39.5	18.9	48.3	20.8	11.2
iso-C _{17:0}	1.9	1.2	1.1	ND	5.6
anteiso-C _{11:0}	tr	ND	ND	ND	tr
anteiso-C _{13:0}	ND	ND	ND	2.0	tr
anteiso-C _{15:0}	26.8	40.8	26.9	51.1	27.1
anteiso-C _{17:0}	3.8	2.9	4.8	6.9	10.0
Unsaturated					
C _{16:1} ω7c alcohol	ND	ND	tr	tr	ND
C _{16:1} ω11c	ND	ND	tr	tr	ND
C _{17:1} ω6c	ND	ND	tr	ND	ND

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain S1-3^T among all species of the genus *Cohnella* and selected species of the genus *Paenibacillus*. Based on 1000 replications, bootstrap percentages above 50% are shown. Bar, 0.01 substitutions per nucleotide position.

observed for other members of the genus *Cohnella* (Kämpfer *et al.*, 2006; Yoon *et al.*, 2007). Strain S1-3^T contained iso-C_{16:0} (39.5%) and anteiso-C_{15:0} (26.8%) as the predominant cellular fatty acids. Strain S1-3^T, *C. ginsengisoli* GR21-5^T, *C. thermotolerans* CCUG 47242^T, *C. yongneupensis* 5YN10-14^T and *C. hongkongensis* HKU3^T showed similar cellular fatty acid profiles, but significant quantitative differences were also found, as shown in Table 2. Strain S1-3^T contained *meso*-diaminopimelic acid in the cell-wall peptidoglycan. The major menaquinone was MK-7. Major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysyl-phosphatidylglycerol (Supplementary Fig. S3).

The physiological and biochemical characteristics and DNA G+C contents that differentiate strain S1-3^T from other species of the genus *Cohnella* are listed in Table 1. It should be noted that, in contrast to *C. ginsengisoli* GR21-5^T, *C. thermotolerans* CCUG 47242^T, *C. yongneupensis* 5YN10-14^T and *C. hongkongensis* HKU3^T, strain S1-3^T was able to grow in 3% NaCl and at 50 °C and to produce acid from many carbohydrates. On the basis of its phenotypic characteristics and its phylogenetic position, the strain represents a novel species of the genus *Cohnella*, for which the name *Cohnella thailandensis* sp. nov. is proposed.

Emended description of the genus *Cohnella* Kämpfer *et al.* 2006 emend. García-Fraile *et al.* 2008

The description is as given by Kämpfer *et al.* (2006) and García-Fraile *et al.* (2008) with the following amendments. Cells are aerobic or facultatively anaerobic and stain Gram-positive or Gram-negative. Some species grow at 10 or 60 °C. Some species grow in the presence of 3% NaCl. The diamino acid in the cell-wall hydrolysates is *meso*-diaminopimelic acid. The DNA G+C content ranges from 49.6 to 61.3 mol%.

Description of *Cohnella thailandensis* sp. nov.

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

Cells of strain S1-3^T are Gram-stain-positive, facultatively anaerobic, motile rods (0.2–0.5 × 1.2–2.5 µm). Subterminal ellipsoidal endospores are observed in swollen sporangia. Colonies are 0.5–1.0 mm in diameter, circular, flat and white after 2 days of incubation on C agar medium. Grows at pH 5 (weakly), pH 6–9 (optimally at 7), 20–50 °C (optimally at 37 °C), in 3 % NaCl and under anaerobic conditions. Does not grow in 5 % NaCl and at 10, 15, 55 and 60 °C. Positive for catalase (weakly), oxidase, hydrolysis of aesculin, gelatin and starch, but negative for methyl-red and Voges–Proskauer reactions, nitrate reduction, indole production, citrate utilization, H₂S production, urease and hydrolysis of L-arginine, casein, DNA, Tween 80 and L-tyrosine. Able to produce acid from D-arabinose (weakly), amygdalin, L-arabinose, arbutin, cellobiose, aesculin, D-fructose, L-fucose, D-galactose, gentiobiose, glucose, α-glucopyranoside, glycogen, lactose, maltose, D-mannitol, D-mannose, melibiose, melezitose, raffinose, L-rhamnose, D-ribose, salicin, sucrose, starch, trehalose, turanose, D-xylose and β-xylopyranoside, but not from N-acetylglucosamine, D-adonitol, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, gluconate, 2-ketogluconate, 5-ketogluconate, glycerol, inositol, inulin, D-lyxose, α-mannopyranoside, D-sorbitol, L-sorbose, D-tagatose, xylitol or L-xylose. MK-7 is the predominant menaquinone. The predominant fatty acids are iso-C_{16:0} and anteiso-C_{15:0}. The diamino acid in the cell-wall hydrolysate is meso-diaminopimelic acid. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysylphosphatidylglycerol. The DNA G + C content of the type strain is 53.3 mol%.

The type strain is S1-3^T (=KCTC 22296^T =TISTR 1890^T =PCU 306^T), isolated from a soil sample collected in Muang district, Nan province, Thailand.

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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