Cohnella xylanilytica sp. nov. and *Cohnella terrae* sp. nov., xylanolytic bacteria from soil

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Two xylan-degrading bacteria, strains MX15-2^T and MX21-2^T, were isolated from soils collected in Nan province, Thailand. Cells were Gram-reaction-positive, facultatively anaerobic, spore-forming and rod-shaped. They contained *meso*-diaminopimelic acid in the cell-wall peptidoglycan. The major menaquinone was MK-7. *iso*-C_{16:0} and *anteiso*-C_{15:0} were the predominant cellular fatty acids. Diposphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysyl-phosphatidylglycerol were the major polar lipids. The genomic DNA G + C contents of strains MX15-2^T and MX21-2^T were 63.0 and 65.1 mol%, respectively. Phylogenetic analysis using 16S rRNA gene sequences showed that strains MX15-2^T and MX21-2^T were affiliated with the genus *Cohnella* and were closely related to *Cohnella thermotolerans* CCUG 47242^T, with 96.5 and 95.6% sequence similarity, respectively. The strains could be clearly distinguished from each other and from all known species of the genus *Cohnella* based on their physiological and biochemical characteristics as well as their phylogenetic positions and levels of DNA–DNA hybridization. Therefore, these two strains represent novel species of the genus *Cohnella*, for which the names *Cohnella xylanilytica* sp. nov. (type strain MX15-2^T = KCTC 22294^T = PCU 309^T = TISTR 1891^T) and *Cohnella terrae* sp. nov. (type strain MX21-2^T = KCTC 22295^T = PCU 310^T = TISTR 1892^T) are proposed.

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, spore-forming, aerobic, motile or non-motile, thermotolerant, rod-shaped bacteria. At the time of writing, the genus *Cohnella* contains nine species, *C. 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, *Cohnella yongneupensis* and *Cohnella ginsengisoli* from wetland and ginseng soils in Korea and *Cohnella thailandensis* from soil in Thailand (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; Khiangam *et al.*, 2010). In this paper, we present the characterization of two novel xylanolytic bacteria belonging to the genus *Cohnella* by means of a polyphasic taxonomic study.

MX15-2^T and MX21-2^T were isolated from soil samples 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. Xylanase-producing capacity of the cultures was detected by using a Congo red overlay

Abbreviations: MR, methyl red; VP, Voges–Proskauer.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MX15-2^T and MX21-2^T are FJ001841 and FJ001842, respectively.

An SEM of strains MX15-2^T and MX21-2^T, two phylogenetic trees showing relationships between strains MX15-2^T and MX21-2^T, known species of the genus *Cohnella*, and other related taxa, and polar lipid profiles of the two strains are available as supplementary material with the online version of this paper.

method, as reported previously (Teather & Wood, 1982; 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 incubation at 37 °C for 2 days. Critical-point-dried cells were observed under a scanning electron microscope. Flagella were stained by the method described by Forbes (1981). Physiological and biochemical characterization was performed using API 20 NE and API 50 CH strips combined with API 50 CHB/E medium (bioMérieux), in accordance with the manufacturer's directions. Tests for catalase, oxidase and urease activity, hydrolysis of casein, DNA, starch, Tween 80 and L-tyrosine, the methyl red (MR)/Voges-Proskauer (VP) reactions, indole production, citrate utilization and H₂S production were performed as described by Barrow & Feltham (1993). Growth under anaerobic conditions on agar plates was investigated using a Gaspak (BBL) anaerobic jar. Growth at pH 5, 6, 7, 8 and 9, in 3 and 5 % (w/v) NaCl and at 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 cell-wall diaminopimelic acid was performed as described by Komagata & Suzuki (1987). For total cellular fatty acid analyses, cells were grown on tryptic soy agar (TSA; Difco) for 48 h at 30 °C, and the standard Microbial Identification System (MIDI) was used for automated GC analyses (Sasser, 1990). Polar lipids were extracted, examined by two-dimensional TLC and identified by using published procedures (Minnikin *et al.*, 1977). Polar lipids were characterized with spray reagents specific for sugars (α -naphthol/H₂SO₄ and anisaldehyde/H₂SO₄), phosphate (Zindzadze), free amino groups (ninhydrin) and quaternary nitrogen compounds (Dragendorff). The spots were identified by referring to previous descriptions of polar lipid profiles from species of the genus *Cohnella* (Kämpfer *et al.*, 2006; Cho *et al.*, 2007; Cai *et al.*, 2010).

DNA was prepared by the method of Saito & Miura (1963). DNA base compositions were determined by reversed-phase HPLC (Tamaoka & Komagata, 1984). Photobiotin-labelled DNA–DNA hybridization was performed in a solution of 2 × SSC and 50 % formamide at 50 °C for 15 h (Ezaki *et al.*, 1989). The 16S rRNA genes of the novel strains were amplified, and the PCR products were purified and sequenced as described previously (Tanasupawat *et al.*, 2004). The sequences of strains MX15-2^T and MX21-2^T (1507 nt and 1553 nt, respectively) were 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 construction of

phylogenetic trees. Phylogenetic trees were constructed by using neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) algorithms in MEGA4 software (Tamura *et al.*, 2007). Confidence values of branches of the phylogenetic trees were determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Cells of strains MX15-2^T and MX21-2^T were Gram-reaction-positive, facultatively anaerobic, motile and rod-shaped. Central ellipsoidal endospores were observed in swollen sporangia (Supplementary Fig. S1, available in IJSEM Online). After incubation on C medium for 2 days, colonies were circular, flat, 1–3.5 mm in diameter and white. The phenotypic characteristics are listed in the species descriptions and in Tables 1 and 2. In the 16S rRNA gene-based phylogenetic tree reconstructed according to the neighbour-joining method, MX15-2^T and MX21-2^T were placed in a monophyletic cluster consisting of all known species of the genus *Cohnella* and some closely related species of other genera (Fig. 1). Trees constructed by the neighbour-joining and maximum-parsimony methods including strains MX15-2^T, MX21-2^T, species of the genus *Cohnella* and related taxa are available as Supplementary Figs S2 and S3. Strains MX15-2^T and MX21-2^T were closely related to each other (97.2 % 16S rRNA gene sequence similarity) and to *C. thermotolerans* CCUG 47242^T (96.5 and 95.6 %, respectively) based on 16S rRNA gene sequence similarity. The two strains showed 93.8–95.2 % similarity with *C. thailandensis* S1-3^T and *C. ginsengisoli* GR21-5^T. The DNA–DNA relatedness between strains MX15-2^T and MX21-2^T was 52.9 % and both strains showed low levels of DNA–DNA relatedness to *C. thermotolerans* CCUG 47242^T (2.1–5.5 %). The genomic DNA G+C contents of strains MX15-2^T and MX21-2^T were 63.0 and 65.1 mol%, respectively, which were in the range of values observed for other members of the genus *Cohnella* (Kämpfer *et al.*, 2006; Khiangnam *et al.*, 2010). Strains MX15-2^T and MX21-2^T contained iso-C_{16:0} (36.1–39.2 %), anteiso-C_{15:0} (31.6 %) and iso-C_{15:0} (7.5–8.3 %) as the predominant cellular fatty acids. These two strains showed similar cellular fatty acid profiles to *C. thermotolerans* CCUG 47242^T, but significant quantitative differences were also found (Table 2). Strains MX15-2^T and MX21-2^T contained *meso*-diaminopimelic acid in the cell-wall peptidoglycan, as do species of the genera *Bacillus*, *Paenibacillus* (Shida *et al.*, 1997) and *Cohnella* as described in the emended genus description by Khiangnam *et al.* (2010). The major menaquinone was MK-7. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysyl-phosphatidylglycerol. Unknown phospholipids and aminophospholipids were also detected (Supplementary Fig. S4). Strains MX15-2^T and MX21-2^T could be differentiated from each other by differences in growth at 50 °C, the hydrolysis of DNA, the assimilation of carbohydrates and acid production from L-arabitol, 5-ketogluconate, α -mannopyranoside, D-mannitol, melezitose and D-tagatose. They were distinguished from

Table 1. Differential characteristics of strains MX15-2^T and MX21-2^T and type strains of related species of the genus *Cohnella*

Strains: 1, MX15-2^T; 2, MX21-2^T; 3, *C. thermotolerans* CCUG 47242^T; 4, *C. thailandensis* KCTC 22296^T; 5, *C. ginsengisoli* GR21-5^T. Data for related type strains were obtained from this study and Khiangngam *et al.* (2010) unless otherwise indicated. All strains are positive for catalase, oxidase, growth at pH 6–9 and 20, 25, 30 and 40 °C, hydrolysis of aesculin, PNPG and starch and acid production from aesculin, amygdalin, D-fructose, glycogen, D-ribose, starch, turanose and β-xylopyranoside. All strains are negative for growth in 5 % NaCl, growth at 60 °C, fermentation of glucose, indole production, hydrogen sulfide production, MR and VP reactions, hydrolysis of L-arginine, casein and L-tyrosine, assimilation of N-acetylglucosamine, adipic acid, capric acid and phenylacetic acid and acid production from N-acetylglucosamine, D-adonitol, dulcitol, erythritol, D-fucose, gluconate, inositol, inulin, 2-ketogluconate and L-sorbose. w, Weakly positive.

Characteristic	1	2	3	4	5
Growth at/in:					
3 % NaCl	w	–	w	+	–
pH 5	–	+	–	–	+
10 and 15 °C	–	–	–	–	+
45 °C	+	+	+	+	–
50 °C	w	–	+	+	–
55 °C	–	–	+	–	–
Citrate utilization	–	–	–	–	w
Nitrate reduction	–	–	–	–	+
Urease	–	–	–	–	+
Hydrolysis of DNA	+	–	–	–	–
Gelatin	w	+	+	+	–
Tween 80	+	+	+	–	–
Assimilation of:					
L-Arabinose	+	–	+	+	–
D-Glucose	+	–	+	+	+
Malic acid	+	–	–	–	w
Maltose	+	–	–	–	–
D-Mannitol	+	–	–	+	–
D-Mannose	+	–	+	+	–
Potassium gluconate	+	+	–	–	–
Acid production from:					
D-Arabinose	+	+	+	w	–
L-Arabinose	+	+	–	+	–
D-Arabitol	+	+	–	–	–
L-Arabitol	–	+	–	–	–
Arbutin	+	+	–	+	+
Cellobiose	+	+	–	+	+
L-Fucose	+	+	–	+	–
D-Galactose	+	+	+	+	–
Gentiobiose	+	+	–	+	+
α-Glucopyranoside	+	+	–	+	–
Glucose	+	+	–	+	+
Glycerol	–	–	–	–	+
5-Ketogluconate	–	+	–	–	–
Lactose	+	+	–	+	+
D-Lyxose	+	+	+	–	–
α-Mannopyranoside	–	+	+	–	–
Maltose	+	+	–	+	+

Table 1. cont.

Characteristic	1	2	3	4	5
D-Mannitol	+	–	–	+	+
D-Mannose	+	+	–	+	+
Melezitose	–	+	–	+	–
Melibiose	+	+	–	+	+
Raffinose	+	+	–	+	+
L-Rhamnose	+	+	–	+	–
Salicin	+	+	–	+	+
D-Sorbitol	+	+	–	–	–
Sucrose	+	+	–	+	+
D-Tagatose	–	+	–	–	–
Trehalose	+	+	–	+	+
Xylitol	+	+	+	–	–
D-Xylose	+	+	–	+	+
L-Xylose	–	–	+	–	–
DNA G + C content (mol%)	63.0	65.1	59 ^a	53.3 ^b	61.3 ^c

Data from: a, Kämpfer *et al.* (2006); b, Khiangngam *et al.* (2010); c, Kim *et al.* (2010).

Table 2. Cellular fatty acid compositions of strain MX15-2^T, MX21-2^T and type strains of related species of the genus *Cohnella*

Strains: 1, MX15-2^T; 2, MX21-2^T; 3, *C. thermotolerans* CCUG 47242^T; 4, *Cohnella thailandensis* KCTC 22296^T; 5, *C. ginsengisoli* GR21-5^T. Data in columns 3–5 were obtained from Khiangngam *et al.* (2010). Values are percentages of total fatty acids. –, Not detected; tr, trace (<1 %).

Fatty acids	1	2	3	4	5
Straight-chain saturated					
C _{10:0}	–	–	tr	–	–
C _{12:0}	–	–	tr	–	–
C _{14:0}	tr	tr	1.5	1.8	5.5
C _{15:0}	2.0	4.5	1.1	5.2	1.5
C _{16:0}	5.4	5.4	6.8	7.7	16.2
C _{17:0}	tr	tr	–	tr	–
Branched saturated					
iso-C _{11:0}	–	–	–	tr	–
iso-C _{14:0}	3.0	3.2	3.4	4.4	3.8
iso-C _{15:0}	7.5	8.3	4.4	7.5	9.2
iso-C _{16:0}	39.2	36.1	48.3	39.5	18.9
iso-C _{17:0}	2.1	2.1	1.1	1.9	1.2
anteiso-C _{11:0}	–	–	–	tr	–
anteiso-C _{15:0}	31.6	31.6	26.9	26.8	40.8
anteiso-C _{17:0}	6.4	5.6	4.8	3.8	2.9
Unsaturated					
C _{16:1} ω7c alcohol	tr	tr	tr	–	–
C _{16:1} ω11c	–	–	tr	–	–
C _{17:1} ω6c	tr	tr	tr	–	–

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strains MX15-2^T and MX21-2^T, known species of the genus *Cohnella*, and other related taxa. Based on 1000 resamplings, bootstrap percentages above 50% are shown. Bar, 0.01 substitutions per nucleotide position.

other species of the genus *Cohnella* by differences in growth in 3% NaCl, at pH 5 and at 10, 15 and 55 °C, citrate utilization, nitrate reduction, the hydrolysis of DNA, gelatin and Tween 80, assimilation and acid production from carbon sources and the DNA G+C contents, as listed in Table 1 (Kämpfer *et al.*, 2006; Khianngam *et al.*, 2010; Kim *et al.*, 2010). Based on their phenotypic characteristics and phylogenetic positions, strains MX15-2^T and MX21-2^T represent novel species of the genus *Cohnella*, for which the names *Cohnella xylanilytica* sp. nov. and *Cohnella terrae* sp. nov., respectively, are proposed.

Description of *Cohnella xylanilytica* sp. nov.

Cohnella xylanilytica (xy.la.ni.ly'ti.ca. N.L. neut. n. *xylanum* xylan; N.L. fem. adj. *lytica* from Gr. masc. adj. *lytikos* able to loose, dissolving; N.L. fem. adj. *xylanilytica* xylan-dissolving).

Cells are Gram-reaction-positive, rod-shaped (0.3–0.5 × 1.4–3.5 µm), facultatively anaerobic and motile by means of peritrichous flagella. Central ellipsoidal endospores are observed in swollen sporangia. After 2 days of incubation on C agar medium, colonies are 1–3 mm in diameter, circular, flat and white. Grows in 3% (w/v) NaCl (weakly), at pH 6–9, at 20–45 °C and at 50 °C (weakly) and under anaerobic conditions. Does not grow in 5% (w/v) NaCl, at pH 5 or at 10, 15, 55 or 60 °C. Positive for catalase and oxidase activity, hydrolysis of aesculin, DNA, starch, Tween 80 and gelatin (weak), assimilation of L-arabinose, D-glucose, malic acid, maltose, D-mannitol, D-mannose and potassium gluconate. Negative for citrate utilization, indole production, H₂S production, MR and VP reactions, nitrate reduction and urease activity, hydrolysis of L-arginine, casein and L-tyrosine and assimilation of N-acetylglucosamine, adipic acid, capric acid and phenylacetic acid. Acid is produced from aesculin, amygdalin, D- and L-arabinose, D-arabitol, arbutin, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, α-glucopyranoside, glucose, glycogen, lactose, D-lyxose, maltose, D-mannitol, D-mannose, melibiose, raffinose, L-rhamnose,

D-ribose, salicin, D-sorbitol, starch, sucrose, trehalose, turanose, β-xylopyranoside, xylitol and D-xylose. No acid production from N-acetylglucosamine, D-adonitol, L-arabitol, dulcitol, erythritol, D-fucose, gluconate, glycerol, myo-inositol, inulin, 2-ketogluconate, 5-ketogluconate, α-mannopyranoside, melezitose, L-sorbose, D-tagatose and L-xylose. MK-7 is the predominant menaquinone. The predominant fatty acids are iso-C_{16:0} and anteiso-C_{15:0}. The cell-wall diamino acid is *meso*-diaminopimelic acid. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysyl-phosphatidylglycerol. Unknown phospholipids and aminophospholipids are present. The genomic DNA G+C content of the type strain is 63.0 mol%.

The type strain is MX15-2^T (=KCTC 22294^T =PCU 309^T =TISTR 1891^T).

Description of *Cohnella terrae* sp. nov.

Cohnella terrae (ter'rae. L. gen. n. *terrae* of the earth).

Cells are Gram-reaction-positive, rod-shaped (0.3–0.5 × 1.5–4.0 µm), facultatively anaerobic and motile by means of peritrichous flagella. Central ellipsoidal endospores are observed in swollen sporangia. After 2 days of incubation on C agar medium colonies are 1–3.5 mm in diameter, circular, flat and white. Grows at pH 5–9, 20–45 °C and under anaerobic conditions. No growth in 3–5% (w/v) NaCl or at 10, 15, 50, 55 or 60 °C. Positive for catalase and oxidase activity, hydrolysis of aesculin, gelatin, starch and Tween 80 and assimilation of potassium gluconate. Negative for citrate utilization, indole production, H₂S production, MR and VP reactions, nitrate reduction, urease activity, hydrolysis of L-arginine, casein, DNA and L-tyrosine and assimilation of N-acetylglucosamine, adipic acid, L-arabinose, capric acid, D-glucose, malic acid, maltose, D-mannitol, D-mannose and phenylacetic acid. Acid is produced from aesculin, amygdalin, D- and L-arabinose, D-arabitol, L-arabitol, arbutin, cellobiose,

D-fructose, L-fucose, D-galactose, gentiobiose, α -glucopyranoside, glucose, glycogen, 5-ketogluconate, lactose, D-lyxose, α -mannopyranoside, maltose, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, starch, sucrose, D-tagatose, trehalose, turanose, β -xylopyranoside, xylitol and D-xylose. No acid production from *N*-acetylglucosamine, D-adonitol, dulcitol, erythritol, D-fucose, gluconate, glycerol, *myo*-inositol, inulin, 2-ketogluconate, D-mannitol, L-sorbose and L-xylose. MK-7 is the predominant menaquinone. The predominant fatty acids are iso-C_{16:0} and anteiso-C_{15:0}. The cell-wall diamino acid is *meso*-diaminopimelic acid. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysyl-phosphatidylglycerol. Unknown phospholipids and aminophospholipids are present. The genomic DNA G+C content of the type strain is 65.1 mol%.

The type strain is MX21-2^T (=KCTC 22295^T =PCU 310^T =TISTR 1892^T).

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