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

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Two xylan-degrading bacteria, strains MX15-2T and MX21-2T, were isolated from soils collected in Nan province, Thailand. Cells were Gram-posi ti ve, facultatively anaerobic, spore-forming and rod-shaped. They contained meso-diaminopimelic acid in the cell-wall pept idoglycan. The major menaquinone was MK-7. iso-C16:0 and anteiso-C15:0 were the predominant cellular fatty acids. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysyl-phosphatidylglycerol were the major polar lipids. The genomic DNA G+C contents of strains MX15-2T and MX21-2T were 63.0 and 65.1 mol%, respectively. Phylogenetic analysis using 16S rRNA gene sequences showed that strains MX15-2T and MX21-2T were affiliated with the genus Cohnella and were closely related to Cohnella thermotolerans CCUG 47242T, 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 MX15-2T =KCTC 22294T =PCU 309T =TISTR 1891T) and Cohnella terrae sp. nov. (type strain MX21-2T =KCTC 22295T =PCU 310T =TISTR 1892T) are proposed.

The genus Cohnella was proposed by Kömpf er 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 coccinus 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; Kömpf er et al., 2006; Cho et al., 2007; Garcia-Fraile et al., 2008; Shiratori et al., 2010; Cai et al., 2010; Kim et al., 2010; Khiangngam 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.

MX15-2T and MX21-2T were isolated from soil samples collected in Muang district, Nan province, Thailand, by the spread plate method on XC agar medium containing (l): 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.
DNA, starch, Tween 80 and L-tyrosine, the methyl red
accordance with the manufacturer’s directions. Tests for
ambiguous nucleotides prior to the construction of
alignment was edited manually to remove gaps and
labelled DNA–DNA hybridization was performed in a
DNA base compositions were determined by reversed-
DNA was prepared by the method of Saito & Miura (1963).
et al. Cai
performed using API 20 NE and API 50 CH strips
(1981). Physiological and biochemical characterization was
described by Forbes and was 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
Gaspack (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 daiminopimelic acid was performed as described by Komaga & 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
(Dragendorf). 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 & Komaga, 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 meth-
ods 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. ther-
moderans 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; Khianngam et al., 2010).
Strains MX15-2 T and MX21-2 T contained iso-C₁₆:0 (36.1–
39.2 %), anteiso-C₁₅:0 (31.6 %) and iso-C₁₅:0 (7.5–8.3 %)
as the predominant cellular fatty acids. These two strains
showed similar cellular fatty acid profiles to C. ther-
moderans CCUG 47242 T, but significant quantitative differ-
ences 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,
Paeibacillus (Shida et al., 1997) and Cohnella as described in
the emended genus description by Khianngam et al.
(2010). The major menaquinone was MK-7. The major
polar lipids were diphasphatidylglycerol, phosphatidylgly-
cerol, phosphatidylethanolamine and lysyl-phosphatidylgly-
cerol. 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 1-
arabitol, 5-ketogluconate, α-mannopyranoside, α-mannitol,
melezitose and D-tagatose. They were distinguished from

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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 Khianngam *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 ascin, amygdalin, β-fructose, glycogen, α-rubin, 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-acetylgulosamine, adipic acid, capric acid and phenylacetic acid and acid production from N-acetylgulosamine, β-adonitol, dulcitol, erythritol, α-fucose, gluconate, inositol, inulin, 2-ketogluconate and D-sorbose. W, Weakly positive.

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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>w</td>
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<tr>
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<td>+</td>
<td>+</td>
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Data from: *a*, Kämpfer *et al.* (2006); *b*, Khianngam *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 Khianngam *et al.* (2010). Values are percentages of total fatty acids. –, Not detected; tr, trace (<1 %).

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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.


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, adic 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, D-glucopyranoside, glucose, glycosgen, lactose, D-lyxose, maltose, D-mannitol, D-mannose, melibiose, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, starch, sucrose, trehalose, turanose, β-xlyopyranoside, xyitol and D-xyllose. No acid production from N-acetylglucosamine, D-adonitol, L-arabitol, dulcitol, erythritol, D-fucose, gluconate, glycerol, myo-inositol, inulin, 2-ketogluconate, 5-ketogluconate, L-mannopyranoside, melezitose, L-sorbose, D-tagatose and L-xyllose. MK-7 is the predominant menaquinone. The predominant fatty acids are iso-C₁₆:0 and anteiso-C₁₅:0. The cell-wall diaminodicarboxylic acid is *meso*-diaminopimelic acid. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysyl-phosphatidylethanolamine. 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 22294T =PCU 309T =TISTR 1891T).

**Description of Cohnella terrae** sp. nov.

*Cohnella terrae* (ter’ae. 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, adic 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, 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-xylenepranoside, xylitol and D-xylene. No acid production from N-acetylglucosamine, D-adenosine, dulcitol, erythritrol, D-fucose, gluconate, glycerol, myo-inositol, inulin, 2-ketogluconate, D-mannitol, L-sorbose and L-xylene. MK-7 is the predominant menaquinone. The predominant fatty acids are iso-C₁₅:₀ and anteiso-C₁₅:₀. The cell-wall diamino acid is meso-diaminopimelic acid. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylyethanolamine 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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