Cohnella luojiensis sp. nov., isolated from soil of a Euphrates poplar forest

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A novel strain, HY-22R^T, was isolated from soil of a Euphrates poplar forest in Xinjiang, China. The cells were Gram-positive-staining, rod-shaped and motile by means of peritrichous flagella. Growth occurred at 10–37 °C (optimum 30 °C), at pH 7.0–8.0 (optimum pH 7.0) and with 0–1 % NaCl. A phylogenetic analysis based on 16S rRNA gene sequences revealed that strain HY-22R^T was closely related to *Cohnella phaseoli* GSPC1^T (96.3 % sequence similarity). The major respiratory quinone was MK-7 and the predominant fatty acids were anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{16:0} and C_{16:0}. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The DNA G + C content was 49.6 mol%. On the basis of the phylogenetic, physiological and chemotaxonomic data, strain HY-22R^T represents a novel species in the genus *Cohnella*, for which the name *Cohnella luojiensis* sp. nov. is proposed. The type strain is HY-22R^T (=CCTCC AB 208254^T =NRRL B-59213^T).

The genus *Cohnella*, a member of the family *Paenibacillaceae*, was proposed by Kämpfer *et al.* (2006) and its description was emended by García-Fraile *et al.* (2008). At the time of writing, it comprises six species, *Cohnella thermotolerans* (Kämpfer *et al.*, 2006), *C. hongkongensis* (Kämpfer *et al.*, 2006), *C. laeviribosi* (Cho *et al.*, 2007), *C. phaseoli* (García-Fraile *et al.*, 2008), *C. yongneupensis* and *C. ginsengisoli* (Kim *et al.*, 2010). Members of the genus *Cohnella* are spore-forming, aerobic and rod-shaped and contain MK-7 as the main menaquinone and iso-C_{16:0}, anteiso-C_{15:0} and C_{16:0} as major fatty acids. In this study, a novel member of the genus *Cohnella* is characterized.

Strain HY-22R^T was isolated from a soil sample of a Euphrates poplar forest in Xinjiang, China. The soil sample was diluted with sterile water and the dilutions were plated onto half-strength R2A (Difco) plates. The strain was isolated after incubation for 2 days at 30 °C.

Growth on various standard bacteriological media was tested, using nutrient agar (NA), tryptic soy agar (TSA), R2A and marine agar (MA) (all from Difco). For the experiments described below, TSA was used as the basal medium. Gram-staining was carried out using the procedure described by Doetsch (1981). The flagellation type was determined by electron microscopy after incubation for 48 h. Endospores were stained according to the Schaeffer–Fulton method (Smibert & Krieg, 1994). Cell

motility was observed using phase-contrast microscopy (Olympus BX51) and the hanging-drop technique with cells grown for 2 days at 30 °C. Growth at 4, 10, 15, 20, 28, 30, 37, 42 and 45 °C and at pH 5.0–10.0, in increments of 0.5 pH units, was assessed on TSA. Oxidase activity was evaluated via the oxidation of 1 % *p*-aminodimethylaniline oxalate and catalase activity was determined by bubble production with 3 % (v/v) hydrogen peroxide solution. Tolerance of NaCl (0–6 %, w/v, in increments of 1 %) was determined. Further biochemical characteristics were determined using the API 20NE, API ID 32GN and API ZYM systems (bioMérieux), according to the manufacturer's instructions.

Cells of strain HY-22R^T were Gram-positive-staining, rod-shaped (0.4–0.6 × 1.2–3.5 µm) and motile by means of peritrichous flagella. Subterminal oval spores appeared within the cells after growth for 7 days. Strain HY-22R^T grew aerobically at 10–37 °C, with an optimum of 30 °C, at pH 6.0–8.0, with an optimum of pH 7.0, and with 0–1 % (w/v) NaCl. The results for oxidase and catalase tests were positive. Colonies were opaque, white, convex and about 1 mm in diameter after growth at 30 °C for 48 h. The strain grew well on TSA, R2A and MA, but did not grow on NA. The phenotypic properties that differentiate strain HY-22R^T from related strains are listed in Table 1 and the species description.

To measure the G + C content of the chromosomal DNA, genomic DNA from the novel strain was extracted and purified as described by Moore & Dowhan (1995) and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HY-22R^T is GQ214052.

Table 1. Differential phenotypic characteristics of strain HY-22R^T and the type strains of phylogenetically closely related *Cohnella* species

Strains: 1, *Cohnella luojiensis* sp. nov. HY-22R^T; 2, *C. phaseoli* KACC 13436^T; 3, *C. hongkongensis* KACC 11644^T; 4, *C. thermotolerans* KACC 11643^T. All data are from this study. +, Positive; (+), weakly positive; –, negative.

Characteristic	1	2	3	4
Growth at/with:				
42 °C	–	+	+	+
2 % NaCl	–	+	+	–
Nitrate reduction	–	+	+	–
Enzyme activity (API ZYM)				
N-Acetyl-β-glucosaminidase	–	(+)	–	–
Acid phosphatase	–	(+)	+	+
Alkaline phosphatase	–	–	+	(+)
Cystine arylamidase	–	–	–	(+)
Valine arylamidase	–	–	(+)	(+)
α-Fucosidase	–	(+)	+	–
α-Galactosidase	+	+	–	+
α-Glucosidase	–	+	+	+
β-Glucosidase	–	+	+	+
Assimilation (API 20NE, API 32GN) of:				
N-Acetylglucosamine	–	+	–	–
L-Arabinose	+	+	–	+
L-Fucose	+	+	+	–
Glycogen	–	+	–	+
Inositol	+	–	–	–
D-Mannitol	+	–	+	+
L-Rhamnose	–	+	+	+
D-Sorbitol	–	–	+	+
Potassium gluconate	–	–	+	–
Potassium 2-ketogluconate	–	+	–	+

degraded enzymically into nucleosides and the G+C content was then determined as described by Mesbah *et al.* (1989) using reversed-phase HPLC (UltiMate 3000; Dionex). Respiratory quinones were isolated from lyophilized cells as described by Collins *et al.* (1977) and were analysed by HPLC as described by Xie & Yokota (2003). For cellular fatty acid analysis, strain HY-22R^T and strains of phylogenetically closely related *Cohnella* species were cultivated on TSA medium at 30 °C for 2 days before harvesting the cell biomass. The fatty acid methyl esters were determined according to the protocol of the Sherlock Microbial Identification System (MIDI) and GC (model 6890; Agilent) using the Microbial Identification software package (Sasser, 1990). Polar lipids were extracted and analysed by the methods of Tindall (1990) by using two-dimensional TLC (silica gel 60 F₂₅₄ plates, layer thickness 0.2 mm, no. 5554; Merck).

The DNA G+C content of strain HY-22R^T was 49.6 mol%. Strain HY-22R^T contained menaquinone 7 (MK-7) as the predominant isoprenoid quinone (99.7%); in addition, a small amount (0.3 %) of MK-6 was detected.

These characteristics were in agreement with those of members of the genus *Cohnella*. The fatty acid pattern of strain HY-22R^T is shown in Table 2. The predominant fatty acids were anteiso-C_{15:0} (57.4 %), iso-C_{15:0} (9.1 %), iso-C_{16:0} (9.1 %) and C_{16:0} (6.2 %), which are also the predominant fatty acids of the type strains of closely related *Cohnella* species (Table 2); however, the proportions of anteiso-C_{15:0} were lower (30.4–36.1 %) and of iso-C_{16:0} were higher (16.3–37.7 %) in the other *Cohnella* type strains. The major polar lipids of strain HY-22R^T were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Two unknown phospholipids, three unknown aminophospholipids and lysyl-phosphatidylglycerol were detected in minor amounts (Fig. 1). This profile is in agreement with the characteristics of *Cohnella* species (Kämpfer *et al.*, 2006).

For phylogenetic analysis, genomic DNA was isolated from a fresh culture of strain HY-22R^T following the methods of Sambrook *et al.* (1989). PCR amplification of the 16S rRNA gene was performed using an established method (Lane, 1991) and the PCR products were sequenced by Invitrogen Biotechnology. A preliminary phylogenetic analysis was performed using the EzTaxon database (Chun *et al.*, 2007). Selected sequences were loaded into the software package MEGA version 4.0 (Tamura *et al.*, 2007) and phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-

Table 2. Fatty acid patterns of strain HY-22R^T and the type strains of phylogenetically closely related *Cohnella* species

Strains: 1, *C. luojiensis* sp. nov. HY-22R^T; 2, *C. phaseoli* KACC 13436^T; 3, *C. hongkongensis* KACC 11644^T; 4, *C. thermotolerans* KACC 11643^T. Values are percentages of total fatty acids and were obtained in this study. –, Not detected or <1 %.

Fatty acid	1	2	3	4
iso-C _{13:0}	–	–	1.4	–
anteiso-C _{13:0}	2.6	1.3	1.2	–
C _{14:0}	1.2	2.1	2.7	1.6
iso-C _{14:0}	1.4	4.2	4.3	3.0
anteiso-C _{15:0}	57.4	35.8	36.1	30.4
iso-C _{15:0}	9.1	11.2	11.8	15.4
C _{16:0}	6.2	13.0	12.9	5.2
iso-C _{16:0}	9.1	16.7	16.3	37.7
C _{16:1} ω11 <i>c</i>	–	–	1.5	–
C _{16:1} ω7 <i>c</i> alcohol	–	1.5	2.2	1.1
iso-C _{17:0}	3.7	2.8	1.7	–
anteiso-C _{17:0}	4.8	2.5	2.9	5.3
C _{17:1} ω6 <i>c</i>	–	–	–	1.0
C _{18:0}	–	3.6	–	–
Summed feature 8*	–	–	–	1.7

*Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 8 consisted of C_{18:1}ω7*c* and/or C_{18:1}ω6*c*.

Fig. 1. Two-dimensional thin-layer chromatogram of polar lipids of strain HY-22R^T. DPG, Diphosphatidylglycerol; LPG, lysyl-phosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unknown phospholipids; PN, unknown aminophosphoglycolipid.

parsimony (Fitch, 1971) methods. Evolutionary distances for the neighbour-joining algorithm were calculated with Kimura's two-parameter model (Kimura, 1980) and the topology of the neighbour-joining tree was evaluated by bootstrap analysis on the basis of 1000 replications (Felsenstein, 1985). Close-neighbour interchange (search level=2, random additions=100) was applied in the maximum-parsimony analysis.

The 16S rRNA gene sequence of strain HY-22R^T shared 93.7–96.3 % similarity (over 1458 nt) with all of the other *Cohnella* strains. The phylogenetic tree from the neighbour-joining analysis illustrates the phylogenetic relationships within the genus *Cohnella* (Fig. 2). Strain HY-22R^T showed the highest 16S rRNA gene sequence similarity

with *C. phaseoli* GSPC1^T (96.3 %). The topologies of phylogenetic trees generated using the maximum-parsimony algorithm were similar to those generated using neighbour-joining analysis (data not shown). According to Stackebrandt & Goebel (1994) and Wayne *et al.* (1987), the levels obtained for 16S rRNA gene sequence similarity between strain HY-22R^T and the type strains of the other *Cohnella* species (≤ 97 %) supported the isolate's genomic distinction.

On the basis of the phenotypic variation and chemotaxonomic and phylogenetic analysis, strain HY-22R^T should be classified in a novel species of the genus *Cohnella*, for which we propose the name *Cohnella luojiensis* sp. nov.

Description of *Cohnella luojiensis* sp. nov.

Cohnella luojiensis (lu.o.ji.en'sis. N.L. fem. adj. *luojiensis* pertaining to Luojia hill, the site of the campus of Wuhan University, where the type strain was characterized).

Cells are strictly aerobic, Gram-positive-staining, rod-shaped ($0.4\text{--}0.6 \times 1.2\text{--}3.5$ μm) and motile by means of peritrichous flagella. Oxidase- and catalase-positive. Oval subterminal spores are formed. Colonies on TSA are opaque, white, convex and about 1 mm in diameter after growth at 30 °C for 48 h. Grows at 10–37 °C (optimum 30 °C), at pH 6.0–8.0 (optimum pH 7.0) and with 0–1 % (w/v) NaCl. With API 20NE and API ZYM, positive for aesculin hydrolysis and esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, leucine arylamidase and α - and β -galactosidase activities, but negative for nitrate reduction, indole production, gelatin hydrolysis, glucose fermentation and alkaline phosphatase, acid phosphatase, lipase (C14), arginine dihydrolase, cystine arylamidase, valine arylamidase, urease, α -fucosidase, α - and β -glucosidase, β -glucuronidase, α -mannosidase, *N*-acetyl- β -glucosaminidase, trypsin and α -chymotrypsin activities. With API 20NE and API ID 32GN, assimilates L-arabinose, L-fucose, D-glucose, inositol, maltose, D-mannitol, D-mannose, melibiose, D-ribose, salicin and sucrose, but not sodium acetate, trisodium citrate, *N*-acetylgluco-

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequence analysis (1458 nt), showing the phylogenetic relationships between strain HY-22R^T and other related taxa. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. Bar, 0.01 substitutions per nucleotide position.

samine, glycogen, L-rhamnose, D-sorbitol, adipic acid, capric acid, itaconic acid, lactic acid, malic acid, phenylacetic acid, suberic acid, propionic acid, valeric acid, sodium malonate, L-alanine, L-serine, L-histidine, L-proline, potassium 2-ketogluconate, potassium 5-ketogluconate, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid or 3-hydroxybutyric acid. The major respiratory quinone is MK-7 and the predominant fatty acids are anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{16:0} and C_{16:0}. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. In addition, lysyl-phosphatidylglycerol, two unknown phospholipids and three unknown aminophospholipids are also detected. The DNA G+C content of the type strain is 49.6 mol%.

The type strain, HY-22R^T (=CCTCC AB 208254^T =NRRL B-59213^T), was isolated from a soil sample from Xinjiang, China.

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