Cohnella arctica sp. nov., isolated from Arctic tundra soil

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A psychrotolerant Gram-reaction-negative, rod-shaped and orange-pigmented bacterium, designated strain M9-62^T, which was motile by means of peritrichous flagella, was isolated from tundra soil sampled near Ny-Ålesund, Svalbard Islands, Norway (78° N). Growth occurred at 4–30 °C (optimum, 25 °C) and pH 5.0–8.0 (optimum, pH 6.0–7.0). Analysis of the 16S rRNA gene sequence of strain M9-62^T placed it in the genus *Cohnella*; sequence similarities of the isolate with type strains of members of related genera ranged from 92.0 to 96.3 %. Strain M9-62^T contained anteiso-C_{15:0} (51.1 %), iso-C_{16:0} (7.5 %) and C_{16:0} (6.1 %) as the major cellular fatty acids and diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysyl-phosphatidylglycerol as the main polar lipids. The major respiratory quinone was MK-7 and the DNA G + C content was 50.3 mol%. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain M9-62^T is considered to represent a novel species of the genus *Cohnella*, for which the name *Cohnella arctica* sp. nov. is proposed; the type strain is M9-62^T (=CCTCC AB 2010228^T=NRRL B-59459^T).

The genus *Cohnella* was proposed by Kämpfer *et al.* (2006) to accommodate Gram-positive, spore-forming, aerobic, rod-shaped bacteria containing MK-7 as the main menaquinone. At the time of writing, the genus *Cohnella* comprised 12 recognized species isolated from various environments such as soil, fresh water, industrial starch production, human blood, a root nodule of *Phaseolus coccineus* and a volcanic pond, namely *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), *C. fontinalis* (Shiratori *et al.*, 2010), *C. luojiensis* (Cai *et al.*, 2010), *C. damuensis* (Luo *et al.*, 2010), *C. thailandensis* (Khianngam *et al.*, 2010a), and *C. xylanilytica* and *C. terrae* (Khianngam *et al.*, 2010b). In this paper, we isolated a psychrotolerant representative of the genus *Cohnella* from high Arctic tundra soil and report the results of a phenotypic, genetic and phylogenetic examination.

Strain M9-62^T was isolated from a soil sample collected from a tundra near the settlement Ny-Ålesund (78° 55' N 11° 58' E), Svalbard Islands, Norway, in July 2009. At the time of sampling, the soil had a salt content of 0.032 %, a pH of 8.0 and a temperature of 8.3 °C at 5 cm below the

surface. The soil sample was diluted serially with a sterile 0.85 % (w/v) NaCl solution and these dilutions were plated onto 0.3 × marine broth 2216 (MB; Difco) agar plates. Single colonies on these plates were purified by transferring them onto new plates followed by additional incubation for 7 days at 25 °C. The isolate was tentatively identified by using partial 16S rRNA gene sequence analysis and stored as lyophilized cells.

Cell morphology was examined by using phase-contrast microscopy (Olympus BX51) and transmission electron microscopy (Hitachi 8100). Flagellation was determined by using transmission electron microscopy of cells from an exponentially growing culture. For this purpose, cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. Endospores were stained according to the Schaeffer–Fulton method (Smibert & Krieg, 1994). The Gram-reaction test was performed on cells grown on 0.3 × MB agar at 25 °C for 1–14 days by using the classical Gram procedure described by Doetsch (1981). Gliding motility was investigated as described by Bowman (2000). Growth under anaerobic conditions was tested on 0.3 × MB agar in a GasPak (BBL) jar at 25 °C for 20 days. Oxidase activity was evaluated using 1 % (w/v) tetramethyl-*p*-phenylenediamine. Catalase activity was determined by measurement of bubble production after the application of 3 % (v/v) H₂O₂ solution. Growth at different temperatures (0, 4, 8, 15, 20, 25, 28, 30, 37 and 42 °C) and pH values (4.0–11.0 at 1.0 pH

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain M9-62^T is HQ315787.

Two supplementary figures are available with the online version of this paper.

unit increments) was investigated on 0.3 × MB agar for up to 1 week. Tolerance to 0.5, 1, 2, 3, 4, 5 and 6 % (w/v) NaCl was assessed on 0.3 × R2A agar (Difco) medium. Growth was also evaluated on several bacteriological media: R2A agar (Difco), 0.3 × R2A agar (Difco), nutrient agar (NA; Difco), MacConkey agar (Difco), tryptic soy broth (TSB) agar (Difco) and 0.3 × MB (Difco) agar at 25 °C. Further biochemical characteristics were determined by using API kits (API ZYM, API 20NE and API ID 32GN) according to the manufacturer's instructions (bioMérieux).

Strain M9-62^T was aerobic, Gram-reaction-negative, rod-shaped (0.2–0.3 × 1.3–2.3 µm) and motile by means of peritrichous flagella (Supplementary Fig. S1, available in IJSEM Online). Oval spores appeared in a subterminal position within the cells after 7 days growth. The isolate was oxidase-positive and showed weak catalase activity. Growth of the strain was observed on 0.3 × MB agar at temperatures between 4 and 30 °C (optimum, 25 °C) and

at pH 5.0–8.0 (optimum, pH 6.0–7.0). The strain showed good growth in the presence of 0.5 % (w/v) NaCl in 0.3 × R2A, but no growth occurred in the presence of ≥1.0 % (w/v) NaCl. Colonies were orange, circular, convex and smooth after growth for 7 days at 25 °C on 0.3 × MB agar. The isolate grew well at 25 °C on R2A agar, 0.3 × R2A agar, NA and 0.3 × MB agar, but not on MacConkey agar or TSB agar. Other characteristics are summarized in the species description and those that differentiate strain M9-62^T from closely related type strains are shown in Table 1.

Genomic DNA was extracted with a Bacteria Genomic DNA Isolation kit (Shanghai Chaoshi Bio Technologies). PCR amplification of the 16S rRNA gene was performed using an established method (Lane, 1991) and PCR products were sequenced by Invitrogen Biotechnology. Sequence similarity was calculated by pairwise alignment obtained from the EzTaxon database (Chun *et al.*, 2007). Sequences were aligned using CLUSTAL_X software (Thompson *et al.*,

Table 1. Differential characteristics of strain M9-62^T and related *Cohnella* species

Strains: 1, M9-62^T; 2, *C. luojiensis* HY-22R^T; 3, *C. thermotolerans* CCUG 47242^T; 4, *C. phaseoli* GSPC1^T; 5, *C. hongkongensis* HKU3^T. Data are from this study unless otherwise indicated. +, Positive; (+), weakly positive; –, negative.

Characteristics	1	2	3	4	5
Temperature range for growth (°C)	4–30	10–37*	20–55†	10–45‡	25–50†
Flagella	+	+*	–†	+‡	–†
Nitrate reduction	–	–	–	+	–
Enzyme activity (API ZYM):					
Alkaline phosphatase	+	+	+	–	+
Lipase (C14)	+	–	+	–	–
Leucine arylamidase	–	+	+	+	+
Acid phosphatase	+	–	+	+	+
α-Glucosidase	+	–	+	+	+
β-Glucosidase	+	–	+	+	+
α-Fucosidase	–	–	–	+	+
Assimilation (API 20NE/32GN) of:					
D-Glucose	–	+	+	+	+
L-Arabinose	–	+	+	+	+
D-Mannitol	–	+	+	–	+
N-Acetylglucosamine	–	+	–	+	–
Potassium gluconate	–	–	+	–	–
L-Rhamnose	–	–	–	–	+
D-Ribose	–	+	–	+	–
Inositol	–	+	–	–	–
Sucrose	–	+	–	+	+
Glycogen	–	–	+	+	–
Salicin	–	+	–	+	–
Melibiose	–	+	–	+	(+)
L-Fucose	–	+	–	+	(+)
D-Sorbitol	–	–	–	–	(+)
DNA G + C content (mol%)	50.3	49.6*	59†	60.3‡	60.9†

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

†Data from Kämpfer *et al.* (2006).

‡Data from García-Fraile *et al.* (2008).

1997). Phylogenetic trees were constructed by neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. An evolutionary distance matrix for the neighbour-joining method was generated according to Kimura's two-parameter method (Kimura, 1980) and close-neighbour-interchange (search level=2, random additions =100) was applied in the maximum-parsimony analysis. The topology of the neighbour-joining tree was evaluated by using the bootstrap resampling method with 1000 replicates (Felsenstein, 1985). MEGA version 4.0 (Tamura *et al.*, 2007) was used for all analyses.

The 16S rRNA gene sequence of strain M9-62^T was a continuous stretch of 1441 bp. Sequence comparisons with 16S rRNA gene sequences from the EzTaxon database revealed that strain M9-62^T had highest similarity with *C. luojiensis* HY-22R^T (96.3%), followed by *C. yongneupensis* 5YN10-14^T (94.7%), *C. phaseoli* GSPC1^T (94.4%), *C. ginsengisoli* GR21-5^T (94.0%), *C. hongkongensis* HKU3^T (93.4%), *C. thermotolerans* CCUG 47242^T (93.1%), *C. xylanilytica* MX15-2^T (92.9%), *C. laeviribosi* RI-39^T (92.4%) and *C. fontinalis* YT-1101^T (92.0%). These values are well below the threshold for demarcating bacterial species (Stackebrandt & Goebel, 1994). The neighbour-joining tree (Fig. 1) showed that strain M9-62^T formed a tight phyletic lineage with type strains of members of the genus *Cohnella*. The overall topology of the maximum-parsimony tree was essentially the same (data not shown).

Cell biomass for the analysis of respiratory quinones and polar lipids was obtained from cultivation on 0.3 × MB at 25 °C. Respiratory quinones were extracted and identified by HPLC (UltiMate 3000; Dionex) as described by Xie & Yokota (2003). For cellular fatty acid analysis, strain M9-62^T and four reference strains (*C. luojiensis* HY-22R^T, *C. thermotolerans* CCUG 47242^T, *C. phaseoli* GSPC1^T and *C. hongkongensis* HKU3^T) were grown on 0.3 × MB agar plates at 25 °C; cells grown at late-exponential phase were used in this study. Harvesting, saponification, methylation and extraction of cellular fatty acids were carried out according to the protocol of the Sherlock Microbial Identification System (MIDI) version 6.0. Separation and identification of fatty acid methyl esters was performed using a Hewlett Packard 6890N GC, with the MIDI Sherlock TSBA6 database (Sasser, 1990). The DNA G+C content of strain M9-62^T was determined by HPLC according to the method of Mesbah *et al.* (1989). Polar lipids were extracted and analysed by two-dimensional TLC (silica gel plates, layer thickness 0.2 mm; Merck) according to Tindall (1990).

Strain M9-62^T contained MK-7 as the predominant respiratory quinone. The major cellular fatty acids of strain M9-62^T were anteiso-C_{15:0} (51.1%), iso-C_{16:0} (7.5%) and C_{16:0} (6.1%), which were consistent with data for recognized *Cohnella* species. However, differences in the fatty acid contents of strain M9-62^T and closely related type strains of members of the genus *Cohnella* demonstrate

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between strain M9-62^T and related taxa. Bootstrap values (%; 1000 replications) greater than 70% are shown at nodes. The sequence of *Alicyclobacillus acidiphilus* TA-67^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid compositions (%) of strain M9-62^T and closely related strains

Strains: 1, M9-62^T; 2, *C. luojiensis* HY-22R^T; 3, *C. thermotolerans* CCUG 47242^T; 4, *C. phaseoli* GSPC1^T; 5, *C. hongkongensis* HKU3^T. Data were obtained in this study. —, Not detected or <1%.

Fatty acid	1	2	3	4	5
anteiso-C _{13:0}	2.5	2.7	—	2.1	3.7
C _{13:0}	1.5	—	—	—	2.0
C _{14:0}	2.8	2.3	3.1	3.1	7.0
iso-C _{14:0}	5.9	3.8	3.4	4.0	7.7
iso-C _{13:0} 3-OH	—	—	—	—	1.3
iso-C _{15:1} -F	—	—	—	—	1.3
anteiso-C _{15:0}	51.1	53.3	26.8	38.6	31.5
iso-C _{15:0}	1.8	5.0	1.0	2.3	1.5
C _{16:0}	6.1	9.4	14.0	14.9	15.3
iso-C _{16:0}	7.5	12.8	26.3	15.0	8.9
C _{16:1} ω11c	4.2	1.2	1.8	3.1	1.9
C _{16:1} ω7c alcohol	6.6	1.9	1.7	4.0	2.6
anteiso-C _{17:0}	1.5	2.5	3.7	3.2	1.5
C _{17:0}	1.6	—	—	3.7	1.2
C _{18:1} ω9c	—	—	1.8	—	2.5
C _{18:0}	—	—	8.0	—	4.7
Summed features*					
1	—	—	—	—	1.1
8	—	—	4.0	—	1.1

*Summed feature 1 contains iso-C_{15:1} H and/or C_{13:0} 3-OH; summed feature 8 contains C_{18:1}ω7c and/or C_{18:1}ω6c.

that strain M9-62^T is not a member of known *Cohnella* species (Table 2). The DNA G+C content of the novel isolate was 50.3 mol%, which is consistent with the range reported for the genus *Cohnella* (49.6–65.1 mol%; Kämpfer *et al.*, 2006; Cai *et al.*, 2010; Khiangam *et al.*, 2010a). The major polar lipids of strain M9-62^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysyl-phosphatidylglycerol (Supplementary Fig. S2, available in IJSEM Online); these polar lipids are characteristic of members of the genus *Cohnella* (Kämpfer *et al.*, 2006). Five unknown phospholipids (PL1 to 5) and three unknown aminophospholipids (PN1, PN3 and PN4) were also detected.

On the basis of the data presented, strain M9-62^T is considered to represent a novel species of the genus *Cohnella*, for which the name *Cohnella arctica* sp. nov. is proposed.

Description of *Cohnella arctica* sp. nov.

Cohnella arctica (arc'ti.ca. L. fem. adj. *arctica* northern, from the Arctic, referring to the site where the type strain was isolated).

Cells are aerobic, Gram-reaction-negative, rod-shaped (0.2–0.3 × 1.3–2.3 µm) and motile by means of peritrichous flagella. Oval subterminal spores are formed. Growth

occurs on R2A agar, 0.3 × R2A agar, NA and 0.3 × MB agar, but not on MacConkey agar or TSB agar. Colonies grown on 0.3 × MB agar are orange, circular, convex and smooth. Growth occurs between 4 and 30 °C with optimum growth at 25 °C. The pH range for growth is pH 5.0–8.0, with optimum growth at pH 6.0–7.0. Growth occurs in the presence of 0.5% (w/v) NaCl, but no growth occurs in the presence of ≥1.0% (w/v) NaCl. Oxidase-positive and shows weak catalase activity. In the API ZYM gallery, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase activities are present, but leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. In API 20NE, positive for aesculin hydrolysis and β-galactosidase activity, and negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urea and gelatin hydrolysis. In API 20NE and API ID 32GN strips, assimilates D-mannose and maltose; does not assimilate D-glucose, L-arabinose, D-mannitol, N-acetylglucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, inositol, sucrose, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, salicin, melibiose, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid or L-proline. MK-7 is the predominant respiratory quinone. The major cellular fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and C_{16:0}. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysyl-phosphatidylglycerol.

The type strain, M9-62^T (=CCTCC AB 2010228^T=NRRL B-59459^T), was isolated from a tundra soil near Ny-Ålesund, Svalbard Islands, Norway. The DNA G+C content of the type strain is 50.3 mol%.

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