Colwellia chukchiensis sp. nov., a psychrotolerant bacterium isolated from the Arctic Ocean

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A novel psychrotolerant bacterial strain, BCw111^T, was isolated from seawater samples from the Chukchi Sea in the Arctic Ocean. Cells of strain BCw111^T were Gram-negative, motile, facultatively anaerobic, curved rods and were able to grow at 0–30 °C (optimum 23–25 °C). Strain BCw111^T had Q-8 as the major respiratory quinone and contained iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (28.13%), C_{16:0} (13.28%) and C_{17:1} (12.90%) as the major cellular fatty acids. The genomic DNA G+C content was 41.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain BCw111^T formed a distinct lineage within the genus *Colwellia* and exhibited the highest 16S rRNA gene sequence similarity with *Colwellia polaris* 537^T (97.8%) and *Colwellia aestuarii* SMK-10^T (97.1%). Based on phenotypic characteristics, phylogenetic analysis and DNA–DNA relatedness, a novel species, *Colwellia chukchiensis* sp. nov., is proposed. The type strain is BCw111^T (=CGMCC 1.9127^T =LMG 25329^T =DSM 22576^T).

The genus *Colwellia*, which belongs to the family *Colwelliaceae*, was originally proposed by Deming *et al.* (1988). At the time of writing, the genus *Colwellia* comprised 11 species: *Colwellia psychrerythraea* (the type species) and *C. hadaliensis* (Deming *et al.*, 1988), *C. demingiae*, *C. hornerae*, *C. psychrotropica* and *C. rossensis* (Bowman *et al.*, 1998), *C. maris* (Yumoto *et al.*, 1998), *C. piezophila* (Nogi *et al.*, 2004), *C. aestuarii* (Jung *et al.*, 2006), *C. polaris* (Zhang *et al.*, 2008) and *C. asteriadis* (Choi *et al.*, 2010). During screening of cold-adapted bacteria from seawater samples from the Chukchi Sea in the Arctic Ocean (73° 00′ 00″ N 169° 32′ 44″ W), we isolated a psychrotolerant bacterium, strain BCw111^T. Phenotypic, genotypic and phylogenetic data indicated that the new isolate could represent a novel species of the genus *Colwellia*.

Seawater at a depth of 60 m was collected for the isolation of bacterial strains during the Second Chinese National Arctic Research Expedition cruise of the icebreaker *Xue Long*. Strain BCw111^T was isolated by the dilution-plating technique on marine agar (MA; Difco) after incubation at 4 °C for 4 weeks. *Colwellia polaris* CGMCC 1.6132^T and *Colwellia aestuarii* CGMCC 1.6965^T were obtained from the CGMCC and used as reference strains. Strains were stored at –80 °C in marine 2216 broth (MB; Difco) supplemented with 30% (v/v) glycerol.

Cell morphology was examined using phase-contrast microscopy (Eclipse 80i; Nikon) and transmission electron

microscopy (JEM-100CX; JEOL). Colony morphology was observed on MA after incubation at 25 °C. Growth at 0–37 °C was investigated in MB for 5–30 days. Growth at pH 4–11 was determined by the method of Zhang *et al.* (2009). Growth with 0–10% (w/v) NaCl (in increments of 1%) was tested in a medium containing (l⁻¹) 5 g MgCl₂, 2 g MgSO₄, 0.5 g CaCl₂, 1 g KCl and 5 g peptone and adjusted to pH 7.5 with KOH (Smibert & Krieg, 1994). Growth under anaerobic conditions was determined by incubation in an anaerobic chamber (AnaeroJar; Oxoid). Hydrolysis of casein, starch, Tween 80 and gelatin was tested as described by Smibert & Krieg (1994) on MA. Gram stain, flagella stain and tests for catalase and oxidase were performed using conventional methods (Dong & Cai, 2001). Substrate utilization, activities of constitutive enzymes and other physiological properties were determined using the API 50 CH, API 20 E, API 20 NE and API ZYM systems (bioMérieux) with inoculum prepared in 3% (w/v) sea salt solution (Sigma). Acid production from carbohydrates was determined using the API 50 CH system with 3% (w/v) NaCl added to the API 50 CHB/E medium. All tests were performed in duplicate. The morphological, physiological and biochemical characteristics of strain BCw111^T are given in the species description and Table 1.

Respiratory quinones were extracted and purified according to Collins (1985) and analysed by HPLC (Wu *et al.*, 1989). For fatty acid methyl ester analysis, cells of strain BCw111^T, *C. polaris* CGMCC 1.6132^T and *C. aestuarii* CGMCC 1.6965^T were harvested from MA after incubation at 25 °C for 48 h. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the Microbial Identification system (MIDI; Sasser, 1990). To

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain BCw111^T is FJ889599.

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

Table 1. Characteristics that differentiate strain BCw111^T from its closest phylogenetic neighbours

Strains: 1, *Colwellia chukchiensis* sp. nov. BCw111^T; 2, *C. polaris* CGMCC 1.6132^T; 3, *C. aestuarii* CGMCC 1.6965^T. Data were obtained in this study unless indicated. All strains are positive for cytochrome oxidase, catalase, alkaline phosphatase and leucine arylamidase and utilize D-glucose as a sole carbon source.

Characteristic	1	2	3
Growth at 30 °C	+	—	+
Growth with 7 % NaCl	+	—	—
Nitrate reduction	+	—	+
Hydrolysis of:			
Aesculin	+	+	—
Gelatin	+	+	—
Starch	—	+	—
Urea	+	—	—
API ZYM results			
Valine arylamidase	+	—	—
β-Glucosidase	—	—	+
Esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase	+	—	+
Utilization of:			
Glycerol, D-fructose, D-mannose, sucrose, D-mannitol, D-sorbitol	—	+	—
L-Arabinose, maltose	—	+	+
Cellobiose, trehalose, D-xylose, salicin	—	—	+
Citrate	+	—	—
Acid production from:			
Lactose, D-fructose, L-arabinose, D-mannose	—	—	+
Glycerol, D-mannitol, starch, sucrose	—	+	—
Melibiose	—	+	+
D-Galactose	+	+	—
Major fatty acids (>10 % of total)*	SF3, C _{16:0} , C _{17:1}	SF3, C _{15:1} , C _{17:1}	SF3, C _{17:1} , C _{15:1} , i-C _{16:0}
DNA G + C content (mol%)	41.3	38.9 ^{a†}	39.3 ^b

*SF3, Summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1ω7c}); i, iso-branched.

†Data taken from: *a*, Zhang *et al.* (2008); *b*, Jung *et al.* (2006).

determine the DNA G + C content of strain BCw111^T, DNA was extracted using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). The G + C content was determined by HPLC according to the method of Mesbah *et al.* (1989) with non-methylated lambda DNA (Sigma) and DNA from *Bacillus subtilis* DSM 402, *Xanthomonas campestris* pv. *campestris* DSM 3586^T and *Streptomyces violaceoruber* DSM 40783 as references. Cells of strain BCw111^T contained ubiquinone-8 (Q-8; 98 %) and ubiquinone-7 (Q-7; 2 %) as the respiratory quinones. The major cellular fatty acids (>10 % of the total) of strain BCw111^T were iso-C_{15:0} 2-OH and/or C_{16:1ω7c} 2-OH (28.13 %), C_{16:0} (13.28 %) and C_{17:1} (12.90 %). The fatty acid profiles of strain BCw111^T, *C. polaris* CGMCC 1.6132^T and *C. aestuarii* CGMCC 1.6965^T are given in Supplementary Table S1 (available in IJSEM Online). The DNA G + C content of strain BCw111^T was 41.3 mol%.

For sequencing and phylogenetic analysis of the 16S rRNA gene of strain BCw111^T, DNA was extracted and purified with a commercial kit (BioDev, Beijing, China) and amplified by PCR using universal primers 8f

(5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3') (Weisburg *et al.*, 1991). The purified PCR product was ligated and cloned into pMD 18-T (TaKaRa) according to the manufacturer's instructions. Sequencing reactions were carried out using an ABI BigDye Terminator 3.1 sequencing kit and an automated DNA sequencer (ABI 3730; Applied Biosystems). The nearly complete (1463 nt) 16S rRNA gene sequence of strain BCw111^T was submitted to public databases to search for similar sequences using the BLAST algorithm. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Sequences were aligned using CLUSTAL X version 1.8 (Thompson *et al.*, 1997) and edited manually using the BioEdit sequence alignment editor version 5.0.9 (Hall, 1999). Phylogenetic trees were constructed using the neighbour-joining and maximum-parsimony methods with Kimura's two-parameter model in MEGA version 4 (Tamura *et al.*, 2007) and the maximum-likelihood method in PHYLIP version 3.69 (Felsenstein, 2009).

The phylogenetic analysis showed that strain BCw111^T grouped with the members of the genus *Colwellia* and formed a distinct cluster with *C. polaris* 537^T and *C. aestuarii* SMK-10^T, which was supported by a bootstrap value of 100 % in the neighbour-joining tree (Fig. 1). Similar tree topologies were found with the maximum-parsimony and maximum-likelihood algorithms (Supplementary Figs S1 and S2). Pairwise analysis revealed that strain BCw111^T exhibited 97.8 and 97.1 % 16S rRNA gene sequence similarity with *C. polaris* 537^T and *C. aestuarii* SMK-10^T, respectively, and <95.9 % 16S rRNA gene sequence similarity with other members of the genus *Colwellia*. Although there is no precise correlation between 16S rRNA gene sequence similarity and species delineation, it is generally recognized that ≥3 % sequence divergence is significant (Stackebrandt & Goebel, 1994).

To differentiate strain BCw111^T from its closest phylogenetic neighbours, DNA–DNA hybridization was carried out using the liquid renaturation method (De Ley *et al.*, 1970) as modified by Huß *et al.* (1983) using a DU800 spectrophotometer (Beckman) with a thermal controller at 65 °C. DNA–DNA relatedness between strain BCw111^T and *C. polaris* CGMCC 1.6132^T and *C. aestuarii* CGMCC 1.6965^T was 42.6±6.5 and 19.8±7.8 %, respectively. These values indicated that strain BCw111^T was distinct from these type strains (Wayne *et al.*, 1987).

The genetic distinctiveness of strain BCw111^T was supported by phenotypic characteristics. In contrast to *C. polaris* CGMCC 1.6132^T and *C. aestuarii* CGMCC 1.6965^T, strain BCw111^T was able to grow with 7 % NaCl, to hydrolyse urea and to utilize citrate and it contained C_{15:0}, C_{18:1ω7c} and larger amounts of C_{16:0} (Table 1 and Supplementary Table S1).

Therefore, on the basis of phenotypic, phylogenetic and genotypic data, strain BCw111^T represents a novel species of the genus *Colwellia*, for which the name *Colwellia chukchiensis* sp. nov. is proposed.

Description of *Colwellia chukchiensis* sp. nov.

Colwellia chukchiensis (chuk.chi.en'sis. N.L. fem. adj. *chukchiensis* named after the Chukchi Sea, the geographical origin of the type strain).

Cells are Gram-negative, psychrotolerant, curved rods (0.5–1.0 × 1.1–4.5 µm). Motile by means of a single polar flagellum. Growth occurs under anaerobic conditions on MA and on MA supplemented with nitrate. Colonies are non-pigmented, convex, circular and smooth with entire edges. Growth occurs at 0–30 °C (optimum 23–25 °C), but not at 33 °C, at pH 5.0–10.5 (optimum pH 6.0–8.0) and with 0.5–8.0 % (w/v) NaCl, but not without NaCl. Catalase- and cytochrome oxidase-positive. Hydrogen sulfide production, indole production and Voges–Proskauer tests are negative. Nitrate is reduced to nitrogen. Casein, gelatin and Tween 80 are hydrolysed. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, valine arylamidase and leucine arylamidase, but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, lipase (C14), α-chymotrypsin, acid phosphatase, trypsin, cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. With API 50 CH, D-galactose, D-glucose and citrate are utilized as sole carbon sources and acid is produced from D-galactose and citrate. The major cellular fatty acids (>10 %) are iso-C_{15:0} 2-OH and/or C_{16:1ω7c}, C_{16:0} and C_{17:1}. The major respiratory quinone is Q-8. The DNA G+C content of the type strain is 41.3 mol%.

The type strain is BCw111^T (=CGMCC 1.9127^T =LMG 25329^T =DSM 22576^T), isolated from the Chukchi Sea in the Arctic Ocean (73° 00' 00" N 169° 32' 44" W).

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Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain BCw111^T and related taxa. Bootstrap values (>50 %) based on 1000 resampled datasets are shown at branch nodes. Bar, 1 % sequence divergence.

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