Colwellia polaris sp. nov., a psychrotolerant bacterium isolated from Arctic sea ice

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A novel psychrotolerant, Gram-negative, aerobic bacterium, designated strain 537^T, was isolated from sea-ice samples from the Arctic. Strain 537^T was able to grow at 4–26 °C, with optimum growth occurring at 20–21 °C. Strain 537^T had Q-8 as the major respiratory quinone and contained iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (22.95 %), C_{15:1} (17.64 %) and C_{17:1}ω8c (13.74 %) as the predominant cellular fatty acids. The genomic DNA G + C content was 38.9 mol%. A phylogenetic analysis based on 16S rRNA gene sequences indicated that strain 537^T formed a coherent cluster within the genus *Colwellia*. The highest level of 16S rRNA gene sequence similarity (97.5 %) exhibited by strain 537^T was obtained with respect to the type strain of *Colwellia aestuarii*. On the basis of phenotypic, chemotaxonomic and phylogenetic properties and DNA–DNA relatedness data, strain 537^T represents a novel species of the genus *Colwellia*, for which the name *Colwellia polaris* sp. nov. is proposed. The type strain is 537^T (=CGMCC 1.6132^T =JCM 13952^T).

The family *Colwelliaceae*, which includes the genera *Colwellia* and *Thalassomonas*, accommodates bacteria that are Gram-negative, seawater-requiring, chemo-organotrophic, curved or straight rods and contain Q-8 as the predominant isoprenoid quinone (Ivanova *et al.*, 2004; Jung *et al.*, 2006). The genus *Colwellia* was originally proposed by Deming *et al.* (1988) with the description of two facultatively anaerobic bacteria, *Colwellia psychrerythraea* and *Colwellia hadaliensis*. Seven further species have since been added to the genus: *Colwellia demingiae*, *C. hornerae*, *C. psychrotropica* and *C. rossensis* (Bowman *et al.*, 1998), *C. maris* (Yumoto *et al.*, 1998), *C. piezophila* (Nogi *et al.*, 2004) and *C. aestuarii* (Jung *et al.*, 2006). During a survey of cold-adapted micro-organisms from Arctic sea-ice samples, we isolated a novel psychrotolerant bacterial strain. In this study, we report the isolation and identification of that strain, designated 537^T. Physiological, biochemical and chemotaxonomic data, together with the results of a phylogenetic analysis based on 16S rRNA gene sequences, indicated that the novel isolate represents a novel species of the genus *Colwellia*.

During the Second Chinese National Arctic Research Expedition cruise of the Chinese ice-breaker M/V *Xue Long* in August 2003, strain 537^T was isolated from sea-ice samples collected from the Canadian Basin (77° 30' 59" N 152° 52' 04" W) using a MARK II ice auger. Sea-ice samples were cut carefully into 10–20 cm sections using a sterile saw and placed in sterile plastic bottles for melting at 4 °C. Strain 537^T was obtained using previously described media and methods (Zhang *et al.*, 2006). *C. aestuarii* DSM 17314^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). The culture was routinely grown on marine agar 2216 (MA; Difco) or marine broth 2216 (MB; Difco) at 27 °C and used as a reference strain.

Cell morphology was examined with a Zeiss Axioplan 2 microscope. Colony morphology was observed on MA after incubation at 20 °C for 24–36 h. The growth temperature was determined with a TN3F temperature-gradient incubator (Advantec). The pH range for growth was determined for the strain in MB at various pH values adjusted with 1 M HCl or 1 M NaOH. General physiological tests were performed using conventional methods (Dong & Cai, 2001). Acid production from carbohydrates was determined as described by Leifson (1963). Biochemical traits were determined using API kits (API 20E, API 20NE and API ZYM; bioMérieux) according to the manufacturer's instructions, except that the cultures

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 537^T is DQ007434.

Cellular fatty acid compositions of strain 537^T and *Colwellia* species are presented in a supplementary table available with the online version of this paper.

were suspended in 3 % (w/v) NaCl solution. Cells of strain 537^T were Gram-negative, motile, curved rods. Colonies on MA were white, smooth, circular and convex with entire margins. Strain 537^T grew aerobically; growth occurred at 4–26 °C and the optimal growth temperature was 20–21 °C. Growth occurred in the presence of 1–6 % (w/v) NaCl and no growth occurred in the absence of NaCl.

DNA was extracted and purified as described by Sambrook *et al.* (1989). The 16S rRNA gene was amplified by using a PCR with two universal primers (Zhang *et al.*, 2006). The purified PCR product was ligated to the vector pMD 18-T (TaKaRa Bio) and cloned according to the manufacturer's instructions. Sequencing reactions were carried out using the ABI BigDye 3.1 sequencing kit (Applied Biosystems) and an automated DNA sequencer (model ABI3730; Applied Biosystems). The BLAST algorithm was used to search the GenBank and EMBL databases for sequences similar to the almost-complete 16S rRNA gene sequence (1501 bp) obtained for strain 537^T. A phylogenetic tree was constructed using Kimura two-parameter and pairwise-deletion model analysis implemented in the program MEGA, version 3.0 (Kumar *et al.*, 2004). The resultant tree topology was evaluated by means of a bootstrap analysis based on 1000 replicates. The phylogenetic analysis (Fig. 1) showed that strain 537^T grouped with the members of the genus *Colwellia*, forming a distinct cluster with *C. aestuarii* DSM 17314^T (97.5 %). The levels of 16S rRNA gene sequence similarity between strain 537^T and other strains used in the phylogenetic analysis were less than 96 %.

Isoprenoid quinones were determined as described previously (Komagata & Suzuki, 1987), using reversed-phase HPLC. For the analysis of whole-cell fatty acid compositions, strain 537^T and *C. aestuarii* DSM 17314^T were grown on MA for 4 days at 20 and 27 °C, respectively. Fatty acid methyl esters were prepared and analysed according to the standard protocol described in the MIDI (Microbial Identification) system (Sasser, 1990). Cells of strain 537^T contained Q-8 as the major respiratory quinone. The predominant cellular fatty acids of strain 537^T were

iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (22.95 %), C_{15:1} (17.64 %), C_{17:1}ω8c (13.74 %), C_{16:0} (7.55 %), iso-C_{12:0} 3-OH (6.45 %) and iso-C_{16:0} (5.69 %); the fatty acid profile resembles those determined for other *Colwellia* species (Bowman *et al.*, 1998; Nogi *et al.*, 2004; Jung *et al.*, 2006) (see Supplementary Table S1, available in IJSEM Online).

The DNA G + C content was determined using the thermal denaturation method, with *Escherichia coli* K-12 as the reference, and DNA–DNA hybridization was performed using the liquid renaturation method (De Ley *et al.*, 1970) as modified by Huß *et al.* (1983). Both experiments were carried out using a DU800 spectrophotometer (Beckman) with a thermal controller. The DNA G + C content of strain 537^T was 38.9 mol% and the DNA–DNA relatedness between strain 537^T and *C. aestuarii* DSM 17314^T was 42.5 %.

Strain 537^T was distinguishable from type strains of *Colwellia* species by using a combination of physiological and biochemical properties (Table 1). On the basis of these results, strain 537^T represents a novel species of the genus *Colwellia*, for which the name *Colwellia polaris* sp. nov. is proposed.

Description of *Colwellia polaris* sp. nov.

Colwellia polaris (po.la'ris. N.L. fem. adj. *polaris* polar, referring to the isolation of the type strain from the Arctic Ocean).

Cells are Gram-negative, psychrotolerant, curved rods, 0.6–0.9 × 0.9–4 µm. Colonies are non-pigmented, convex, circular and smooth with entire edges. Growth occurs at 4–26 °C and pH 5.0–10.0; optimum growth occurs at 20–21 °C and approximately pH 6.0–9.0. Grows aerobically and produces catalase and cytochrome oxidase. Growth occurs in the presence of 1–6 % (w/v) NaCl and no growth occurs in the absence of NaCl. Negative for indole production and in the Voges–Proskauer test. Negative for nitrate reduction and the production of hydrogen sulfide.

Fig. 1. Neighbour-joining phylogenetic dendrogram, based on 16S rRNA gene sequences, for strain 537^T and related species. Bootstrap percentages (based on 1000 resampled datasets) greater than 50 % are shown at branch points. GenBank accession numbers are shown in parentheses. Bar, 2 % sequence divergence.

Table 1. Phenotypic characteristics that differentiate strain 537^T from other *Colwellia* species

Taxa: 1, strain 537^T; 2, *C. aestuarii* (data from Jung *et al.*, 2006); 3, *C. psychrerythraea* (Deming *et al.*, 1988; Bowman *et al.*, 1998); 4, *C. demingiae* (Bowman *et al.*, 1998); 5, *C. hornerae* (Bowman *et al.*, 1998); 6, *C. psychrotropica* (Bowman *et al.*, 1998); 7, *C. rossensis* (Bowman *et al.*, 1998); 8, *C. maris* (Yumoto *et al.*, 1998); 9, *C. piezophila* (Nogi *et al.*, 2004). +, Positive; –, negative; v (+), variable (type strain positive); NG, no growth on test medium; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9
Motility	+	+	+	+	+	+	–	+	+
Growth at 30 °C	–	+	–	–	–	–	–	–	–
Hydrolysis of:									
Aesculin	+	–	+	+	+	–	NG	ND	ND
Gelatin	+	–	v (+)	–	–	–	–	+	+
Starch	+	–	+	+	+	–	+	–	–
Casein	+	+	+	+	+	+	–	–	–
Urea	–	–	v (+)	–	–	+	+	ND	ND
Tween 80	+	+	+	–	+	+	NG	+	ND
Utilization of:									
Glucose	+	+	v (+)	–	–	–	+	+	ND
Cellobiose	–	+	–	–	–	–	–	ND	–
Fructose	+	–	–	–	–	–	+	–	–
Acetate	+	–	+	+	+	+	+	ND	ND
Acid production from:									
Mannitol	+	–	–	–	–	–	–	ND	–
Melibiose	+	+	–	–	–	–	–	–	ND
Lactose	–	+	–	–	–	–	–	ND	–
Sucrose	–	+	–	–	–	–	–	–	–
Fructose	–	+	–	–	–	–	–	–	–
Arabinose	–	+	–	–	–	–	–	–	–
DNA G + C content (mol%)	38.9	39.3	35–38	37	39	42	38	39.4	38.5–39.1

Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, urease, agarase, chitinase, lecithinase, esterase (C4), esterase lipase (C8), lipase (C14), α -chymotrypsin, acid phosphatase, trypsin, naphthol-AS-BI-phosphohydrolase, cystine arylamidase, valine arylamidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase and *N*-acetyl- β -glucosaminidase. Positive for gelatinase, caseinase, amylase, alkaline phosphatase and leucine arylamidase. The following substrates are utilized as sole carbon sources: glucose, mannitol, D-mannose, sucrose, D-sorbitol, L-arabinose, maltose, fructose, D-galactose, melibiose, glycerol and pyruvate. The following substrates are not utilized as sole carbon sources: cellobiose, xylose, melezitose, galactitol, erythritol, salicin, lysine, valine, glycine, cysteine, citrate, hippurate, malate, succinate and tartrate. Acid is produced from mannitol, maltose, melibiose, D-galactose and glycerol. The predominant cellular fatty acids are iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c (22.95 %), C_{15:1} (17.64 %), C_{17:1} ω 8c (13.74 %), C_{16:0} (7.55 %), iso-C_{12:0} 3-OH (6.45 %) and iso-C_{16:0} (5.69 %). The major respiratory quinone is Q-8. The DNA G + C content of the type strain is 38.9 mol%.

The type strain, 537^T (=CGMCC 1.6132^T =JCM 13952^T), was isolated from sea ice collected from the Canadian Basin in the Arctic Ocean.

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