**Moritella dasanensis** sp. nov., a psychrophilic bacterium isolated from the Arctic ocean

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An aerobic, motile, Gram-negative, ice-active substance-producing, rod-shaped psychrophile, designated strain ArB 0140ᵀ, was isolated from seawater collected from near a glacier in Kongsfjorden, Svalbard Archipelago, Norway. Phylogenetic analysis using 16S rRNA gene sequences indicated that strain ArB 0140ᵀ showed a distinct phyletic line within the genus *Moritella*. Characteristic chemotaxonomic data [predominant isoprenoid quinone, Q8; major fatty acids, C₁₄:₀, C₁₄:₁, C₁₆:₀, C₁₆:₁ and C₂₂:₆ (docosahexaenoic acid; DHA)] also corroborated the affiliation of strain ArB 0140ᵀ to the genus *Moritella*. The maximal growth rate of the novel strain was observed at 9 °C, with a maximum temperature for growth of 18 °C. The genomic DNA G + C content was 46.9 mol%. Based on the data obtained from this polyphasic study, including DNA–DNA relatedness, physiological and biochemical tests and ice-controlling activity, strain ArB 0140ᵀ was found to be genetically and phenotypically different from other recognized species of the genus *Moritella*. Therefore strain ArB 0140ᵀ represents a novel species, for which the name *Moritella dasanensis* sp. nov. is proposed. The type strain is ArB 0140ᵀ (=KCTC 10814ᵀ=KCCM 42845ᵀ=JCM 14759ᵀ).

Ice-active substances (IAS) are macromolecular substances found from numerous Antarctic terrestrial and aquatic organisms that affect the shape of ice crystals by binding to the growing ice crystals (Raymond, 2000; Raymond & Fritsen, 2000, 2001). These substances are slightly different from antifreeze proteins or ice-structuring proteins in that they do not significantly lower the freezing point of the sample in which they are contained. The ability to secrete IAS is widely distributed amongst living organisms and has been shown to occur in various sea diatoms, lichens, cyanobacteria (*Nostoc* sp. and *Phormidium* sp.), green algae (*Prasiola* sp.), mosses (*Bryum* sp.) and the nematode *Panagrolaimus davidi* (Raymond et al., 1994; Raymond, 2000; Raymond & Fritsen, 2000; Raymond & Knight, 2005; Wharton et al., 2005). Various species of Antarctic ice fish (the notothenioids) also possess IAS (glycoproteins) in their blood.

Species of the genus *Moritella* occur in seawater, fish farms, marine sediments and the abyssal ocean (Steven, 1990; Nogi et al., 1998; Urakawa et al., 1998; Nogi & Kato, 1999; Benediktsdottir et al., 2000; Xu et al., 2003). At the time of writing, the genus *Moritella* consists of six species. Non-piezophilic *Moritella marina* was isolated from seawater or sediment of the North Pacific Ocean (Urakawa et al., 1998). Two piezophilic species, *Moritella japonica* and *Moritella yayanosii* were isolated from the Japan Trench and Mariana Trench, respectively (Nogi et al., 1998; Nogi & Kato, 1999). The psychrotolerant species, *Moritella viscosa*, pathogenic for Atlantic salmon parr, was isolated from the lesions or the internal organs of fish (Benediktsdottir et al., 2000). Recently, two psychropiezophilic bacteria, *Moritella profunda* and *Moritella abyssi* were isolated from the deep-sea of the eastern tropical Atlantic (Xu et al., 2003). Since most of the strains in this genus have been isolated from the deep-sea where the temperature favours psychrophilic micro-organisms, psychrophyll is a characteristic of this genus, with some species also being piezophilic. In this study, taxonomic and phylogenetic analyses of a novel psychrophilic bacterial strain isolated from the open sea off the coast of Svalbard, Norway, are presented.
The IAS-producing bacterial strain ArB 0140T was collected from surface seawater near a glacier at Kongsfjorden, Svalbard Archipelago (78° 55’ N 11° 56’ E). The temperature was 3 °C. The sample was stored in an ice cooler and was subsequently diluted in sterilized artificial seawater and spread onto marine 2216 agar (MA; Difco) at 3 °C. The strain was isolated by serial inoculation. The isolated colony was routinely cultivated for three weeks at 3 °C. Strain ArB 0140T was Gram-negative, motile and non-spore-forming. Cells ranged from 2–7 μm in length and 0.8–1.2 μm in diameter (see Supplementary Fig. S1 available in IJSEM Online). Three *Moritella* strains were used as reference strains in this study; *Moritella marina* ATCC 15381T, *Moritella viscosa* NCIMB 13584T and *Moritella japonica* JCM 10249T were grown and maintained as previously described (Steven, 1990; Nogi et al., 1998; Benediktsdottir et al., 2000; Xu et al., 2003).

Growth of strain ArB 0140T was tested on marine broth 2216 (MB; Difco), diluted MB (10 %), nutrient broth, tryptic soy broth (TSB; Difco) and Zobell’s broth. The pH range for growth was determined by incubating cells in MB for 3 days, using a waterbath (RW-0525G; Jeio TECH) for temperatures of −3 and −1 °C and a temperature gradient incubator (TVS 126MA; Advantec) for temperatures of between 5 and 30 °C. The optimal NaCl concentration for growth was determined by incubating cells at 11 °C in Bacto tryptic soy broth containing 1–12 % (w/v) NaCl at 1 % increments. The pH range for growth was determined by incubating cells at 11 °C in MB containing 3 % NaCl at pH values of 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 or 10.0. Motility was examined with an optical microscope using the hanging-drop technique (Skerman, 1967). The morphological and physiological characteristics were investigated with cells grown on MA or MB with 3 % NaCl added at 9 °C. For the physiological tests, API kits (bioMérieux) were used with slight modifications. A final concentration of 3 % NaCl was added to the inoculum medium provided in the kits since strain ArB 0140T grew optimally at this salt concentration. The API 50 CHB kit was used to test for acid production from carbohydrates; the API 20NE kit for nitrate and nitrite reduction, indole production, arginine dihydrolase, urease, aesculinase, gelatinase, motility and non-spore-forming. Cells grown on MA (see Supplementary Fig. S1 available in IJSEM Online) were C14 : 0, C14 : 1, C16 : 0 and C16 : 1. Docosahexaenoic acid (DHA; C22 : 6), the major fatty acid of this strain grown on MA (see Nogi et al., 1998 for sample analysis) were C14 : 0, C14 : 1, C16 : 0 and C16 : 1. Docosahexaenoic acid (DHA; C22 : 6), the characteristic fatty acid of the genus *Moritella*, was also present (Delong et al., 1997; Kato et al., 1998). In addition, the major respiratory isoprenoid lipoquinone (Komaga & Suzuki, 1987; Nogi et al., 1998) was Q-8, as found for other species of the genus *Moritella* (Nogi & Kato, 1999; Benediktsdottir et al., 2000; Xu et al., 2003).

Genomic DNA was extracted from bacterial cells and the 16S rRNA gene was amplified by the methods of Rainey et al. (1992). The 16S rRNA gene sequence of strain ArB 0140T was determined as described by Chun & Goodfellow (1995). The resulting almost-complete 16S rRNA gene sequence was analysed against class *Gammaproteobacteria* reference sequences using the PAUP version 4.0 (Sinauer Associates) software package. The analysis placed our isolate within the cluster comprising species of the genus *Moritella* and joined *Moritella* sp. (GenBank accession no. AY700605) with a bootstrap value of 99.33 % (Saitou & Nei, 1987). From this analysis, strain ArB 0140T was found to cluster within the genus *Moritella* (Fig. 1). The 16S rRNA gene sequence showed 99.33 % similarity with *Moritella viscosa* JCM 10249T (GenBank accession no. AY700605), *Moritella marina* ATCC 15381T and *Moritella marina* DSM 9775T. The optimal growth temperature was 9 °C, based on the Ratkowsky growth model analysis (Ratkowsky et al., 1983) of the data obtained, the notional minimum, optimum and maximum growth temperatures were −11.9, 9 and 17.8 °C, respectively. A graph showing the growth rate of strain ArB 0140T over the temperature range studied is available as Supplementary Fig. S2 in IJSEM Online. No growth was observed in the medium containing less than 1.5 % NaCl or above 5 % NaCl. The optimal concentration of NaCl for growth was 2.5 %. Slow or no growth was detected below pH 4.5 and above pH 10.5. Sucrose and glucose were not fermented. The metabolism of strain ArB 0140T was aerobic. Strain ArB 0140T was psychrophilic and halophilic. Strain ArB 0140T was cultivated for 2 days at 3 °C in MB and harvested at 8000 g for 10 min. Fatty acid methyl esters were analysed by GLC according to the Microbial Identification system (MIDI, 1999). Isoprenoid quinone was extracted according to the method of Minnikin et al. (1984) and analysed by HPLC as previously described in Collins (1985). The fatty acid profile showed that the major cellular fatty acids of this strain grown on MA (see Nogi et al., 1998, for sample analysis) were C14 : 0, C14 : 1, C16 : 0 and C16 : 1. Docosahexaenoic acid (DHA; C22 : 6), the characteristic fatty acid of the genus *Moritella*, was also present (Delong et al., 1997; Kato et al., 1998). In addition, the major respiratory isoprenoid lipoquinone (Komaga & Suzuki, 1987; Nogi et al., 1998) was Q-8, as found for other species of the genus *Moritella* (Nogi & Kato, 1999; Benediktsdottir et al., 2000; Xu et al., 2003).
rRNA gene sequence of strain ArB 0140\textsuperscript{T} shared sequence similarities of 98.1–98.8\% with the type strains of the recognized species of the genus Moritella, including \textit{M. abyssi} (98.8\%), \textit{M. marina} (98.7\%), \textit{M. profunda} (98.5\%), \textit{M. japonica} (98.1\%) and \textit{M. viscosa} (98.1\%).

The G+C content of the genomic DNA was determined as described previously (Tamaoka & Komagata, 1984). The DNA was hydrolysed and analysed by reverse-phase HPLC.

DNA–DNA hybridization between strain ArB 0140\textsuperscript{T} and other species of the genus Moritella was performed according to the method of Ezaki \textit{et al.} (1989). The G+C content of the DNA of strain ArB 0140\textsuperscript{T} was 46.9 mol\%.

DNA–DNA hybridization was performed to determine the genomic relatedness between strain ArB 0140\textsuperscript{T} and two other species of the genus Moritella. Strain ArB 0140\textsuperscript{T} exhibited mean values of DNA–DNA relatedness to the type strains of \textit{M. abyssi} and \textit{M. marina} of 45.69\% and 43.46\%, respectively. The determined values were well below the threshold value of 70\% accepted for the differentiation of species within a particular genus (Wayne \textit{et al.}, 1987).

Phenotypic characteristics differentiating strain ArB 0140\textsuperscript{T} from the six recognized species of the genus Moritella are shown in Table 1.

To examine any ice-modifying activity, strain ArB 0140\textsuperscript{T}, \textit{M. abyssi} JCM 11436\textsuperscript{T}, \textit{M. marina} ATCC 15381\textsuperscript{T} and \textit{M. japonica} JCM 10249\textsuperscript{T} were grown in MB at 4\,\textdegree C. Supernatants of culture media were obtained by centrifugation and used directly for the assay. Two assay systems were employed to characterize ice-modifying activity. Firstly, ice-pitting activity was measured which exploits the tendency of IAS to form pits on the basal planes of growing ice crystals (Raymond, 2000; Raymond & Fritsen, 2000). Secondly, hexagonal ice crystal formation during crystal growth from a single seed crystal was observed using nanolitre osmometry as described previously (Wharton \textit{et al.}, 2005). Strain ArB 0140\textsuperscript{T} exhibited ice-modifying activity but other species of the genus Moritella examined did not (see Supplementary Fig. S3 in IJSEM Online).

\textbf{Description of Moritella dasanensis sp. nov.}

\textit{Moritella dasanensis} (da.san.en.sis. N.L. fem. adj. dasanensis pertaining to the Korean Arctic Dasan station where the type strain was isolated).

Cells are Gram-negative, rod-shaped, non-spore-forming and motile by means of a single, unsheathed, polar flagellum. Cells are 0.8–1.2\,\mu m in diameter and 2.0–7.0

\begin{table}[ht]
\centering
\small
\begin{tabular}{|l|c|c|c|c|c|}
\hline
\textbf{Characteristic} & \textbf{1} & \textbf{2} & \textbf{3} & \textbf{4} & \textbf{5} & \textbf{6} \\
\hline
Temperature (\textdegree C) for maximum growth rate & 18 & 10 & NG & 2 & 4–6 & 5 \\
\hline
DNA G+C content (mol\%) & 42.5 & 45 & 44.6 & 41.4 & 41.6 & 46.9 \\
\hline
Gelatinase & + & + & + & – & – & + \\
\hline
\hline
Acid produced from: & & & & & & \\
\hline
Cellobiose & + & – & – & – & + & – \\
\hline
D-Galactose & + & – & – & ± & + & – \\
\hline
Glycerol & + & + & – & – & – & – \\
\hline
Maltose & + & + & – & – & – & + \\
\hline
\hline
\hline
\hline
Utilization as carbon source: & & & & & & \\
\hline
\hline
Cellobiose & + & – & – & – & + & – \\
\hline
d-Galactose & + & – & – & + & + & + \\
\hline
Glycerol & + & + & – & + & + & + \\
\hline
Maltose & + & – & – & – & – & + \\
\hline
Trehalose & – & – & – & – & – & + \\
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\end{tabular}
\end{table}
μm in length. On MA, colonies are smooth, flat, and cream coloured. Psychrophilic and halophilic. The temperature range for growth in MB is from −3 to 17 °C. The optimal growth temperature is 9 °C. No growth occurs above 18 °C. The optimal NaCl concentration for growth is around 2.5 % (w/v). No growth occurs in the absence of NaCl. The optimal pH for growth is pH 8. Catalase and cytochrome oxidase tests are positive. Nitrate is reduced to nitrite but no gas is produced. No indole is produced from tryptophan. Does not produce acid from arabinose, inositol, D-lactose, raffinose, sucrose, d-sorbitol, trehalose, cellulose, D-galactose, glycerol, D-mannitol, D-mannose or xylitol. Produces acid from maltose. The following substrates are utilized for growth: trehalose, D-arabinose and glycero. Does not grow on sucrose or D-sorbitol as sole carbon sources for growth. The DNA G+C content of the type strain is 46.9 mol%. The major isoprenoid quinone is Q-8. Predominant cellular fatty acids are C14:0, C16:0, C16:1 and C22:6. The type strain, strain Ab 0140T (=KCTC 10814T=KCCM 42845T=JCM 14759T) was isolated from surface seawater off the near shore of Kongosfjorden in the Svalbard Archipelago, Norway.

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References


