

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

Hak Jun Kim,¹ Soyoung Park,¹ Jung Min Lee,¹ Seungil Park,¹ Woongsic Jung,¹ Jae-Shin Kang,² Hyung Min Joo,¹ Ki-Won Seo¹ and Sung-Ho Kang¹

Correspondence
Sung-Ho Kang
shkang@kopri.re.kr

¹Department of Applied Polar Sciences, Korea Polar Research Institute, KORDI, 7-50 Songdo-dong, Incheon 406-840, Republic of Korea

²Biological Resources Research Department, National Institute of Biological Resources, Environmental Research Complex, Incheon 404-170, Republic of Korea

An aerobic, motile, Gram-negative, ice-active substance-producing, rod-shaped psychrophile, designated strain ArB 0140^T, 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 0140^T showed a distinct phyletic line within the genus *Moritella*. Characteristic chemotaxonomic data [predominant isoprenoid quinone, Q8; major fatty acids, C_{14:0}, C_{14:1}, C_{16:0}, C_{16:1} and C_{22:6} (docosahexaenoic acid; DHA)] also corroborated the affiliation of strain ArB 0140^T 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 0140^T was found to be genetically and phenotypically different from other recognized species of the genus *Moritella*. Therefore strain ArB 0140^T represents a novel species, for which the name *Moritella dasanensis* sp. nov. is proposed. The type strain is ArB 0140^T (=KCTC 10814^T=KCCM 42845^T=JCM 14759^T).

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 ice 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, 2003; Wharton *et al.*, 2005). Various species of Antarctic ice fish (the notothenioids) also possess IAS (glycoproteins) in their blood.

Abbreviations: DHA, docosahexaenoic acid; IAS, ice-active substances.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Moritella dasanensis* sp. nov. ArB 0140^T is EF192283.

A scanning electron micrograph of cells of strain ArB 0140^T, a graph showing the growth rate of the strain over a range of temperatures and images showing the ice-modifying activity of strain ArB 0140^T and other species of the genus *Moritella* are available as supplementary figures with the online version of this paper.

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, psychrophily 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 0140^T 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 0140^T 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 15381^T, *Moritella viscosa* NCIMB 13584^T and *Moritella japonica* JCM 10249^T 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 0140^T was tested on marine broth 2216 (MB; Difco), diluted MB (10%), nutrient broth, tryptic soy broth (TSB; Difco) and Zobell's broth. The temperature 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 0140^T 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, gelatinase, aesculinase, β-galactosidase and assimilation of sole carbon sources and the API ZYM kit was used to test for other enzymic activities.

Strain ArB 0140^T grew at –3–18 °C. 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 0140^T 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 0140^T was aerobic. Strain ArB 0140^T was psychrophilic and halophilic.

Strain ArB 0140^T 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 C_{14:0}, C_{14:1}, C_{16:0} and C_{16:1}. Docosahexaenoic acid (DHA; C_{22:6}), 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 (Komagata & 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 0140^T 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 0140^T was found to cluster within the genus *Moritella* (Fig. 1). The 16S

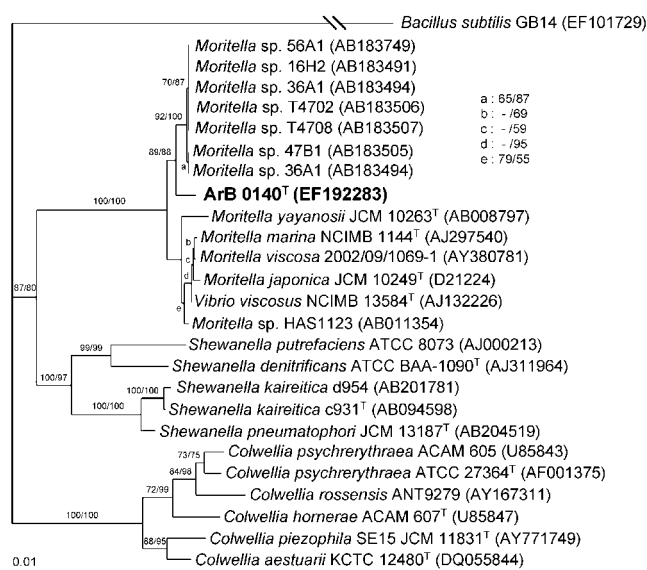


Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence analysis showing relationships between strain ArB 0140^T and other members of the class *Gammaproteobacteria*. The tree was plotted using the neighbour-joining method. The values of bootstrap percentages from neighbour-joining and maximum-parsimony analyses are shown on branch nodes and were calculated from 1000 replications. Bar, 0.01 nucleotide substitutions per position.

rRNA gene sequence of strain ArB 0140^T shared sequence similarities of 98.1–98.8% with the type strains of the recognized species of the genus *Moritella*, including *M. abyssi* (98.8%), *M. marina* (98.7%), *M. profunda* (98.5%), *M. japonica* (98.1%) and *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^T and other species of the genus *Moritella* was performed according to the method of Ezaki *et al.* (1989). The G+C content of the DNA of strain ArB 0140^T was 46.9 mol%. DNA–DNA hybridization was performed to determine the genomic relatedness between strain ArB 0140^T and two other species of the genus *Moritella*. Strain ArB 0140^T exhibited mean values of DNA–DNA relatedness to the type strains of *M. abyssi* and *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 *et al.*, 1987).

Phenotypic characteristics differentiating strain ArB 0140^T from the six recognized species of the genus *Moritella* are shown in Table 1.

To examine any ice-modifying activity, strain ArB 0140^T, *M. abyssi* JCM 11436^T, *M. marina* ATCC 15381^T and *M. japonica* JCM 10249^T were grown in MB at 4 °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 *et al.*, 2005). Strain ArB 0140^T exhibited ice-modifying activity but other species of the genus *Moritella* examined did not (see Supplementary Fig. S3 in IJSEM Online).

Description of *Moritella dasanensis* sp. nov.

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 µm in diameter and 2.0–7.0

Table 1. Physiological and biochemical characteristics of strain ArB 0140^T and other species of the genus *Moritella*

Strains: 1, *M. marina* ATCC 15381^T; 2, *M. japonica* JCM 10249^T; 3, *M. yanosii* JCM 10263^T; 4, *M. profunda* JCM 11435^T; 5, *M. abyssi* JCM 11436^T; 6, ArB 0140^T. Data for other species of the genus *Moritella* are from Xu *et al.* (2003). +, Positive; –, negative; ±, weak response after 2 weeks; NG, no growth. All strains are positive for catalase and oxidase activities. All strains contain Q-8 as the major isoprenoid quinone. All strains reduce nitrate to nitrite but not to gas. None of the strains produce acid from arabinose, inositol, D-lactose, raffinose, sucrose, D-sorbitol or trehalose. None of the strains grow on sucrose or D-sorbitol as sole carbon sources.

Characteristic	1	2	3	4	5	6
Temperature (°C) for maximum growth rate	18	10	NG	2	4–6	5
DNA G+C content (mol%)	42.5	45	44.6	41.4	41.6	46.9
Gelatinase	+	+	+	–	–	+
Indole production	–	–	–	–	+	–
Acid produced from:						
Cellobiose	+	–	–	–	+	–
D-Galactose	+	–	–	±	+	–
Glycerol	+	+	–	–	–	–
Maltose	+	–	+	–	+	+
D-Mannitol	–	–	+	–	+	–
D-Mannose	–	–	+	–	–	–
Xylose	–	–	+	–	–	–
Utilization as carbon source:						
D-Arabinose	–	–	–	–	–	+
Cellobiose	+	–	–	–	+	–
D-Galactose	+	–	–	+	+	–
Glycerol	+	+	–	+	+	+
Maltose	+	–	–	–	+	–
Trehalose	–	–	–	–	–	+
Xylose	–	–	+	–	–	–

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, cellobiose, D-galactose, glycerol, D-mannitol, D-mannose or xylose. Produces acid from maltose. The following substrates are utilized for growth: trehalose, D-arabinose and glycerol. 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 $C_{14:0}$, $C_{14:1}$, $C_{16:0}$, $C_{16:1}$ and $C_{22:6}$.

The type strain, strain ArB 0140^T (=KCTC 10814^T=KCCM 42845^T=JCM 14759^T) was isolated from surface seawater off the near shore of Kongsfjorden in the Svalbard Archipelago, Norway.

Acknowledgements

We would like to thank Dr Han-Gu Choi for helping us with phylogenetic analysis of the 16S rRNA genes. This work was supported by the KOPRI grant No. PE07060 to S.-H. K.

References

- Benediktsdottir, E., Verdonck, L., Sproer, C., Helgason, S. & Swings, J. (2000). Characterization of *Vibrio viscosus* and *Vibrio wodanis* isolated at different geographical locations: a proposal for reclassification of *Vibrio viscosus* as *Moritella viscosa* comb. nov. *Int J Syst Evol Microbiol* **50**, 479–488.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- Collins, M. D. (1985). Analysis of isoprenoid quinones. *Methods Microbiol* **18**, 329–366.
- DeLong, E. F., Franks, D. G. & Yayanos, A. A. (1997). Evolutionary relationships of cultivated psychrophilic and barophilic deep-sea bacteria. *Appl Environ Microbiol* **63**, 2105–2108.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Kato, C., Li, L., Nogi, Y., Nakamura, Y., Tamaoka, J. & Horikoshi, K. (1998). Extremely barophilic bacteria isolated from the Mariana Trench, Challenger Deep, at a depth of 11,000 meters. *Appl Environ Microbiol* **64**, 1510–1513.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- MIDI (1999). *Sherlock Microbial Identification System, Operating Manual*, version 3.10. Newark, DE: MIDI, Inc.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Nogi, Y. & Kato, C. (1999). Taxonomic studies of extremely barophilic bacteria isolated from the Mariana Trench and description of *Moritella yayanosii* sp. nov., a new barophilic bacterial isolate. *Extremophiles* **3**, 71–77.
- Nogi, Y., Kato, C. & Horikoshi, K. (1998). *Moritella japonica* sp. nov., a novel barophilic bacterium isolated from a Japan Trench sediment. *J Gen Appl Microbiol* **44**, 289–295.
- Rainey, F. A., Dorsch, M., Morgan, H. W. & Stackebrandt, E. (1992). 16S rDNA analysis of *Spirochaeta thermophila*: its phylogenetic position and implications for the systematics of the order *Spirochaetales*. *Syst Appl Microbiol* **15**, 197–202.
- Ratkowsky, D. A., Lowry, R. K., McMeekin, T. A., Stokes, A. N. & Chandler, R. E. (1983). Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *J Bacteriol* **154**, 1222–1226.
- Raymond, J. A. (2000). Distribution and partial characterization of ice-active molecules associated with sea-ice diatoms. *Polar Biol* **23**, 721–729.
- Raymond, J. A. & Fritsen, C. H. (2000). Ice-active substances associated with Antarctic freshwater and terrestrial photosynthetic organisms. *Antarct Sci* **12**, 418–424.
- Raymond, J. A. & Fritsen, C. H. (2001). Semipurification and ice recrystallization inhibition activity of ice-active substances associated with Antarctic photosynthetic organisms. *Cryobiology* **43**, 63–70.
- Raymond, J. A. & Knight, C. A. (2003). Ice binding, recrystallization inhibition, and cryoprotective properties of ice-active substances associated with Antarctic sea ice diatoms. *Cryobiology* **46**, 174–181.
- Raymond, J. A., Sullivan, C. W. & DeVries, A. L. (1994). Release of an ice-active substance by Antarctic sea ice diatoms. *Polar Biol* **14**, 71–75.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Skerman, V. B. D. (1967). *A Guide to the Identification of the Genera of Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.
- Steven, S. E. (1990). *Molecular systematics of Vibrio and Photobacterium*. PhD thesis, University of Maryland, College Park, MD, USA.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Urakawa, H., Kita-Tsukamoto, K., Steven, S. E., Ohwada, K. & Colwell, R. R. (1998). A proposal to transfer *Vibrio marinus* (Russell 1891) to a new genus *Moritella* gen. nov. as *Moritella marina* comb. nov. *FEMS Microbiol Lett* **165**, 373–378.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Wharton, D. A., Barrett, J., Goodall, G., Marshall, C. J. & Ramlov, H. (2005). Ice active proteins from an Antarctic nematode, *Panagrolaimus dividi*. *Cryobiology* **51**, 198–207.
- Xu, Y., Nogi, Y., Kato, C., Liang, Z., Ruger, H. J., De Kegel, D. & Glansdorff, N. (2003). *Moritella profunda* sp. nov. and *Moritella abyssii* sp. nov., two psychropiezophilic organisms isolated from deep Atlantic sediments. *Int J Syst Evol Microbiol* **53**, 533–538.