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Isolation of novel psychrophilic bacteria from Arctic sea ice

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Abstract The phylogenetic diversity of culturable psychrophilic bacteria associated with sea ice from the high latitude regions of Canadian Basin and Chukchi Sea, Arctic, was investigated. A total of 34 psychropilic strains were isolated using three methods of (ⅳ) dilution plating (at 4 ℃), (㈦) bath culturing (at - 1 ℃) and dilution plating and (㈣) cold shock (- 20 ℃ for 24 h), bath culturing and dilution plating under aerobic conditions. Sea-ice samples were exposed to -20 ℃ for 24 h that might reduce the number of common microorganisms and encourage outgrowth of psychrophilic strains. This process might be able to be introduced to isolation psychrophilic bacteria from other environmental samples in future study. 16S rDNA nearly full-length sequence analysis revealed that psychrophilic strains felled in two phylogenetic divisions, γ-proteobacteria (in the genera *Colwellia*、*Marinobacter*、*Shewanella*、*Glaciecola*、*Marinomonas* and *Pseudoalteromonas*) and Cytophaga-Flexibacter-Bacteroides (*Flavobacterium* and *Psychroflexus*). Fifteen of bacterial isolates quite likely represented novel species (16S rDNA sequence similarity below 98%). One of strains (BSi20002) from Canadian Basin showed 100% sequence similarity to that of *Marinobacter* sp. ANT8277 isolated from the Antarctic Weddell sea ice, suggesting bacteria may have a bipolar distribution at the species level.

Key words Psychrophilic; Novel bacteria; Sea ice; Actic

1 Introduction

In recent years, the study of psychrophilic organisms has been intensified, fueled in part by the realization that the physiological and biochemical adaptation to cold environments of psychropiles may have considerable potential for biotechnological applications (D'Amico *et al*. 2006; Georlette *et al*. 2004). A large number of psychrophiles were isolated from permanently cold environments from the deep sea to high mountains and the polar regions which cover a wide range of phylogenetic diversity. Polar sea ice is a primarily originating environment for psychrophiles (Bowman 2001; Brinkmeyer 2003; Deming 2002; Helmke and Weyland 2004; Staley and Gosink 1999). Furthermore, a considerable number of novel genera and species have been successfully isolated and characterized from sea ice (Bowman *et al*. 1997; Bowman *et al*. 1998; Junge *et al*. 2002; Groudieva *et al*. 2004).

Most researches of the sea ice psychrophiles were carried in Antarctica (Bowman *et*

al 1997; Brinkmeyer *et al* 2003). In Arctic, attention was paid to fjords of Spitzbergen (Groudieva et al 2004), Fram Strait (Brinkmeyer *et al* 2003; Helmke and Weyland 2004), Norwegian Sea (Helmke and Weyland 2004), and Chukchi Sea (Junge 2002). However, no information was available about psychropiles in the high latitude regions of Canadian Basin.

In the present study, diversity of culturable psychrophilic bacteria associated with sea ice in Canadian Basin and Chukchi Sea was investigated. Isolation and molecular phylogenetic analysis of Arctic strains were performed in order to discover novel bacteria and expand our knowledge on biogeography of psychrophilic bacteria.

2 Materials and methods

2.1 Sampling stations and sample collection

Samples were collected using a MARK II ice auger during the Second Chinese National Arctic Research Expedition cruise of the USCGC icebreaker Xue Long into the Canada Basin and Chukchi Sea in August 2003 (Fig. 1). During sampling and processing, careful attention was paid to maintaining sterile conditions. The ice cores were cut into 10-cm to 20-cm sample sections using a sterile saw. The ice sections were melted at 4 ℃ in equal volume unamended, pre-filtered (0.2 μm pore size) and autoclaved natural seawater (NSW, from 5 m below the ice).

2.2 Bacterial isolation

Bacterial strains were isolated using three different methods of (ⅳ) spreading plate method: 100 μL of each dilution (1∶10) ice-melt sample was spread onto the surface marine 2216 agar (DIFCO laboratories, Detroit, MI) and incubated for 30 to 60 days at 4 ℃; (㈦) bath culture and spreading plate method: 1mL of sample was added to 9 mL of NSW and incubated for 30 days at −1 ℃, then spreading plate method was used to isolate bacterial strains from the pre-cultured samples; (㈣) cold shock, bath culture and spreading plate method: samples were exposed to −20 ℃ for 24 h, then bacterial strains isolated by bath culture and spreading plate method under aerobic conditions. Isolates were selected according to the morphological difference of colonies and purified by streaking technique. All pure strains were grown in Marine 2216 broth (DIFCO laboratories, Detroit, MI) and preserved at −80 ℃ in 20% (V/V) glycerol.

2.3 Temperature tolerance of the strains

Freezing stored strains were inoculated onto marine 2216 agar. Upon the colonies reached the desirable size, they were transferred using a sterilized wire loop onto marine 2216 agar, and incubated at 0, 5, 10, 15, 20, 21, 25 and 30 ℃. Growth was monitored under a stereoscope (Leica Microscopy Systems Ltd, Heerbrugg, Switzerland) at the 64 fold magnification.

2.4 Seawater requirements and sodium chloride tolerance

Bacterial isolates were tested for their requirement of seawater in growth and sodium chloride tolerance. Such testing was carried out using modified Marine 2216 media including 2216/NSW (prepared with the natural seawater), 2216/ASW (prepared with the artificial seawater) and 2216/DI water (prepared with deionized water), or 2216E prepared with different concentration of NaCl solution ranging from 0 to 150 g/L. The pH of each medium was 7.8. The different media were inoculated with 100 μL pre-culture and incubated at 5 ℃ with slight shaking (30 rpm) for 48 and 96 h. Growth was determined by means of OD at 600 nm.

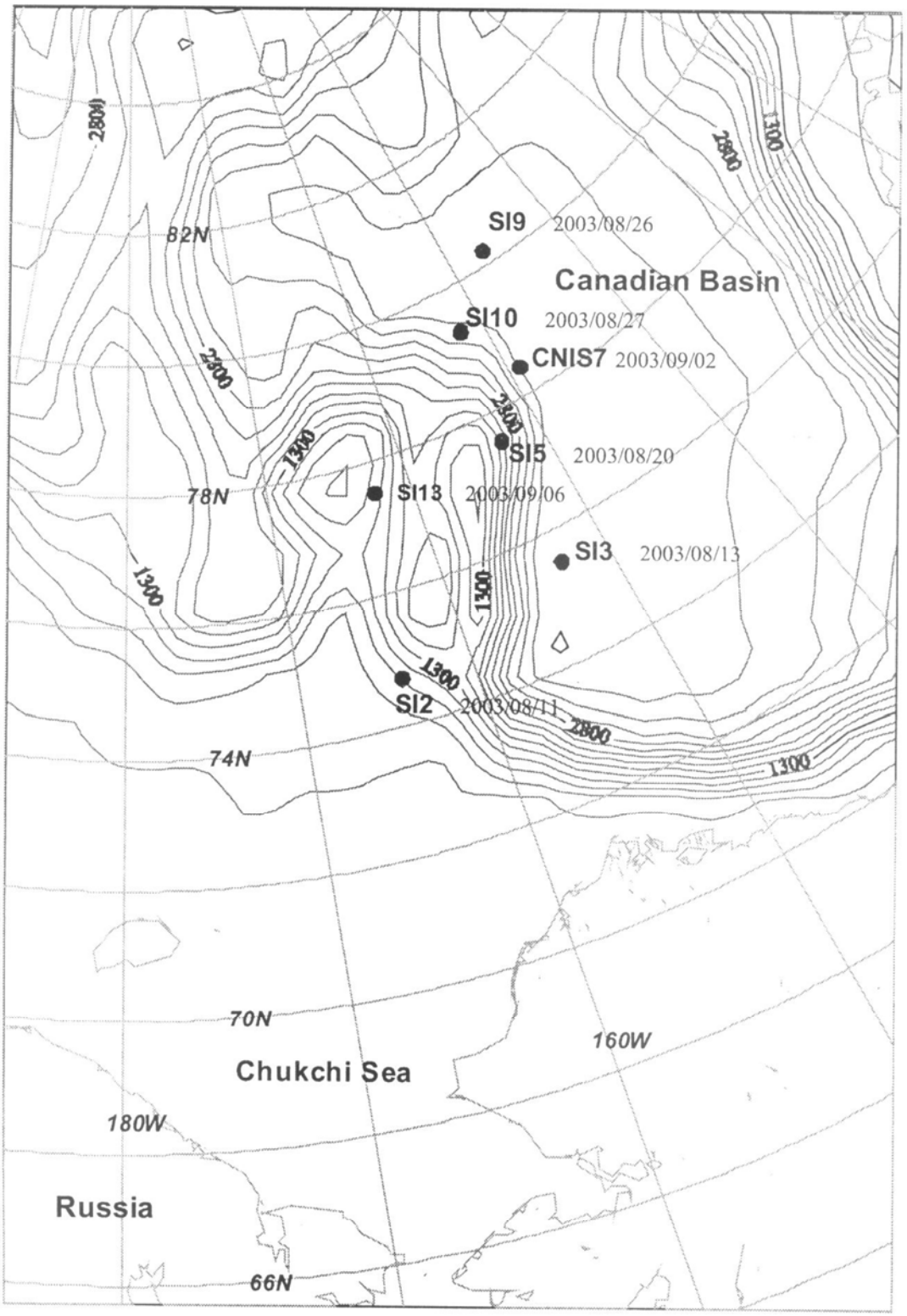


Fig 1 The sampling stations in the Canadian Basin and Chukchi Sea (adapted from Li *et al* 2005).

2.5 *DNA extraction, PCR amplification and Sequencing of PCR products*

Bacterial genomic DNA extraction, nearly full-length 16S rDNA amplification, PCR products purification, and purified PCR products sequencing was performed as described by Yu *et al* (2005). The 16S rDNA nucleotide sequences of the bacterial isolates reported in this study have been submitted to the Genbank and EMBL data bank. The accession numbers were given next to the organisms in Table 1.

2.6 *Phylogenetic analysis*

The 16S rDNA nucleotide sequence of bacterial isolate was submitted to GenBank and EMBL to search for similar sequences using the BLAST algorithm. Evolutionary distances were calculated by the method of Kimura two-parameter calculation model. The phylogenetic tree was constructed using Kimura 2-parameter and pairwise-deletion model analysis implemented in the program MEGA version 3.1 (Kumar *et al* 2004). The resultant tree topologies were evaluated by bootstrap analysis based on 1000 replicates.

3 Results and Discussion

3.1 *Isolation of psychrophilic bacteria from Arctic sea ice*

The term 'psychrophilic' first time used by Schmidt-Nielsen was to define a bacterial type which had the ability to reproduce at 0 ℃ (Schmidt-Nielsen 1902 cited in Helmke and Weyland 2004). In this early definition the minimum growth temperature was only considered, and some confusion arose because of the lack of differentiation between 'cold-loving' and 'cold-tolerant' adaptation types. Morita ended the confusion with a new definition in 1975. He assigned the term 'psychrophilic' to those microorganisms whose minimum, optimum, and maximum growth temperatures are at or below 0, 15 and 20 ℃, respectively, while microorganisms with a higher growth optimum and maximum were called 'psychrotrophic' (nowadays replaced by 'psychrotolerant') (Morita 1975). This definition is still commonly used today. Although Morita's definition has in principal been proven to be useful, the cardinal temperatures should be reconsidered on the basis of increased numbers of cold adapted bacteria isolated from various habitats and in the light of new ecological data. Considering the quite a number of bacteria living in the different cold environments which surpass the 20 ℃ limit by only few degrees, Helmke and Weyland (2004) proposed to introduce an additional group of 'moderate psychrophiles' with a minimum growth temperature of ≤0 ℃ and a maximum growth temperature ranging from 20 ℃ to 25 ℃. In Arctic sea ice a considerable number of moderate psychrophilic bacteria were found (Helmke and Weyland 2004). So we appoint the term 'psychrophilic' for those bacteria which meet Morita's definition or Helmke and Weyland's definition in this study.

A total of 34 psychrophilic strains were isolated from Arctic sea ice using three different methods. We found that of 34 strains 9 were isolated by method ⅳ belonged 4 different genera, 10 by method ㈦ belonged to 3 genera, and 15 by method ㈣ belonged to 7 genera

(Table 1). The cold shock process resulted in even higher psychrophilic isolates. Only about 40% of Arctic sea-ice isolates were psychrophilic bacteria based on results of Groudieva *et al* (2004) and our study (date not shown). Sea-ice samples were exposed to −20 °C for 24 h that might reduce the number of common microorganisms and encourage outgrowth of psychrophilic strains.

All isolates had been shown to have an obligate growth requirement for salt, and grew better on media prepared with the natural seawater than on those prepared with the deionized water. Phylogenetic analysis of the nearly complete 16S rDNA sequences of these isolates indicated that they were closest homologous to some bacteria from marine environments. Those results confirmed these strains as bona fide marine organisms. A majority of psychrophilic isolates were slight halophiles, growing over a relatively wide range of salinities. Concurrently, the salinity within sea ice was known to vary widely on both temporal and spatial scales during the ice formation process (Ackley and Sullivan, 1994). The temperature with the combination of salinity stress might primarily control on the selection of psychrophilic bacteria in the sea ice environment.

3.2 Phylogenetic analysis

All of the psychrophilic isolates fell into two phylogenetic groups: the γ-subclasses of Proteobacteria and the Cytophaga-Flexibacter-Bacteroides (CFB) group (Table 1). Thirty two of strains were affiliated with the γ-subclasses of Proteobacteria and two with the CFB group. Within the γ-subclasses of Proteobacteria, the isolates were belonged to genera *Colwellia*, *Glaciecola*, *Marinobacter*, *Marinomonas*, *Pseudoalteromonas*, and *Shewanella*. And other two isolates falling into CFB group were identified as the members of genera *Flavobacterium* and *Psychroflexus*. Fifteen of strains quite likely represented novel species (16S rDNA sequence similarity below 98% (Stackebrandt and Goebel 1994; Suau *et al* 1999; Paster *et al* 2001)). But the novel bacteria at a higher taxonomic level were not found.

Within the genus *Colwellia*, two candidates for novel species existed among our strains. Strain BSi20517 was phylogenetically closely related to *Colwellia hornerae* ACAM 607T with 97.9% similarity and to other members of this genus with 95.8—97.7% similarity. Strain BSi20537 showed 98.0% of similarity to *C. aestuarii* KCTC 12480^T and 94.3—96.3% of similarity to other members of this genus (Fig. 2a).

Strains BSi20138 and 170 with 99.8% sequence identity fell in the genus *Glaciecola*. Strain 170 was selected as a novel species candidate, which was phylogenetically closely related to *Glaciecola mesophila* KMM 241^T with 96.8% similarity and to other members of this genus with 93.3-96.3% (Fig. 2b).

One large group consisting 8 strains, represented by isolate BSi20001, was found to belong to the genus *Marinobacter*. They possessed high 16S rDNA sequence similarity of 99.5-99.9% to each other. The type strain BSi20001 was phylogenetically closely related to *Marinobacter maritimus* CK47^T with 97.4% similarity and to other members of this genus with 94.0-96.0% (Fig. 2c).

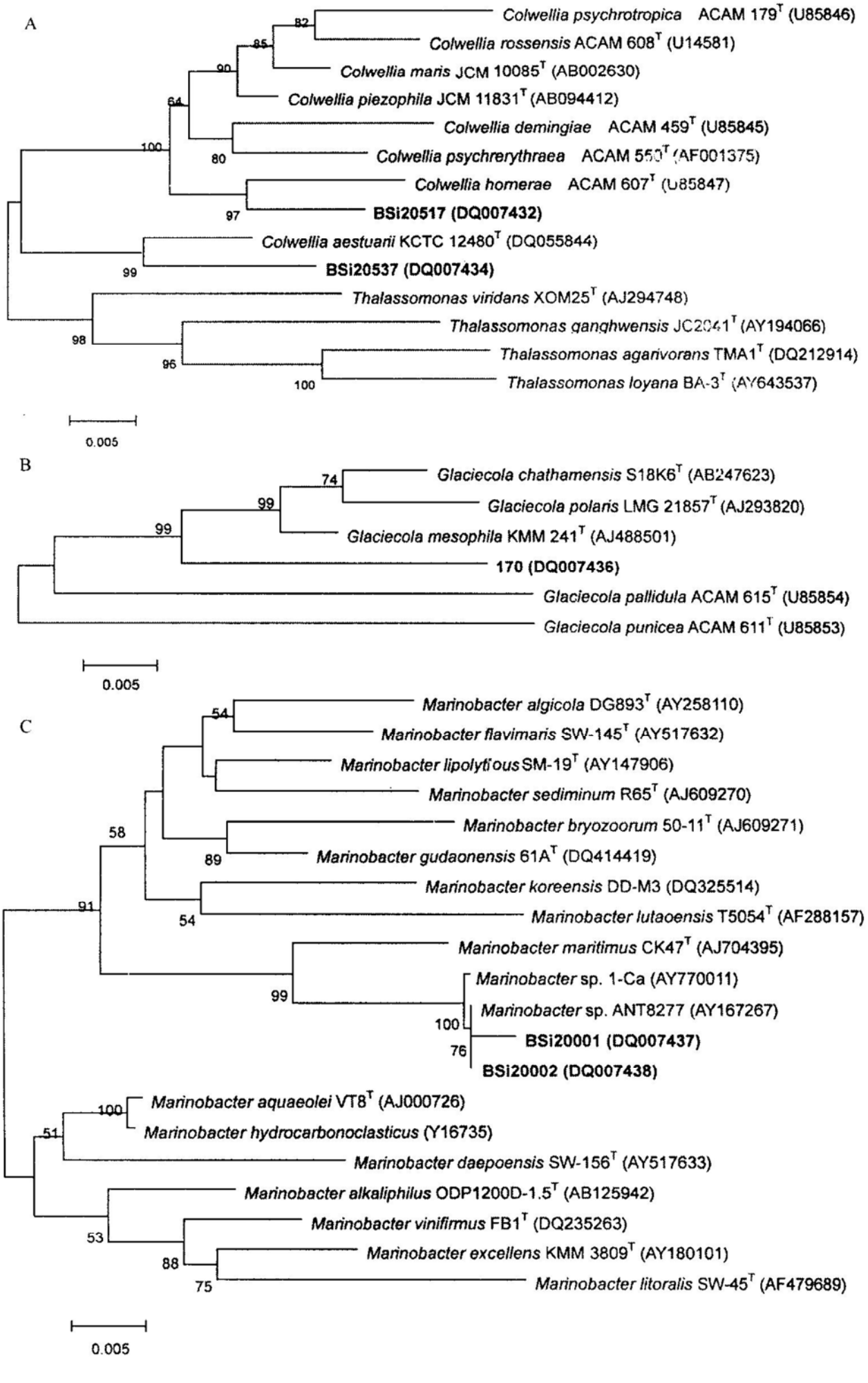
The last novel species candidate was found in the genus *Marinomonas*. Strain BSi20328 clustered with *Marinomonas polaris* CK13T (96.7% sequence similarity), and showed similarity of 93.5-96.7% to other species in this genus (Fig. 2d).

Table. 1 Isolation methods, temperature characteristics, salt tolerances and phylogenetic relationships of psychrophilic bacterial isolates from Arctic sea-ice

Isolates (Genbank accession no.)	Isolation method	Optimal growth Temperature (℃)	Maximal growth temperature (℃)	Sequence similarity (%)	16S rDNA identification (closest species)	Salt (NaCl) tolerance (‰)	Sampling station
γ-Proteobacteria							
BSi20003 (DQ060387)	(Ⅰ)	10	20	98.3	*Colwellia hornerae* ACAM 607^T (U85847)	10 ~ 50	SI9
BSi20004 (DQ060388)	(Ⅰ)	10	20	98.4	*C. hornerae* ACAM 607^T (U85847)	10 ~ 50	SI10
BSi20008 (DQ060389)	(Ⅲ)	10	20	98.2	*C. hornerae* ACAM 607^T (U85847)	10 ~ 50	SI5
BSi20043 (DQ060390)	(Ⅲ)	10	20	98.4	*C. hornerae* ACAM 607^T (U85847)	10 ~ 50	SI3
BSi20045 (DQ060391)	(Ⅲ)	10	20	98.6	*C. hornerae* ACAM 607^T (U85847)	10 ~ 50	SI2
BSi20074 (DQ060393)	(Ⅰ)	15	25	98.3	*C. hornerae* ACAM 607^T (U85847)	10 ~ 50	SI13
BSi20076 (DQ060394)	(Ⅰ)	10	20	98.3	*C. hornerae* ACAM 607^T (U85847)	10 ~ 50	CNIS7
BSi20091 (DQ060395)	(Ⅰ)	15	25	98.4	*C. hornerae* ACAM 607^T (U85847)	10 ~ 50	SI5
BSi20095 (DQ007429)	(Ⅰ)	15	25	98.4	*C. hornerae* ACAM 607^T (U85847)	10 ~ 50	SI2
BSi20169 (DQ007430)	(Ⅱ)	10	20	99.0	*C. rossensis* ACAM 608^T (U14581)	10 ~ 50	SI9
BSi20497 (DQ007431)	(Ⅱ)	10	20	98.2	*C. hornerae* ACAM 607^T (U85847)	10 ~ 50	SI3
BSi20517 (DQ007432)	(Ⅱ)	10	20	97.9	*C. hornerae* ACAM 607^T (U85847)	10 ~ 50	SI3
BSi20520 (DQ007433)	(Ⅱ)	10	20	98.0	*C. hornerae* ACAM 607^T (U85847)	10 ~ 50	SI3
BSi20537 (DQ007434)	(Ⅱ)	15	25	97.9	*C. aestuarii* KCTC 12480^T (DQ055844)	10 ~ 50	SI5
BSi20660 (DQ060396)	(Ⅲ)	10	20	98.5	*C. hornerae* ACAM 607^T (U85847)	10 ~ 50	SI10
BSi20138 (DQ492706)	(Ⅲ)	10	15	96.8	*Glaciecola mesophila* KMM 241^T (AJ488501)	10 ~ 60	SI10
170 (DQ007436)	(Ⅲ)	10	15	96.8	*G. mesophila* KMM 241^T (AJ488501)	10 ~ 60	SI9
BSi20001 (DQ007437)	(Ⅰ)	10	20	97.4	*Marinobacter maritimus* CK47^T (AJ704395)	5 ~ 60	CNIS7
BSi20002 (DQ007438)	(Ⅲ)	10	20	97.6	*M. maritimus* CK47^T (AJ704395)	5 ~ 60	SI9

Strain	Isolation method	Optimal growth Temperature (℃)	Maximal growth temperature (℃)	Sequence similarity (%)	16S rDNA identification (closest species)	Salt (NaCl) tolerance (‰)	Sampling station
γ-Proteobacteria							
BSi20018 (DQ060399)	(Ⅲ)	10	20	97.5	*M. maritimus* CK47^T (AJ704395)	5~60	SI5
BSi20032 (DQ060400)	(Ⅲ)	10	20	97.6	*M. maritimus* CK47^T (AJ704395)	5~60	SI10
BSi20033 (DQ060401)	(Ⅲ)	10	20	97.4	*M. maritimus* CK47^T (AJ704395)	5~60	SI3
BSi20041 (DQ060402)	(Ⅱ)	10	20	97.5	*M. maritimus* CK47^T (AJ704395)	5~60	SI2
BSi20422 (DQ060403)	(Ⅱ)	10	20	97.4	*M. maritimus* CK47^T (AJ704395)	5~60	SI2
BSi20675 (DQ007440)	(Ⅱ)	10	20	97.4	*M. maritimus* CK47^T (AJ704395)	5~60	SI13
BSi20328 (DQ492749)	(Ⅲ)	15	25	96.7	*Marinomonas polaris* CK13^T (AJ833000)	5~60	CNIS7
BSi20062 (DQ007441)	(Ⅰ)	15	25	99.3	*Pseudoalteromonas elyakovii* KMM162^T (AF082562)	5~80	CNIS7
BSi20044 (DQ492714)	(Ⅰ)	15	25	98.9	*Shewanella livingstonensis* LMG19866^T (AJ300834)	5~60	SI9
BSi20586 (DQ007443)	(Ⅱ)	15	25	99.8	*S. livingstonensis* LMG19866^T (AJ300834)	5~60	CNIS7
BSi20587 (DQ060406)	(Ⅱ)	15	25	99.8	*S. livingstonensis* LMG19866^T (AJ300834)	5~60	CNIS7
BSi20593 (DQ007444)	(Ⅲ)	15	25	99.7	*S. livingstonensis* LMG19866^T (AJ300834)	5~60	CNIS7
BSi20607 (DQ007445)	(Ⅲ)	15	25	98.9	*S. livingstonensis* LMG19866^T (AJ300834)	5~60	CNIS7
Cytophaga- Flexibacter-Bacteroides							
BSi20510 (DQ007435)	(Ⅲ)	15	25	98.9	*Flavobacterium degerlachei* LMG 21915^T (AJ557886)	5~60	SI3
BSi20642 (DQ007442)	(Ⅲ)	10	20	98.4	*Psychroflexus torquis* ACAM623^T (U85881)	15~40	SI3

(Ⅰ) spreading plate method, (Ⅱ) bath culture and spreading plate method, (Ⅲ) cold shock, bath culture and spreading plate method *Colwellia hornerae* ACAM 607^T and *C. rossensis* ACAM 608^T from coastal Antarctic sea-ice diatom assemblages, *C. aestuarii* KCTC 12480^T from a tidal flat sediment in Korea, *Glaciecola mesophila* KMM 241^T from marine invertebrate specimens, *Marinobacter maritimus* CK47^T and *Marinomonas polaris* CK13^T from sea water off the subantarctic Kerguelen islands; *Pseudoalteromonas elyakovii* KMM162^T from from the Far-Eastern mussel *Crenomytilus grayanus*, *Shewanella livingstonensis* LMG19866^T from Antarctic coastal marine environments, *Flavobacterium degerlachei* LMG 21915^T from microbial mats in Antarctic lakes, and *Psychroflexus torquis* ACAM623^T from Antarctic sea ice.


A
Colwellia psychrotropica ACAM 179T (U85846)
Colwellia rossensis ACAM 608T (U14581)
Colwellia maris JCM 10085T (AB002630)
Colwellia piezophila JCM 11831T (AB094412)
Colwellia demingiae ACAM 459T (U85845)
Colwellia psychrerythraea ACAM 55?T (AF001375)
Colwellia homerae ACAM 607T (U85847)
BSi20517 (DQ007432)
Colwellia aestuarii KCTC 12480T (DQ055844)
BSi20537 (DQ007434)
Thalassomonas viridans XOM25T (AJ294748)
Thalassomonas ganghwensis JC2041T (AY194066)
Thalassomonas agarivorans TMA1T (DQ212914)
Thalassomonas loyana BA-3T (AY643537)
82
85
90
64
100
80
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99
98
96
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0.005
B
Glaciecola chathamensis S18K6T (AB247623)
Glaciecola polaris LMG 21857T (AJ293820)
Glaciecola mesophila KMM 241T (AJ488501)
170 (DQ007436)
Glaciecola pallidula ACAM 615T (U85854)
Glaciecola punicea ACAM 611T (U85853)
74
99
99
0.005
C
Marinobacter algicola DG893T (AY258110)
Marinobacter flavimaris SW-145T (AY517632)
Marinobacter lipolyticus SM-19T (AY147906)
Marinobacter sediminum R65T (AJ609270)
Marinobacter bryozoorum 50-11T (AJ609271)
Marinobacter gudaonensis 61AT (DQ414419)
Marinobacter koreensis DD-M3 (DQ325514)
Marinobacter lutaoensis T5054T (AF288157)
Marinobacter maritimus CK47T (AJ704395)
Marinobacter sp. 1-Ca (AY770011)
Marinobacter sp. ANT8277 (AY167267)
BSi20001 (DQ007437)
BSi20002 (DQ007438)
Marinobacter aquaeolei VT8T (AJ000726)
Marinobacter hydrocarbonoclasticus (Y16735)
Marinobacter daepoensis SW-156T (AY517633)
Marinobacter alkaliphilus ODP1200D-1.5T (AB125942)
Marinobacter vinifirmus FB1T (DQ235263)
Marinobacter excellens KMM 3809T (AY180101)
Marinobacter litoralis SW-45T (AF479689)
54
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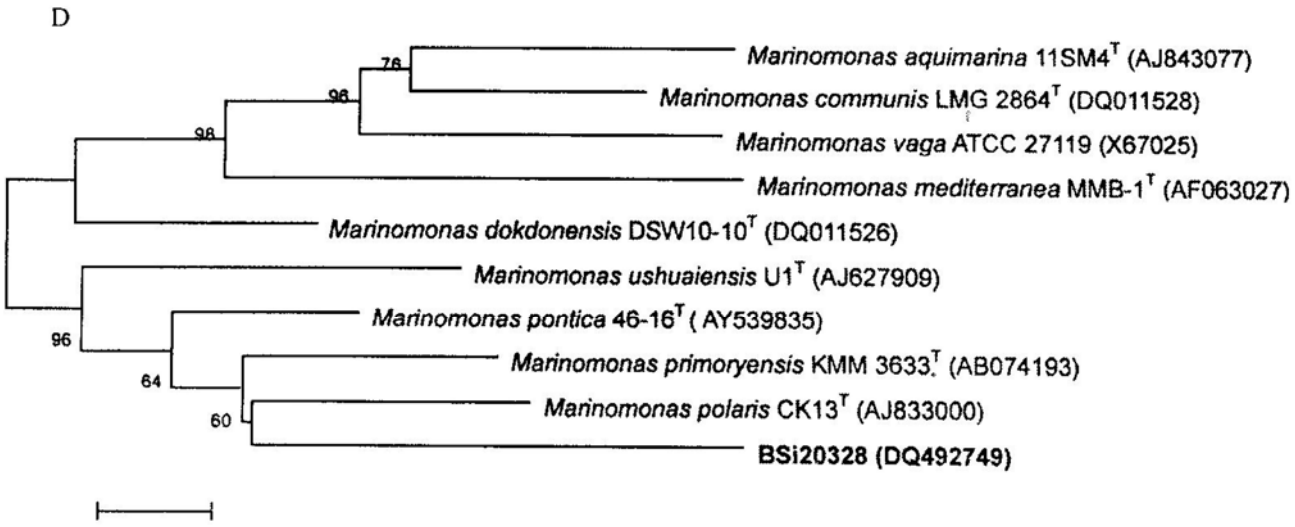

Fig 2 Phylogenetic dendrogram of novel psychrophilic bacterial isolates and related species based on 16S rRNA gene homology. The tree was constructed using the neighbor-joining method. Numbers at nodes represented percentage levels of bootstrap support (%) based on a neighbor-joining analysis of 1000 re-sampled datasets. GenBank accession numbers of 16S rRNA sequences are given in the parentheses. Scale bars correspond to a 0.5% divergence in nucleotide sequence.

The polar sea ice bacteria were particularly appropriate to study regarding bacterial biogeography because of the dispersal of psychrophilic bacteria between the north and south poles being extremely difficult. Staley and Gosink (1999) suggested that bacteria had a bipolar distribution at genus level, but were endemic to the north or south polar environment at species level. However, Junge *et al* (2002) discovered for the first time an Arctic isolate (*Shewanella frigidimarara*) which showed 100% 16S rDNA sequence (about 550 bp) similarity to the same isolate from Antarctica. Furthermore, one of our strains (BSi20002) from Canadian Basin was phylogenetically closely related to the Antarctic Weddell sea ice isolate *Marinobacter* sp. ANT8277 with 100% sequence similarity at 1493bp level (Fig 2C). Therefore the earlier suggestion of Staley and Gosink should be revised. The same species of bacteria also occurred at both Arctic and Antarctica.

4 Conclusions

1) Sea-ice samples were exposed to −20 °C for 24 h that might reduce the number of common microorganisms and encourage outgrowth of psychrophilic strains. This process might be able to be introduced to isolating psychrophilic bacteria from other environmental samples in future study.

2) Five candidates for novel species were found in our psychrophilic isolates.

3) Based on our results and that of Junge *et al*, the earlier suggestion of Staley and Gosink should be revised. The bacteria not only had a bipolar distribution at genus level, but also at species level.

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References

Ackley SF, Sullivan CW (1994): Physical controls on the development and characteristics of Antarctic sea ice biological communities—a review and synthesis. Deep-Sea Res., 41: 1583–1604.

Bowman JP, McCammon SA, Brown MV *et al* (1997): Diversity and association of psychrophilic bacteria in Antarctic sea ice. Appl. Environ. Microbiol., 63(8): 3068–3078.

Bowman JP, McCammon SA, Brown JL *et al* (1998): *Glaciecola punicea* gen. nov., sp. nov. and *Glaciecola pallidula* gen. nov., sp. nov.: psychrophilic bacteria from Antarctic sea-ice habitats. Int. J. Syst. Bacteriol., 48: 1213–1222.

Bowman JP (2001): Methods for psychrophilic bacteria. In: Marine Microbiology, Paul J (ed.), Acad. Press, San Diego, 591–613.

Brinkmeyer R, Knittel K, Jørgens J *et al* (2003): Diversity and Structure of Bacterial Communities in Arctic versus Antarctic Pack Ice. Appl. Environ. Microbiol., 69(11): 6610–6619.

Deming JD (2002): Psychrophiles and polar regions. Current Opinion in Microbiology, 5: 301–309.

D'Amico S, Collins T, Marx JC *et al* (2006): Psychrophilic microorganisms: challenges for life. EMBO reports, 7: 385–389.

Georlette D, Blaise V, Collins T *et al* (2004): Some like it cold: biocatalysis at low temperatures. FEMS Microbiol. Rev., 28: 25–42.

Groudieva T, Kambourova M, Yusef H *et al* (2004): Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen. Extremophiles, 8(6): 475–488.

Helmke E, Weyland H (2004): Psychrophilic versus psychrotolerant bacteria—occurrence and significance in polar and temperate marine habitats. Cell Mol. Biol., 50(5): 553–561.

Junge K, Imhoff F, Staley T *et al* (2002): Phylogenetic Diversity of Numerically Important Arctic Sea-Ice Bacteria Cultured at Subzero Temperature. Microb. Ecol., 43(3): 315–328.

Kumar S, Tamura K, Nei M (2004): MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief Bioinform., 5: 150–163.

Li H, Yu Y, Chen B *et al* (2005): Molecular genetic diversity of bacteria in the bottom section of arctic sea ice from the Canada Basin. Acta Oceanologica Sinica, 24(6): 153–161.

Morita RV (1975): Psychrophilic bacteria. Bacteriol. Rev., 39: 146–167.

Paster BJ, Boches SK, Galvin JL *et al* (2001): Bacterial diversity in human subgingival plaque. J. Bacteriol., 183(12): 3770–3783.

Stackebrandt E, Goebel BM (1994): Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Bacteriol., 44(4): 846–849.

Suau A, Bonnet R, Sutren M *et al* (1999): Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. Appl. Envir. Microbiol., 65(11): 4799–4807.

Staley J, Gosink (1999): Poles apart: Biodiversity and biogeography of sea ice bacteria. Annu. Rev. Microbiol., 53: 189–215.

Yu Y, Li H, Zeng Y *et al* (2005): Isolation and phylogenetic assignation of actinomycetes in the marine sediments from the Arctic Ocean. Acta Oceanologica Sinica, 24(6): 135–142.