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Phylogenetic analysis of cultivable bacteria isolated from Arctic sea-ice

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Abstract Phylogenetic analysis based on 16S rDNA of 8 strains of cultivable bacteria isolated from Arctic sea-ice was studied. The results showed that strain BJ1 belonged to genus *Planococcus*, which was a genus of low mole percent G+C gram-positive bacteria; strain BJ6 belonged to genus *Burkholderia* of β-proteobacteria and the rest 6 strain all belonged to γ-proteobacteria, of which strain BJ8 was a species of *Pseudoalteromonas*, strain BJ2-BJ5 and BJ7 were members of genus *Psychrobacter*. Phylogenetic analysis also indicated that bacteria of genus *Psychrobacter* of the isolates formed a relatively independent phylogenetic cluster in comparison with other bacteria belonged to genus *Psychrobacter*.

Key words Arctic sea-ice; cultivable bacteria; phylogenetic analysis

1 Introduction

Existence of sea ice is an ephemeral feature of polar regions, making it one of the major biomes on the planet. Many planktonic organisms, including viruses, bacteria, algae, protists, flatworms and small crustaceans, stick to, or are caught among, ice crystals rising through the water when surface water freezes in autumn. Subsequently, as the ice grows and consolidates, the organisms become trapped within the brine channels. Hence, a diverse group of organisms is almost instantaneously confined to a new habitat that is quite different from the one from which they are recruited[1-4]. The relative development and complexity of the Sea-ice Microbial Communities (SMCO) is primarily determined by physical forces. Changes in salinity are the dominant factor in external chemistry to influence the biological assemblages within the sea ice. In any case, sea ice is a biologically active habitat and where on a per volume basis, biomass is more productive than that in the underlying pelagic zone[4,5].

Bacterial activity and populations in sea ice are tightly dependent upon algal primary productivity, playing an important role in secondary mineralization of dissolved and particulate organic matter[6]. At certain times, bacteria can dominate within sea-ice habitats; in general, though they make up only a small proportion of the total SMCO biomass. Most bacteria isolated from sea ice have found to be pigmented, highly cold-adapted with both

free-living and epiphytic bacteria present, some are able to form gas vesicles. Extensive cultivation and identification of bacteria from SMCO indicated that about half of the taxa isolated are psychrophilic while the rest are psychrotrophic. Most taxa isolated from sea ice belong to γ-proteobacteria and the Cytophaga-Flavobacterium-Bacteroids (CFB) division[4,7,8].

8 strains of bacteria were isolated from the sea-ice samples collected during Chinese 2nd Arctic Scientific Expedition, their phylogenetic positions were identified by neighbor-joining method based on 16S rDNA alignment and analysis.

2 Materials and Methods

2.1 *Sample collection and isolation of cultivable bacteria*

Sea-ice was collected during Chinese 2nd Arctic Scientific Expedition (location: 73° 35'N, 168°45'W; time: August 5, 2003). The sample was kept in aseptic plastic ware at -20 ℃ until cultivable bacteria were isolated. The culture medium and procedure of isolation was as Lin[9].

2.2 *Amplification and sequencing of 16S rDNA*

The procedure of DNA preparation and performance of PCR amplification of 16S rDNA was as Yang[10].

Amplified rDNA were separated by electrophoresis on 1.0% (W/V) agarose gel and then purified by DNA extraction kit (Takara). The PCR fragments were cloned into vector pMD-18T (Takara), followed by transformation into *Escherichia coli* DH5α. Restriction analysis was adopted to confirm the white colonies contained the plasmid with the insert. Positive clones were kept at -80 ℃ in LB medium containing 15% glycerin and 100μg/ml ampicillin.

Sequencing was completed with two primers, R (RV-M, 5' GAGCGGATAACAATTTCA CAGG 3') and F (M13-47, 5' CGCCAGGGTTTTCCCAGTCACGAC 3'). Clone sequences were edited by DNAMAN and about 1.5 kb of the sequences were used in the following analysis.

2.3 *Phylogenetic analysis*

The sequences of 16S rDNA obtained from 8 strains were compared to 16S rDNA sequences available in the GenBank by BLAST search (http://www.ncbi.nlm.nih.gov/blast/blast.cgi). 16S rDNA sequences were aligned using the Clustalw multiple-alignment program (ClustalW.). Sites involving gaps were excluded from all analysis. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.0[11].

3 Results and Discussion

3.1 *Isolation of cultivable bacteria and analysis of physiological and biochemical character-*

istics

Arctic sea-ice had highly active members despite the extreme conditions in sea-ice. 8 strains were isolated according to their growth on plates and identified by their distinct colony morphology including size, pigment and growth time. One remarkable result of sea-ice microorganisms was that the cultivable fraction and the PCR-detected fraction of bacteria in sea-ice strongly overlapped. The cultivability of Arctic sea-ice bacteria can reach 62%, according to Junge *et al* whereas common cultivability for most sea-water bacteria was less than 0.01%[12]. This character favored isolation of cultivable bacteria from Antarctic and Arctic sea-ice. The physiological and biochemical character of the 8 strains of bacteria isolated from Arctic sea-ice were summarized in table 1. All the isolates were gram-negative except BJ1. All the isolates were aerobic or facultative anaerobic. Indole test, H_2S test, MR test, VP test and ONPG test were negative for all strains.

Table 1. Physiological and biochemical character of the 8 strains of bacteria isolated from Arctic sea-ice

strain / characteristics	BJ1	BJ2	BJ3	BJ4	BJ5	BJ6	BJ7	BJ8
morphology	bulbiform	rod shape	rod shape	rod shape	rod shape	rod shape	rod shape	rod shape
size	1.0~1.2μm	1.0×2.8μm	0.8×2.2μm	1.0×3.0μm	0.7×2.0μm	0.9×2.7μm	0.5×1.6μm	1.0×2.2μm
Gram reaction	G^+	G^-	G^-	G^-	G^-	G^-	G^-	G^-
oxygen	aerobic	facultative anaerobe	facultative anaerobe	facultative anaerobe	facultative anaerobe	aerobic	facultative anaerobe	facultative anaerobe
catalase	+	+	+	+	+	+	+	+
oxidase test	−	+	−	+	−	−	−	+
nitrate reduction	−	+	+	+	+	+	+	−
gelatin liquefaction	+	+	+	−	−		+	−
indole test	−	−	−	−	−	−	−	+
H2S test	−	−	−	−	−	−	−	−
MR	−	−	−	−	−	−	−	−
VP	−	−	−	−	−	−	−	−
ONPG	−	−	−	−	−	−	−	−
urease	−	−	−	−	−	−	−	−
lysine decarboxylase	−	−	−	−	−	−	−	+
ornithine decarboxylase	−	−	−	−	−	−	−	+
arginine decarboxylase	+	+	+	+	+	+	+	+
arginine dihydrolase	+	+	+	+	+	+	+	−

3.2 16S rDNA sequencing and analysis

Amplified 16S rDNA of 8 strains of bacteria were sequenced. The sequences, with about 1.5 kb in length, were aligned and compared with the 16S rDNA sequences from GenBank by BLAST search. 8 closest match reference strains were listed in Table 2. The 16S rDNA sequences of 8 strains were deposited to GenBank nucleotide sequence database with the accession numbers DQ298404-DQ298410 and EF627986.

Table 2 Homology comparison of 16S rDNA sequences of the 8 strains of bacteria isolated from Arctic sea-ice

strain	accession number	Closest match(% similarity)	accession number	Phylum
BJ1	DQ298405	*Planococcus southpolaris* strain 840r(98.52)	AJ314747	Firmicutes
BJ2	DQ298406	*Psychrobacter* sp. DVS150R(99.60)	AY864645	γ-Proteobacteria
BJ3	DQ298407	*Psychrobacter* sp. DVS150R(99.93)	AY864645	γ-Proteobacteria
BJ4	DQ298408	*Psychrobacter* sp. DVS150R(99.86)	AY864645	γ-Proteobacteria
BJ5	DQ298409	*Psychrobacter* sp. DVS150R(99.73)	AY864645	γ-Proteobacteria
BJ6	EF627986	*Burkholderia* sp. oral clonrAK168(98.79)	AY005032	β-Proteobacteria
BJ7	DQ298410	*Psychrobacter cibarius* strain JG-220(99.61)	AY639872	γ-Proteobacteria
BJ8	DQ298404	*Pseudoalteramonas* sp. 2-3-6-2(99.80)	AY383040	γ-Proteobacteria

16S rDNA sequences of strains BJ2-BJ5 and BJ7 were 99% similar to that of genus *Psychrobacter*, strain BJ1, BJ6 and BJ8 were 98%, 98% and 99% similar to that of genus *Planococcus*, *Burkholderia* and *Pseudoalteramonas* respectively. In general, the 16S rDNA sequence homolog should be higher than 98% among species, while no less than 93% ~ 95% among genus[13, 14]. The strains BJ2-BJ5 and BJ7 may be ranged as members of genus *Psychrobacter*. Strain BJ1, BJ6 and BJ8 may be categorized as a member of genus *Planococcus*, genus *Burkholderia* and genus *Pseudoalteramonas* respectively.

3.3 Phylogenetic analysis

A phylogenetic tree of the 8 strains constructed with the neighbor-joining method of program MEGA3 was shown in Fig.1. Strain BJ2-BJ5 and BJ7 all belonged to γ-Proteobacteria, Pseudomonadales, Moraxellaceae, *Psychrobacter*. Strain BJ8 belonged to γ-Proteobacteria, alteromonadales, Pseudoalteromonadaceae, *Pseudoalteramonas*. Strain BJ1 belonged to bacteria, Firmicutes, Bacillales, Planococcaceae, *Planococcus*. Strain BJ6 belonged to Bacteria, β-Proteobacteria, Burkholderiales, Burkholderiaceae, *Burkholderia*.

16SRNA sequence analysis of bacteria in Antarctic sea-ice revealed that psychrophilic strains belonged to the genera *Colwellia*, *Shewanella*, *Marinobacter*, *Planococcus*, and novel phylogenetic lineages adjacent to *Colwellia* and *Alteramonas* and within the *Flexibacter-Bacteroides-Cytophaga* phylum. Psychrotrophic strains were found to be members of the genera *Pseudoalteramonas*, *Psychrobacter*, *Halamonas*, *Pseudamonas*, *Hyphamonas*, *Sphingamonas*, *Arthrobacter Planococcus*, and *Halobacillus*[8]. Sea-ice bacteria were mainly categorized into 4 kinds: Proteobacteria, CFB, low mole ratio G+C gram-positive bacteria and high mole ratio G+C gram-positive bacteria[3]. Brinkmeyer *et al*.[15] conducted a comprehensive assessment of bacterial diversity and community in Arctic and Antarctic pack ice through cultivation and cultivation-independent molecular techniques. The results showed that γ-Proteobacteria was the dominating group in their sea-ice sample, and a few β-pro-

teobacteria was detectable. Our results were consistent with conclusions above. The 8

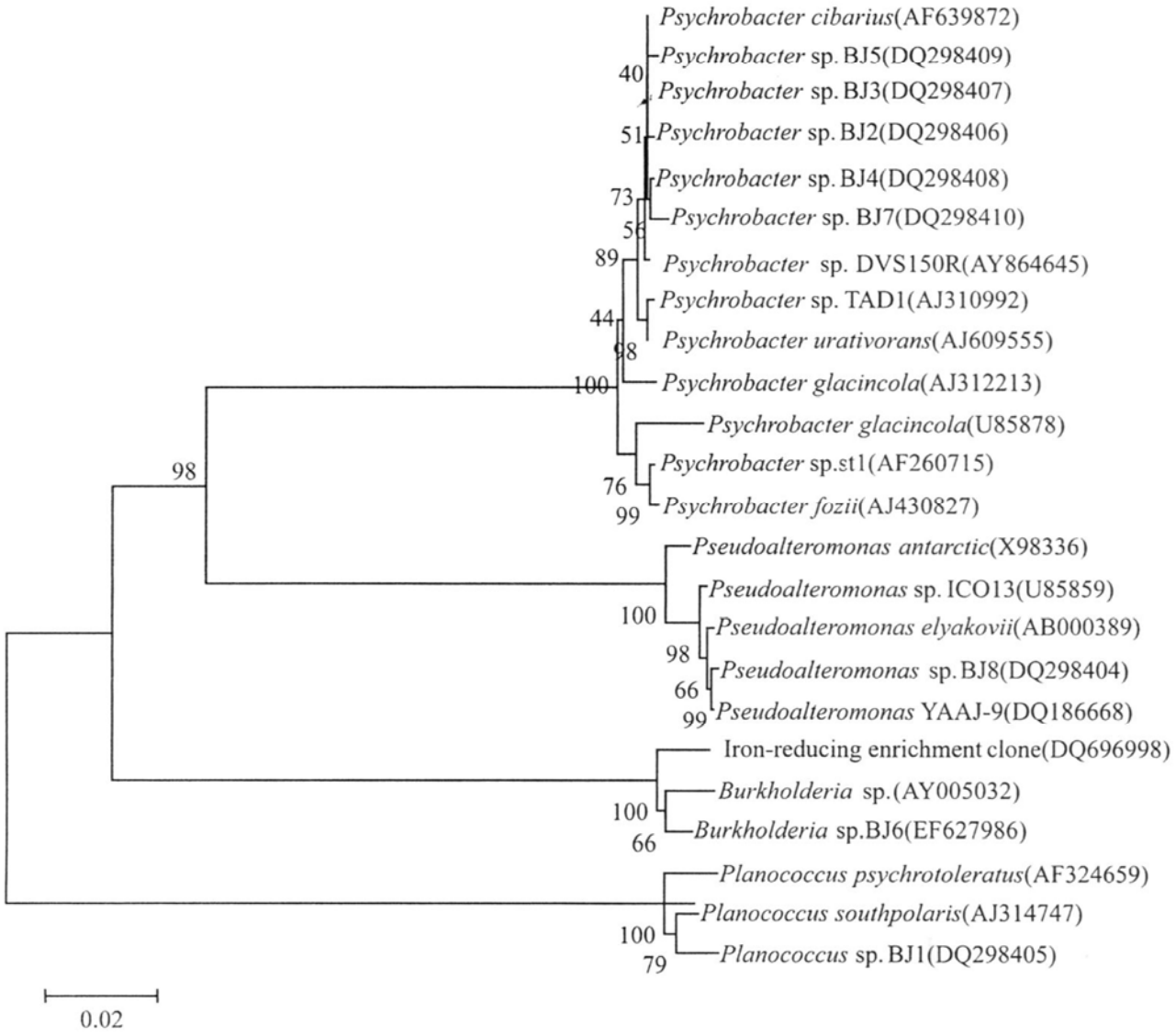

Fig. 1 Phylogenetic tree of 8 strains of bacteria isolated from Arctic sea-ice

stains belonged to genus of *Psychrobacter*, *Burkholderia*, *Pseudoalteromonas* and *Planococcus respectively*, of which *Psychrobacter* and *Pseudoalteromonas* belong to γ-proteobacteria, *Burkholderia* belonged to β-proteobacteria, *Planococcus* belong to low mole ratio G + C gram-positive bacteria. *Psychrobacter*, *Pseudoalteromonas* and *Planococcus* were all members of gram-negative psychrotrophic genera.

Bacteria of Genus *Psychrobacter* were the main species of cultivable bacteria isolated from the Arctic sea-ice sample in our study. The genus *Psychrobacter* was created by Juni & Heym to accommodate a group of non-motile, oxidase-positive, non-pigmented, chiefly psychrotolerant, often found as diploforms, measuring 0.4-1.8 by 0.4-0.8 μm in size, Gram-negative rods or coccobacilli isolated from the skin of fish and chickens and from various processed foods[16]. *Psychrobacter* is a genus within the gamma-proteobacteria, which belongs to Pseudomonadales, Moraxellaceae. These bacteria live in extremely cold habitats, such as Antarctic ice, soil, and sediments, as well as in deep sea environments (http://microbewiki.kenyon.edu/index.php/Psychrobacter).

Phylogenetic analysis also indicated that cultivable bacteria of genus *Psychrobacter* of

our study formed a relatively independent phylogenetic cluster in comparison with other bacteria belonged to *psychrobacter* genus. However, the general similarity of bacterial phylotypes in Arctic and Antarctic pack ice implied that the same selective mechanisms occur at the both poles. Analysis at the conservative gene level of 16S rDNA was not sufficient to determine if the same species occurred at the both poles. Other analytical methods such as DNA-DNA hybridization could elucidate diversity that was not detected by 16S rDNA gene sequencing[15]. It was still uncertain whether Arctic sea-ice might have a relatively particular microbial population unless other more detailed phylogentic analysis was carried out.

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References

[1] Thomas DN, Dieckmann GS (2002): Antarctic sea ice—a habit for extremophiles. Science, 295: 641-644.

[2] Staley JT, Gosink JJ (1999): Biodiversity and biogeography of sea ice. Annu Rev Microbiol, 53: 189-215.

[3] Yu Y, Li HR, Chen B *et al* (2005): Advance of research for sea-ice bacteria. Chinese Journal of Polar Research (in chinese), 17(1): 75-85.

[4] Brown MV, Bowman JP (2001): A molecular phylogenetic survey of sea-ice microbial communities. FEMS Microbiology Ecology, 35: 267-275.

[5] Arrigo KR, Worthen DL, Lizotte MP *et al* (1997): Primary production in Antarctic sea ice source. Science, 276: 641-644.

[6] Kotteier ST, Sullivan CW (1987): Sea ice microbial communities. VIII. Bacterial production in annual sea ice of McMurdo Sound. Antarct Mar Ecol Prog Ser, 35: 175-186.

[7] Gleitz M, Grossmann S, Scharek R *et al* (1996): Ecology of bacterial and diatom assemblages in water associated with melting summer sea ice in the Weddle Sea. Antarctic Science, 8: 135-146.

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

[9] Lin XZ, Shen JH, He PQ (2005): Effects of salt on the growth of bacteria isolated from Arctic ice. Chinese Journal of Polar Research (in chinese), 17(2): 121-126.

[10] Yang XX, Lin XZ, Bian J *et al* (2004): Screening and Phylogenetic Analysis of Antarctic Bacteria Producing Low-temperature Lipase. Acta Oceanologica Sinica, 22(4): 643-650.

[11] Kumar S, Tamura K, Nei M (2004): MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics, 5: 150-163.

[12] Mock T, Thomas DN (2005): Recent advances in sea-ice microbiology. Environmental Microbiology, 7(5): 605-619.

[13] Devereux R, He SH, Doyle CL *et al* (1990): Diversity and origin of Desulfovibrio species: phylogenetic definition of a family. J Bacteriol, 172: 3609-3619.

[14] Fry NK, Warwick S, Saunders NA *et al* (1991): The use of 16S ribosomal RNA analyses to investigate the phylogeny of the family Legionellaceae. J Gen Microbiol, 137: 1215-1222.

[15] Brinkmeyer R, Knittel K, Jurgens L *et al* (2003): Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. Appl Environ Microbiol, 69(11): 6 610-6 619.

[16] Núria Bozal, M. Jesús Montes, Encarna Tudela *et al* (2003): Characterization of several Psychrobacter strains isolated from Antarctic environments and description of Psychrobacter luti sp. nov. and Psychrobacter fozii sp. nov. International Journal of Systematic and Evolutionary Microbiology, 53: 1093-1100.