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| 보 문 |

Diversity and Physiological Characteristics of Culturable Bacteria from Marine Sediments of Ross Sea, Antarctica

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남극 로스해 퇴적물로부터 분리된 세균의 다양성 및 생리학적 특성

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The affiliations and physiological characteristics of culturable bacteria isolated from the sediments of Ross Sea, Antarctica were investigated. Sixty-three isolates obtained by cultivation were grouped into 21 phylotypes affiliated with the phyla *Actinobacteria* and *Bacteroidetes* and with the classes *Alphaproteobacteria* and *Gammaproteobacteria* by phylogenetic analysis of 16S rRNA gene sequences. Based on phylogenetic analysis (<98.65% sequence similarity), approximately 49% of total isolates represented potentially novel species or genus. Among them, extracellular protease, lipase, and exopolysaccharide activities at 10℃ or 20℃ were detected in approximately 46%, 25%, and 32% of the strains, respectively. Forty-three isolates produced at least one type of extracellular material and 21 of them produced at least two extracellular protease, lipase, and/or exopolysaccharides. Our findings indicate that culturable bacterial diversity present within the marine sediments of Ross Sea, Antarctica may contribute to the hydrolysis of the major organic constituents which is closely related with carbon and nitrogen cycling in this environment.

***Keywords*:** cold-active enzymes, cultivation, exopolysaccharides, marine sediments

The fraction of benthic bacteria may account for 10% to 30% of the Earth's total biomass and approximately 70% of the global prokaryotic biomass (Whitman *et al*., 1998; Li *et al*., 2009). In addition, benthic bacterial communities in the ocean play significant roles in biogeochemical cycles and in the remineralization of organic materials (Ravenschlag *et al*., 2001; Li *et al*., 2009).

Among the diverse mechanisms that enable microorganisms to survive and thrive in cold environments are adaptive alterations in cellular proteins and lipids (Russell, 1998; Gerday *et al*., 2000). In polar environments, extracellular enzymes secreted by cold-adapted microorganisms play a crucial ecological role in nutrient cycling (Staley and Herwig, 1993; Vazquez *et al*., 2004). These cold-active enzymes are also highly valuable because of their potential biotechnological applications that reduce the energy required for chemical processes (Groudieva *et al*., 2004) and the potential use of exopolysaccharides from polar fungi and bacteria as cryoprotectants (Selbmann *et al*., 2002; Kim and Yim, 2007). Thus, the ability of the newly isolated psychrophilic or psychrotolerant strains to produce a broad spectrum of cold-active enzymes and other materials is of great interest for both fundamental research and a broad range of industrial, agricultural, and medical applications (Yu *et al*., 2011).

New molecular techniques have enabled cultivation-independent approaches to be widely adopted in research into microbial diversity (Bai *et al*., 2006). Nonetheless, previous studies on benthic bacterial communities by culture-independent methods revealed that many of the OTUs detected had low similarities with known strains, indicating their unknown functions and physiologies (Bowman and McCuaig, 2003; Polymenakou *et al*., 2005; Webster *et al*., 2006). This observation highlighted the necessity for cultivation, to isolate previously undescribed strains and to understand their physiological characteristics. Determination of the physiological characteristics

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Table 1. Sampling sites and sample collection information.

Sample ID	Sampling site	Water depth (m)	Sampling method	Sampling date
Sed1	74°38'46.20"S/ 164°13'24.60"E	156	Box corer	2011-02-06
Sed2	76°06'17.10"S/ 169°12'45.36"E - 76°41'03.60"S/ 169°11'30.66"E	375	Dredge	2011-02-08
Sed3	74°37'30.61"S/ 164°14'56"E	54	Grab sampler	2011-02-11

of culturable microorganisms can provide information on their ecological roles of at least some members of the microbial community (Jiang *et al*., 2006). In addition, the culture-based approach is appropriate for assembling a collection of microorganisms for biochemical, genetic, and physiological studies (Jiang *et al*., 2006).

In this study, we present the taxonomic affiliations of bacterial isolates recovered from the sediments of Ross Sea, Antarctica to gain insights into the nature of the culturable bacterial diversity. Additionally, the physiological characteristics of the obtained bacteria were investigated.

Materials and Methods

Samples and isolation of bacterial strains

Marine sediments were collected in 2011 from three sites in Ross Sea, Antarctica by a box corer, dredge, or grab sampler (Table 1). The samples were suspended in 20% glycerol and preserved at -80℃ until use. For cultivation of bacterial isolates, the samples were serially diluted up to 10^{-3} in sterilized sea water and 100-µl aliquots of the diluted sample suspensions were spread on four kinds of medium followed by incubation at 10℃ for 12–22 days. The media used in these cultivations were: (1) marine agar (MA, Difco, USA), (2) 1/10 diluted marine agar (0.1× MA), (3) marine R2A (MR2A, Difco), and (4) 1/10 diluted marine R2A (0.1× MR2A). After incubation, colonies from the agar plates were picked on the basis of their morphology and subcultured in fresh agar medium until pure isolates were obtained. Pure cultures of the bacterial isolates were deposited in the Polar and Alpine Microbial Collection (PAMC) (Lee *et al*., 2012).

Examination of physiological characteristics

Cell suspensions cultured in marine broth medium (Difco) at 10℃ with shaking at 120 rpm were used to investigate the physiological characteristics of the respective isolates. Growth temperature and the production of extracellular protease, lipase, and exopolysaccharides were determined using the replica plating methods of Lee *et al*. (2012). The temperature-dependent growth response was studied by replica plating of cell suspensions with a 96-pin replicator (VP-408B, V&P Scientific, USA) followed by a 7-day incubation at 4℃, 10℃, 15℃, 20℃, 25℃, 30℃, or 37℃. Growth was evaluated by scoring the size and turbidity of the colony, as described by Lee *et al*. (2012). Protease and lipase secretion was examined by replica plating of cell suspensions onto 0.1× MA plates supplemented with 1% skim milk (Difco) or 1% tributyrate (Sigma, USA), respectively. The plates were incubated for 7 days at 10℃ and 20℃, respectively. Enzyme secretion was scored based on the ratio of colony size to the width of the clear zone surrounding the colony. Exopolysaccharide (EPS) production was recognized by the formation of ropy colonies (Macura and Townsley, 1984) at 20℃. The color of the isolates was determined visually.

Identification of bacterial isolates

Bacterial strains were identified based on the sequence similarity and phylogenetic analysis of 16S rRNA gene sequences. Bacterial genomic DNA was extracted using the LaboPass tissue mini kit (Cosmogenetech, Korea). The 16S rRNA gene was PCR-amplified with two universal primers, 27F; 5′-AGA GTT TGA TCM TGG CTC AG-3′ and 1492R; 5′-GGT TAC CTT GTT ACG ACT T-3′, as described by Lane (1991). PCR was carried out using the method described by Lee *et al*. (2012). PCR products were purified using the LaboPass PCR purification kit (Cosmogenetech) and sequenced with the same primers used for amplification. The sequence of the 16S rRNA gene was compared with those of type strains available in the EzTaxon-e database (Kim *et al*., 2012) to find closely related species and to choose reference sequences for the phylogenetic analyses. Phylogenetic trees were reconstructed by the neighbor-joining method (Saitou and Nei, 1987) based on the distance matrix generated according to Kimura's two-parameter model (Kimura, 1980) and using phydit ver. 3.2 (http://plaza.snu.ac.kr/~jchun/phydit/). The confidence level of the tree topology was evaluated by bootstrap analysis using 1,000 sequence replications. The species affiliation of a bacterial isolate was determined when the isolate formed a monophyletic group with the reference species and based on a 98.65% or higher similarity (Kim *et al*., 2014). The sequences were submitted to NCBI GenBank under the accession numbers KJ475136-KJ475197 and KF977035.

Results

Phylogenetic identification of the isolates

Sixty-three isolates, affiliated with *Actinobacteria*, *Bacteroidetes*,

Table 2. Taxonomic assignments and physiology of the bacterial isolates.

Species name	Similarity (%)	Temperature range (℃)	Protease*		Lipase*		EPS production	Pigment characteristics	Sample ID	PAMC No.	Accession no.
			10℃	20℃	10℃	20℃					
Actinobacteria											
Cryobacterium psychrotolerans	99.3	10-15	3	2	0	0	No	Yellowish cream	Sed3	27129	KJ475137
Marisediminicola antarctica	100.0	4-30	0	0	0	0	No	Apricot	Sed3	27228	KJ475136
Bacteroidetes											
Algibacter sp.	97.2	4-15	0	0	0	0	No	Yellow	Sed1	27237	KJ475138
Flavobacteriaceae sp.	95.9	4-30	0	0	2	2	No	Yellow	Sed1	27105	KJ475139
Flavobacterium gelidilacus	100.0	10-37	2	2	2	3	No	Yellow	Sed3	27103	KJ475144
Flavobacterium gelidilacus	99.6	4-25	0	0	0	0	Yes	Yellow	Sed3	27227	KJ475154
Flavobacterium sp.	99.4	10-30	4	1	0	1	No	Yellow	Sed3	27098	KJ475140
Flavobacterium sp.	98.9	4-15	0	2	0	0	No	Reddish	Sed3	27099	KJ475141
Flavobacterium sp.	99.2	10-15	3	0	0	0	Yes	Yellow	Sed3	27101	KJ475142
Flavobacterium sp.	99.0	10-20	3	1	0	0	Yes	Yellow	Sed3	27102	KJ475143
Flavobacterium sp.	99.1	4-30	3	3	2	2	No	Yellow	Sed3	27115	KJ475145
Flavobacterium sp.	99.1	4-30	4	3	0	0	No	Yellow	Sed3	27122	KJ475146
Flavobacterium sp.	99.1	4-20	3	0	0	0	No	Yellow	Sed3	27123	KJ475147
Flavobacterium sp.	99.1	4-20	3	0	0	0	Yes	Yellow	Sed3	27124	KJ475148
Flavobacterium sp.	99.1	10-20	4	2	0	0	No	Yellow	Sed3	27125	KJ475149
Flavobacterium sp.	99.2	4-20	3	0	0	0	No	Yellow	Sed3	27131	KJ475150
Flavobacterium sp.	99.1	4-20	4	3	0	0	Yes	Yellow	Sed3	27133	KJ475151
Flavobacterium sp.	99.2	4-20	4	3	0	0	No	Yellow	Sed3	27134	KJ475152
Flavobacterium sp.	99.1	4-20	4	2	0	0	Yes	Yellow	Sed3	27207	KJ475153
Lacinutrix sp.	97.5	4-10	0	0	0	0	No	Yellow	Sed1	27137	KF977035
Polaribacter sp.	97.9	10-30	0	0	0	0	Yes	Yellow	Sed1	27095	KJ475155
Polaribacter sp.	97.9	4-30	0	0	2	2	Yes	Yellow	Sed1	27096	KJ475156
Polaribacter sp.	98.1	10-15	1	0	0	0	Yes	Reddish	Sed3	27100	KJ475157
Psychroserpens damuponensis	99.2	4-30	0	0	0	0	Yes	Yellow	Sed2	27240	KJ475165
Psychroserpens sp.	97.6	10-20	0	0	0	0	No	Reddish	Sed1	27104	KJ475158
Psychroserpens sp.	97.8	4-30	0	0	1	1	Yes	Reddish	Sed1	27106	KJ475159
Psychroserpens sp.	97.0	10	0	0	0	0	No	Greenish Yellow	Sed3	27130	KJ475160
Psychroserpens sp.	97.3	4-20	4	2	0	0	Yes	Yellow	Sed1	27206	KJ475161
Psychroserpens sp.	97.7	10-15	3	3	0	0	No	Reddish	Sed1	27216	KJ475162
Psychroserpens sp.	97.1	10-15	0	0	0	0	No	Yellow	Sed1	27220	KJ475163
Psychroserpens sp.	97.8	4-30	0	0	0	0	No	Reddish	Sed1	27238	KJ475164
Winogradskyella sp.	98.5	4-30	0	0	1	2	Yes	Apricot	Sed1	27097	KJ475166
Winogradskyella sp.	98.5	4-30	0	0	2	1	Yes	Apricot	Sed1	27107	KJ475167
Winogradskyella sp.	98.5	10-30	0	0	0	0	Yes	Reddish	Sed1	27217	KJ475171
Winogradskyella sp.	98.6	10-15	0	0	0	0	No	Reddish	Sed1	27221	KJ475172
Winogradskyella sp.	97.0	10-20	3	1	0	0	No	Yellow	Sed3	27136	KJ475168
Winogradskyella sp.	97.1	10-15	0	0	0	0	No	Yellow	Sed3	27139	KJ475169
Winogradskyella sp.	97.0	10-37	0	0	0	0	Yes	Yellow	Sed3	27140	KJ475170
Alphaproteobacteria											
Loktanella salsilacus	99.9	4-30	3	0	0	0	Yes	Beige	Sed3	27121	KJ475175
Loktanella salsilacus	100.0	4-30	0	0	0	0	No	Beige	Sed3	27229	KJ475181

Table 2. continued

Species name	Similarity (%)	Temperature range (℃)	Protease*		Lipase*		EPS production	Pigment characteristics	Sample ID	PAMC No.	Accession no.
			10℃	20℃	10℃	20℃					
Loktanella sp.	95.7	4-30	0	0	1	0	Yes	Beige	Sed1	27117	KJ475173
Loktanella sp.	95.7	4-30	0	0	1	0	Yes	Beige	Sed1	27118	KJ475174
Loktanella sp.	97.0	10	0	0	0	0	No	Beige	Sed1	27126	KJ475176
Loktanella sp.	97.0	4-20	3	3	0	0	No	Beige	Sed3	27132	KJ475177
Loktanella sp.	96.7	4-20	4	2	0	0	No	Cream	Sed3	27135	KJ475178
Loktanella sp.	96.8	10-15	0	0	0	0	No	Beige	Sed1	27138	KJ475179
Loktanella sp.	96.4	10-15	3	2	0	0	No	Beige	Sed1	27223	KJ475180
Loktanella sp.	97.1	10-25	0	0	0	0	No	Beige	Sed3	27241	KJ475182
Octadecabacter antarcticus	99.9	10-25	0	0	0	0	No	Beige	Sed1	27224	KJ475183
Octadecabacter antarcticus	99.9	10-25	0	0	0	0	No	Beige	Sed1	27225	KJ475184
Roseovarius sp.	97.0	10-20	0	0	0	0	No	Beige	Sed3	27236	KJ475185
Sulfitobacter litoralis	100.0	4-30	2	0	0	0	No	Beige	Sed2	27120	KJ475187
Sulfitobacter litoralis	99.6	4-30	2	2	0	2	No	Cream	Sed2	27109	KJ475186
Sulfitobacter sp.	98.1	10	0	0	0	0	No	Beige	Sed1	27222	KJ475188
Sulfitobacter sp.	98.1	10-15	0	0	0	0	No	Beige	Sed1	27232	KJ475189
Gammaproteobacteria											
Psychrobacter luti	99.8	4-30	4	3	2	1	Yes	Cream	Sed3	27116	KJ475194
Psychrobacter luti	100.0	4-30	2	2	0	0	No	Beige	Sed2	27119	KJ475195
Psychrobacter nivimaris	99.9	4-30	0	1	2	0	No	Cream	Sed2	27108	KJ475190
Psychrobacter nivimaris	99.9	4-30	0	0	1	2	No	Cream	Sed2	27110	KJ475191
Psychrobacter nivimaris	99.8	4-30	0	0	1	2	No	Cream	Sed2	27111	KJ475192
Psychrobacter nivimaris	99.9	4-30	1	0	1	1	No	Cream	Sed2	27112	KJ475193
Psychrobacter submarinus	99.9	10-30	0	0	0	0	No	Cream	Sed2	27239	KJ475196
Shewanella gelidimarina	99.7	4-10	0	0	0	0	No	Apricot	Sed1	27094	KJ475197

* The scores (from 1 to 4) represent the degree of production, with a higher number implying better production of protease and lipase

Alphaproteobacteria, and *Gammaproteobacteria*, were obtained from three sediment samples (Table 2 and Fig. 4). Two of the isolates belonged to the phylum *Actinobacteria*, represented by the genera *Cryobacterium*, and *Marisediminicola*; 36 isolates belonged to the phylum *Bacteroidetes*, represented by the genera *Algibacter* (1 isolate), *Flavobacterium* (15 isolates), *Lacinutrix* (1 isolate), *Polaribacter* (3 isolates), *Psychroserpens* (8 isolates), *Winogradskyella* (7 isolates), and the unidentified genus of family *Flavobacteriaceae* (1 isolate); 17 isolates of the class *Alphaproteobacteria* belonged to the genera *Loktanella* (10 isolates), *Octadecabacter* (2 isolates), *Roseovarius* (1 isolate), and *Sulfitobacter* (4 isolates); and 7 to the genus *Psychrobacter* and 1 member of the genus *Shewanella* of *Gammaproteobacteria*. Among these isolates, the largest groups in terms of the number of isolates recovered were those belonging to the genera *Flavobacterium* (15 isolates), *Loktanella* (10 isolates), *Psychroserpens* (8 isolates), and *Winogradskyella* (7 isolates), and *Psychrobacter* (7 isolates). Isolates affiliated with the genera *Psychroserpens* and *Sulfitobacter* were obtained from all three sediments. The overall similarity of the isolates to known type strains ranged from 95.7% to 100% and 31 isolates (49.2% over total strains) had <98.65% 16S rRNA gene sequence similarity with the nearest type strains (Table 2).

Physiological characteristics

The growth temperature range of the 63 isolates and their production of extracellular enzymes, such as protease and lipase, and polymers were determined. Most of the isolates (86%) produced apricot, beige, yellow, or red pigments readily recognizable with the unaided eye (Table 2). As the temperature increased from 4℃ to 10℃, the number of growing strains increased from 39 to 63 and then gradually decreased from 15℃ (Fig. 1). Most of the isolates grew well between 10℃ and 15℃ but only three strains could grow at 37℃ (Fig. 1).

Extracellular protease activities were detected in 29 isolates, belonging to the genera *Cryobacterium*, *Flavobacterium*, *Polaribacter*, *Psychroserpens*, *Winogradskyella*, *Loktanella*,

Sulfitobacter, and *Psychrobacter* (Table 2). Strains belonging to the genera *Flavobacterium* (45%), *Psychrobacter* (19%), and *Loktanella* (13%) accounted for a large proportion of the extracellular protease producers (Fig. 2A). Proteolysis at 10℃ and 20℃ was detected in 27 and 21 isolates, respectively (Table 2). Nineteen isolates showed extracellular protease activities both at 10°C and 20℃ and higher extracellular protease activity was observed at 10℃ than 20℃. In particular, 21 isolates, belonging to the phylotypes *Cryobacterium psychrotolerans*, *Flavobacterium* sp., *Psychroserpens* sp., *Winogradskyella* sp., *Loktanella salsilacus*, *Loktanella* sp., and *Psychrobacter luti* (Table 2 and Fig. 4), had high extracellular protease activity (score 3≥) at 10℃ (Fig. 3A).

Sixteen isolates, belonging to the genera *Flavobacterium*, *Polaribacter*, *Psychroserpens*, *Winogradskyella*, *Loktanella*, *Sulfitobacter*, *Psychrobacter*, and unidentified genus of the family *Flavobacteriaceae*, had extracellular lipase activity, with strains of the genera *Psychrobacter* (31%), *Flavobacterium* (19%), *Loktanella* (13%), and *Winogradskyella* (13%) accounting for a large proportion of the extracellular lipase producers (Fig. 2B). Fourteen isolates exhibited enzyme activity at 10℃ and 13 isolates exhibited enzyme activity at 20℃ (Table 2). Among the 11 isolates with extracellular lipase activity at both 10℃ and 20℃, score-based enzyme activity was higher at the higher temperature (Fig. 3B). There were no isolates that had an extracellular lipase activity of score 4 and only one strain, PAMC 27103, had an extracellular lipase activity of score 3 at 20℃. This strain had 100% similarity with *Flavobacterium gelidilacus* (Table 2 and Fig. 4).

The 20 isolates that produced exopolysaccharides at 20℃ were affiliated with the genera *Flavobacterium*, *Polaribacter*, *Psychroserpens*, *Winogradskyella, Loktanella*, and *Psychrobacter* (Table 2). Among them, strains belonging to the genera *Flavobacterium* (30%) were the most abundant followed by *Winogradskyella* (20%), *Loktanella* (15%), *Polaribacter* (15%), and *Psychroserpens* (15%) (Fig. 2C). Approximately 43 isolates (70%) produced at least one extracellular enzyme or exopolysaccharide, and the isolate, PAMC 27116, with 99.2% similarity to *Psychrobacter luti*, produced three of them (Table 2).

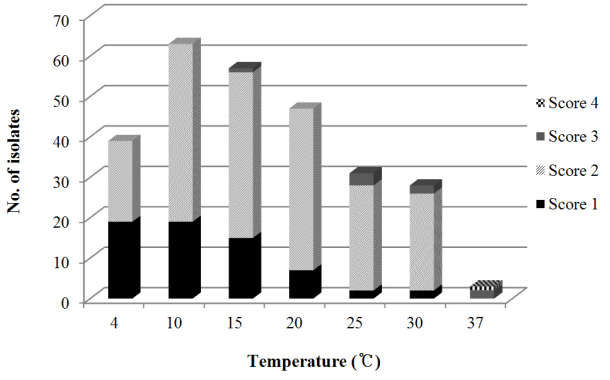

Fig. 1. Effect of temperature on bacterial growth. The scores (from 1 to 4) represent the degree of growth, with a higher number implying better growth.

Discussion

In the sediments from Ross Sea, Antarctica, both the spatial distribution of the organic matter composition and bacterial densities have been reported (Fabiano and Danovaro, 1998; Fabiano and Pusceddu, 1998; Pusceddu *et al*., 2000; Baldi *et al*., 2010). The bacterial community of Ross Sea sediments, as determined by T-RFLP analysis, revealed the predominance of bacteria belonging to *Gammaproteobacteria*, *Deltaproteobacteria*, *Bacteroidetes*, and *Acidobacteria* (Baldi *et al*., 2010). To the best of our knowledge, this is the first report on the diversity and physiological characteristics of culturable bacteria in Ross Sea sediments. The culturable bacteria present in the samples

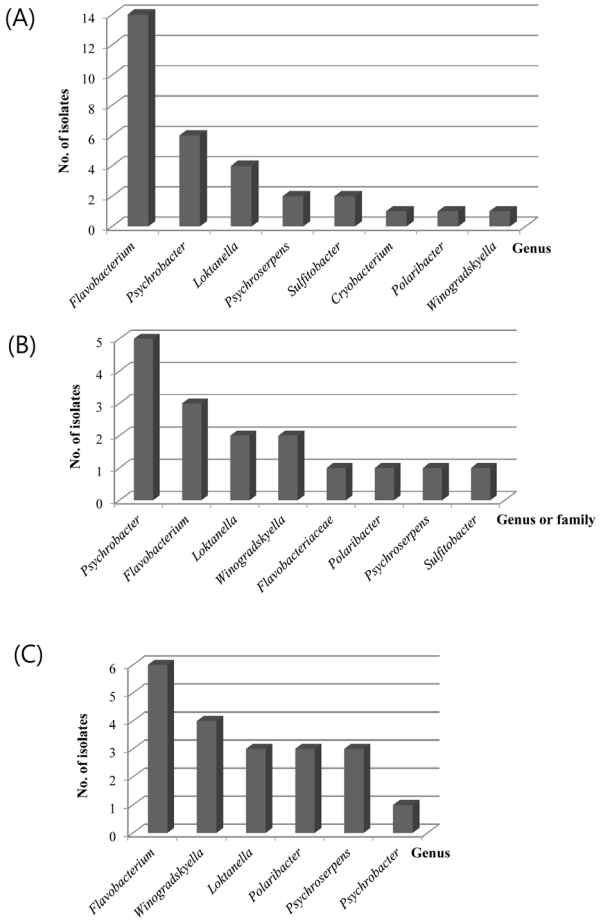

Fig. 2. Abundance of isolates, according to genus or family, that produce extracellular protease (A), lipase (B), and exopolysaccharides (C).

were affiliated with 16 genera (Table 2) and many of them (70%) produced extracellular protease, lipase, and/or exopolysaccharides. Most of the organic matter in benthic communities is produced as high-molecular-weight polymeric compounds, which before they can be incorporated into microbial cells must be degraded by a series of extracellular hydrolytic enzymes (Yu *et al.*, 2011). The finding in this study, that a large proportion of the isolates produced at least one type of extracellular enzymes, points to the significant roles played by benthic microorganisms in the biogeochemical cycles of Ross Sea sediments. In addition, analyses of temperature-dependent growth showed that the number of isolates capable of growing at 10℃ was highest, indicating an adaption at the stable, low temperature of the bottom sediments in the Southern Ocean (Helmke and Weyland, 2004).

The predominance of the phylum *Bacteroidetes* is known in the previous studies of bacterial communities from marine sediments of Antarctica (Bowman and McCuaig, 2003; Baldi *et al.*, 2010). In this study, 59% of total recovered isolates belonged to the phylum *Bacteroidetes*. Among them, the large percentage (67%, 29 isolates out of 43) of extracellular-material-producing strains were affiliated with the genera *Flavobacterium*, *Polaribacter*, *Psychroserpens*, *Winogradskyella*, and unidentified genus of the family *Flavobacteriaceae* and it is consistent with the well-established finding of biopolymer hydrolysis by this phylum (Kirchman, 2002). In addition, extracellular protease or lipase production by isolates assigned to the genera *Cryobacterium*, *Loktanella*, *Psychrobacter*, and *Sulfitobacter*, belonging to *Actinobacteria*, *Alphaproteobacteria*, and *Gammaproteobacteria* (Table 2), reflects the importance of these strains in the hydrolysis of organic constituents.

Twenty-one isolates, affiliated with the genera *Flavobacterium*, *Polaribacter*, *Psychroserpens*, *Winogradskyella*, *Loktanella*, *Sulfitobacter*, and *Psychrobacter* produced at least two extracellular proteases, lipases, and/or exopolysaccharides. Of these isolates, members of the genus *Flavobacterium* (33%) clearly dominated. The strains of the genus *Flavobacterium* are known for their specialized roles in the uptake and degradation of the high-molecular-mass fraction of dissolved organic matter and in remineralization processes, both in freshwater and in marine ecosystems (McCammon and Bowman, 2000). They have been frequently reported in oligotrophic and eutrophic Antarctic freshwater, terrestrial samples, and marine sediments, suggesting their wide diffusion in Antarctica and their ecological roles in macromolecule hydrolysis (McCammon *et al.*, 1998; McCammon and Bowman, 2000; Humphry *et al.*, 2001; Van Trappen *et al.*, 2003, 2005; Yi *et al.*, 2005; Yi and Chun, 2006; Michaud *et al.*, 2012).

Although culturable bacteria do not fully represent the bacterial communities of the environment, many candidate novel species with <98.65% similarity to known strains, were recovered in this study. This result demonstrates that relatively simple cultivation methods can be used to isolate as-yet-undescribed taxa and they can be used for expanding the knowledge on the unknown functions and physiologies of bacterial OTUs obtained by molecular techniques. In addition, our finding that culturable bacteria produce cold-active enzymes indicates that the isolated strains contribute to the hydrolysis of major organic constituents and are therefore involved in carbon and nitrogen cycling at the low temperature of sediments of Ross Sea, Antarctica.

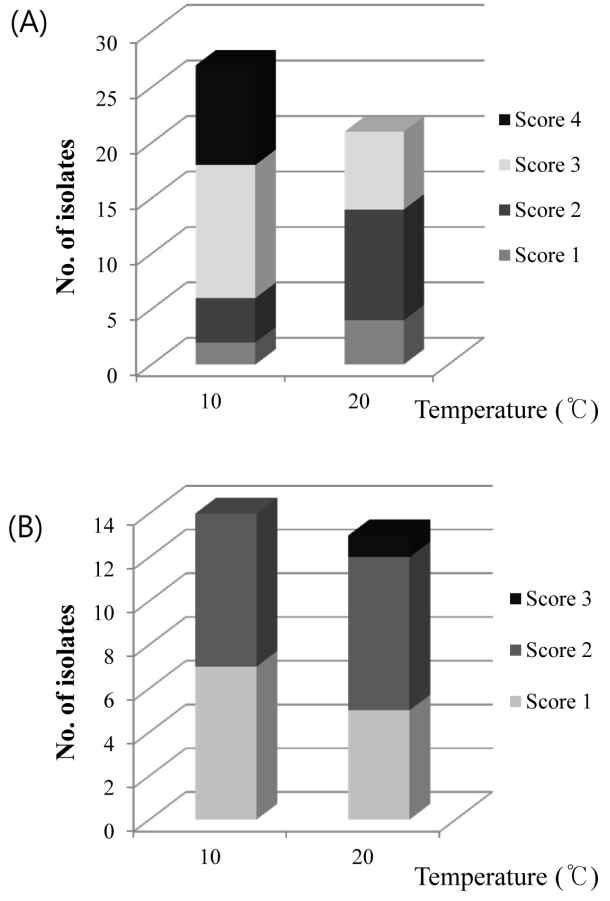

Fig. 3. Frequency of bacterial isolates with extracellular protease (A) and lipase (B) activities. The scores (from 1 to 4) represent the degree of production, with a higher number implying better production.

적 요

남극 로스해의 퇴적물로부터 배양을 통해 분리한 균주의 분류 및 생리학적 특성 분석을 수행하였다. 분리 세균 63균주의 16S rRNA 유전자 염기서열을 이용한 계통분류학적 분석 결과, 이들

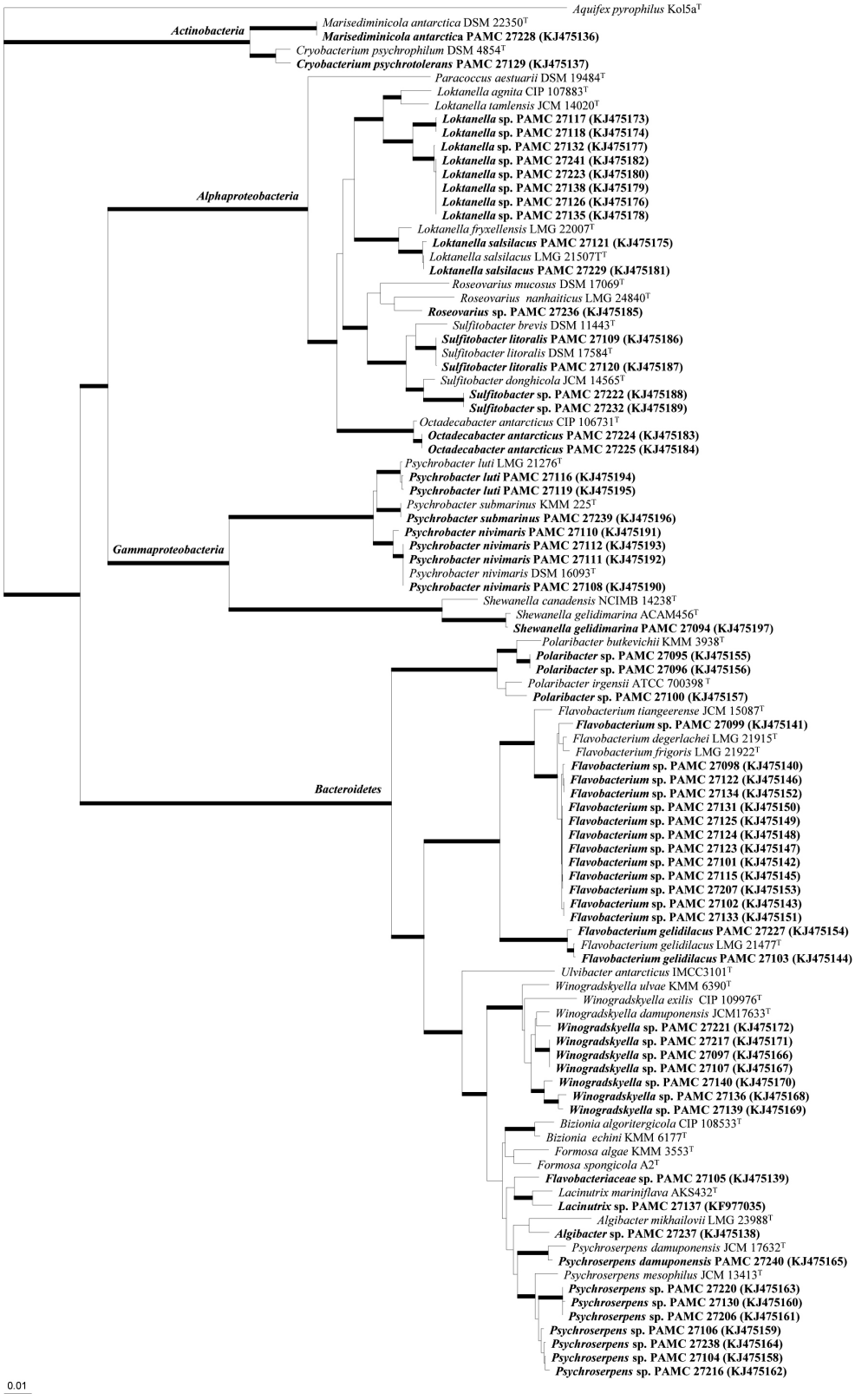

Fig. 4. Neighbor-joining tree of isolates with closely related reference species, based on the 16S rRNA gene sequences. Representative isolates for each phylotype are indicated by bold letters, and branches supported by high bootstrap values (>70%) as thick lines. Bar, 1 nucleotide substitutions per 100 nucleotides.

은 *Actinobacteria*, *Bacteroidetes*, *Alphaproteobacteria* 및 *Gammaproteobacteria* 내의 21개의 파일로타입(phylotypes)에 속하였다. 98.65% 염기서열 유사도를 기준으로, 약 49%의 균주가 잠재적으로 신종 또는 신속 후보인 것으로 나타났다. 분리된 균주 중, 각각 46%, 25% 및 32%의 균주가 세포외 단백질분해효소, 지질분해효소 및 외부다당체 생성에 대한 활성을 나타냈다. 43개의 균주는 최소 1개의 세포외 분비 물질을 생산하였고, 이들 중 21개 균주는 최소 2개의 세포외 단백질분해효소, 지질분해효소 또는/및 세포외다당체를 생성하였다. 이러한 결과는 남극 로스해 퇴적물 내의 배양된 세균 군집이 해당 환경에서 탄소와 질소와 관련된 유기물질의 가수분해에 영향을 미치고 있다는 것을 시사한다.

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