Korea Polar Research Institute, KORDI

24-26 September, 2008

The 15th International Symposium on Polar Sciences

Polar Ecosystems: Biodiversity and Adaptation

Programme and Abstracts

Contents

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About KOPRI

History

2008	Present	The 15th International Symposium on Polar Ecosystems is held in Incheon.
2007	May	Dr. Hong Kum Lee appointed KOPRI's Director-General.
2006	May	The International Symposium on Antarctic Sciences held in Korea.
	April	Move to Incheon Free Economic Zone in Songdo, Incheon
2004	1 July	Korea Polar Research Institute(KOPRI), KORDI was established
	1 May	Dr. Yeadong Kim appointed KOPRI's first Director-General.
	16 April	KOPRI was separated from KORDI and became an independent polar research institution
2003	September	Polar Sciences Laboratory was expanded to Korea Polar Research Institute
2002	27 June	Korean National Committee on Polar Research (KONPOR) was established.
	29 April	The Arctic <Dasan> Station was inaugurated.
	25 April	Korea joined the International Arctic Science Committee (IASC).
2000	July	Polar Research Center was renamed as Polar Sciences Laboratory.
1995	May	The 19th Antarctic Treaty Consultative Meeting (ATCM) was held in Seoul.
1990	July	Korea acceded to SCAR as a regular member.
	June	Polar Research Laboratory was expanded and renamed as Polar Research Center.
1989	October	Korea joined Antarctic Treaty as a Consultative Party
1988	November	The 1st International Symposium on Antarctic Sciences was held in Seoul.
	17 February	The Antarctic <King Sejong> Station was inaugurated.
	January	The first Korea Antarctic Research Program (KARP) team conducted survey in the vicinity of King Sejong Station.
1987	December	Korea joined the Scientific Committee on Antarctic Research (SCAR) as an associated member, and consturuction of King Sejong Station began.
	August	Korean National Committee on Antarctic Research (KONCAR) was established.
	April	A survey team was dispatched to the King George Island to search for a station construction site.
	March	Polar Research Laboratory was established at the Korea Ocean Research and Development Institute (KORDI).
	January	Ministry of Science and Technology (MOST) disclosed a plan to build the first Korean Antarctic Research Station on King George Island
1986	November	Acceded to the Antarctic Treaty as its the 33rd signatory

Programme

Wednesday, September 24

OPENING CEREMONY

09:00 Registration
09:30 Opening Remarks & Welcoming Address / Group Photo

PLENARY LECTURE Ⅰ

Chair : Sung-Ho Kang

10:00 Michael Stoddart	The census of Antarctic marine life; IPY and beyond
10:50 Coffee Break	

POLAR ECOSYSTEMS Ⅰ

Chair : In-Young Ahn

11:10 Peter Convey	Antarctic terrestrial ecosystems: sentinels of change on Gondwanan to Anthropocene time scales
11:35 Arve Elvebakk	A revision and a molecular phylogeny of the austral and partly bipolar lichen genus Psoroma
12:00 Takeshi Naganuma	Merge as an IPY core coordinating activity and for post-IPY
12:25 Lunch	

MECHANISM OF COLD ADAPTATION

Chair : Soon Gyu Hong

14:00 Kwang-Hwan Jung	Adaptation of proteorhodopsin variants from the surface of the Arctic ocean
14:25 John Day	Protistan and cyanobacterial survival at low and ultra-low temperatures
14:50 Rainer Kiko	Ecophysiology of metazoans living inside sea ice
15:15 Coffee Break	
15:35 Gwang Hoon Kim	Radiosensitivity and antioxidant activity of the arctic algae inrelation with cold acclimation
16:00 Yoo Kyung Lee	What genes are expressed in polar plants?

16:25 Jae-Seong Lee	Heat shock protein (*Hsp*) gene responses of the intertidal copepod *Tigriopus japonicus* to environmental stress
16:50	Poster Session
18:30-20:30	Reception by Director-General of KOPRI (Korea Polar Research Institute)

Thursday, September 25

PLENARY LECTURE Ⅱ

Chair : Joung Han Yim

09:00 Tadayuki Imanaka	Interesting microorganisms in the Antarctica
09:50 Coffee Break	

CLASSIFICATION OF POLAR ORGANISMS

Chair : Sang Hoon Lee

10:10 Soon Gyu Hong	Introduction to KOPRI biological database
10:35 Peter Doran	Astrobiology and lakes of the McMurdo Dry Valleys, East Antarctica
11:00 Jang-Cheon Cho	Understanding bacterial diversity in the Arctic ocean by plateculturing, extinction culturing, and 16S rDNA cloning
11:25 Jeong-Hoon Kim	Biodiversity and phylogeny of Skuas from the Antarctia
11:50 Lunch	

POLAR ECOSYSTEMS Ⅱ

Chair : Hyung Chul Shin

13:30 SeungHyun Son	Seasonal and Annual variation of the primary production in the Bering and the Chukchi Seas using the Ocean Color and the sea-observing measurements.
13:55 Yasuhiko Naito	A new approach for complete detection of feeding behavior of marine animals using very small accelerometer
14:20 Veronica Fuentes	Influence of increasing suspended particulate matter concentration on benthic and planktonic Antarctic filter-feeding organisms: a relation with glacier melting
14:45 Coffee Break	

BIOTECHNOLOGY OF POLAR ORGANISMS

Chair : Yoo Kyung Lee

15:10 Hyuncheol Oh	Bioactive secondary metabolites from Antarctic lichens
15:30 Angela Kőhler	Biological effects of contaminants on health of Arctic organisms-urgent needs and problems for the implementation of a monitoring program
15:55 Jae-Seoun Hur	Biological activity of lichen-forming fungi
16:20	Poster Session
18:30-20:30	Banquet (at Hotel Ramada Songdo)

Friday, September 26

09:00 Excursion to Seoul

Abstracts - Oral Presentations

Wednesday, September 24

Plenary Lecture I

Chair : Sung-Ho Kang

The Census of Antarctic Marine Life: IPY and Beyond

Michael Stoddart
Chief Scientist, Australian Antarctic Division
Project Coordinator, CAML

'The Census of Antarctic Marine Life' (www.caml.aq) is a major Antarctic contribution to the International Polar Year 2007/08. Its objectives are to better understand the biological diversity of the waters around Antarctica and to provide a robust benchmark of diversity against which future environmental change can be assessed. In addition it seeks to provide answers to evolutionary questions about the consequences of geographic isolation and adaptation to cold conditions. In addition it is a major field project of the Census of Marine Life (www.coml.org), funded by the Alfred P Sloan Foundation, New York.

Over a dozen dedicated marine science research voyages have participated in the Census over the past two Antarctic summers, with many scientists participating. Three hundred and fifty sites were sampled and 24,000 nautical miles sampled for plankton. Apart from the discovery of large numbers of undescribed species, which will be described and named over the coming years, the CAML has collated distributional data on 11,286 taxa and has compiled over half a million geo-referenced data points and made it available through the Scientific Committee on Antarctic Research's Marine Biodiversity Information Network (www.scarmarbin.be).

Of particular interest are observations that the level of endemism of Antarctic marine species is not as great as previously thought; that parts of the fauna appear to be truly circum-Antarctic; that the Antarctic Circumpolar Current together with the overturning circulation has played a strong part in the speciation of octopus of the genus *Pardelene*; and that the sub-Antarctic sea spider fauna appears to have had its origins in Antarctic waters and further northwards spread is accompanied by speciation.

CAML's data will play a vital part in future deliberations by the Convention on the Conservation of Antarctic Marine Living Resources on identification of marine protected areas, in support of Resolution 61/105 (Sustainable fisheries) adopted by the General assembly of the UN in 2007.

This paper outlines the scope and achievements of the Census to data, and looks forward to what will come after it.

Session 2 : Polar Ecosystems Ⅰ

Chair : In-Young Ahn

Antarctic terrestrial ecosystems: sentinels of change on Gondwanan to Anthropocene time scales

Peter Convey

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Antarctic terrestrial ecosystems are often overlooked. They contribute a tiny proportion of the area of Antarctica, have low species diversity and biomass, and biological communities composed of what are often seen as insignificant groups of lower plants, micro-invertebrates and microbial groups. However, recent research has demonstrated that they carry important signals of the fundamental changes experienced by the Antarctic continent on Pleistocene, Miocene and Gondwana-breakup timescales, relevant to the glacial and geological reconstruction of the continent's history. Coming to the present day, contemporary terrestrial ecosystems provide a powerful tool in identifying the biological consequences of the various anthropogenic environmental change processes affecting the planet.

A revision and a molecular phylogeny of the austral and partly bipolar lichen genus Psoroma

Arve Elvebakk[1], E. Robertsen[2], Chae Haeng Park[3], Anton Liaimer[2] & Soon Gyu Hong[3]

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Psoroma is a heterogeneous genus in the lichen family Pannariaceae. Within its family, it is characterized by having green algae as a primary photobiont and cyanobacteria as secondary photobionts localized to particular structures known as cephalodia. A lichen with two symbiotic partners, in addition to its major mycobiont, is referred to as tripartite.

Psoroma in a wide sense has an almost global distribution. It is found in polar areas (6 species are at present recognized from Antarctica), alpine areas, Paleotropic, montane forests (from Madagascar to Japan/Tahiti/N. Australia), and has a concentration of species in former southern Gondwanaland territories, southern South America and New Zealand/southern Australia. Thus, it is unique in having species concentrations in such different areas of the world, and most certainly an old evolutionary history related to major events in the earth's history, maybe at least during the last 100 mill. years.

Psoroma in a strict sense should be restricted to 15-20 mostly brownish species, concentrated to soil and to arctic-alpine or steppe areas. Such biomes are not the oldest ones, and *Psoroma* s. str. may not be the most ancient part of the complex. To define different parts of this heterogeneous group and their evolutionary relationships, molecular-based studies are needed. We have now initiated a cooperation and have a plan of addressing various subgroups of the complex, some related to the neighbouring genus *Pannaria.* Most of these groups potentially represent new and yet undescribed genera.

The topic of the first manuscript are species forming yellowish-green patches of squamules resting on a thin, black mat of fungal hyphae ('prothallus') on cortex in austral areas. Our ITS and LSU-based analysis produced a phylogenetic tree which shows, when constructed on a sufficient number of sequences, two distinct groups, in addition to the reference groups *Psoroma* s. str. and *Pannaria*. One is yellow due to usnic acid, a unique character within the family, and contains two widespread species, one of them with an erroneously adapted name. This group will be described as a new genus, and may contain two species in addition, which will be studied separately. The other group is strongly yellow-green when wet, but weakly green when dry. However, this is not due to any secondary compound, but to lack of the mostly brownish melanins present in most species of *Pannaria* s.str. Thus, this is the colour of the

chlorobiont, and it deteriorates when the chlorobiont dies. This is also a new genus, and it differs from *Psoroma* s. str. also in anatomical structures. It includes probably two closely related species, one from the Chilean Juan Fernández Islands, the other from moist Patagonian forests.

Our tree aimed for the first paper is based on 38 own sequences. However, an almost equally large number of additional sequences have been made already. They show very interesting patterns also for additional groups, but these need to be supported by additional samples. The general impression is that this group of 'psoromoid' lichens (*Psoroma* in a wide sense) is much more diverse than thought previously, indicating that it had a long history in areas previously united into southern parts of Gondwanaland.

MERGE as an IPY core coordinating activity and for post-IPY

Takeshi NAGANUMA[1] and Annick WILMOTTE[2]

1: School of Biosphere Science, Hiroshima University, Japan

2: Institute of Chemistry, University of Liege, Belgium

An integrated activity "Microbiological and Ecological Responses to Global Environmental Changes in Polar Regions" (MERGE) has been proposed to the International Polar Year (IPY) 2007-2008, and endorsed by the IPY committee as a Coordination Proposal (No. 55). MERGE is an umbrella hosting a number of original IPY proposals. Three key questions have been selected to yield scientific achievements efficiently. Prokaryotic and eukaryotic organisms in terrestrial, lacustrine and supraglacial habitats are targeted in terms of: 1) Diversity and biogeography, 2) Food webs and ecosystem evolution, and 3) Linkages between biological, chemical and physical processes in the supraglacial biome.

The MERGE umbrella respects priorities of achievements by members; however, MERGE also aims at yielding syntheses from the three selected themes. That is, the MERGE umbrella does not necessarily take a top-down approach but a bottom-up approach, and each bottom-up achievement comprises part of a holistic view. As a result, MERGE will submit a few holistic conclusions to IPY after major activities have finished. This lecture is to encourage more participations and contributions of Korean scientists.

Many scientists from Asian countries have already been the member of MEGRE, and are encouraged to utilize MERGE to achieve each Arctic scientist's own goal. MERGE will prepare two key lists of wishes and offers and try to match them for filed activities. This "matchmaking" may be extended to extra-MERGE activities, including the existing and planned projects.

In order to share data and samples, common methodologies are vitally needed. For example, common PCR primer sets to characterize macro-/microflorae should be used among us. An international program "Regional Sensitivity to Climate Change" (RiSCC) of the Scientific Committee for Antarctic Research (SCAR) discussed over this issue; however, the RiSCC protocols do not necessarily cover latest molecular techniques. We may have to develop "MERGE standard protocols", in close collaboration with the RiSCC successor program, viz., Evolution and Biodiversity in Antarctica: the Response of Life to Change (EBA). That is, MERGE is, in a sense, a sister and focused activity of EBA.

Building a formal data archive is also a mission of MERGE. Data collected under the MERGE umbrella should be stored in a "formal data archive". We have not shaped the structure of the archive yet. In addition, Education, Outreach and Communication (EOC) is an important

aspect of IPY. MERGE should construct easily accessible paths for general public. International efforts to develop data archives and EOC paths should be essential in MERGE.

Session 3 : Mechanism of Cold adaptation

Chair : Soon Gyu Hong

Adaptation of proteorhodopsin variants from the surface of the Arctic ocean

Kwang-Hwan Jung

Sogang University, Dept of Life Science

Proteorhodopsin (PR), a retinal-containing seven transmembrane helix protein that functions as a light-driven proton pump, was discovered in the genome of marine bacteria. There are two types of PRs and they have three major differences between GPR and BPR. First, GPR absorbs green light (525nm, pH7), while BPR absorbs blue light (490nm). Second, GPR shows a fast photocycle rate (t1/2, 50 ms), while BPR shows a slower photocycle rate (t1/2>300 ms). Third, GPR exhibits higher light-induced proton pumping activity than BPR. Eighteen PR variants were isolated using PCR from the surface of the Arctic Ocean of Korean Arctic Research Station Dasan. Their absorption maxima were between 517-546 nm at pH7. Interestingly, 10 isolates contain the retinal-binding site member Tyr200 (in MBP) which is replaced with Asn. They show a slow photocycle, more blue shifted absorption maxima than GPR at pH 10, and relatively larger ΔH and ΔS between ground and O intermediate than GPR. It is suggested that the variants might modulate their photocycling rate to control proton pumping efficiency from environmental temperature changes.

Protistan and cyanobacterial survival at low and ultra-low temperatures

John Day

Culture Collection of Algae and Protozoa, Scottish Association for Marine Science, Dunbeg, Argyll, UK

Water is nature's solvent and its behaviour at subzero temperatures is the key to survival in and/or recovery from the "frozen state". Understanding survival in natural and artificial cryogenic environments requires multidisciplinary knowledge, especially regarding the effect of temperature on water behaviour and the formation of its different states, which comprise liquid, solid (ice) and vitreous (glass) forms.

At cold (<0^{o}C) and ultra-cold (<-80^{o}C) the environment impose severe stresses on any organism, with the effects on biological processes and structure being dependant not only on temperature, but also on water content and nature of the environmental niche. Injuries at these temperatures may be categorised as resulting from the stresses associated with chilling, ice formation (both extra- and intracellular) and due to solution/ dehydration effects resulting from an increased osmotic potential as liquid water is converted to solid "ice". In order to survive, any organisms must have the capacity to avoid, adapt to, or be inherently resistant to the effects of these stressors.

This paper discusses the survival of micro-algae (eukaryotic protists and cyanobacteria) in polar niches, the permafrost and at ultra-low temperatures when subjected to cryopreservation.

Ecophysiology of metazoans living inside sea ice

Rainer Kiko, Kramer M, Lucassen M, Schnack-Schiel S
Diplom Biologist, Institute for Polar Ecology, Kiel

Sea ice is a unique habitat. It is permeated by small brine channels, which house a diverse fauna and flora. Surface, interior, bottom and sub-ice habitats characterised by different environmental factors are associated with sea ice and play different roles in the life cycles of sympagic (ice-associated) species. Several copepod and one acoel turbellarian species are the dominant members of the Antarctic sympagic meiofauna. Other species, recently identified in pack ice, are the ctenophore Callianira antarctica and rhabditophore turbellarians. The nudibranch Tergipes antarcticus was refound in this habitat – the occurrence of this species in Antarctic pack ice has only been mentioned in the original species description in 1903 and never since. With temperature extremes of down to -22 °C, sea ice represents one of the coldest habitats on earth. Coupled to the temperature, the brine salinity can vary between S = 220 at -22 °C and S = 2-3 near 0 °C ice temperature. In-situ studies show that copepods and turbellarians cope well with temperatures down to -4 °C and corresponding salinities of 69. The sympagic calanoid copepod Stephos longipes is well adapted to a temperature range of at least -1.2 °C (S = 25) to -3.1 °C (S = 55). This species is slightly hyperosmotic to seawater in a salinity range from 25 to 45 and exhibits a thermal hysteresis of 0 °C to -3.8 °C (median -1.0 °C). Analysis of a cDNA-library of S. longipes enriched for genes expressed at -3.1 °C revealed the presence of an antifreeze protein. Furthermore, a high representation of genes involved in transcription and translation in this library indicates that large structural rearrangements are essential for an adaptation to osmotic and temperature stress. Future applications of molecular biological methods to questions of sea ice research will be proposed.

Radiosensitivity and antioxidant activity of the arctic algae in relation with cold acclimation

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Filamentous arctic algae (*Zygnema* sp), psychrophilic algae (*Zygnema cruciatum* and *Spirogyra varians*) were analyzed for relations between radiosensitivity and antioxidant activity. Arctic alga (*Zygnema* sp.) cultivated at two different temperatures (4℃ and 20℃) were exposed to various does rates of gamma ray irradiation (10 kGy/ hr). Warm condition (20℃) cultivated *Zygnema* sp. could not survive below 1 kGy. Cold codition (4℃) acclimated *Zygnema* acquired a radioresistance and survival dose rate was increased to 5 kGy. When the antioxidant contents of methanolic extract were simultaneously analyzed, filamentous algae accumulated polyphenolic compounds and antioxidant (3 fold than non-cold acclimated algae), were detected. Increment of antioxidant content was in directly proportional to radioresistance. Isolation of the radioresistant related compounds were performed and the possible structures were predicted using ^{1}H, ^{13}C-NMR. The compounds were identified as galloylglucose and flavonoid derivate. The radical scavenging activities of the isolated compounds were 100% to 150% in comparison with ascorbic acid.

What Genes are Expressed in Polar Plants?

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Polar plants have to endure drought as well as low temperature. Because water is frozen in polar region, plants have to overcome not only cold stress but also water stress. Other important factors for plant growth in polar region are day length and irradiance. Due to the high latitude of the continent, considerable differences in the levels of solar radiation and day length between summer and winter occur. The conjunction of high irradiance and low temperature may damage the photosynthetic apparatus, causing a reduction in photosynthesis known as the photoinhibition. Therefore polar plants may have some defense mechanisms that help them survive under the harsh conditions. Deschampsia anatarctica is the only monocot that thrives in the tough conditions of the Antarctic region. It is an invaluable resource for the identification of genes associated with tolerance to various environmental pressures. In order to understand characteristics of gene expression in polar plants and to identify genes that are differentially regulated between greenhouse-grown and Antarctic field-grown plants, we have carried out expressed sequence tags (ESTs) analysis. Antarctic plants were collected and greenhouse plants served as controls. Two different cDNA libraries were constructed with these plants. A total of 2,112 cDNA clones was sequenced and grouped into 1,199 unigene clusters consisting of 243 consensus and 956 singleton sequences. Using similarity searches against several public databases, we constructed a functional classification of the ESTs into categories such as genes related to responses to stimuli, as well as photosynthesis and metabolism. Real-time PCR analysis of various stress responsive genes revealed different patterns of regulation in the different environments, suggesting that these genes are involved in responses to specific environmental factors.

Keywords: Abiotic Stress; Antarctic; Deschampsia antarcitca; Expressed Sequence Tags (ESTs); King George Island; Quantitative Real Time Reverse Transcription PCR (qRT-PCR)

Heat shock protein (*Hsp*) gene responses of the intertidal copepod *Tigriopus japonicus* to environmental stress

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The induction of heat shock proteins (Hsps) is considered as an important protective, ecophysiologically adaptive, and genetically conserved response to environmental stress in all organisms. Among the Hsps, the heat shock protein 70 (Hsp70) family members are most extensively studied for their characterization from and induction in response to environmental stressors in a range of species. We studied expression of ten *Hsp* transcripts in response to heat treatment in an intertidal marine copepod *Tigriopus japonicus* and observed that expression of *Hsp70* was more pronounced than other *Hsps*. Subsequently, cDNA and genomic sequences of *T. japonicus Hsp70* (*TJ-Hsp70*) were worked out by molecular cloning techniques and phylogenetic relationship was analyzed. The bacterial expression of *TJ-Hsp70* and its expression in response to metal and endocrine-disrupting chemical (EDC) exposures were also studied. The *TJ-Hsp70* transformed bacteria showed increased thermotolerance compared to bacteria with vector only. All the trace metals (viz., copper, silver, and zinc) caused a concentration-dependent increase in the expression of *Hsp70* transcripts. Effect of EDCs on *Hsp70* expression was differential. While 4-nonylphenol (NP) and 4-*t*-octylpheno (OP) caused downregulation, bisphenol A (BPA) caused upregulation. The promoter region of the genomic *Hsp70* sequence contained putative xenobiotic response elements (XREs) indicating that *TJ-Hsp70* regulation not only by temperature but also by xenobiotics. These findings suggest that in *T. japonicus,* Hsp70 has a conserved role of thermotolerance and its expression in response to xenobiotics exposure appears to be a protective response.

Thursday, September 25

Pleanary Lecture II

Chair : Joung Han Yim

Interesting microorganisms in the Antarctica

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The presenting author had the opportunity to participate in the 46th Japanese Antarctic Research Expedition (JARE46) from November 28, 2004 to March 24, 2005. During the expedition, a total of 261 water, soil and rock samples were collected from various sites in inland and coastal areas of the continent. Taking into consideration the various environmental conditions from which the samples were obtained, we initially focused on several parameters for screening microorganisms. These include low temperature (psychrotrophs/philes), high salt concentration (halophiles), and low nutrient availability (oligotrophs/philes). Cultivation was performed under both aerobic and anaerobic conditions. At present, from 20 different growth media, we have isolated over 1000 different microorganisms based on growth characteristics, and colony morphology and color. 16S rRNA sequences have been determined for approximately 500 of these strains. A number of strains displayed low sequence similarity, less than 95% identical, to previously characterized bacteria, suggesting that these strains may be representatives of novel genera. A low number of halophilic archaea have also been isolated. We also examined the presence of photosynthetic organisms, and observed photoautotrophic growth from a rock sample. 16S rRNA sequencing indicated that the organism was a cyanobacterium. Several heterotrophic bacteria seemed to grow in a dependent manner along with the cyanobacterium. In the presentation, I will present the latest status of our findings and introduce several examples of the extremophiles from Antarctica.

Session 2 : Classification of Polar organisms

Chair : Sang Hoon Lee

KOPRI BioDB: a database to serve information of polar biodiversity and bio-resources

Soon Gyu Hong, Min Chul Lee and Hong Kum Lee

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Biodiversity information and bio-resources collected from polar areas are invaluable resources that were acquired by expensive and time-consuming processes. To make information and bio-resources more useful, it is very important to share them in science community and in public. In order to fulfill the tasks efficiently, database linked to the samples, strains and other materials should be constructed. 'KOPRI BioDB' was constructed to make it easy to exchange information with Joint Committee on Antarctic Data Management (JCADM), Ocean Biogeographic Information System (OBIS), Global Biodiversity Inforamtion Facility (GBIF), and Korean Biodiversity Information Facility (KBIF). The database contains information for the environmental parameters, geographical origin, taxonomy, and physiological characteristics such as optimal media, growth temperature, and production of enzymes. The database was designed to upload and download data by Excel format. Searching for microbial strains using various keywords such as environmental parameters, geography, taxonomy, and physiology are available. The database will be available in public next year after examining the stability and reliability of the database and server system.

Astrobiology and lakes of the McMurdo Dry Valleys, East Antarctica

Peter T. Doran

University of Illinois at Chicago

Operationally, the search for life beyond Earth can be regarded as a search for liquid water, since all life as we know it depends on liquid water. Most available data implies that water in its liquid form is currently rare in our solar system, with the only confirmed reserves residing on Earth. There are indications that liquid water may be present beneath thick ice covers of the Jovian moon Europa, water vapor is spewing from the surface of Saturn's moon Enceladus suggesting a subsurface reservoir and there is considerable evidence that Mars may have had liquid water at or near the surface throughout its climatic history. In all the examples above, the liquid water, if present, would reside very closely with ice. Additionally on Mars, subsurface water would almost certainly be hypersaline due to the long contact time with the local geology. For this reason, the cold desert environment of the dry valleys creates conditions that have frequently been used as an analog for Mars present and past. The presence of liquid water under thick ice covers on early Mars has been suggested as an important possible habitat for early life. The impact of thick ice covers on these extreme ecosystems, and the techniques used to study them can also guide our development of strategies for exploring the potential water bodies on the icy moons of the outer planets.

This presentation will provide an oversight of the dry valleys as a planetary analog for astrobiology, with a focus on the ice covered lakes,. Two recent NASA-funded projects will be discussed. The first sampled a hypersaline brine beneath a 20 m ice cover in a lake which has been sealed from the atmosphere for nearly 3000 ^{14}C yrs. The second is in progress and has as its goal to develop an Autonomous Underwater Vehicle (AUV) which will survey the entire water body of West Lake Bonney in the dry valleys and produce 1) a high resolution sonar map, 2) a 3-D biogeochemical sensor dataset which includes bottom imagery, light, temperature, salinity, pH/ORP, dissolved organic matter, chlorophyll-a and turbidity and 3) a photo mosaic of the underwater portion of Taylor Glacier.

Understanding bacterial diversity in the Arctic ocean by plate culturing, extinction culturing, and 16S rDNA cloning

Jang-Cheon Cho

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To get more comprehensive understanding about the diversity of Arctic marine bacterial community, two different culture-dependent approaches and a culture-independent analysis have been performed for five surface seawater samples collected from the coastal region near to Dasan Arctic Station in Ny-Ålesund, Svalbard, Norway. For the culture-independent analysis, DNAs were directly extracted from 1-3 liters of seawater samples and community 16S rRNA genes were cloned. Sequences of 524 total clones belonged to the *Alphaproteobacteria* (32%), *Betaproteobacteria* (5%), *Gammaproteobacteria* (12%), *Actinobacteria* (12%), *Bacteroidetes* (29%), *Cyanobacteria* (5%), and *Verrucomicrobia* (4%). The SAR11, SAR86, OM42, RCA clades dominated the 5 seawater samples. SSU clones from other phylogetetic groups were not clearly differed among the samples, but several intriguing phylogenetic lineages represented habitat-specific abundance. For the conventional standard dilution plating, 100 μl of seawater samples were directly spread onto 1/10 R2A and marine agar 2216, and further incubated at 10˚C for 2 months. By using a conventional dilution-plating method, 398 bacterial strains were obtained from five different seawater samples. Based on 16S rRNA gene sequence analyses, the colony-forming bacterial strains were assigned to the *Alpha-, Beta-, Gammaproteobacteria, Bacteroidetes,* and *Actinobacteria.* The culturability in dilution-to-extinction method was very low; only 35 extinction cultures were obtained among 960 culture-wells screened. None of extinction cultures formed visible colonies in several oligotrophic agar media. Based on 16S rRNA genes between the direct 16S rRNA gene cloning and the dilution plating, it was found that there have been huge differences between the two approaches; for example, members of the SAR11 clade could not be recovered by the plating approaches. However, among the 35 extinction cultures, 12 new strains (34.3%) belonging to the SAR11 clade were obtained. Moreover, 3 novel strains belonged to the SAR11 subgroup 3, which does not have any cultured representatives yet. We are currently analyzing genomes and physiology of the new SAR11 isolates, which will eventually show how these organisms have been well adapted to polar environments.

[This study was supported by a grant from Korea Polar Research Institute (KOPRI)]

Diversity and Phylogeny of Skuas from the Antarctica

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Among eight taxa of skuas in the world, brown (*Catharacta lonnbergi*) and south polar skuas (*C. maccormicki)* breed in the Antarctic region. Although the classic taxonomy of skuas has regarded brown and south polar skuas as separated species, recently taxonomic levels of two taxa are under dispute. The southern breeding range of brown skuas was overlapped with northern breeding range of south polar skuas in South Orkney Islands, South Shetland Islands, Antarctic peninsula and so on. Not only hybridization from mixed pairs, but also backcross between male hybrid and female brown skuas were observed there. Even though they can distinguished by morphological characteristics, incomplete reproductive isolation seems to be a worthwhile subject to reinvestigate their taxonomy and phylogeny. To study the diversity and phylogeny of skuas, we compared their body size and sequence data for mitochondrial cytochrome b (cytb), cytochrome c oxidase I (COI), and intron of nuclear Z-chromosome-linked chromo-helicase binding protein gene (CHD1Z) from both taxa and hybrids. All measured brown (n = 49) and south polar skuas (n = 91) were completely separated with body size each other. However, existence of hybrids, which had intermediate body size of both taxa, confused morphological classification. In addition, they were not distinguished with mitochondrial DNA sequence data. Interspecific variation (0-0.98 %) of cytb was similar to intraspecific variation (0-0.83%) and there were no fixed species-specific substitutions. Herbert and his colleagues suggested a threshold of 10 x the mean intraspecific difference to identify bird species. If the mean interspecific variation is over the threshold, they can be classified as separated species. In our study, the mean interspecific differences (0.1-0.2%) were smaller than the threshold (1.47%), thereupon brown and south polar skuas could not be discriminated with the 10 x rules. Consistent with mtDNA, we did not also detect significant interspecific variations in the CHD1Z (intron A) sequence data. Our morphological and molecular evidences did not support that brown and south polar skuas are assigned into two separated species. The implications for the taxonomy, phylogeny and biogeography of the southern hemisphere skuas and related species within the Stercorariidae are discussed.

Session 3 : Polar Ecosystems Ⅱ

Chair : Hyung Chul Shin

Seasonal and Annual variation of the primary production in the Bering and the Chukchi Seas using the Ocean Color and the sea-observing measurements.

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It has been widely studied that reduction of sea ice in the Arctic Ocean due to the large scale climate change such as global warming would cause significant influence on the marine ecosystem. Since the field measurement in estimating oceanic primary productions are temporally and spatially restricted, satellite estimation of ocean primary production is a more effective method to provide the large scale maps of the primary production over the Artic ocean waters with high spatial and temporal resolution. 10-years ocean color data from the NASA Sea-viewing Wide Filed-of-view sensor (SeaWiFS) are used to estimate the primary production within the water column in the Bering-Chukchi Sea using an existing satellite-based primary production model. In-situ measurements in the Bering-Chukchi Sea are also used to compare with the satellite estimation. In this study, we demonstrate the seasonal and annual variation of the primary production in the Bering-Chukchi Sea and investigate whether there is any influence of environmental variation on the phytoplankton production.

A new approach for complete detection of feeding behavior of marine animals by using very small accelerometer

Yasuhiko Naito

National Institute of Polar Research

It was extremely difficult to study foraging behavior of endothermic animals especially in in the polar oceans because foraging took place underwater covered by the thick ice. Recent advanced “Biologging Science” revealed their diving behavior in terms of diving physiology while less has been studied on their foraging behavior. Most studies of foraging behavior have been made by inferring dive profile analysis.

Generally physiological O_2 regime strictly limits the underwater foraging activity of endothermic animals, which might have enhanced their ability of efficient O_2 use and unique underwater foraging strategies particularly animals that forage in ice-covered sea may developed unique adaptive behavior. To understand their underwater foraging behavor, information on feeding behavior and it’s associated behavior is essential. However accurate measurements of feeding events have not been made and development such method was left as challenging subject in foraging study. Some methods have been developed though; development of further reliable and practicable method has been encouraged.

To broaden the methodological choice in the field study we tried to develop a new mandible movement method by using miniaturized accelerometers. Assuming that the animals would use mandible to capture their prey underwater, accelerometer attached on their mandible may detect mandible movements. However signals from mandible accelerometer may include noises associated with feeding behavior which was removed by frequency filtering method (FFT). A new method was tested by captive seals and dolphins resulting that the method is reliable to determine the timing of prey capture in both bite feeding and suction feeding.

Advantage of this acceleration method is that the accelerometer provides not only feeding information but also information on stroke, body posture (angle) and body mass change, which allow us to understand the complex system of eco-physiology of polar endothermic animals.

Influence of increasing suspended particulate matter concentration on benthic and planktonic Antarctic filter-feeding organisms: a relation with glacier melting

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There is strong evidence that the majority of polar glaciers of the Antarctic Peninsula are rapidly retreating. Melt water flow into seas causes, among others, a strong near-shore sedimentation. The increasing of suspended particulate matter in Antarctic coastal affects its physical, chemical and biological characteristics. In order to estimate the effect of sediment concentration on an Antarctic coastal ecosystem, the metabolic rates of 3 ascidians species and the Antarctic krill (*Euphausia superba*) were measured under different conditions in Potter Cove (King George Island). The most abundant ascidians species in Potter Cove: *Ascidia challengeri, Cnemidocarpa verrucosa* and *Molgula peduncula* had similar patterns of respiratory response to increasing sediment concentration, showing a gradual increment of their metabolic rate at medium levels up to a limit concentration. However, this limit concentration was markedly different among the species, 200, 100 and 15 $mg \cdot l^{-1}$ for *A. challengeri*, *C. verrucosa* and *M. pedunculata* respectively. Indicating different tolerances to sediment load. The daily ingestion rate of the Antarctic krill, decreases while the sediment concentration increase, independently of the phytoplankton concentration. With sediments concentrations similar to that found in Potter Cove, actually in summer (maximal melting period), the feeding capacity of the species was notably affected.

Session 4 : Biotechnology of Polar organisms

Chair : Yoo Kyung Lee

Bioactive Secondary Metabolites from Antarctic Lichens

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Current paradigm in the generation of drug-like leads emphasizes the concept of achieving high molecular diversity, and natural products have long been recognized to have high chemical diversity, biochemical specificity, and other favorable structural characteristics as leads for drug discovery. Considering a number of reports stating that many natural product resources are largely unexplored, it would be important to investigate the chemistry of untapped natural resources such as microbes from unexplored habitats or relatively less screened organisms for their potential. In a line with this concept, we have recently initiated the investigation of secondary metabolites from lichens and mosses collected from Antarctica as potential sources of new bioactive agents.

In the course of these studies, several classes of lichen metabolites including usnic acid derivatives, cyclic depsipeptide, depsides, and depsidones, along with benzonaphthoxanthenones from the Antarctic moss were encountered. Details of the isolation, structure elucidation and biological activity of these compounds will be presented.

Biological effects of contaminants on health of Arctic organisms-urgent needs and problems for the implementation of a monitoring program

Angela Kőhler

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The Arctic is estimated to contain at least 25% of the world's undiscovered petroleum resources (Ahlbrandt, 2002, AMAP, 2007). By 2010, annual volumes of 150 million tonnes of oil may be shipped by sea as a result of the development of oil production and transportation infrastructure in the Barents region alone (Frantzen and Bambulyak, 2003). For example, Shtockman gas deposit are estimated at 3.2 billion cubic metres of gas and 31 million tonnes of condensate. As energy consumption and air pollution are directly linked, any increase in oil and gas related activities is likely to result in increased pollutant emissions. Oil spills, whether from blowouts, pipeline leaks or shipping accidents, pose a tremendous risk to arctic ecosystems. These ecosystems are characterised by a short productive season, low temperatures, and limited sunlight. As a result, it can take many decades for them to recover from habitat disruption, sediment disturbance and not least oil spills. The problem is particularly acute in ice-infested waters.

There are two major gaps of knowledge and available technologies to limit potential risks of population-wide impacts by oil spills and gas leakages: 1. Monitoring tools to detect detrimental effects of oil spills from various sources on health of marine arctic organisms. 2. Effective method for containing and cleaning up an oil spill in ice conditions. In this talk we will focus on monitoring strategies for oil pollution accidents as well as chronic long term exposure scenarios. These strategies are already implemented in the frame of the Oslo Paris Convention (OSPAR) for the North Sea and North East Atlantic and Mediterranean regions (UNEP, Barcelona Convention) and need to be transferred to Arctic organisms.

What is needed to fill critical gaps of knowledge for the protection of the Arctic before gas and oil exploration and exploitation starts? With respect to selection of indicator organisms, test organisms should encompass key ecologically important species in food webs. Ideally contaminant body residues should be determined so that they can be linked to effects of biomagnification in the foodweb and related health effects. As ***acute effects biomarkers*** indicating contact of organisms to oil related chemicals, induction of detoxifying enzymes such as EROD activity, induction CP4501, GST activity, antioxidant enzymes: SOD, CAT, GPX and GR DT-diaphorase are suitable tools. As ***longterm effects biomarkers***, lipid peroxidation/oxidative stress, micronuclei formation as indication for genotoxic effects and histopathology including cancer diagnosis are recommended by the ICES Working Group of Biological Effects of Contaminants (OSPAR). Parallel to acute and longterm biomarkers, the measurement of lysosomal membrane stability is advised as biomarker integrating effects on cell (mal)function by various classes of oil components sensitively reflecting the onset and

progression of toxicant induced tissue pathologies.

For the successful implementation of an Arctic monitoring programme, acquisition of data on seasonal biomarker/ bioresponse/detoxification patterns of pressure- and cold-adapted indicator species are essential as a baseline. ***Status quo*** determination of biomarkers response, biodiversity, body burden of PAHs and other chemicals as well as water and sediment contamination before oil and gas exploitation activitey start is a prerequisite for correct scientific interpretation of pollution effects and legal claims against producers.

Biological activity of Lichen-forming fungi

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Lichens are symbiotic organisms composed of a fungus (mycobiont) and phototrophic algal partners, namely green algae and/or cyanobacteria (photobiont/cyanobiont). Lichen-forming symbiosis is followed about one fifth of all fungi. About 14,000 lichen species have been reported until now. Lichens, as pioneer plants, have adapted to extreme ecological conditions, being dominant at high altitudes, in Arctic boreal and also tropical habitats.

They produce characteristic secondary metabolites, lichen substances, which seldom occur in other organisms. Numerous lichen metabolites (e.g., aliphatic acids, desides, desidones, dibenzofurans, and pulvinic acid derivatives) have also been found to exert a wide range of biological activities. Clearly there is not a single role for these compounds, and besides having natural functions as light screens, metal chelators, water repellents or as defensive agents against parasites, there are other interesting effects. For example, antimicrobial, antifungal, antiviral, antiprotozoal, antiproliferative, antioxidant and anti-inflammatory were found in *in vitro* studies on human and animal cell lines. Lichens apparently evolved several biosynthetic pathways to produce an amazing diversity of phenolic compounds, mainly polymalonate-, shikimate- and mevalonate-derived metabolites. Most of these metabolites are produced by the fungus, in symbiosis or in the aposymbiotic state. Lichen metabolites are often structurally unique, with only a small number of them being found in other fungi and higher plants. Nevertheless, the potential of fungal spp. obligate symbionts in lichen have long been neglected by mycologists and overlooked by agrochemical industry because of its slow growth in nature and difficulties in their artificial cultivation. Furthermore, the lichen biomass in the spontaneous flora is not sufficiently voluminous to allow its economic application. The large-scale industrial production of the lichen metabolites has never been accomplished. However, use of lichen-forming fungi can overcome the disadvantage of natural lichen extracts for industrialization of their metabolites because of their much faster growth and larger production of the metabolites in culture than the natural thalli.

Far from an academic question, understanding the potential of lichen-forming fungi has implication for bioprospectors searching for efficient methods to uncover novel secondary metabolites. Molecular biotechnology for mass production of lichen substances will be feasible in the near future. This presentation is to highlight progress, challenges and frontiers in the study of lichen-forming fungi application in Korea. For this purpose, we discuss cultural characteristics of lichen-forming fungi and screening of their biological activity. Recent development of lichen-forming fungal transformant is also discussed.

Key Words: biological activity, lichen bioresources, lichen-forming fungi, secondary metabolites, transformation

Abstracts – Posters

Wednesday, September 24

1. Polar Ecosystems

PE-1

Variations of oceanic CO2 and air-sea CO2 exchange in the eastern Indian sector of the Southern Ocean in austral summer

Shin-ichiro Nakaoka[1,2], Takakiyo Nakazawa[2], Hisayuki Yoshikawa-Inoue[3], Shuji Aoki[2], Gen Hashida[1], Tsuneo Odate[1] and Mitsuo Fukuchi[1]

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The Southern Ocean is an important region for anthropogenic CO2 removal, mainly due to its geographically large area with high wind speeds, and biological activities. We observed pCO2air and pCO2sea in the eastern Indian sector of the Southern Ocean (south of 50˚S, 140˚-150˚E) from November 2001 to March 2002, using 4 research vessels to estimate the air-sea CO2 flux and examine the relationship between pCO2sea variation and biological activities. pCO2sea changed by as much as 200 μatm over the 5-month period in the Seasonal Sea Ice Zone (SSIZ), due mostly to very low values in January caused by upwelling from subsurface layers related to cyclonic eddies, stratification of water column, and strong biological activities. The results indicate that the variation of oceanic physics affect not only oceanic biological activities but also carbon cycle. The monthly oceanic CO2 uptake in the area covered by this study (1.5 x 1012 km2) was estimated to be in the a range from of 1.5 x 10-3 GtC month-1 to 2.4 x 10-3 GtC month-1, with a mean flux value of 1.9 x 10-3 GtC month-1.

PE-2

Foraging habitat of two species of penguins in relation to marine environment around King George Island, Antarctica

Nobuo Kokubun[1], Akinori Takahashi[1,2], Yoshihisa Mori[3] and Hyoung-Chul Shin[4]

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Marine top predators rely on food resources that fluctuate dynamically in various spatial and temporal scales. Understanding how the foraging behaviour of these predators interacts with heterogenous marine environment would provide key information about their ecological response to local environmental change. Chinstrap and gentoo penguins are the major top predators that breed on King George Island, Antarctica. We investigated their foraging locations and diving behavior using recently developed GPS-depth data loggers. The study was conducted during the chick-guarding period of both species, from December 2006 to January 2007. We successfully obtained GPS and dive data for 19 chinstrap and 14 gentoo penguins. The foraging location together with diving behavior and bathymetric information indicated that gentoo penguins frequently performed benthic dives reaching to sea floor (28 % of all dives deeper than 5 m) in nearshore region, whereas chinstrap penguins frequently used pelagic layer in both nearshore and offshore regions. Diving parameters such as diving efficiency also suggested that benthic layer is a profitable foraging habitat for gentoo penguins. On the other hand, foraging property of birds together with *Chl_a* distribution from satellite images suggested that offshore pelagic regions were potentially profitable habitat for chinstrap penguins. These results suggest that chinstrap and gentoo penguins have developed their behavioural strategies to feed efficiently in pelagic and benthic habitat, respectively. The foraging behaviour and reproductive success of chinstrap and gentoo penguins may reflect the environmental conditions of pelagic and benthic habitat in the Antarctic coastal marine ecosystem.

PE-3

A new pumping system designed to collect animals under the sea-ice: MASMA

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MASMA (MAngueraSubMArina in Spanish) (Fig.1) is a new zooplankton pump prototype.. The system consists of a motor driven centrifuge pump (0,40 m^3 min^{-1}) connected to a plankton filtration system. Filtration is carried out through a zooplankton net (the mesh size of the net can be decided depending on the target animals), which is located inside an airtight container and placed upstream the centrifuge pump. High volumes of water are transported to the container through a 2 inch diameter tube (up to 50m length); this allow the concentration of the animals in the net before the water reach the pump. Animals turned out to be in natural physical condition.

The prototype was tested during the Antarctic winter expedition ANT XXIII/6 on board of the RV "Polarstern". During the expedition the system was used to collect animals (mainly *Euphausia superba* larvae and juveniles) under the sea-ice (droved by divers or from a rubber boat) and from Polarstern working-deck.

The handling for the diver is carried out by means of the plastic tube that could be used as a modified vacuum cleaner to catch distinct and selected parts of the populations (Fig. 1).

The system is especially useful to sample in places difficult to access and where the animals are patchy distributed, as for example craves under the sea-ice. Due to the gentle collection of specimens, animals could be used for aquaria experiments, which always require animals in good physiological conditions.

PE-4

Ecosystem Responses to Climate Change on a High Arctic Glacier Foreland: Overview for the New Project

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Current climate change predictions indicate that warming will be more pronounced at high latitudes in the Northern Hemisphere (IPCC 2001, ACIA 2005). Because of the large carbon stocks present in the Arctic soils and the possible feedback effect on global atmospheric CO^2, the impact of climate change on carbon balance of Arctic ecosystems has received considerable attention by a number of scientists. On the other hand, on a High Arctic glacier foreland in Ny-Alesund, Svalbard, Nakatsubo et al. (2005) reported that the soil organic layer is very thin even in the later stages of succession. If this thin organic layer decreases as a result of climate change, it would profoundly affect the structure and function of whole ecosystems. Thus, understanding the ecosystem carbon cycle is needed to predict future ecosystem response to climate change on the High Arctic glacier foreland. Ecosystems in the glacier foreland have historically received the effect of glacial advance and retreat. Recent decades, glaciers throughout much of the Northern Hemisphere have lost mass (ACIA 2005). In the Greenland, it has been reported that 17% expansion of the melt region was observed for 10 years (1992-2002) (Comiso and Parkinson, 2004). Such glacial retreat provides new habitat for plant colonization and hence organic carbon accumulation. Therefore, to predict future ecosystem response to climate change on the High Arctic glacier foreland, it is also important to consider future glacial retreated area as well as present one.

In this report, we introduce a new project "Ecosystem Responses to Climate Change on a High Arctic Glacier Foreland". This project aims 1) to estimate changes of glacial retreat and vegetation distribution for past 30 years using remote sensing data and 2) to construct a compartment model to evaluate structure and function of various vegetation types. At the same time, we will investigate the relationship between carbon cycle and ecosystem development. Finally, we will predict changes in the distribution and function of the ecosystem on the future glacier foreland.

STUDY SITE

The study site is located on the glacier retreated area of East Brogger Glacier, Ny-Alesund, Svalbard, Norway (79^{o}N, 12^{o}E) and Oobloyah valley, Ellesmere Island, Canada (81^{o}N, 83^{o}E). In case of the glacier retreat area in Ny-Alesund, the annual mean air temperature and amount of

precipitation in this area are -5.5°C and 362 mm, respectively. The glacier shows net negative

INTRODUCTION

balance for the last 30 years (Lefauconnier et al. 1999) and retreated more than 30 m for two years (2003-2005). Plant succession was observed on the glacier foreland. Vegetation cover and species composition was described by Nakatsubo et al. (2005). Vegetation cover was less than 10% from tip of the glacier to about 1 km distance. At this site, a few moss species and purple saxifrage, *Saxifraga oppositifolia* were dominant. More than 1 km apart from tip of the glacier, vegetation cover increased rapidly and surpassed 40%. The dominant moss and vascular plant species at this site were *Sanionia uncinata* and *Salix polaris*, respectively. This area might occur land-uplift before thousands years after deglaciation because many seashell fossils were found around there.

OVERVIEW OF THE PROJECT

The project consists of three scientific articles, *plot-based study*, *remote sensing observation* and *model analysis*.

Plot-based study

Plot-based study is conducted to clarify plant species composition, biomass, photosynthesis and respiration characteristics, biochemical composition of leaves, spectral profile of reflected radiance for various vegetations, soil respiration and soil carbon and nitrogen contents under various vegetations. For plant ecophysiological characteristics, some plant species such as *Salix polaris*, *Sanionia uncinata* (moss) and *Cetrariella delisei* (lichen) have been investigated (Nakatsubo et al. 1998; Muraoka et al. 2002; Uchida et al. 2002, 2006). In addition, soil microbial biomass and activity have been also studied (Bekku et al. 2003, 2004a,b; Yoshitake et al. 2006).

dating result of seashell fossils, it is assumed that some amounts of carbon might exist as fossil carbon in deeper soil horizons. Therefore, to clarify carbon dynamics in deeper soil horizons, we investigate existence of the fossil carbon and its biological decomposition.

Remote sensing observation

To know the scale of expanding area of deglaciated terrain, satellite data and aerial photos are used to observe position of glacial tip for the past 30 years. The results of plot-based studies such as plant species composition and biomass, and the data of spectral profile of reflected radiance are used to obtain a suitable algorithm to identify vegetation type and plant biomass on the deglaciated area. Distribution changes of the vegetation for the past 30 years on the deglaciated area are estimate using remote

In a preliminary study of spectral profile of reflected radiance for various vegetations, it was revealed that the biomass distribution in this region can be evaluated by remote sensing. On the other hand, we also found the relationship between vegetation cover and soil carbon content. At well developed vegetation site, by sensing data and the algorithm. In addition, we will make a microtopographical, radiation and soil water content map to investigate the relationships

between vegetation and environmental factors on the glacier foreland.

Model analysis

A Compartment model of the carbon cycle is constructed to analyze various ecosystem characteristics. Relationships between microtopograph, microclimate and vegetation structure and function are investigated using remote sensing data and the compartment model. Then, we will reconstruct the history of ecosystem carbon dynamics for the past 30 years on the glacial retreated area to evaluate ecosystem response on the environmental factors. After that, future ecosystem responses to climate change on the glacier foreland will be predicted

TOWARD IPY PROJECT

In order to clarify the ecological change of tundra plants at the foreland of glaciers, retreating because of warming climate, a comparison between ecological features in Ny-Alesund, Svalbard with maritime climates and in Oobloyah valley, 80˚51’N, 82˚50’W, Ellesmere Island,Canada with those of continental climates is significant for evaluating response to climate change in the high Arctic. Some studies concerning to vegetation in Oobloyah valley have been already undertaken (Okitsu, et a., 2004, Osono, et al, 2006, Mori et al, 2006), but the study on carbon balance of the Arctic ecosystem has just started. The most important achievement will be to gather high quality data by long-term observation in the Arctic in order to predict global and Arctic environmental changes. Further, it is important to continue the observation over more than ten years in order to understand the implications of changes and to assess the environmental affect. Toward the International Polar Year (IPY 2007-2008), we undertook the research project, “TUNDRACYCLE” which started in full-scale in 2007 to 2008, using monitoring stations placed as a circum-Arctic observatory network. In the period, we proceeded the study on the changes of vegetation and soil along the glacier foreland and environmental changes as seen by aerial photography over a long term.

PE-5

A Study on the Life-Cycle of *Tisbe furcata* s. l. from Ny-Alesund, Svalbard, Arctic

Kanghyun Lee, Seunghan Lee, Dong Ju Lee and Wonchoel Lee

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Ny-Alesund in the Kongsfjorden has received a lot of research attention as a site for exploring the impacts of climate change. Korean Arctic Station, Dasan, is an important Arctic site for monitoring and detecting future environmental change. The genus *Tisbe* contains more than 60 described species and occurs worldwide especially in shallow marine water. *Tisbe furcata* s. l. is dominant along the coasts of Ny-Alesund. This species shows rapid increase during summer indicationg its ability of quick response to the environmental factors such like temperature, light and salinity. Therefore *Tisbe furcata* s. l. can be a good indicator for environmental change in Arctic region. As a primary step, we analyzed life-cycle of *Tisbe furcata* s. l. from Ny-Alesund. Laboratory experiment revealed that the duration of the development of eggs was 10 days, and the development time from the first nauplius stage to the first copepodite stage was 20 days. Mean egg pouching time was about 4 days and the egg production rate were 15.3 indiv.female^{-1}. Arctic *Tisbe furcata* s. l. has a long life-cycle and a low reproduction rate than those in its congeners dwelling in the temperate area. Ultraviolet radiation is a critical factor to survive for a tiny copepods livingg in tidal pools, especially in arctic summer. A series of exposing experiments of Ultraviolet to *Tisbe furcata* s. l. will be planned to discover the important of Ultraviolet radiation on the organisms in the Arctic area.

PE-6

Effect of UV radiation on the food quality of marine ecosystem in Antarctic and Arctic regions

Sunyong Ha, Kyunghoon Shin, Hyongmin Joo, Sungho Kang, Inyoung Ahn

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We investigated the change of new organic compounds using 13C tracer for effect of UV radiation to in situ phytoplankton at the one station of Marian Bay in KingGeorge Island, Antarctica during December, 2005 and Ny-Alesund of Spitsbergen Island in DaSan station, Arctic Ocean during August, 2006. We experimented on the effect of UV radiation for fatty acids composition of phytoplankton by in situ incubation for 72 hours using UV radiation transmitted in quartz bottles and little transmitted in polycarbonate bottles. The incubated phytoplankton exposed to UV radiation at both regions showed the decreased polyunsaturated fatty acids productions rather than cut-off UV radiation. In contrast, newly produced cells in phytoplankton under UV radiation had an increased proportion of the saturated fatty acids. But we could not find significantly the effect of UV radiation on the bulk fatty acids composition. So, UV radiation affected strongly newly assimilated carbon into fatty acids of phytoplankton. In particular, C20:5(EPA) and C22:6(DHA) were susceptibly influenced by the exposed UV radiation during the incubation experiment in Antarctica. The results in this study demonstrates the inhibitive effect of UV radiation on in situ phytoplankton in Antarctica and Arctic, indicating the decrease of polyunsaturated fatty acids. As a result, the food quality deterioration of Antarctic marine ecosystem can be occurred by continually exposed UV radiation on Antarctica region.

PE-7

Metal accumulation in sea urchins from an Arctic fjord (Kongsfjorden, Svalbard) and an implication of biomagnification through algal diet

In-Young Ahn, Jungyoun Ji, Hyun Park

KOPRI

The sea urchin Strongylocentrotus spp. are the dominant grazers in shallow rocky subtidal habitats of Kongsfjorden, an Arctic fjord in Svalbard, preferentially grazing on the laminarian kelps. In addition to their ecological importance, they have a wide geographic distribution, occurring in the North Atlantic, North Pacific as well as Arctic Oceans, thus suggesting a potential utility as biomarkers for marine pollution monitoring. In this study the sea urchins were collected from Kongsfjorden during the periods of summer months, and tissue concentrations of trace metals were determined. Metal concentrations were also determined in their preferential diet, the laminarian kelps such as Laminaria spp. and Alaria esculenta and ambient seawater to investigate metal transfer at low trophic levels in Arctic marine ecosystem. The results show strong metal accumulation capacity of Strongylocentrotus spp. and implication of biomagnifications of some metals through their algal diet.

PE-8

PCR Analysis of Gut contents of Bivalves from the Polar Ocean

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Study of the dietary habits of marine invertebrate is essential to understand ecological processes, energetics, and natural history. It is still poorly known about the diet of polar invertebrates compared with large vertebrate. It is just because of difficulty of analysis methods. PCR technique was recently applied but still has a few problems such as contamination of host sample or observer's DNAs or suppression of DNA with low copy number. In present study, we collected two clams from the polar ocean. Laternula elliptica was collected from the antarctic ocean and one unknown clam was sampled from arctic region. The phyto-specific 16S rDNA PCR primers was used to isolate phytoplankton sequence and previously designed universal nuclear rDNA PCR primers used to identify unknown species and analyze PCR efficiency. Phylogenetic tree was constructed to understand evolutional relationship among the recovered sequence and quantification of each species are underway. Only if database of DNA sequence information and quantification techniques are improved, this technique will be one of the most powerful techniques to understand marine ecological science.

PE-9

Climate forcing of alpine tundra ecosystems in southwest Yukon: A Canadian contribution to the International Polar Year

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Of the four circumpolar sub-regions defined in the 2004 Arctic Climate Impact Assessment, Alaska and the Canadian Yukon have experienced the most dramatic warming, resulting in major ecological impacts. Using detailed field data from the southwest Yukon, this IPY research project combines new and ongoing field studies with the development of a spatio-temporal computational model of climate-forced land surface -ecosystem interactions in the study region. In particular, we are examining how treeline, tundra vegetation and the life history and behaviour of alpine dwelling mammalian herbivores (collared pikas, hoary marmots, arctic ground squirrels, Dall sheep) respond to variable weather conditions and climate warming. The evidence so far suggests influences of both small-scale and large-scale climate forcing on alpine terrestrial ecosystem dynamics in these subarctic mountain environments.

PE-10

ARAON - Korean research icebreaker

Won Jun Kim

Korean Ice-Breaker R/V, Korea Polar Research Institute, KORDI

The Korea's first icebreaker(Araon), which is scheduled to be completed by the end of 2009, is currently under construction. She is about 6,950-ton icebreaker and designed for operation in one-meter-thick-multiple-year ice condition (KR PL-10) with 3 knot speed per hour and will be equipped with twin Azimuth propulsion units driven by diesel-electric propulsion plant. She will accommodate up to 85 persons, including 25 crew members.

Following a circumspective feasibility test in 2003, the basic design and general arrangement of vessel had been produced in 2004 and 2005 respectively. At the beginning 2007, Hanjin Heavy Industry, Inc. has won the contract for constructing an icebreaker and the steel-cutting ceremony was held in Jan. 2008.

After keel-laying in May of 2008 and launching and delivery in 2009, she is planned to be commissioned for scientific research and logistic purposes in both Antarctic and Arctic region. Korea expects that the icebreaker would enhance the capability of conducting scientific research in polar region with upgraded efficiency and quality. Currently Korea is operating one "over-wintering" station, the King Sejong on King George Island in the Antarctic, and it also has research facilities, the Dasan, in NyAlesund, Svalbard Islands in the Arctic.

2. Mechanism of Cold adaptation

CA-1

Effect of Trehalose of Boar Sperm on Cold Shock from Freezing Process

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Cryopreservation of boar sperm is continually researched in biotechnology for conservation and reproduction of breeds. It is important to control the temperature of each stage on cryopreservation. It's also necessary to find the optimal cryoprotectant concentration and chemical elements of the extender. Recently, a lot of studies use many kind of materials for cryoprotectant such as Antifreeze Peptide(AFP), Amides and glycerol. Glycerol is commonly using cryoprotectant these days. However, the glycerol has critical cytotoxic property such as osmotic pressure. It can give irreversible damage to live cell. In other study, the fluidity of goat sperm membrane is enhanced by trehalose. The modification of membrane fluidity can reduce the cell damage from freezing and thawing procedure. Our study focused on the positive effect of trehalose to the viability, chromatin integrity and motility of boar sperm. The boar semen was separated sperm from the semen by Hulsen solution. Then, centrifuged for 3 minutes at 2400g, 15 degree of C. Pellet was diluted with the prepared first extender. BF5, first extender, was mixed with glycerol and threhalose for second extender. Only glycerol added extender was control group. The extender and sperm solution put into straw and then froze in liquid nitrogen. Extender with glycerol and trehalose, was compared with only glycerol extender. Sperm viability was checked by WST1 assay and chromatin structure was checked by acridine orange staining. We used a microscope to count motile sperm. In cases of glycerol and trehalose, they showed positive effect to sperm motility, viability and chromatin structure. In this study, glycerol and trehalose added extender showed more effective to cryopreservation of boar sperm. It suggests that trehalose is effective to reduce freezing damage on boar sperm and can use for assist the cryoprotectant which reduce the cryoinjury.

CA-2

Cytoskeletal changes of Zygnema cruciatum (Chlorophyta, Zygnematales) during cold acclimation process

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Cytoskeletal changes were observed in a psychrophilic alga *Zygnema cruciatum* during the cell division using FITC-conjugated phallacidin and anti-α-tubulin. The distribution of actin filaments changed at each stage of the cell division. At the initial stage of the nuclear division the actin filaments accumulated at the centre of the nucleus and associated with chromosomes until telophase. During cytokinesis a ring shaped actin filaments appeared at the cleavage furrow suggesting that the actin filaments plays an important role both in nuclear division and cytokinesis. The microtubules were arranged spirally beneath the protoplasma membrane during the interphase but they were observed only at the nuclear region from prophase to telophase. At the prophase a massive accumulation of microtubule occurred at the nuclear region and it developed into mitotic spindle during the metaphase. During the cold acclimation of *Zygnema cruciatum* the distribution of microtubules changed. As known, most tubulin dimers cannot polymerize into microtubules at temperatures below 4℃ but the cells of *Z. cruciatum* could divide and grow at this temperature. This ability may require molecular adaptation of the tubulin gene to low temperature. cDNA encoding α-tubulin gene of *Z. cruciatum* was isolated. The gene had a typical modified site of amino acid sequence reported in the psychrophilic species. Northern blot analysis showed that higher transcription of α-tubulin gene occurred at 4℃ than at 20℃. Interestingly, the transcription level of the β-tubulin gene of *Z. cruciatum* was not affected by the temperature conditions.

CA-3

Purification and characterization of the antioxidants accumulated during the cold acclimation of a freshwater green alga, *Spirogyra varians*

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When *Spirogyra varians* was cultured at low temperature (4℃) the antioxidant activity in methanolic extracts increases over time course. HPLC analysis of the extract showed four new compounds produced during cold acclimation process. The radical scavenging activities of the isolated compounds were 100% to 150% in comparison with ascorbic acid. Purification of the compounds was performed and the possible structures were predicted using H-NMR. The compounds were similar to gallic acid or flavonoid derivate which is involved in shikimate pathway. The activity of antioxidant enzymes (Catalase and Superoxide dismutase) was observed either unchanged or reduced during incubation at low temperature (4℃) suggesting *Spirogyra varians* overcome cold stress using above compounds rather than antioxidant enzymes. A shikimate pathway related gene involved in the cold acclimation process of *Spirogyra varians* was isolated and possible role of the gene for the production of the antioxidant compounds was discussed.

CA-4

In silico analysis of archaeal genomic sequences of global transcriptional regulator acting for environmental adaptation

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The transcriptional regulator TrmB family members (TrmB, TrmBL1 and TrmBL2) were characterized as the sugar-specific sensing regulator for sugar ABC transporters (maltose and maltodextrins), glycolysis and gluconeogenesis from hyperthermophilic euryarchaeon *Pyrococcus furiosus*. The molecular characteristics of TrmBL1 were indicated that it has dual functions as a repressor for maltodextrin ABC transporter and glycolysis and as an activator for gluconeogenesis especially in promoter of fructose-1,6-bisphosphatase. TrmBL2 with missing sugar binding domain was highly conserved in other Thermococcales strains. Thus, transcriptional regulators of TrmB family member are central regulator for the expression control of different sugar transporters as well as sugar metabolic pathways to adapt environmental exchange Here, we demonstrate that the in silico analysis of archaeal genome sequences for searching a global transcriptional regulator as like TrmB family members.

CA-5

Structural study of Cold Shock Proteins from *C. psychrerythraea* and *X. oryzae*

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Cold shock proteins (Csps) are a subgroup of the cold-induced proteins on reduction of the growth temperature below the physiological temperature. They preferentially bind to single-stranded nucleic acids to translational regulation via RNA chaperoning. In order to investigate the structural stability of Csps from psychrophilic bacteria, *Colwellia psychrerythraea* and mesophililc bacteria, *Xanthomonas oryzae*, we determined three dimensional structures of CpCspC (*Colwellia psychrerythraea CspC)* and XOCsps (XOO1510, XOO1870 *Xanthomonas oryzae* Csps) by homology modeling. The results implies that in the case of psychrophile, two salt bridges ([Glu21-Lys3] and [Lys3-Glu50]) and hydrophobic interaction (Val49-Val65) may play important roles in structural stability. Also CD data indicated that the structural stability of Csps strongly depends on pH due to protonation of charged residues. CpCspC was expressed in *E. coli* with pET-11a vector system and purified by ion exchange and size exclusion chromatography. Expression and purification of Csps in M9 minimal media will be optimized to get ^{15}N, ^{13}C labeled proteins with high purity. Further study will be carried out to investigate the tertiary structure and dynamics of Csps.

CA-6

Effects of environmental heat stress on the thermoregulatory responses in the Antarctic bivalve *Laternula elliptica*

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Antarctic bivalve, *Laternula elliptica*, adapted for survival under extremely cold and stable environmental conditions. To address the possibility that such cold-adapted species retain a highly conserved cellular response to heat stress, we determined the full-length cDNA sequences of two heat shock protein, HSP70 and HSP90, and three antioxidant defense proteins, peroxiredoxin V, peroxiredoxin VI and glutathione S-transferase, from this species. We also studied the transcriptional expression pattern of these five stress-related genes exposed to thermal stress with real-time PCR. These indicate that HSP70 and HSP90 may play an important role in mediating thermal stress and tolerance, and also suggest that PrxV, PrxVI and GST play protective roles against oxidative stress caused by thermal exposure.

CA-7

Analysis of cDNA Library of Psychrophilic Green Alga KOPRI AnM0046

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Diatoms and other microalgae in sea ice are major primary producers in marine ecosystem of polar regions. Out of more than 100 microalgal strains isolated from Dasan Station, Arctic and King Sejong Station, Antarctica, four Antarctic strains (three marine strains and one freshwater strain) and one Arctic strain were further investigated on physiological changes by growth temperatures. A green alga, KOPRI AnM0046 was shown to be the most psychrophilic strain in five polar strains.

In order to study and obtain the pool of genes for KOPRI AnM0046, two different sets of cDNA libraries for KOPRI AnM0046 were constructed. One set of cDNA library was constructed in normal culture condition (2℃, 80umol m-2s-1 and continuous shaking), while the other was done in cold shock condition (-2℃, 80umol m-2s-1 and continuous shaking).

We analyzed approximately 1,000 EST data from each condition. All of the EST data were included in the fields of KOG categories. The most abundant EST data of KOPRI AnM0046 were related to function of posttranslational modification, protein turnover, and chaperones in the category of cellular processes and signaling.

KOPRI AnM0046 is shown to have various genes in a number of different functions by analyzing the cDNA library. We are going to analyze the gene functions of this green alga and study the chaperones and other genes related to cold shock stress.

Thursday, September 25

1. Classification of Polar organisms

CP-1

Pseudomonas pyramimonadae sp. nov., isolated from a culture of the Antarctic green alga *Pyramimonas gelidicola*

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A Gram-negative, strictly aerobic bacterium, designated CL-AP6^T, was isolated from a culture of a green alga *Pyramimonas gelidicola* established from the Antarctic. Cells of strain CL-AP6^T were straight rods and motile by means of single polar flagellum. Strain CL-AP6^T grew with 1–3% NaCl (optimum 2%) and at 4-30°C (optimum 25°C) and pH 6.3–8.8 (optimum pH 8.0). Analysis of the 16S rRNA gene sequence of strain CL-AP6^T revealed that it was a member of the genus *Pseudomonas* and most closest to *Pseudomonas pertucinogena* NBRC 14163^T (95.1% sequence similarity) and to other members of the genus *Pseudomonas* (< 95.0% sequence similarity). Phylogenetic analyses based on the 16S rRNA gene sequence showed that it belonged to the genus *Pseudomonas*, forming a robust cluster with the *Pseudomonas pertucinogena* group. The major isoprenoid quinone was Q-9 and the major cellular fatty acids were $C_{18:1}\omega7c$ (40.8%), summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, 28.1%), $C_{16:0}$ (8.4%) and $C_{12:0}$ (7.3%). The DNA G+C content was 60.0 mol%. The phylogenetic analysis, physiological and biochemical data showed that strain CL-AP6^T was classified in the genus *Pseudomonas* as a novel species, for which the name *Pseudomonas pyramimonadae* sp. nov. is proposed. The type strain is CL-AP6^T (= KCCM 90073^T).

CP-2

Maribacter antarcticus sp. nov., a novel psychrophilic bacterium isolated from a culture of the Antarctic green alga Pyramimonas gelidicola

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A psychrophilic, Gram-negative, dark-orange-pigmented bacterium, designated CL-AP4T, was isolated from a culture of a green alga Pyramimonas gelidicola from the Antarctic ocean. Strain CL-AP4T grew optimally at temperature of 10°C, in the presence of 3–4% sea salts and pH 8. The 16S rRNA gene sequence analysis revealed that strain CL-AP4T belonged to the family Flavobacteriaceae with Maribacter arcticus as its closest relative (similarity of 97.2%). The following chemotaxonomic characteristics support the affiliation of strain CL-AP4T with the members of the genus Maribacter: iso-C15:0 (17.2%), iso-C15:1 (16.8%) and iso-C17:0 3-OH (14.9%) as the dominant fatty acids. MK-6 is the major menaquinone. DNA G+C content is 37.1 mol%. Phylogenetic analyses of the 16S rRNA gene sequences showed that strain CL-AP4T formed a robust clade with M. arcticus. However, the DNA-DNA relatedness between CL-AP4T and M. arcticus was 10%, suggesting that they are genomically distinct species. In addition, strain CL-AP4T differed phenotypically from M. arcticus by optimum growth temperature, hydrolysis of starch, Tweens 40 and 80 and productions of certain enzymes. On the basis of the results of the polyphasic analysis, strain CL-AP4T was classified in the genus Maribacter as a novel species, for which the name Maribacter antarcticus sp. nov. is proposed. The type strain is CL-AP4T (= KCCM 90069T = JCM 15445T).

CP-3

MOLECULAR IDENTIFICATION OF NOVEL ISOLATES OF THE SAR11 CLADE 3 CULTURED FROM ARCTIC SEAWATER

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Dilution-to-extinction culturing using seawater samples collected from coastal region near to Dasan Arctic Station in Ny-Ålesund, Svalbard, Norway, yielded 33 extinction cultures from 960 culture wells. None of extinction cultures formed visible colonies on several oligotrophic solid media. In order to get 16S rRNA, 16S-23S intergenic, and proteorhodopsin sequences, we adopted touch-down PCR instead of conventional nested PCR approaches because it showed enhanced results for obtaining SAR11 sequences. In addition multiple displacement amplification of genomic template DNA using rolling circle phi29 replicase was set up for one-step amplification of target amplicons. Among the 33 extinction cultures, 22 new strains (66.6%) belonging to the SAR11 clade were obtained based upon the small subunit rRNA phylogeny. Eighteen strains were closely related to previously cultured SAR11 isolates such as HTCC1062 in the SAR11 subgroup 1. Interestingly, 4 novel strains belonged to the SAR11 subgroup 3 that does not have any cultured members yet. Proteorhodopsin sequences from subgroup 3 was green-light adapted but formed a different cluster from those of HTCC1062 and blue-light adapted groups. Intergenic sequences of 16S-23S from subgroup 3 formed a distinct cluster that is clearly separated from members of the SAR11 clade. This study proclaims the multiple displacement amplification as a practical alternative to amplify constrained genomic DNA targets. Such an amplification of DNA approach successfully overcame low nucleic acid yields owing to limited biomass yields of novel isolates from SAR11 subgroup 3.

[This study was supported by a grant from Korea Polar Research Institute (KOPRI)]

CP-4

Community composition of Arctic seawater bacterioplankton revealed by 16S rRNA gene cloning

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We analyzed the phylogenetic composition of the uncultured bacterial community in 5 Arctic coastal seawater samples collected around Dasan station by using 16S rRNA genes.

Sequences of 524 total clones belonged to *α-proteobacteria* (32%), β-*proteobacteria* (5%), γ-*proteobacteria* (12%), *Actinobacteria* (12%), *Bacteroidetes*(29%), *Cyanobacteria* (5%), and *Verrucomicrobia* (4%).

Clones of the SAR11 clade were less abundant in coastal (PSW1) and intermediate (PSW2) zones compared to those from near open ocean (PSW3), and glacia-melting zones (PSW4 and PSW5).

The SAR11 subclades 2 & 3 were found in PSW3 & 4 while the SAR11 subclade 1 could be found abundantly in every samples analyzed.

The OM42 and RCA cladess dominated any region except for PSW3.

Interestingly, uncultured *Flavobacteraceae* were widely distributed in the samples.

Clones of the γ-proteobacterial SAR86 clade could be demarcated except for PSW2 whilst methylotrophic β-proteobacterial OM43 clones were pertinent to PSW3, PSW4, and PSW5.

SSU clones from other phylogetetic groups were not clearly differed among the samples, but several intriguing phylogenetic lineages represented habitat-specific abundance.

CP-5

Cultured Representatives of Uncultivated Marine Bacterial Groups Obtained using Conventional Dilution Plating and Dilution-to-Extinction culturing from the Arctic Seawaters

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Polar region provides potential benefits and offers new insights into the illogical mechanisms of adaptation to and tolerance in the cold environments. To overview the diversity of culturable Arctic bacteria, the surface Arctic seawaters were sampled from the coastal region near to Dasan Arctic Station in Ny-Ålesund, Svalbard, Norway. For the conventional standard dilution plating, 100 μl of seawater samples were directly spread onto M-R2A, M-10 R2A and M-PY, and further incubated at 10˚C for 2 months. Frozen glycerol suspensions, prepared immediately after collecting seawaters, were further used for a dilution-to-extinction culturing. By using a conventional dilution-plating method, 398 bacterial strains were obtained from five different seawater samples. Based on 16S rRNA gene sequence analyses, the colony-forming bacterial strains were assigned to the *Alpha-, Beta-, Gammaproteobacteria, Bacteroidetes,* and *Actinobacteria.* Approximately 23.5% of the colony formers showed less than 97% 16S rDNA sequence similarity to validly published species and comprised novel taxa. The culturability in dilution-to-extinction method was very low; only 33 extinction cultures were obtained among 960 culture-wells screened. None of extinction cultures formed visible colonies in several oligotrophic agar media. Among the 35 extinction cultures, 12 new strains (34.3%) belonging to the SAR11 clade were obtained. Eighteen strains were closely related to previously cultured SAR11 isolates such as HTCC1062 in the SAR11 subgroup 1. Interestingly, 3 novel strains belonged to the SAR11 subgroup 3, which does not have any cultured representatives yet. This study proclaims again the practical aspects of dilution-to-extinction culturing to bring oligotrophic bacterial lineages to the laboratory.

[This study was supported by a grant from Korea Polar Research Institute (KOPRI)]

CP-6

Summary of Polyphasic Taxonomy on Marine Bacteria Isolated from the Antarctica

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The Antarctica is one of the most extreme environment, harsh condition and cold temperature, and is major source of cold-adapted microorganisms. Understanding the diversity and taxonomic relationships of culturable marine bacteria has meanings due to their important microbial functions and environmental specificity. To overview bacterial diversity dwelling in the Antarctic Ocean, seawaters were sampled from the coast of Maxwell Bay, King George Island, Antarctica. A total of 416 bacterial strains were obtained using a microcolony observation combined with conventional dilution-plating on copiotrophic(marine agar 2216) and oligotrophic(M-1/10R2A) culture media at three different growth temperatures(3°C, 8°C, 20°C) from the Antarctic seawaters. Based on 16S rRNA gene sequence-based phylogenetic analyses, all the strains were primarily assigned to the *Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes, Betaproteobacteria*, and *Actinobacteria.* The phylogenetic analyses also revealed that approximately 43% of the isolates showed less than 97% 16S rDNA sequence similarity to validly published species and comprised novel taxa. Of the novel isolates, eight novel strains were classified using polyphasic taxonomy. Among them, one novel family, one novel genera and four novel species were described as follows the names; *Granulosicoccus antarcticus* gen. nov., sp. nov. in *Granulosicoccaceae* fam. nov., *Robiginitomaculum antarcticum* gen. nov., sp. nov., *Hahella antarctica* sp. nov., *Sejongia marina* sp. nov., *Ulvibacter antarcticus* sp. nov., and *Lewinella antarctica* sp. nov. Two genera, *Antarcticicola litoralis* gen. nov., sp. nov., *Antarcticimonas flava* gen. nov. sp. nov., are further characterized using polyphasic taxonomy and phenotypic properties.

[This study was supported by a grant from Korea Polar Research Institute (KOPRI)]

CP-7

Phytoplankton community structure in Kongsfjorden, Svalbard

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Kongsfjorden is a glacial fjord in the Arctic that is influenced by both Atlantic and Arctic water masses. This area is affected by large range of environmental factors, and it is important to measure, characterize and monitor the ecological status. The size structure and species composition were investigated during 2006 to 2008, with special attention to small phytoplankton (cells <20 μm in diameter). To aid the interpretation of phytoplankton data, hydrographic data were also collected. Measurements made on the phytoplankton included size fractionated chlorophyll, photosynthetic pigments by HPLC, and cell counts by inverted microscopy. These data were also compared with the lab controlled phytoplankton culture collections in Korea polar institute.

CP-8

Evolution and geographical distribution of the lichen genus *Usnea* in Antarctic

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Phylogenetic relationships of *Usnea* species collected from different localities in Antarctic, including Leningradskaya, Russkaya, Lindsey Island, Mt. Moses, Maish Nunatak and King Jeorge Island, were reconstructed based on combined sequences of ITS and 28S rDNA. From the tree, geographical isolation of *Usnea* species in Pacific coast of continental Antarctic was not evident. Instead, samples from long distance were clustered together and contained rDNA sequences of high similarity, implying that *Usnea* species can be easily transferred and widely distributed in Pacific coast of continental Antarctic. However, *Usnea* species from King Jeorge Island looked isolated from those of continental Antarctic. *Usnea* species with close phylogenetic relationships showed variation in intron possession pattern, implying that introns are easily lost or obtained. However, sequences of introns were generally well conserved in the same phylogenetic lineages. Sharing of same type of intron by lichens from different geographical origin supported the hypothesis of easy geographical distribution of lichen species in Antarctic continent.

CP-9

Molecular characterizations of the Arctic jellies from Svalbard (79°N), Norway, by ribosomal DNA sequences

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Gelatinous plankton organisms (jellyfishes) frequently observed in Arctic coastal waters of Svalbard (79°N) in summer, and belong to cnidarians and ctenophores morphologically. Species identification, however, remained unclear due to lack of knowledge. In this study, we determined 18S and 28S ribosomal DNA sequences of five jellies (five genera: *Beroe, Bolinopsis, Cyanea, Leuckartiara*, and *Leuckartiara*) from Svalbard coastal waters in August 2006. Comparative analyses of the sequences revealed that two cryptic jellies were *Bolinopsis infundibulum* (Ctenophora; Cyclocoela) and *Mertensia ovum* (Ctenophora; Typhlocoela) but we could not identify the remaining three genera to species level. On the molecular data, they belonged to *Beroe* sp. (Ctenophora; Typhlocoela), *Cyanea* sp. (Cnidaria; Scyphozoa), *Leuckartiara* sp. (Cnidaria; Hydroida), respectively according to their morphological characters. Phylogenetic analysis showed that the Arctic jellies formed the independent clade, respectively. This suggests that the Arctic jellies may possess a little genetic variation, compared to the same species from other regions (e.g. temperate, tropical). These may provide genetic characteristics of the Arctic jellies using rDNA for making molecular comparisons of jellies.

CP-10

The complete mitochondrial genome of the Arctic sympagic gammaridean *Onisimus nanseni* Sars 1900 (Crustacea; Amphipod)

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The life of gammaridean amphipod *Onisimus nanseni* is closely associated with the Arctic sea ice, and they play an important role in the macrofauna community. Although knowledge about their biology and ecology has increased during recent years, their genetic information is completely unknown. In this study, we report the complete mitochondrial genome of *O. nanseni* with emphasis on a highly rearranged mitogenomic organization. The genome is 14,734 bp in length and contains 2 rRNAs, 22 tRNAs, 13 protein-coding genes; however, the genome does not have an apparent non-coding region (e.g. control region), excluding 194 bp between tRNA-Val and -Met. In addition, the mitochondrial genome is high AT content (70.3%), particularly in control region (78.9% AT). Considering structures of typical arthropods and available mitogenomes, *O. nanseni* has highly rearranged mitogenome gene order. This report provides first information on the complete mtDNA sequence from the amphipods.

CP-11

The complete mitochondrial genome of Arctic sea urchin *Strongylocentrotus* sp. (Echinoidea; Strongylocentrotidae) from Svalbard coastal waters, Norway

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The Arctic sea urchin *Strongylocentrotus* sp. has a circumpolar distribution and has been identified as a dominant species in Arctic habitats. Recently, the species has increased in Svalbard coastal waters in high densities. Although they are considered as *S. droebachiensis,* molecular data suggests they have different genotypes each other. In this study, we report the complete mitochondrial genome sequence of *Strongylocentrotus* sp. from Arctic coastal waters of Svalbard, Norway, in 2007. The genome is 15,711 bp in length and contains 2 rRNAs, 22 tRNAs, 13 protein-coding genes; the genome does not have an apparent non-coding region, excluding 126 bp between tRNA-Thr and -Pro. The AT content of the mitogenome was 59.0%. Upon structural comparisons, the Arctic sea urchin has perfectly identical gene order with other *Strongylocentrotus* species. Similarity showed that the Arctic sea urchin has divergent genotype, compared to mitogenomic genes of other *Strongylocentrotus* (e.g. 96.5% of COI amino acid with *S. droebachiensis*; 96.5%, *S. pallidus* ; 93.6%, *S. purpuratus*). These provide first information on the complete mtDNA sequence from the Arctic sea urchin.

CP-12

Isolation and identification of psychrophilic bacteria in the vicinity of the Korean King Sejong Antarctic Station

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Marine psychrophilic bacteria that inhabit seawater at below 4℃, play essential roles in marine ecosystem. To investigate their distribution in the Antarctic region, we have isolated 35 strains of polar bacteria originated from Antarctic sea water, fresh water in the vicinity of the King Sejong Korean Antarctic Station located at King George Island (62°S, 58°W) during November and December, 2005. To examine their growing temperature ranges, we measured average diameter of colonies formed at various temperature ranging from 4℃ to 37℃. Among them, 10 strains have formed large colonies at low temperatures and shown the highest growth rate at 4℃ or 15℃, and formed no colony above 20℃. We have carried out molecular identification using 16S rRNA sequence as a marker. As a result, 8 strains were identified as belonging to genus *Flavobacterium,* 12 strains to genus *Psychrobacter* speceis like *P. arcticus, P. glacincola,* and *P. luti.* Also, nine Antarctic strains showing low sequence similarity (<97%) with known speices are candidates for new species or genus. In this study, we isolated 35 psychrophilic or psychrotolerant bacteria from Antarctic region and they would be useful biological sources for studying cold adaptation of living organisms.

CP-13

Abundance of bacteria in Lakes Baikal (Russia) and Khuvsgul (Mongolia) by CARD-FISH and FISH methods

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Lakes Baikal and Khuvsgul are unique ecosystems with oligotrophic status and surrounded area. More than 80 % of flora and fauna is not found other place, so there would be unique microorganisms. For grouping the bacteria in aquatic ecosystem, FISH (fluorescence in situ hybridization) method is widely used. But, most cases in both lakes, about 40 % of DAPI-bound cells are not classified into target group. This phenomenon is caused by low fluorescence intensity. CARD-FISH method is developed for overcoming this problem of FISH. We employed CARD-FISH for detection and quantification of eubacteria in three cultured strains and in Lake Baikal and Khubsgul and compared the result with that of FISH with monolabeled probe. The permeabilization process in this study was carried out with lysozyme and achromopeptidase. The permeabilizaiton of E. coli and Pseudomonas fluorescens were suitable at 0.78 mg/ml lysozyme solution for 10 min and that of Bacillus megaterium was at 20 mg/ml lysozyme solution for 1 h. In case of the environmental samples, it was proper at 10 mg/ml lysozyme solution for 1 h and 60 U/ml achromopeptidase treatments for 30 min. The tyramide dilution rate 1:50 was optimum to detect eubacteria in cultured strains whereas 1:20 was optimum to detect it in natural environment. The detection rates of eubacteria with FISH (average, 90.1%) and CARD-FISH (average, 92.6%) were not greatly different. All of the three strains were cultured under rich substrates state, so they might be detected well in both FISH and CARD-FISH. In other hand, the detection rates showed the difference in Lake Baikal and Khuvsgul between FISH and CARD-FISH method. In Lake Baikal, hybridized cells by depths ranged from 57 to 66% (FISH) and from 68 to 76% (CARD-FISH), respectively. In Lake Khubsgul, eubacteria populations by depths were detected from 49 to 63% in FISH and from 63 to 71% in CARD-FISH. Although detection rates were different between both methods, the vertical distributions of eubacteria showed similar patterns except at 235m depth of Lake Khubsgul. From 100m to 235m depth of Lake Khuvsgul, hybridized cells of eubacteria increased by CARD-FISH whereas that of eubacteria abundance decreased by FISH. At 235m depth of Lake Khuvsgul, therefore, the difference rate between CARD-FISH and FISH was highest, which was 19%. The CARD-FISH method led to a significant improvement in

detection rates compared to conventional FISH method. It is debatable whether current FISH method can quantitatively detect all bacterial abundance because the rRNA content varies with metabolic activity in prokaryotic cells.

CP-14

Pedobacter svalbardensis, a psychrophile isolated from Svalbard archipelago

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A red-coloured, aerobic bacterium, strain AB 15T was isolated from the Arctic pond. It was found to be a Gram-negative, oxidase-positive, rod-shaped organism. The organism optimally grew at 18.6℃, pH 6 in sea -salt-free Zobell's broth without NaCl. Phylogenetic analysis based on 16S rRNA gene sequences indicated that this strain was related to the genus Pedobacter, showing 16S rRNA gene sequence similarity of 95% with respect to type strains of Pedobacter terrae, Pedobacter koreensis, Pedobacter sandarakinus, Pedobacter suwonensis, respectively. Assimilation of carbohydrate, enzyme activity and other biochemical properties were checked by using API 20NE, API ZYM, API 50CH, API 20E kit (bioMériux). On the basis of phenotypic properties and phylogenetic distinctiveness, strain AB 15T represents a novel species of the genus Pedobacter.

2. Biotechnology of Polar organisms

BP-1

Hypothermic preservation effect on cell lines and some kinds of primary cultured cells from mice

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Hypothermic preservation is used widely to preserve graft organs or cells in low temperature. There have been many reports about hypothermic preservation of organs or some kinds of cells, especially hepatocytes. The University of Wisconsin (UW) solution is frequently used in hypothermic preservation and showed effect. Antifreeze proteins (AFPs) had been reported to protect cells in low temperature from hypothermic damage. Some sugars are essential to cell function. They act as antioxidants and antioxidants are important in cold storage. So, to improve the preserving capacity, we tried to modify the solution. Modification method was adding some sugar (fucose, mannose, and xylose) or polysaccharide (fucoidan) or AFP to UW solution. We used 5 types of cells; HepG2 cell line as a cancerous hepatocyte sample, 293T cell line as a normal kidney cell sample, isolated hepatocyte, isolated lung cell, and blood cells from adult mice. After one-day-incubation under condition of 37 ℃ and 5% CO2, hypothermic preservation at 4℃ experiments were performed for 24-72 hours. Cells were divided into 9 groups by preservation solution; DMEM media, D-PBS, UW solution, UW solution with sugars (fucoidan, fucose, mannose, and xylose respectively) or AFP, and DMEM media with DMSO. The cell viability was assessed by LDH assay, WST-8 assay, CYP1A1, and immunohistochemistry(IHC). Modification of UW solution had improved the preservation efficiency. Most sugars and AFP added have a tendency that improves the capacity of preserving solution. However, media with DMSO did not affect the efficiency of storage. By varying concentration of sugars and AFP, the capacity of preservation effect may change. So, further study about the most proper condition will be needed.

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BP-2

Effect of rapid-freezing conditions on mammalian cells

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Cryopreservation is beneficial for prolonging the viability of living cell by reducing their metabolic rate. The main problem during that is the formation of intracellular ice, which can lead to cell damage and developmental arrest. To optimize the process of cryopreservation over the cell damage we compared the procedure of freezing way, specifically, factors related to rapid-freezing conditions. Also we examine the efficiency of various sugar extenders.

Mammalian cell lines including NCI-H292, HEPG2, SNU354 were frozen in culture media with various condition. Cell was diluted in each of the two cryoprotectants (DMSO and UW) × four extenders (0.9% NaCl, 5% glucose, 5% fucose, 5% mannose). Diluted cell was seeded in 96-well plates and frozen. Cell viability was evaluated after thawing and activation with a total of four different activation media (distilled water, 0.15% NaCl, 0.29% NaCl or 1% NaHCO3). To compare viability after thawing we stained cells by trypan blue. Considerable number of cells was counted in sample, and the survival rate was defined as the ratio of viable to total cells. And to determine the metabolic efficiency, we used MTT assay, CCK-8, CYP141, LDH and analyzed cell viability. In experiment, cell cryopreserved in 5% glucose and UW, presented and show that cells can successfully cryopreserved. A beneficial protective role of glucose on cell survival was found, similar to that of mannose, a recognized cryoprotectant. The results suggest that glucose as a sole additive may provide effective protection for mammalian cells during freezing.

Evaluating and optimization of these rapid-freezing condition will help to minimize sources of injury, maximize survival, and contribute to the improvement of optimized cryopreservation protocol for cell preservation.

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BP-3

Crystallization and preliminary X-ray crystallographic studies of the *rho* class glutathione *S*-transferase from the Antarctic clam *Laternula elliptica*

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Glutathione *S*-transferases are involved in phase II detoxification process and catalyze the nucleophilic attack of the tripeptide glutathione on a wide range of endobiotic and xenobiotic electrophilic substrates. The *rho* class of glutathione *S*-transferase from *Laternula elliptica* was overexpressed in *Escherichia coli*, purified, and crystallized with substrates, glutathione and 1-chloro-2,4-dinitrobenzene (CDNB). The diffraction data were collected to 2.20 Å for the glutathione-complexed crystals and 2.00 Å for the CDNB-complexed crystals using a synchrotron radiation source. Both crystals belong to the C-centered monoclinic space group C2. The unit cell parameters for CDNB-complexed crystals are a = 89.66, b = 59.27, c = 55.45 Å, β = 124.52˚. The asymmetric unit contained one molecule, with a corresponding V_m of 2.36 $Å^3Da^{-1}$ and a solvent content of 47.8%.

BP-4

Expression of Recombinant Endochitinase of Antarctic *Sanguibacter sp.* KCTC 13143 in *Pichia pastoris*

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Chitin composes the cell walls of some animals and microbes, including insects, crustaceans, and fungi. Chitinases break down glycosidic bonds in chitin. In combination of exochitinases, endochitinases are considered as important enzymes in biomedical industry for producing particularly N-acetylglucosamine (NAG) and others. Endochitinase chi21702 was isolated from Antarctic *Sanguibacter sp.* KCTC 13143 and well characterized in our lab previously. The gene for this enzyme was obtained from the genomic DNA and the sequence was determined successfully. The methylotrophic Yeast *Pichia pastoris* expression system was applied to develop the production process of the enzyme since this system is known to facilitate the purification of the recombinant enzymes secreted to the culture media. The *Pichia* system expressed the recombinant Antarctic endochitinase successfully and revealed enzymatic activity using colloidal chitin as a substrate. The expressed protein showed higher molecular weight than theoretical one due to maybe post-translational modification, presumably glycosylation. This presentation introduces unique characteristics of chitinases from Antarctic bacteria and suggests a potential for the development of biomedical applications.

Keywords: Endochitinases, Antarctic, Pichia, Recombinant, NAG

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BP-5

Functional Expression and Characterization of Recombinant Chitinase from Antarctic Psychrophilic Bacterium *Sanguibacter antarcticus* KCTC 13143

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A psychrophilic *Sanguibacter antarcticus* KCTC 13143 has been previously isolated from sea sand on King Sejong Station in Antarctica. An cold-active endochitinase gene, designated *chi21702*, from KCTC 13143 was PCR- amplified, cloned into pEXP5-CT/TOPO vector, and overexpressed in *Eschrichia coli* BL21(DE3) without its signal-peptide and with an C-terminal His-tag. The recombinant chitinase (R-Chi21702) was approximately 56 kDa in size, most of which was converted to inclusion body having no chitinae activity against oligomers of N-acetylglucosamine. In next step, refolding experiment for the inert chitinase was carried out using optimized refolding conditions. The refolded R-Chi21702 was then purified using a Ni-NTA column, which restored its chitinase specific activity (5.0 U/mg). The optimal reaction temperature and pH of R-chitinase were determined to be 40 ℃ and pH 8.0, respectively.

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BP-6

Purification and Characterization of Endochitinase from Antarctic Bacterium *Sanguibacter antarcticus* KCTC 13143

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A psychrotrophic bacterium *Sanguibacter antarcticus* KCTC 13143 was previously isolated from sea sand on King Sejong Station in Antarctica. In this study we purified and characterized a cold-adapted chitinase from this bacterium. The chitinase was produced in ZoBell medium containing 0.4% swollen chitin at 25℃, and purified by two-step using a hydrophobic interaction chromatography (phenyl-sepharose) and a gel filtration chromatography. The purified chitinase had specific activities of 23.28 and 12.57 U/mg toward the substrates *p*NP-$(GlcNAc)_2$ and *p*NP-$(GlcNAc)_3$, respectively, while had no activity for *p*NP-GlcNAc. This results indicated that the chitinase from KCTC 13143 have an endo-specific activity. Temperature and pH for the optimal chitinase activity were determined to be 37 ℃ and pH 7.6, respectively. Moreover, the chitinase activity at 0 ℃ remained approximately 40% lower than at the optimal temperature. This enzyme was stable at 10-37 ℃ and pH 4-10, respectively. Also it was stable in the presence of various metal ions and detergents. Thus, the above characteristics of this chitinase shows that it could be developed as a useful cold-active biocatalyst in commercial processes.

BP-7

In vitro Antioxidant activity of the extract and isolated compounds from the Antarctic lichen *Ramalina terebrata*

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Antioxidant agents, which inhibit the destructive actions of free radicals or reactive oxygen species (ROS), are widely used as food additives as well as in medicines and cosmetics. Antioxidant agents derived from natural sources are believed to be safer than synthetic compounds. In order to identify a new potential source of natural antioxidant compounds, we evaluated various antiradical and antioxidant activities of a methanol-water (70:30) extract of seven species of the Antarctic lichen including *Ramalina terebrata* Hook and Taylor (Ramalinaceae). The experimental data showed that even the crude extract of *R. terebrata* exhibited potential antiradical activities against DPPH free radical (IC_{50}-.11.2 μg/mL against the commercial standard BHA, 4.97 μg/mL). After the application of various chromatographic techniques, Usnic acid, Usnamine A, Usnamine B, Usnamine C and a yet to be identified compound [L5-C, M.W. 254 (M+H)] were isolated. Usnic acid did not show any antiradical activity against ABTS and DPPH free radicals. L5-C showed strong *in vitro* antiradical activities against DPPH and ABTS free radicals (IC_{50}s, 0.9 μg/mL against 4.97 μg/mL of BHA and 1.5 μg/mL against 46.35 μg/mL of trolox, respectively). The isolation, characterization and antioxidant capacity of these active crude and purified constitutes of *R. terebrata* are presented.

BP-8

Anti-wrinkle and skin bleaching materials produced from the Antarctic moss *Polytrichastrum alpinum*'s filamentous cell

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Polytrichastrum alpinum mosses growing under natural conditions were collected in the vicinity of the Korean King Sejong Antarctic Station (62°13.18.1"S; 058°47'24.2"W), on the Barton Peninsula of King George Island, during Antarctic summers (December 2005). Previous to experiment, we investigated a MeOH extract of a sample of *P. alpinum*. Crude extract had seen anti-wrinkle and skin bleaching effect. For anti-wrinkle and skin bleaching product yield-up, we were treated plant growth regulators (PGR) for induced *P.alpinum*'s filamentous cell (F/C) in solid Murashige-Skoog (MS) medium (pH 5.8; 3% sucrose). Induced *P.alpinum*'s F/C was subcultured in treated same concentration PGRs in liquid BCDATG medium. *P.alpinum*'s F/C is very well growing in 0.1ppm 1-Naphthaleneacetic acid (NAA), 0.01ppm 6-Benzylaminopurine (BA) and 0.5ppm 2,4-Dichlorophenoxyacetic acid (2, 4-D) in BCDATG medium.

BP-9

Cryoprotective properties of exopolysaccharide (P-21653) produced by the Antarctic bacterium, *Pseudoalteromonas arctica* KOPRI 21653 in Red Blood Cell

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25 bacterial strains that secrete mucous materials were isolated from sediment obtained from King George Island, Antarctica. Seven of these strains proved capable of producing cryoprotective exopolysaccharides. The strain KOPRI 21653 was selected for the further study. KOPRI 21653 was identified as *Pseudoalteromonas arctica* as the result of 16S rRNA analysis. The exopolysaccharide, P-21653, was purified completely from the KOPRI 21653 cell culture via column chromatography and protease treatment. The principal sugar components of P-21653 were determined to be galactose and glucose, at a ratio of 1:1.5, via GC-MS analysis. The cryoprotective activity of P-21653 was characterized via a red blood cell (RBC) LDH assay. In the presence of 0.5% (w/v) P-21653, the dead cell ratio of RBC was as low as 20.83±2.83% over three repeated freeze-thaw cycles. The dead cell ratio of RBC increased to 26.1%, respectively, in five repeated cycle conditions; however, the dead cell ratios of RBC were grater over (20.83±2.83 - 42.11±7.27 %) in the presence of 0.5 - 0.2% (w/v) P-21653. In addition, at much lower concentrations (0.2 - 0.5%), P-21653 resulted in dead cell ratios of RBC were more less than generally employed as a RBC cryoprotectant (glycerol), which were utilized at the recommended concentrations (40%). The biochemical characteristics of exopolysaccharide P-21653 reflect that this compound may be developed as a useful cryoprotectant for use in medical applications.

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