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Antarctic cooperation between Romania and Korea 2015-2017

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Summary

Romania, through this IP, presents the development of the Cooperation between Romania and the Republic of Korea, represented by the National Institute of Research and Development for Biological Sciences (NIRDBS) and the Korea Polar Research Institute (KOPRI). This cooperation started in 2015 and was strengthened in 2016, when KOPRI Antarctic Station King Sejong hosted the Romanian Research Scientific Expeditions (ROICE) with Governmental support, in Antarctica.

ROICE 2015 and ROICE 2016 were conducted with the purpose to investigate the Antarctic ecosystems by studying the microbial communities from different habitats, in order to identify and characterize their specific microcosms. Equally, they aim to study the effects and adaptations of organisms in extreme environments. Additionally, during the 2016 fieldwork season, the isotopic composition of waters in the southern peninsulas of the King George Island was analyzed.

Introduction

The National Institute of Research and Development for Biological Sciences is an important research life science institute in Romania, which promotes fundamental and applied research. Its mission is to excel in life sciences research, through integrated research approaches in Biological Sciences and, in collaboration with Romanian and international partners, to understand the life processes and solve various issues regarding health, food, society and environment.

The Arctic and Antarctic Research Department was established with the mission to strengthen the research area of the Environment and Biodiversity pillar from NIRDBS and to contribute to the development of polar research through a specialized domain.

The authorized representative of the National Institute of Research and Development for Biological Science is involved in national and international Antarctic bodies. Dr. Manuela Elisabeta Sidoroff, as Vice-President of the National Commission for Antarctic Research (NCAR) established within Romanian Academy, the representative scientific organism of Romania in the Antarctic Treaty System, is now the Romanian ATCM Contact Point responsible by the deal with scientific data. Also, the leader of ROICE teams, Dr. Cristina Purcarea, is the Scientific Secretary of NCAR, and she is the Romanian delegate to SCAR.

During previous Antarctic Treaty System Meetings attendance, NIRDBS identify partners to collaborate on scientific and logistic grounds. Thereby, NIRDBS initiated and developed a partnership with KOPRI, based on the Scientific Priorities of SCAR and the ATCM/CEP Recommendations, especially the recommendation of the clauses in the *73rd paragraph ACTM XXIX final report*.

Executive Summary

The National Institute of Research and Development for Biological Sciences and the Korea Polar Research Institute signed the Framework Agreement for a 5 (five) years period that allowed researchers from our institute to benefit of the KOPRI facilities. The Framework Agreement aims to develop close cooperative relationships in Polar sciences, between the two parties, in both scientific and logistic directions, in the spirit of the Antarctic Treaty and that of the Madrid Protocol.

Based on scientific partnership between the National Institute of Research and Development for Biological Sciences, Arctic and Antarctic Research Department, and Korea Polar Research Institute for the period 2015-

2020, there were organized two scientific expeditions ROICE 2015 - ROICE 2016, in King George Island, West Antarctica.

These expeditions took place during 03 – 23 February 2015/2016.

The members of the ROICE - Romanian Scientific Expeditions in Antarctica are scientific researchers specialized in molecular microbiology of extremophiles, human biology and also in geology. The team of the first expedition was formed of three researchers. They initiated a direction of Antarctic research in partnership with Korean researchers during ROICE 2015 expedition, which was complemented with two other research directions (human biology and geology) during the following year expedition (ROICE 2016), with the participation of five Romanian researchers.

In this cooperation, both the KOPRI *Division of Polar Life Sciences* and *Division of Polar Logistic* assured the scientific and logistical assistance for the research team of NIRDBS.

The initiation and development of the partnership between NIRDBS and KOPRI were possible due to the direct involvement of Dr. Yeadong Kim, former President of KOPRI, Dr. Ho-Il Yoon, current President of KOPRI, and Dr. Hyoung Chul Shin, Head of the Department of International Cooperation.

Scientific Cooperation

Considering the recognized vulnerability of Antarctic environments to climate change and pollution, and the increased interest in psychrophilic and psychrotolerant microorganisms due to their important biotechnological potential, our research team was interested in continuing the research directions started in the ROICE 2015 expedition. For a comprehensive evaluation of the effect of climate change on the glacier retreat and the transfer of airborne microorganisms in Antarctic habitats, a multiannual investigation is essential. Also it was studied the climate effects on the expedition members. These studies were started in the first ROICE expedition, ROICE 2015 and continued and improved for the ROICE 2016 team.

The ROICE 2016 continued the major objectives described in the ROICE 2015 expedition, namely:

- Investigating the impact of glacier retreat on perennial ice-embedded microbial communities.
- Investigating the snow microbial diversity as a possible indicator of an air transfer of microorganisms and pollutant agents.
- Isolation and characterization of cold-adapted enzymes.
- Study of the effects and adaptation of the human body to extreme conditions.

In addition, isotopic investigation of the various water reservoirs in the southern peninsulas of the King George Island was initiated in order to determine the timing of groundwater and lake recharge and streamflow generation.

The research activity focused on:

- 1) Spatial and temporal variation of microcosms from glacier ice and forefield from King George Island in relation with their physicochemical parameters. Re-sampling perennial ice and glacier forefield for a comparative analysis of microbial diversity and metabolism. A SSU rDNA metabarcoding approach will be used.
- 2) Resilience of subglacial water flow microcosms from King George Island: spatial and temporal variations of the structural and functional microbial diversity of underglacier streams. Re-sampling subglacial water flowpaths at the interface permafrost/glacier on King George Island for structural and functional diversity analyses based on SSU rRNA and functional genes. Stable isotope (O and H) investigation in order to identify its origin and flow history.
- 3) Dynamics of snow-embedded microcosm on King George Island. Re-sampling snow from previously studied areas. A Next Generation Sequencing approach will be undertaken, together with in silico predictions of metabolic activity and quantification of target functional genes.
- 4) Identification of novel sampling points for projects 1-3.
- 5) Screening of the permafrost and ice microbiota from King George Island for cold-adapted enzymes for biotechnological applications.

- 6) Structure-function relationship in pyrimidine and arginine biosynthetic extremozymes from KOPRI microbial library isolated from King George Island.
- 7) Investigating the adaptation of the human body to extreme environmental conditions, where these weather parameters (wind speed, air pressure, air temperature, relative humidity, solar radiation) influence the human red blood cells viability. Collaborative study with the KOPRI research team. Comparative flow cytometry studies with new criteria of haematological parameters (the study was conducted on human erythrocytes). The human erythrocytes were studied before and after the two expeditions, and the results were compared. We analyzed the shape, size and number of erythrocytes and their viability by measuring the intracellular esterase activity with Calcein-AM.
- 8) Stable isotope (O and H) analyses of the snow, ice, permafrost, lake and river waters in King George Island.

The scientific support was provided by the KOPRI research team from King Sejong Station, King George Island, NW Antarctica, with field trip and laboratory logistics for sampling and preliminary analyses, and by organizing scientific seminars to identify areas of joint research. NIRDSB expresses all the gratitude to Dr. Soon Gyu Hong, Principal Investigator at King Sejong Station and to Dr. Ok-Sun Kim, Senior Research Scientist at KOPRI. Personal at Bellingshausen and Carlini Stations helped with water sampling.

Results

1. Identification and morphological description of cyanobacteria and algae in the microbial communities developed on snow.

Until now, 14 taxa were identified: 2 cyanobacteria (*Aphanothece* sp. and *Phormidium autumnale*) and 12 green algae. The taxa were identified in live or fixed samples. The algal communities are dominated by green algae: *Chlamydomonas nivalis*, *Chloromonas* sp., *Raphidonema nivalis*, *Koliella* sp., *Stichococcus bacillaris* and *Klebsormidium* sp. The identification and morphological description will be continued by repeated examinations of enrichment cultures performed on different growth media.

For most of the taxa that are identified or in the course of identification a good iconography was obtained (approx. 1400 digital photographs).

An elemental analysis (EDX) was also performed that showed two main types of particles: aluminosilicates and mixed particles (aluminosilicates with organic residues).

2. Isolation of cryophilic and cryotolerant bacterial strains for future biotechnological applications.

Isolation of different bacterial strains from snow and soil were carried out on solid R2A medium. From each sample, 100 μ l were spread onto the surface of the plate. Pure strains were isolated based on distinctive colony morphology (shape, size pigmentation). A total of 35 pure strains were obtained: 3 from soil and 32 from filtered snow.

Bacterial colonies from the ice, subglacial water and soil samples cultivated on R2A medium at different temperatures were isolated and identified by 16S rRNA gene PCR amplification and sequencing. Morphological characterization of isolates was carried out by optical microscopy and scanning electron microscopy (SEM).

3. Investigation of microbial diversity from glacier ice, snow and underglacier streams, by 16S rDNA and ITS MiSeq Illumina sequencing

The microbial biodiversity from snow samples was investigated by a metagenomic approach using the Next Generation Sequencing method in collaboration with the Microbial Diversity laboratory from the Norwegian University of Life Sciences.

DNA from samples of ice and subglacial water, the structural diversity of prokaryotic microbial communities (bacteria and archaea) and eukaryotes (fungi) is being determined by the metagenomic analysis through sequencing using Illumina MiSeq (Next-Generation Sequencing) of the gene 16S rRNA, respectively ITS2 of 93 samples, in cooperation with Korean partners (Dr. Soon Gyu Hong - Kopriva).

4. Geochemical analyses of subglacial ice and water samples was carried out in order to correlate the diversity of the microbiomes habitats corresponding to the composition of the carbon / nitrogen and trace element substrate.
5. Investigation of the influence of ecological conditions from Antarctica on human erythrocytes viability provided preliminary results on understanding adaptation mechanisms at cellular level under extreme conditions, with possible future applications to improve the life and health in general.

The adaptation to living conditions in Antarctica and the impact of stress conditions on hematology parameters, particularly on the biology of the human erythrocyte, at morphological, physiological and molecular level, is under investigation, for practical applications in blood storage by cryopreservation, cold storage at 4°C, pressure, etc.

6. Interactions between surface waters in King George Island, Antarctica – a stable isotope perspective

It was the first study of the isotopic composition of surface waters in the southern peninsulas (Barton, Fildes, Weaver and Potter) of King George Island, Antarctica. It has collected > 200 samples of snow and snowmelt, water (lake, river and spring), ice (glacier ice and permafrost) from the four peninsulas in February 2016 and analysed them for their oxygen and hydrogen stable isotopic composition.

Conferences

- European Geosciences Union General Assembly, Vienna, April 23-28, 2017, “*Interactions between surface waters in King George Island, Antarctica – a stable isotope perspective*”, Perşoiu, A., Bădăluță, C.
- First APECS Balkan Meeting, Kardzhali, Bulgaria, 1-2 October 2016, “*Microbial diversity of glacial habitats from King George Island (NW Antarctica)*” Corina Ițcus
- SCAR Open Science Conference 2016 – Kuala Lumpur, Malaysia, 21-31 August
 - “*Microbial community structure in different snow types from King George Island, Antarctica*”, Cecilia Chiriac, Corina Ițcuș, Cristina Purcărea, Nicolae Dragoș, Knut Rudi, Sorin Marius Avramescu, Irina Fierascu, Lucian Barbu-Tudoran, Iris Tusa, Manuela E. Sidoroff, Cristian Coman
 - “*Spatial Diversity of Glacier Ice Bacteria from King George Island (NW Antarctica)*”, Corina Ițcus, Cristian Coman, Constantin Marin, Tue Kjærgaard Nielsen, Lars Hansen, Iris Tusa, Manuela E. Sidoroff, Cristina Purcarea
 - “*Influence of ecological conditions from Antarctica on human erythrocytes viability. Preliminary report*” Iris Tusa, Manuela Sidoroff, Daniela Bratosin
- IUBMB “STANDARD” - Modern Biotechnologies in Sustainable Development of the Danube Delta, Murighiol, Tulcea, Romania “*Bacterial diversity in glaciers from King George Island, Antarctica*” 31 May – 2 June, 2016, Ițcuș C, Marin C, Coman C, Tușa IM, Sidoroff ME, Purcărea C
- International Symposium of the “Young Researchers in BioSciences”, Cluj-Napoca, Romania, 22-26 July 2015 “*Screening of bacterial diversity on glaciers from King George Island, Antarctica*”, Ițcus C., Coman, C., Tusa, I.M., Sidoroff, E.M., Purcarea, C.
- APECS World Summit 2015 “*The Future of Polar Research*” Sofia, Bulgaria, June 6-8, Ițcus, C.
- The article “*ROICE 2015 Romanians reconquered Antarctica*” - The science and technology magazine - number 45, April 2015 - interview with Dr. Manuela Sidoroff

Dr. Cristina Purcarea, the scientific leader of ROICE expeditions, was elected member of the board of the group SCAR Antarctic Peninsula Linking Science (LAPES) created in 2016 and coordinated by Dr. Soon Gyu Hong (KOPRI) and Dr. Angelica Casanova - Katny (INACH). Both Dr. Purcarea and Dr. Cristian Coman are members of this SCAR work group.

The two Romanian researchers are also members of another SCAR workgroup created in 2016 that is focused on studying *The stability and reconstruction of ecological ecosystems Antarctic following the action factors*, coordinated by Charles Lee (U of Waikato, NZ), Chun Wie Chong (International Medical U, Malaysia) Jeff Bowman - Earth Observatory, USA,.

Based on their Antarctic research expertise, two of the ROICE team members, Dr. Cristina Purcarea, and Dr. Cristian Coman, recently obtained financing from the Romanian Ministry of Research and Innovation for coordinating research projects, as follows:

1. Experimental Demonstration Project PN-III-P2-2.1-PED-2016-0116 *"Novel aldehyde dehydrogenase from Antarctic bacterium as highly efficient catalyst for low temperature biosensing and biotechnologies - COLDSSENSOR"* (2017-2018) – Project director - Dr. Cristina Purcarea
2. Experimental Demonstration Project PN-III-P2-2.1-PED-2016-1170 *"Antarctic bacteria against human pathogens: a quest for new antimicrobial compounds - AntarcticPharma"* (2017-2018) – Project director – Dr. Cristian Coman

Logistic Cooperation

Logistics cooperation refers at:

- Logistic assistance in preparing the expeditions in accordance with the KOPRI procedures.
- Logistic support in the preparation phase of the expedition regarding the transport of the Romanian team and the equipment to be transported for field trips and sampling.
- Transfer of the Romanian expedition team members from Punta Arenas, Chile to Antarctica and return through the travel agency responsible for the KOPRI scientists and foreign visitors.
- Providing accommodation and daily meals for the Romanian researchers in King Sejong Station.
- Providing a laboratory infrastructure at KOPRI's station for sampling and partial processing of biological material.
- Provide logistical support for field sampling from various locations and habitats of King George Island, Antarctica (e.g., boat transportation to the neighboring peninsulas).
- Logistic support on environmental protection in ASPA No. 171, in 6 ASPAs and 1 ASMA.
- Logistic support for the health and safety of the Romanian team assured by the presence of a medical doctor in the King Sejong Station that monitored the team members' vital functions.
- NIRDBS ensures the research facilities for processing the samples from Antarctica and for realising subsequent research and publishing the results.

Conclusion

Cooperation between the *Romanian National Institute of Research & Development for Biological Science* and the *Korea Polar Research Institute* contributes to the participation of Romanian scientists to Antarctic research, promoting interactions with international working groups focused on Polar science, in view of answering highly sensitive questions on the dynamics of Antarctic ecosystems.

Also strong ties between Romanian and Korean researchers leading to high lever joint researches which will contribute to knowledge of the development of the mechanisms polar, as well as increasing visibility of the researchers and ours institute.