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## **Cooperation between Romania and Korea (ROK) in Antarctica**



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Information Paper is submitted by Romania under agenda item 13, “Science Issues, Scientific Cooperation and Facilitation”.

## 1. Summary

Romania, through this IP, presents 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). Romania started investigating both the impact of glacial retreat on the ice microcosms from King George Island, considering the aggressive glacier melting for the last decade in this Antarctic region, and how are the Antarctic habitats affected by the transfer of microbial cells and pollutant agents, with the purpose to develop Romanian research directions in polar sciences.

## 2. Introduction

The National Institute of Research & Development for Biological Sciences is an important structure in the life sciences research in Romania.

In 2014 the Arctic and Antarctic Research Department was established within NIRDBS through a ministerial decision. The mission of the newly founded department is 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.

In order to accomplish its mission, NIRDBS participated, as observer, at the XXXIII SCAR Delegates Meeting, held in Auckland, New Zealand, between 1st and 3rd of September 2014.

At that meeting, NIRDBS initiated a partnership with KOPRI, based on the Scientific Priorities of SCAR and the CEP Recommendations, especially the recommendation of the clauses in the *73rd paragraph ACTM XXIX final report*.

## 3. 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 the KOPRI facilities. The Framework Agreement aims to develop close cooperative relationships in polar sciences, between the two parties, in both scientific and logistic field, in the spirit of the Antarctic Treaty and that of the Madrid Protocol.

Based on this agreement, NIRDBS, supported by the Romanian Government and having the scientific endorsement of NCAR from the Romanian Academy, has organized the Romanian Scientific Expedition with Governmental support, in Antarctica, ROICE 2015. The King Sejong Antarctic Station of KOPRI, on King George Island in West Antarctica, hosted the Romanian researchers between 3<sup>rd</sup> and 23<sup>rd</sup> of February 2015.

The members of the Romanian Scientific Expedition in Antarctica are scientific researchers specialized in microbiology and biodiversity.

From the KOPRI side, the Division of Polar Life Sciences and the Division of Polar Logistic assured 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, President of KOPRI, and Dr. Hyoung Chul Shin, Head of the Department of International Cooperation.

#### 4. Scientific Cooperation

Considering the vulnerability of the Antarctic environment to climate change and pollution, as well as the increasing interest in the study of the cryophilic and cryotolerant microorganisms in the Antarctic ecosystems, briefly studied in the past, and due to the important medical and biotechnological potential, the main research topics of ROICE 2015 are:

1. Investigation of the impact of climate changes on the diversity of microbial communities and their role in balancing the glacial ecosystem following glaciers melting.
2. Study of microbial diversity in Antarctic habitats, with implications in the transfer of microbial strains and of pollutant agents.
3. Isolation of microbial strains and extremozymes from the Antarctic habitats, with possible applications in bionanotechnology.
4. Study of the effects and adaptation of the human body to extreme conditions.

The scientific support was provided by several researchers from King Sejong Station for selecting the optimal points for sampling the biological material and also 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.

The samples taken during the ROICE 2015 expedition and the activities performed during the Antarctic Research Programme were processed following 3 major goals:

- 1) Investigation of microbial communities from snow, ice, subglacial water flowpaths and soil.
- 2) Identification and screening of microbiota from King George Island for cold-adapted enzymes used in nanotechnologies and isolation of bacterial strains for future biotechnological applications.
- 3) Determination of phylogenetic and metabolic diversity of microbial communities based on SSU and LSU rRNA gene through Next Generation Sequencing technique and metagenomics/metatranscriptomic analyses.

#### Preliminary results:

Melted ice and water samples were cultivated on R2B and BBM media at 4°C and 15°C. The colonies obtained will be further used for screening of applicative enzymatic activities. Glutaraldehyde-fixed samples are currently analysed by Scanning Electron Microscopy. Total DNA was extracted for determining the bacterial/archaeal community structure through a metagenomic approach using the Next Generation Sequencing method based on the 16S rRNA gene. Ongoing investigations also cover physical and chemical characterization of collected samples, to determine the microbiome dependence on environmental parameters at the interface glacier/soil/water.

The morphological characterization of the cyanobacteria and algae isolated from snow, resulted in the identification of 14 taxa: 2 cyanobacteria and 12 green algae. An elemental analysis (EDX) was also performed that showed two main types of particles: aluminosilicates and mixed particles. Also, several pure strains were isolated from soil and filtered snow based on distinctive colony morphology (shape, size pigmentation).

The second part of the Antarctic research is focused on the influence of environmental conditions on the viability of human erythrocytes in Antarctica, as a haematological adaptation.

Romanian researchers considered that the human red blood cells can be excellent models for studies on environmental adaptations. These adaptations can be on morphological, physiological or molecular level. Cell lesions and viability of cells related to senescence and apoptosis of nucleated cells, including nucleated erythrocytes, are well known and led to the definition of cell viability criteria like mitochondrial membrane potential ( $\Delta\Psi_m$ ) loss, cysteine protease activation, chromatin condensation and fragmentation and propidium iodide uptake. These criteria are not applicable to human RBCs due to their lack of nucleus, mitochondria and other organelles.

Recently NIRDBS researchers devised a new flow cytometric assay for the measurement of viability and ageing of human RBCs using calcein-AM for measurement of esterase activity. The loss of calcein fluorescence is associated with a decrease in the amount of ATP, another marker of RBC senescence and

apoptosis which is classically considered as an indicator of viability for RBCs. In addition, we observed that the loss of esterase activity was an early event which occurs prior to the phosphatidylserine exposure.

The preliminary results obtained by comparing the data collected from the members of the ROICE expedition, before and after the 3 weeks period spent in Antarctica, demonstrates a clear influence on the viability of erythrocytes, a decrease in their number being observed, which can mean a faster aging of these elements. It is difficult to say at this point which of the environmental factors caused the viability changes. No morphological changes were observed.

## **5. Logistic Cooperation**

KOPRI has provided logistical support for NIRDBS since the early stage of organizing the Romanian Expedition.

Logistics cooperation refers at:

- Logistic assistance in preparing the expedition 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 neighbouring 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.

## **6. Conclusion**

In the frame of the 5-year partnership with KOPRI for Antarctic collaborative investigations, these projects could be continued and enhanced by extending the investigations regarding the retreat phenomenon to a broader glacier area of the island. An increased number of snow sampling points could increase the knowledge regarding the spreading of pollutant agents (chemical, biological) in the Antarctic habitats through air. The technical results obtained will be discussed with the Korean partners in order to be further used in correlation with other studies performed in King George Island.