AMBIO / ANTAR-IMPACT meeting

15th of December 2008

Report

University of Liège, Sart Tilman campus, Liège, Belgium

Context

The **AMBIO** project started two years ago, officially with the Kick-off meeting which took place at the Belgian Royal Library (Brussels) on the 26-27/03/2007. During the first two years, samples were collected from Antarctica during the MERLIN expedition and from international collaborators. Cultivation and molecular work has begun by the three partners and the first results started to be published (Zakhia *et al*. 2007). Besides, many activities related to the International Polar Year programs were done by the partners and the AMBIO website was constructed (www.ambio.ulg.ac.be).

The **ANTAR-IMPACT** project started in the beginning of 2007 with the BELARE expedition. Dr. D. Ertz collected samples from the region around the Belgian Polar Station "Princess Elisabeth" before its construction. The molecular and morphological characterization already started on these samples. In addition to future samples from the same regions, a comparison will assess the impact of the station on the Antarctic ecosystems.

These two projects are closely linked as they are conducted by almost the same partners and they share similar users committee. For this reason a joint meeting was organized on the 15th of December 2008 at the University of Liège, Liège, Belgium. The day was divided into two parts: the morning session with the members' presentations and the afternoon workshop with seminars given by several users.

Morning session: members' presentations

Welcome and presentation of the meeting: Dr. Annick Wilmotte (C, AMBIO & ANTAR-IMPACT)

The opening session started with Dr Annick Wilmotte, who welcomed the audience, reminding during her talk the context of AMBIO and ANTAR-IMPACT, in the frame of the International Polar Year program MERGE (Microbiological and Ecological Responses to Global Environmental Changes in Polar Regions), and introducing the different partners and participants, in relation to the Workpackages. The talk started with a special emphasis on the microbial character of the Antarctic continent. The molecular tools allowed a revolution in the exploration of the molecular diversity of both cultured and uncultured microorganisms. The project is framed in the current scientific discussions:

- Is ecology of microorganisms driven by the same factors as eukarya?
- Do endemic microbial taxa exist? (or due to their small size, they can be everywhere?)
- To explain the biogeography of microorganisms

The Antarctic continent is the best place to address these questions: it is a remote place, under extreme conditions. However, it presents a gradient of environmental conditions, from harsh (continental biotopes) to milder (Antarctic Peninsula) ones.

Our aim is to ultimately generate molecular diversity data, which we can deposit in a database and to know better about the communities turnover and learn about the biodiversity patterns.

Then Dr. Wilmotte talked about the objectives, work packages and work to be done in the second phase (starting January 2009). Finally, she presented the ANTAR-IMPACT project that contributes to the evaluation of the environmental impact of the construction and functioning of

the future Belgian Polar Base “Princess Elisabeth” on the Antarctic ecosystems, with its aims and work packages.

Molecular Diversity of Antarctic Cyanobacteria: Mr. Pedro De Carvalho Maalouf (P1, AMBIO)

P. De Carvalho Maalouf started by explaining the different techniques used in the AMBIO project in order to study the diversity of cyanobacteria in Antarctica (PCR, DGGE, cloning, sequencing). He then presented the work that has been done previously (F. Zakhia) in the cyanobacteria laboratory on the Sample 41 coming from the Antarctic Peninsula. This was the first report of the diversity in a bi-laminated mat. Some results from the work on West Ongul lakes (East Antarctica) were also presented. These, in addition to other samples, were used to construct a phylogenetic tree. Three new – potentially endemic – OTUs were discovered. A clone library was also constructed with the sample WO4, it showed a rather low diversity (2 OTUs). The presentation ended with the work to be done in the second AMBIO phase.

First Biological Assessment of Utsteinen with Focus on Lichens: Dr. Damien Ertz (National Botanical Garden, ANTAR-IMPACT)

During the BELARE expedition in February and January 2007 to the region of the future Belgian Polar Base Princess Elisabeth, Dr. Ertz did a mapping of the Lichens and bryophytes. He talked about the importance of a Petrel population (150 couples) and its impact on the lichen communities by providing them with a source of nutriments. After identification of the present organisms, they were differentiated in cosmopolitan, bipolar and endemic species. Two potentially new species were discovered: *Trapelia* sp. and *Lecidella* sp. The ITS sequencing of the *Lecidella* specimens showed that they are all genotypicaly identical. When observing other samples, Damien found *Myriospora* and lichenologous fungi on *Physia dubia*. This campaign also provided 52 samples that are now being analyzed by the different partners for the diversity of bacteria, cyanobacteria, diatoms, green algae, rotifers and tardigrades. One mite and 1 collembola were found.

This presentation led to a discussion on the importance of having an undisturbed monitoring site on which studies could be held in parallel on different organisms. It was referred to the ASPA-SSI site at Rothera that could be used as example. It was discussed that there was a need for a physical boundary to stop regular access to such a reference monitoring site. This could be indicated by bamboos in the ice on both sides, with a cord. Drums could also be used, but afterthoughts were that they could be quite dirty.

ANTAR-IMPACT. Diversity of the Surroundings of Princess Elisabeth Antarctic Station: Mr. Rafael Fernandez Carazo (P1, ANTAR-IMPACT)

R. Fernandez Carazo presented the work that was done on cyanobacteria on the Belgian Polar Base “Princess Elisabeth” samples that were brought by D. Ertz. DGGE with semi-nested PCR

was used in order to increase the specificity and have more reliable results. Two methods of DNA extraction were also tested and the Smalla *et al.* (1993) method was chosen. On the twelve analyzed samples, a relatively low diversity was found compared to other coastal samples. A high degree of endemism and three yet undiscovered OTUs were found. All samples but one shared at least one OTU, which is in concordance with the theory of distribution of species in near-by habitats. Finally, R. Fernandez Carazo brought up the importance of the preservation of these sites that hold unique biodiversity against the introduction of alien species.

Exploring Heterophic Bacterial Diversity of Antarctic Samples through Cultivation: Miss Karolien Peeters (P3, AMBIO)

The work on the cultivated bacterial diversity of nine samples coming from different Antarctic regions was presented. The plate counts results as well as the molecular characterization by Rep-PCR and partial sequencing of the 16S rRNA gene of the isolates show a large diversity in the samples (especially for PQ1 sample from Pourquoi-Pas Lake) and between the samples. TM2 from Forlidas Pond is very different from TM4, Lundström Lake, in agreement with the observations on cyanobacterial diversity. There are several clusters and separate isolates that have sequence similarities with known taxa below 97% and 95% revealing the presence of probable new species and even new genera. The future work will aim to sequence the complete 16S rRNA gene for *at least* one representative per cluster and to do a detailed characterization of selected new groups with a view to describing them. (Real-Time) PCR tests will be optimized for fast screening a large number of samples for some specific groups.

Aaike discussed that the samples came from very diverse sites, and were chosen in fact to maximize the diversity. Concerning the temperature of isolation, not many isolates were restricted to 4°C, the majority was isolated at 15°C and the ones from 20°C were the same as at 15°C. Interesting was that the DGGE did not give the same diversity as the cultivation work. Of course, with the molecular methods, it cannot be ruled out that some DNA comes from dead organisms or just blown in by winds.

Hidden Levels of Phylodiversity in Antarctic Green Algae & Uncultivated Diversity of Green Algae and Bacteria: Influence of Regional vs. Environmental Conditions: Dr. Aaike De Weever (P2, AMBIO)

The first part of the presentation underlined the phylodiversity studies done by P2 on green algae. Current knowledge (work of Broady in 1996 on terrestrial diversity based on morphology) was that most diversity was cosmopolitan and there were few endemics. After the isolation of the strains, their microscopic characterization and ARDRA screening, the 18S rRNA gene of 61 strains was sequenced. They were grouped into 14 taxa and a phylogenetic tree was constructed, it revealed the long Antarctic isolation of the microorganisms. For the time scale, it is estimated that the divergence of green algae took place 700-1200 million years ago. 18S rDNA results showed that there is a distinct Antarctic green algae flora. As *Chlorella* and *Scenedesmus* are two clades with identical 18S rDNA sequences and are detected in most regions sampled, these will

be studied in detail using more detailed markers such as ITS. Ecophysiological work on the strains will be carried out by Sophie.

The second part of the presentation focused on the selection and analysis of 83 samples coming from 70 lakes for studying the importance of regional vs. environmental factors in shaping green algal, diatom and bacterial community composition. The selection was carried out by stratified random sampling, aiming to find samples similar in environmental conditions to others in other regions. Multivariate analysis of DGGE data showed that the distribution of the samples was mainly explained by the environmental variables, yet a small percentage of the variation was explained by regional variables (although not significantly for bacteria). Finally, the importance of obtaining environmental data for each studied region was underlined, as this is needed for evaluating the importance of regional vs. environmental factors in microbial communities.

It was noted that we miss the environmental data for the Belgian Basis, what makes the multivariate analysis impossible. It was asked about pH values. They range from 6 to 8. The sulfate is linked to salinity.

Lunch

Afternoon workshop: general talks

Antarctica's Biological History – Insights from Terrestrial Ecosystems: Dr. Peter Convey (BAS)

Dr. Convey talked about the ice evolution on Antarctica, its geographical isolation and its specific biology. The high level of endemism (100% of the nematode species) as well as the separation of the continent into 10 biological regions was underlined. To explain the long persistence of organisms, refuges are needed. They could be nunataks, and do not need to have been the same all the time. Finally, Dr. Convey put forth the importance of joining biological data to the glaciology and evolution of Antarctica. This would enable to improve the ice sheets models.

Interestingly, the mosses do not show the same pattern and seem to be recent colonisers.

Climate Change Effects on Antarctic Terrestrial Ecosystems: Dr. Ad Huiskes (NIOO-KNAW)

The effect of climate change on Antarctic ecosystems is being studied with the TARANTELLA project. Open Top Chambers were used to mimic a global warming scenario and studies are in course on different vegetation and soil communities as well as arthropods. The experiment has run for 3 years and the OTC will stay after that. The talk ended with the importance of comparing the results of the studies (13 countries involved) using different kinds of Open Top

Chambers. There were large differences between locations, thus a potential for a large response over longer periods.

After a question, it was noted that the plastic was transparent to UV-B but could block UV-C.

Antarctica: White and Wild: For How Long? Alexandre de Lichtervelde (CCAMLR and IWC Commissioner)

The meeting ended with a more political talk by Mr. De Lichtervelde who started with the history of Antarctica and the birth of the Antarctic Treaty System followed by the policy-making and the governments involved. The focus is now on the conservation of Antarctica (climate change, tourism, pollution, exotic species...). The talk ended with the importance of the surrounding countries and the international collaboration on the scientific as well as the politic levels.

INTERNAL DISCUSSIONS

1) Phase 1:

a) PAE will not make clone libraries on algae but rather DGGE-based study. The resolution is less than expected and bands at the same heigth might come from different organisms. Moreover, there is a special difficulty with algae and protists: lack of specific PCR primers

b) For bacterial clone libraries, PAE will agree with LMG on the basis of the cultivated diversity, early 2009.

c) LMG will do the 16S rRNA of remaining isolates, that will probably give new taxa. They will think of more specific tests to carry out in larger sets of samples.

d) ULg will do the DGGE of the samples studied also in Gent, and drop the ones of Gibson (too late) and Borghini (too late). Frederic subsampled Syowa and Schirmacher Oasis. The Borghini samples will be used in Phase 2.

2) Phase 2:

a) PAE is thinking about doing T-RFLP for bacteria, and ITS sequencing (probe?) for Chlorella and Scenedesmus.

b) LMG will stop the cultivation, will do Real Time Quantitative PCR for specific groups, describe the taxonomy of the new taxa and integrate the results.

c) Ulg will use the same samples as Gent for the cultivation of cyanobacteria, and start the Real Time Quantitative PCR.

d) New samples to come: Belgian Basis, Macquarie Islands (Dana Bergstrom, Australia), Byers (Bart Van de Vijver) and South Georgia (Dom Hodgson). Macquarie and South Georgia are from Subantarctic Islands, where we have no samples yet.

3) Publication strategy

a) PAE: - DGGE MICROMAT (problems with referees and data on DGGE gels of cyanobacteria, Elie should write to Arnaud and Stana to see if they can help)

- Green algae cultures

- Limnology of Syowa Oasis/Schirmacher Oasis

- Uncultivated diversity by DGGE (all organisms)

- Clones versus cultures (PAE/LMG)

b) LMG : samples of Belgian Basis, comparison with clone libraries

c) ULg: - Transantarctic Mountains

- Belgian Basis, DGGE + microscopy

d) choice of co-authorship? The people who sampled and the ones contributing data (see algorithm of BAS, that was given by Dom for MICROMAT).

Think about the IPY meeting in 2010 in Oslo.

Addendum: Participant list

Name	Institute	Fuction
Boistos, Sophie	University of Ghent, Belgium	PhD student, P
Convey, Peter	BAS, UK	Dr., scientific visitor
De Carvalho Maalouf, Pedro	University of Liège, Belgium	PhD student, P
de Lichtervelde, Alexandre	Federal Ministry of the Environment, Belgium	Follow-up committee
De Wever, Aaike	University of Ghent, Belgium	Dr., Postdoc, P
Ertz, Damien	National Botanic Garden of Belgium	Dr., P
Fernandez Carazo, Rafael	University of Liège, Belgium	PhD student, P
Huiskes, Ad	NIOO - KNAW, The Netherlands	Dr., scientific visitor
Mano, Marie-José	University of Liège, Belgium	PhD student, P
Moermans, Coraline	National Botanic Garden of Belgium	Technician
Namsaraev, Zorigto	University of Liège, Belgium	Dr., Postdoc, P
Peeters, Karolein	University of Ghent, Belgium	PhD student, P
Savichtcheva, Olga	University of Liège, Belgium	Dr., Postdoc, P
Simon, Patricia	University of Liège, Belgium	Technician, P
Van Isaker, Nathalie	IPF, Belgium	User
Vancauwenberghe, Maaike	BELSPO, Belgium	Manager
Vereecke, Claudine	University of Ghent, Belgium	BCCM manager, follow-up committee
Verleyen, Elie	University of Ghent, Belgium	Dr., P
Vyverman, Wim	University of Ghent, Belgium	Prof., P
Wilmotte, Annick	University of Liège, Belgium	Dr., C

P: partner

C: coordinator

BCCM: Belgian Collection of Microorganisms

IPF: International Polar Foundation

BELSPO: Belgian Federal Science Policy

N.B. the following persons apologized for their absence: Dr. Gibson, Dr. Pearce, Prof. Naganuma, Prof. Marinelli, Prof. Vincent, Prof. Quesada, Dr. Chapelle, Dr. Hodgson, Dr. Danis, Dr. Bosschaerts, Dr. Seghers and Dr. Sabbe.

Annex: program meeting

AMBIO / ANTAR-IMPACT meeting

15th of December 2008, University of Liège, Sart Tilman campus, Liège, Belgium

Morning session: members' presentations - *room 2.71, building B6c*

09h50 **Welcome and presentation of the meeting**
Dr. Annick Wilmotte, Cyanobacteria group, University of Liège, Belgium

10h00 **Molecular Diversity of Antarctic Cyanobacteria**
Mr. Pedro De Carvalho Maalouf, Cyanobacteria group, University of Liège, Belgium

10h20 **First Biological Assessment of Utsteinen with a Focus on the Lichens**
Dr. Damien Ertz, National Botanic Garden of Belgium, Brussels, Belgium

10h30 **ANTAR-IMPACT. Diversity in the Surroundings of Princess Elizabeth Antarctic Station**
Mr. Rafael Fernandez Carazo, Cyanobacteria group, University of Liège, Belgium

10h50 **Exploring Heterotrophic Bacterial Diversity of Antarctic Samples Through Cultivation**
Miss Karolien Peeters, Laboratory of Microbiology, University of Ghent, Belgium

11h20 **Uncultivated Diversity of Green Algae and Bacteria: Influence of Regional vs. Environmental Factors**
Dr. Aaike De Weever, Laboratory of Protistology and Aquatic Ecology, University of Ghent, Belgium

12h00 **Discussion**, questions and suggestions

12h30 **Lunch** (sandwiches and beverages)

Afternoon workshop - *amphitheatre A.4, building B7b*

14h00 **Antarctica's Biological History – Insights from Terrestrial Ecosystems**
Dr. Peter Convey, British Antarctic Survey, Cambridge, United Kingdom

14h45 **Climate Change Effects on Antarctic Terrestrial Ecosystems**
Dr. Ad Huiskes, Unit for Polar Ecology, Netherlands Institute of Ecology, Yerseke, The Netherlands

15h30 **Antarctica: White and Wild: How long for?**
Mr. Alexandre de Lichtervelde, Federal Public Service Health, Food Chain security and Environment - *National Contact point for the Committee for Environmental Protection of the Antarctic Treaty - CCAMLR and IWC Commissioner*