



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 nutriment. 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 genotypically 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 political 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 height 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*

Appendix 2

AMBIO/ANTAR-IMPACT/BELDIVA meeting

9 December 2009

Report



AMBIO / ANTAR-IMPACT / BELDIVA meeting 2009

**9th of December 2009, University of Liège, Belgium
Sart Tilman campus, building B6a, room 2.47**

Morning session

10h00 Welcome, presentation of the projects and the meeting
Dr. Annick Wilmotte (ULg) and Dr. Elie Verleyen (PAE)

10h10 Cyanobacterial diversity and distribution in aquatic microbial mats assessed by DGGE
Mr. Pedro De Carvalho Maalouf (ULg)

10h25 ANTAR-IMPACT/BELDIVA, cyanobacterial diversity and plans for the 2010 field campaign
Miss Marie-José Mano (ULg) and Dr. Zorigto Namsaraev (ULg)

10h45 Antarctica, characterized by a specific green algal flora?
Dr. Aaike De Wever (PAE)

11h00 Bacterial community composition in the surroundings of the Belgian Base
Miss Dagmar Obbels (PAE)

11h10 Culturable bacterial diversity in antarctic samples
Miss karolien Peeters (LM)

11h25 SCAR-MarBIN/ANTABIF projects
Dr. Bruno Danis (VUB)

11h35 Discussion around the projects

13h00 LUNCH

Afternoon workshop : "Antarctic climate, biodiversity and invasions"

14h00 Aliens in Antarctica: Quantifying propagules inadvertently carried into the Antarctic
Dr. Ad Huiskes (BAS)

14h30 Biological invasions in the Antarctic
Dr. Pete Convey (NIOO-KNAW)

15h00 HOLANT
Dr. Elie Verleyen (PAE)

15h30 The Belgian Biodiversity Platform: a science - policy interface on biodiversity research
Dr. Hendrick Segers (VUB)

15h50 Conclusion and internal discussion

Context

Belgium is building an expertise in research on polar microbial ecology via the collaboration between 1) the Cyanobacteria group of the ULg led by Dr. Wilmotte, 2) the Protistology and Aquatic Ecology research group of Prof. Dr. Vyverman from UGent, 3) the Microbiology Laboratory of Prof. Dr. Willems also from Ugent and 4) the National Botanical Garden with Prof. Dr. Bart van de Vijver and Dr. Damien Ertz.

This year's meeting was a great opportunity to join the 3 BELSPO research projects: AMBIO, Antarctic Microbial BIOdiversity: the influence of geographical and ecological factors, ANTAR-IMPACT (Expertisepool on inventarisation and evaluation of the environmental impact of the Antarctic Research Station 'Princess Elisabeth') and BELDIVA (Belgian Microbial Diversity Exploration near the Princess Elisabeth Station, Antarctica).

The day was divided in two parts: during the morning session, the partners presented their advances and results in front of the users committee. In the afternoon, a workshop entitled "Antarctic climate, biodiversity and invasions" was given for a wider audience. Just before lunch, we had the opportunity to listen to the presentation of Dr. Danis on the SCAR-MarBIN and ANTABIF projects. We also had a discussion about the non-native species in Antarctica, and discussed the answers to give to a questionnaire posted on the forum of the Committee for Environmental Protection.

Morning session: partners' presentations

Welcome, presentation of the projects and the meeting (Dr. Wilmotte, ULg, and Dr. Verleyen, PAE)

Dr. Wilmotte inaugurated the day with underlining the coincidence of this year's meeting with two events: the 150th anniversary of Darwin's "The Origin of Species" and the Copenhagen summit for climate change.

As ULg coordinates AMBIO and ANTAR-IMPACT, she started by drawing the context of the AMBIO project that fits in the frame of the "everything is everywhere, but the environment selects" hypothesis of Baas Becking. With the ongoing advances in molecular tools, the main objectives of AMBIO are to understand and describe microbial biogeography in Antarctica. Also we will enlarge 16S databases with new representatives and the culture collections of microorganisms. Then, Dr. Wilmotte presented the structure of the project, the various work packages with an emphasis on the outreach and dissemination.

The ANTAR-IMPACT project was launched with respect to the necessity of evaluating the environmental impact of the construction and functioning of the Belgian research station "Princess Elisabeth". This is, until now, the unique opportunity to evaluate such an impact by having a pre-construction sampling and a post-construction monitoring. Dr. Wilmotte also presented the objectives and work packages of the project.

Then Dr. Verleyen presented the BELDIVA project as PAE is the coordinator. The main task of this project is to collect samples for microbial research. The exploratory field campaign was done in 2008-2009 and a collaboration with Prof. Donnay (ULg) has started in order to map the regions visited. Finally, he presented the plans for the 2010 field campaign (03.01.2010 to 14.02.2010) where they will visit the region near the "Princess Elisabeth" station and the Schirmacher Oasis.

Cyanobacterial diversity and distribution in aquatic microbial mats assessed by DGGE (Mr. De Carvalho Maalouf, ULg)

The intermediary results of AMBIO regarding the study of the cyanobacterial diversity were presented. Briefly, the DNA from all of the 83 samples to be analyzed in common by the 3 partners was extracted. The extract migrated on a gel with a denaturing gradient and all obtained bands were excised. The bands of 46 samples were sequenced and analyzed. A distance tree was constructed and the bands that shared more than 97.5% of similarity were

grouped into operational taxonomic units (OTUs). These results show a strong degree of endemism (36%) of the cyanobacterial taxa in the studied lakes corroborating previous similar studies. Moreover, the endemism seems to be at different geographic levels: we find OTUs that are restricted to a particular region. Also, the East Antarctic region has specific endemic flora compared to the Antarctic Peninsula. On the other hand, 8 of the 21 cosmopolitan OTUs seem to be restricted to "cold" environments such as high altitudes, glaciers, polar and alpine environments.

ANTAR-IMPACT/BELDIVA, cyanobacterial diversity and plans for the 2010 field campaign (Miss Mano and Dr Namsaraev, ULg)

Miss Mano presented the results obtained with the samples from the 2007 (15 samples) and 2009 (65 samples) BELARE expeditions. The samples came from 5 different locations in an area of 50 km around the Princess Elisabeth station. The area of the Sør Rondane Mountains harbors a relatively high cyanobacterial diversity in spite of permanently negative air temperatures. The highest biodiversity was found in areas protected from katabatic winds and in granitic areas. Sixteen morphotypes were found by microscopical analysis with the highest diversity observed in Pingvinane (11 morphotypes). An efficient method of DNA extraction from polysaccharide rich samples was developed for the molecular analysis of these terrestrial samples. Currently 15 OTUs were found : 3 "new" (previously unknown), 5 "Antarctic" (potentially endemic) and 7 cosmopolitan.

Dr. Namsaraev presented the plans for the 2010 expedition. To simulate global changes Open-Top Chambers will be installed to increase the local temperature. To estimate a potential of cyanobacteria for long and short-range transport by air, an experiment of air filtration will be performed. A measurement of chlorophyll fluorescence during a daily cycle will be made to estimate changes in photosynthetic efficiency. Finally different samplings of biofilms, rock surfaces, cryoconites will be done and also the sampling sites will be characterized with microsensors (T°, humidity, light...). These different experiments will give us environmental information to understand the cyanobacterial diversity in the Sør Rondane Mountains.

The audience made some remarks, as being cautious with the wordings 'native' and 'cosmopolitan', asked how long the OTCs will stay (several years), and whether the snow accumulation will be measured (only temperature and humidity)

Antarctica, characterized by a specific green algal flora? (Dr. De Wever, PAE)

Dr. De Wever presented the results on the work on the Green Algal diversity (part of AMBIO). First, 150 strains were isolated from fresh samples and their 18S rRNA gene was partially sequenced. They were also analyzed by microscopy and ARDRA in order to choose 30 strains for which the 18S rRNA was fully sequenced.

Bacterial community composition in the surroundings of the Belgian Base (Miss Obbels, PAE)

The bacterial and green algal diversity in samples obtained near the Utsteinen nunatak (field campaign 2007) was studied using denaturing gradient gel electrophoresis (DGGE) analysis. The most dominant DGGE-bands were sequenced and identified using BLAST-analysis. Patterns in the microbial community composition were studied using ordination analysis. DGGE-analysis on 24 samples using bacteria specific primers did not reveal clear differences in bacterial community composition between different substrates. The uncultivated diversity of green algae was studied using two sets of primers. The DGGE-data obtained using the Euk528f-CHL002r primers, which were applied on eleven samples, suggest that rock samples harbor a different green algal community compared to aquatic and microbial mat

samples. The second analysis on green algae, a nested PCR using the Euk1A-Euk516r-GC after the Euk1A-CHL002r primers, applied on 23 samples also suggests a relationship between species composition and the substrate type.

The use of nested PCR and the specificity of PCR primers was discussed.

Culturable bacterial diversity in antarctic samples (Miss Peeters, LM)

In the Lab. of Microbiology, nine samples from different regions were investigated using a culture-dependent approach. Between 254 and 573 isolates were obtained for each sample and dereplicated with the rep-PCR fingerprinting technique. Despite the relatively large amount of isolates that was taken, the rarefaction curves comparing the number of delineated rep-clusters with the total number of isolates show that not all diversity was recovered.

The preliminary identification obtained with partial 16S rRNA gene sequencing shows a large diversity. *Actinobacteria* and *Alphaproteobacteria* are well represented in all samples and *Bacteroidetes* and *Betaproteobacteria* were found in lower amounts whereas *Deinococci*, *Gammaproteobacteria* and *Firmicutes* were only isolated from some samples. Some of the isolates, especially in the *Sphingobacteria* and *Deinococci* show rather low 16S rRNA gene sequence similarity with known species, which means they may represent new taxa.

Comparing the sequences obtained from the isolates with the sequences from the extracted DGGE-bands (PAE team) shows that both techniques are complementary and that some of the isolates obtained may be also present in other samples.

The genus *Flavobacterium* was chosen for a detailed study because of the expertise acquired for this group in previous work. Phenotypic and genotypic (16S rRNA gene and *gyrB* gene) analyses were performed and results indicate that 17 groups of isolates may represent new species. These will be further investigated in order to describe them and specific primers are designed to study their distribution in a larger amount of samples.

SCAR-MarBIN/ANTABIF projects (Dr. Danis, VUB)

Dr. Danis presented the new ANTABIF project (www.biodiversity.aq) which is a very interesting initiative following the SCAR-MarBIN. In deed, this will a public, free access database of all antarctic (terrestrial and limnetic) biodiversity data. This project is built in collaboration with the AAD and EBA. Some characteristics of this database is the link with GenBank and the possibility to add sequences with accession numbers. It is quite flexible as it can accept various kind of data (biology, taxonomy, physico-chemistry, location...). A meeting with Bruno will be held during the beginning of 2010 in order to start the process of submission of our data in ANTABIF.

Discussion about the ANTAR-IMPACT Workshop

A Workshop should be organized at the end of the project, as a wrap up in 2010: could we combine with another meeting to get more attention and more participants?

The ALIENS project will finish in July 2010 at the SCAR meeting in Buenos Aires. It will get a special session also in Oslo. TARANTELLA will also finish as ALIENS

So, we remain with EBA and MERGE: microbial diversity

We also discussed about the IPY Belgian meeting planned on 26 May, but it is close to Oslo and maybe not so good.

Maybe that some SCAR funding would be available if there was a link with EBA or general microbiology community through David Pearce (?)

Conclusion: get in touch with EBA to know if we could organize something together. Otherwise, we will organize a small meeting, as we still have some coordination money and could ask a small support to BELSPO.

Questions

Add: Changes in microbial present? Need coordinates ! Not present for Dry valleys, but available

for ridge (grid)

Enclosure: needs to be clearly marked !!!

Discussion about Non Native Species

How to make the distinction between natural and anthropic introduction/colonization?

We will study the uncultivated diversity in air samples. However, we can expect a very low amount of material - collaboration with Alexander Mangold of the Meteorological Institute (use their spare material). The chemical composition of the filtrate also interesting to determine the origin of the air masses (e.g. NaCl when the air comes from the sea)

The placing of OTC is discussed. They will be positioned on biofilms but also in one bare place (Teltet nunatak)

There is a discussion on the questions raised by Y. Frenot for the Forum of the Committee for Environmental Protection of the Antarctic Treaty. We read them and give recommendations for the answers.

Afternoon workshop: “Antarctic climate, biodiversity and invasions”

Biological invasions in the Antarctic (Dr. Convey, BAS)

Dr. Convey talked about the risks of invasions in Antarctica by non native species *via* human vectors. Antarctica’s isolation by marine and atmospheric barriers has protected the continent from human impacts until the last two centuries. Human activity initiated over last 100-250 year. Currently, 5000 research staff visit the continent yearly, and 30000 tourists visit successive sites. Human impacts are concentrated in particular areas, mostly ice-free regions. Introduction and establishment of alien species occurs inevitably with human presence. The greatest impacts to date occurred in the subantarctic, Scotia Arc and the Antarctic Peninsula. These non-native species can bring new trophic functions, synergies, and capacity for drastic alteration to ecosystems and threaten indigenous biodiversity. There is also a critical lack of baseline biodiversity data and monitoring effort, for both land and sea. Until the IPY Aliens program in 2007, there were just many anecdotes, but no concerted monitoring and few data. The current environmental change trends is likely to further lower barriers to establishment and invasion.

Aliens in Antarctica: quantifying propagules inadvertently carried into the Antarctic (Dr. Huiskes, NIOO)

Currently, a major issue for ecologist is the problem of the homogenization of biota. One of the main causes of this phenomenon is the invasion of non-native species (NNS) which is, in the case of Antarctica, often due to human activities. The problem can be summarized in three points (1) humans carry propagules, (2) alien species are only detected after establishment, and (3) climate change may increase the establishment of NNS. No real effort has been produced in order to assess and quantify the flux of propagules going in -or out- of Antarctica *via* human transport. This is why the Aliens IPY project was launched. The aim is to “scan” the visitors going into Antarctica by vacuuming their gear and clothing and asking them to fill a questionnaire to know about the places they visited before and whether they had the same clothes/gear. The results of the questionnaire and sampling collected from the visitors showed that 30% of them carried plant seeds. A total of 250 species were found on the visitors. Researchers and other national program personnel are the groups that carry the largest amount of seeds, more than tourists and ship's crews! With these results we will better understand the vulnerability of Antarctica and develop measures preventing the introduction of NNS.

Holocene climate and ecosystem changes in coastal east and maritime Antarctica - HOLANT (Dr. Verleyen, PAE)

The HOLANT project aims to (1) know the timing, duration and magnitude of Holocene climate anomalies in coastal areas in the maritime and east antarctic regions and how are these anomalies related to climatic events recorded in inland locations (ice cores) and (2) to elucidate how Holocene climate changes affect regional ice sheet/glacier dynamics and the diversity of primary producers in antarctic lakes.

There is an overall warming trend in the Antarctic continent, specially higher in the Antarctic Peninsula and West Antarctica, together with an increased wind speeds in East Antarctica. This temperature increase led to increased primary productivity in lakes. Different climate optima are identified in ice cores, marine sediment cores and lake sediment cores, but it is clear is that during the late Holocene a climate optimum was present which can be used as a past analogue. As the current climate anomaly is amplified in lakes, the HOLANT project is particularly interested in studying changes in lacustrine communities in response to past climate variability.

Five sampling sites were studied in both East and West Antarctica. Dr.Elise Verleyen showed how the study of these lakes allows us to observe the marine to lacustrine shift and the climate optima at the late Holocene.

Turnover patterns during recent decades exceed the natural variability of at least the past 4000 years in some regions (Margarie Bay and the Lasermann Hills). In West Antarctica, diatom communities in Pourquoi-Pas Island shows recent changes with the reappearance of species which were abundant immediately following lake isolation when marine derived nutrients were still high.

In East Antarctica, an increase in wind speeds was observed leading to an increase in salinity. Using transfer functions, Dr. Verleyen showed how this part of the continent was dried out, with an increase of halotolerant diatoms in those lakes. He also made an overview of the endemism rates in different Antarctic locations and the factors that structure these communities.

Finally the speech concluded that (1) recent climate and ecosystem changes beyond natural variability of the past 4-6 ka BP and (2) that the East Antarctic Ice Sheet likely responded to Holocene climate variability.

The Belgian Biodiversity Platform: a science – policy interface on biodiversity research (Dr. Segers, VUB)

Dr. Segers started by presenting the mission of Belgian Biodiversity Platform which is i) to inform and communicate about the belgian biodiversity research and ii) to promote and valorize it. The tasks of the platform are to facilitate free access and exchange of all primary biodiversity data, provide access to biodiversity research information, facilitate interdisciplinary scientific cooperation and to serve as an interface between the researchers and the scientific policy organizations. Then he talked about the Global Biodiversity Information Facility which has a belgian portal and an Antarctic node which is dedicated to the Antarctic biodiversity data. Finally he talked about the various ways of promoting biodiversity research in Belgium and Europe, and mostly how to inform people about it (www.biodiversity.be, workshops, flyers, thematic forums...).

Internal discussion

After the meeting was over, members of the three partner laboratories had an internal technical discussion. The first topic of the discussion was the advance in the analysis of the samples in each laboratory and the publication strategies that will be adopted for each project.

Concerning the AMBIO project, we also discussed about our respective pyrosequencing plans for the uncultivated diversity. For the cultivated diversity, the ITS gene will be used for green algae and both 16S rRNA and ITS will be analyzed for cyanobacteria.

Some technical aspects of the sampling campaign were also discussed. Zorigto explained briefly the experiments that he will do in Antarctica and the regions that he will visit for sampling.