Next-Generation Sequencing at the Poles

21-23 November 2012
Université de Liège, Academic Hall, *place du 20-Août*, Liège, Belgium

A SCAR-EBA Workshop, including APECS young scientist contributions
Workshop of the BelSPo project CCAMBIO

FOREWORD

Dear Participants of the CCAMBIO workshop 'Next-generation sequencing at the Poles',

In May 2012, the BELSPO project CCAMBIO officially began. It unites 5 Belgian laboratories and 2 foreign partners from the British Antarctic Survey (UK). Our focus is to study the microbial diversity and biogeography of Antarctic aquatic mats.

As we are going to use next-generation sequencing methods extensively, we decided that it would be best to consult with the wider scientific community, sharing best practices when using these new methodologies. Thus, we looked for speakers working in the Polar Regions, as we hoped to come to common guidelines for experiments and their analyses. This will facilitate data comparisons aiming at formulating more global assessments, allowing a wider relevance of our conclusions through cooperation. The Round table at the end of the day will be the occasion to synthesize our ideas and could be the basis for an 'opinion paper' to propose to the wider Polar community.

In addition, it seemed that we should have more than just a theoretical discussion and combine the workshop with a training session. Fortunately, three of our speakers volunteered to share their knowledge of two frequently used analysis programmes (MOTHUR and QIIME) and 21 participants have registered for this 2-day training course. One Teaching Assistant will also join to help.

To decrease our Carbon footprint and allow international colleagues to join the workshop on 21 November from a distance, we will have videoconference connections with colleagues working on polar microbial diversity at the Institute of Ecology of the University of Innsbruck, Austria, and at the Center for Nuclear Energy in Agriculture (CENA), University of São Paulo, Brazil. As shown by the paper of Hervé Philippe (2008), polar scientists can send a symbolic message to the authorities by decreasing their own environmental footprint. To repeat the words of a great thinker: 'Be the change that you want to see in the world' (Gandhi).

So, WELCOME to Liège and we hope that you will enjoy the workshop and the training course.

The organising committee

Annick Wilmotte & Haywood Dail Laughinghouse, University of Liège, BE
Anne Willems & Bjorn Tytgat, University of Ghent, BE
Elie Verleyen & Wim Vyverman, University of Ghent, BE
Bruno Danis, Institute of Natural Sciences, Brussels, BE
Alison Murray, Desert Research Institute, NV, USA

Philippe H (2008) Less is more: decreasing the number of scientific conferences to promote economic degrowth. Trends in Genetics 24: 265-267.

SPONSORS

Belgian Science Policy Office – Sponsor of the CCAMBIO project

The International Council for Science - Scientific Committee on Antarctic Research – SCAR, Evolution and Biodiversity in Antarctica – EBA program

Fund for Scientific Research Flanders – Contact Group Eco-evolution

Belgian Society for Microbiology

Universté de Liège

Beckman Coulter Genomics

Roche

Workshop Next-Generation Sequencing at the Poles

DAY 1 – 21 November 2012

8:45 Registration
9:15 Introduction by **Annick Wilmotte** (University Liège, Belgium)

Session I: *Chairperson: Dail Laughinghouse (University Liège, Belgium)*
9:25 **David Pearce** (British Antarctic Survey, Cambridge, UK): What metagenomics can tell us about microbial diversity?
10:05 **Antonio Quesada** (Autonomous University Madrid, ES): Biocomplexity of microbial mats. Can we cross the borders with high-throughput sequencing techniques?

10:45 Coffee/Tea

Session II: *Chairperson: Anne Willems (Ghent University, Belgium)*
11:05 **Alison Murray** (Desert Research Institute, Reno, NV, USA): Different approaches – same story? Studies of Antarctic Peninsula bacterioplankton through a rapid evolution in DNA sequencing technology
11:45 **Jean-François Ghiglione** (Laboratoire d'Océanographie Microbienne, Banyuls sur mer, FR): Pole to pole biogeography of surface and deep marine bacterial communities

12:25 Lunch & Poster Session

Session III: *Chairperson: Wim Vyverman (Ghent University, Belgium)*
13:55 **George Kowalchuk** (Netherlands Institute of Ecology, Wageningen, Netherlands) – Tracking short and long-term microbial responses to environmental change
14:35 **Thomas Pommier** (Université de Lyon 1, Lyon, France) – Investigating microbial diversity: Pitfalls and promises of NGS approaches
15:15 **Craig Herbold** (University of Waikato, Hamilton, New Zealand) – Avoid the fool's gold in the cold. Lessons learned from sequencing artificial microbial communities

15:55 Coffee/Tea

Session IV: *Chairperson: Elie Verleyen (Ghent University, Belgium)*
16:15 **Étienne Yergeau** (McGill University & National Research Council, Montréal, Canada) – Metagenomic analyses of Canadian high Arctic soils

16:55-17:45 Round Table Discussion and Closing remarks
Moderator: **Alison Murray** (Desert Research Institute, USA)

Workshop Next-Generation Sequencing at the Poles

Practical Training Session on the use of the bioinformatics pipelines Qiime and MOTHUR
Teachers: **Craig Herbold (CT), Alison Murray (AEM), Thomas Pommier (TP)**; Assistant: **Bjorn Tytgat**

November the 21st PM
1. Meet with students who bring their own data, to set up their data sets (limited to 6 samples) to run overnight through Amplicon Noise.

November the 22nd AM
Morning Schedule:
1. Questions in Microbial Ecology (~ 45 minutes) (TP)
• What can NGS help you learn?
• When do you NOT need NGS?
• How to organize your data to answer your key questions?

2. Participant and data set introductions (~ 30-45 minutes) (ALL)

3. Presentation of the Mothur Data Analysis Pipeline (1 hr) (TP, AEM, CH)
• Considerations and introduction to Mothur and the steps required for start to finish data analysis
• Data quality screening
• Resampling
• Alignment
• Clustering
Mothur vs. Qiime: Pros and cons. (CH)

Afternoon Schedule
30 minutes on bash and command line –> hand out + Mothur syntax (AEM)
clean your data (1h) split in several groups
align your data (30 minutes) split in several groups
clustering your data (2h.) split in several groups

November the 23rd AM
Morning Schedule:
1. OTU and beta diversity commands (TP)

Afternoon Schedule:
2. Questions on tutorial (AEM + CH)

ACKNOWLEDGEMENTS

This workshop would have not been possible without the participation of many persons who, each in their own way, have helped us. Many thanks to:

- Dominique Dehareng for the web page and the registration system

- Paola Catanzaro for all the (many) bookkeeping issues

- Fabienne Julémont for the badges and hotel registrations

- Caroline Bortuzzo for help with the food and drinks

- Tracy Beujean for taking care of the installation outside the Academic Room

- Richard Fischer for being our photographer

- Mr Thomas Duquesne for taking care of the videoconferences and recording

- Ms Mariella Guadagnano for helping to find available lecture rooms and video equipment

- Ms Hélène Leduc for helping with the reservation of the Academic Room, the parkings, etc

ABSTRACTS OF POSTER PRESENTATIONS

Depth-related changes in microbial diversity and active microbial communities of Fennoscandian deep terrestrial subsurface aquifers

M. Bomberg, M. Itävaara

VTT Technical Research Centre of Finland, P.O. Box 1000, FIN-002044 VTT, Finland

malin.bomberg@vtt.fi

Finnish deep groundwater is stratified due to the evolution history of the Baltic Sea and the most recent glaciation. The salinity increases with depth while the organic carbon decreases. Sulphate and methane are present in separate groundwater strata. In this study, deep bedrock aquifers in Western Finland were investigated. Groundwater samples from 11 fracture zones between 300 and 800 m depth were obtained from different boreholes. The microbial diversity of the groundwater was characterized by targeting the archaeal and bacterial 16S rRNA genes and the active microbial populations were resolved from the rRNA fractions by high throughput 454 pyro-sequencing. The microbial community structure changed with depth. Between 300 and 450 m depth the bacterial community was dominated by delta- and epsilon-proteobacteria, while alpha-, beta- and gamma-proteobacterial lineages dominated below 450 m. At 800 m the community was dominated by fermenting Firmicutes and Actinomycetes. Most archaea were methanogens, although Thermoplasmatales-archaea dominated at specific depths. Methanosarcinales archaea were the prevailing group between 300 and 450 m, while Thermoplasmatales and hydrogenotrophic methanogens and SAGMEG/SAGMA1 archaea were more common below 450 m depth. Specific environmental conditions, such as the salinity gradient, organic carbon and availability of sulphate or iron clearly affected the diversity and activity of the microbial communities at different depth. The sulphate-methane zone at approximately 400 m depth showed especially high diversity and zones enriched in *dsr*B or *mcr*A gene copy numbers could be identified. Despite the important role of the SRB and methanogens in subsurface environment these groups contribute with only a small fraction of the total microbial communities at the different studied depths. In contrast, novel putative sulfur oxidizing and nitrate reducing groups appeared to dominate and methylotrophic groups were common only at a specific depth. CO_2-fixing groups were more abundant between the depths of 300 to 450 m, after which they gave way to N_2-fixing bacteria. The forces driving the Fennoscandian deep subsurface microbial communities are yet not known. However, there is a clear stratification of both the microbial community compositions and the putative functions of the detected microbial groups in this deep groundwater system. It is possible that the different aquifers have completely different microbial communities, which have adapted over time, and that the change in the microbial communities is more due to isolation of aquifers in bedrock pockets than to depth.

Diversity of metal resistance genes in sedimentary bacterial populations

Stéphanie Roosa, Melissa Laghdass, Ruddy Wattiez, David C. Gillan

Laboratory of Proteomics and Microbiology, UMons, Belgium

stephanie.roosa@umons.ac.be

Introduction: Large amounts of trace metals have been introduced in the aquatic environment due to the increasing industrialization. To resist such harsh conditions, bacteria have developed several defense mechanisms coded by genes mainly located on plasmids. These genes encode for specific resistance systems to a variety of metal ions. In this work, we focused on 3 different genes: *czcA*, *arsB* and *copA* for respectively Co-Zn-Cd, As and Cu resistance. The aim of the present research was to design degenerate primer sets to target these 3 genes and to study their diversity in 4 sediment samples featuring contrasting metal levels. The selected stations included **station 130** in the North Sea & **MetalEurop** in the Deûle River (high metal levels), the Scarpe River in **Râches** and the Sensée River in **Férin** (low metal levels). In future research, these primer sets will be used to quantify the number of resistance genes by qPCR in different sedimentary environments.

Methods: Primers were designed on the basis of an alignment of different genes and proteins found in GenBank and their specificity was tested using PCR and the bacterium *Cupriavidus metallidurans* CH34. Genetic diversity of the metal resistance genes in the 4 environments was then assessed using a bar-coded pyrosequencing method (Beckman Coulter-Genomics). Raw sequences were sorted using QIIME to remove primers and TCMID and to delete short sequences (< 100 pb). Chimera were detected with MOTHUR (uchim command), leading to the creation of a file with unique sequences. These sequences were then assigned using BLASTX (NCBI) to determine the functions targeted with our primers and with BLASTN to evaluate genetic diversity. Sequences with identity scores greater than 97% identity were resolved at species level, greater than 95% at genus level, greater than 90% at family level, greater than 85% at order level, greater than 80% at class level and greater than 77% at phyla. All sequences below this percentage identity were not taken into account for subsequent analyses. Data analysis and visualizations were performed with MEGAN and PermutMatrix.

Results: The specificity of each primer set for targeted genes was greater than 99% for the assigned sequences. It can be concluded that primer sets are sufficiently specific for the 3 resistance genes to be quantified in bacterial populations by qPCR. Proteobacteria are mainly targeted using all primer sets. A greater diversity was found within *arsB* and *czcA*. For *copA*, only 8 different genera were represented.

Characterization of microbial biodiversity in the pelagic zone of alpine lakes using high throughput sequencing

Stephan Blank[1], Li Deng[1,2], Hannes Pröll[3], Norbert Niklas[3], Christian Gabriel[3], Rainer Kurmayer[1]

[1] *University of Innsbruck, Research Institute for Limnology Mondsee*
[2] *Helmholtz Zentrum München, Dep. of Groundwater ecology*
[3] *Austrian Red Cross, Blutzentrale Linz, Linz*

rainer.kurmayer@uibk.ac.at

Understanding the effects of climate change on biodiversity is one of the major aims in ecology. The influence of rising air temperature is expected to be particularly pronounced in alpine ecosystems as they are typically inhabited by cold-adapted stenothermic species which would become extinct. In a similar vein it has been suggested that alpine lakes with a low average water temperature during summer are most vulnerable to a warmer climate, as a sudden rise in temperature would significantly shift species occurrence towards more opportunistic species such as bloom-forming cyanobacteria.

In order to characterize bacteria community composition in relation to average water temperature we used ultra-deep sequencing of the 16S rRNA gene as amplified from five alpine lakes of the Niedere Tauern region (1700-2118 m a.s.l.) during the years 2009-2011. The lakes differed significantly in summer mean water temperature by approx. 4°C which is independent from the altitude but rather due to local influences. In parallel physical and chemical parameters were recorded and plankton community composition was determined by counting under the microscope. For each lake five samples during summer months July and August were obtained. The sequences were processed and analysed using the QIIME pipeline. A total of 272,571 sequences including hypervariable region V3 and 594,260 including V6 (average length 534 bp) were obtained resulting in 9,366 OTUs and 16,751 OTUs (97% similarity).

In general the lakes were of an oligotrophic state (1.8-7.7µ L^{-1}) and the absolute bacterial numbers were correlated with phytoplankton biomass as indicated by chlorophyll a (R^2 = 0.17 0.67; average R^2 = 0.39). Maximal Chlorophyll a values coincided with highest phototrophic cyano¬bacterial abundance.

Ultra-deep sequencing revealed that the most important phyla comprised Proteobacteria (51.6% (V3); 55.8% (V6)), Bacteroidetes (21.6%; 18.2%), Actinobacteria (8.8%; 9.3%) and (non-chloroplast) cyanobacteria (4.7%; 4.4%). The highest cyanobacterial abundance in more productive lakes was confirmed and OTUs assigned to *Cyanobium* (0 75.9% abundance) and *Synechococcus* (23.9 63.2% abundance) were identified most frequently in the year 2011. Other cyanobacteria such as *Limnothrix* and *Anabaena* occurred specifically in some lakes.

Surprisingly, the lowest species diversity was observed in the lake with the lowest average summer mean temperature (~8°C) implying that specific cold-adapted species might competitively exclude bacterial species occurring in warmer lakes. Indeed the respective communities differed significantly and consistently in bacterial community composition suggesting that the annual variation in growth period (e.g. ice cover duration) within lakes did not lead to variation in bacterial community composition.

Protist diversity in the Southern Ocean revealed by molecular tools

C. Wolf and K. Metfies

Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

Christian.Wolf@awi.de

The objectives of this study were the establishment of molecular approaches in the diversity investigation of eukaryotic protists in the Southern Ocean and the delivery of a comprehensive and taxon detailed overview of protist assemblages in the Pacific sector of the Southern Ocean, especially in the Amundsen Sea. The molecular approaches used to achieve these goals were automated ribosomal intergenic spacer analysis (ARISA) and 454-pyrosequencing.

First, the hypothesis that distinct protist community assemblages characterize large-scale water masses was tested. The composition and biogeography of late summer eukaryotic protist assemblages along a transect from the coast of New Zealand to the eastern Ross Sea was determined. ARISA and 454-pyrosequencing were used in combination with flow cytometry and pigment measurements via high performance liquid chromatography (HPLC) to study the protist assemblage. We found distinct biogeographic patterns defined by the different oceanic regions. Different water masses harboured different microbial communities, and environmental gradients limited their dispersal. Picoeukaryotes were of minor importance throughout the investigated transect and were nearly absent south of the Polar Front. Dinoflagellates, Syndiniales, and small stramenopiles dominated the Subantarctic Zone, whereas the importance of diatoms increased southwards, in the Polar Frontal Zone, the Antarctic Zone and the Subpolar Region. South of the Polar Front, haptophytes were the dominating group.

Second, the investigation focused on the Amundsen Sea to see if the protist community assemblages vary in different areas of a single large-scale water mass. The composition and structure of late summer eukaryotic protist assemblages along a west east transect in the Amundsen Sea were analysed. ARISA and 454-pyrosequencing were combined with HPLC. Characteristic communities offshore and inshore were revealed, but the differences were weaker, compared to those found along the north south transect. In general, total chlorophyll a and microeukaryotic contribution were higher in inshore samples. Picoeukaryotes were also of minor importance. Diatoms were the dominating group across the entire area, at which *Eucampia* sp. and *Pseudonitzschia* sp. were dominating inshore and *Chaetoceros* sp. was dominating offshore. At the eastern most station, the assemblage was dominated by *Phaeocystis* sp. Under the ice, ciliates showed their highest and haptophytes their lowest abundance.

This study sheds light on the use and applicability of several molecular methods for the investigation of protist assemblages in polar waters. It delivers a comprehensive and taxon detailed overview of the eukaryotic protist composition during the austral summer in the Pacific sector of the Southern Ocean, especially in the Amundsen Sea. This study constitutes as groundwork for future investigations of protist assemblage changes in this area.

Boom Clay pore water, home of a diverse microbial community

K. Wouters, H. Moors, P. Boven, N. Leys

SCK•CEN, Insitute for Environment, Health and Safety, Expert Group for Molecular and Cellular Biology, Unit for Microbiology

katinka.wouters@sckcen.be

The Boom Clay layer located at 230 m depth under the Mol site of SCK•CEN (Belgium) is presently investigated as potential host formation for the disposal of high-level nuclear waste. Using the HADES underground laboratory of SCK•CEN in this clay layer, Boom Clay and Boom Clay Pore Water (BCPW), has been studied in this regard for over two decades. In this study, the possibility of biological factors, microbes, interacting with future radioactive waste in Boom Clay is addressed. Similar to the previous characterization of the 'average' BCPW chemical composition, the primary aim of the presented microbiological study was to determine a representative BCPW bacterial community which can a.o. be used in laboratory studies. Secondly, the in situ activity and the metabolic properties of members of this community were addressed, aiming to assess their survival and proliferation chances in repository conditions.

In a first approach to address microbial presence, scanning electron microscopy (SEM) was performed and total microbial DNA of the community was extracted from ten BCPW samples from different clay layers. By polymerase chain reaction (PCR) on the highly conserved bacterial 16S rRNA genes in this DNA pool and subsequent sequencing and bio-informatic analysis, operational taxonomic units (OTUs) could be assigned to the bacterial community. In a second approach, microbial activity and metabolic capacity in BCPW samples was assessed by analysis of intracellular adenosine triphosphate (ATP) and cultivation in relevant, anaerobic media by most probable number technique (MPN). In a third approach, individual microbial strains were isolated, propagated and identified, in order to evaluate specific properties of cultivated subpopulations.

Based on SEM and DNA analysis, it became clear a large diversity of microbes were present and abundant in the clay pore water. A core bacterial community (CBC) of Boom Clay pore water samples was characterized, representing six bacterial phyla present in all BCPW samples. A combination of BCPW from three piezometer filters was selected as a representative microbial community inoculum for future lab scale experiments. This microbial community was proven to be not merely present, but also alive and active. Even without the addition of extra substrates for growth, microbial activity was indicated in sampled BCPW. Moreover, the bacteria were clearly capable of proliferation on a range of growth substrates.

The omnipresence of such a diverse and in situ active microbial community in Boom Clay pore water samples is surprising. Microbial contamination during piezometer installation and survival of introduced species during several years in stringent conditions are therefore considered quite credible. On the other hand, the indicated diversity of strict anaerobic micro-organisms with specific properties like sulphate reduction and sporulation invites speculations that indigenous micro-organisms, living in the clay since many centuries, will account for at least part of the observed viable community. The interaction of this clay microbial community with the waste disposal facility and sensor systems installed, and/or the radioactive waste itself, now needs to be further investigated.

The CCAMBIO project: responses of the aquatic microbial mats to Climate Change

The CCAMBIO members (in alphabetical order): Pete Convey[1], Bruno Danis[2], Haywood Dail Laughinghouse[3], Dagmar Obbels[4], David Pearce[1], Igor Stelmach Pessi[3], Bjorn Tytgat[5], Bart Van de Vijver[6], Elie Verleyen[4], Wim Vyverman[4], Anne Willems[5], Annick Wilmotte[3]

[1]*British Antarctic Survey, High Cross, Madingley Road, Cambridge, UK*
[2]*Royal Institute of Natural Sciences, Rue Vautier, Brussels*
[3]*Cyanobacteria group, Centre for Protein Engineering, Liege University, 4000 Liège, Belgium*
[4]*Protistology and Aquatic Ecology, Biology Department, Ghent University, Krijgslaan 281-S8, 9000 Ghent, Belgium*
[5]*Laboratory of Microbiology, Dept. of Biochemistry & Microbiology, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium*
[6]*National Botanical Garden of Belgium, Meise, Belgium*

awilmotte@ulg.ac.be

CCAMBIO ('Climate Change and Antarctic Microbial Biodiversity') is 4-year project funded by the Belgian Science Policy Office within the framework of the Science for a Sustainable Development call. CCAMBIO aims to study the diversity, biogeographic zoning, evolutionary history, and genomic make-up of lacustrine microbial mat communities in the Antarctic Realm in order to assess their resilience and responses to global change. It will be a collaborative effort to study microbial communities from Antarctic lakes along a wide geographic and climatic gradient using next-generation sequencing (NGS) methodologies.

The specific objectives are as follows:
1. To extend and improve existing sample collections of lacustrine microbial communities by conducting field campaigns to the understudied sub-Antarctic Islands Iles Crozet and South Georgia, the Antarctic Peninsula and the Maritime Antarctic Islands, thanks to collaboration with other National Antarctic Programmes
2. To quantify the degree and nature of microbial bioregionalisation in the Antarctic Realm using in-depth inventories of microbial biodiversity (cyanobacteria, selected groups of bacteria, and protists).
3. To test evolutionary hypotheses on the origin, diversification rate and range dynamics of selected taxa.
4. To study the overall genomic make-up and biochemical properties of a microbial mat community along environmental gradients to assess the contribution of the different taxonomic/functional groups to the functioning of the consortium in response to changes.
5. To explore the potential of microorganisms and functional genes/groups as early warning indicators for global change through modelling the distribution of focal taxa and functional groups in response to climate and environmental change.

Conservation of the Polar cyanobacterial diversity: the BCCM/ULC culture collection

A. Wilmotte[1], M. Renard[1], Z. Namsaraev[1], H.D. Laughinghouse[1], W.F. Vincent[2], R.W. Castenholz[3], F. Marinelli[4], J. Komarek[5], J. Elster[5]

1 - University of Liege, Centre for Protein Engineering, Liege, Belgium.
2 - Université Laval, Department of Biology, Quebec, Canada;
3- University of Oregon, Department of Biology, Eugene, USA ;
4 - University of Insuburia, DBSM, Varese, Italy
5 – University of South Bohemia, Ceske Budejovice, Czech Republic

awilmotte@ulg.ac.be

Since 2006, the BCCM (Belgian Co-ordinated Collections of Microorganisms) has supported the elaboration of a research collection of polar cyanobacteria. Since 2010, the purpose of the project has become the integration of a new public collection into the BCCM consortium, and the construction of a Quality Management System.

The research collection includes 125 unicyanobacterial strains, most of them coming from different Antarctic (South Victoria Land, East Antarctica, Transantarctic Mountains...) regions. They were isolated from different biotopes (microbial mats, ice shelves, hypolithic and endolithic habitats, cryoconites ...). The three main orders of cyanobacteria (Chroococcales, Oscillatoriales, Nostocales) are represented. The taxonomic characterisations include the sequencing of the 16S rRNA gene and the spacer between the 16S and 23S rRNA genes (ITS).

The public collection presently includes 45 strains, as listed on the BCCM/ULC catalogue (http://bccm.belspo.be/db/ulc_search_form.php) and it will progressively grow. An ISO9001 certificate was obtained for the deposition and distribution of strains.

Strain P*lectolyngbya hodgsonii* ANT.PROGRESS2.2 (ULC009) is the type strain of the newly characterized genus *Plectolyngbya* (Taton et al. Polar Biology, 2011). This illustrates the interest of the isolation of a diversity of strains to improve the taxonomy of the cyanobacteria.

Several Antarctic strains were already screened for bioactivity against pathogenic bacteria and fungi (Taton et al. J. Phycol, 2006; Biondi et al. J. Appl. Microbiol, 2008). About 120 chemically diverse extracts were generated from 48 strains and screened by antimicrobial assays and cytotoxicity tests. Besides the high percentage of cytotoxic activities, they showed a widely distributed activity versus Gram positives, and few but very interesting activities against fungal pathogens. 17 strains were bioactive against the Gram-positive *Staphylococcus aureus*, or the fungi *Aspergillus fumigatus* and *Cryptococcus neoformans* and 25 were cytotoxic against HeLa cells. The bioactivities were not in accordance with the phylogenetic relationships, but rather strain-specific. These data confirm the renewed interested for biotechnological exploitation of cyanobacteria.

Role for urea in the metabolism of polar *Archaea*

L. Alonso-Sáez[a,b], A.S. Waller[c,] D.R. Mende[c], K. Bakker[d], H. Farnelid[e], P. Yager[f], C. Lovejoy[g], J-É. Tremblay[g], M. Potvin[g], F. Heinrich[a], M. Estrada[h,] L. Riemann[i], P. Bork[c], C. Pedrós-Alió[h], S. Bertilsson[a]

[a]Department of Ecology and Genetics, Limnology, Uppsala University, Norbyvägen 18D, 75236 Uppsala, Sweden; [b]Centro Oceanográfico de Gijón, IEO, Avda. Principe de Asturias 70bis, 33212 Gijón, Spain; [c]European Molecular Biology Laboratory, Meyerhofstrasse 1, 699117 Heidelberg, Germany; [d]Ecology and Evolutionary Biology Department, University of Michigan, 48109 Ann Arbor, Michigan; [e]Department of Natural Sciences, Linnaeus University, 39182 Kalmar, Sweden; [f]School of Marine Programs, University of Georgia, 30602-3636 Athens, Georgia; [g]Departement de Biologie, Université Laval, G1V 0A6 Quebec, Canada [h]Institut de Ciències del Mar, CSIC, P. Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain; [i]Marine Biological Section, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark.

laura.alonso@gi.ieo.es

Archaea are key microorganisms in polar oceans but their metabolic features are largely unknown in these environments. We collected data on abundance and metabolic activity of Marine Group I *Thaumarchaea* in Arctic and Antarctic waters in different seasons, including the wintertime. As previously observed, *Archaea* grew throughout the winter, increasing their abundances one order of magnitude from January to March 2008. Yet, in situ single-cell measurements revealed an unexpected low metabolic activity, i.e., less than 5% of thaumarchaeal cells took up leucine or bicarbonate, inconsistent with both recognized heterotrophic and autotrophic archaeal lifestyles. To resolve how *Archaea* obtain energy and carbon for growth, we analyzed a metagenome collected during the Arctic winter, when the *Thaumarchaeota* population was at its maximum of abundance (18% of cell counts). The metagenomics analysis showed that archaeal *amoA* genes were abundant, indicating that polar *Archaea* have the potential for ammonia oxidation. Furthermore, the presence of archaeal genes involved in urea transport and degradation suggested that polar *Archaea* may use urea as an alternative source of energy for growth. Quantitative PCR analysis confirmed that most polar *Thaumarchaeota* had the potential to oxidize ammonia and a large fraction of them had ureases, enabling the alternative use of urea when ammonia is scarce. Therefore, the degradation of urea may be an alternative for *Thaumarchaeota* and other microorganisms exposed to the low-energy conditions of dark polar waters.

Community diversity in protists along gradients in the region of the Vestfold Hills, Antarctica

Sylvie Tesson*, Ramiro Logares**, Karin Rengefors*

** Aquatic Ecology, Lund University, Sölvegatan 37, SE-22362 Lund, Sweden*
*** Institut de Ciències del Mar, CSIC, Passeig Marítim de la Barceloneta, 37-49, 08003 Barcelona, Spain*

Sylvie.Tesson@biol.lu.se

Community diversity of protists in the region of the Vestfold Hills has been investigated previously using Sanger sequencing and cloning methods. These studies screened a minute fraction of the biodiversity as culture methods were used before sequencing. Previous analyses give the big picture of protists diversity but in general underestimate it. We are investigating the community diversity in protists in 16 lakes and one marine site along different environmental and geographical gradients (e.g salinity, connectivity, degree of isolation…) using a high-throughput sequencing method (i.e. 454) and the v4 region of the rSSU. We want to understand which factors shape the community diversity and elucidate the role of the geographical factor on the diversity pattern observed. Light microscopy observations have been performed to verify the taxonomic estimations and proportions. I will discuss our preliminary results from the region and the accuracy of the methods.

LIST OF PARTICIPANTS

Lastname	Firstname	Country	E-mail
Ahmed	Mohamed	Belgium	mahmed@sckcen.be
Alonso Saez	Laura	Spain	laura.alonso@gi.ieo.es
Beujean	Tracy	Belgium	
Biersma	Elisabeth	United Kingdom	elisebiersma@gmail.com
Blank	Stephan	Austria	stephan.blank@uibk.ac.at
Bomberg	Malin	Finland	malin.bomberg@vtt.fi
Calusinska	Magdalena	Luxembourg	calusins@lippmann.lu
Carnol	Monique	Belgium	m.carnol@ulg.ac.be
Convey	Peter	United Kingdom	pcon@bas.ac.uk
Cornet	Luc	Belgium	luc.cornet@ulg.ac.be
Delhalle	Laurent	Belgium	laurent.delhalle@quality-partner.be
Dvorak	Petr	Czech Republic	p.dvorak@upol.cz
Fischer	Richard	Belgium	richard-fischer@live.com
Ghiglione	Jean-François	France	ghiglione@obs-banyuls.fr
Goux	Xavier	Luxembourg	goux@lippmann.lu
Grigorescu	Alina	Belgium	agrigorescu@ulg.ac.be
Hardge	Kristin	Germany	kristin.hardge@awi.de
Herbold	Craig	New Zealand	cherbold@waikato.ac.nz
Huet	Julia	Netherlands	j.huet@nioo.knaw.nl
Kowalchuk	George	Netherlands	G.Kowalchuk@nioo.knaw.nl
Kunath	Benoit	Belgium	benoit.kunath@student.umons.ac.be
Laghdass	Melissa	Belgium	melissa.laghdass@umons.ac.be

Lastname	Firstname	Country	E-mail
Lara	Yannick	Belgium	larayan2000@yahoo.fr
Larose	Catherine	France	catherine.larose@ec-lyon.fr
Laughinghouse	Dail	Belgium	hdliv@hotmail.com
Maalouf	Pedro	Belgium	pedromaalouf@aol.com
Malchair	Sandrine	Belgium	S.Malchair@ulg.ac.be
Mano	Marie-José	Belgium	marie-jose.mano@ulg.ac.be
Monsieurs	Pieter	Belgium	pmonsieu@sckcen.be
Moors	Hugo	Belgium	hmoors@sckcen.be
Murray	Alison	USA	Alison.Murray@dri.edu
Newstead	Rebekah	United Kingdom	osua23@bangor.ac.uk
Obbels	Dagmar	Belgium	dagmar.obbels@ugent.be
Pearce	David	United Kingdom	dpearce@bas.ac.uk
Pessi	Igor	Belgium	ispessi@ulg.ac.be
Polain	Stephanie	Belgium	S.Polain@ulg.ac.be
Pommier	Thomas	France	thomas.pommier@univ-lyon1.fr
Quesada	Antonio	Spain	antonio.quesada@uam.es
Redondo Nieto	Miguel	Spain	miguel.redondo@uam.es
Renard	Marine	Belgium	mrenard@ulg.ac.be
Roosa	Stephanie	Belgium	stephanie.roosa@umons.ac.be
Sheridan	Christopher	Belgium	christopher.sheridan@umons.ac.be
Simonin	Marie	France	simonin.marie@gmail.com
Sohlberg	Elina	Finland	elina.sohlberg@vtt.fi
Souffreau	Caroline	Belgium	caroline.souffreau@bio.kuleuven.be
Strunecky	Otakar	Czech Republic	otakar.struncky@gmail.com

Lastname	Firstname	Country	E-mail
Taminiau	Bernard	Belgium	bernard.taminiau@ulg.ac.be
Tesson	Sylvie	Sweden	Sylvie.Tesson@biol.lu.se
Tytgat	Bjorn	Belgium	bjorn.tytgat@ugent.be
Van de Putte	Anton	Belgium	antonarctica@gmail.com
Verleyen	Elie	Belgium	elie.verleyen@ugent.be
Vyverman	Wim	Belgium	wim.vyverman@ugent.be
Wilkinson	Paul	United Kingdom	p.t.wilkinson@open.ac.uk
Willems	Anne	Belgium	anne.willems@ugent.be
Wilmotte	Annick	Belgium	awilmotte@ulg.ac.be
Wolf	Christian	Germany	Christian.Wolf@awi.de
Wouters	Katinka	Belgium	katinka.wouters@sckcen.be
Yergeau	Etienne	Canada	Etienne.Yergeau@cnrc-nrc.gc.ca
Zifcakova	Lucia	Czech Republic	zifcakoval@gmail.com