



---

# Census of Antarctic Marine Life (CAML)

## Microbes Workshop - Abstracts

*31 May 2006, Innsbruck Austria.*

---

### 1. Marine population genomics analysis by MLST

Francisco Rodriguez-Valera

The cataloguing of prokaryotic diversity presents an inherent problem of defining units of representation of such diversity. The species concept, the ecotype and the biogeography associated to such entities are still largely unknown for bacteria with the possible exception of some pathogenic species. Here the development of Multi-locus sequence typing (MLST) has been instrumental in advancing in such understanding. However for pathogenic and saprophytic bacteria the problem will always be compounded with the host population biology. In marine bacteria there is now a opportunity to advance enormously along the same lines. The marine habitat represents a nearly ideal model since it is relatively easy to sample reproducibly and representatively and is less complex at the microniche level. I propose here one specific study that could help the fundamental issues of microbiology mentioned above. Briefly it would involve selecting some representative taxa of marine bacteria and apply a Multilocus Sequence Typing (MLST) scheme to all of them. Actually it could be considered complementary to the Moore Foundation to sequence at least 80 strains (pure cultures) of marine bacteria since having the genome of one strain simplifies enormously the task of developing good MLST scheme. The degree of genetic diversity, sexuality, and geographic ecological distribution are all essential attributes that cannot be disclosed by sequencing one single strain. The project would require the participation of a number of scientists knowing the ecological and biological attributes of the selected strain, ideally they would have already isolates from different ecological niches colonized by the species and geographic locations e.g. Atlantic, Pacific, Mediterranean, Antarctic. Alternatively campaigns to collect samples and isolates could be carried

out to complete collections as much as possible, but this can be done simultaneously to the rest of the project (i.e. no need to wait to have all the isolates to start). Not less than 30 strains per taxon would be required, ideally about 100. From the genomes the genes to be sequenced can be easily selected on the grounds of conservation (essential rather than adaptive), function (different, independent) and location in the genome. In principle the process is identical to the one followed for bacterial pathogens.

## **2. (Meta) genome mining for novel protein diversity**

Golyshin P.N., Timmis K.N. (GBF Braunschweig, Germany),  
Ferrer M. (ICP-CSIC, Madrid, Spain) and Yakimov M.M. (IAMC-CNR, Messina, Italy)

The mining and characterisation of new enzymes from extremophiles has a great potential for "white biotechnology/green chemistry", biocatalytic processes for the production of high value chemicals serving as building blocks for the synthesis of biologically-active compounds, like pharmaceuticals, agrochemicals and pesticides. On the other hand, a mechanistic understanding how these proteins function under the hostile conditions of environment will shed new light on adaptation mechanisms of microbial cells and extend our knowledge on the limits to life on Earth.

We perform function-based mining of genomes of individual extremophilic microorganisms and of "metagenomes" from entire communities from extreme habitats (a few examples will be given). Of special interest to us are hydrocarbonoclastic microorganisms and marine oil-degrading microbial communities in polar regions. To circumvent problems of expression/thermo-inactivation of cold-adapted enzymes, we created a psychrotolerant *E. coli* strain which grows at temperatures as low as 0°C and which we use as a host for the "cold" screening of expression libraries for new hydrolytic enzymes.

## **3. Microbial Diversity of Maritime ecosystems in Antarctic Peninsula.**

Nakayama, C.R., Vazoller R.F., Pellizari, V.H.

The objective of the Environmental Microbiology Laboratory (LMA/USP) in Antarctica is the study of biodiversity and biogeography of aquatic ecosystems of the Antarctic Peninsula. The studies recently proposed by the group consider a broad range of groups of microorganisms, such as bacteria, archaea, virus, yeast and filamentous fungi. Microbial community structure analysis based on molecular tools as denaturing gradient gel electrophoresis (DGGE), FISH and clone library of 16S rDNA, *rpoB* and functional genes have been done to give us an understanding of functional and biogeographic relationships. These data are vital for an improved understanding of ecosystems processes and the role that microorganisms play in the Antarctic environment. Besides the culture-independent studies, efforts have been made to cultivate not yet cultured microorganisms and physiological, taxonomic studies and exploitation of

bacteria and fungi will be developed. The occurrence of virus in birds and mammals of Peninsula will be covered during the polar year as part of the epidemiological studies of viral diseases spread over a wide geographical range. Also, studies on diversity and ecology of microorganisms involved in the methane cycle (methanogenic archaea and methanotrophic bacteria), associated to *in situ* determination of methane, are on course in order to characterize these communities and obtain data that may help predicting possible impacts of the global warming on the production of this greenhouse gas in the Peninsula. Finally, as microbial diversity data obtained for Antarctic Peninsula are not yet organized and accessible, the creation of a database to display the activities, isolated strains and location of culture collections, as well as publications and gene sequences generated by Brazil, Uruguay and Argentina will be developed to compare the biodiversity and biogeography of Antarctic Peninsula microorganisms.

#### **4. Exploring protistan diversity in Antarctic coastal waters**

Astrid Schnetzer and David Caron

Assessing the ecological role of protists requires a basic knowledge of species abundances and composition *in situ*. Little is known about the diversity of small (<20 µm) Antarctic protists (especially protozoa), despite their pivotal role in biogeochemical processes within microbial communities. Novel molecular, culture-independent methods indicate protistan diversity has been substantially underestimated. Many new species (and their ecologies) are yet to be described. The identification and enumeration of these novel protistan species is now being accomplished through a combination of traditional approaches (microscopy) and cutting edge molecular techniques (targeting single cells and whole communities) which allow to link morphological and genetic features. In addition, the cultivation of these species is providing invaluable insight into the range of abiotic conditions that these organisms can tolerate. Finally, how protistan diversity impacts energy and nutrient cycling within Antarctic microbial communities can be assessed.

#### **5. Fungal biodiversity in Antarctic marine environment**

Silva Sonjak & Nina Gunde-Cimerman

The Arctic and Antarctic regions have been mainly investigated regarding the presence of psychrophilic bacteria, archaea and algae. Recent scientific reports have shown as well sporadic presence of different fungi in permafrost layers, soil and ice. Low numbers of colony forming units (CFU) of fungi were detected on non-selective, mesophilic general media. Active microbial growth in extremely cold environments is under the influence of ice formation and consequently of little biologically available water. Thus, water

activity in habitats such as snow, sea ice and glacier ice, is the dominant factor in external chemistry that influences microbial activity. Therefore, isolation conditions were designed to accommodate xerotolerant/halotolerant fungi from ice by using media with high concentrations of salt or sugar and thus low water activity. These media should give selective advantage to microorganisms adapted to ice. In this way a surprisingly high fungal CFU per litre of water was detected in Arctic coastal environment, ranging from 1000-3000/l in seawater, 6000-7000/l in melted sea ice and up to 13.000/l in melted glacier ice. The dominant taxa were ascomycetous and basidiomycetous yeasts, melanized fungi, mainly represented by the genera *Cladosporium* and *Aureobasidium* and different filamentous fungi with the dominant genus *Penicillium*. The taxonomic studies of the isolates revealed the existence of several new species.

## **6. Environmental genomics of Antarctic marine bacterioplankton using a large contig sequencing approach.**

Alison Murray, Joseph Grzymski, Edward DeLong, Hugh Ducklow.

Cold ocean regions are the norm on this planet with 79% of the world's ocean volume below 1000m and 5°C, with some expansive regions such as the Southern and Arctic oceans remaining constantly below 0°C. In addition to the water column, sea ice, forms over the Southern ocean annually doubling the size of the continent (>20million km sq), and covers a large portion of the Arctic ocean. The biomass in these waters and sea ice environments is dominated by a diverse community of bacteria and archaea. These organisms have combated the challenges to survival in these systems largely by modifications coded for in their genomes. The changes required are slight – in terms of gene content, though are significant in terms of gene modification. The goal of this project is to generate genome sequence data for Antarctic marine bacterioplankton assemblages from late winter and summer waters that will benefit three critical areas of study in Antarctic marine microbial ecology: (i) phylogenetic and genomic diversity of Antarctic bacterioplankton and comparisons with temperate analogs (ii) evolutionary history of Antarctic marine bacterioplankton populations (iii) structural adaptation of microbial genomes to permanently cold environments. This project will contribute substantially to the field of marine microbial diversity and evolution in polar environments through end-sequence generation from two environmental fosmid libraries (17,200 clones) derived from bacterioplankton assemblages collected in late austral winter and summer and whole fosmid sequencing of 150 fosmids from each library. The sequence information will make a significant contribution to a new international Antarctic Census of Marine Life program that aims to characterize diversity in the Antarctic marine environment at all biological levels over the next five years. The genes identified (est. 34,000 partial and 7000 complete ORFs) will also be used to design gene expression detection systems (microarrays and RT-PCR) and environmental proteomics to better understand Antarctic bacterioplankton community structure, cellular function and survival in subzero waters.