



Census of Antarctic Marine Life (CAML)

Microbes Workshop – Minutes

31 May 2006, Innsbruck Austria.

In attendance: Daniel Delillie (France), Christine Foreman (USA), Peter Golyshin (Germany), Luigi Michaud (Italy), Angelina Lo Guidice (Italy), Roberta Marinelli (USA), Alison Murray (USA), Cristina Nakayama (Brazil), Francisco Rodriguez-Valera (Spain), Astrid Schnetzer (USA), Silva Sonjak (Slovenia).

Schedule of Presentations and brief notes

9:30 – 9:50am Marine population genomics analysis by MLST.
Francisco Rodriguez-Valera

Dr. Rodriguez's talk discussed using MLST to understand the evolution of marine bacterial populations that have widespread distribution. He described the required traits of an organism to select for such a study. Discussion following his talk suggested that a MLST analysis would be a great IPY-CAML oriented program.

9:50 – 10:10 am (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)

Dr. Golyshin's interested in mining metagenomes. He described the technologies used for this, including using Lambda phage vectors as expression systems. They can clone unknown DNA and survey the clones for activities of interest. They've worked with one Antarctic microorganism and are interested in collaborating with Antarctic researchers to study both genomes of cultivable organisms as well as metagenomic DNA from Antarctica.

10:10 – 10:30 am Microbial Diversity of Maritime ecosystems in Antarctic Peninsula.
Nakayama, C.R., Vazoller R.F., Pellizari, V.H.

Dr. Nakayama described the Brazilian research program on King George Island where they have been focused primarily on marine sediments, environmental monitoring, and bioremediation. They have used E. coli assays as indicators of fecal pollution and studied microbiota enriched for under various hydrocarbons. The Brazilians have a new IPY program that was recently funded studying Antarctic Microbiology, including community structure, isolation of cultivated organisms, and screening of functional genes.

11:00 – 11:20am Exploring protistan diversity in Antarctic coastal waters.
Astrid Schnetzer and David Caron

Dr. Schnetzer discussed the molecular approach that her group uses for characterizing protistan organisms in natural systems. The diversity of protists in Antarctic waters is understudied. She discussed the utility of using the 18S rRNA gene for organisms less than 5 μm . Outstanding questions are similar to those for bacteria and archaea – are there endemic species? Schnetzer and Caron have a recently funded program to study diversity of protists in the Ross Sea.

11:20 – 11:40am Fungal biodiversity in Antarctic marine environment.

Silva Sonjak & Nina Gunde-Cimerman

Ms. Sonjak discussed the work that she and Dr. Gunde-Cimerman have been working on characterizing fungi from ice collected at Svalbard. They have found many cosmopolitan fungi, as well as some novel strains. They use a culture-based approach, and found that the diversity of fungi reflects Aeolian depositions. Most of their results thus far have been from ice/subglacial environments. Marine fungi are little studied in the Antarctic, they would be very happy to collaborate with CAML microbial scientists during the IPY. They are interested in studying the cryo-adaptations that marine fungi have (freezing resistance, UV resistance, induction of sporulation).

11:40 – 12:00pm Environmental genomics of Antarctic marine bacterioplankton using a large contig sequencing approach.

Alison Murray, Joseph Grzymiski, Edward DeLong, Hugh Ducklow

Dr. Murray shared the work that she and colleagues are doing with environmental genomic DNA from the Antarctic Peninsula waters. They have a program funded to study the genome content and diversity of bacterioplankton in winter and summer environmental DNA libraries.

1:00 – 1:30 French Microbiology Program in the SubAntarctic

Daniel Delillie

Dr. Delillie shared the results from recent years of microbial ecology research at Kerguelen Island and Dumont d'Urville research stations. The French stations stay open year round, and have gathered a significant amount of data. They have studied microbial diversity in sea ice and in the water column, where they have found higher diversity in the sea ice. They also have substantial efforts in characterizing organisms capable of hydrocarbon degradation and UV resistance.

Afternoon: Group discussions of priorities for microbial diversity research in Antarctic marine environments. Discussion of specific methods required for Antarctic marine microbial research.

Ideas for CAML-Microbes on the web

- Organize a sample exchange forum
- Organize an analysis exchange forum

Synopsis of discussion of CAML-Microbes methods:

- Recommend primer pairs for clone libraries for bacteria, archaea, and eukarya
- Dr.s' Schnetzer, Golyshin, and Rodriguez-Valera will share methodological schema from their presentations
- Protocols for DGGE, TRFLP, and QPCR will be useful.
- Recommend standard sample volumes and collection methods for sea ice

Methods – detailed notes:

1. Sea ice

- Sampling must be randomized
- Mesh 200 μm , 80 μm , prefilters 5.0 μm and 3.0 μm ; filters 0.45 μm (bacteria are best collected in the $< 1.0 \mu\text{m}$ fraction – and this will miss the chain-forming bacterial cells).
- Key to look for “attached” bacteria.
- Lots of sterile containers are needed for melting
- First – cut away the outer edges with sterile snow saw; or dissolve away outer diameter of the core with ethanol or peroxide
- Melt the sea ice at room temperature and sample every 10 cm. Add sterile (or artificial) seawater at room temperature to melt to sea ice. This will help eliminate major changes in salinity as the ice melts.

2. Sediment samples

- MoBiokit is recommended
- Mini-box cores are useful for sampling anaerobic organisms (if the sediment is soft enough)
- Grabs, piston cores and gravity cores are other options – each with their own issues for sterilization and sample handling

3. Water column samples

- Sample at 10m and above the thermocline; then at the chlorophyll maximum and deeper waters
- Isolates – sampled from seawater or sea ice are best cultured immediately from fresh sample.
 - Can apply various selective media
 - Useful to characterize the 16S rRNA genes immediately

4. Surface and organism-associated microbes

- Fish gut/intestines – can squeeze out the contents; dilute them, then solicate to detach
- Sponge – need to section the sponge tissue [diver-collected samples are preferred]
- Dredges are another method of sampling – need to wash the fish or invertebrates with sterile water, then for culture-based approach use sterile mortar and pestle to grind, and then plate.
- Beware of sponge spicules and mucus from many invertebrates

Random cautionary notes:

- use low cycle numbers for clone library
- always filter formalin before using it to preserve samples

Methodological Approaches:

1. Q-PCR – use either crude lysate or purified DNA
2. 454 Pyrosequencing: Variable region 6 rRNA gene sequencing – ICOMM protocol
[note: ICOMM recently received a grant from the Keck Foundation to support sequencing of selected samples to sample the “rare biosphere” in microbial communities. This approach can sequence > 60K sequences per sample. The reads are short - < 100 bp.]
3. Isolates
 - Be aware of shipping regulations from different Antarctic stations and through various countries.
 - Do as much of the enrichments in Antarctica
4. Cellular enumeration
 - Flow cytometry is ideal – filter onto polycarbonate filters; fix with glutaraldehyde or para-formaldehyde for 12 hrs at 4C, then wash in sterile PBS.
 - Direct counts are also possible – easier for station based sampling; or prepare at sea and count later (store at -20C)

Discussions:

Large culture collections:

Shivadji (India) has a collection with > 2000 strains, many of which have not been characterized.

The Italians have a collection that's going to be part of the National Museum of Antarctica (NMA-IT)

There are a number of Antarctic bacterial cultures collections. We will investigate the potential to generate metadata concerning these collections. An ideal census project would synthesize the following which could be set up as a legacy for CAML and future researchers: 16S rRNA gene sequences, ITS sequences, fosmid-end reads, and isolate collections. This all would require good organization – recognized by all!

Biogeography

- encompasses the concepts of endemism and population structure
- gene exchange between the N and S poles
- the 16S rRNA gene does not represent the genome in this case – other genes are needed to tell if there's genetic exchange; whole genome sequences are the most valuable
- possible to accumulate data on different isolates
- there's also a replacement problem – as the life of a species can be a million years long
- Have to be aware of genetic selection – pressures from the environment influencing the synonymous / non-synonymous mutation ratio

Diversity links to ecosystem role and function

- links to function are key
- stable isotope probing holds promise here

Antarctic marine environment has an unprecedented spatial/temporal resolution in the high latitude environment - though this area is understudied