

## Lake Vostok DEEP ICE Project

**Project 6: Microbiological study of deep glacier ice in Antarctica** (in the frame of: Study of climate and environmental changes by investigation of deep glacier ice cores and sediments in Antarctic lakes and offshore seas)

**Project 7: Study of subglacial Lake Vostok, Antarctica** (molecular biology study)

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## PREFACE

At present we work on a huge range of microbes in trying to find out/identify those occur in both DEEP glacial and accreted (lake water) ice samples originating from different horizons of Vostok ice cores. My group focuses only on molecular biology study but I have working contacts with specialists (on ice etc.) affiliated to AARI (Arctic and Antarctic Research Institute) and RAE (Russian Antarctic Expedition), both Saint Petersburg, and Dr Abyzov from Microbiology Institute, Moscow. Especial interest is paid to fishing ice-entrapped DNA using random-primered PCR approach.

What we study now in short of living beings (both glacial and accreted ice cores):

**Of bacteria** (now only 16S rDNA- v3 region; semi-nested PCR; two different sets of universal primers) - representatives of 16 divisions (all culturable) and cyanobacteria (along with pollen) (separately). Further more divisions including those for unculturable bacteria – about 20 divisions) will be included.

**Of archaea** (now only 16S rDNA- v3 region; semi-nested PCR) - representatives of all three well-known divisions.

**Of fungi** (ITS region and D1/D2 region of nuclear LSU rDNA) – representatives of all four divisions (Asco-, Basidio-, Zygo-, and Chytridiomycetes).

At the same time because of known limitations of 16S rDNA approach (e.g, not possible to make designed true universal primers which could cover all microbes known and even unknown) we also work on **just DNA** (any DNA; dissolved DNA) by adjusting/attenuating PCR conditions for gene-specific (rDNA) PCR and implementing UP-PCR (random-like primered PCR) technique.

### Main Objectives:

- **Study of microbial diversity and microbe identification in an isolated environment in the East Antarctica (ice cores from a boring hole drilled towards the subglacial Lake Vostok, the Lake Vostok itself, Russian station Vostok)**
  - **Looking for ancient nowadays non-existing microbial species**
  - **Looking for unknown living beings with no homologous sequences available**
- **Study of microbial biogeography in terms of Staley and Gosink (1999) (e.g., polar endemic species like *Polaribacter spp.* of Gosink et al., 1998)**
- **Study of gene evolution (protein/enzyme/RNA encoding genes of interest)**

- **Development of new methodology and advanced molecular tools** to facilitate recovering and identification of relic (surviving) and other microbes inhabiting extreme (space-like) conditions

### Sampling:

- Samples from **glacier ice**
- Samples from **subglacial lake** (**accreted ice**, water, sediments)

### Molecular tools:

**Polymerase Chain Reaction (PCR)** using **gene-specific** and **random** primers

- **PCR amplification of evolutionary conservative genes** (e.g., **rDNA**, **gyrB**) followed by their sequencing and phylogenetic analyses allows to identify organism using databases established
- **Universally Primed PCR (UP-PCR) amplification of random-like primer driven loci** followed by their sequencing and hybridization analyses (in microarrays format) allows to identify any organism through the comparative study of genomic structure and DNA signatures

Both techniques will be applied to the **bulked** microbial DNA (no needs for culturing)

**Hybridization of genes of interest with an environmental METAGENOME cloned** - Rondon et al. (2000) AEM 66: 2541-2547

### Research Rationale:

#### **rDNA PCR approach**

- In studying **biodiversity** (e.g., **V3** [and V6] regions of bacterial SSU rDNA)
- In inferring **global phylogenetic networks** allowing to make a phylogenetic placement of new microbes regarding those whose rDNA sequences are available

#### **Degenerate primers PCR approach**

- In looking for unknown microbes with no homologous sequences available

### UP-PCR approach

- In studying **biodiversity (at species level)** by testing **spatio-temporal samples** in cross Dot Blot Hybridization using UP-PCR products
- In studying **intraspecies genetic diversity, species limits** and local (at **species level**) **phylogenetic relationships**
- In studying **microbial biogeography** by testing field samples for occurrence of **microbes of interest** using their UP-PCR amplified DNA as the 'label'

Our main approaches in molecular biological studying DEEP ICE CORES, Lake Vostok, Antarctica

### CONTAMINATION ISSUE

1. To decontaminate ice cores use specialized reagents/solutions like **Biohit Biocontrol** (in addition to washing water) and specific gas (e.g., **ozone**) treatment which both do not require thorough washing afterwards
2. Pay attention to **DOC contents** of **core-washing water** (even commercially available sources may contain microbes at starvation-survival state or their DNA)
  - Everywhere use **control** (artificial) **ice core** prepared from the core-washing water
3. **Take care mostly for DNA rather than cells.** Try to affect this foreign DNA somehow (e.g., MOP along with UV irradiation, ozone treatment) to prevent any **PCR amplification** with this DNA
4. When work on cells collected by filtering try to **decrease number of processing steps** in order avoid contamination and loss of material (e.g. through extra steps concerning DNA extraction, purification etc.)
5. In contamination issue pay attention to both '**laboratory environment**' (including healthy/diseased personal) and '**drill-in-site environment**' (compounds like **kerosene** used in drilling process directly in Antarctica)

6. In overcoming contamination issue when using phylogenetically conservative primers use **PNA mediated PCR clamping** approach to avoid dominated or undesirable (occurred in your environment) microbes

|                                    |
|------------------------------------|
| <b>BIODIVERSITY STUDYING ISSUE</b> |
|------------------------------------|

1. Make focus on **dissolved DNA** rather than on microbial cells (visible somehow)
  - Pay attention to **0.2 µm filterable bacteria (survival-starvation forms)**
2. Perform a **special effort in adapting PCR conditions** (diff. loci, diff. primers, diff. polymerases, cycling conditions, PCR buffer contents – enhancers, aptamers etc.)
  - Apply UP-PCR technique (ours) and work specifically (gene-specific PCR with SCAR primers) on unknown DNA (to prove the lake source of microbes by monitoring glacier and accreted ice cores)
  - Use **PNA-mediated PCR clamping** in selective (biased) amplification of target DNA of interest (e.g., von Wintzingerode et al., 2000)
3. In studying glacial ice cores make focus on those originating from **neighboring INTERGLACIALS** as possible source for new life-forms
4. In Biodiversity studies additionally use:
  - **DNA array (microarray) analysis** (species (gene)-specific oligonucleotide probes and UP-PCR products)
    - array for human commensals and pathogens
    - array for crude oil (kerosene) metabolizing microbes
  - **Whole-cell FISH** (fluorescent in situ hybridization) (species (gene)-specific oligonucleotide probes)
  - **BAC method** (in cloning **METAGENOME**)
5. Preferably work on **microbes which do not occur in the lab** and especially do not **occur/inhabit healthy/diseased human skin/mucous surfaces etc.** but vice-verse **can occur in an environment to study**
  - When find a microbe of interest (identify microbe by DNA sequence) to validate its **ICE SOURCE**:
    - always test lab environment and persons directly involved in ICE CORES handling for that microbe/DNA using gene-specific PCR approach (by DNA array analysis)
    - try to perform replicated study in another lab (in Antarctica)

- When find a microbe of interest (identify microbe by DNA sequence) to validate its **Lake Vostok WATER SOURCE**:
  - test corresponding glacier ice cores (which can be involved (by melting and re-freezing) in generation of accreted ice) for that microbe/DNA using gene-specific PCR approach (by DNA array analysis)
  - try to perform replicated study in another lab

Testing DEEP ICE cores (lake **Vostok**, Antarctica) for microbes: **Molecular Biology Study**

|   | Glacial Ice                   |          |                               |                               |                         |                               |                               |        | Basal (Accreted) Ice |                               |        |          |                     |        |        |
|---|-------------------------------|----------|-------------------------------|-------------------------------|-------------------------|-------------------------------|-------------------------------|--------|----------------------|-------------------------------|--------|----------|---------------------|--------|--------|
|   | 1 <sup>st</sup> inter-glacial |          | 1 <sup>st</sup> inter-glacial | 2 <sup>nd</sup> inter-glacial | 2 <sup>nd</sup> glacial | 3 <sup>rd</sup> inter-glacial | 4 <sup>th</sup> inter-glacial | (???)  |                      | 1 <sup>st</sup> inter-glacial |        |          |                     |        |        |
| Microbes to test under specified PCR conditions | Spring Ice 0.0 m              | K1 (Lab) | 54 m                          | 1500 m                        | 2046 m                  | 2446 m                        | 3000 m                        | 3483 m | K2 (Lab)             | I/1 0.0 m                     | 3546 m | K3 (Lab) | 3556 m - max. cells | 3571 m | 3604 m |
| <b>Bacteria I</b>                               | +                             | +        |                               |                               | +                       |                               |                               | +      |                      |                               |        |          |                     |        |        |
| Bacteria II                                     |                               |          |                               |                               |                         |                               |                               |        |                      |                               |        |          |                     |        |        |
| <b>Cyano</b>                                    |                               | -        |                               |                               | -                       |                               |                               | -      | -                    | +*                            | -      |          |                     | -      | -      |
| <b>Archaea</b>                                  |                               |          |                               |                               |                         |                               |                               |        | +                    | -                             | -      |          |                     | -      | -      |
| <b>Unknown DNA/Archaea prs</b>                  |                               |          |                               |                               |                         |                               |                               |        | -                    | -                             | -      |          |                     | +      | -      |
| <b>Fungi I</b>                                  |                               | -        |                               |                               | +                       |                               |                               | +      |                      |                               |        |          |                     |        |        |
| <b>Fungi II</b>                                 |                               |          |                               |                               |                         |                               |                               |        | -                    | -                             | -      |          |                     | -      | -      |
| <b>Unknown DNA/Fungi II prs</b>                 |                               |          |                               |                               |                         |                               |                               |        | -                    | +**                           | -      |          |                     | -      | +**    |

\* unidentified (related to high plant [Nothophagus?] pollen)

\*\* at least four different sequences

2046 Cold period

I/1 Warm period

**Bacteria I** – old primers

**Bacteria II** – new (much broader) primers

**Unknown DNA** – no matches in databases available

**Fungi I** – one primer set and approach

**Fungi II** – another primer set and approach (better?)

### Projects: How they are for now

1. **Project** ‘Cold’ atmospheric cores (m) – 963, 2046
2. **Project** ‘Warm’ atmospheric cores (m) – [Spring Ice], 0.0[I/1], 54, 1265<sup>Delayed</sup>, 1500, 2446, 3000
  - **Sub-Project** Within interglacial period – 1265 and 1500 - **Delayed**
3. **MEGA-Project** ‘Cold’ - ‘Warm’ atmospheric cores [+ 3483]
4. **Project** Basal (lake water accreted) ice cores (m) - 3546, 3556, 3571, 3586, 3604
5. **MEGA-Project** Basal cores – ‘Cold’+‘Warm’ atmospheric cores