Report for short term research visit at GEUS (21.02.2012 to 12.03.2012)

Objectives: RNA isolation from the oil polluted rhizosphere and bulk soils sampled during the winter months (snow cover), Quantification of catabolic genes (Ring hydroxylating dioxygenases and alkane hydroxylases) from cDNA and DNA.

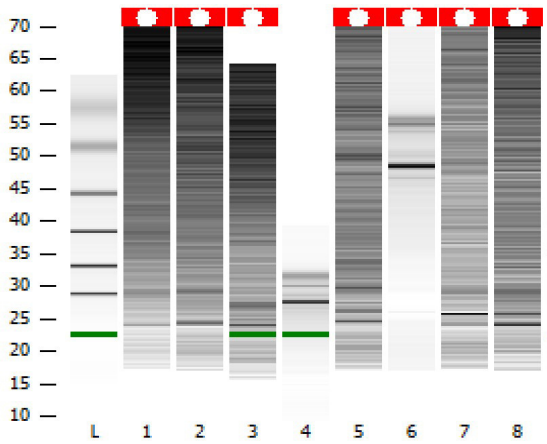
Results

A) Sample collection and processing:

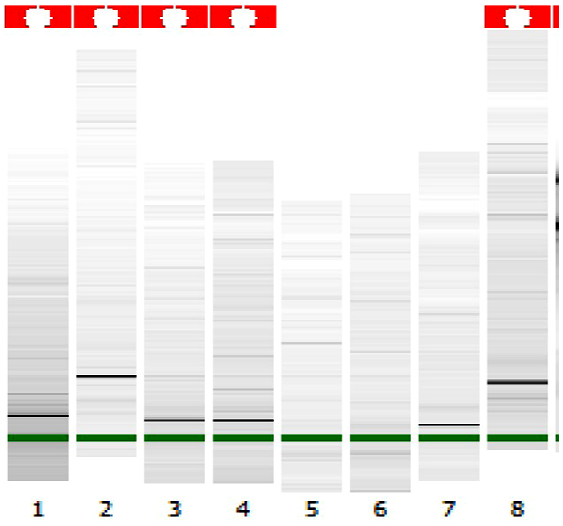
Samples were collected from a long term phytoremediation experiment at the Plant breeding station of Finnish Forest Research Institute, Läyliänen, Finland. Soil samples from petroleum hydrocarbon polluted *Populus* rhizosphere and unplanted soils were collected at 2 time points- a) November (before snow, ambient air temperature: 5˚C) and b) February (about 40 cms snow, ambient air temperature: -10˚C). Samples were immediately snap shot frozen in liquid nitrogen and stored at -80˚C until further use. Freeze drying of samples was performed at GEUS.

B) Selection of method for RNA extraction from polluted frozen soil:

Jacob'smethod and RNA Power Soil Kit, MO BIO



1. Jacob's method



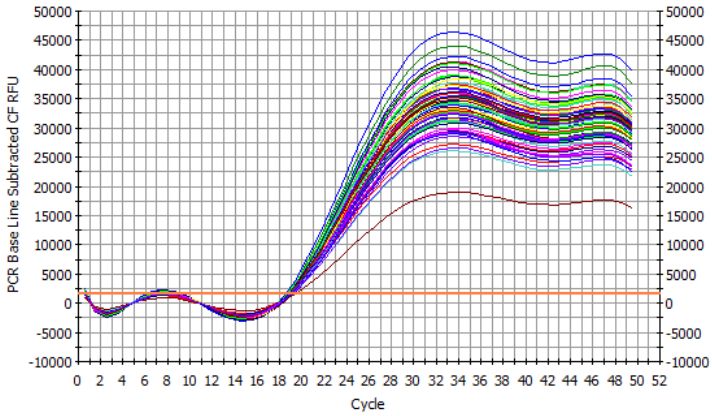
2. RNA Power Soil Kit, MO BIO

Fig. 1 and fig. 2 show the Bioanalyzer results from Prokaryote Total RNA Pico assay. The yield and quality of RNA was better with Jacob's method as compared to the RNA Power Soil Kit. But even with Jacob's method, the RNA was degraded in most of the samples. We decided to follow the Jacob's method for RNA extraction.

- Extraction of RNA and DNA was performed from 54 samples which included polluted and clean rhizosphere and bulk.
- In most of the extracts , RNA was either partially degraded or was very low in concentration (8-20 picograms).

C. Inhibition assay:

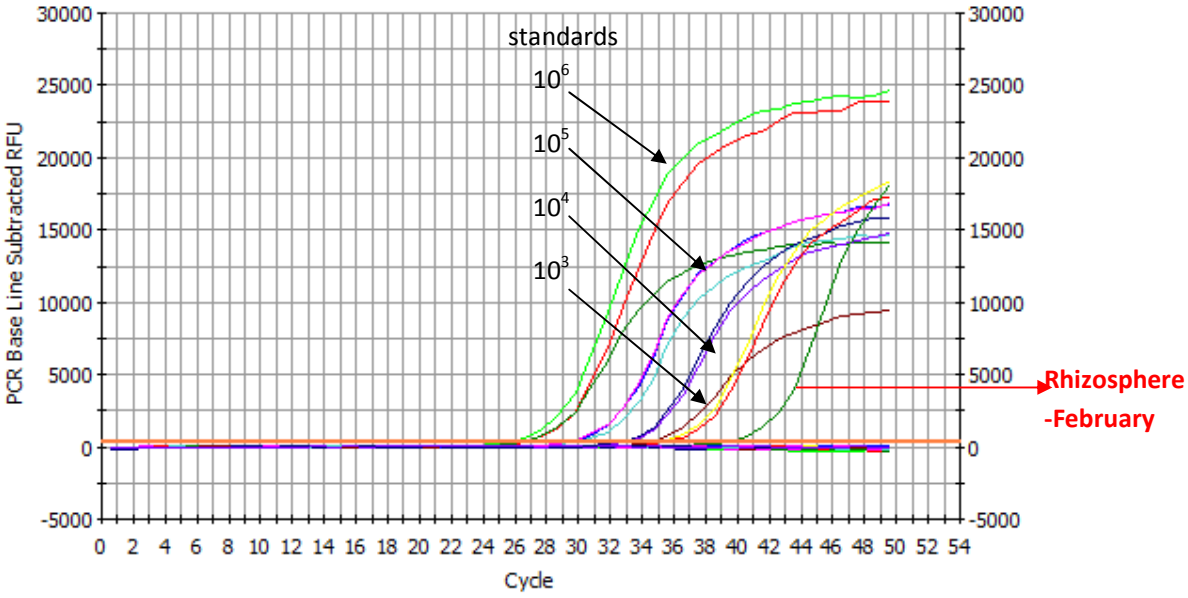
To check the presence of PCR inhibitors in the soil RNA/DNA extracts, inhibition test was carried out. pCR 2.1-TOPO plasmids were added at a concentration of 10^6 per reaction, qPCR was done with the M13 primers and 1 µl of the soil DNA (and cDNA) extract.By comparing the mean Ct values obtained from the soil DNA and cDNA dilutions with the plasmid controls, it was concluded that there were no potential PCR inhibitors.



D. 16S qPCR

qPCR for 16S rRNA gene was carried out using 341 F and 518R primers. Gene copy numbers ranged from **10^7 -10^4 per µl of cDNA** from most of the samples.

E. Gram-Negative Ring hydroxylating dioxygenase –qPCR



qPCR was performed for the ring hydroxylating dioxygenase genes from gram negative bacteria . (Primers: Cébron et. al. 2008, J Microbiol Methods.)

Only one of the replicates from one sample showed a positive signal, therefore the quantification was not possible. This sample was cDNA from the field rhizosphere clone 287.

F. Gram-Positive Ring hydroxylating dioxygenase –qPCR

qPCR was performed for the ring hydroxylating dioxygenase genes from gram positive bacteria . (Primers: Cébron et. al. 2008, J Microbiol Methods.)

No positive signals were seen in any samples except the positive controls.

G. Conclusion:

Based on the bio-analyzer and qPCR results, it can be said that the RNA extraction procedure needs further optimization.

H. Future work:

I have started repeating the RNA extractions and have so far successfully amplified alkane hydroxylase and bphC genes from the cDNA (using conventional PCR). We have done 454 sequencing of the 16SrRNA gene amplicons from the winter samples recently which will help us identify the main bacterial groups in frozen polluted soils in winter months.