Scientific Report of the Short-Term Scientific Mission (STSM)

Topic: Molecular analysis to study microbial communities in deep sea sediments

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Purpose of the STSM

Expedition 351 of the International Ocean Discovery Program (IODP) recovered a 1461 m-long sedimentary sequence of Amami-Sankaku Basin (ASB) that records the volcanic eruptions related to the subduction of the Pacific lithospheric plate under the Philippine Sea plate. The subduction-related volcanic debris have accumulated in the ASB for the last c. 50 million years, and now sit below the Philippine Sea at a water depth of 4700 m. In this expedition, 25 sediment samples were collected for onshore microbiological analyses at selected depths, until 880 m below seafloor (mbsf).

In the University of Aveiro, molecular biology analyses have been done which include epifluorescence microscopy counts of prokaryotic cells with Acridine Orange Direct Counts, identification of active bacteria community using a nested PCR approach to amplify the 16S rRNA gene sequence, and characterization of Bacteria community structure using a 16S based barcoded pyrosequencing approach.

In order to complement the microbiological dataset produced so far in the University of Aveiro, this STSM was held in ETH Zürich to implement new extraction methods for further molecular biology analyses which include next-generation sequencing of bacterial and archaeal 16S rRNA gene sequences, and the preliminary analysis and interpretation of the corresponding DNA sequence data. In addition, interstitial water samples were extracted to quantify the concentration of dissolved nitrate which is a potentially important electron acceptor in these highly oligotrophic sediments.

Description of the work carried out

During the five weeks that I stayed in ETH Zurich, I have performed the DNA extraction of 25 samples collected in Expedition 351 of IODP and their preparation for next-generation sequencing of bacterial and archaeal 16S rRNA gene sequences.

First, I did a quantification PCR (qPCR - Real time PCR) in DNA samples previously extracted in Aveiro to have an idea of the number of bacterial and archaeal 16S rRNA gene copies and

subsequently comparing with the results obtain after using a new extraction method implemented by Doctor Mark Lever. After that, a few sub-samples of each sample were chosen and the same variations in the method described in Lever et al., 2015 were tested. To check the extraction procedure, qPCR analysis of Bacteria and Archaea 16S rRNA gene copies number were performed. The extraction procedure was performed in a clean room and a blank sample was always included for contamination control.

After obtaining the DNA extracted, the next step was to prepare DNA amplicon library to next-generation sequencing of bacterial and archaeal 16S rRNA gene sequences using Illumina MiSeq Personal Sequencer at Genetic Diversity Centre (GDC; http://www.gdc.ethz.ch/) located in the Department of Environmental Systems Sciences at ETH Zürich. The samples were prepared according to procedures implemented by GDC. Briefly, extraction samples were first amplified using a boost PCR and then a tailing PCR was made using primers with barcodes. After that, a first clean up (AMPure beads) was done and Qubit quantification was performed to verify product quantity. In the next step, the Nextera XT index adaptors with the barcode were attached to the amplicon (Index PCR) and then a second clean-up was made. To control the cleaned up sample a Qubit quantification was realized. The last step consisted in the library normalization (putting the libraries in the same concentration) and then pooling. Finally, the pooled library was quantified with the Qubit and qPCR to check if the concentration was between 2 and 4 nM.

The next step is to run the samples in Illumina MiSeq Personal Sequencer, which will be done in the next 2 weeks, after the end of this STSM.

Preliminary results

Unfortunately it was not possible to do all the work planned in the five weeks. We had some problems in the extraction which needed to be solved, particularly due to contamination. Nevertheless, the main purpose of this proposal was mostly completed. Moreover, in this STSM I have acquired new and important skills in molecular biology.

The main result of this STSM was the obtained amplicon library to next-generation sequencing of bacterial and archaeal 16S rRNA gene sequences using Illumina MiSeq. The qPCR results (Figure 1) show that samples extracted in ETH Zurich have a higher bacterial and archaeal gene copies number comparing with those obtained in the University of Aveiro. These results suggest that in these samples the extraction method tested in ETH Zurich is more efficient than the kit method used in the University of Aveiro.

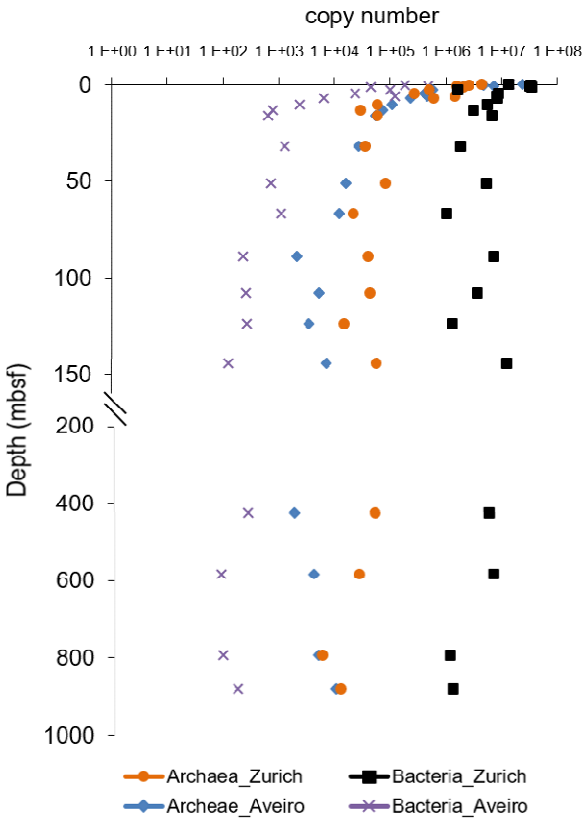

Figure 1: Bacterial and archaeal 16S rRNA gene copy number

Future collaborations including publications

The results of this STSM are going to be integrated in the post-cruise work carried out by Clara Sena, who participated in IODP Expedition 351, and together with Mark Lever, from ETH Zürich, and other colleagues from the University of Aveiro, we will compare the results attained now with the results already published for deep-sea areas with active fluid flow, in order to identify the major differences in the microbial communities and biogeochemical processes that occur in contrasting geological contexts, in terms of fluid flow in deep-sea sediments. In this context, a scientific paper will be prepared to be published in an international journal of the Citation Index.

References

Lever MA, Torti A, Eickenbusch P, Michaud AB, Šantl-Temkiv T and Jørgensen BB (2015) A modular method for the extraction of DNA and RNA, and the separation of DNA pools from