

Metatranscriptomic Analysis of the Post-Eruptive Bacterial Community from the Hydrothermal Vent Field at 9°N on the East Pacific Rise



Jessica Ricci and Costantino Vetriani

Inst. of Marine and Coastal Science and Dept. of Biochemistry and Microbiology, Rutgers Univ.



Introduction

Hydrothermal vents are a unique extreme environment characterized by high temperatures, high pressures, and toxic chemicals (Figure 1). In 2006, an eruption occurred at the vent field at 9°N on the EPR. An initial study showed that *Epsilonproteobacteria* dominate the community that colonizes the newly formed vent. While *Epsilonproteobacteria* are known to thrive at deep-sea vents (1), little is known about what processes these bacteria undertake to colonize these sites. A metatranscriptomic approach will be taken to identify these processes.

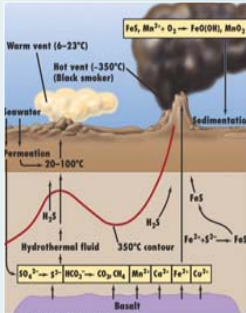


Figure 1. Hydrothermal vent (Brock Biology of Microorganisms 11/e © 2006 Pearson Prentice Hall, Inc.)

Objectives

- To determine the genes being expressed by the bacterial community at the time of colonization of a hydrothermal vent
- To optimize a metatranscriptome procedure

We hypothesize that genes involved in biofilm production (Figure 3), metal detoxification, and diverse metabolisms are being expressed at the time of colonization.

Method

A metatranscriptome is a library of transcripts or mRNA from an entire community of microorganisms. This analysis will tell us what processes these organisms are carrying out at a given time. Enrichment of mRNA, which encodes functional genes, is the objective of this procedure (Figure 2). To avoid biases the samples will be pyrosequenced (2).

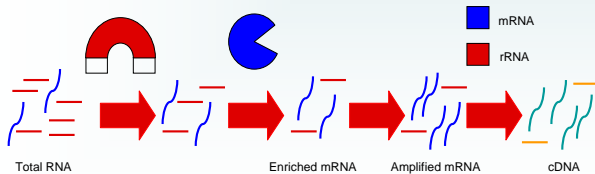


Figure 2. Diagram of metatranscriptome method

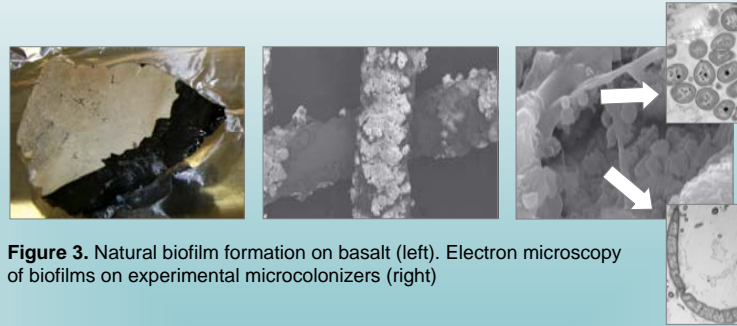


Figure 3. Natural biofilm formation on basalt (left). Electron microscopy of biofilms on experimental microcolonizers (right)

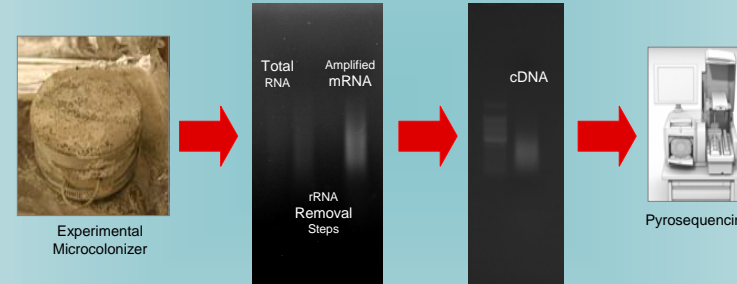
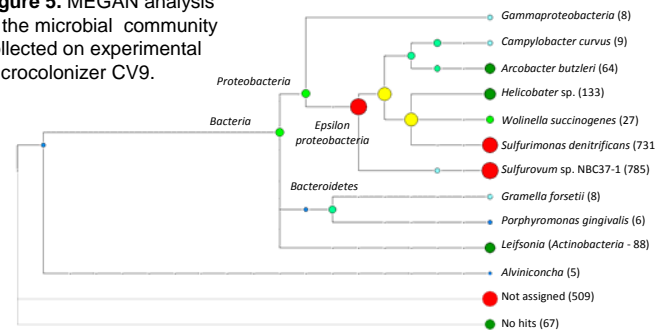


Figure 4. Visualization of the metatranscriptome procedure using experimental microcolonizer CV41 (left). An equal molar amount of each RNA sample was loaded on a denaturing gel (center left). 2 µl of cDNA was loaded on a 1% agarose gel (center right).

Figure 5. MEGAN analysis of the microbial community collected on experimental microcolonizer CV9.



In collaboration with Boris Wawrik, University of Oklahoma

Results

The gel electrophoresis visualization of the metatranscriptomic procedure for CV41 illustrates a decrease in the RNA smear after rRNA removal and an increase after mRNA amplification (Figure 4). The cDNA produced was validated by pcr (Figure 6). MEGAN analysis of pyrosequencing data from pilot sample CV9 revealed the microbial community composition (Figure 5).

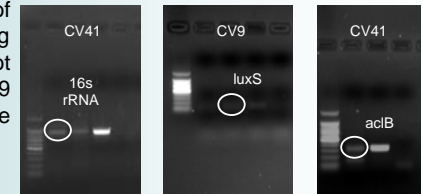


Figure 6. The presence of 16s, luxS, acIB genes was confirmed

Conclusions

- cDNA ranging in length from 100-900 bp was produced by a metatranscriptome procedure
- The presence of the functional genes luxS (biofilm) and acIB (metabolism) were confirmed by pcr
- The CV9 metatranscriptome was consistent with a previous study which indicated that *Epsilonproteobacteria* dominate the microbial community there

Future Work

- Pyrosequence pilot sample CV41
- Analyze pyrosequencing data from CV41 using MEGAN

References

1. Campbell, B., Engel, S., Porter, M., & Takai, K. (2006) The versatile ε-proteobacteria: key players in sulphidic habitats. *Nat. Rev. Microbiol.* 4, 458-469.
2. Ronaghi, M. (2008) Pyrosequencing sheds light on DNA sequencing. *Genome Research.* 11, 3-11.

