Origin of Microbial Communities in Flowing Hot Spring Sediments

Ryan Hagar[1], Areej Almasary[2], Dr. Rick Bizzoco[3]
1) Southwestern, 2) King Saud University 3) SDSU

Introduction

Previously, it was thought that nothing could live within the boiling hot, acidic waters of a hot spring. This was later to be shown incorrect in the early 1970's when Thomas Brock discovered *Sulfolobus* in an acidic hot spring at 93ºC pH 1.4 in Yellowstone. The year before that,1969, he discovered *Thermus aquaticus (Taq),* an organism classified as a thermophile, which lives between 45ºC and 80ºC. Hyperthermophiles live at 80ºC or above. An enzyme from Taq was later used in polymerase chain reaction (PCR), a method of duplicating DNA.

In current science there is a controversy over the origin of microorganisms in sediments of flowing hot springs. Our hypothesis is that the origin is from subsurface waters flowing into the springs, rather than from some other source. Our samples have been taken from Lassen and Yellowstone National Parks from waters at temperature of 71.6ºC-93.5ºC and pH 1.08-6.7. Our approach included methods to demonstrate viability of subsurface microorganisms and to use PCR and sequencing for their identification. Comparison of these results with sediment populations suggests a contribution of the subsurface waters to the surface communities.

Aims

•To evaluate the contribution of subsurface waters to surface sediment microbial communities.

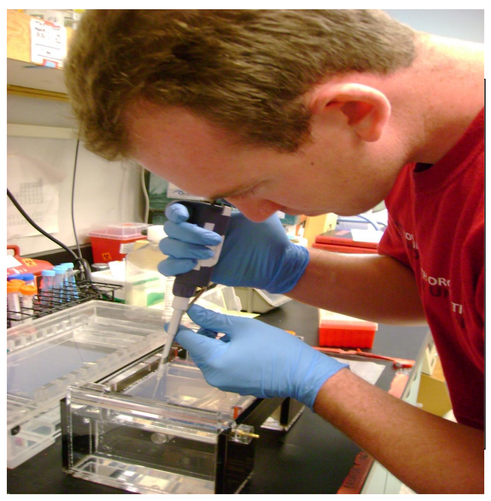
•To discover species that inhabit the subsurface waters and surface sediments.

Methods

In order to test our hypothesis, samples of water and sediment are taken at the spring origin and downstream sediments. These samples are chilled, brought to the lab, and grown on plates of Gelrite by incubation at 70ºC or 55ºC. After single colonies are isolated on Gelrite plates, DNA is extracted by lysozyme and detergent lysis. Next, DNA is purified on mini columns and analyzed for purity and DNA concentration. PCR is used to increase the DNA extracted from each sample. Afterwards the DNA is analyzed by gel electrophoresis to determine amplification of the 16S rRNA gene sequence. PCR products are cleaned on mini columns and sent to the MicroChemical Core for sequencing. The resulting sequences are trimmed, aligned in greengenes and analyzed by BLASTn (NCBI), for probable identifications.

Readings being taken from Lassen Volcanic National Park.

Above: Sara is standing between Voldemort Spring on the left and Toto Spring to the right, Lassen Volcanic Nation Park.
Below: Ryan is preparing an electrophoresis gel.



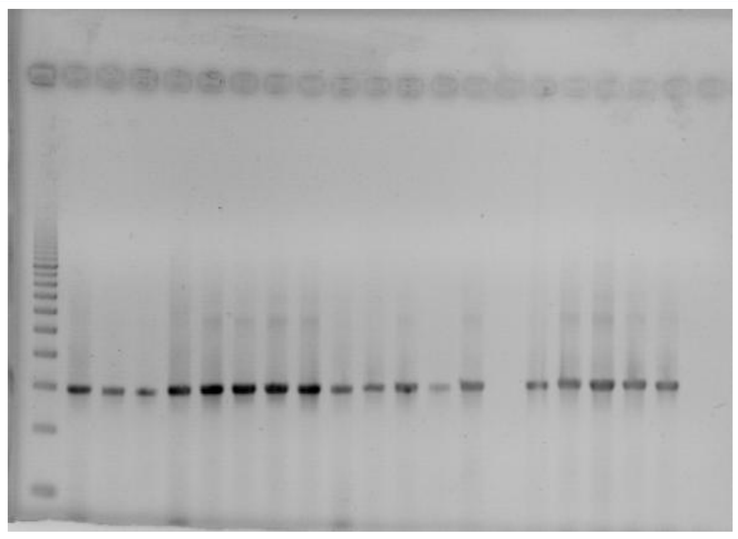
Results (origin water)

Lassen Volcanic National Park

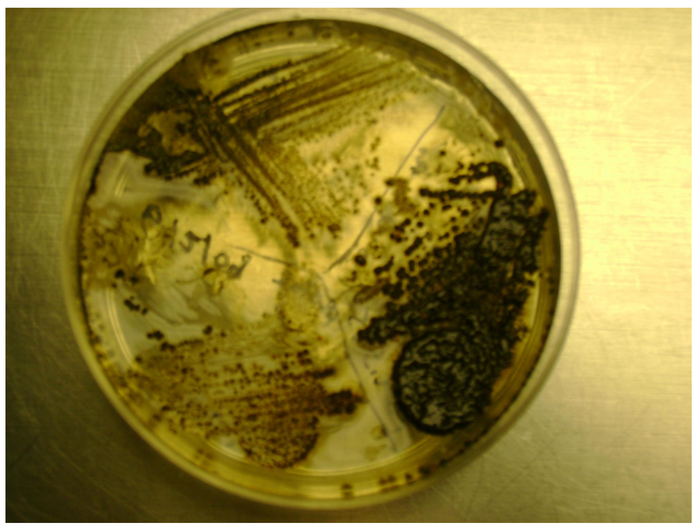
Site	Organism	%ID
Toto Spring:		
	Alicyclobacillus acidocaldarius	97%
	Thermomonas hydrothermalis	98%
	Thermomonas hydrothermalis	98%
	Alicyclobacillus acidocaldarius	98%
	Alicyclobacillus acidocaldarius	97%
	Alicyclobacillus acidocaldarius	99%
	Paenebacillus pabuli	97%
	Burkholderia	96%
	Alicyclobacillus	96%
Voldemort Spring:		
	Alicyclobacillus	85%
	Alicyclobacillus	84%
Devil's Kitchen:		
	Alicyclobacillus pomorium	82%
	Burkholderia	99%
	Alicyclobacillus pomorium	82%
	Alicyclobacillus pomorium	86%
	Alicyclobacillus	83%

Yellowstone National Park

Amphitheatre Springs 104:		
	Alicyclobacillus acidocaldarius	96%



Isolates obtained from Yellowstone and Lassen were grown, DNA-extracted, PCR amplified, and analyzed by agarose gel electrophoresis. Bands represent amplified 16S rRNA gene sequences. Far left lane, 500 kb ladder. All bands have approximately the same molecular weight (1500 kb). Last two lanes on right represent E. coli(+) and water (-), respectively.



Three water samples were taken from the origin shown at the far lower left (column 1) and plated on to Gelrite solid medium and streaked for isolation. The dominant, but not the only culture that grew from the waters, turned orange then black. The black colony form is shown in the three samples above. It was sequenced (in other studies)as described in the methods section of this poster and falls into the phylogenetic group, Micromonospora, a bacterium with fungus-like morphology.

Conclusion

We found that the diversity provided some coverage, but not all sediment organisms were grown from the origin waters. Only about 1-5% of existing diversity has been cultured in the laboratory.

Acknowledgements

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