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Survey of Protist Genetic Diversity in the Hydrothermal Environments of Lassen Volcanic National Park N-063

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Introduction

Hydrothermal environments support diverse prokaryote assemblages, but less is known about eukaryotes, or hydrothermal food webs. Culture-based studies suggest protists might possibly be found up to 60-70 C (Tansey and Brock, 1978), and many acidophilic taxa are well known (Packrof and Woelfl, 2000).
Recent reports of novel protist rRNA genes in extreme environments, such as acidic or anoxic sites (Amaral Zettler et al., 2002, Edgcomb et al., 2002, Dawson and Pace, 2002, Baker et al., 2003) suggest that diversity might be higher than previously thought.
Here, we present data of a survey of eukaryal rRNA diversity in the Lassen Volcanic National Park (LVNP) hydrothermal environments.

Questions:

1. What eukaryotes reside in the hydrothermal features of LVNP? Do protists dominate diveristy?
2. Are the sites dominated by heterotrophic or autotrophic organisms?
3. How does pH and temperature influence what protists are found in the hydrothermal features?

Methods

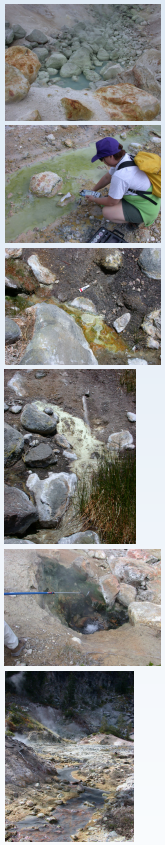
Study Sites: We sampled mud pots, springs, and mats from sites at Upper Sulfur Works, Bumpass Hell, Boiling Springs Lake and Devil's Kitchen (**figs. 1, 2**).

DNA extraction and quantification: Sediment and mat samples were dewatered and resuspended in Tris buffer, pH 8. We compared a modified CTAB/chloroform extraction with Wizard (Promega) purification, and several soil DNA kits (UltraClean kit MoBio Labs, FastDNA, BIO101). Denaturing Gradient Gel Electrophoresis (DGGE) was used to compare extraction methods. For most samples, we combined extracted DNA by multiple methods to minimize extraction bias. DNA was quantified fluorimetrically with PicoGreen (Molecular Probes).

PCR Amplification: We amplified eukaryal SSU rDNA with several primer combinations (**table 1**) using standard PCR conditions.

Cloning and Sequencing: PCR products were cloned using the TOPO-TA cloning kit (Invitrogen). Inserts were reamplified directly from colonies, screened by RFLP (RspI and HaeIII), and unique restriction patterns sequenced using ABI Big Dye 3.1 on an ABI 310 sequencer.

Figure 1: Location of LVNP (red square) and Bumpass Hell site.



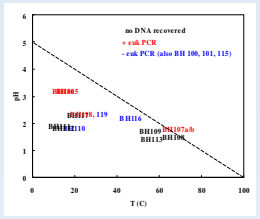


Figure 2a: PCR success vs. pH and temperature for Bumpass Hell

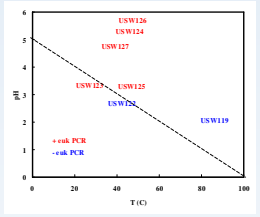


Figure 2b: PCR success vs. pH and temperature for Upper Sulfur Works

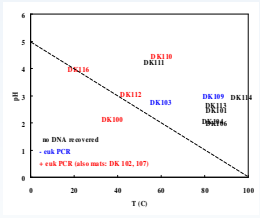


Figure 2c: PCR success vs. pH and temperature for Devil's Kitchen

DNA Source	pH	T	DNA µg/ml*	Eubact 16S 27/534	Archaeal 16S 751/1204	Eukaryote 18S 4/516 82/1390	82/516	615/1200	
BH107	1.8	68	7.5	+++**		++	++		+++
BH100	1.7	30	152	+++	+		+/-		+
BH101	1.7	30	33	+++	+++				+
BH118	2.2	19	40	+++		+	++	+++	++
BH105	3.2	15	423/26	+++		++	+++	+++	+++
BH106	3.2	15	304/22	++		++	+++	+++	
DK108	3	85	13	+++	+++	+/-	+/-		+
DK109	3	85	1.4/20	+++	+++	+			
DK110	4.5	60	2.4	+++	+++	+	+++	+/-	++
DK112	3	45	297/100	+++	+++	+	++		++
DK116	4	22	312/58	+++	+	+++	+++	+++	+++
DK102	mat		13	++	+++		+	+++	++
DK105	mat		454/58	+++	+/-	+	+	++	++
DK107	mat		8/10.7				+++	+++	+++
USW119	2	85	6/32	+	+++	+/-			
USW126	6	45	66/7	+++		+++	+++	+++	+
USW124	5	44	37/35			+	+++	+++	+++
USW125	3	44	76/2.6	++		+	+++	+++	+++
USW127	5	36	303/4	+++		++	+++	+++	+++
USW123	3	25	94/34	++	+	+/-	++	+	+++

*DNA extractions for CTAB/UltraClean DNA kit shown where both used
**+++ = strong PCR product, etc

Table 1: PCR results with different primer sets. Blank=primer not used. +++ = relative amount of PCR product.

Table 2 a-c: Blast Identity from clone libraries

DNA Source	pH	T	# clones	BLAST Identity
BH100	1.7	30	2	*Platyophrya vorax* (ciliate)
			3	Uncultured Rio Tinto Chlorphyte
			1	*Parauronema longum* (ciliate)
			1	*Bodo edax* (ciliate)
			6	*Philasterides* (ciliate)
			2	*Chlorella* (chlorophyte)
			1	*Dunaliella* (chlorophyte)
BH105	3.2	15	4	Uncultured Rio Tinto Chlorphyte
			6	*Aulacoseira ambigua* (stramenopile)
			1	*Pinnularia* (stamenopile)
			1	*Microspora* (chlorophyte)
BH106	3.2	15	6	*Pinnularia* (stramenopile)
			4	*Aulcoseira baicalensis* (stramenopile)
			5	*Pinnularia* (stramenopile)
			1	*Staurastrum punctulatum* (desmid)
BH107	1.8	68	12	*Aulacoseira ambigua* (stramenopile)
			1	*Powellomyces variabilis* (chytrid)
			1	Cercozoa (unculture Rio Tinto)
			1	*Pinnularia* (stramenopile)
			1	*Poteriochromonas* (stramenopile)
			1	*Chloromonas* (chlorophyte)
			1	*Englemanniella* (amoeba)
			2	Chysophyte
BH118	2.2	19	1	*Chlamydomonas* (chlorophyte)

DNA Source	pH	T	# clones	BLAST Identity
DK102	mat		12	*Chlamydomonas* (chlorophyte)
DK105	mat		7	Hexapod
			1	*Echinamoeba thermarum* (amoeba)
			1	*Pichia* (chytrid)
DK107	mat		1	Hexapod
			1	*Acanthamoeba castellanii*
			1	*Chlorella* (chlorophyte)
DK110	4.5	60	2	*Sclerotium* (basidiomycete)
			6	*Podura* (hexapod)
			4	*Pinnularia* (stramenopile)
DK112	3	45	5	*Pinnularia* (stramenopile)
			1	*Klebsormidium nitens* (chlorophyte)
			2	*Chlorella* (chlorophyte)
DK116	4	22	4	*Pinnularia* (stramenopile)
			1	*Chlorella* (chlorophyte)
			1	hexapod

DNA Source	pH	T	# clones	BLAST Identity
SW105	5.1	15	1	*Monosiga ovata* (choanoflagellate)
SW117	2.2	34	2	*Chlamydomonas* (chlorophyte)
USW123	3	25	1	Rio Tinto clone
			6	Stenostomum leucops (platyhelminth)
			1	mite
USW124	5	44	6	*Hartmannella* (amoeba)
			1	*Pinnularia* (stramenopile)
			2	*Scenedesmus subspicatus* (chlorophyte)
USW125	3	44	6	*Pinnularia* (stramenopile)
USW126	6	45	2	*Chlamydomonad* (chlorophyte)
			1	*Chlorella* (chlorophyte)
			8	*Bacillaria paxillifer* (stramenopile)
			2	nemotode
USW127	5	36	1	*Rhizophydium* (fungi)
			14	*Pinnularia* (stramenopile)
			1	*Metopus palaeformis* (alveolate)

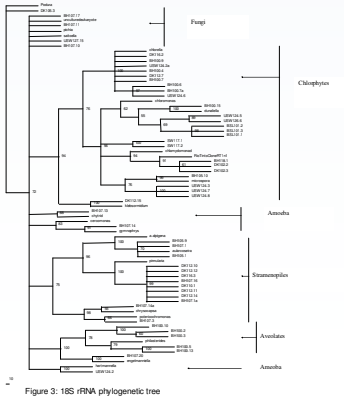


Figure 3: 18S rRNA phylogenetic tree

Results

1. Bumpass Hell environments are more acidic and cooler than Devil's Kitchen and Upper Sulfur Works, which show similar ranges (**fig. 2**).

2. DNA extractions ranged from 0 to 454 mg/ml. Many mud pots gave low or no DNA recoveries (**table 1**). Bacterial 16S rDNA was amplified successfully from most sites, while Archaeal 16S rDNA was amplified mostly at Devil's Kitchen

3. Eukaryal 18S rDNA was amplified successfully from most samples except those above 65 C (**fig. 2, table 1**). We found primer combinations 82/516 worked better than 4/516 (**table 1**).

4. Based on clones, acidophilic protists dominate eukaryotes in LVNP hydrothermal environments. Many gave 90-98% homology to known acidophilic taxa, or environmental clones from other acidic sites (Rio Tinto, Spain). Most sites showed phototrophic assemblages, dominated by stramenopiles, chlorophytes, and some chrysophytes. Heterotrophic taxa included diverse alveolates, amoebae, and fungi. Occasional metazoa (hexapods, nematodes, platyhelminths) were detected in low temperature, less acidic environments, especially in mats.

5. Based on the phylogeny, the clones cluster in groups of chlorophytes, stramenopiles, ciliates and fungi (**fig. 3**).

Conclusions

1. Diverse eukaryal rRNA genes were found in many LVNP hydrothermal environments. Protist taxa dominate sequences, with fungi and some metazoa appearing in cooler environments and mats.

2. Protist communities appear largely photosynthetic, typically mats and benthic assemblages in shallow streams consisting of acidophilic diatoms and chlorophytes. Interestingly, *Cyanidium*, a dominant acidophilic rhotophyte found at other hydrothermal sites (Tansey and Brock, 1978), was only found in Boiling Springs Lake. Heterotrophic protists, especially aveolates and ciliates, were found, but we suspect that some delicate cells may have ruptured during pH neutralization.

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