Survey of Protist Genetic Diversity in the Hydrothermal Environments of Lassen Volcanic National Park N-063
Patricia B. Brown*; Gordon V. Wolfe**
California State University, Chico
pbrown12@mail.csuchico.edu
gwolfe2@csuchico.edu

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 eukaryotic taxa are well known (Paice and Woold, 2000). Recent reports of novel protist rRNA genes in extreme environments, such as acidic or anoxic sites (Amor Zettel 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 diversity?
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?

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 Archea 16S rDNA was amplified mostly at Devil’s Kitchen.
3. Eurkaryal 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% BLAST identity. Here, we present data of a survey of eukaryal rRNA diversity in the Lassen Volcanic National Park (LVNP) hydrothermal environments. Many gave 90-98% BLAST identity.
5. Based on the phylogeny, the clones cluster in groups of domains.

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 dewaxed 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 (Rap and Hasl), and unique restriction patterns sequenced using ABI Big Dye 3.1 on ABI 3700 sequencer.

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, Gyanelas, a dominant acidophilic phototrophs found at other hydrothermal sites (Tansey and Brock, 1978), was only found in Boiling Springs Lake. Heterotrophic protists, especially asclastes and ciliates, were found, but we suspect that some delicate cells may have ruptured during pH neutralization.

References


Acknowledgements
