A Survey-Driven Study of Microbial Mats in Joseph's Coat Thermal Basin, Yellowstone National Park

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ABSTRACT

We comprehensively surveyed thermal features in Joseph's Coat Basin, identifying two red layer communities among 155 aqueous sites. We compared 16S rRNA libraries amplified using two primer sets (R-GNS- and *Chloroflexacaea*-specific) from these sites, demonstrating novel primer amplification and bias. Analyzing survey and molecular data allowed us to define sub-regions within the basin and suggest potential dispersal patterns.

Introduction

General bacterial and archeal 16S rRNA studies performed at individual sites in Yellowstone National Park have revealed remarkable microbial diversity. Representative study sites include Obsidian Pool (4-6, 10) and Octopus Spring (11, 13-15) (Figure 1, Panel 1). In contrast, we have targeted 16S rRNA studies to Green Nonsulfur bacteria (GNS), retrieving diverse red GNS (R-GNS) sequences from five red layer communities in southern Yellowstone (Figure 1, Panel 1), Red Layer Microbial Observatory sites (RLMOs) (2). A similar GNS-targeted study was recently performed at Mushroom Spring, Yellowstone National Park (Figure 1, Panel 1), using *Chloroflexacaea*-specific primers (8). To date, the only cultured R-GNS is Roseiflexus castenholzii, a red filamentous phototroph from Japan (3).

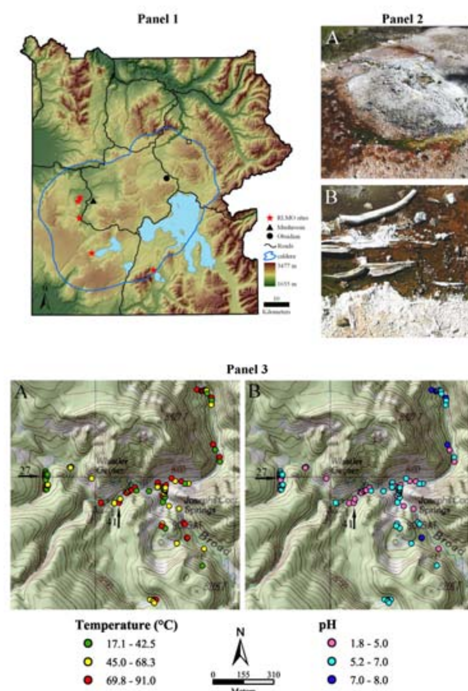

Figure 1: (Click on Image for Full-Screen View) Panel 1: ArcGIS Map of Yellowstone National Park (data obtained from Yellowstone Spatial Analysis Center Website [www.nps.gov/yell/technical/gis/] and USGS Website [http://volcanoes.usgs.gov/yvo/LvlMap.html]) indicating locations of RLMOs from this study and relevant research sites from other cited studies. The square representing JC corresponds to the regions shown in Panel 3. Panel 2: Photographs from Joseph's Coat Thermal Basin. (A) RLMO JC27; (B) RLMO JC41. Panel 3: ArcGIS Map of Joseph's Coat Basin with RLMO sites JC27 and JC41 indicated. (A) Source Temperature Dataset. (B) Source pH Dataset.

We were afforded the opportunity to comprehensively survey Joseph's Coat (JC) basin, a thermal region on the leading edge of the Yellowstone caldera (Figure 1, Panel 1), with the Yellowstone Spatial Analysis Laboratory (12). Among 155 aqueous sites in JC, we identified two red communities that, unlike other Yellowstone red layer communities, formed distinct surface layers. From these samples, we characterized and compared GNS 16S rRNA libraries amplified using two primer sets (R-GNS-specific and *Chloroflexacaea*-specific).

Joseph's Coat survey

JC is located in the canyon carved by Broad Creek (Figure 1). Photographs, GPS, temperature and pH information was gathered at the source for all 155 aqueous JC sites (7) (Figure 1, Panel 3). JC27 and JC41 supported red surface mats (5-10 mm thick) in their run-off channels (Figure 1, Panel 2). JC27, 500 m upstream of JC41, was situated in a drainage that contained primarily low-temperature (17.1-68.3°C), neutral features (pH 5.2-7). JC41 was located in the center of JC where most sources were 45-91°C and pH 1.8-5. Owing to difficult site access, samples had to be stored at ambient conditions for 1-3 days prior to further analysis. JC mats were composed of predominantly red-orange filaments that contained Bchl a (data not shown).

PCR-derived GNS libraries from JC RLMOs

Genomic DNA was prepared as previously described (2). We subjected each sample to PCR amplification with two primer sets: R-GNS-specific 77FGNS/953RRED (2) and *Chloroflexacaea* -specific CCR-344-F/CCR-1338-R (8). These will heretofore be referred to as R and C, respectively. Amplified products were cloned and screened as described (2). Sequences were determined for all inserts and compared with the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) (1). One sample (JC41/2001) would not yield PCR product with R primers after several attempts. In 2002, we retrieved R-amplified product from JC41 after removing lower mat portions we speculated contained problematic mineral deposits.

In screening GNS libraries, we observed a useful EcoRI RFLP that distinguished GNS inserts (Figure 2): green and non-phototropic GNS inserts contained an EcoRI site (Figure 2A, lanes 2, 3, 5, 6); R-GNS inserts lacked this site (Figure 2A, lanes 4, 7-9; Figure 2B, lanes 2-6, 8, 9, 11). This RFLP was ascertained while screening JC27/2001-R and -C libraries, which contained both RFLP types. Consequently, we performed sequence analysis on the most overall clones from these two libraries (Table 1). JC27/2002-C contained similar RFLP distributions (Figure 2A, Table 1). In contrast, three libraries (JC41/2001-C, JC27/2002-R, JC41/2002-R) contained only R-GNS RFLPs among 20-40 clones screened (representative data for JC27/2002–R, Figure 2B). We subjected 5-7 clones from each to

sequence analysis, confirming R-GNS similarity (Table 1). The library obtained from JC41/2002-C contained predominantly inserts bearing EcoRI sites. Of eight representative clones selected for sequence analysis, seven were non-phototrophic GNS-like (EcoRI site present) and 1 was R-GNS-like (EcoRI site absent) (Table 1).

Site Year	Mat Temp.	Source Temp.	Mat pH	Source pH	Library Amplification	R-GNS	G-GNS	Non-R/G GNS
JC27 2001	45	41.2	8	6.05	CCR-344-F CCR-1338-R[a]	11	5	2
					77FGNS 953RRED[b]	15	10	0
JC41 2001	37.7	85.4	7.5	7.94	CCR-344-F CCR-1338-R	5	0	0
					77FGNS 953RRED	NP	NP	NP
JC27 2002	53	ND	7.5	ND	CCR-344-F CCR-1338-R	4	6	2
					77FGNS 953RRED	7	0	0
JC41 2002	61.5	ND	7.5	ND	CCR-344-F CCR-1338-R	1	0	7
					77FGNS 953RRED	6	0	0

Table 1: JC Site and Clone Summary. [a]Nubel et al. 2001. [b]Boomer et al. 2002. NP = no product. ND = not done.

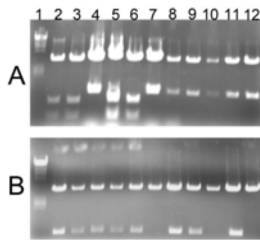

Figure 2: Representative library screens from JC21 (2002) generated with 77FGNS/953RRED (2) (Panel A) and GNS-specific CCR-344-F/CCR-1338-R (8) (Panel B) and screened for insert using EcoRI, which cleaves at vector sites flanking all inserts (2). Lane 1 in both cases is standard marker (Lambda/HindIII) and all lanes contain vector (pCR® 2.1-TOPO, 3.9 kb). Three clones (B, lanes 7, 10, 12) contain no insert. Inserts derived from G-GNS and non-phototroph GNS clones all contained an EcoRI site (A, lanes 2, 3, 5, 6).

Sequences retrieved from JC RLMO samples using these amplification strategies suggested that both primer sets amplified more diversity than previously reported. R-GNS-specific 77FGNS/953RRED (2) also amplified G-GNS-like products. *Chloroflexaceae*-specific CCR-344-F/CCR-1338-R (8) also amplified products most similar to chemotrophic GNS. New nucleotide sequence accession numbers are AY349513-AY349592.

Phylogenetic analyses.
JC phototroph GNS-like sequences and a relevant dataset of GNS sequences were uploaded to the Biology Workbench (version 3.2, San Diego Supercomputer Center, University of California San Diego [http://workbench.sdsc.edu/]). This dataset was aligned as previously described and trimmed to a length that was common to dataset sequences (bases 348-1025 of E. coli) (2). Phylogenetic trees were generated using parsimony methods and tested for robustness with bootstrap resampling as previously described (2). A majority-rule consensus tree was also generated using 1000 most parsimonious trees. There were 303 usable characters out of 727 total in this analysis. The results of these analyses were combined and are presented in Figure 3.

Phylogenetic analysis (Figure 3) revealed two clusters, R-GNS and G-GNS (100% and 77% bootstrap support, respectively). Within R-GNS, R-amplified R-GNS clustered separately from C-amplified R-GNS (majority-rule consensus value of 100%). C-amplified R-GNS sequences from Mushroom Spring (9) grouped between JC-R and JC-C clusters, supporting previous observations that R-GNS sequences often display site-specific grouping (2). Within the G-GNS group, phylogenetic analyses did not reveal such primer-associated patterns. Given that sequences from both sites and years were found together within nearly all primer-specific clusters, we hypothesize that the stream between JC27 and JC41 may serve as a transport mechanism.

Figure 3: : (Click on Image for Full-Screen View) Maximum Parsimony tree of JC RLMO GNS sequences. New JC RLMO community-16S rRNA sequences with accession numbers in parentheses are underlined. In each case, the name indicates the RLMO site (JC27 or JC41), the collection year (01 or 02), the primer set used for PCR amplification (R = 77FGNS/953RRED; C = CCR-344-F/CCR-1338-R), followed by the accession number. Sequences of cultured GNS isolates are indicated in italics with GenBank accession numbers in parentheses. Relevant sequences of uncultured GNS isolates, both RLMO-derived and non-RLMO-derived, are included with GenBank accession numbers in parentheses. Bold numbers represent bootstrap percentages. Filled circles at branch points represent a 100% consensus among 1000 most parsimonious trees. Italicized numbers represent consensus percentages which were less than 100%. Brackets refer to the following groups from this study: (1) R-amplified R-GNS; (2) C-amplified R-GNS; (3) R-amplified G-GNS; (4) C-amplified G-GNS. The bar represents 10 nucleotide changes.

Conclusions.
One goal of this study was to comprehensively survey Joseph's Coat thermal basin, a remote and poorly studied region. Defining this dataset enabled us to map potential niches and transport mechanisms within JC. However, without downstream community data, source data alone is not particularly informative. Survey photographs (data not shown) were useful provided they included downstream communities. Another limitation is the lack of specific water

chemistry information, which would have been useful for better defining niches and selection factors. Despite being located in distinct regions within JC basin, both JC RLMOs contained a similar array of GNS-like sequences. That one of these RLMOs was upstream from the other suggest that water dispersal may play a role in defining downstream habitats, a current focus of study in our lab. Finally, in comparing libraries generated using two different GNS primer sets, we observed that different primer sets amplified distinct R-GNS populations. Although these findings re-confirm the difficulty of designing specific primers given emerging diversity, they also indicate that specific primers can nonetheless yield new information about diversity.

Website and Database Information

The RLMO database can be found at: (www.wou.edu/~boomers/research/allresearch.html).

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