PROJECT DESCRIPTION - NSF-MO/RUI

4. Project Description and Results from Prior Support

Rationale

Hot spring microbial mat ecosystems model early life and are typically supported by filamentous phototrophs (Schopf, et al., 1987, Walsh, et al., 1985). Known filamentous photosynthetic bacteria fall into one of two divisions: oxygenic Cyanobacteria and deep-rooting anoxygenic Green Non-Sulfur (GNS) bacteria (Oyaizu, et al., 1987, Woese, 1987). In contrast with chlorophyll-containing Cyanobacteria, GNS bacteria comprise a less-studied, metabolically diverse group of chemotrophs and phototrophs, the latter of which contain bacteriochlorophylls (Bchl) (Pierson, et al., 1992). Prior to 1998, the paucity of GNS molecular data made understanding this lineage challenging and intriguing (Hugenholtz, et al., 1998). At this time, molecular studies of GNS chemotrophs suggested novel diversity and phylogeny: several dozen uncultivated, activated sludge-derived GNS-like sequences were re-designated members of then-new candidate division TM7 (Hugenholtz, et al., 1998) and comparable studies of Herpetosiphon isolates suggested new relationships within the GNS division (Sly, et al., 1998).

In 1998, representative GNS phototroph isolates included green Chloroflexus and Oscillochloris, and orange Heliothrix (Pierson, et al., 1992). A limited number of culture-independent studies suggested that Heliothrix-like organisms were more widespread than appreciated. Based on physiology, microscopy, and pigment data, Castenholz described "Rabbit Creek Red" (RCR) in Yellowstone (Castenholz, 1984) and our subsequent surveys suggested additional RCR-like communities throughout the park (Boomer, 1999, Boomer, et al., 2000). Ward retrieved two Heliothrix-like sequences from Yellowstone's Octopus Spring. These environmental sequences, along with ribosomal genes of cultured GNS, represented the only 16S rRNA information about GNS phototrophs in GenBank in 1998. In 1999, Hanada isolated Roseiflexus, a red GNS bacterium, from alkaline hot springs in Japan (Hanada, et al., 2002). Given these data, we proposed to establish, describe, and compare Yellowstone "Red Layer Microbial Observatories" (RLMO) based on the following objectives (Boomer, 1999).

(1) *Retrieve 16S rRNA sequences from Yellowstone mats with red filamentous bacteria*
(2) *Design and employ red GNS-specific oligonucleotide primers and probes*
(3) *Broaden Yellowstone surveys to describe the distribution and diversity of red GNS bacteria*
(4) *Isolate Bchl-associated light-harvesting proteins from red GNS bacteria*
(5) *Attempt to culture red GNS bacteria using Roseiflexus methods*

As an RUI proposal, this project would involve undergraduates via research-driven curriculum in Molecular Biology and independent study projects, including team survey trips in Yellowstone. I also proposed using RLMO materials to develop a new research-driven course in Computational Biology and highschool-oriented education outreach activities.

Results from Prior NSF Support

I have received two NSF awards: (1) Improved Laboratory Instrumentation Grant DUE-9851322 (1998); and (2) Microbial Observatories/Research at Undergraduate Institutes Grant MCB-0074452 (2000-2003).

Objective One: 16S rRNA Analysis of Yellowstone Red Layer Communities

Table I summarizes Yellowstone RLMO-derived 16S rRNAs retrieved during this project to date. GNS-like sequences from RLMO communities were most similar to Roseiflexus and formed a distinct cluster within GNS. Subgrouping within this cluster was site-specific (Boomer, et al., 2001, Boomer, et al., 2002, Lodge, et al., 2002, Williams, et al., in preparation). Originally, we hypothesized that clustering correlated with Bchl signatures; however, as with Purple Proteobacteria, this hypothesis was not supported (Boomer, et al., 2002, Glaeser, et al., 1999). Thermal (75-95° C), neutral (pH 7) sourcewater supporting three RLMO features contained GNS sequences and filaments, supporting our hypothesis that RLMO communities are seeded by sourcewater (Williams, et al., 2002, Williams, et al., in preparation).

Table I: Yellowstone RLMO 16S rRNA Sequences

Site	# GNS Sequences	# Non-GNS Sequences	Published	GenBank
Hillside II Spring[a]	10	6	2002	2001
Spray Geyser	10	0	2002	2001
Fairy Spring[a]	7	0	2002	2001
Witch Pond	7	6	2002	2001
Shoshone/Western	10	0	2002	2001
RCR Sample	0	12	no	2001
Joseph's Coat	14	0	in preparation	pending
Imperial Run-Off[a]	7	0	in preparation	pending
Hillside II Source[b]	10	0	in preparation	pending
Spray Source[b]	11	0	in preparation	pending
Imperial Source[b]	11	0	in preparation	pending
Totals	97	24		

a Cloning, screening, and partial sequencing by students in Molecular Biology (Biology 475)
b Carried out by summer research students, Peter Williams (2001-2) and Terry Manning (2002-3)

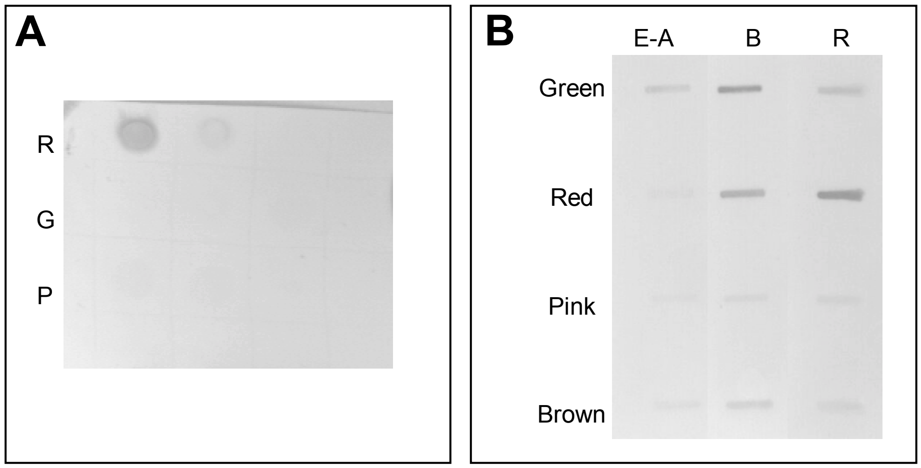
Objective Two: Red-orange GNS-specific probes

Based on new data, we designed GNS-specific primers (77FGNS and 953RRED) for PCR, using them to generate all but three Yellowstone libraries (Boomer, et al., 2002). Interestingly, these primers amplified three novel green-like sequences from Imperial sourcewater, indicating that they are not truly red-GNS-specific and that green GNS diversity is higher than appreciated (Williams, et al., in preparation).

In the last year, Danny Lodge (research assistant, former undergraduate) and I have developed hybridization methods using 953RRED. Hopes to develop a fluorescent *in situ* hybridization (FISH) system proved impossible because of natural autofluorescence by low levels of cohabiting Cyanobacteria. Consequently, we altered our strategy to a blot-based DIG label system. 953RRED-DIG hybridized control DNAs as predicted; application of 953RRED-DIG to total RNA from sectioned mat layers of three RLMO (Hillside, Fairy, Imperial) suggested that red GNS sequences primarily localize with red layers (Figure 1) (Lodge, et al., 2002). However, given aforementioned sourcewater results, we can not rule out 953RRED binding to novel green

GNS organisms. Cluster-specific red GNS probes are being developed based on 16S rRNA loops that exhibit site-specific variation (Boomer, et al., 2002).

Figure 1: GNS Probe Studies



Panel A Dot blot of 16S rRNA genes hybridized to 953RRED-DIG. Row R: Hillside II red GNS clone 10 (1 µg – 10 ng dilution series). Row G: 1 µg each - Chloroflexus, uncultivated Green Sulfur and Cyanobacteria (both Yellowstone-derived). Row P: 1 µg each – three uncultivated Proteobacteria (Yellowstone-derived).

Panel B Slot blot of total RNA from Imperial Run-Off RLMO mat layers hybridized to three DIG-labeled 16S rRNA probes. Column E-A: ARC/EUK-1373R, an Archea- and Eukaryote-specific probe (Hugenholtz, et al., 1998); Column B: BAC-924R, a Bacteria-specific probe (Hugenholtz, et al., 1998). Column R: 953RRED-DIG.

Unknown diversity makes probe design a daunting process, exacerbated by the fact that full-length 16S rRNA sequences remain unavailable for orange Heliothrix (Weller, et al., 1992), which enigmatically still groups among green GNS (Boomer, et al., 2002). Thus, we set out to clone full-length Heliothrix. Unfortunately, original cultures were lost and we resurveyed two Oregon springs that harbored Heliothrix-like filaments (Castenholz, personal communication). We retrieved six novel, full-length red-like GNS-like sequences from Kah-nee-tah, Warm Springs (eastern Cascades). Mats from Borax Lake (Alvord Desert) provided one full-length Heliothrix-like sequence. Oregon RLMO data were generated, in large part, by undergraduates in Molecular Biology (2002) (Lodge, et al., in preparation).

Objective Three: Yellowstone Biogeography and Biodiversity

As proposed, we surveyed Shoshone, Potts, and White Creek Basins and will survey the Bechler Plateau in 2003; only Shoshone contained a flourishing RLMO (Boomer, et al., 2002). Additionally, the Yellowstone GIS Lab lead a survey of remote, off-trail Joseph's Coat thermal basin (August, 2002); undergraduate research students and I also participated in this work. Otherwise high-acid (pH 2-4), Joseph's Coat contained two uniquely alkaline RLMO with distinct red GNS sequences (Lodge, et al., 2002). Finally, inspired by Bonheyo's Mammoth-based sourcewater studies (Bonheyo, et al., 2001, Potera, 2001), undergradute Peter Williams (2001-2) performed aforementioned sourcewater studies at three established RLMO (Hillside, Spray, Imperial) (Williams, et al., 2002, Williams, et al., in preparation). Undergraduate Terry Manning (2002-3) is continuing this exciting project.

Objective Four: Light Harvesting Genes

With just over one year left on this grant, we have put no effort into this objective because this question was addressed by the Madigan lab using Purple Proteobacteria, both cultured and environmental isolates (Achenbach, et al., 2001). Plans to apply light-harvesting gene-specific primers to Roseiflexus were discussed at the 2002 ASM General Meeting (Achenbach, personal communication) and I look forward to sharing RLMO material as their studies progress.

Objective Five: Red Layer Culture Work

Given Hanada's successful culturing of Roseiflexus (Hanada, et al., 2002), we applied these culture methods to three Yellowstone RLMO samples (Hillside, Spray, Fairy) to no avail (Boomer, et al., 2002). Interestingly, filaments from the aforementioned Kah-nee-tah RLMO proliferated in this media through one passage. We routinely propagate Roseiflexus in our lab as a control for hybridization studies and so we believe our results do not reflect poor media preparation or lab technique.

Education Outcomes

Education outcomes of this grant include new research-driven curricula for three undergraduate course labs, summer research experiences, and education outreach - all summarized in the RUI Impact Statement section of this grant. In sum, 83 undergraduates and 65 pre-college students have been impacted by this project (Table II). All education materials are available free, on-line via my university/RLMO website (Boomer, ongoing). Molecular Biology (Biology 475) curricula have been published and presented locally, nationally, and commercially (Boomer, et al., 2001, Boomer et al., 2002, Li-Cor, 2002).

Table II: Impact of RLMO-Based Education Projects

Kind of Education	Students (1999-Present)
Research-Driven Curricula	
Molecular Biology (Biology 475)	23 Undergraduates
Computational Biology (Biology 301)	9 Undergraduates
Microbiology (Biology 331)	34 Undergraduates
Summer Research Experiences	
Summer Field Survey Trips	10 Undergraduates
Summer Stipend Recipients	4 Undergraduates
Education Outreach	
Undergraduate Mentors/Assistants	3 Undergraduates
Pre-College Participants	65 Middle/Highschool students
Total	*83 Undergraduates; 65 Pre-College*

Research and Management Plan

Given results and outcomes described in the previous section, I propose the following objectives for this RLMO/RUI project renewal: (1) Implementation of new molecular and geochemical survey methodology and equipment; (2) Expanded biodiversity surveys and longitudinal studies; (3) Improvements to RLMO-based curricula and education outreach; and (4) Development of an improved on-line database for all aspects of this project.

OBJECTIVE ONE: Implementation of new molecular and geochemical survey methodology

New Molecular Survey Methods - in situ PCR and DGGE

Improved understanding of red GNS diversity has facilitated the development of GNS-specific probes (Boomer, et al., 2002, Lodge, et al., 2002). Given new tools, I propose implementing two powerful molecular survey methods: *in situ* PCR and denaturing gradient gel electrophoresis (DGGE). These new methods will be included in all RLMO studies proposed, including a longitudinal study of four established RLMO (Objective Two) and new research-based lab curricula (Objective Three).

In situ PCR will enable us to fix mat layer samples (homogenates and whole-mat thin-sections) on slides and directly amplify red-GNS targets (DNA or RNA) using specific primers, DIG-labeled dNTPs, and a slide-adaptable thermal cycler (Ausubel, et al., 1997). Amplified targets will contain incorporated DIG-label that can be visualized and documented within cells using an anti-DIG alkaline phosphatase conjugate and existing microscopy methods (Tani, et al., 1998). Such data will be significant for the following reasons: First, GNS sequences may, for the first time, be directly linked with specific filamentous members of the mat. Second, whole mat hybridization studies will better address the following questions: Is there a gradient of GNS expression or GNS diversity within a given red layer? Do some red GNS sequences penetrate upper green layers? Indeed, whole mat hybridization studies will likely reveal additional unique niches for GNS diversity, suggesting additional phylogenetics studies.

DGGE provides an effective means to assess microbial community diversity based on mutational and topological differences between 16S rRNA genes (Muyzer, et al., 1998). In implementing this method, we will employ reverse-transcriptase-mediated PCR (RT-PCR) to amplify total 16S rRNA from defined mat sections. An alternative set of standard internal 16S rRNA general bacterial primers will, as recommended, be modified with a GC-clamp and used for this step (Casamayor, et al., 2002). Product will be separated using DGGE and hybridized with GNS-specific probes. These data will be significant for the following reasons: First, positional GNS diversity may be analyzed to address whether there are unknown GNS niches within red and non-red sections of a given mat community. Second, relative abundance of GNS organisms may be assessed both temporally and as a function of site chemical parameters described in the next section. However, this method is not truly quantitative given potential selection during DNA extraction or amplification. Third, DNA bands may be directly isolated, cloned, and subjected to DNA sequence analysis, permitting the discovery of new GNS variants localized in specific regions of a given mat at a given point in time.

New Geochemical Survey Methods

As recognized by the American Academy of Microbiology, it is important that more molecular microbiologists become involved in surveying geochemical information about microbial habitats, whether for databasing efforts, improving media development, or making better inferences about retrieved sequence information (Nealson, et al., 2001). For example, our GNS-like sequences phylogenetically cluster in a site-specific manner (Boomer, et al., 2002, Lodge, et al., 2002) but groups do not correlate with any of the limited community features we currently survey (temperature, pH, pigment signatures). Park-based surveys perform equally limited site analysis (Stoner, et al., 2001) and, with the exception of Joseph's Coat, park survey teams have yet to database any RLMO sites. Consequently, our 2002 undergraduate survey team (Melissa Boshee, Terry Manning, and Peter Williams) performed a preliminary assessment of four RLMO sourcewater and mat homogenates using eleven simple, available colorimetric test kits. Data suggested, in several cases, site-specific chemical variation, supporting our working hypothesis that chemical determinants correlate with observed molecular diversity (Table III).

Given these promising preliminary data, we propose performing a more extensive survey of site chemical parameters using a more precise portable spectrophotometric system. Site sourcewater and mat homogenates will be analyzed, databased, and compared, with the hope of correlating site-specific molecular diversity with site chemistry - and potential changes over time in terms of both. Proposed chemical signatures will include metals, nitrogen compounds, sulfur componds, dissolved oxygen, silica, and chlorine (Gold, 1992, Reysenbach, et al., 2002). These new methods will be applied to all RLMO studies proposed in Objective Two. New methods troubleshooting will be performed by survey team members during the summer of 2003 and a new undergraduate summer research course experience will be developed around RLMO longitudinal studies (Objective Three).

Table III: Chemical Anlaysis of RLMO Sourcewater vs. Mat Communities

	Fairy		Imperial		Spray		Hillside	
	Source	Mat	Source	Mat	Source	Mat	Source	Mat
Sulfide	0.01	0	0.1	0.5	0.14	0	0.01	3
Nitrate	1.4	45	2	40	2.3	30	1.7	105
Sulfate	15	0	25	0	17	0	8	75
Silica	28	54.5	17.6	92	246	61	20.2	88.5
Free Ammonia	0	0.5	0	0	0	0	0.09	3
Molybdenum	0.1	10	0	0	1	0	0	22.5
Aluminum	0	13	0.217	0.25	0.009	10.2	0	18.525
Copper	0	0	0	1	0	0	0	3
Total Iron	0.03	5.5	0.06	1.5	0.06	3.5	0.09	6
Manganese	0	0.3	0.012	0	0.002	0.2	0.018	1.2
Zinc	0.08	22.5	0.11	7.5	0.33	7.5	0.05	18.75

All values reported in mg/L

Finally, in collaboration with the Thermal Biology Institute (TBI), sourcewater and mat homogenates will also be subjected to advanced aqueous and solid state chemical analyses (see supporting letter, Supplementary Documentation). Given that samples must be transported to the TBI for analysis, summer students will have the opportunity to tour these facilities and learn about alternative chemical, spectroscopic, and microscopic methods. It should be noted that TBI staff will perform actual chemical analyses, providing data to my lab after the sample drop-off and educational tour.

OBJECTIVE TWO: Expanded biodiversity surveys and longitudinal studies

Undergraduate field experiences in Yellowstone will include longitudinal surveys of four established RLMO, analysis of four "red-negative" sites, and new Yellowstone RLMO surveys. All data described below will be archived in the proposed new, improved RLMO on-line database (Objective Four).

Longitudinal Surveys of Four RLMO Sites

RLMO sites will include easily accessible Fairy, Spray, Hillside, and Imperial features. Anecdotally, we have noted that source features and RLMO communities at most of these sites have physically changed over time (e.g. altered thermal output or source relocation with concomitant movement or thickness changes in the supported RLMO community). For this study, site analysis and collection will be performed by teams of six undergraduates over four consecutive summers during the second week of July (2004-2007). In the field, student teams will perform site, source, and mat community documentation, including proposed new chemical analyses (Objective One). Students will precisely measure, section, and photo-document mat communities into different-colored layers that will be analyzed upon return to the lab (based on observations over the last 3-5 years, each mat will be dissected into 2-3 layers total). In the lab, students will analyze mat sections and pigments using existing microscopy and spectroscopy methodology, and using newly proposed methods for *in situ* PCR (Objective One). Total RNA will be extracted from each layer and subjected to two fates: (1) RT-PCR/DGGE with GNS-probe hybridization (Objective One) and (2) total RNA slot-blotting with GNS probe hybridization. Annual RLMO sample sections will be developed into 16S rRNA libraries and analyzed by undergraduates in my improved Molecular Biology course, offered every spring (Objective Three).

Analysis of Red-Negative Sites

New RLMO surveys will assess four different "red-negative" samples (one collected each summer) for the presence of red or atypical green GNS sequences in our effort to understand potential origins, transfer, and selection of GNS bacteria. Sites will be selected on the basis of having no visible red layer and/or having non-RLMO-associated pHs and/or temperatures. Recommended, accessible sites include the Firehole River near Ojo Caliente (neutral pH, cold), Mound Spring in Sentinal Meadow (neutral pH, near-boiling), Imperial Mudpot (high acid, warm), Rabbit Source Run-Off (green mats only, despite typical RLMO pH and temperature). In the field, student research teams will perform site and source documentation, including newly proposed chemical analysis (Objective One). In this case, however, "red negative" samples will be frozen and I will use them to generate 16S rRNA libraries for analysis during the improved molecular microbiology unit of Microbiology (Objective Three).

New RLMO Discovery

Finally, given ongoing discovery of novel GNS sequences throughout Yellowstone and Oregon, three “new” thermal regions in Yellowstone will be assessed for new RLMO communities: (1) Smoke Jumper Basin (Madison Plateau); (2) Violet and Highland Hot Springs Basin (Central Plateau); and (3) Wapiti Lake/Hot Springs Basin (Mirror Plateau). All comprise backcountry sites that are geographically distant (10-30 miles) from known RLMO communities. The first two involve single-day hikes and will be completed during the new proposed summer course trip (Objective Three). The latter involves a multi-day backpack and will comprise a separate trip with only experienced students and leaders; additional funding for this trip will be supplied by participants and campus support. New RLMO samples will be used to generate 16S rRNA libraries by independent study students and/or used in education outreach projects during the regular academic year.

Yellowstone Permit Status

Since 1997, I have maintained ongoing permits to conduct microbiology research in Yellowstone National Park (Boomer, 1997-present).

OBJECTIVE THREE: An Improved RLMO Education Program

Expanded Summer Research and Field Experiences

Current funding supports two students' travel to Yellowstone and research/thesis development. Unfortunately, I turn away 6-8 interested students each summer, either because I lack additional travel/research funds or because many students need, at the very least, part-time summer employment to earn money for college tuition during the regular academic year. To address this need and facilitate more repetitive longitudinal RLMO studies, I am replacing my previous summer stipend program with an expanded field and research course experience that will support six undergraduates. This program will be scheduled so students who have family or financial obligations can plan and participate accordingly (Table IV). Each student will prepare a required web-based portfolio and results will be made available on the RLMO website and database. Students will have the option to continue projects and/or give talks or posters during the regular academic year via available independent study credit options.

Table IV: Summer Field and Research Experience Schedule

Week	Activity	Times
One	Introduction to Field Methods and Equipment	Wednesday, Thursday 9-3
Two	Yellowstone Field Survey and Collection	All week
Three	Pigment, Microscopy, in situ PCR	Monday-Wednesday 9-3
Four	RT-PCR, DGGE	Monday-Wednesday 9-3
Five	Probes and Hybridization	Monday-Wednesday 9-3

Improved Microbiology Curriculum

I am currently restructuring my majors-level Microbiology course (Biology 331), a core course required for all biology majors (32-40 students per year). Specific changes include the following:

(1) Microbiology will be converted to a two-track option in Applied/Environmental (Biology 390, to be offered every fall to 16-20 students) and Medical Microbiology (Biology 391, to be offered every winter to 16-20 students); (2) both track lab components will be increased from one to two 3-hour sessions; (3) both tracks will now mandate pre-requisites in Genetics, Evolution, and Cell Biology; and (4) both track labs will involve parallel curricula that will equally emphasize culture-dependent and culture-independent methodology. In terms of this project, I propose to integrate RLMO research into the new Applied/Environmental Microbiology core option (Biology 390). During the first five weeks of this course, I will emphasize culture-dependent isolation methods and phenotypic testing based on current protocols from my existing class (coliforms, phototrophs, nitrogen fixers, antibiotic-producers, and amylase-producers – all from local sources). During the last five weeks, students will work with the RLMO project, analyzing "red negative" 16S rRNA clone libraries (Objective Two). Specifically, they will screen provided libraries for insert-bearing clones, determine the DNA sequence of 16S rRNA clones, and identify clones using BLAST and computer-based phylogenetic methods.

Improved Molecular Biology Curriculum

Given new curricula in my Microbiology course, I will make the following improvements to my existing research-based program in Molecular Biology, offered every spring for up to 16 students. In this case, students will use established methods to generate their own libraries from annual longitudinal study samples (Boomer, et al., 2002); additionally, students will perform new methods in RNA isolation, RT-PCR, DGGE, and nucleic hybridization.

Improved Education Outreach

Despite limited, nonspecified commitments to education outreach in our current grant, research assistants, mentees, and I have served 65 diverse local middle and highschool science students through a variety of mechanisms (Table II and RUI Impact Statement). These activities have been facilitated by on-campus Upward Bound Programs and Oregon Junior Academy of Science Events, and by the off-campus Saturday Academy (Oregon State University-affiliated). Given my great interest in improving research and education impact at the pre-college level, I am requesting funds to support an education coordinator, in addition to release time that will enhance my ability to facilitate outreach.

The proposed outcome of this education outreach program will be an official program of regular, scheduled activities that will draw primarily from RLMO-based activities but also coordinate other activities executed by members from our division's Environmental Science Institute (ESI). This project will be modeled after Saturday Academy but remain an independent, campus-based program. Each spring, the education coordinator and I will distribute a sign-up sheet to ESI faculty for the following academic year. Based on feedback, a schedule of activities will be generated by the end of spring term. Newsletters containing this schedule and related information will be distributed to our campus Upward Bound office and eight local highschool science programs on a quarterly basis, each advertising events for the year with sign-up/contact information. Each term, the education coordinator will lead 2-3 three-hour both existing and new RLMO-based activities and the rest of slots will be filled by other ESI members. At least eight other division professors have instructed comparable, voluntary outreach modules; thus, interest in a grassroots program is high, provided a reliable mechanism for scheduling and advertising events. It should be stressed that the RLMO coordinator will only act as a scheduler and liaison – not an instructor or preparator – for non-microbiology related activities. Instructors in the series will be asked to complete and on-line activity description, to be compiled for an education website about this program, and to report assessment based on existing RLMO templates. In addition to scheduled campus activities, we will advertise and accommodate off-campus class visits when feasible. Finally, we will develop all of the above modules into credit-based teacher workshops, offering them 3-6 weekends a year. Precedent for such a program exists at this time: a three-part

"Biotechnology" workshop series I offered with support from the Waksman Foundation attracted six masters candidate teachers and four undergraduate education majors.

OBJECTIVE FOUR: An Improved RLMO Database

The current RLMO database is a rudimentary on-line collection of linked HTML documents and tables (Boomer, ongoing). Stipend recipient and former Computational Biology student Melissa Boschee (2002-3) is working with on-campus collaborators Dr. Bryan Dutton and Matt Drury (undergraduate computer services technician) to develop an on-line, searchable database using Oracle and Arc-IMS. Melissa's goal is to create a long-term datafile structure and populate this template with current, available information from the four RLMO targets for proposed longitudinal studies. Templates will serve to analyze current project information and allow for the organized addition of long-term project data based on new objectives in this proposal. On-line HTML-based entry forms have been developed for database management that can be updated by me and future research students over time (Boomer, ongoing). The datafile structure for the Improved RLMO database includes the following elements: (1) GIS-referenced Site Data (photography, pH, temperature, and chemical data – all linked to timepoint information); (2) Mat Layer Data (thickness measurements, pigment spectroscopy, microscopy images, in situ PCR images, and DGGE/hybridization data – all linked to site, layer, and timepoint information); (3) DNA Information (GenBank flatfile information, BLAST result data, and general information about known genera that are similar to RLMO community members – all linked to site, layer, and timepoint information); and (4) Phylogenetic Trees (annual site information will be analyzed against a defined dataset and presented as a graphic image – all linked to site and timepoint information). The improved RLMO Database will be permanently housed on a server at WOU, linked to my existing RLMO website that serves to archive curriculum, student-generated websites, and outreach activities/programs.