PROJECT DESCRIPTION

BACKGROUND AND SIGNIFICANCE

Moderately thermophilic (45-60°C) microorganisms such as those thriving in hot spring microbial mats are hypothesized to be modern homologs of the most ancient forms of life on earth (Schopf and Packer, 1987; Walsh and Lowe, 1985). Typical hot spring communities are largely composed of filamentous photosynthetic bacteria arranged in layers according to light-harvesting and metabolic properties. For example, many mats in Yellowstone National Park consist of a surface layer of cyanobacteria and an underlayer of Chloroflexus (Pierson and Castenolz 1974, 1992). Phylogenetically, these bacteria comprise diverse genera: Chloroflexus is an anoxygenic Green Non-Sulfur Bacterium, an ancient lineage of eubacteria (Oyaizu et al. 1987). In contrast, oxygenic cyanobacteria represent a distinct phylogenetic lineage that is predicted to have arisen significantly after the Green Non-Sulfur Bacteria (Woese, 1987). While these phototrophs visibly appear to be the predominant organisms in the mat community, a remarkable diversity of microorganisms, many entirely novel, has been observed via the application of molecular methodology to hot spring communities (Ward et al., 1998).

To date, surprisingly few anoxygenic filamentous phototrophic bacteria have been characterized. Those described can be broadly divided into two groups: genera with both bacteriochlorophyll (Bchl) a and c and genera with only Bchl a. The latter include Chloroflexus and some species of Oscillochloris. Chloroflexus has been observed in hot springs, hypersaline, marine, and freshwater habitats (Pierson and Castenolz 1974, 1992; Pierson et al., 1994). Chloroflexus typically carries out photoheterotrophy and displays unique intracellular membrane vesicles called chlorosomes. Similar Bchl-a and -c-containing Oscillochloris have been isolated from non-thermal freshwater environments (Keppen et al., 1994).

Filamentous bacteria containing only Bchl a have been observed but not fully isolated from freshwater thermal environments (Pierson et al., 1984). Heliothrix was observed in hot springs in Oregon (Kahneeta, Warm Springs) where it forms a bright orange layer on the surface. It has not been successfully cultivated in axenic culture, although one successful co-culture with the chemotrophic bacterium, Isosphaera pallida was obtained (Pierson et al., 1985) . Cells contain no distinctive ultrastructural membranes commonly associated with phototrophic bacteria (Pierson et al., 1985). Phylogenetically, Heliothrix is most closely related to Chloroflexus (Pierson et al., 1985; Weller et al., 1992).

Dr. Richard Castenholz first observed an unusual bacterium called "Rabbit Creek Red" (RCR), that formed a deep red layer within a microbial mat near Rabbit Creek in Yellowstone National Park (Castenholz, 1984); we continued to study it for over a decade from 1981 until its demise in 1996 when the spouting geyser source dried up . The Rabbit Creek mat was composed of three layers: a top layer of cyanobacteria, a second layer of Chloroflexus, and a deep third red layer that contains RCR. RCR consisted of filamentous cells with complex internal membranes called lamellae. RCR contained Bchl a as its primary photosynthetic pigment (methanol extract pigment absorbance maxima = 770 nm); the in vivo absorption maxima for the RCR Bchl a pigment-protein complex was 915 and 804 nm. RCR was photoheterotrophic, as shown by the uptake of radiolabeled acetate by mat suspensions in different conditions of light . RCR maximally incorporated acetate in light wavelengths of 840 and 910 nm. These conditions reflected the light energy that actually penetrated to the red layer within the mat, as demonstrated by spectrophotometric measurements determined in vivo. Metabolism was unaffected by sulfide. In contrast with Heliothrix oregonensis, RCR was exposed to limiting concentrations of oxygen and light intensity. Only one other organism, a recently identified unicellular purple non-sulfur bacterium, Roseospirillum parvum, displays Bchl a-protein maxima beyond 900 nm. Roseospirillum, a member of the Proteobacteria lineage, was isolated from hypersaline mats and, like RCR, contains extensive lamellae. Notably, Roseospirillum was designated a new genus and species in part because of its novel and distinctive Bchl a pigment-protein absorbance properties (Glaeser and Overmann, 1999). A summary of these characteristics is shown in Table I (Boomer et al. Submitted).

TABLE I

Feature	RCR	Heliothrix	Roseospirillum
Mat habitat			
temperature	40° C	45° C	20-30°C
pH	8.1	8.2	7.9 (and 1-2% saline)
color/position	deep red 8 mm	surface orange layer	deep brown (variable)
oxygen	limiting	saturated	variable
Cell Structure			
cell shape	filamentous	filamentous	unicellular vibroid
cell dimensions	1 X 7 µm	1 X 7 µm	0.5-2.2 µm
membranes	lamellae	none	lamellae
Pigments			
Bchl a	770 nm	770 nm	770 nm
Bchl a-Protein	840 and 910 nm	795 and 865 nm	806 and 911 nm
Metabolism	photoheterotroph non-sulfur	photoheterotroph non-sulfur	photoheterotroph non-sulfur
Classification	unknown	Green Non-Sulfur	Proteobacteria

Taken together, these data suggest that RCR may represent a new species of Heliothrix or a new genus entirely. While RCR displays similarities to Heliothrix and Roseospirillum, it is distinct. Despite several years of attempts, RCR was never isolated successfully in axenic culture or grown in co-culture; nor have DNA methods (16S rRNA amplification and sequence analysis) been applied to this community, although glutaraldehyde fixed specimens have been preserved and may be utilized for such analysis.

Owing to the demise of the Rabbit Creek Spouter in 1996, we began surveying Yellowstone National Park for new representatives of Heliothrix-like organisms. Over the summers of 1998 and 1999, a total of eleven undergraduate research students and I performed surveys of several thermal basins in Yellowstone National Park. Data gathered on these trips was used to generate the schematic map shown in Figure 1 which illustrates sites that harbored Heliothrix-like organisms (we also ruled out many regions that fail to harbor Heliothrix-like bacteria, as noted in Figure 1). A general overview of park thermal features and basins is summarized in Bryan, 1995.

Phenotypic criteria used for positive site designation (Heliothrix-like organisms present) included the temperature and pH of the habitat and macroscopic analysis of the mat for obvious deep red layers. In the field, students worked as a team to map sites using global positioning system equipment, to characterize habitats using pH and temperature sensors, to record data and drawings of the mat structure in formal notebooks, and to coordinate labeling and documentation of collected biological samples as required by the Yellowstone Center for Resources (Figure 2). Following each field trip, one or two research students worked under my direction to characterize mat communities and pigment/pigment-protein complexes from mat samples. All data have been combined and are summarized in Table II. Red/orange layers from samples observed to contain both filaments and Bchl a were carefully dissected into multiple aliquots and frozen at -80°C for future DNA analysis. Some of these data have been reported in Boomer et al. (Submitted).

HYPOTHESIS AND OBJECTIVES

The identity of each Yellowstone red layer organism and its relationship with both Heliothrix and other Yellowstone isolates remains to be determined. The most distinctive features of the Yellowstone isolates are (1) the high content of lamellae of RCR and (2) the diversity of in vivo absorption maxima for pigment-protein complexes among nearly all Yellowstone isolates (the most notable being above 900 nm).

FIGURE 1

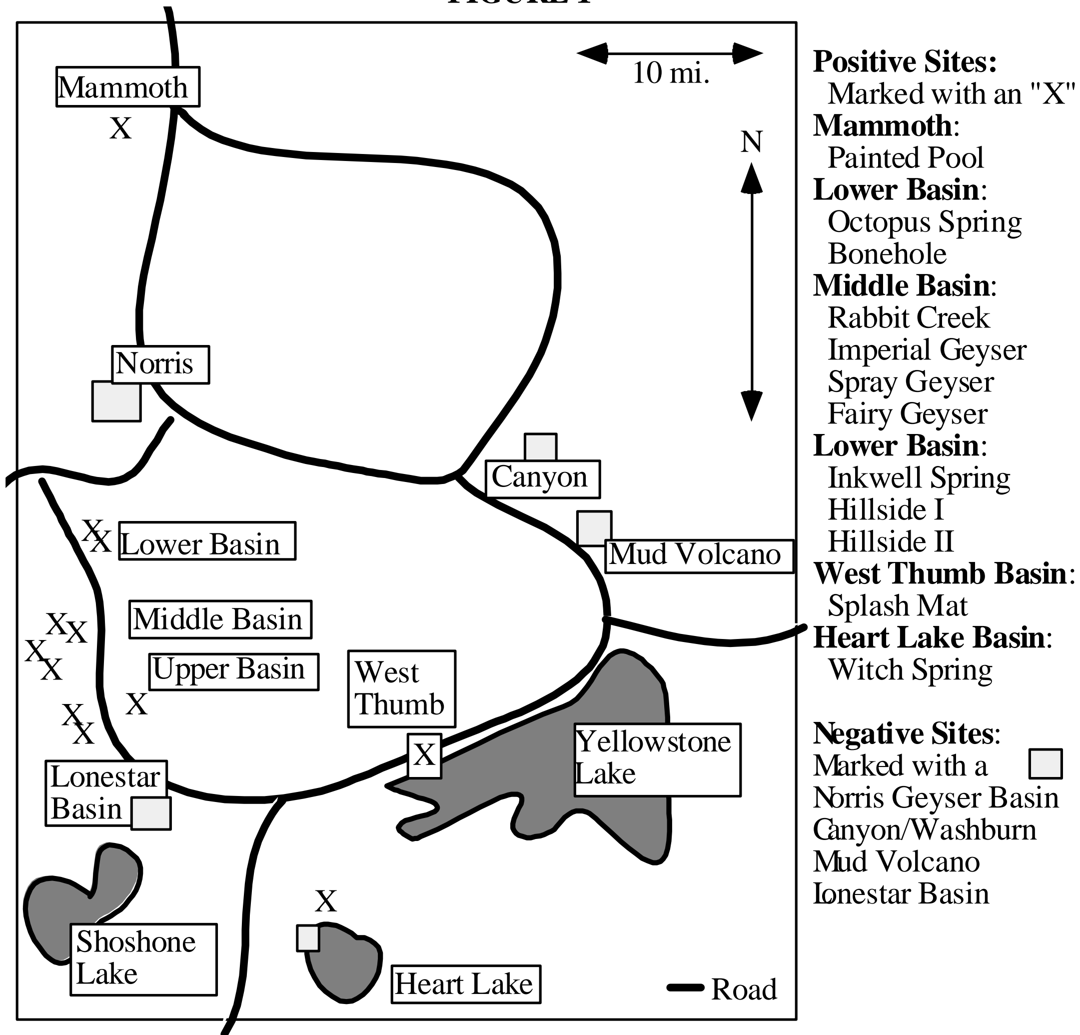

TABLE II

Specific Site	Region	Temp.	pH	Bchl a	Bchl a-protein
Heliothrix oregonenesis (for comparison)		45°C	8.2	770 nm	795, 865 nm
Painted Pool	Mammoth	42°C	8.1	771 nm	796, 867 nm
Hillside I	Upper Geyser Basin	52°C	8.0	771 nm	803, 886 nm
Hillside II	Upper Geyser Basin	54°C	8.5	771 nm	802, 881 nm
Inkwell	Upper Geyser Basin	48°C	8.7	770 nm	801, 880 nm
Rabbit Creek	Middle Geyser Basin	40°C	8.1	770 nm	840, 910 nm
Imperial Geyser	Middle Geyser Basin	62°C	8.5	771 nm	743, 884 nm
Spray Geyser	Middle Geyser Basin	45°C	9.0	769 nm	801, 905 nm
Fairy Geyser	Middle Geyser Basin	54°C	8.0	769 nm	804, 901 nm
Bonehole	Lower Geyser Basin	62°C	8.2	771 nm	797, 886 nm
Octopus Spring	Lower Geyser Basin	58°C	8.2	771 nm	798, 887 nm
Splash Mat	West Thumb Basin	50°C	7.9	770 nm	799, 882 nm
Witch Spring	Upper Heart Lake	63°C	8.0	769 nm	796, 884 nm

With regards to (1), we hypothesize, based on observations in other anoxygenic phototrophs, both purple Proteobacteria and Green Non-Sulfur bacteria, that transcription-level expression differences mediate observed structural differences (Elsen et al., 1998; Bowman et al., 1999; Tadros et al, 1993; A Gall and B Robert, 1999; Madigan and Brock, 1997). With regards to (2), we hypothesize that distinct absorption properties of pigment-protein complexes is mediated by distinct light-harvesting proteins and, hence, DNA sequence-level differences. The utility of photosynthesis-related proteins in assessing phylogeny has been described and, as proposed in Objective Four (See below), we intend to incorporate similar approaches in the analysis of Heliothrix and Heliothrix-like Yellowstone isolates (Xiong et al., 1998; Allen and Williams, 1998).

To date, none of the Yellowstone isolates has been purified in culture (Boomer et al., Submitted). Because these organisms have proven difficult to isolate, we aim to use molecular approaches to identify what appear to be novel, significant, and widespread components of hot spring microbial mats in Yellowstone National Park. In so doing, we hope to significantly add to the sparse database of Heliothrix relatives. Presently, national DNA databases (Ribosomal Database Project and GenBank) contain only two representatives of Heliothrix. One was derived from the Heliothrix oregonensis co-culture (Weller et al., 1992). The other was derived from Octopus Spring, Yellowstone while screening the mat population for diversity using standard eubacterial 16S rRNA probes (Weller et al., 1992). Based on all of our preliminary studies and the published results of others, we have proposed the following specific hypotheses and objectives (Boomer, 1998, 1999).

Hypotheses:

(1) Heliothrix-like organisms thrive in many mat communities in Yellowstone National Park.

(2) Heliothrix-like organisms are more widely distributed than previously thought.

(3) Yellowstone-derived Heliothrix-like isolates represent, minimally, a new species.

Objectives:

(1) To characterize PCR-amplified 16S rRNA sequences from Yellowstone mat communities that harbor Heliothrix-like organisms.

(2) To design and employ Heliothrix-specific oligonucleotides.

(2) To broaden ecological surveys of thermal mats in Yellowstone National Park in order to characterize the distribution and diversity of Heliothrix-like organisms.

(3) To isolate and compare Bchl-a-associated light-harvesting proteins from Heliothrix and Heliothrix-like organisms, using these proteins as secondary markers by which to phylogenetically assess diversity among members of this lineage.

Figure 2: Student Research Team, Hillside I Spring

SPECIFIC METHODS AND PRELIMINARY RESULTS (INCLUDES "RESULTS FROM PRIOR NSF SUPPORT STATEMENT" DESCRIPTION)

Objective One: 16S rRNA Analysis

Specific methods for objective one were established by me while working with Jim Staley and Brian Hedlund (Dept. of Microbiology, University of Washington) over winter and spring break (1998-9) using mat samples collected during the 1997 field research trip. During winter and spring term (1999) research students and I, either in the context of Independent Study Coursework or a course research project in Molecular Biology Lab (Biology 475), successfully employed these methods to extract total genomic DNA from mat communities at Hillside II Springs (Upper Basin), Fairy Spring (Middle Basin), and Bonehole Spring (Lower Basin). Extraction methods were adapted from Kerkoff (Brian Hedlund, personal communication). We utilized standard eubacterial 16S rRNA primers (forward primer = GCGGATCCGCGGCCG CTGCAGATGTTGATCCTGGCTCAG; reverse primer = GGCTCGAGCGGCCGCCCGGG TTACCTTGTTACGACTT) to amplify a 1.2 kb fragment that corresponded to the predicted 16S rRNA gene product (primers provided Brian Hedlund/Jim Staley; sequence based on

Reysenbach et al., 1994; PCR Supermix reagents from Gibco Inc.). Amplified products from only Bonehole and Fairy Springs were directly cloned into the pCRII-TOPO vector (Invitrogen, Inc.).

In May 1998, I was awarded an NSF-ILI grant that furnished my laboratory with a small-scale automated DNA sequencer (Li-Cor, Inc.). Thus, upon cloning 16S rRNA genes, students utilized the DNA sequencer to obtain sequences for six Bonehole isolates and one Fairy Spring isolate. They submitted each sequence to the National Center for Biotechnology's (NCBI) internet-based BLAST program to determine which sequences in the database most strongly resembled their clones (Altschul et al., 1997). Three isolates demonstrated strong homology (greater than 45% homology) to other uncultured Yellowstone-derived thermophiles. The third demonstrated strong homology (80%) to Aeromonas. The remaining two, however, demonstrated limited homology (less than 10%) to miscellaneous bacterial 16S rRNA sequences in the database. A summary of these results is shown in Table III below.

TABLE III

Clone	**Closest Sequence** **(% Homology)**	**Location** **(*Yellowstone-derived)**	**Reference**
Bonehole 16	uncultured eubacteria (60%)	Obsidian Pool*	Graber et al., unpublished
Bonehole 17	uncultured eubacteria (46%)	Octopus Spring*	Reysenbach et al., 1994
Bonehole 7	Clostridium-like (22%)	Octopus Spring*	Ward et al., 1992
Bonehole 6	Nitrospira-like (55%)	Obsidian Pool*	Hugenholtz et al., 1998
Bonehole 4	Clostridium cluster (1%)	various	Lawson et al., unpublished
Bonehole 2	uncultured eubacteria (10%)	Mummy, Alps	Cano et al., unpublished
Fairy 1	Aeromonas (80%)	various	Kaku et al., 1999

In the mean time, research mentees and I are troubleshooting two recommended approaches to select Heliothrix-like organisms prior to DNA isolation. Both rely on the observation that Heliothrix-like organisms form distinctive long filaments and are thus significantly larger than unicellular mat cohabitants (it is noteworthy that all organisms in Table III are homologous to small unicellular eubacteria). Specifically, these approaches include: (1) passage of mat homogenates through 10-20 micron nitrocellulose filters; and (2) differential centrifugation of mat homogenates (recommended by Brian Hedlund, Niki Parenteau, and Bev Pierson, personal communication).

Objective Two: Heliothrix-specific oligonucleotides

In order to ultimately circumvent the lengthy cloning process described in Objective One, we intend to design and utilize genus- and/or species-specific primers. The utility of these oligonucleotides will be two-fold: first, we will employ them in previously described PCR methods to facilitate cloning and sequence analysis. Second, we will conjugate primers to fluorescent tags and apply them in situ, thereby directly and visually correlating these specific rRNA sequences with mat community inhabitants. Methods for the design and application of labeled primers to fixed cyanobacterial and anoxygenic bacterial communities have been described (Tuschak et al., 1999; Schonhuber et al., 1999).

We were reluctant to design and employ such primers as our first step because, with only two representative Heliothrix-like sequences in the database, we felt it would be difficult to design appropriate oligonucleotides (that is, we were concerned that we may "cast too specific a net"). Additionally, the two Heliothrix or Heliothrix-like 16S rRNA sequences in the database represent only partial clones, specifically the first 875 base pairs of the 1500 base pair gene (Weller et al., 1992).

Objective Three: Biogeography and Biodiversity

A primary goal in this study is to assess the biogeography and biodiversity of Heliothrix-like organisms in Yellowstone National Park. To date, we have identified twelve isolated mat communities in the park which, based on preliminary characterization, harbor diverse Heliothrix-like organisms (Figure 1, Table II). Objectives One and Two will determine the genetic diversity of these sites. Objective Three supports ongoing survey work to describe and assess additional mat communities in the park. This goal will specifically support summer field research studies that involve student team research as described previously.

Unsurveyed thermal basins of interest include: (1) Shoshone Basin; (2) Bechler Plateau region above Shoshone; (3) Gibbon Group features, south of the Norris region; (4) Lakeshore and Potts Hot Spring Basins, both near West Thumb; and (5) White Creek Drainage springs in the Lower Basin (Bryan, 1995).

Objective Four: Isolation and characterization of light-harvesting proteins

Yellowstone isolates contain Bchl a pigment-protein complexes that distinct in vivo absorption properties (Table II), including, in many cases, unusual absorption beyond 900 nm. Absorption spectra measured in vivo assess both bacteriochlorophyll and associated proteins as they exist in the photosynthetic units of the cell. The significance of in vivo absorption variation may thus reflect protein, and hence DNA, differences between isolates. Consequently, the comparison of

light-harvesting proteins, and respective gene sequences, may provide a valuable additional marker by which to assess diversity and identity among these anoxygenic phototrophs.

Photosynthetic units are composed of reaction centers and antenna complexes (Thorner et al, 1983). Antenna complexes capture light and transfer energy to the reaction center. Antenna complexes contain bacteriochlorophylls complexed with specific light-harvesting proteins. Among bacteriochlorophyll a-containing purple Proteobacteria, the best-studied anoxygenic phototrophs, there are two distinct light-harvesting complexes (LHI and LHII), each consisting of distinct proteins. In most purple Proteobacteria, LHI contains a Bchl a pigment-protein complex that absorbs 870 nm light; LHII contains a Bchl a pigment-protein complex that absorbs 800-850 nm light. In both LHI and LHII, Bchl a is bound to two protein subunits, alpha and beta, both encoded by a gene cluster called the pucAB operon. Different genera of purple phototrophic eubacteria, including Rhodobacter, Rhodovulum, Rhodopseudomonas, and Rhodospirillaceae, contain genetically similar but varying numbers of puc operons. These data are summarized in Tadros and Drews, 1990; Tadros et al, 1993; Tadros et al., 1995; Hagemann et al, 1997.

Despite 16S rRNA sequence homology with Green Non-Sulfur Bacteria, Heliothrix oregonenesis strongly resembles purple phototrophic members of the Proteobacter lineage in terms of being composed of only Bchl a. Specific similarities between the unusual Bchl a-protein absorption spectra of some Yellowstone isolates and the recently described Roseospirillum provide additional evidence for these significant comparisons (Glaeser and Overmann, 1999, Table I).

We hypothesize that the light-harvesting proteins (and genes) of Purple Proteobacteria and Heliothrix are homologous but genetically distinct. Thus, by analogy to Rhodopseudomonas palustris, Heliothrix LHI corresponds to Bchl a pigment-protein complexes that absorb 795nm light; LHII with Bchl a pigment-protein complexes that absorb 865 nm light. Furthermore, we hypothesize that the unique absorption properties of Heliothrix-like Yellowstone isolates reflects additional variation. To date, however, no studies have addressed light-harvesting proteins in Heliothrix; Sakuragi et al. recently reported the successful isolation of pigment-associated proteins in Chloroflexus (Sakuragi et al., 1999). Using methods described in these systems, we intend to address this question first in Heliothrix oregonensis (owing to the fact that it grows in co-culture with non-phototrophic Isosphaera pallidum). We will then apply methods to Yellowstone populations. We will now discuss our specific goals and strategies.

To study light-harvesting proteins in Heliothrix oregonensis, we will use a two-armed strategy that consists of (1) a biochemical and reverse genetics approach and (2) a purely molecular/DNA-based approach. For (1), we will sonicate Heliothrix co-cultures, releasing pigment-protein complexes. Following simple centrifugation, crude sonicated extracts will be subjected to sucrose-gradient ultracentrifugation and reverse phase HPLC (Note: Western Oregon University Dept. of Chemistry has an HPLC, see Equipment List). Specific activity based on predicted pigment-protein absorption properties will be assessed following each of these methods using a spectrophotometer. Final fractions displaying appropriate activities will be characterized via SDS PAGE gel electrophoresis and terminal amino acid sequence analysis. Oligonucleotide probes based on these sequences will then be applied, using PCR, to genomic DNA from Heliothrix co-cultures to isolate putative gene cluster(s) that encode respective proteins. We will then apply these probes to genomic DNA representing Yellowstone isolates. Methods for these approaches have been successfully used to isolate light harvesting proteins and respective gene sequences from Rhodobacter sulfidophilus, Rhodovulum sulfidophilum, and Rhodopseudmonas palustris, all distinct genera of purple Proteobacteria (Tadros MH et al, 1993; Tadros MH et al, 1995; Tadros MH et al., 1995; Hagemann GE et al., 1997). To date, centrifugation and SDS PAGE methods have been used to characterize pigment-associated proteins in Green Non-Sulfur Chloroflexus (Sakuragi et al., 1999).

For approach (2), we will design oligonucleotides based on conserved regions of Proteobacteria genes encoding known light harvesting proteins. We will apply these primers directly to genomic DNA from Heliothrix co-cultures. Our rationale for this approach is based on (a) our hypothesis that light-harvesting proteins are conserved in both of these Bchl a-containing phototrophs and (b) this method has been successfully applied across genera lines within the Proteobacter lineage (Tadros et al., 1993; Hagemann GE et al., 1997).

While our specific aim in Objective Four is to characterize and evaluate light harvesting protein genes in terms of identification and phylogenetic utility, we recognize the evolutionary implications and additional genetic studies that could be performed with regards to these systems. We hope, in fact, that results from these studies will lead to genetic regulation studies and an improved understanding of the evolutionary relationships between the phylogenetically distinct Green Non-Sulfur and Proteobacteria lineages.

BUDGET JUSTIFICATION

The budget I have requested will allow for the implementation of the hypothesis-driven undergraduate research proposed above (See also: Impact Statement, Supplementary

Documents). My modest lab is a reflection of the University's primary mission: to provide undergraduate instruction and research experiences for students that emphasize education (See also Certification of Eligibility, Supplementary Documents). I perform a pivotal role in this regard, carrying a teaching/contact load with an average of seventeen to twenty-two hours per week. To date, I have supervised short-term (one to two quarter long) research projects. I have successfully incorporated molecular research projects into my majors microbiology (Biology 331) and molecular biology course (Biology 475), although progress is, admittedly, slow and punctuated.

University support for this research includes PCR and HPLC facilities, basic molecular biology and protein equipment and reagents for both my lab and for research-based teaching labs, matching funds for a DNA sequencer, and has included limited travel support for students and I to perform summer field research in Yellowstone. In order to address what are important and exciting hypotheses based on extensive preliminary data, to provide the opportunity for more students to work on this project, to publish data in a timely manner, and to increase my chances at obtaining future grants and professional development-related awards, I am requesting funds for a lab assistant/technician, summer research stipends for undergraduate research mentees, a concomitant increase in general perishable reagents (primarily cloning, sequencing, PCR, and protein analysis), and a gel imaging system.

I am requesting a technician, promoting this as an opportunity for a Western graduate, rather than a post-doctoral fellow. This is in recognition of the absence of a graduate-level environment for training post-docs at the University. Technician duties will include carrying out research objectives, assisting with lab and database organization/ordering, training research mentees using the DNA sequencer, and providing research reagents for molecular and computational biology labs. In the past two years, I have had to turn away two qualified research mentees who would have benefited from such a position in terms of gaining additional experience and training for their goals, working as technicians in molecular biology labs and eventually attending graduate school.

I am requesting summer stipends for two students annually. Stipends would support a twelve-week summer research experience, including field studies, that would lead to the writing and public defense of a senior thesis. From my own training (I received a Murdock summer stipend through the University of Puget Sound), I know that this experience is invaluable in terms of scientific training and professional development. Unfortunately, it has been my experience with mentees at Western that financial support is the primary factor that diminishes summer

participation in independent study work towards the development of a thesis project. Most students spend their entire summer working non-science jobs to pay for tuition during the academic year. This problem is exacerbated by the fact that Western is located in a college town of nine thousand people with no science-based lab job opportunities. Because of these two limitations, most students lack a fundamental perception that summer research is a viable option that will enhance their education. Attempts to gain competitive Murdock grants have not been successful, in part because our department has only recently attained a critical mass of faculty involved in undergraduate research project. As a department, we have not attempted to compete for NSF-REU funding both for that reason and because the student body at Western is primarily from Oregon.

To be eligible for consideration for my summer stipend program, students would be required to submit a formal proposal to me in the spring term based on their understanding of provided literature about the project, a basic literature review they conduct, and their specific goals for the thesis project. Summer stipend monies would be given with the contingency that (a) formal research notebooks would be maintained and evaluated every two weeks for progress and organization; (b) a senior thesis that summarized the project would be written and approved by me and two other faculty readers by the end of the academic year following the summer research project (if the student is in the Honors Department, more extensive Honors-specific thesis requirements would be met) and (c) at least one public defense/presentation of the work would be required in the spring term following the summer research project (acceptable forum include: the campus-wide Academic Excellence Week, Division Seminar, or a formal Honors Defense). Students will also be encouraged to present work at local (Oregon Science Academy, American Society for Microbiology Northwest) and national meetings (American Society for Microbiology). Consequently, I have requested some support for travel to meetings in this budget.

Finally, I am requesting one substantial piece of equipment: a gel-imaging system (Fotodyne Archiver System, $10,000). Currently, we are only able to run mini-gels and a view them on a 10 X 12 cm screen UV light box with no working camera; students literally hand-draw the images in their notebooks. This system is, simply, unacceptable for the numbers of clones we intend to analyze and the organization and documentation that must accompany these results. Competitive campus faculty development grants offer up to $3,000 for such equipment, which could cover part of this equipment expense. This, however, has not been included in the budget statement.