

4. Project Description

Results from Prior NSF Support

I have received two NSF awards. Improved Laboratory Instrumentation (ILI) Grant DUE-9851322 (1998) funded the acquisition of a Li-Cor DNA sequencer and improvements to Molecular Biology (Biology 475). Research at Undergraduate Institutes Microbial Observatories (RUI-MO) Grant MCB-0074452 (2000-2003) funds my ongoing Red Layer Microbial Observatory (RLMO) research program that uses DNA methods to identify uncultured hot spring bacteria from Yellowstone National Park (6). The latter project (Grant MCB-0237167), for which I have been recommended but am pending renewal confirmation (2003-7), supports and will continue to support research-driven curricula in Molecular Biology and pre-college education outreach, the impact of which totals 83 undergraduates and 65 pre-college students over the last three years. ILI and RUI-MO curricula have been disseminated nationally and locally (3-5), and are available on my professional website (<http://www.wou.edu/rlmo>).

Project Description

In 2001, two national colloquia published similar conclusions about higher education training in microbiology that advocated increased lab experience and assessment, improved training in computer technology using on-line databases, and application of less selective molecular diagnostic techniques, many of which have not translated from research or clinical settings into classrooms. One of these colloquia produced the report: "Bioterrorism Threats to Our Future, The Role of the Clinical Microbiology Laboratory in Detection, Identification, and

Confirmation of Biological Agents" (18), the other: "Geobiology: Exploring the Interface Between the Biosphere and the Geosphere" (17). The broad discipline of microbiology, as applied to health or applied and environmental issues, can be methodologically united by the theme of understanding diversity through molecular diagnostic techniques and databasing efforts. It is the goal of this proposed CCLI project to improve learning in our microbiology majors core course through laboratory experiences that enable students to master traditional culture-driven strategies and molecular diagnostic approaches while discovering microbial diversity from relevant local field sites.

New lab curricula will facilitate the following learning outcomes:

- (1) Improved synthesis, analysis, and understanding of scientific data because projects and methods are continuous, related, and comparative***
- (2) Improved hands-on connections to lecture information about molecular diagnostics***
- (3) Improved on-demand summative assessment using digital images generated directly from lab projects over time***

New lab curricula will facilitate the following project goals and objectives:

- (1) To equally emphasize culture-dependent and -independent methods as applied to local field sites that address broadly applicable questions about microbial diversity***
- (2) To enhance student access to and learning through computer technology in the lab in terms of data collection, archiving, and analysis***
- (3) To introduce students to molecular diagnostic epifluorescence microscopy in the context of inquiry-driven experiences***
- (4) To disseminate new curricula and project results through publications, meetings, on-line course materials, and on-line student-generated web-based lab projects***

The Current Situation at Western Oregon University

Proposed new microbiology curricula will impact all biology majors (30-60 per year) at Western Oregon University (WOU), the oldest public institute of higher education in Oregon (average annual enrollment = 4500). Historically devoted to the specific training of teachers, the WOU mission has, over the last ten years, enlarged to emphasize both teacher training and an undergraduate liberal arts education. Biology majors earn one of three degree options: general biology, biology education, or specialized pre-professional. Over the past five years, most microbiology majors have assumed professions in health science (33%), local secondary education (25%), and research or biotechnology (9%), as summarized in Table I.

Table I: Microbiology Students (1997-2002)

<u>Term</u>	<u>Secondary Education</u>	<u>Research Biotech.</u>	<u>Forensic Science</u>	<u>Health Medicine</u>	<u>Government</u>	<u>Non-Science or Unknown</u>	<u>Totals</u>
Fall 1997	2	1	0	4	0	10	17
Spring 1998	0	1	0	6	0	4	11
Fall 1998	1	0	0	2	1	3	7
Spring 1999	3	1	0	7	1	2	14
Fall 1999	5	2	1	3	0	3	14
Spring 2000	6	0	0	3	1	0	10
Fall 2000	4	0	3	2	0	1	10
Spring 2001	2	2	0	1	0	4	9
Fall 2001	4	3	3	5	0	2	17
Spring 2002	4	1	2	9	0	1	17
Totals	31	11	9	42	3	30	126

All biology majors complete a core that includes one year of 200-level introductory biology and all of the following 300-level courses: Genetics, Ecology, Evolution, Cell Biology, and Microbiology. Currently, most majors leave our program with no lab experience using molecular methods. Our 200-level introductory series lab, taught using a subset of labs from Morgan & Carter's Investigating Biology (15), is based on canned exercises that, in terms of molecular content, expose students to hands-on enzyme experiments and dry lab evaluations

of protein gels. Although we lack molecular equipment to fully develop the 200-series (annual enrollment since 1997 = 80-100), campus-based grantsmanship has improved computer technology in this series. Beyond the 200 series, core courses in Genetics and Evolution have no lab components. Cell Biology is limited to light microscopy and enzymed-oriented assays. Ecology labs are field-based and deal with macrobiological data.

Microbiology, my primary core course, is taught using Brock Biology of Microorganisms (14) and original or adapted on-line lab protocol developed by me. Course lectures cover comparative cell and molecular biology, metabolism as a function of diversity and ecology, medical microbiology, and biotechnology. Laboratory methods, described next and in Table II, are limited relative to contemporary diagnostic capabilities. During weeks 1-2, students complete canned laboratory exercises that facilitate mastery of basic microbiology methods and tools using provided lab strains. During weeks 3-7, students complete two culture-based isolation projects (river and soil) based on methods adapted from available protocols (2, 11, 16, 19). The river project compares bacteria from four local river systems, all of which have been impacted by agricultural and industrial waste. Students plate water samples on selective media, retrieving 50-70% Pseudomonas (Boomer unpublished). Using culture-based phenotypic tests, available taxonomic keys (13, 19), and a bioremediation assay adapted by me from a primary research article (12), student teams attempt to identify ten specific river isolates. The soil project uses culture-based approaches to isolate four groups of microorganisms: antibiotic-producing Streptomyces, nitrogen fixing Rhizobium and Azotobacter, anoxygenic phototrophs, and amylase-producing Bacillus. In contrast with the

comparative class river project, each team performs only one of the four different soil projects using many different sources. As with the river project, identification is based on culture-based phenotypic tests and teams typically report inconclusive results, in large part because we lack equipment for more precise molecular diagnostics.

Weeks 8-10 comprise, for most students, the only hands-on molecular experience that WOU biology majors experience. All materials and protocols are derived from my RLMO research project (3). During weeks 8-9, students analyze cloned ribosomal RNA genes from my extensive "unknown" hot spring bacteria library, assessing population diversity via restriction fragment length polymorphisms (RFLP) analysis. During week 10, students analyze previously-derived ribosomal RNA gene sequences and phylogenetic trees, learning about bacterial diversity and on-line computer databases.

Current lab assessment, based on National Science Education standards (7), encompasses the following: week 1-2 labs are summatively evaluated via individual formal lab reports and on-demand exam questions. For long-term summative evaluation of projects (weeks 3-7), students maintain formal notebooks and design web-based projects using provided templates that assemble digital images, results tables, analysis, and discussion. Weeks 8-10 labs are only assessed summatively via on-demand final examination questions.

Beyond the core series, only my elective majors course in Molecular Biology (Biology 475) offers hands-on experience with molecular methods (average annual enrollment since 1998 =

6). Unfortunately, many students become interested in molecular biology late in their academic careers and elect not to complete the lengthy elective pre-requisites for this course (two non-lab-based biochemistry courses). Recently (Spring 2002), I offered a one-time special topics course in Microbial Biotechnology that included no pre-requisites beyond the 200 series; this course attracted 13 students, only two of whom would have had the pre-requisites to take Molecular Biology. Based on these experiences, I contend that Microbiology lab offers an appropriate platform for curriculum improvements that will serve a larger audience of interested students while integrating molecular diagnostic techniques and inquiry- and research-driven approaches.

Detailed Project Plan

To facilitate improved learning, Microbiology will be restructured from its current schedule (3 lectures and 1 three-hour lab per week) to a more lab-intensive program (3 lectures and 2 two-hour labs per week). The new lab curriculum will be divided into the following three units (summarized in Table II): (1) Culture-Based Identification of Microorganisms; (2) Rapid Diagnostic Fluorescent in situ Hybridization (FISH) Microscopy; and (3) Molecular Microbiology. This proposal will allow the development and implementation of Unit 2, as well as impact Unit 1 methods. New units will be comprehensively assembled by student teams into web-based projects, described at the end of this section.

Table II – Current versus Revised Lab Syllabus

<u>Week</u>	<u>Current 331 Course</u>	<u>Revised 390 Course</u>
1	Aseptic Techniques	1a: Aseptic Techniques, Media Preparation 1b: Sampling, Enrichment Inoculation

2	Gram Staining, Selective Media	2a: River Enumeration, Sub-Culturing
		2b: River ID tests, Bioremediation Assays
3	RLMO Computational Taxonomy	3a: Soil Sub-Culturing
		3b: <u>Bacillus</u> ID tests, Enzyme Assays
4	Project Media Preparation	4a: <u>Streptomyces</u> ID tests, Antibiotic Assays
		4b: <u>Azotobacter</u> ID tests
5	Project Inoculation	5a: Soil and River microscopy fixaton
		5b: FISH analysis – control practice
6	River ID Testing	6a: FISH analysis – soil and controls
		6b: FISH analysis – river and controls
7	Soil-Specific Testing	7a: RLMO plasmid isolation
		7b: RLMO RFLP Digestion
8	Project Catch-Up	8a: RLMO RFLP Electrophoresis
		8b: RLMO DNA Sequence Reactions
9	RLMO Plasmid Isolation	9a: RLMO DNA Sequence Loading
		9b: RLMO DNA Sequence Editing, BLAST
10	RLMO RFLP Analysis	10a: RLMO Computational Taxonomy
		10b: Comprehensive Lab Final

(1) Culture-Based Identification of Microorganisms – Weeks 1-4

Traditional culture-based approaches use defined media to isolate and identify microorganisms. In mastering these methods, students directly learn basic media preparation, dilution and enumeration, pure culture maintenance, and phenotypic testing for the purpose of taxonomic identification. Existing river and soil project curricula effectively teach most of these approaches in the context of relevant local field projects. Some specific changes – described next - are proposed to improve learning and to better integrate this project into new Unit 2 methods. In terms of existing river project curricula, students will collect and filter (0.45 micron) whole river samples (200-500 ml), capturing bacteria in a non-selective fashion. Filtered bacteria will be fixed and enumerated using both simple light microscopy techniques and proposed new methods in Rapid Diagnostic FISH-based Microscopy (Unit 2).

In contrast with the current soil project, new soil curricula will involve all teams learning and comparing common strategies to isolate three ubiquitous soil bacteria (Streptomyces, Bacillus,

and Azotobacter) from four field sites. A single soil sample will thus be divided and used for four purposes: (a) Amylase-producing Bacillus: 1 g soil will be subjected to two parallel enrichments in starch media, one at high and one at low temperature conditions. Enrichments will then be transferred and grown on starch indicator plates to assay for amylase production. (b) Nitrogen fixing Azotobacter: 150 g soil will be selected in nitrogen-free media prior to plating on nitrogen-free agar plates. Azotobacter represent the only aerobic nitrogen fixer obtained using these historic methods. (c) Streptomyces: 1 g will be subjected to a 6X10-fold dilution series and diluents will be directly plated on Streptomyces-enrichment media and assayed for antibiotic production; (d) Non-selective Assessment: dilutions described in (c) will be fixed and enumerated using both simple light microscopy counting techniques and proposed new methods in Rapid Diagnostic FISH-based Microscopy (Unit 2).

(2) Rapid Diagnostic FISH Microscopy – Weeks 5-6

Diagnostic epifluorescence microscopy utilizes fluorescent-labeled DNA or antibody probes to bind and indicate target molecules. Fluorescent probes have broad applications in cell biology, genetics, and microbiology. DNA-based methods are known as FISH; a review of these methods, as applied to microbial diversity assessment, was published by Amann et al.

(1). To improve curriculum for this unit, I will adapt a cell biology curriculum developed by Douglas Kline (Kent State University, NSF-DUE Award 9551341): “Cell Biology – Advanced Microscopy for the Teaching Laboratory”

(<http://dept.kent.edu/projects/cell/labs.html-ssi>). Briefly, Dr. Kline employs epifluorescence video microscopy and fluorescent antibody probes to explore the structure and function of

eukaryotic cells by labeling specific cellular proteins. In contrast with his methods, our microbial FISH studies differ in three ways: First, our probes are made of DNA oligonucleotides and will hybridize with complementary DNA and RNA in the bacterial cytoplasm. Second, our aim is identification of whole cells – not sub-cellular structures; thus, microscopic resolution and layering capacity are not as serious issues. Third, our probes are applied to dead, fixed cells – not living, metabolically active cells; thus, manipulation and fixation procedures are straight-forward and we do not require real-time video microscopy. Specific fixation and FISH-based procedures for bacterial preparations are widely published (1) and have been successfully used in one “canned” undergraduate teaching lab with which I am directly familiar (8).

In the proposed course revision, students will characterize both non-selective filtrates and pure culture-derived isolates from river and soil, alongside prepared control organisms, using available fluorescent DNA probes, summarized thoroughly in Amann et al. (1). River samples will be hybridized with available probes that label: all Bacteria, all Gamma Proteobacteria phylum (includes enteric bacteria), only Pseudomonas species, and only enteric Enterococcus. Soil samples will be hybridized with available probes that label: all Bacteria, Gram Positive High-GC organisms (primarily Streptomyces), and Bacillus only. At this time, no Azotobacteria-specific genetic probes are cited in the literature. I will consider developing Azotobacteria-specific oligonucleotides based on available pure cultures, RNA sequence information, and widely-used ribosomal motifs recommended for probe design (1). Requested equipment that will facilitate the implementation of these new classroom methods

include two teaching-grade digital epifluorescence microscopes to be networked to four lab-dedicated computer workstations.

(3) Molecular Microbiology – Weeks 7-10

Culture-based methods grow only 1-5% of microbial diversity present in a given environment (1, 9, 10). Less selective molecular approaches – such as FISH-based microscopy using phylogenetic probes (Unit 2) or PCR-based cloning of microbial DNA sequences directly from an environmental sample (Unit 3) - have provided unsurpassed insights into microbial diversity. DNA information and computational databases provide the current keys to analyzing and archiving molecular diversity information. In this final unit, I will implement my ongoing RLMO curricula to guide "extremophile" microbial diversity discovery. Specifically, during weeks 7-10, each student will analyze 2 novel hot spring-derived clones via existing plasmid isolation and RFLP assessment, enhanced methods in DNA sequence analysis, and computational assessment of retrieved DNA sequences. While it would have been ideal to analyze river and soil project materials in molecular unit, lengthy cloning and screening procedures would not have been feasible given time constraints. Necessary equipment – including dedicated lab computers, an additional lab-dedicated PCR machine, and a DNA sequencer upgrade – to facilitate these goals will be provided via my MO/RUI grant in 2003.

Web-Based Projects

As with current web-based projects, student teams will assemble raw data and digital images - both macroscopic pictures of plates and methods, and new microscopic images - from Units 1-2 into meaningful tables that reflect methods, results, and discussion sections of primary scientific literature. Currently employed web-based templates will be expanded to include new tables that summarize non-selective enumeration and FISH-based microscopy. A second website template will be assembled by individual students based on the improved molecular unit (Unit 3). Digital images of RLFP data will be directly linked with student-derived DNA sequence information, NCBI-BLAST similarity results, and a referenced discussion of the broad significance of each top BLAST result. All web-based projects will be available via an on-line class database, linked from my professional website.

Experience and Capability of the Principal Investigator

New lab curricula will be implemented, assessed, and disseminated by me. I earned my doctoral degree in Microbiology from the University of Washington (1996) studying molecular evolution and pathogenesis of retroviruses. I earned undergraduate Biology and English degrees at the University of Puget Sound, completing research describing novel hot spring bacteria as part of an Honors thesis under the direction Dr. Bev Pierson. I continue this research at WOU in collaboration with Dr. Pierson. Promoted to Associate Professor of Biology in 2002, I have six years of formal teaching experience that includes the following regularly-offered courses: Microbiology, Majors Introductory Biology I (Biology 211), Molecular Biology, Molecular Virology (Biology 420), and Emerging Diseases (Biology 440).

In conjunction with my RLMO research project, I have coordinated, participated in, and developed assessment tools for pre-college outreach projects and credited teacher workshops (3-5). Additionally, I have supervised over a dozen independent undergraduate research projects, seven undergraduate field research teams in Yellowstone, and a full-time research assistant. In terms of specific methods and equipment experience relevant to this proposal, I have demonstrated experience using epifluorescence microscopy methods from both graduate level research and teaching positions. I have three years of experience using digital microscopes/cameras and computers (imaging, website construction, NCBI/BLAST instruction, and phylogenetics) in teaching lab settings for all aforementioned courses.

Evaluation Plan

In accordance with recommended project evaluation strategies (20), evaluation of new curricula will include three levels of assessment: (1) Planning and Planning Evaluation; (2) Summative and Formative Student Evaluation; and (3) Summative Project Assessment. Specific details of each of these levels will now be described.

(1) Planning and Planning Evaluation: Between Fall 2003 and Winter 2004, I will troubleshoot new equipment and develop on-line protocols and evaluation tools outlined below. The first offering of the new course will be Spring 2004. Formative evaluations will be reviewed and protocol modifications will be made over Summer 2004. Similar analysis will occur after Fall 2004. Thorough program assessment will take place during Summer 2005 and dissemination will follow (see Dissemination Plans).

(2) Summative and Formative Student Evaluation: Students will be assessed using three strategies in class: graded materials that include a variety of summative assessment formats, un-graded pre- and post-tests that examine background in relation to mastery, and formative pre- and post-surveys that examine student attitudes. Each strategy will now be described.

Summative Graded Materials. Graded materials will include: (a) three lecture-only exams that contain "on-demand" questions (in contrast with the current course that includes four lecture/lab exams); (b) one writing assignment that requires students summarize three primary research articles, each focusing on a different infectious agent using a specific molecular method (consistent with the current course); (c) "over-time" laboratory projects that include all-term lab notebooks and web-based projects (in contrast with the current four-week-only notebook and web assignment); (d) two entirely new in-lab exams that will be visual and interactive in the sense that students will discuss, identify, and/or analyze data and images produced in the lab and presented using the requested overhead projection system.

Pre/Post-Mastery Tests. This new mechanism of formative evaluation aims to compare a student's background knowledge with his/her mastery during the course. Administered the first and last days of lecture, mastery tests will, via matching and short answer questions, test knowledge based equally on lecture and lab. Students will understand the purpose of the tests, including that it has no bearing on their course grades.

Formative Pre/Post-Attitudinal Surveys. This new mechanism of formative evaluation is designed to assess student expectations (both in the course and beyond the course) and perceptions about the efficacy, significance, and relevance of the new lab curriculum. Two different surveys will be composed to address all of these issues and administered the first and last day of lab. Surveys will include both quantitative scoring and short answer questions to facilitate meaningful analysis and dissemination.

(2) Summative Project Assessment: Comprehensive project assessment will include two components: (a) post-course student tracking; and (b) post-project evaluation of goals with an emphasis on dissemination. Given the two-year time-line of this project, in combination with the fact that most students will take microbiology during their third or fourth academic year, summative post-course tracking will likely be limited to following students through their academic careers at WOU; in as many cases as possible, the author will track their pursuits and accomplishments post-graduation. In terms of post-project assessment, all evaluation tools will be analyzed against project goals and disseminated via a variety of mechanisms outlined below. Anonymous summaries of un-graded formative materials will be databased on-line. For privacy issues, graded materials will only be disseminated as a comprehensive publication following the two-year study. At this time, the author will evaluate the project for consideration as a nationally disseminated adoption and adaptation project.

Dissemination of Results

New curricula, results, and assessment will be presented at local Oregon Academy of Science Meetings (February 2006) and the national American Society of Microbiology (ASM) General Meeting (May 2006). Thereafter, the author will submit this work to the ASM Microbiology Education Journal. The author will continue to develop and provide free, internet-accessible curriculum and methods with links to appropriate organizations (e.g. ASM, NSF, and CUR).