A Laboratory Class Exploring and Classifying Anoxygenic Phototrophic Bacteria Using Culture-Based Approaches, Microscopy, and Pigment Analysis

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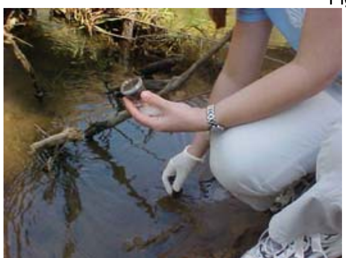
Abstract
In this exercise, students set up bottle enrichments for phototrophic microbes using river sediment and then, using representative controls, they then view and describe wet mounts of representative oxygenic algae and cyanobacteria, and anoxygenic purple bacteria. Following 2-3 weeks incubation, students characterize pigmented enrichments using basic microscopy and pigment extraction and analysis, the latter of which requires a spectrophotometer. Using provided tables and their text, they classify enriched phototrophs using pigment absorption data, cell shape/organization, and growth conditions such as the presence/absence of sulfur.

Figure One – Student collection of local river mud/sediment samples for phototroph enrichment.


Luckiamute River,
Helmick Park, Oregon

Willamette River,
Independence, Oregon

Gentle Creek,
Independence, Oregon

Rickreall Creek,
Rickreall, Oregon

Activity

INTRODUCTION

Learning Objectives.
Upon completion of this activity, students will be able to (1) better understand anoxygenic phototrophic bacteria based on direct enrichment and observation from local soil or river environments; (2) compare and contrast anoxygenic phototrophs with more familiar oxygenic phototrophs (e.g. plants/algae); (3) generate and use molecular pigment spectrum data; (4) combine a variety of microbial phenotypes (pigment profiles, microscopy, growth conditions) to classify unknown phototrophs from natural environments.

Background.
This laboratory exercise has been carried out at Western Oregon University. It was included as one component of our poster entitled " A General Microbiology Lab Curriculum Featuring Culture-Dependent and -Independent Approaches and Computer-Based Project Presentations" at the 2004 American Society for Microbiology General Meeting. Here, it received considerable attention from undergraduate microbiology instructors interested in environmental enrichment lab exercises. Although comparable undergraduate pigment-based exercises exist, most involve oxygenic plants or algae (3, 4, 5). We have been unable to find examples of undergraduate labs that combine bacterial culture-driven procedures with pigment assessment for the purpose of classification. Although these approaches are less exact for genus-level identification than, say, solving "unknown" Enteric isolates using media-based tests and phenotypic charts, they provide valuable models for enriching from a natural mixed population, for exposure to relevant culture-independent phenotypic assessment (e.g. spectrophotometry and microscopy), and for studying environmentally important and beneficial microbes.

This curriculum was first implemented in 1997 for the laboratory component of General Microbiology (Biology 331), a course that all Biology Majors are required to take. Students complete this laboratory exercise during the first month of the 10-week course, concurrent with extensive lectures about microbial diversity, ecology, and metabolism (with an entire lecture devoted to microbial phototrophs and diversity). The course requires Brock Biology of Microorganisms (2), an invaluable resource for bacterial diversity - particularly during this exercise. To take this course, students have also had to pass one year of introductory Principles of Biology (Biology 211-213), the laboratory component of which includes pigment assessment of spinach extracts using a spectrophotometer (3).

The same instructor (Boomer) who has developed and delivered lectures also runs the laboratory; first-person portions of this report refer to Boomer. Students receive all instruction materials at the beginning of the term. During the first laboratory session, students collect local mud and/or river samples and set up replicate bottle enrichments using both sulfur and non-sulfur media. Two to three weeks later, the typical time it takes bottles to develop visible color (either green or purple/red), students perform pigment isolation and microscopy on positive enrichments. Pre-lab lecturing is limited to an overview of specific methods and a review of microbial phototrophs, emphasizing key phenotypic traits they will use to identify their enriched isolates to the phylum level.

PROCEDURE

Materials.

This exercise can be run with 2-4 people working together. Each team needs:

Session One

4 g freshwater mud or soil source (sandy mud or soil should be avoided)
4 clear glass bottles with screw-caps or tight-fitting corks (ours hold 200 ml liquid)
Microscopy controls - Spirogyra, Anabaena, Rhodospirillum- for microscopic viewing*
Basic light microscopy supplies (microscope, standard slides, and coverslips)
1L of each of the following liquid media (protocol written for 200 ml bottles, some extra for spillage):

Nonsulfur Phototroph Media	Sulfur Phototroph Media

NaHCO3: 5.0 g NaCl: 2.0 g (NH4)2SO4: 1.0 g K2HPO4: 0.5 g MgSO4: 0.1 g yeast extract: 2.0 g Na2S-9H2O: 0.1 g	NH4Cl: 1.0 g KH2PO4: 1.0 g MgCl2: 0.5 g NaHCO3: 2.0 g Na2S-9H2O: 1.0 g NaCl: 1.0 g

**These samples are all available fresh from biological supply companies (e.g. Ward's). Alternatively, prepared slides may be used, although these have been stained during preservation and colors do not accurately reflect the natural color of living cells.*

Session Two
Basic light microscopy supplies (microscope, standard slides, and coverslips)
Micro-centrifuge (capable of 10,000 g) that holds 1-2 ml micro-centrifuge tubes
Pipettes that fit into bottles above (disposable plastic pipettes are adequate)
Micro-centrifuge tubes (this procedure is written for 1-2 ml)
Methanol (with gloves, waste beakers, and hood for evaporation/disposal)
Spectrophotometers and appropriate cuvettes*

*Given the variety of available spectrophotometers, a complete discussion of this piece of equipment is beyond the scope of this curriculum. The least expensive spectrophotometers - used by freshman in our introductory biology courses - require manual setting, repetitious zeroing between data collection, and data recording/graphing by hand; Morgan/Carter provides a thorough explanation of basic spectrophotometer operation (3). More expensive spectrophotometers - which advanced students use in this course lab - are fully digital, allow simultaneous analysis of the experimental sample and the blank, and provide automatic graphical output of the data. Instructors should carefully research their options if they do not currently own a spectrophotometer and cuvettes.

Student Version.

Lab Session One - Collection and Enrichment, **APPENDIX ONE** (see end of this report for worksheet)

APPENDIX ONE: Anoxygenic Phototrophs Week One - Collection and Enrichment

Introduction to Anoxygenic Phototrophs
Anoxygenic phototrophs include members of the following bacterial phyla: Proteobacteria (both Purple Sulfur and Nonsulfur), Green Nonsulfur (also known as Chloroflexi), Green Sulfur (also known as Chlorobi), and Gram Positives (<u>Heliobacteria</u>). Review your text and lecture notes about phototrophs, contrasting the major features of oxygenic photosynthesis (as performed by plants, algae, and cyanobacteria) with those of anoxygenic photosynthesis (as performed by the bacteria listed above). Today, you will set up enrichments for anoxygenic phototrophs. In 2-3 weeks, you will use the following phenotypic traits to classify them: sulfur utilization, pigments absorption data, and cell morphology.

ACTIVITIES - ENRICHMENTS USING MUD

Bottle Enrichments
4 g from 1 of 4 local rivers will be collected per team - optional field trip before lab
Add 1 g mud to each of 2 bottles - one labeled S (sulfur) and other NS (nonsulfur) plus your initials
Fill "S" to TOP with SULFUR MEDIA (cap/cork tightly); repeat with NONSULFUR MEDIA
Place in light (40W bulb, 10-15 cm) and grow 2-3 weeks
During enrichment, take time each last session to note and record color changes

ACTIVITIES - MICROSCOPY/DIVERSITY

Phototroph Wet Mounts
Wet mounts are good for pigmented microbes such as <u>Spirogyra</u>, <u>Anabaena</u>, and <u>Rhodospirillum</u>
Adding too much material/water will result in the inability to focus (everything moving too much)
Using a pipette or tweezers, withdraw SMALL amount of sample - make sure you see colored filaments
Place filaments in the middle of the slide, add half a drop of sourcewater, and cover with a coverslip
View <u>Spirogyra</u> and <u>Anabaena</u> using 40X, noting color, shape, cell organization, and motility
View <u>Rhodospirillum</u> using 40X; you may be able to view at 100X without oil
Complete colored drawings in the space provided, signifying the entire field of view and relative sizes
When finished, complete concept check information on the attached worksheet

Lab Session Two - Pigment Assessment, **APPENDIX TWO** (see end of this report for worksheet)

APPENDIX TWO: Anoxygenic Phototrophs Week Two - Pigment Assessment

Photosynthetic Pigment Analysis
All phototrophs use membrane-bound pigments to capture light. Oxygenic phototrophs use chlorophyll (Chl) and anoxygenic phototrophs use bacterichlorophyll (Bchl). Pigments can be described by their light absorbance properties (also known as absorbance maxima, A-max) Using bottle enrichment conditions, you should have selected for pigmented purple or green anoxygenic phototrophs.

ACTIVITIES - ENRICHMENT CHARACTERIZATION AND SAMPLING

Bottle Enrichments
Fully describe the macroscopic features of each bottle, disregarding bottles with no color at this time
If there is color, withdraw or scrape 1 ml pigmented material using a pipette for further analysis
Place in carefully labeled tubes, noting where each sample was taken from (bottle, kind of media)
Proceed to pigment analysis and microscopy after sampling from bottle enrichments

ACTIVITIES - PIGMENT ANALYSIS AND MICROSCOPY

Pigment Analysis - Methanol Extraction
Methanol is toxic - wear gloves and collect methanol in waste beakers - NOT down sink.
Centrifuge all colored samples collected above to pellet cells - 5 minutes at 8-10 g (10,000 rpm)
Decant aqueous liquid, removing as much as possible by inverting on a paper towel 2-5 minutes
Add 1 ml methanol and resuspend, agitating 2-5 minutes - methanol should begin to appear colored
Wrap in foil and place on ice for at least 20 minutes - during wait, proceed to microscopic analysis…

Microscopic Analysis
For this exercise, repeat wet mount procedures as performed during the first session of this lab
Using a pipette, withdraw SMALL amount of sample - make sure you see color when you do so
Place sample in the middle of the slide and cover with a coverslip
View each using the 40X or 100X, noting color, shape, cell organization, and motility

Pigment Analysis - Spectrophotometry
Centrifuge final extracts to pellet cell debris - 10 minutes at 8-10 g (10,000 rpm)
Carefully decant top liquid to a new centrifuge tube, discarding the tube with the debris pellet
Keep on ice and wrapped in foil until ready to measure; prepare a methanol blank control
Analyze as directed using a spectrophotometer, measuring the absorbance between 400-800 nm
Graph data - absorbance (Y axis) vs. wavelength (X axis) - and determine the absorption maxima
Turn in well-labeled print-outs with your classification table on the attached worksheet

There are 7 major Bchl, each associated with a distinct group of anoxygenic bacteria, summarized below (adapted from Brock's Microbiology, Tenth Edition).

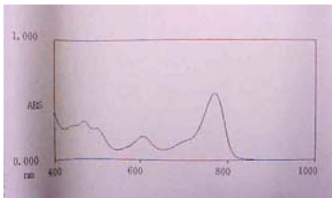
Bchl	Anoxygenic Bacteria Association	A-max/methanol	Cell Organization and Shape(s)
a*	Purple/Proteobacteria – Sulfur & Nonsulfur	770 nm	Unicellular spirilla, rods, spheres, curved rods, ovals
b	Purple/Proteobacteria – Sulfur & Nonsulfur	794 nm	Unicellular spirilla, rods, spheres, curved rods, ovals
c	Green Sulfur/Chlorobi	660-669 nm	Mostly spheres or rods, sometimes branching rods
c-s	Green Nonsulfur/Chloroflexi	667 nm	Non-branching filamentous rods
d	Green Sulfur/Chlorobi	654 nm	Mostly spheres or rods, sometimes branching rods
e	Green Sulfur/Chlorobi	646 nm	Mostly spheres or rods, sometimes branching rods
g	Gram Positive/Heliobacteria (green)	765 nm	All rods - most unicellular but sometimes bundled

Instructor Version.
This class extends over two sessions that are separated by a mandatory 2-3 week enrichment wait period. Each session is 2-3 hours in duration. For this course, students have previous experience using centrifuges, spectrophotometers, and microscopes. Although these students typically take 2 hours to complete these exercises, students lacking comparable experience should be provided 3 hours. If students have not been exposed to information about photosynthetic microbial diversity, an additional hour of lecture time should be developed to address these topics.

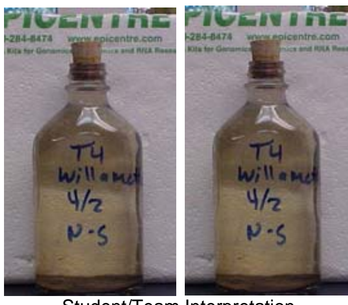
Session One
In session one, students set up bottle enrichments for anoxygenic phototrophs (both sulfur and non-sulfur), and review/view control phototrophs: oxygenic algae (Spyrogyra) and cyanobacteria (Anabaena), and anoxygenic purple nonsulfur bacteria (Rhodospirillum). I provide interested students with the option of collecting river sediment/mud, a 2-3 mile driving excursion to local rivers an hour before lab begins. The 3-5 interested volunteer participants typically enjoy this optional field trip. Instructors who do not have access to nearby rivers can alternatively collect from any moist soil area. This exercise is messy and I try to provide student teams with large plastic containers over which they can pour and inoculate materials. I also recommend that students wear gloves during the collection and inoculation steps given that, minimally, local rivers harbor agricultural run-off from many farms, both dairy and heavily-fertilized grass seed operations. I also carry anti-bacterial wipes to collection sites for students to wash off. Representative student-derived photographs of mud collection at the four local rivers or creeks (Luckiamute, Willamette, Rickreall, and Gentle) we sample, taken with digital cameras provided for class use and intended only for simple presentations (i.e. low-resolution), are shown in **FIGURE ONE**. A representative student-derived photograph of bottle enrichments are shown in the top panel of **FIGURE TWO**.

Figure Two – Bottle enrichments over time and sample spectrophotometry data.

Bottles - Lab One
Willamette River/Independence Park, Independence, Oregon



Willamette Pigment Data
Y axis = absorption; axis = wavelength
A-max: 467 nm, 769 nm; Pigment(s): carotenes, Bchl a



Student/Team Interpretation
Media: Nonsulfur
Visible Color: Negative/Colorless
No Additional Analysis

Student/Team Interpretation
Media: Sulfur
Visible Color: Positive/Purple
Cell Shape/Organization: large rods, unicellular
A-max: 467 nm, 769 nm
Pigment(s): carotenes, Bchl a
Probable Phylum: Proteobacteria/Purple Sulfur
Potential Genera: Lamprobacter, Chromatium

After enrichments are set up, students view and draw the three aforementioned control phototrophs. Although students have performed basic microscopy before, it is often necessary to review good wet mount technique and the kinds of information these preparations provide. Students accurately draw the entire field of view for a given sample, emphasizing the relative size and shape/organization of the microbial samples. Comparing large, filamentous eukaryotic Spyrogyra (with its visible chloroplasts) with smaller, filamentous prokaryotic Anabaena at the same magnification typically provides meaningful evidence about the differences between these two cell types. Observant students will notice abundant heterocysts in the Anabaena sample, reminding them about nitrogen fixation (previously covered in lecture and lab). Viewing spiral, unicellular, and motile Rhodospirilla is frustrating for some students. Although liquid cultures of this microbe appear macroscopically purple/red, it is challenging to see this color microscopically. Its size

and unicellular organization, however, will be evident, contrasting with both the algae and cyanobacteria. Given previous lectures, I avoid assisting students with the "Concept Review Check" summary table in favor of making them synthesize information from their notes and text. A sample key is provided for this worksheet below.

	Spirogyra	Anabaena	Rhodospirillum
Microbial Domain	Eukaryote	Prokaryote/Bacteria	Prokaryote/Bacteria
Microbial Phylum	Algae	Cyanobacteria	Proteobacteria
Primary Pigment(s)	Chl a and b	Chl a	Bchl a
Specialized Membranes?	Chloroplast with thylakoid	Lamellae	Lamellae
Oxygenic or Anoxygenic?	Oxygenic	Oxygenic	Anoxygenic
Number of Photosystems	2 - I and II	2 - I and II	1 - I
Typical Habitat(s)	Freshwater mesophile	Freshwater mesophile	Freshwater or soil mesophile

Session Two
Over the 2-3 weeks between sessions, students should have made at least 1 weekly record of their enrichments in their term-long informal notebooks. In general, most retrieved cultures (60%) are purple, with a 50:50 split between sulfur and nonsulfur. Our most frequent failures have involved sandy inoculum, prevalent at one local river site that I now avoid. Many bottles change dramatically over time, turning black, then slightly green, and then fully purple/red. Occasionally, we have also retrieved green bottles that, upon closer inspection (in our case, under UV), reveal fluorescent chlorophylls (i.e. Cyanobacteria or Algae). This finding indicates one of two possibilities: (1) that bottles were not capped properly, allowing oxygen into the system; or (2) that oxygenic phototrophs were performing photosynthesis using only PSII (which doesn't require oxygen).

In general, 1-2 students should analyze 1 enrichment sample. In most cases, team enrichments adequately provide 2-3 samples, allowing larger teams to divide into smaller groups for pigment and microscopy exercises. Given that bottle phototrophs often form biofilms on the glass, students will be challenged by the retrieval process - which involves careful pipette scraping/extracting. Many students incorrectly assume that they can simply place a pipette in the center of the bottle, squeeze the bulb, and withdraw the same color they see through the glass. Students also need to allow solid material to settle at the end of the pipette before adding it to the micro-centrifuge tube or their final collection will be too dilute. In the event that their first sample attempt is too dilute, students can centrifuge the sample (using the same settings as those for methanol extraction), discard the supernatant, and add more sample. In any case, collection is highly variable, requiring manual skill and patience. Thus, students should be encouraged to show the instructor their final sample before proceeding.

Although students have few problems with methanol extraction procedures, they often have questions about their wet mounts, which typically appear less uniform as compared with previously-viewed controls. Initially, I steer them toward making certain they are focused on a visibly colored mass, as opposed to an open and dispersed area where color is not as discernable. Once students concentrate on these colored regions - emphasizing cells along their peripheries - they have always reported unicellular rods or ovals. To date, we have yet to isolate any filaments or spirilla. If students seem extremely frustrated and confused about shape, I have them prepare and view heat-fixed smears and simple stains of their samples.

Instructors will have to make necessary time amendments for spectrophotometer analysis. If students have never used a spectrophotometer and if only manual spectrophotometers are available, instructors should add 45-60 minutes to the second lab session. It is also possible to freeze methanol extracts for a few days (so long as they are wrapped in foil and not exposed to light), allowing instructors to add a third lab session if need be. If this option is used, extracts should be analyzed within one week.

Beyond the Lab Homework Activities
Once student teams have completed both lab sessions and worksheets, instructors may wish to provide or assign additional individual student activities to encourage a broader understanding of these organisms in the environment, in an evolutionary context, and in current research. Three recommended activities include: (1) Describing specific ecological role(s) their isolates perform in the environment, using both text information and internet resources they search and cite; (2) Speculating on the evolutionary significance of their team isolates, using both text information and internet resources they search and cite; and (3) Locating and summarizing a current primary research article that involves 1 specific genus that is related to one of their isolates. For the third activity, students should be specifically encouraged to use www.asm.org, selecting publications/journals. For environmental and general research papers, I typically recommend searching titles or abstracts of Applied and Environmental Microbiology and J. of Bacteriology using a specific genus name. Students should be required to print/attach his/her selected article and, minimally, briefly summarize the questions being addressed, major findings, and what they think is significant about the research.

Safety Issues.
Pathogenic Organisms:
As stated, local river beaches we collect from contain, minimally, coliforms from untreated agriculture/dairy run-off; we know this because we collect and enumerate coliforms from adjacent river water during another lab exercise performed earlier in the term (typically, we observe coliforms on the order of 10^{2-3}/L). Consequently, students are required to wear gloves (we use non-latex given allergy issues) and clean hands immediately (either using anti-bacterial wipes in the field or washing hands in the lab) while handling these potential pathogens. All materials contaminated with mud inoculum are bagged and autoclaved immediately. A key reason we favor of bottle enrichments over Winogradsky Columns (described later in the Possible Modications section) is because the latter involve substantially more inoculum and potential exposure to pathogens. Although moist soil provides an alternative inoculum, this probably also contains potential pathgens (e.g. manure, animal feces, etc.) and the same precautions need to exercised during collection, inoculation, and disposal.

Methanol:
Prior to using methanol, I review safety issues concerning this toxic solvent. In particular, I stress that methanol can be fatal if ingested (because it readily converts to formaldehyde, a highly toxic tissue fixative that most students have heard about) and can cause skin and eye irritations. Given these safety issues, students - according to Material Safety Data Sheet (MSDS) recommendations - are required to wear gloves (again, we use non-latex) when handling this solvent. Each table is provided with transfer pipettes and only a small amount of methanol (3 ml) that I have previously aliquoted. As recommended for all materials, students are strongly cautioned to keep all microfuge tubes capped as they work to avoid unnecessary exposure and contamination. Each table is also providing a labeled waste beaker and required to place all methanol waste in this container during the lab. At the end of the lab, teams move their waste to our lab fume hood for methanol evaporation/disposal.

ASSESSMENT and OUTCOMES

Suggestions for Assessment.

Individual students each turn in completed copies of the Microscopy and Concept Check Worksheet (15 pts). Student teams turn in completed Phenotypic Charts with attached pigment data print-outs (15 pts). The combined value of this assignment is 10% of the lab assignment grade (30/300 total points). Additionally, 10% of the lab exam (15/150 total points) covers this lab exercise.

Field Testing.
Since being developed in 1997, approximately 160 junior- or senior-level undergraduate Biology Majors have completed this curriculum. Most (50-60%) students were pursuing careers in the health sciences. The remaining students sought careers in secondary education and research (academic, government, or biotechnology).

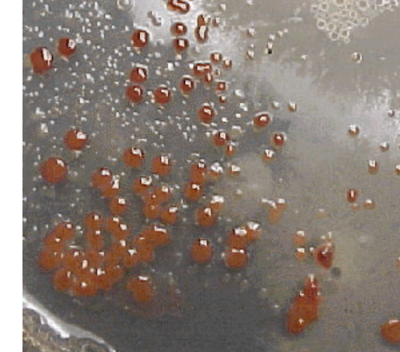
Student Data.
Since 2004, we have completed assessment of lab curricula in General Microbiology, which serves a maximum of 16 students per term. Twenty-two students rated this curriculum on a 10-point scale in Fall 2003 and Spring 2004, employing a 10-point scale, as summarized below:

Please Rate The Statement: This Lab…	Average Rating
Made Connections Beyond Microbiology	8.4
Improved My Awareness of Microbial Diversity	9
Improved My Interest in Microbial Diversity	7.7
Enhanced My Interest In Scientific Research	8.1
Enhanced My Ability To Use Computers	7.5
Exposed Me To New Technology	8.1
Enhanced My Organizational Skills	8.2
Enhanced My Writing Skills	7.7
My Overall Rating Of This Lab Is	9

SUPPLEMENTARY MATERIALS

Figure Three – Modification using Winogradsky Columns, candlejars, and plate subculture methods.

Winogradsky Columns, Bottle Enrichments, Candlejar, Subculture Plates



Candle Jar-derived plates of purple (left) and green (right) primary plates

Possible Modification.
This exercise can be modified in many ways, some based on different methods, equipment, and available time, and some based on analyzing research specimens from thermal photosynthetic mats in Yellowstone (1).

Modification One - The Winogradsky Column
Developed by S. Winogradsky in late 1800s to study sulfur cycling bacteria, the Winogradsky column provides a simple microbial ecosystem for observing and enriching both oxygenic and anoxygenic phototrophs. Winogradsky columns are well-described in most textbooks and set-up procedures are included in many microbiology lab manuals (2, 6). Although we recommend it for set-up and observational purposes (based on actively carrying it out from 1997-2000, **FIGURE THREE**, top panel), we have found that it is difficult to work with in terms of performing post-enrichment sampling for microscopy and pigment analysis. In general, we have found that columns typically develop two zones of activity over the course of 2-3 weeks: an upper oxic region that is green (cyanobacteria and/or algae) and a lower anoxic region that is green or purple (anoxygenic green or purple bacteria that usually - but not always - represent sulfur groups). This modification would add 30-60 minutes to each session.

Modification Two - Pure Cultures
It is also possible to use streak-plating methods to purify colonies of bottle-enriched phototrophs. For this modification, agar plates (20 g agar per liter) corresponding to each kind of media (sulfur and nonsulfur) must be prepared in advance of session two. Using loops, students streak colored material from bottles onto appropriate agar plates (3-4 replicates are recommended per student). Because these are anoxygenic phototrophs, they must be grown in the absence of oxygen. We have successfully used both inexpensive candle jars as well as anaerobic chambers (e.g. GasPak Jar Systems available from Fisher Scientific). When we have done this lab modification, most teams require at least two week-long sub-culturing times to fully purify phototrophs. A candle jar set-up with two resulting primary plates is shown in **FIGURE THREE**.

Modification Three - Alternative Phototroph Sources
Because we are involved in a long-term research project to identify new thermophilic phototrophs from nonsulfur bacterial mat communities in Yellowstone, we also have students analyze original research samples using pigment analysis and microscopy, both light and UV/fluorescence (where Chl fluoresce red under UV, Bchl emit green to no fluorescence). The particular communities we study are composed of 2 distinct layers of phototrophs: top/surface cyanobacteria (typically unicellular Synechococcus) and lower/deeper red filaments (new members of the Chloroflexi phylum). This ecosystem layering mirrors similar structures found in deep lakes, sediments, and Winogradsky Columns. Some representative images and data are shown in **FIGURE FOUR**.

Acknowledgements.
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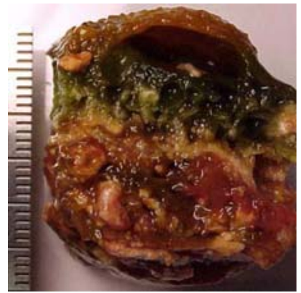
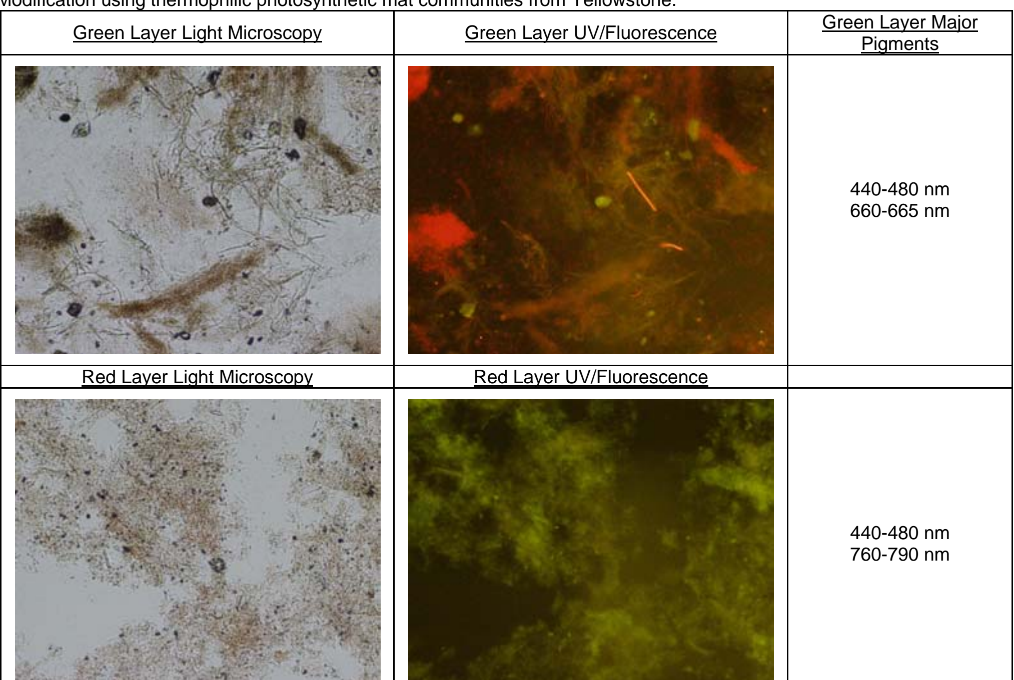
Figure Four – Modification using thermophilic photosynthetic mat communities from Yellowstone.

Yellowstone Sampling Site



Whole Mat: Green Upper, Red Lower Layer



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Microscopy and Concept Check Worksheet Name: ______________________________

Spyogyra, 40X Objective	Spyogyra Concept Check	
	Microbial Domain	
	Microbial Phylum	
	Primary Pigment(s)	
	Specialized Membranes?	
	Oxygenic or Anoxygenic?	
	Number of Photosystems	
	Typical Habitat(s)	

Anabaena, 40X Objective	Anabaena Concept Check	
	Microbial Domain	
	Microbial Phylum	
	Primary Pigment(s)	
	Specialized Membranes?	
	Oxygenic or Anoxygenic?	
	Number of Photosystems	
	Typical Habitat(s)	

Rhodospirillum, 40X Objective	Rhodospirillum Concept Check	
	Microbial Domain	
	Microbial Phylum	
	Primary Pigment(s)	
	Specialized Membranes?	
	Oxygenic or Anoxygenic?	
	Number of Photosystems	
	Typical Habitat(s)	

Phenotypic Chart - Classification

Team Names: ____

As a team, complete the following to assign probable identities to your samples. For bottles that did not produce visible color, write N/A.

	Bottle 1	Bottle 2	Bottle 3	Bottle 4
River Source				
Sulfur or Nonsulfur				
Visible Color				
Cell Shape/Organization				
A-max - (2 highest peaks)				
Pigments Present - (relate to A-max)				
Most Probable Phylum				
1-2 Possible Genera (consult text)				