



Biology 507 - In Lab Workshop Photosynthetic Microbes from Local Rivers & Beyond



National Science Foundation
Western Oregon University
Yellowstone National Park

Course Description/Syllabus

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Current Schedule

April 16, 2005 – 9 a.m. to 2 p.m.

April 23, 2005 – 9 a.m. to 2 p.m.

Total: 10 hours = 1 credit (\$45 per credit)

Course Description: Students will learn about photosynthetic microbes, emphasizing those that live naturally in local rivers and Yellowstone hot springs. Students will prepare enrichment bottles and Winogradsky columns from local river mud, assessing for the development of ancient non-oxygen-evolving photosynthetic bacteria. Students will extract pigments from bacterial samples and measure pigment absorbance, an important tool for identifying photosynthetic bacteria. Finally, students will view a variety of photosynthetic microbes using a variety of microscopic techniques. During each session, there will be 30-60 minutes lecture-style presentation and approximately 4 hours hands-on lab work. There will be some wait steps during the lab for short breaks and lunch. Following lab, students will use available computers to document labwork and assemble a Word document-based portfolio using a provided template. Specifically, students will complete Word-based templates of their projects that will be posted on-line as part of ongoing project portfolios (75% course grade). A short, in-class essay exam will be given during the last hour of each class (25% course grade). A traditional percentage system will be used for grading (90-100 = A; 80-89 = B; 70-79 = C; 60-69 = D; and less than 60 = F).

Schedule of Activities Session One

1. Introduction to Microbial Diversity & the RLMO Program
2. Photosynthetic Microbes
3. Mud Enrichment
4. Microscopic Diversity

Microbial Diversity

Prokaryotes: Bacteria and Archaeobacteria

Eukaryotes: Fungi, Algae, and Protozoa

Culture-Dependent Identification

Plate, grow, isolate microbes using media

Identify isolates - media-based tests

Naming traditionally requires "pure cultures"

These grow only 1-5% of natural inhabitants.

Culture-Independent Identification

Extract/describe biological molecules

e.g. DNA, proteins/enzymes, pigments

Apply of specific probes or antibodies (Ab)

Assess environment for metabolic processes

NSF/MO

Enhance awareness of microbial diversity

Discover new ways to describe new microbes

RLMO - photosynthetic bacteria, Yellowstone

MO projects worldwide - soil, open ocean, polar, urban (homeland security issues)...

Photosynthetic Microbes

Algae - eukaryotic

Cyanobacteria - bacteria

Purple - bacteria/Proteobacteria

Green Sulfur - bacteria, unique phylum

Green Nonsulfur - bacteria, unique phylum

Heliobacteria - bacteria/Gram Positive

Halophiles - archaea... next week

Algae/Cyano. = oxygenic; *others* = anoxygenic

Overall Reaction - Oxygenic/Plant-Like

Light + pigment: $H_2O \rightarrow O_2 + (H^+) + (e^-)$
ATP synthesis via e- transport chain (ETC)
ATP used to fix CO_2 ("dark reaction")

Summary Reaction

$H_2O + CO_2$ yields $O_2 + glucose + ATP$
We say that this is "oxygenic" photosynthesis.

Oxygenic Membrane Systems

Plants/Algae - chloroplast with thylakoid
Cyanobacteria - simple stacks, no organelles

Algae Diversity

Green - cellulose walls, fresh/marine
Euglena - no wall, most fresh
Dinoflagellates - cellulose walls, marine - toxins
Golden - silica walls, fresh/marine - toxins
Brown - cellulose walls, marine
Red - cellulose walls, marine

Cyanobacteria Diversity

Widespread habitats - deserts to sea ice
Typical bacterial walls - peptidoglycan
Unicellular/Non-N₂-Fixing - Synechococcus
Filamentous/non-N₂-Fixing - Oscillatoria
Filamentous/N₂-Fixing - Anabaena

Overall Reaction - Anoxygenic Bacteria*

Light + pigment: $H_2S \rightarrow S + (H^+) + (e^-)$
ATP synthesis via e- transport chain (ETC)
ATP used to fix CO_2 ("dark reaction")

Summary Reaction

$H_2S + CO_2$ yields $S + glucose + ATP$
Sulfur tolerate/use HIGH sulfur. Nonsulfur low.

*Archaeal photosynthesis TOTALLY different.

Anoxygenic Membrane Systems

Purples - lamellae (simple stacks)
Green - chlorosomes (vesicles)
Heliobacteria - none, all on cell membrane

Purple Bacteria Diversity

Anoxic lakes, springs, mud - some thermal
High levels H_2S (sulfur) or lower (nonsulfur)
MANY shapes - cocci, rods, spirilla, ovals...
e.g. genus - Rhodospirillum

Green Sulfur Diversity

Anoxic sulfur lakes, sediments - few thermal
MANY shapes - cocci, rods, ovals...
e.g. genus - Chlorobium

Green Nonsulfur Diversity

Hot spring mats, marine salt marshes
All filamentous, with cells being large rods
e.g. genus - Chloroflexus

Heliobacteria (Gram Positive) Diversity

Anoxic lakes, sediments - few thermal
Closest relatives: Bacillus and Clostridium
Rods that, like relatives, form spores
Also can ferment chemicals, like relatives

Winogradsky Column Enrichments

1980s - mud mixed with C source, buffer...
Packed into graduated cylinder placed in light
Sustains oxygenics (top), anoxygenics (middle)

Sealed Bottles Enrichments

1 tsp mud plus known media (S or no S)
Tightly corked and placed in light

Other Ways to Exclude Oxygen

Candle jars - just like they sound
Shake tubes - tricky to make, get cultures out of
Commercial anaerobic jars - expensive

Microscopy - Standard Wet Mount

Agents live, applied with liquid to slide/coverslip
No need for stain - agents pigments, large
Algae, Cyanobacteria - use 20-40X objective

Fixed Slides/Simple Stain

Agents dead, dried and heated to just slide
Agents extremely small, pigment light
Other prokaryotes - need oil/100X

ACTIVITIES - ENRICHMENTS USING MUD

Winogradsky Column

Mud and water will be collected from the front of the building - measure temperature and pH
Sift through mud - removing stones and crushing clumps - and sort/remove 200 ml "clean" mud
Mix with 1-2% calcium carbonate buffer and 1-2% $CaSO_4$ - you will need to calculate and weigh
Now add 50-100 ml moistened shredded paper, grass, leaves, or sawdust (anything with cellulose)
CAREFULLY pack this mix into provided glass cylinder (NO air pockets) - stop 5 cm from top

Place 0.5-1 cm moistened shredded paper over top, cover with sourcewater (fill to 1-2 cm from top)
Cover with plastic wrap and place in light (40W bulb, 10-15 cm away) - grow indefinitely

Bottle Enrichments

Add 1 tsp raw mud from above to each of 2 bottles - one labeled S and other NS plus initials
Fill one to TOP with sterile SULFUR MEDIA (cork tightly); repeat with NONSULFUR MEDIA
Incubate IN LIGHT at room temperature - photodocument starting and final bottles

Typically, both bottles and columns develop best over 2-3 weeks. They can also be kept growing almost indefinitely. The Winogradsky Columns are yours to keep growing.

ACTIVITIES - MICROSCOPY/DIVERSITY

Wet Mounts

Pigmented cells can be viewed live using “wet mount” procedures. Wet mounts are good for assessing color but, if done poorly or with motile organisms, they will appear fuzzy and moving. Adding too much material/water will result in the inability to focus (everything moving too much) and more cleaning because you will have sample everywhere. If dissatisfied, consider staining (next optional procedure).

Procedures

Using a pipette or tweezers, withdraw SMALL amount of sample - you should see colored filaments
Place filaments in the middle of the slide, add half a drop of sourcewater, and cover with coverslip

Fixing and Simple Stain

Heat-fixing kills cells and makes them stick to the slides during the staining/rinsing steps. Preparing smears is the most time-consuming part of this procedure so set these up early. After smears are complete, a single stain is applied to the preparation. Typically used for non-pigmented cells, stains allow one to determine size and shape. Because stained samples are typically not viewed with coverslips, however, one can also view them at 100X using oil immersion (i.e. more detail).

Procedures

Place 1 loopful of visible sample on each slide. DO NOT confuse LOOPFUL and DROPFUL.
Set slides aside to COMPLETELY air-dry. In the meantime, proceed with the following....
After you have finished ALL other exercises, pass dried slides through a flame three times to heat-fix.
Flood heat-fixed smear with malachite green and let stand 30 seconds
Rinse with water bottle (2-3 seconds) and gently blot dry before viewing

Basic Microscopy Refresher

Focus PERFECTLY on 10X and then 40X (you may begin on 4X but it is usually not useful)
Because we are doing wet mounts of fairly large cells, the 100X will not be of use
Light can be adjusted using the iris, the condenser, and various filters inserted below the condenser
Using lens paper and one drop lens cleaner, wipe objectives after each use; dry

There are 2 kinds of microscopes available - fairly expensive stand-alones and fairly cheap digitals. Both give accurate color through the oculars. The camera on the digitals, however, severely reddens and dulls real color. Thus, you are strongly advised to view and record observations on more expensive scopes before capturing the images. Playing with the light - as noted above - does help with more accurate digital images.

Using Digital Microscopes - NS204

Turn on the microscope and camera - green AND red switches (back of scope)

Make sure metal pull-bar on right/upper part of scope is pulled all the way out
Double-click on "Motic Images Plus 2.0" program icon - desktop of computer
Activate camera by selecting File/Capture Still Image - image of live feed should come up
Adjust as suggested above, always using computer screen to assess focus
Once satisfied, click "capture" - a new captured image icon will appear to the right (a .sfc file)
To edit captured image, double click .sfc file and - when satisfied - File/Save As .jpg (to desktop)
You will have to ultimately copy to a floppy disk to transfer back to NS201 machines

Make sure you record the magnification at which you shoot each image as this may help you interpret relative size better.

Inserting a Scale Bar

With your .sfc open, pull down Measure/Calibration Table and choose the objective you used
Re-select Measure, this time pulling to Line
Click (without releasing mouse button) on image and drag a line; release button when done
A line with the scale (in microns) will appear above your line
If you don't like it, select Edit/Undo and start over again