



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



National Science Foundation
Western Oregon University
Yellowstone National Park

Schedule of Activities Session Two

1. Photosynthetic Pigments, Archaea
2. Pigment Analysis Set-Up
3. Follow-Up - Last Week
4. Curriculum Discussion/Essay

Photosynthetic Pigments - Location

Specific proteins hold in membrane - nonpolar
Species-specific proteins define real absorption
Naked pigment uses discrete value - e.g. 770
Species-specific proteins alter - e.g. 800, 900
Different methods for naked vs. protein-bound

Chlorophylls (Chl) - Oxygenic

Plants - green a and b

Algae - green a/b, gold/brown a/c, red a/d

Cyanobacteria - green a only

All, when excited by UV, fluoresce.

Accessory Pigments - Oxygenic

Carotenes - yellow or red, WIDESPREAD

Xanthophylls (brown), phycoerythrin (red)

Bacteriochlorophylls (Bchl) - Anoxygenic

Purple bacteria - Bchl a

Green bacteria - Bchl c

Heliobacteria - Bchl g

Accessory Pigments - Anoxygenic

Carotenes - yellow or red, WIDESPREAD

Phycocyanin - blue, similar to phycoerythrin

Many pathogens have carotenes - neutralize H₂O₂.

Isolating Naked Pigments

Because nonpolar, organic solvents involved

Methanol for research-grade work - GLOVES

Acetone acceptable/available - harsh

Because cell walls, you will pulverize sample

Petroleum ether (jar/hood) - chromatography

Data useful for major grouping assignments

A spectrophotometer allows one to skip chromatography.

Isolating Protein/Pigments IN Membrane

Disrupt cells using just pulverization - expensive

Fragmented pigments called "in vivo"

Data useful for grouping and species variation

Archaeal "Photosynthesis" - Class By Itself

Remarkably, these are commercially available.

Why Unique?

Bacteriorhodopsin (Brho) pigment in membrane

Light causes Brho to physically move H⁺ out

This generates proton motive force - ATPase

NO ETC involved in this process

Brho homologous to eukaryotic rhodopsin.

Halophiles

5.5+ M salt - many different ions and pH

Evaporation ponds, salt lakes, salted foods

Halobacterium = phototropic halophile

Many other Halophiles are not photosynthetic.

Photosynthesis and Evolution

Hot earth and the purple origin theory

Ancient earth - 100°C but dark

Heat gives off infrared - 900 nm or more

Emerging purple Bchl absorb this

What does the tree of life support?

Green nonsulfur most ancient - purple members

Phototrophy evolved many times

Chloroplast most similar to Cyanobacteria

ACTIVITIES - PIGMENT ANALYSIS SET-UP

Sample Preparation

Methanol is toxic to skin - wear gloves and collect methanol in waste beakers - NOT down sink.

Each person will be provided one microbial phototroph sample for analysis
Add 0.5 methanol, agitate, and observe for changes... that's why you have to pulverize - NEXT
From this point on, keep sample on ice and foil-wrapped as much as possible to preserve pigment

Sample Pulverizing (Bead-Beating - BB) and Methanol Extraction

Each person will be given a BB tube half full of glass beads and some methanol
Transfer your methanol sample to this tube and bead-beat sample for 1 minute as shown
Wrap in foil and place on ice for 20 minutes to fully develop extraction process from pulverized cells
Proceed to other finishing last week's lab and understanding the next apparatus and steps.

With a large class, a dedicated blender is a reasonable substitute - although cleaning it carefully between uses is necessary if you want clean data and peaks later. Extract can also be frozen overnight - but keep covered/dark. Procedures work well with spinach, carrots...

Chromatography

Understand the apparatus, which I will go over in lab - what is polar, what isn't, why it moves...
Using a dedicated capillary tube, dot extract on paper (along line, above triangle cut)
Allow to dry and repeat - repeat at least 5 times until line is visible with color of extract
When complete and dry, suspend chromatography paper as shown in tube
ONLY the tip of the triangle should touch the petroleum ether - NOT the line
Develop until "front" is 1 cm from top (about 10-15 minutes) - proceed to next steps

Petroleum ether should not be inhaled or touched. It needs to be kept in a closed jar, preferably in a fume hood. Acetone is a reasonable substitute for acetone-extracted samples.

Spectrophotometric Analysis

Centrifuge sample at full speed for 5 minutes; decant liquid only to a new tube
Prepare a methanol only blank - why...
Gather blanks, methanol waste beaker, samples (on ice), and cuvettes

- I will demonstrate basic running of spectrophotometer and individuals can proceed
- if in doubt or if you need to start over, hit "main menu"
- remember to select load first and then "chlorophyll program" (DO NOT DELETE ANYTHING)
- you may need to rescale data to obtain better resolution
- when ready, print final graph and find peaks/print data for all peaks
- understand what the two axes mean

Make sure you compare class data and analyze everything in your discussion.