

303. CHROMATIC ADAPTATION

The light-harvesting pigments of phototrophs are responsible for the initial capture of light energy. Within certain constraints, these pigments determine the wavelengths of light that the phototroph will be able to use. The light-harvesting pigments function by absorbing light of a particular wavelength and re-emitting light of another, longer, lower-energy wavelength which is eventually absorbed by the photosynthetic reaction center chlorophyll.

Each light-harvesting pigment has a characteristic absorption spectrum, absorbing light of particular wavelengths. Some cyanobacteria are able to produce different light-harvesting pigments depending on the wavelengths of light available to them. This ability to adjust pigment composition in response to the available light spectrum is called **chromatic adaptation**.

The natural habitat for those cyanobacteria capable of chromatic adaptation is lakes. Dissolved and suspended organic matter in lakes absorbs the entering light, especially light of longer wavelengths. Higher-energy blue light can penetrate further into the lake than can red light. We would expect chromatic adaptation to enable cyanobacteria to use light of shorter wavelengths when growing deeper in the lake than when growing at the lake surface.

The light harvesting pigments of these cyanobacteria are **phycocyanin**, which is blue-green in color and has an absorption peak at 670 nm and **phycoerythrin**, which appears red and has an absorption peak at 570 nm. Which of these pigments would you expect to predominate in cyanobacteria grown under blue or green light? Which would you expect to predominate in cyanobacteria grown under red light? In this exercise, you will test your predictions.

Purpose

To observe the phenomenon of chromatic adaptation and to investigate the relationship between the color of the light harvesting pigments and the wavelengths of light used by the phototroph.

Materials: *Fremyella*; 3 25 X 150 mm test tubes, each containing 10 ml BG-11 medium; 15 g sucrose (table sugar); red, green and blue cellophane paper; 4 18 mm test tubes; spectrophotometer; four cuvettes; lights

Using aseptic technique, inoculate each of three tubes of BG-11 medium with 1 ml of *Fremyella*. Wrap two tubes with three layers of cellophane of the colors you selected. Leave the third tube unwrapped. Incubate the cultures under fluorescent light at room temperature until they are brightly colored (up to three weeks).

In large test tubes, mix 3.5 ml of each cell suspension with 5 g of sucrose. Also prepare a spectrophotometer blank by mixing 3.5 ml of water with 5 g of sucrose. Dissolve the sucrose by mixing with a vortex mixer and, if necessary, heating the tubes in a warm water bath. The sucrose makes the density of the suspending medium equal to the density of the cells. Without the sucrose, light bouncing off of the cells would interfere with the spectrophotometric measurement of the pigments. Record the absorbance of each sample every 25 nm from 350 to 700 nm.

With this method, chlorophyll *a* has absorbance peaks at 400-450 nm and 650-700 nm. Phycoerythrin has a peak at approximately 550 nm, and phycocyanin at 600-650 nm. For each pigment, the higher the peak, the more pigment present. (We cannot compare amounts of

different pigments without knowing how much light each pigment absorbs at the same concentration.)

Cleanup

Wash the cuvettes thoroughly in hot soapy water and invert them in the test tube rack to dry. Wipe the test tubes with ethanol to remove the labels. Carefully place the test tubes in a slanted basket on the discard cart. Clean all of the sucrose off of the balance and counter tops.

Recipes

BG-11 Medium

NaNO ₃	1.5 g/l	Trace metal mix	
K ₂ HPO ₄	0.04 g/l	H ₃ BO ₃	2.86 g/l
MgSO ₄ ·7H ₂ O	75 mg/l	MnCl ₂ ·4H ₂ O	1.81 g/l
CaCl ₂ ·H ₂ O	36 mg/l	ZnSO ₄ ·2H ₂ O	0.222 g/l
citric acid	6 mg/l	Na ₂ MoO ₄ ·2H ₂ O	0.39 g/l
ferric ammonium citrate	6 mg/l	CuSO ₄ ·5H ₂ O	0.079 g/l
EDTA (disodium salt)	1 mg/l	Co(NO ₃) ₂ ·6H ₂ O	49.4 mg/l
Na ₂ CO ₃	20 mg/l		
Trace metal mix	1 ml/l		

Text References

Light and Pigments, POH p. 163-168

Additional References

- Packer, Lester and Alexander Glazer, eds. 1988. "Cellular Differentiation: Hormogonia and Baeocytes [22]" Methods in Enzymology. Volume 167: Cyanobacteria. (San Diego: Academic Press)
- Stanier, R. Y., J. L. Ingraham, M. L. Wheelis and P. R. Painter. 1986. p. 360 *In* The Microbial World. Prentice-Hall, New Jersey.
- Truper, H. G. and N. Pfennig. 1981. Characterization and identification of the anoxygenic phototrophic bacteria, p. 299-312 *In* M. P. Starr, H. Stolp, H. G. Truper, A. Balows and H. G. Schlegel. The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria. Springer-Verlag, New York.

303. CHROMATIC ADAPTATION (12 PTS.)

Name _____

Lab day and time _____

PRELAB PREPARATION:

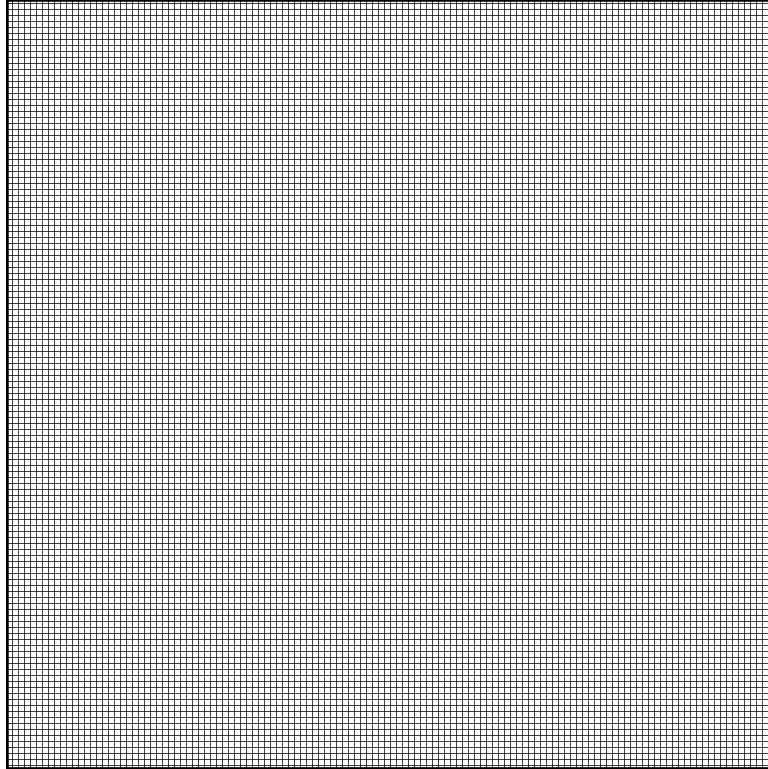
1. Procedural outline:
2. What two colors of light will you use to grow your cyanobacteria? Defend your selection.
3. What effects do you predict the filters you have selected will have on the amount or types of pigments the bacteria produce?
4. What wavelength will allow the fastest growth of the bacteria?

RESULTS:

5. Spectrophotometry Data Table:

Color	Ultra-violet			Blue		Blue-Green		Green		Yellow		Orange		Red	
Wave-length	350	375	400	425	450	475	500	525	550	575	600	625	650	675	700

6. Plot the absorbance of each culture against wavelength.



7. What pigments were present in each culture?

8. Did you observe chromatic adaptation? Were the results compatible with your predictions? Explain any discrepancies.

