

Topic 16. The Cyanobacteria

Domain: Bacteria / Cyanobacteria

Genus: *Oscillatoria*

Genus: *Anabeana*

Domain: Bacteria.

Bacteria is one of two domains of prokaryotes. Based on the sequences of ribosomal DNA, and other molecular evidence, the bacteria are only distantly related to the other domain of prokaryotes, the Archaea. As prokaryotes, bacteria do not have membrane bound organelles, a nucleus or an endoplasmic reticulum. Metabolically bacteria are versatile, and includes members that are heterotrophic, chemoautotrophic, and photosynthetic. Ecologically bacteria are critical in the role of nitrogen fixation, decomposition, primary production, and, as mutualistic symbionts and pathogens. In the Eukarya, both mitochondria and chloroplasts were derived from endosymbiotic bacteria.

Kingdom: Cyanobacteria.

The Cyanobacteria are only one clade of photosynthetic bacteria. Cyanobacteria differ from other clades of photosynthetic bacteria in that they utilize two different light capturing complexes of pigments working in tandem (photosystems I and II), which allows them to take electrons from water. This generates oxygen, and the cyanobacteria were responsible for transforming our atmosphere from a reducing to an oxygenating one. Some members of the group fix nitrogen. Globally the cyanobacteria are ecologically important both as primary producers and for their nitrogen fixation. Members process only chlorophyll a. Accessory pigments include carotenoids, and phycobilins (phycoerythrin and phycocyanin).

I. Genus: *Oscillatoria*.

The cells of *Oscillatoria* form filaments, but the cells are not dependent on one another for survival. Every cell is functionally the same.

Procedure: At the front bench is a saucer of soil. The greenish-black film on the soil is mostly made up of the cyanobacterium *Oscillatoria*. There are many other interesting organisms, however, such as diatoms, euglenoids, and nematodes on the soil.

To prepare a slide, take a small piece of this film with a needle, place it in a drop of water on your slide, mix it around with your needles and cover with a coverslip. *Oscillatoria* is the filamentous organism that constitutes the matrix of the organic material on the soil.

Draw a filament of *Oscillatoria*. Label cells and filament.

_____ mm
Diameter fov = _____ mm

II. Genus: *Anabaena*.

The cells of *Anabaena* form filaments, but unlike *Oscillatoria*, their cells are not all the same. In particular, *Anabaena* produces **heterocysts** which function to fix nitrogen. The heterocysts and the vegetative cells are mutually dependent on each other for survival. As a nitrogen fixer, *Anabaena* forms mutualistic relationships with certain plant species.

Procedure: *Anabaena* grows inside pockets of the tissues of the water fern *Azolla*. Take a small piece of tissue from *Azolla*, add it to a drop of water on a microscope slide, and tear it apart as finely as possible using teasing needles. Prepare a wet mount. Press down on the wet mount with the but end of your teasing needles several times to squeeze the underlying plant tissue. Through your microscope, filaments of *Anabaena* should be clearly visible in the medium around the fern tissue.

Draw a filament. Be sure that you observe, draw and label at least one heterocyst.

_____ mm
Diameter fov = _____ mm

The water fern *Azolla* forms a mutualistic* relationship with a species of *Anabaena*. The plant provides a habitat for the cyanobacterium in pockets in its tissue. The

plant, in turn, benefits from the nitrogen fixed by *Anabaena*. Because of this relationship, *Azolla* is a valuable *green manure* crop in flooded rice paddies in eastern Asia. While the fields are flooded, the plant forms dense mats over the water's surface. When drained, the plant and its symbiont decay, liberating nitrogen to the soil.

* A mutualistic relationship is a physically intimate relationship between organisms of different species where each member benefits from the presence of the other.

III. Determining the Frequency of Heterocysts in *Anabaena* Grown in Complete vs Nitrogen Deficient Media

Introduction: Heterocysts are expensive to make. They cannot divide and are doomed to die without offspring. It should be adaptive to make fewer when nitrogen is abundant.

Procedure: We have two cultures of *Anabaena* at the front. One is in a medium that is complete. The other is in a medium completely deficient in nitrogen (N_2 is the only source of nitrogen in the culture). Make a wet mount of each (sample from the bottom). Count two hundred cells. Make a hash mark on the appropriate line for each heterocyst encountered of the 200 counted. Calculate the frequency of heterocysts in each culture and record below. Draw examples of filaments from each culture and note if there are any obvious differences between them.

Complete Culture (hash marks) _____

Incomplete Culture (hash marks) _____

Frequency in complete culture (#heterocysts/200) _____

Frequency in incomplete culture (#heterocysts/200) _____

Drawing (complete)

Drawing (incomplete)

Record your data on the board to share with your lab.

IV. Determining Heterocyst Distribution

Introduction: Heterocysts fix nitrogen. If only atmospheric nitrogen (N_2) is available to the organism, then the other cells of *Anabaena* are completely dependent on the heterocysts for survival. In this activity we will determine just how far from a heterocyst a regular, vegetative, cell can exist.

Procedure: Make a wet mount of *Anabaena* growing in the incomplete medium. Sample 16 intervals between neighboring heterocysts. For each interval, count the number of cells between the heterocysts, and record these counts below. If a cell is obviously undergoing cell division count it as two cells.

| | | | |
|------------|--|-------------|--|
| Interval 1 | | Interval 9 | |
| Interval 2 | | Interval 10 | |
| Interval 3 | | Interval 11 | |
| Interval 4 | | Interval 12 | |
| Interval 5 | | Interval 13 | |
| Interval 6 | | Interval 14 | |
| Interval 7 | | Interval 15 | |
| Interval 8 | | Interval 16 | |

What is the average number of cells between heterocysts for your sample?

What is the farthest distance, measured by intervening vegetative cells, any cell in your count is from a heterocyst?

Record your data on the board to share with your lab.

V. Stromatolites - Demonstration at the front bench.

These layered sedimentary rocks are a byproduct of the metabolic activity of microorganisms, especially of filamentous cyanobacteria. Stromatolites are found in rock layers some of which are dated at over two billion years old. Until 600 million years ago the cyanobacteria dominated the landscapes of Earth, and were responsible for initiating the oxygen rich atmosphere that exists today. They formed extensive reefs composed of stromatolites are still produced today, but only in environments which are too harsh for their predators to survive.



Topics for discussion

1a. How are the Cyanobacteria similar to the chloroplasts of plants?

1b. How are they dissimilar?

2a. Where are the photosystems found in Cyanobacteria?

2b. Where are they found in the chloroplasts of plant cells?

3. The heterocyst is the site of nitrogen fixation in the Cyanobacteria. This process requires anoxic conditions.

a. From your text - does any type of photosynthesis occur in the heterocyst?

b. How does the heterocyst survive if it can't fix carbon?

c. How do the other cells in a filament of *Anabaena* benefit from the nitrogen fixed by the heterocysts.

4. Fossils of the same form as *Anabaena* and *Oscillatoria* have been discovered that are over 2 billion years old. Speculate about why certain forms persist while others are subject to rapid evolutionary change.