The Cyanobacteria or “blue-green algae”

The cyanobacteria, also known as cyanophyta due to their photosynthetic properties, were once viewed as primitive algae, also referred to as blue-green algae. However, the absence of a nucleus and circular arrangement of the DNA throughout the cell place them firmly within the Subkingdom Prokaryota. The cyanobacteria are therefore closely related to the bacteria (Division Schizonta) but are differentiated from the Schizonta by their ability to photosynthesize and their utilization of chlorophyll a. These features link the cyanobacteria to both the bacteria and the algae. The occurrence of accessory pigments such as phycobilins (red) and carotenoids (orange, yellow) in addition to the green chlorophyll a generally impart a blue-green appearance on the cells. Pigment percentages and distributions may also in rare instances produce species that range from dirty yellow to red, or dark brown to black. Whatever their color, cyanobacteria are photosynthetic, and so can manufacture their own food.

Similar to other bacteria the cyanos are ubiquitous due to their small size and resistant prokaryotic cellular organization. Explosive growths, mats or blooms are frequently found in soil and aquatic environments and some cyanobacteria may be toxic (Anabaena, Lyngbya and Nostoc) while others are used in nutrient supplements (Spirulina). Unlike other bacteria or algae the cyanobacteria are one of very few groups of organisms that can convert inert atmospheric nitrogen into an organic form, such as nitrate or ammonia. It is these "fixed" forms of nitrogen that plants need for their growth, and must obtain from the environment. Nitrification cannot occur in the presence of oxygen, so nitrogen is fixed in specialized cells called heterocysts. These cells have an especially thickened wall that contains an anaerobic environment.

Diversity of morphology may be seen among the 150 extant genera, though this diversity is reasonable subtle compared to other organisms. Cyanobacteria may be uni-cellular or colonial and both versions may show a covering of mucilage. Colonial cyanobacteria may be filamentous (simple, mixed and branched) and in rare occasions form irregular or cuboidal cell aggregates and in same cases plates or sheets.

1. Gloeocapsa – a few-celled colonial cyanobacterium occurring in either single (rarely) or clustered cells enclosed in concentric layers of mucilage. Typically, clusters of two to four are seen. These organisms are predominantly terrestrial though there are also aquatic species (Gloeocapsa crepidinum).

A. Prepared slide of Gloeocapsa – observe on dissecting microscope first in order to find location of the cell aggregations (blue matrix due to stain utilized in slide preparation). Once you have found the cell aggregates general location transfer the slide to a compound microscope and observe at 4, 10 and 40x ocular magnification.

Draw several aggregates and label the sheath and protoplasm. How are they distinguished from each other? (consider delineation, color and appearance)

The chromoplasm contains the photosynthetic components, the centroplasm contains the genetic material. Base on this information can you discern the chromoplasm and centroplasm? If so, how?
B. Prepare a *Gloeocapsa* wet mount using a drop of cultured water (containing the cyanobacteria) on a glass slide. Cover carefully with a coverslip to avoid air bubbles (presence of air bubbles will make it very hard to see these small organisms). Repeat the viewing sequence explained in A and note the differences between the live and preserved organisms.

What color is the live *Gloeocapsa*? Why the difference in color between live and preserved materials?

Can you still distinguish sheath from protoplasm? How? What is the reason for being able to differentiate the difference?

Can you distinguish organelles? If so which organelles are discernible? If not, why?

2. *Oscillatoria* – Genus of blue-green algae most common in freshwater environments with some marine species (generally genus *Trichodesmium*). This unbranched filamentous alga, occurring singly or in tangle mats particularly found in polluted environments, derives its name from its motion, which is thought to result from a secretion of mucilage that pushes the filament away from the direction of excretion. Reproduction is by fragmentation in which dead concave cells (separation disks) separate sections of the filament (hormogonia). When present, the mucilage sheath is very thin.

A. Prepared *Oscillatoria* slide. Observe at increasing magnifications (up to 40X ocular magnification).

Draw the outline of a filament. How are the cells in a filament arranged? Are all cells equally colored? *Oscillatoria* reproduces asexually through a process called hormogonia (trichome becomes detached, glides away to form a new filament), is the coloration of the cells representative of this process, if so how?

What about the cell arrangements within a filament? What are the shapes of the cells? What do you think their cross section will look like? *Oscillatoria* may also show asexual reproduction through fission. Observe cells at the tip of a trichome? Do they differ from the rest of the cells? If so, how?

B. Observe the form, true color and movement (very slow) of *Oscillatoria* in a water culture. Prepare a wet mount (see procedure above for *Gloeocapsa*). Make your observations at 10x ocular magnification.

Use a ruler to determine the diameter of the field of view at 10x ocular magnification. Examine and identify the millimeter markings along one edge of the ruler. Place the ruler on the stage so that it covers half of the stage opening. While on Low power (10x), look through the eyepiece. Focus on the edge of the ruler using the coarse adjustment. Adjust the position of the ruler so that the view starts with a ruler line. Count the squares across one field of view. One millimeter is the distance from the middle of one line to the middle of the next mark. Remember, 1mm = 1000 micrometers.

Sketch the outlines of the trichomes (unbranched filaments) at time 0 and 5 minutes in the field of view. Have the filaments moved? If so what is their rate of movement?
3. **Spirulina** – An undifferentiated, helical filamentous cyanobacterium found in freshwater and marine waters. Previously this organism was thought to be uni-cellular because microscope resolution could not distinguish cellular cross-walls. Spirulina is a type of blue-green algae that is rich in protein (45-65% dry weight), vitamins, minerals, and carotenoids, antioxidants that can help protect cells from damage. It contains nutrients, including B complex vitamins, beta-carotene, vitamin E, manganese, zinc, copper, iron, selenium, and gamma linolenic acid (an essential fatty acid) and therefore provides a food source or nutritional supplement.

A. Prepared *Spirulina* slide.
What is the cell organization of *Spirulina* compared to *Oscillatoria*? Are the filaments constructed in a similar manner? Are the cells of *Spirulina* visually smaller or larger than those of *Oscillatoria* (make sure that you observe them at the same magnification or else you cannot compare approximate sizes)? What does cell size have to do with filament shape?

B. Prepare a wet mount of *Spirulina* from a seawater culture and prepare a sketch. Can you view the cellular cross walls at all magnifications? If not, what magnification is required to view them?

What are the structural similarities to *Oscillatoria*? Name all that may apply.

What are visual differences between *Spirulina* and *Oscillatoria*?

Observe the live specimen for 5 minutes. Is there another biological phenomenon that closely associates *Spirulina* with *Oscillatoria*?

4. **Gloeotrichia** - Filamentous - colonial; trichomes heteropolar with basal heterocytes and apical hair-like ends with own sheaths, united radially into gelatinous, hemispherical or spherical colonies, which are microscopic up to several cm in diameter, olive green, yellow-green, brown or dark blue-blackish. The whole colony enveloped by a fine or firm slime; trichomes always oriented with heterocytes into the centre of the colony. Trichomes rarely false branched (during trichome division), the branches separate soon from the mother trichome, but remain parallel and radially located within the colonial slime and form their own gelatinous sheaths. Colonies are joined to the substrate or free floating. Trichomes uniserial, rarely with intercalar heterocytes (developing before or during trichome disintegration), constricted or unconstricted at the cross walls, more or less straight or coiled. Sheaths are always present, but sometimes gelatinize within the mucilage of colonies, especially near apical parts of trichomes (near the margin of a colony).

A. Prepared slides of *Gloeotrichia*.

Observe the filamentous colony on the slide. Find the basal heterocytes. What are their functions?

Observe the apical hair-like ends. Are they enclosed in their own sheath or lacking mucilage? Do you observe any branching?
B. Make a wet mount of a *Gloeotrichia* culture. After observing the true color, stain the colony with Azure B by removing the water from the slide (place Kimwipe® to the edge of the coverslip to draw water out) and replacing it with Azure B (add a drop close to one side of a coverslip and hold a Kimwipe® to the opposite end to pull the liquid across the slide without removing the cover).

What is the true color of the colony? Are the filaments oriented parallel to one another or is there a different type of orientation?

Is the colony mucilage covered or naked? How can you tell?

5. *Anabaena* – a differentiated, filamentous genus of nitrogen-fixing blue-green algae with beadlike or barrel-like cells and interspersed enlarged spores (heterocysts), found as plankton in shallow water and on moist soil. There are both solitary and colonial forms, the latter resembling a closely related genus, *Nostoc*. In temperate latitudes during the summer months, *Anabaena* may form water blooms and can be used as an indicator of pollution state of an aquatic habitat. *Anabaena* trichomes resemble a “pearl necklace” with the vegetative cells of similar size. In addition, you may find larger, empty looking cells, heterocysts, may be visible. If the colony you are looking at is aging you may also find another cell type, the akinetes, which are larger than the vegetative cells and yellow to reddish in color.

A. Prepare a wet mount of *Anabaena*.

What are similarities between *Anabaena*, *Oscillatoria* and *Spirulina*. What are differences?

Find a heterocyst on the colony and compare this cell type to the vegetative cells. What is the difference between these two cell types? Does their function and therefore their cellular requirements explain that difference? How?

In your specimen, which cells are more frequent? Heterocysts or akinetes? What does this tell you about the age and functionality of the culture?

View representative pictures of *Anabaena* akinetes. How can you explain the color of the cells? What is the function of akinetes? What does that tell you about the environmental conditions for the organism in the picture?

6. *Scytonema* – filamentous, thallous cyanobacterium forming solitary branched filaments or mats on the substrate. Filaments free or in fascicles, sometimes densely coiled, creeping on the substrate or with erected branches, commonly falsely branched, with one or (obligatory) two lateral branches. Branching initiates after trichome disintegration by help of necridic cells between two heterocytes, usually not at heterocytes; both branches grow parallel, aside or in crossing position; the filaments make sometimes typical loop-like lateral formations before branching, in which tops of the trichomes later divide. Trichomes isopolar, cylindrical, not diversified in basal and apical parts, uniseriate, usually with solitary heterocytes, constricted (rarely not constricted) at cross walls; terminal parts of branches cylindrical or slightly widened,
with rounded apical cell; middle parts of trichomes sometimes with elongated, cylindrical cells. Sheaths firm, limited, parallel or diverging, lamellate, usually yellow-brown (colored by scytonemin) in some parts. Cells pale or olive-green, usually with solitary, irregularly disposed granules or with granular content, rarely yellowish or pinkish colored; apical cells sometimes with large vacuoles. Heterocytes intercalar, solitary, rarely in pairs, cylindrical or barrel-shaped. Akinetes were several times mentioned, but not proved and well described.

A. Prepared slide of *Scytonema*.

How are the cells compared to *Anabaena*, *Spirulina* and *Oscillatoria*?

Are the filaments straight, helical or in part falsely branched?

Do you find heterocysts? If so, how does the structure of the heterocysts compare to those of *Anabaena*? What does that tell you about the overall shape of the heterocysts in different species? (View also pictures provided in class to answer this question)

7. *Stigonema* - filamentous thallous cyanobacterium; thallus wooly or crusty, composed from free, coiled, true branched filaments, usually attached to the substrate, not diversified distinctly in basal filaments and branches. Trichomes two or multiseriate (only in young trichomes and at ends of branches uniseriate), sometimes very thick, irregularly laterally true branched (T- and V-type of branching), irregularly coiled, sometimes narrowed (with less number of cell rows) towards the ends, apical cell is sometimes smaller than the other ones. Sheaths thin or thick, limited, later wide, lamellate and usually yellowish-brown; around cells in old parts of filaments sometimes special envelopes (trichomes disintegrating in separated cells within filaments). Cells barrel-shaped or roundly irregular, connected usually by one pore ("pit connections") one with another, which disappear in some segments of trichomes; cell content blue-green or olive green, usually with prominent solitary granules. Heterocytes intercalar, rarely lateral, in a similar form as neighboring vegetative cells. Akinetes not known. Sometimes chroococcoid cell clusters arise.

A. Prepared slide of *Stigonema*.

Are the cells similar or different compared to the other thallous form (*Scytonema*)? If so, how?

How do the filaments differ compared to *Scytonema*.

Are heterocysts present or absent? What does that tell you about the abilities of the organism.

8. *Rivularia* - heteropolar filaments, differentiated into basal and apical parts, simple, joined parallel into firm strata. At the beginning of the vegetation cycle hemispherical or spherical, later sometimes vast, flat, macroscopic, irregular strata, up to several cm or dm in diameter and several mm thick strata are layered (sometimes with several layers), with densely agglomerated trichomes, oriented by their bases with heterocytes to the substrate and by the apical hair-like parts to the surface of the colony. Strata are gelatinous up to leathery or intensely incrusted by calcium carbonate, always covering the substrate. Trichomes +/- cylindrical, constricted or
unconstricted at the cross walls, dividing at intercalar heterocyst: the divided trichomes separate one from another, but remain parallel located within the mother sheaths in the colony. Hairs are composed from the narrow, long, hyaline cells. Sheaths firm, sometimes lamellate, colorless or yellow-brown, enveloping all the "daughter" trichomes.

A. Prepared slides.

View the slides and compare the cell structure, shape and composition (i.e. what cell types) to the structure of *Anabaena*, *Spirulina* and *Oscillatoria*.

What distinguishes this colony from the shape and form of the other colonies?

9. *Nostoc* – filamentous, thallous cyanobacterium. The thallus may be micro- or macroscopic, gelatinous, amorph or spherical, later irregularly spherical, lobate, smooth or warty on the surface, filamentous or forming flat gelatinous or "paper-like" (when dry) colonies, usually with distinct periderm on the colonial surface. Filaments within colony irregularly coiled and loosely or densely agglomerated, sometimes more gathered in peripheral layer; sheaths around trichomes present, but visible usually only in the periphery of colony or in young colonies, wide, fine mucilaginous, confluent with colonial mucilage, sometimes yellowish-brown. Trichomes isopolar, of the same width along the whole length, apical cells morphologically not different from other cells; cells cylindrical, barrel-shaped up to almost spherical (forming moniliform trichomes; variability of cell-size and -shape sometimes distinct in one and the same species). Heterocytes solitary, develop in trichomes terminally or intercalary. (their frequency or absence is dependent on nitrogen metabolism); trichomes in principle metameric. Akinetes arise apoheterocytic, oval, little larger than cells; almost all cells between heterocytes change successively in akinetes towards heterocytes. Nostoc has special life (vegetation) cycle, during which forms several special and characteristic stages.

A. Prepared slides of *Nostoc*. This slide contains filaments only, not globular colonial organisms.

Draw and describe the structure, cell types and their functions of *Nostoc* filaments.

B. Live *Nostoc* filaments. Make a wet mount and compare the structure and composition of these filaments to *Anabaena*. What are similarities? What are differences? What do these tell you about the age, state and function of these colonies?

C. Live *Nostoc* ball cultures. If the balls are large enough, observe under a dissecting microscope first. Can you discern the filaments inside?

Allow one of the balls to sit outside water for one hour. How has the ball changed? What is its appearance?

Make a wet mount and observe using the compound microscope at increasing magnifications. What is the cell composition of the filaments? What does that tell you about the functionality?