Kingdom Eubacteria, Archaebacteria with Cyanobacteria

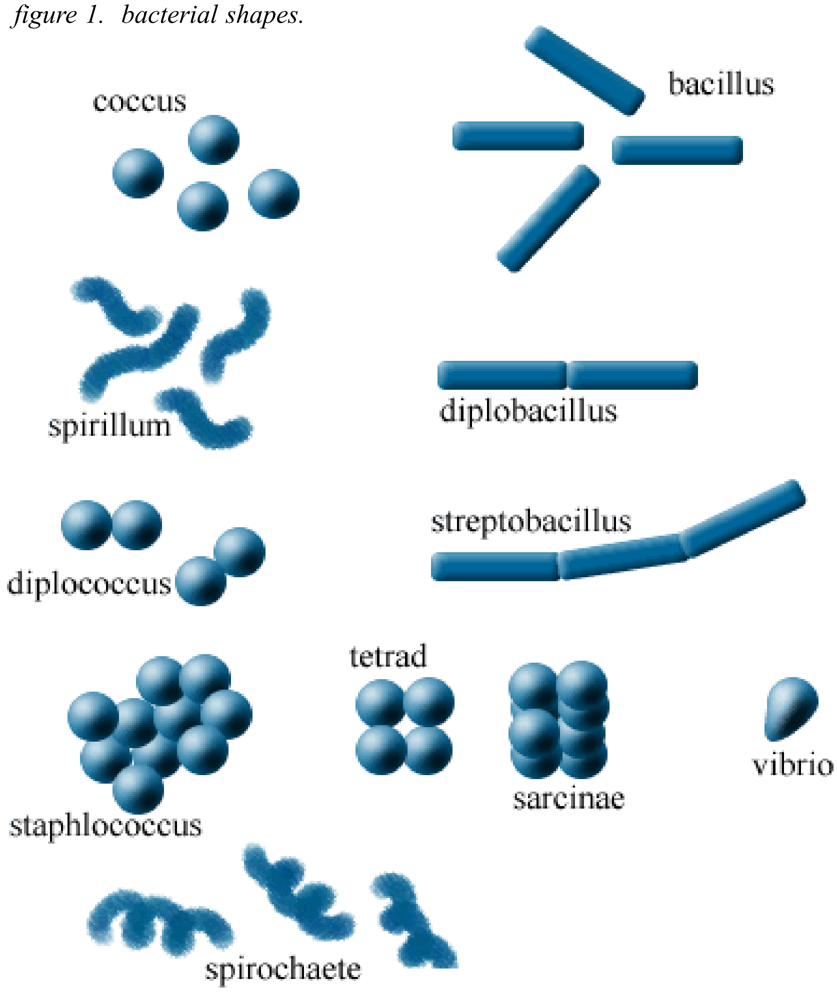
Once, scientists placed all life forms into two kingdoms: Plantae and Animalia. Included in the Kingdom Plantae were bacteria, blue-green algae, fungi, and protistans. Today, the consensus is life can be divided into six kingdoms: Euacteria, Archaebacteria, Protista, Fungi, Plantae and Animalia. Note that even this breakdown of life forms does not include viruses, prions and viroids. We'll stick with the six kingdom approach for this exercise.

Eubacteria

This is a large grouping of organisms, far more extensive than Archaebacteria. Campbell (1993) provides the following breakdown.

- Actinomycetes
- Chemoautotrophs
- Cyanobacteria (a separate lab)
- Endospore forming bacteria
- Enteric bacteria
- Mycoplasmas
- Myxobacteria (separate lab)
- Nitrogen fixing bacteria
- Phototrophic bacteria
- Pseudomonads
- Rickettsias and chlamydias
- Spirochaetes

figure 1. bacterial shapes.



Bacterial Shapes

Bacteria come in all shapes and sizes. However, three basic forms are recognized: coccus, bacillus, and spirillum. Coccal shapes are spherical, bacillial are rod shaped and spirillum is a twisted rod shape. Variations on these forms are numerous. Two coccal forms stuck together are called diplococcal, while clusters of bacteria are indicated by the prefix "strepto-", for example, streptococcus. Chains of bacteria are indicated by the prefix "staphlo-" as in staphlococcus. Groups of eight cocci are called sarcinae while comma shaped bacteria are called vibrios.

Actinomycetes

Although the suffix "mycetes" refers to fungi, these have been removed from the fungal classification and placed in the Eubacteria. These are most often found in soils, as *Streptomyces* and *Mycobacterium.* **Observe the prepared slide of *Streptomyces*,** a Gram+, nonmotile organism with coenocytic hyphae. From this genus, we produce the antibiotic streptomycin.

Chemoautotrophic Bacteria

Many of you have sensed at least one species of these if you have been in the Keys and smelled the rather disagreeable odor of rotten eggs as you drive Highway 1. The smell is hydrogen sulfide H_2S, a compound used by some forms to produce energy. It is noticeably worse when someone disturbs mangroves. Example genera include *Nitrobactera* and *Nitrosomonas*. These bacteria oxidize inorganic substances to produce energy for food production. Some of the inorganic materials used are NH_3, NO_3^-, H_2S, S, and Fe^{+3}. They utilize these inorganic materials, along with carbon in carbon dioxide to manufacture carbohydrates and other compounds. **If prepared slides are available, observe the characteristics of this group.**

Endospore Forming Bacteria

These bacteria form internalized spores which may "hatch" when environmental conditions permit. They may be either aerobic or anaerobic and typically are Gram positive (more on this later), flagellated rods. Genera include *Bacillus* and *Clostridium*. *Clostridium* is the bacterium that causes gangrene. **Observe a prepared slide of *Clostridium botulinum***, rod-shaped and which produces powerful exotoxins. This bacterium causes botulism or food poisoning.

Enteric Bacteria

For a while, little was known about these bacteria since they are facultatively anaerobic and are difficult to culture (at least some forms). The most well known is *Escherichia coli* which lives in our small intestine in a symbiotic relationship producing vitamin K for us. They are Gram negative. Some are quite harmless, and in the case of the *E. coli* that inhabits our small intestine, quite beneficial. Other strains of *E. coli* and other genera are quite pathogenic. Other genera include *Salmonella* (responsible for *Salmonella* poisoning) and *Vibrio cholerae*, responsible for the potentially deadly disease cholera. **Observe the prepared slide of *Vibrio*, a Gram- rod.**

Mycoplasmas

First, unlike all the rest of eubacteria, these don't have any cell walls. They are essentially a mass of protoplasm, albeit a very small mass of protoplasm (0.10-0.25 µm), some of the smallest of all cells. These are saprobic and animal pathogens for the most part, however, they have been implicated in lethal yellowing of palms here in south Florida.

Myxobacteria

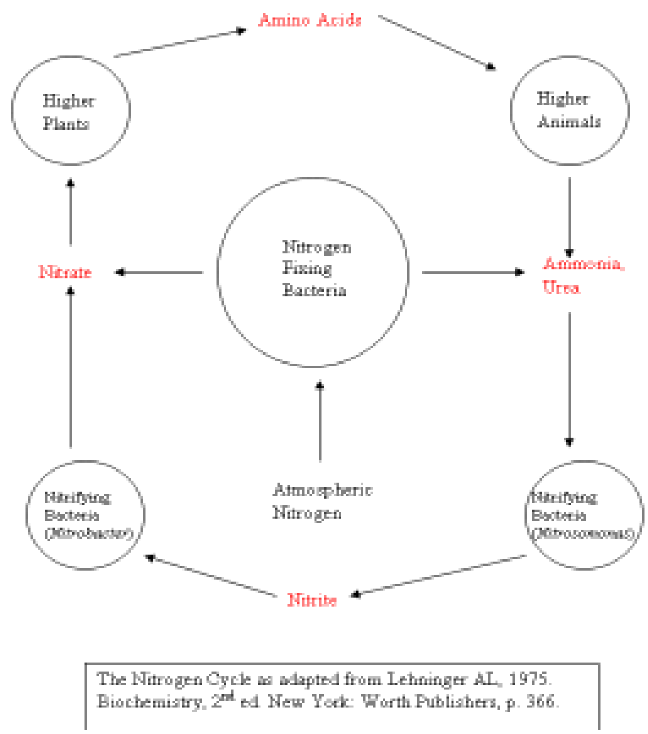
Strange is the best description of these. They are groups of individual cells that feed over a substrate and move by gliding. Once environmental conditions are harsh, the individual cells congregate to form "fruiting" structures which eventually release spores. More will be discussed about this group later in another lab. A typical genus is *Myxococcus*.

Nitrogen Fixing Bacteria

The most studied of these include mutualistic species that live in nodules on the roots of leguminous plants (beans). They are capable of removing atmospheric nitrogen (N_2) and converting it to a form more usable as nitrites and nitrates. In addition to mutualistic species, there are free-living species as well. Typical genera are *Azotobacter* and *Rhizobium*.

Observe the prepared slide of *Rhizobium leguminosarum*, a Gram–, motile rod found in the nodules of roots of legumes. This bacterium fixes atmospheric nitrogen.

Figure 2: The Nitrogen Cycle.



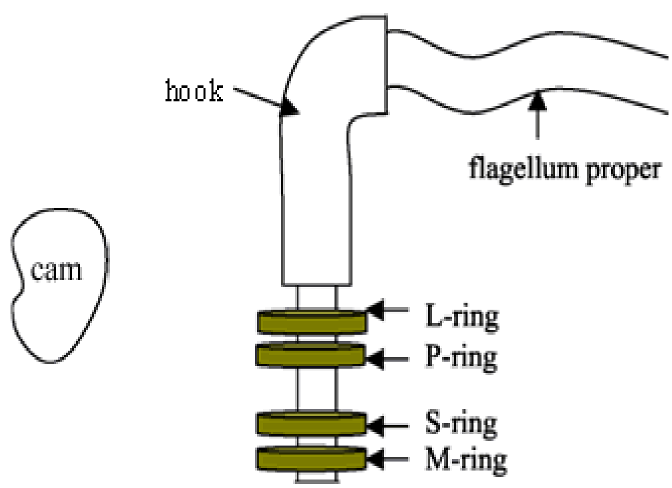
Phototrophic Anaerobic Bacteria

In lecture, you will hear about the differences between cyanobacteria and eubacteria. Much of the comparison involves the photosynthetic process of this group with cyanobacteria. There are numerous groups within this, including purple sulfur bacteria, green sulfur bacteria and the genera *Rhodospirillum*.
Observe the prepared slide of *Rhodospirillum rubrum*, a Gram–, motile, photosynthetic spiral. Upon exposure to light, this organism turns pink.

Pseudomonads

Probably, an entire course could be offered in this diverse group, particularly the genus *Pseudomonas*. This genus is found in virtually all aquatic and soil habitats. The genus is characterized by rod-shaped, Gram negative cells that have flagella "tufted" at one end (lophotrichous). They are chemoheterotrophs.

figure 3. Bacterial flagellum.



Many bacteria (not cyanobacteria) produce flagella. Neither bacteria nor cyanobacteria produce cilia. Both may produce a slime or mucous coat around themselves for protection. The flagellum is composed of a protein called flagellin and episolon-N-methyl lysine (Wistreich and Lechtman 1980). The flagellum is constructed of several rings, a hook-like structure and a long filament. It is attached to the inner and outer membranes (the outer only if present). **Observe the prepared slide of the flagellar stained bacterium.**

Rickettsias and Chlamydias

All of these are obligate parasites with Gram negative walls. Usually there are at least two hosts (arthropod and mammal). *Rickettsia rickettsia* is the causative agent of Rocky Mountain Spotted Fever (the vector is the deer tick) and *Chlamydia* is a pathogenic bacterium passed between birds and humans. If a specimen is available, **observe *Rickettsia* and *Chlamydia***.

Spirochaetes

These are helical cells that have cork-screw like movements due to their internal flagellar filaments. They may be free-living or parasites as *Treponema pallidum*, the causative agent of syphilis. The original test for syphilis was to look for the bacterium after treatment with a dye which fluoresced. More sophisticated tests are now available. **Observe the prepared slide of *Treponema pallidum*.**

Archaebacteria

Although the term *achaio* comes from the Greek for ancient, Archaebacteria probably evolved, like Bacteria and eukaryotes from a common ancestor. However, Archaebacteria are more related to the eukaryotes than to the Bacteria.

Several features separate the archaebacteria from eubacteria.

- no peptidoglycan in cell walls
- unique lipid bilayer of cell membranes
- RNA polymerase more similar to eukaryotes
- ribosomal protein more similar to eukaryotes (Campbell 1993).

Archaebacteria also grow in extreme habitats as salt flats, hot, acidic aquatic environments and anaerobic environments. From this, Campbell (1993) breaks the Archaebacteria down into 3 subgroups: methanogens, extreme halophiles, and thermoacidophiles.

Methanogens

Methanogens are noted for their use of H_2 to reduce CO_2 into methane gas (CH_4). Methanogens may be obligately anaerobic and may actually be destroyed by exposure to oxygen, a principle many water treatment facilities put into play when they aerate sewerage.

Methanogens produce marsh gas, which may be seen bubbling up from stagnant bodies of water (either that or it's oxygen from photosynthesis). You can actually collect the gas and burn it off and many a person thinks they have seen UFO's in the Everglades when in reality it's burning marsh gas.

There is also plenty of methane produced by cattle and termites which is pumped into the atmosphere. Some suggest the huge cattle population may actually be cause for global warming by placing more methane into the atmosphere.

Halophiles

Look for these in salt water environments where the salt concentration is far above the ocean, as the Great Salt Lake in Utah and the Dead Sea in the Middle East. Sea water may have a salt concentration of around 3.3% and halophilic Archaebacteria may survive 10 times the salt concentration of the sea (Campbell 1993).

Thermoacidophiles

As the name implies, these bacteria may withstand quite hot temperatures and low pH. It is not unusual for these forms to survive temperatures of around 80° Celsius and a pH of 2 (Campbell

1993).

Observe any genera of Archaebacteria available. Compare them to the Eubacteria just observed.

Cyanobacteria

In the past, Cyanobacteria were grouped with plants and called blue-green algae. The word cyan comes from the Gr *kyanos* which means blue. Evidence today from electron microscopy and biochemistry suggest they are more bacteria-like (Bold 1973). They are ubiquitous; occurring in aerial, terrestrial, and aquatic habitats. There are approximately 150 genera with 1,500 species (Bold 1973).

Many botanists argue the blue-greens are indeed plants and not bacteria. Their beliefs are based on three characteristics of blue-greens: (1) bacteria do not contain chlorophyll *a* while the blue-greens do, (2) the blue-greens are more differentiated than bacteria and (3) bacteria do not produce oxygen during photosynthesis while plants do.

Reasons for including the Cyanobacteria in the bacteria group include (1) they have a cell wall composed of murein, similar in nature to the cell walls of bacteria (2) they have a single strand of DNA for a genome and (3) it is a prokaryotic cell (Bold 1973). See *table 1* on page 8 for a comparison.

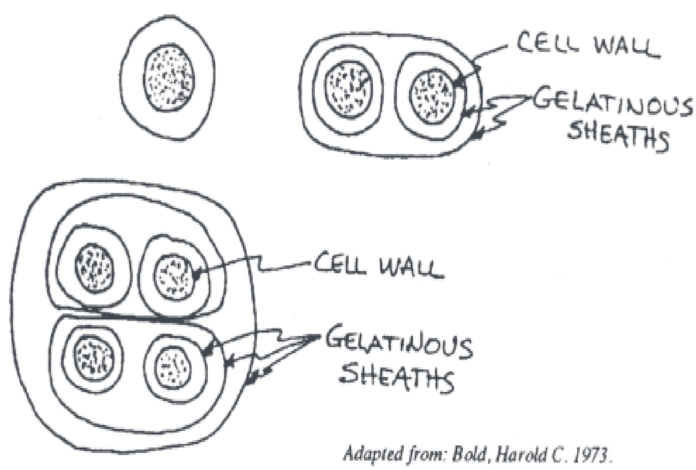
What do you think? Is it a bacterium, archaebacterium, or blue-green alga?

Pigments

The presence or absence of pigments in species is a critical factor in identification. Pigments associated with the cyanobacteria include the water insoluble pigments chlorophyll *a* and the carotenoids. Water soluble pigments include phycocyanin, phycoerythrin, and allophycocyanin.

Gelatinous Sheath

One characteristic shared by all members of the Cyanobacteria is the presence of a layer of slimy material of varying thickness and consistency, the gelatinous sheath. Electron microscopy has determined it is composed of "fibrillar material embedded within an amorphous matrix" (Bold 1973). It can be shown by staining the alga in diluted India ink or methylene blue (Bold 1973). **Obtain a living culture of *Gloeocapsa* and make a wet mount**. Observe first without stain and then stain with methylene blue to see the gelatinous sheath. Save the slide for comparison with *Chroococcus.*



Adapted from: Bold, Harold C. 1973. Morphology of plants, 3rd. ed. New York. Harper & Row. 139.

GLOEOCAPSA

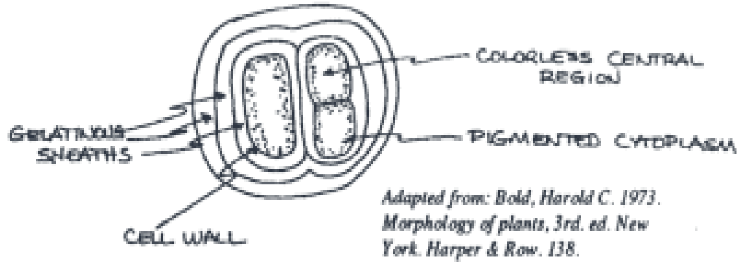
Gloeocapsa (Gr. *gloia*, glue + L. *capsa*, a box or case) is best found on moist rocks in shady areas, walls, and on the outside of flower pots. It gives the appearance of a light green paint on the surface of the object. You generally do not find *Gloeocapsa* growing as a single cell due to the abundant cell division and the gelatinous sheath.

Food Storage

The photosynthetic storage product of plants is starch. However, there are many varieties of starch. Cyanobacteria form cyanophycean starch in structures called alpha granules (Bold 1973). This starch is actually glycogen (Bold 1973) more appropriately associated with animal starch (and in some bacteria).

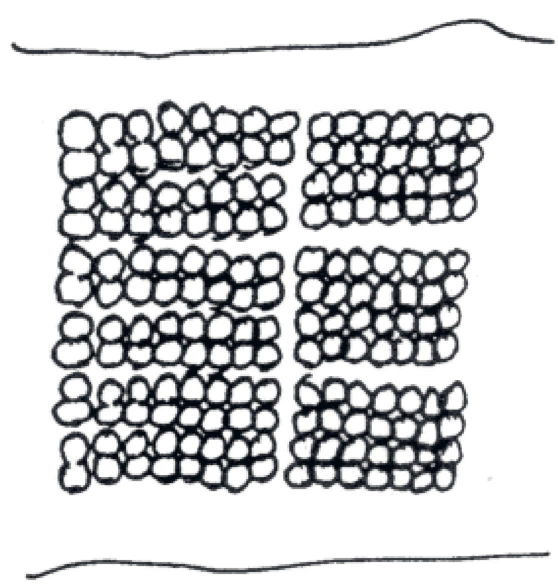
Growth Patterns

The patterns of growth exhibited by the blue-greens take several forms. *Chroococcus* is unicellular. *Gloeocapsa* is unicellular also but thick gelatinous sheathes prevent the cells from separating and thus *Gloeocapsa* appears colonial. **Make a wet mount of *Chroococcus***, stain it with methylene blue and compare it to *Gloeocapsa*.



Adapted from: Bold, Harold C. 1973. Morphology of plants, 3rd. ed. New York. Harper & Row. 138.

Merismopedia forms flat plates of cells and *Lyngbya* and *Gleotrichia* form filamentous structures.



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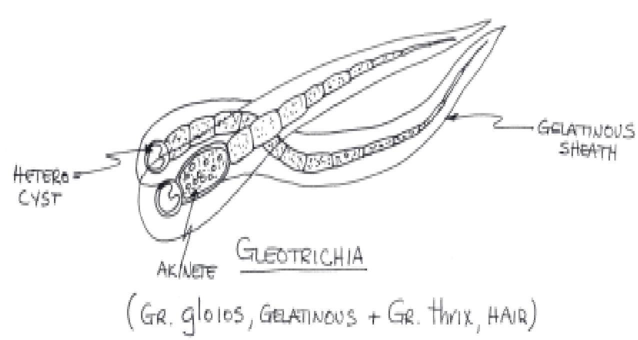
Nostoc (name used by Paracelsus) is also commonly known as star jelly or witches' butter (Bold 1973). It is often found growing as a jelly-like mass on the ground. To the layperson, *Nostoc* is virtually identical to another filamentous genus, *Anabaena* (Gr. *anabainein*, to arise). To the scientist, the way to tell the difference is the more abundant gelatinous sheath in *Nostoc* and the size of hormogonia. A hormogonium consists of a series of cells divided from another series of cells by dead cells.

Both *Nostoc* and *Anabaena* produce akinetes and heterocysts. Both are capable of nitrogen fixation like some of the eubacteria. Akinetes are spores and are highly resistant to environmental stresses and some have germinated after many years in storage (Bold 1973). The heterocysts also serve as the boundaries for hormogonia and are capable of fixing nitrogen.



Adapted from Bold HC. 1973. Morphology of plants, 3rd ed. New York: Macmillan. p 140.

Make a wet mount of either *Nostoc* or *Anabaena*, whichever is provided. Observe and compare to a prepared slide of the same. Try to identify the following: cell wall, akinete, heterocyst, sheath, hormogonium.

Anabaena and another blue-green, *Microcystis*, are the main species found in water blooms in the Everglades and Lake Okeechobee. Water blooms are rapid algae growth that often results in oxygen depletion and death of aquatic life, especially fish.

How is it a plant which evolves oxygen during photosynthesis can deplete oxygen in a lake and cause fish kills?

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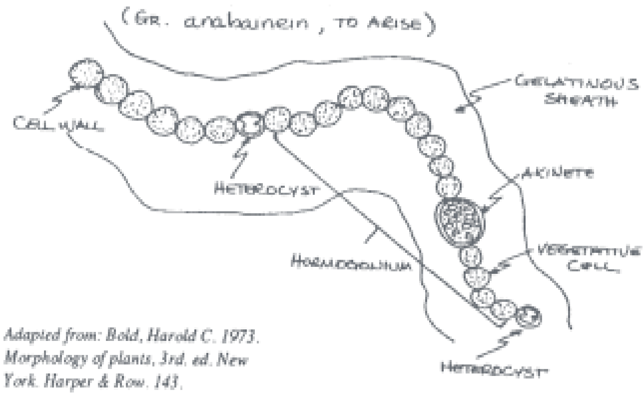

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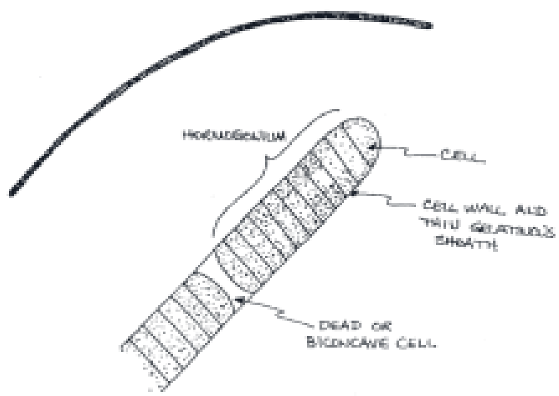
Anabaena may also be found as an endophyte (living inside another plant). The common water fern *Azolla* is found in many of South Florida's canals growing as a green "carpet" on the surface. Inside the fronds of the fern, you may find *Anabaena*. It seems to be a mutualistic relationship in that *Anabaena* is capable of nitrogen fixation. Apparently, *Anabaena* provides nitrates and nitrites for the fern and the fern provides a place for *Anabaena* to live.



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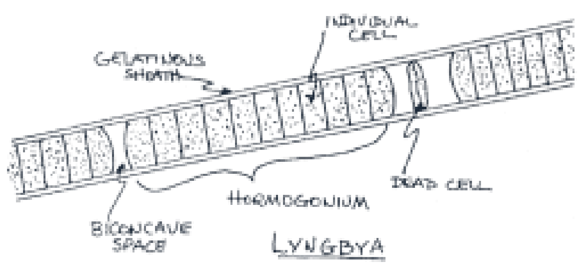
Oscillatoria (L. *oscillare*, to swing) is aptly named. It performs an oscillating motion in aquatic suspension. **Make a wet mount and observe the gentle back and forth swaying of the filaments.** *Oscillatoria* is most often found in floating mats or strings in aquatic habitats or on damp soil (Bold 1973). It has a very thin sheath and cells are divided by hormogonia. Note the dead or biconcave cells.



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Lyngbya (named in honor of the Danish phycologist Lyngbye) is difficult to distinguish from *Oscillatoria* except for one main feature. The gelatinous sheath in *Lyngbya* is extensive whereas it is quite thin in *Oscillatoria*. *Lyngbya* does not produce the oscillating motion seen in *Oscillatoria* but it does produce hormogonia separated by dead cells. Observe a prepared slide of both *Oscillatoria* and *Lyngbya* and compare the two.

Table 1. Comparison of Bacteria and Cyanobacteria from Pritchard and Bradt (1984).

STRUCTURE/FUNCTION	BACTERIA	CYANOBACTERIA
Membrane Bound Organelles	none	none
DNA	no histones	no histones
Ribosomes	sedimentation coefficient 70s	sedimentation coefficient 70s
Cell Wall	muramic acid, diaminopimelic acid and other organic acids	muramic acid, diaminopimelic acid, glucoasamines, alanine, glutamic acid
Lysozyme Reaction	dissolves cell wall	dissolves cell wall
Sexual Activity	bacterial genetic recombination (transformation, transduction, or conjugation0	recombination in a few mutant species
Nitrogen Fixation	some bacterial species	some species, especially those with heterocysts
Light Receptive Molecules	bacteriochlorophylls	chlorophyll *a*, carotenoids, phycobilins
Photosynthetic Environment	anaerobic and aerobic	anaerobic and aerobic
Photosynthetic By-Products	hydrogen, sulfur, organic compounds	oxygen
Materials Required for Photosynthesis	H_2, H_2S, CO_2, organic compounds, light	H_2O, CO_2, minerals, light
Vegetative Reproduction	spores, fission, fragmentation	spores, fission, fragmentation of hormogones, akinetes

References Cited

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