CHAPTER 05: STUDY of the MAJOR BACTERIAL GROUPS

5.1. PHOTOSYNTHETIC BACTERIA

Photosynthetic organisms are differentiated from all other forms of life by their ability to derive their cellular energy not from chemical nutrients, but from the energy of the Sun itself. A number of different microbial types are able to carry out photosynthesis, which we can regard as having two distinct forms: oxygenic photosynthesis, in which oxygen is produced and anoxygenic photosynthesis, in which oxygen is not generated.

Bergey's Manual places photosynthetic bacteria into seven major groups distributed between five bacterial phyla. (1) The phylum *Chloroflexi* contains the green non-sulfur bacteria, and (2) the phylum *Chlorobi*, the green sulfur bacteria. (3) The cyanobacteria are placed in their own phylum, *Cyanobacteria*. (4) Purple bacteria are divided between three groups. Purple sulfur bacteria are placed in the γ-proteobacteria, families *Chromatiaceae* and *Ectothiorhodospiraceae*. The purple non-sulfur bacteria are distributed between the α-proteobacteria (five different families) and one family of the β-proteobacteria. Finally, the Gram-positive heliobacteria in (5) the phylum *Firmicutes* are also photosynthetic.

5.1.1. OXYGENIC PHOTOSYNTHETIC BACTERIA (CYANOBACTERIA)

5.1.1.1. Introduction

Oxygenic photosynthetic bacteria, called **oxyphotobacteria**, forms a single taxonomic and phylogenetic group. Two orders are contained in this group of the oxygenic phototrophic bacteria, *Cyanobacteriales* and *Prochlorales*. The primary photosynthetic pigment in both cases is chlorophyll *a*, but prochlorophytes also possess chlorophyll *b*, making them very similar to the green algae. Presumably, the *Prochlorobacteria* are more closely related to the green algae than are the *Cyanobacteria*. Clearly, there is a phylogenetic relationship among the photosynthetic organisms.

The key characteristic that defines these oxygenic photosynthetic prokaryotes is the presence of two photosystems (PSII and PSI), as well as the use of H_2O as the electron donor in photosynthesis. While some species or strains may exhibit facultative photo- or chemo-heterotrophy, all known members are capable of photoautotrophy, utilizing CO_2 as the primary source of cell carbon.

Cyanobacteria, previously known as **blue-green algae**, are **obligate phototrophic** bacteria. Their name derives from the distinctive blue-green (or cyan) pigmentation they possess, and they carry out the same kind of ***oxygenic photosynthesis*** as algae and green plants. They were long controversial in their classification due to their photosynthetic ability, which led them to be associated with algae and often occupy the same ecological niches. However, they later proved to have a typically prokaryotic cellular structure and no genomic relation to algae. They have an outer membrane and a thin peptidoglycan wall typical of Gram-negative bacteria.

This group of microorganisms is the largest and most diverse group of photosynthetic bacteria. Cyanobacteria (or **cyanophytes**) are photosynthetic organisms that use water as a source of electrons for reducing power and liberating oxygen (O_2), in the same way as plants and eukaryotic algae.

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

Early members of the *Cyanobacteria* evolved when the oxygen content of the earth's atmosphere was much lower than it is now, and these organisms are thought to have been responsible for its gradual increase, since photosynthetic eukaryotes did not arise until many millions of years later. Fossil evidence indicates that, at the time when *Cyanobacteria* appeared, the atmosphere contained only about 0.1% dioxygen. When eukaryotic plants that produce dioxygen emerged, the dioxygen concentration had increased and exceeded 10%. This increase likely resulted from the photosynthetic activity of cyanobacteria. The air we breathe today contains about 20% dioxygen.

5.1.1.2. Classification

The *Cyanobacteria* are placed in volume 1 of the second edition of *Bergey*, along with the Archaea, the deeply branching bacteria, the Deinococcus-Thermus group, and the green sulphur and green non-sulphur bacteria.

The classification of cyanobacteria is still unsettled. *Bergey's Manual of Systematic Bacteriology* divides the cyanobacteria into five subsections with 56 genera. Cyanobacterial diversity is reflected in the G + C content of the group, which ranges from 35 to 71%. The various genera are distinguished using cell or filament morphology and reproductive patterns. Some other properties important in cyanobacterial characterization are ultrastructure, genetic characteristics, physiology and biochemistry, and habitat/ecology (preferred habitat and growth habit).

Subsection I: contains unicellular rods or cocci. Most are nonmotile and all reproduce by binary fission or budding: *Chroococcus*, *Gloeothece*, *Gleocapsa*, *Prochlorococcus*, *Prochloron* and *Synechococcus*.

Subsection II: is also unicellular, though several individual cells may be held together in an aggregate by an outer wall: *Pleurocapsa*, *Dermocarpella* and *Chroococcidiopsis*. Members of this group reproduce by multiple fission to form spherical, very small, reproductive cells called **baeocytes,** which escape when the outer wall ruptures. Some baeocytes disperse through gliding motility.

The other three subsections contain filamentous *Cyanobacteria*. Filaments are often surrounded by a sheath or slime layer.

Subsection III: forms unbranched trichomes composed only of vegetative cells, no heterocysts, binary fission in a single plan, fragmentation: *Lyngbya*, *Oscillatoria*, *Prochlorothrix*, *Spirulina* and *Pseudanabaena*.

Subsection IV: produce heterocysts in the absence of an adequate nitrogen source and also may form akinetes. It forms unbranched filaments (binary fission), fragmentation to form hormogonia: *Anabaena*, *Cylindrospermum*, *Aphanizomenon*, *Nostoc*, *Scytonema* and *Calothrix*.

Subsection V: produce heterocysts in the absence of an adequate nitrogen source and also may form akinetes. It divides in a second plane to produce branches or aggregates, hormogonia formed: *Fischerella*, *Stigonema* and *Geitleria*.

5.1.1.3. Specialized cells and differentiation

a. Heterocysts and N_2-fixation

- Heterocysts are unique to *Cyanobacteria* (Fig. 01);

- When *Cyanobacteria* are deprived of both nitrate and ammonia, their preferred nitrogen sources, around 5 to 10% of the cells develop into heterocysts;

- The *Cyanobacteria* of Subsections IV and V produce heterocysts (develop very thick cell wall) in trichomes at intervals (intercalary) or at their termini (terminal) through the differentiation of vegetative cells;

- Within these specialized cells, photosynthetic membranes are reorganized and the proteins that make up photosystem II and phycobilisomes are degraded. Photosystem I remains functional to produce ATP, but no oxygen is generated, **nitrogenase** (extremely oxygen sensitive) is synthesized, and N_2-fixation proceeds. The thick heterocyst wall slows or prevents O_2 diffusion into the cell.

- Heterocyst structure and physiology ensure that it remains anaerobic;

- It is dedicated to nitrogen fixation and does not replicate;

- It obtains nutrients from adjacent vegetative cells and contributes fixed nitrogen in the form of amino acids;

- The concentration of nitrogen in the medium above a certain level results in complete inhibition of heterocyst production;

- Heterocyst formation increases under conditions of low light intensities. Blue green light inhibits while red light promotes heterocyst formation;

- Nitrogen fixation also is carried out by some *Cyanobacteria* that lack heterocysts. Some fix nitrogen under dark, anoxic conditions in microbial mats;

- Planktonic forms such as *Trichodesmium* fix nitrogen and contribute significantly to the marine nitrogen budget.

b. Akinetes

- Akinetes ("resting spores") are produced by many *Cyanobacteria* in Subsection IV (Fig. 01);

- Many (but not all) of the heterocystous cyanobacteria produce akinetes, particularly under conditions of nutrient deficiency and/or light limitation;

- Akinetes differentiate from vegetative cells, grow, and accumulate a thick wall surrounding the old wall;

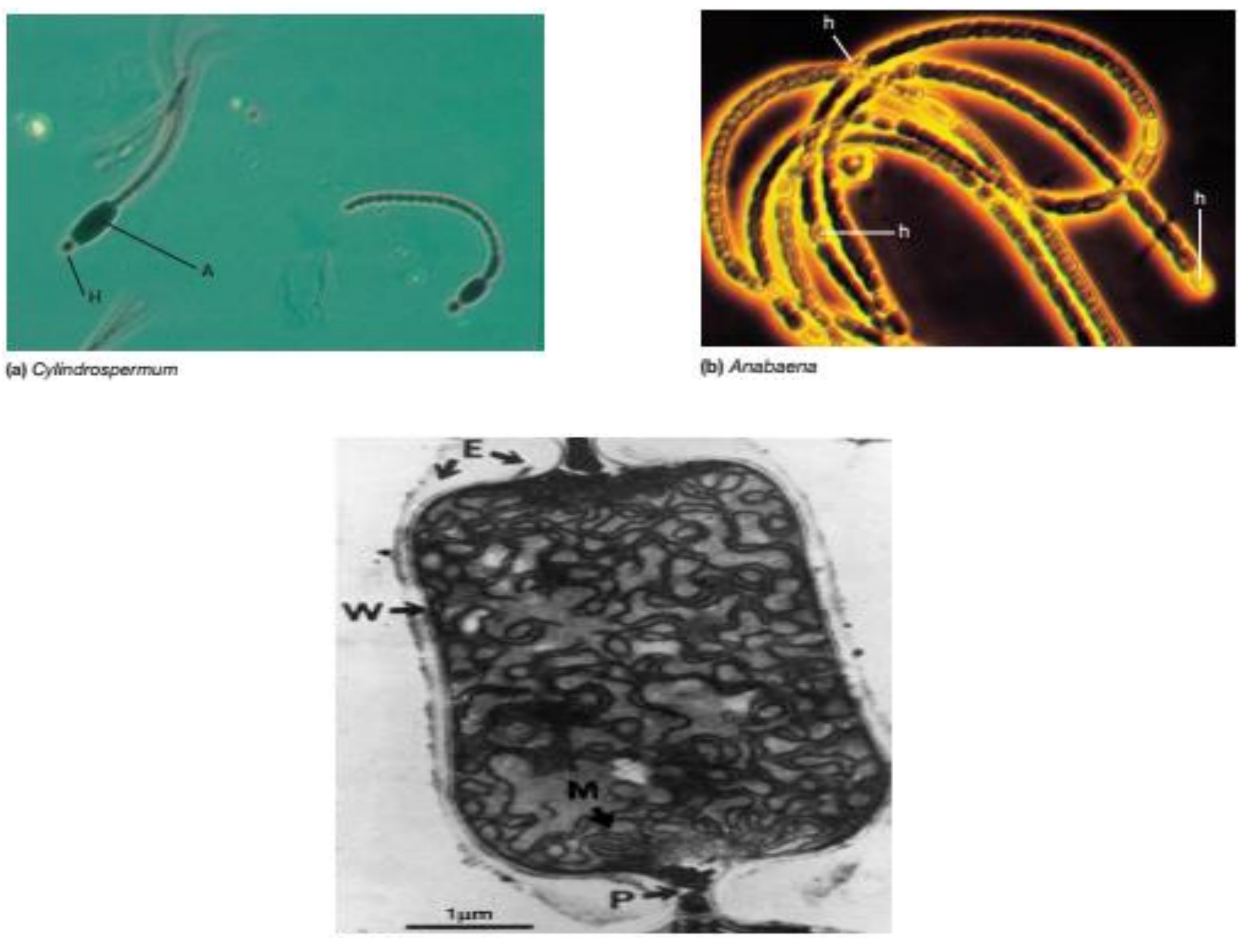
- Akinetes are specialized, dormant, thick-walled resting cells that are resistant to desiccation;

- Akinetes accumulate cyanophycin, glycogen, lipids, and carotenoid pigments, but photosynthetic pigments disappear;

- The pattern of akinete formation is generally related to the induction of heterocysts, with akinetes found either adjacent to or always distant from these structures;

- They are involved in survival, and long-term storage in anoxic sediments and do not require a resting period before germination;

- They, often, germinate to form new filaments.



(a) *Cylindrospermum* (b) *Anabaena* (c) *Anabaena* heterocyst

Figure 01: Examples of Heterocysts and Akinetes.

[(a) *Cylindrospermum* with terminal heterocysts (H) and subterminal akinetes (A) (3500). (b) *Anabaena*, with heterocysts. (c) An electron micrograph of an *Anabaena* heterocyst. Note the cell wall (W), additional outer walls (E), membrane system (M), and a pore channel to the adjacent cell (P)].

c. Hormogonia

- An hormogonium is simply a short chain of cells, the cells usually differ somewhat from the vegetative cells of the trichome (Fig. 02);
- Hormogonia are often defined as chains of 5-15 cells with cell diameters less than those of the vegetative trichomes;
- Short sections of trichome (filament) formed as a result of breaks in long filaments;
- Their formation ensures multiplication ;
- Cell division does not occur until the hormogonial stage ends its dispersal period;
- Hormogonia may form in specific regions of the parent organism;
- The formation and liberation of hormogonia appears to be a timed process associated with environmental conditions (e.g., phosphorus repletion) or with particular stages of a morphogenetic cycle.

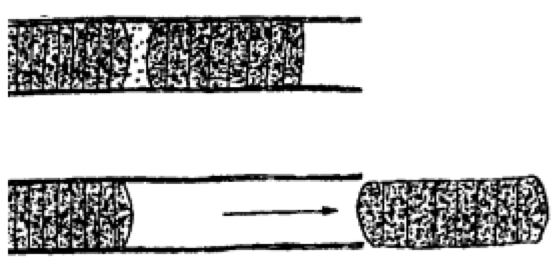


Figure 02: Fomation of hormogonia in *Oscillatoria*.

d. Baeocytes

- These are small reproductive cells found in Subsection 2 of Cyanobacteria;
- They are formed as a result of multiple fissions in a considerably enlarged cell, which is surrounded by a fibrous layer of exopolysaccharide;
- In *Dermocarpa*, for example, binary divisions lead to the formation of 4 to 1000 baeocytes (Fig. 03)

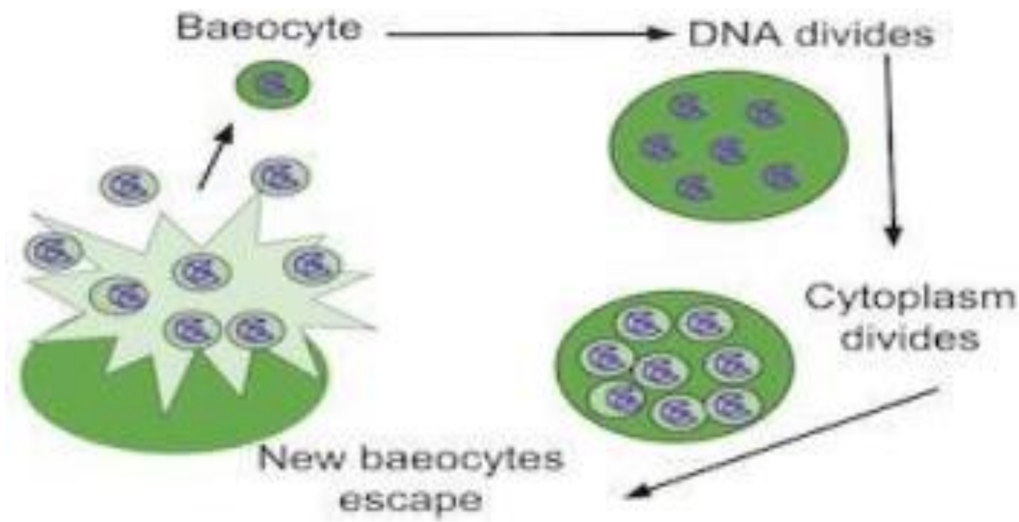


Figure 03: Baeocytes production.

5.1.1.4. Nutrition in cyanobacteria

The cyanobacteria in general are **obligate photoautotrophs** because they can't grow in darkness even in the presence of organic nutrients in the substrate. The reserve food material is stored in the form of cyanophycean starch (a product of photosynthesis). The capacity of *Cyanobacteria* to assimilate and metabolize exogenous organic compound is very limited and they can't use organic compounds as a source of energy.

5.1.1.5. Motility

Fimbriae (or pili) occur abundantly with diverse patterns in many *Cyanobacteria*. Although prokaryotic flagella have never been demonstrated in *Cyanobacteria*, gliding (sliding) motility is known in a large variety of *Cyanobacteria*, but mainly in filamentous forms (e.g. *Oscillatoria, Spirulina)*. In this form of propulsion, contact with a solid or semisolid material is required. The movement is also accompanied by a clockwise or anti clock wise rotation of a trichome and is specific for a species. In *Oscillatoriaceae* the velocity of movement is usually well over 2µm.sec^{-1} and ranges up to 11 µm sec^{-1}.

Many cyanobacterial species use gas vacuoles to position themselves in optimum illumination in the water column, a form of **phototaxis**. Although cyanobacteria lack flagella, about 1/3 of the marine *Synechococcus* strains swim at rates up to 25 mm/sec by an unknown mechanism. Swimming motility is not used for phototaxis; instead, it appears to be used in chemotaxis toward simple nitrogenous compounds such as urea.

5.1.1.6. Structure and cellular composition

Following structures are found in a prokaryotic cyanophyceae cell (Fig. 04).

a. Sheath

Cell in some species possess an external diffluent delicate mucilaginous sheath. The sheath is usually thick and slimy but in a few forms such as *Allacystis montana* it is extremely delicate. Sheath serves to hold the cell colonies together. The sheath may become lamellated or stratified and sometimes pigmented. In the latter case, it is usually yellowish or brownish in color. Usually it is colorless. The slimy nature of the sheath endows the cell with great water absorbing and water retaining capacity favoring its survival under conditions of desiccation.

b. Cell wall

Cell possess a distinct cell wall which is relatively thick and two layered i.e. outer and inner layers. Cell wall is found between the mucilagenous sheath and cell membrane. Pectin is found in the form of Calcium and Magnesium pectate. The longitudinal wall of *Oscillatoria* bears pores. Some endosymbionts viz. *Cyanophora* and *Glaucocystis* lack cell wall and contain plasma membrane made up of lipoprotein.

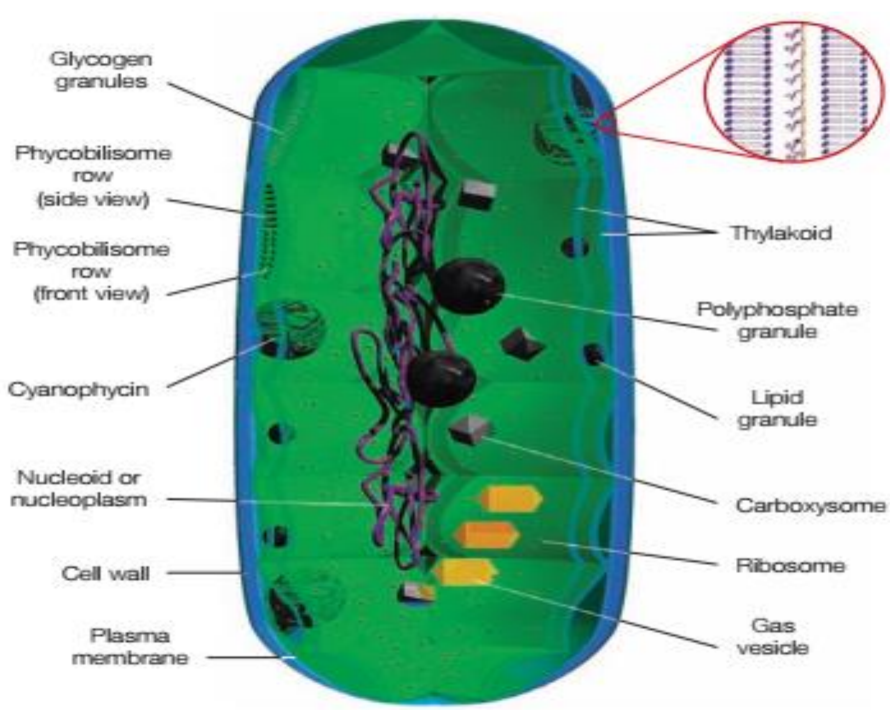


Figure 04: Cyanobacterial cell structure.

c. Protoplast

The protoplast of **Cyanophyta** is a less differentiated structure as it lacks an organized nucleus with distinct surrounding membrane, endoplasmic reticulum, membrane bound plastids, mitochondria, Golgi apparatus, and sap vacuole. Cytoplasm undergoes no cyclosis and streaming movements. It appears to be divided into two regions: centroplasm and chromoplasm (not separated from each other).

- **Chromoplasm:** this is the outer pigmented zone of the cell protoplast. The structure of chromoplasm is considered homogenous, finely alveolar, or reticular structure. The chromplasm contains a considerable amount of imbibed water and sticks to the wall, a number of non-living inclusions as reserve food materials which may be of proteinaceous nature (cyanophycean granules) or carbohydrate nature (cyanophycian starch), oil droplets as well as lipids, pseudo vacuoles (*Anabaena* and *Polycystis*) and many types of pigments.

- **Centroplasm:** it forms the central area of the protoplast of a cyanophyte cells. It forms about 1/4 to 1/2 the volume of the cell and consist of a relatively dense mass of material which is considered nuclear in nature.

d. Nucleoid

It is found in the centroplasm portion of the cell. The DNA fibres are spreaded irregularly in the nucleoid. The fibres lack histones and protamines. The genome is 2nm in width and 1.6×10^9 dalton in its molecular weight. In modern terminology, **genophore** is the name suggested for such a primitive nucleus.

e. Other cell inclusion

In the cytoplasm of *Cyanobacteria*, there are many other components and "inclusions," they include:

- **Glycogen (polyglucose) granules**, which are either ovoid or elongated and rod-shaped, and usually located between the thylakoids. Polyhydroxybutyrate granules are present in a few cyanobacteria. It used as the reserve of carbohydrate.

- **Cyanophycin granules**, which are polymers of arginine and aspartic acid. These "structured granules" are often large enough to be detectable by light microscopy (500 nm diameter). They are apparently unique to *Cyanobacteria*, although some species lack them. Functionally, they serve as reserves of nitrogen.

- **Carboxysomes** (polyhedral bodies), which are large angular structures composed largely of ribulose bisphosphate carboxylase/oxygenase (RUBISCO) (enzyme of Calvin cycle used to assimilate carbon dioxide), apparently serving as reserves of this carboxylating enzyme.

- **Polyphosphate (volutin) granules**, reaching 100-300 nm in diameter. With transmission electron microscopy, they appear as spherical, electron-dense or porous structures, depending on fixation and staining methods. It used to store phosphate.

- **Gas vacuoles:** recent observations have shown that the gas vacuoles (or pseudo-vacuoles) common in planktonic *Oscillatoria* and *Anabaena* species are of irregular shape and consist of cylindrical vesicles with conical ends stacked in arrays. These vesicles are bound by single membrane. The membrane is permeable to common gases. the gas-vacuoles are more commonly produced under low intensity of light and then suddenly collapse under high intensity of light. This result due to increased rate of photosynthesis which produces more quantity of sugar and increases the osmotic pressure so that there is a quick collapse of the gas-vacuoles at the surface level of water in the planktonic species. After collapse the filaments sink down at the bottom of water-reservoir. Thus, gas vacuoles have a great ecological importance and serve to regulate the buoyancy of the planktonic forms. In *Oscillatoria agardhii*, the gas vacuoles are produced under anaerobic conditions.

- **Thylakoids and photosynthetic pigments:** embedded in the cytoplasm within the plasma membrane are elongate flattened sacs named the **lamellae or thylakoids**. They tend to be concentrated and organized in parallel arrays near the periphery of the protoplast. It contains pigments and thus constitute the photosynthetic apparatus of the cyanophyte cells. The thylakoid bears the photosynthetic pigment like **chI-*a*, β-carotene** and lipid globules. **Phycobilisomes** particles are present on the surface of thylakoid. The particles contain the water-soluble pigment **C-phycocyanin** and **C-phycoerythrin**. It’s suggested that the photosynthetic lamellae provide also the sites for cellular respiration and hence should be termed as photosynthetic respiratory membrane.

In some blue green algae, the visual color of thallus depends upon the quality and type of light. For example, in the presence of red light the thallus of *Oscillatoria* appears green in color while in yellow light it appears blue. Thus, this quality of thallus to change its color according to the color of light it receives is called as **complementary chromatic adaptation** or the **Goidukov phenomenon** called after the name of its Russian discoverer, Goidukov. This chromatic adaptation helps the algae to maximum utilize the available light for photosynthesis.

5.1.1.7. Morphology

The thallus ranges from unicellular to coenobial and filamentous forms. Filamentous forms may be unbranched or branched. Architecturally the thallus may be a solitary cell or a colony.

- **Unicellular:** the thallus is a unicell which may be spherical or oval eg. *Chroococcus*, *Synechococcus* and *Gloeocapsa.* Actual unicellular forms however are not many because the copious secretion of mucilage by the daughter cells results in the daughter cells, remaining together after division.

- **Colonial forms:** the cells after division remain attached by their walls or are held in a common gelatinous matrix to form a loose organization of cells which is termed as a colony.

- **Filamentous:** the cells are arranged end to end in a row which is a result of repeated cell divisions in a single plane and in a single direction forming a chain or a thread known as the **trichome**. The trichome together with its mucilaginous sheath is called a **filament**. In most cases a single filament has a single trichome, but in some cases more than one trichome may occur within the common sheath. The trichomes are **homocystous** (made up of uniform cells e.g. *Oscillatoria*) or **heterocystous** (contains some special cells called **heterocyst** e.g. *Rivularia*).

Trichomes may be branched or unbranched (simple, e.g. *Anabaena, Nostoc, Oscillatoria*). The simple trichomes may be either free floating (e.g. *Spirulina)* or aggregated into colonies (eg. *Anabaena* and *Nostoc).* Some time, unbranched trichomes show false branching (eg. *Scytonema).* A cell of a trichome dies and one or both ends of the trichome at the point of the dead cell grow out as false branches. However, branched trichomes can have simple branching (*Westiella*) or heterotrichous (*Mastigocoleus*).

5.1.1.8. Reproduction

The *Cyanobacteria* reproduce by simple and primitive methods of reproduction which are vegetative (fission, fragmentation an hormogonia) and asexual (akinetes, endospores, exospores, nannocytes and hormospores or hormocyst). Sexual reproduction is absent however genetic recombination has been reported.

- **Fission:** the unicellular cyanophyceae (ex. *Synechocystis)* reproduce by this method called **binary fission**. This is the chief method of multiplication in the unicellular forms. The nuclear division precedes the cell division. The protoplast divides into two equal halves. In the middle of the cell, a ring-like septum arises from the inner layer of the cell wall and gradually grows inwards like a diaphragm. Each daughter protoplast comes to possess a continuous plasma membrane. The completed septum or cross wall then thickens and finally divides into two layers. A constriction appears at the surface of the cross wall of the dividing cell exposing the fission between the daughter cells externally. The separation of daughter cells thus starts at the periphery which proceeds towards the axis of the dividing cell gradually separating the two-daughter cells functioning as independent individuals.

- **Fragmentation**: this plays an important role in non-filamentous and filamentous colonies. Reaching a certain size, the colony breaks up into small parts. Each part/ fragment is the beginning of a new colony which increases in size by repeated cell division. The fragmentation may also occur by mechanical means. It may result from the bites of the animals feeding on trichomes or stress caused by water currents or death of certain cells.

- **Hormogonia:** the trichomes of filamentous genera breaks up within the gelatinous sheath into short sections or segments of one-many uniform living cells. These short length cells of trichome are called hormogonia or hormogones (Fig. 02). The hormogones are delimited either by the formation of heterocyst or by the development of bi-concave separation disc or necridia (dead cell). The hormogones may be 2-3 cells or several cells long.

- **Akinetes:** these are thick walled resistant resting cells which are frequently developed singly next to a heterocyst. These spore like structure contain the entire protoplast of the cell and have the original parent cell wall as tile outer portion of the spore wall. it was observed that phosphorous deficiency of water resulted in increased production of akinete bearing trichomes in *Anabaenopsis raciborskii.* These akinetes are modified ordinary vegetative cells. Cell increase in size and accumulates food reserves. They are yellow brown in color, cell wall is highly resistant and is often differentiated into two layers.

With the onset of conditions (favorable) akinetes absorb moisture, the thick and resistant wall softens and the protoplast awakens to activity.

5.1.1.9. Occurrence

Blue-green algae are found in all parts of the world from the tropics to the polar regions, and from the oceans to the top of the mountains. Majority of genera are found both in fresh as well as in brackish (saltish) water.

High temperature, intense light and humid environment is required for the growth of thallus. The members of blue green algae are found in hot and humid environment, moist or alkaline land, along with this they require high intensity of light. The members of blue green algae grow extensively in the paddy fields of tropical and temperate climate region eg. *Anabaena*, *Nostoc*, *Tolypothrix*, and *Cylindrospermum* grow actively and fix atmospheric nitrogen.

Some members of cyanobacteria are found in fresh water lakes, ponds and various types of water reservoirs. Many members grow extensively on the surface of fresh water and form water blooms e.g. *Microcystis* and *Arthospira.* Such algae growth emits bad odor and render water obnoxious and undrinkable.

Some blue green algae when abundant, may also impart their color to water. For example, *Trichodesmium erythraeum* is responsible for red color (red pigments) of the red sea. The extensive growth of this algae imparts the expression of red colored water of the sea.

Some blue green algae live symbiotically with different organisms, eg. *Anabaena* and *Nostoc* in *Anthoceros* (Bryophyta) thallus and *Anabaena* in *Cycas* (coralloid) root. Some members live with fungi forming lichens. A few blue green algae eg. *Oscillospira* and *Anabaeniolum* live endozoically (growing inside animals) in the digestive tract of mammals, including man, while some others live in unicellular animals.