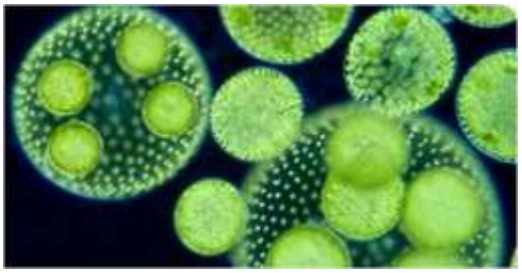
جامعة جنوب الوادي
South Valley University

Practical ecology and physiology of algae

For 3rd year

Chemistry & Microbiology Students

Faculty of Science



By

Staff members of

Botany & Microbiology Department

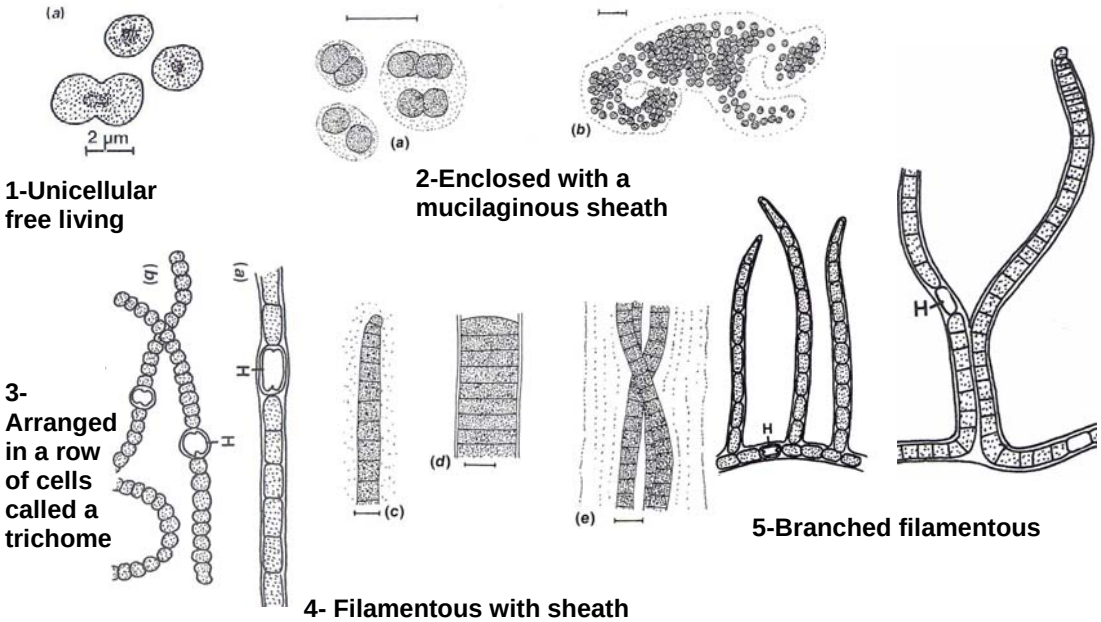
Phycology (Practical part)

Lab (1 +2)
Cyanobacteria
Kingdom: Monera
Division:Eubacteria
Class: Cyanobacteria

Prokaryotic that contains chlorophyll a, phycobiliproteins, glycogen as storage product and cell walls consisting of amino acids and amino sugars.

Morphology
1- unicellular free living
2-Enclosed with a mucilaginous envelope
3-Arranged in a row of cells called a trichome
4- Filamentous: the trichome (one or more) is surrounded by a sheath.
5-Branched filamentous which could be uniseriate (one row of cells) or multiseriate (more than one row)

Morphology of Cyanobacteria



Vision and Mission of the faculty

Vision

The faculty of science seeks to achieve academic community and student dominated by science, realization, culture and challenge, where all aspects are in continuing dialogue, graduating alumni equipped with information that qualifies them to be productive and creative.

Mission

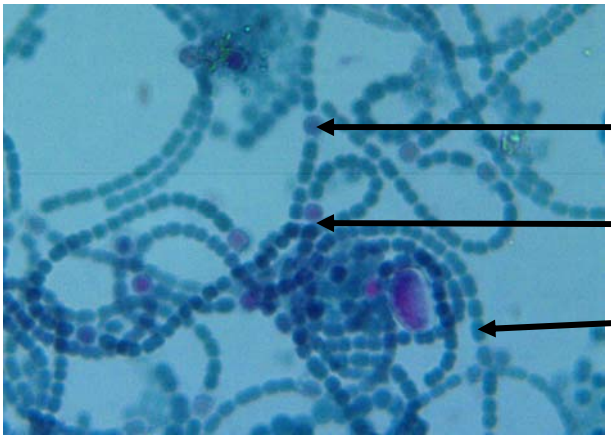
The faculty aims to excel at local level and regional throughout:

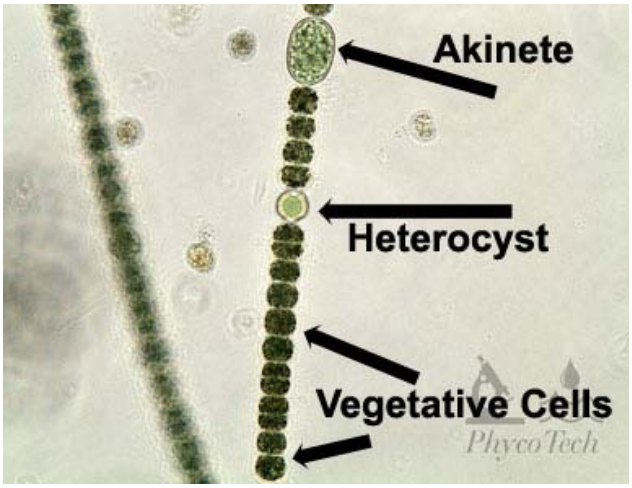
- Providing distinguished educational service to provide the market labor with graduates of high efficiency.
- Cooperating with universities and scientific institutions, regional and international.
- Academic research studies, and purposeful applied
- Providing community services and distinguished scientific consulting for South Valley community
- Training and continuous improvement in the academic field to keep pace with scientific progress

Course Syllabus :

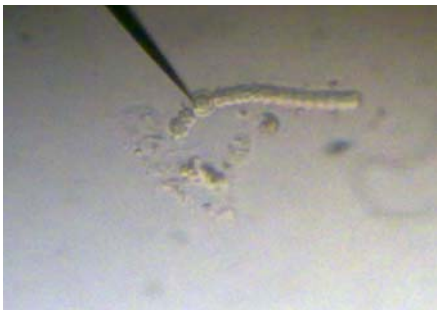
- **INTRODUCTION**
  - **ALGALPRODUCTION:**
  - Algae cultures of limited volume (Batch culture)
  - Algal Growth in Continuous Culture
  - Microalgae Isolation Techniques
  - Indices of growth of algae
  - Inorganic Nutrients of Algae
  - Algal Nutrition
- **PHOTOSYNTHESIS**
- **PLASTID STRUCTURE IN ALGAE**
- **CARBON DIOXIDE FIXATION BY ALGAE**
- **NITROGEN FIXATION IN ALGAE**

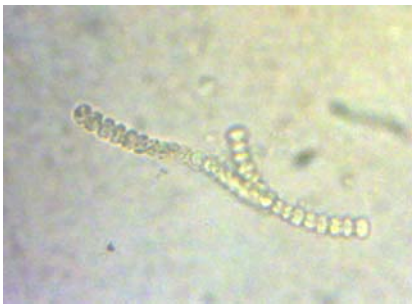
Anabaena

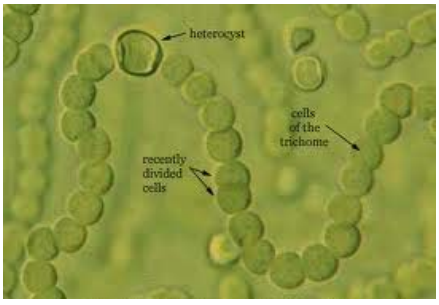




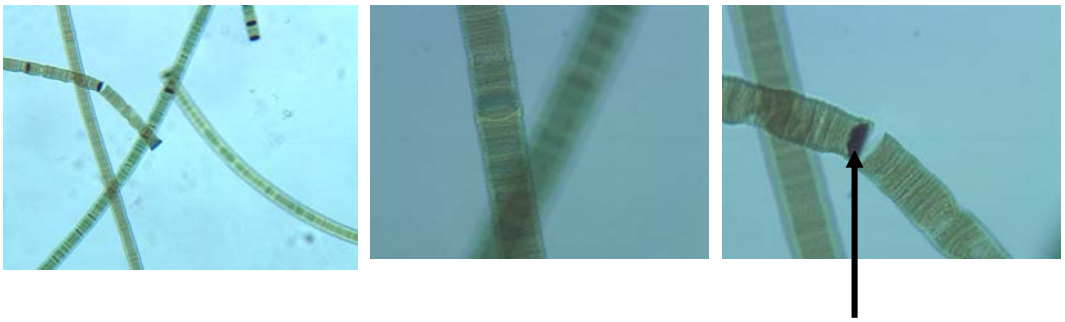
Nostoc (from specimen)



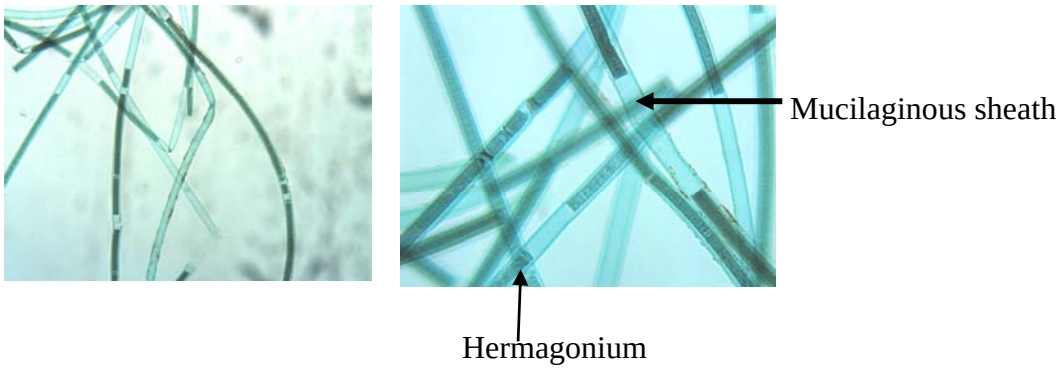




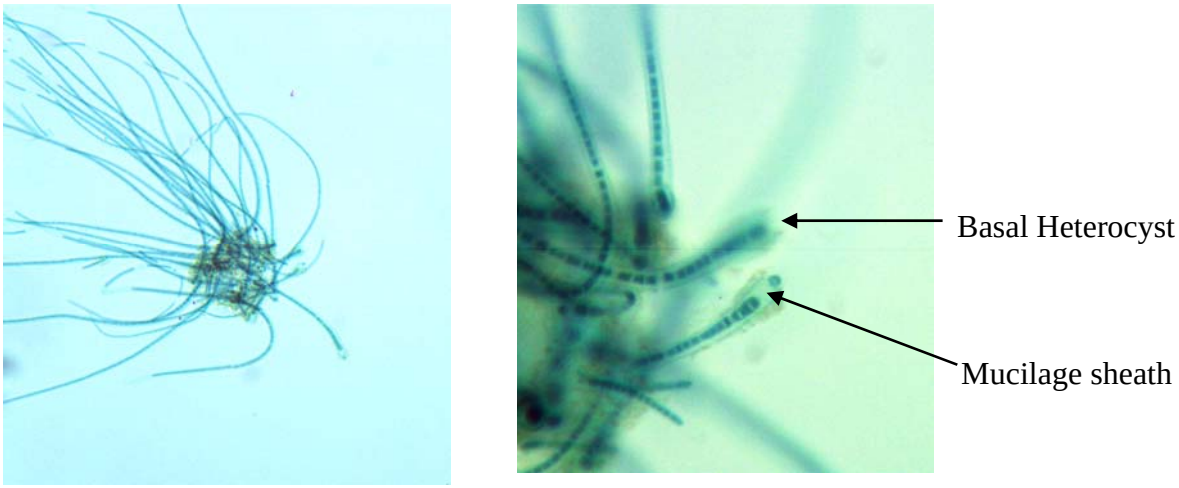
Oscillatoria



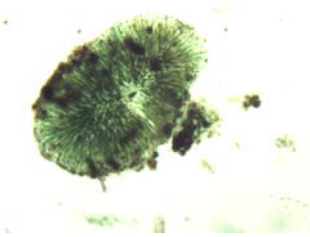
Lyngbya

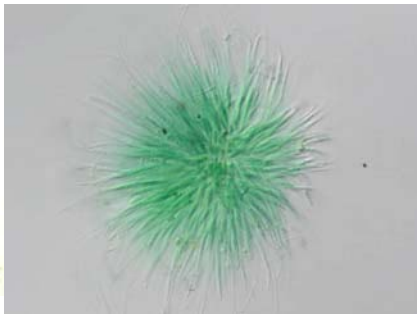


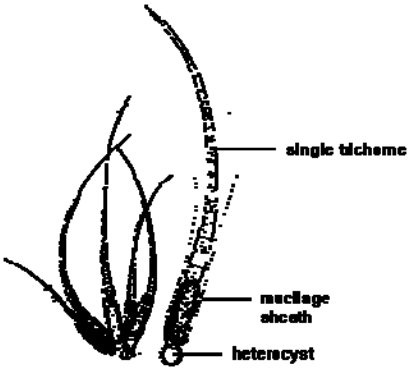
Rivularia



Gloeotrichia

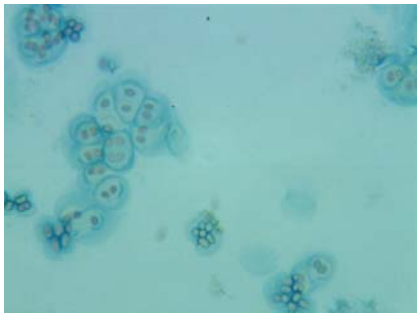


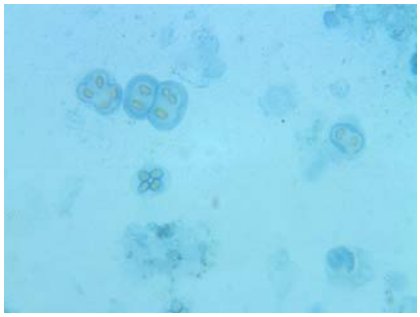


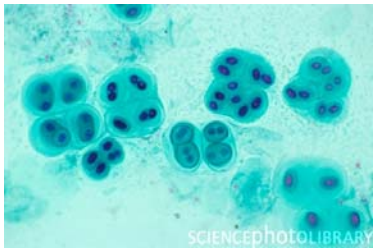


Note the polarity of filaments and the basal hetercysts and akinates

Gloeocapsa

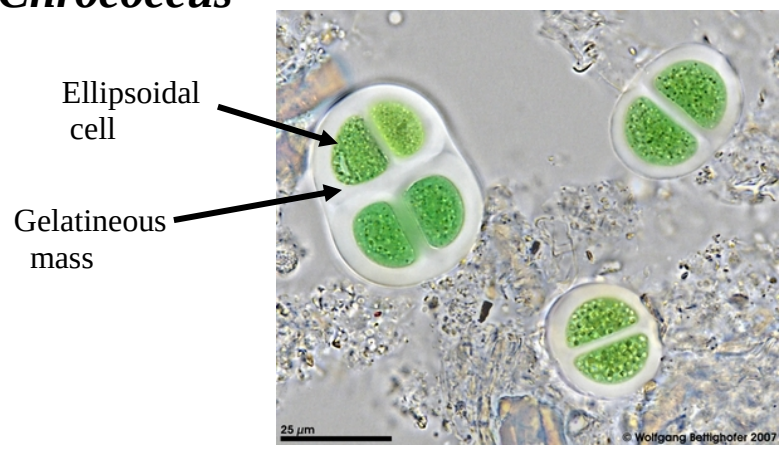




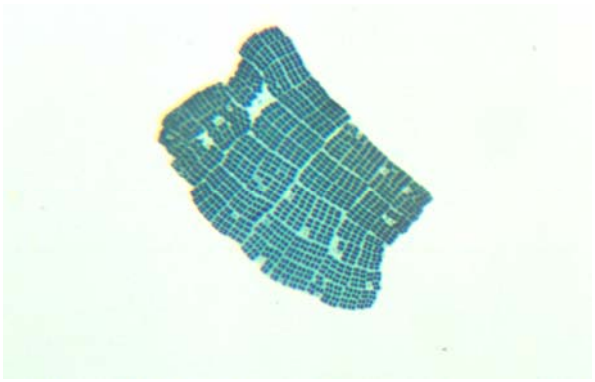


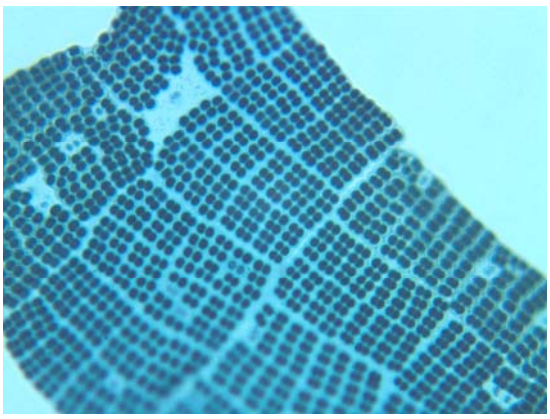
Note the mucilage sheath and the
Spherical and hemispherical cells

Chrococcus



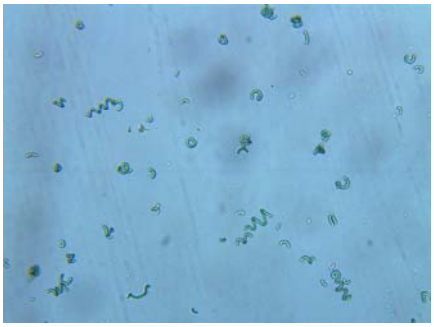
Merismopedia



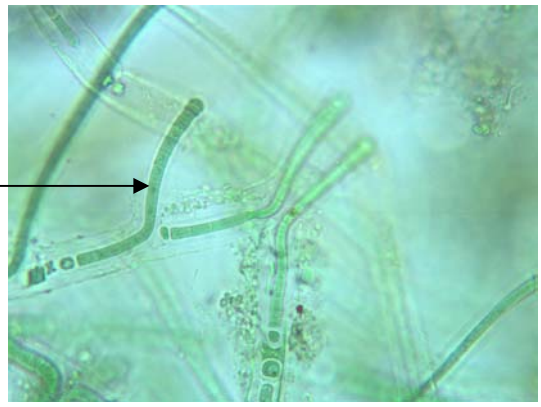


- Cells are ellipsoidal.
- The arrangement is due to the limitation of cell division from two sides only.
- The division results in increase in colony size rather than in multiplication o the individual.

Spirulina



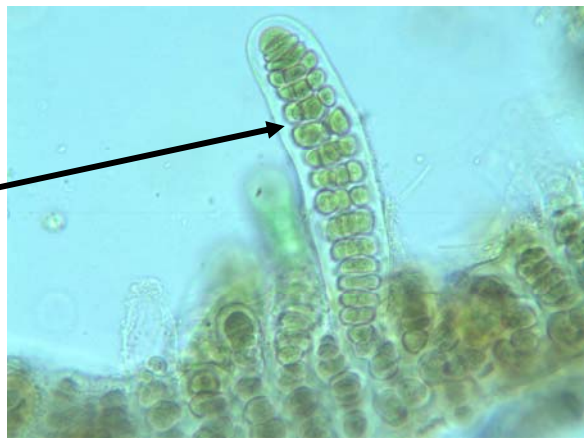
Scytonema



Stigonema

As it appears in water

True branch



Multiseriate filaments

Lab (3)

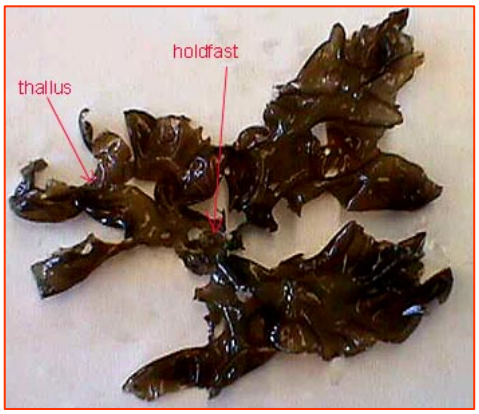
Kingdom Protista (Eukaryotes)

Algae

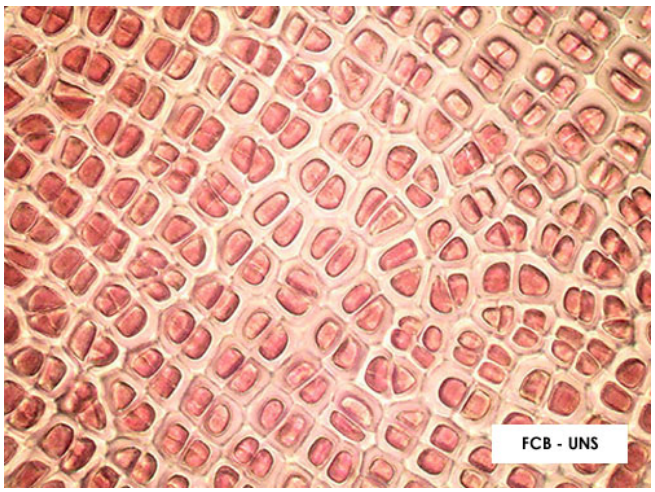
Division: **Rhodophyta**

- **Rhodophyceae** (red algae) comprise the only class in the division.
- Lack flagellated cells.
- Have chlorophyll a and d, phycobiliproteins,,
- Floridean starch granules are the storage product (outside chloroplast).
- No chloroplast ER.
- The majority of seaweeds are red algae (~400 species which is more than all other seaweeds groups).
- They live at depth as great as 200 m.
- About 200 sp. Are found in freshwater with smaller size than seaweeds.

(1) ***Porphyra***, commonly known as **nori**, is the most widely consumed seaweed in the world. This alga attaches itself to the rocks by multicellular rhizoidal attachments, usually disc-shaped. The thalli begins life as uniseriate filaments but this stage is eventually replaced by parenchymatous sheets of cells (1 to 2 cells thick).



As seen in the sea



As seen under the microscope: Note the parenchymatous appearance of uninucleated cells

(2) ***Nemalion*** spp. This red algae grows as a slender and sometimes branched "worm" on rocks in the intertidal zone, especially where there is very active water. The algae are softly cartilaginous because of the rather firm mucilage in which the filaments are encased

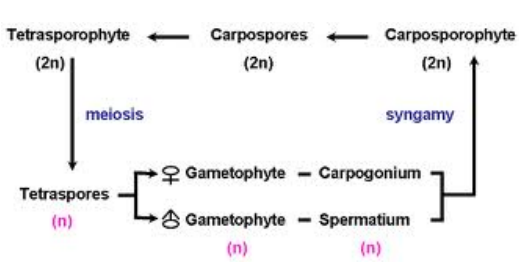
Nemalion helminthoides as seen in sea water

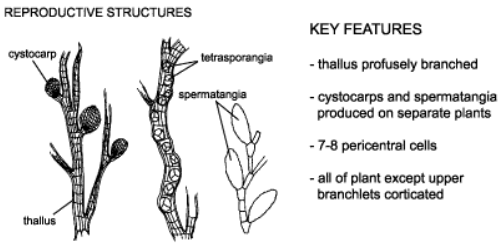
Carpogonial branches with carpospores
Note the phcoerythrin

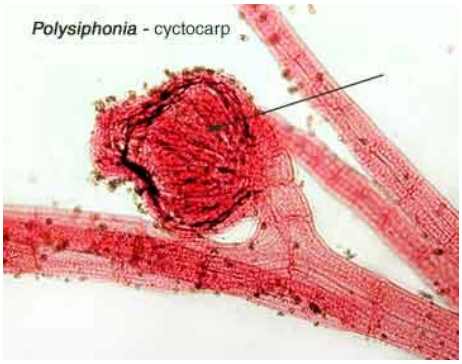
(3) *Polysiphonia*

Polysiphonia has separate male and female gametophytes that are identical in appearance. The tetrasporophytes resemble the gametophytes in size and form.

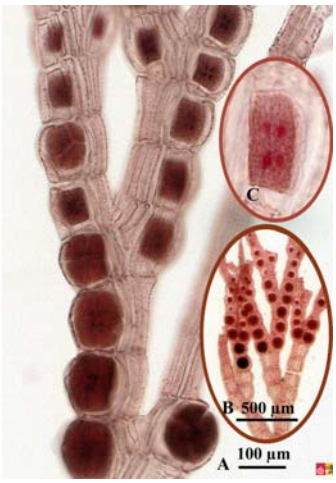
Polysiphonia elongate as seen in sea water







Polysiphonia Cystocarp



Carpospores with tetraspores

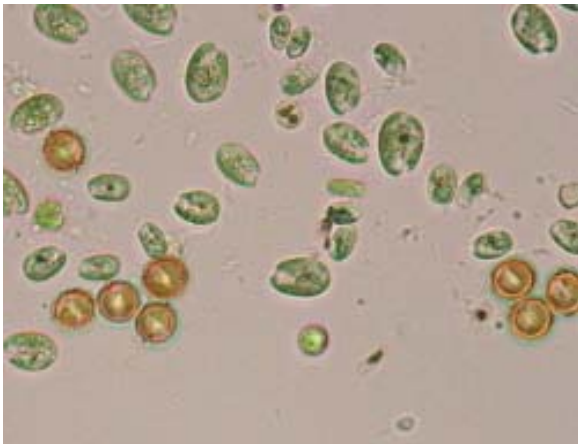
Multiseriate

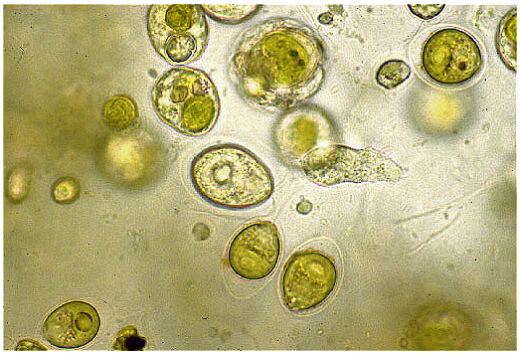
Spermatangia

Division Chlorophyta

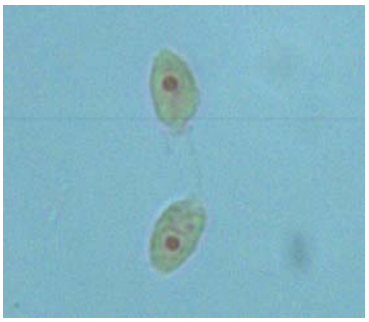
- have chlorophylls a and b.
- form starch with the chloroplast, usually in association with a pyrenoid. The Chlorophyta thus differ from the rest of the eukaryotic algae in forming the storage product in the chloroplast instead of in the cytoplasm.
- No chloroplast endoplasmic reticulum occurs around the chloroplasts.
- The Chlorophyta are primarily freshwater; only about 10% of the algae are marine, whereas 90% are freshwater.
- Some orders are predominantly marine, whereas others are predominantly freshwater or exclusively freshwater. The freshwater species have a cosmopolitan distribution, with few species endemic in a certain area.
- In the marine environment, the green algae in the warmer tropical and semi-tropical waters tend to be similar everywhere in the world. This is not true of the Chlorophyta in the colder marine waters; the waters of the Northern and Southern hemispheres have markedly different species.

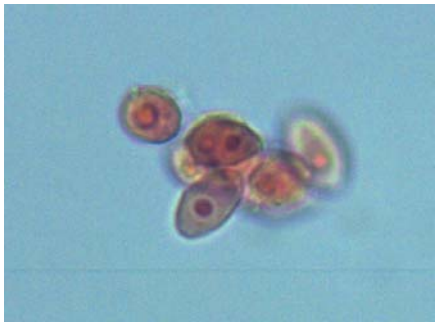
Chlamydomonas

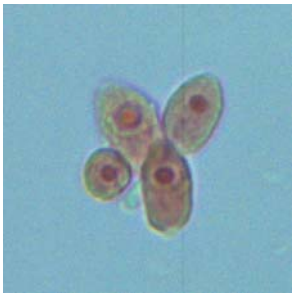




Note the flagella, the pyrenoids and the cup-shaped chloroplast

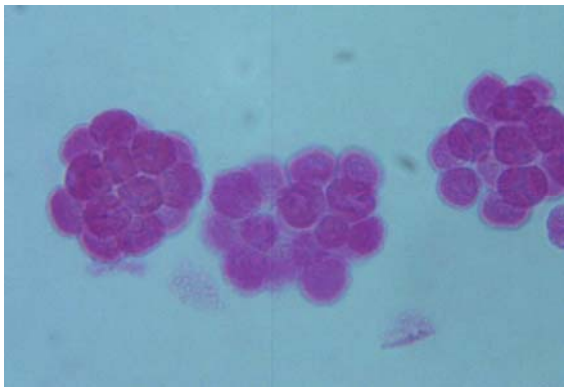


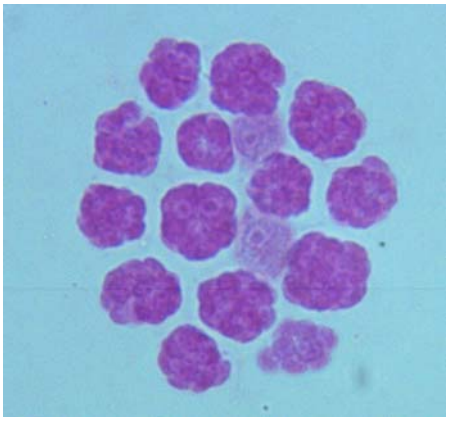




- *Chlamydomonas* is a unicellular motile alga with two flagella.
- Each cell contains single massive chloroplast
- The chloroplast may contain one or more pyrenoids.

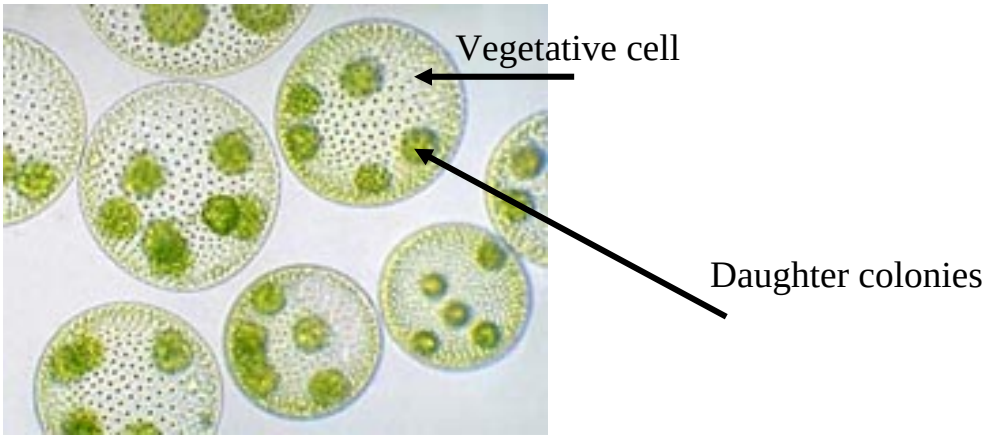
Pandorina

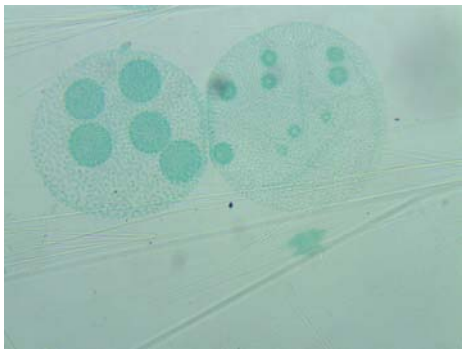


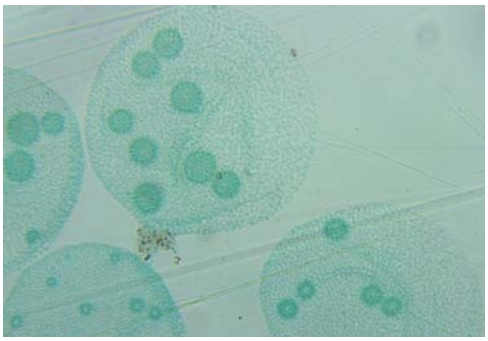


- *Pandorina* consists of 16-like *chlamydomonas* cells.
- Arranged in an almost solid, ovoidal Colony.
- Each cell is flattened at its anterior pole and narrowed posteriorly.
- The chloroplast is massive and contains a basal pyrenoid.
- The single nucleus lies in the colorless central cytoplasm.
- All of the cells in the colony are similar in size.
- After attaining the maximum size characteristic of the species, the colonies sink to the bottom of the pond and initiate autocolony formation (miniature of a parental colony).
- In autocolony formation, each of the parental cells undergoes repeated nuclear and cytoplasmic division until miniature 16-celled colonies are produced.
- The minute cells of the autocolonies then develop flagella, and the coloy begins to move slowly within the matrix of the parent colony until liberated by its dissolution.

Volvox



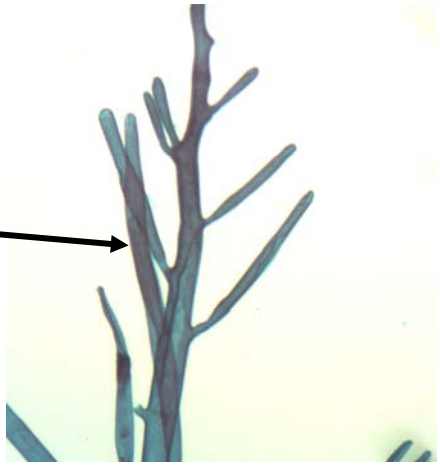




- *Volvox* consists of thousands of *chlamydomonas* like cells.
- The protoplasts of the individual cells are connected by delicate protoplasmic extensions.
- Duaghtr colonies reproduce sexually by formation of zygote, and asexually by formation of gonidia.
- The remaining cells are purely vegetative and disintegrate when the adult colony liberates its daughter colonies.

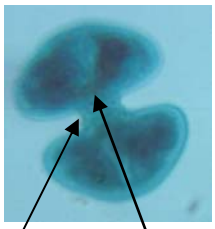
Bryopsis

Lateral branch

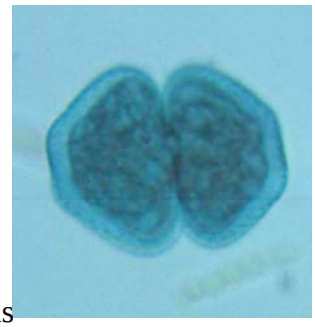


- Grows attached to rocks in shallow marine waters.
- Growth is apical.
- Coenocytic and tubular type of body organization
- At maturity, certain of the branches become segregated from the main axis by the formation of the septa and become transformed into gametangia.

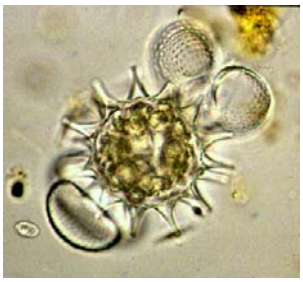
Desmids *Cosmarium*

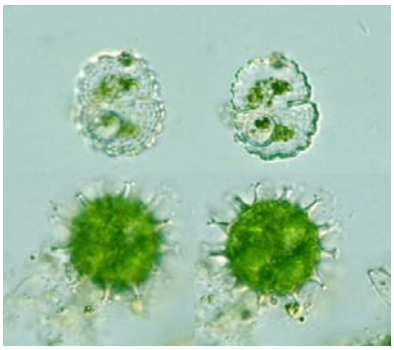


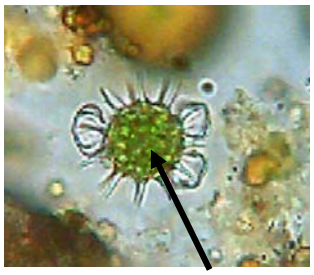
Sinus Isthmus



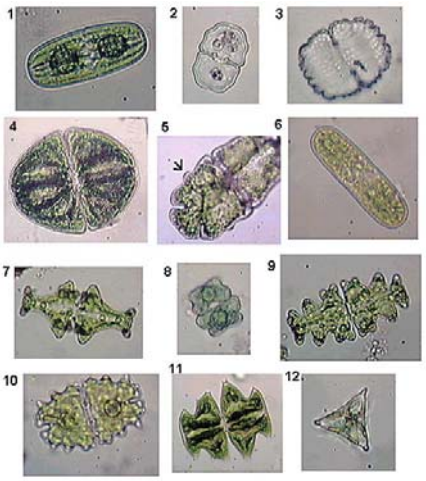
2 semi-cells



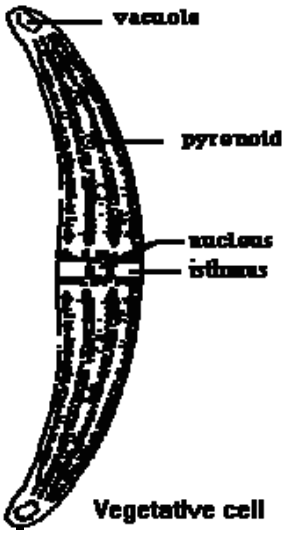


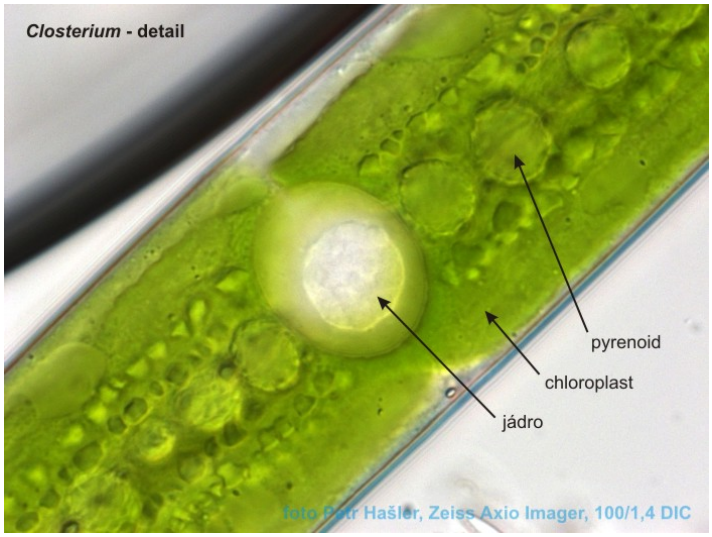


Zygote

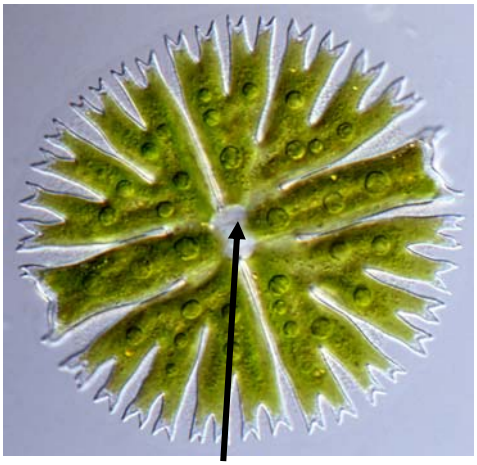


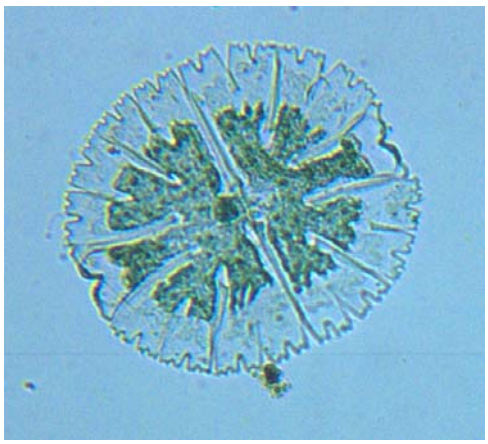
Desmids *Closterium*



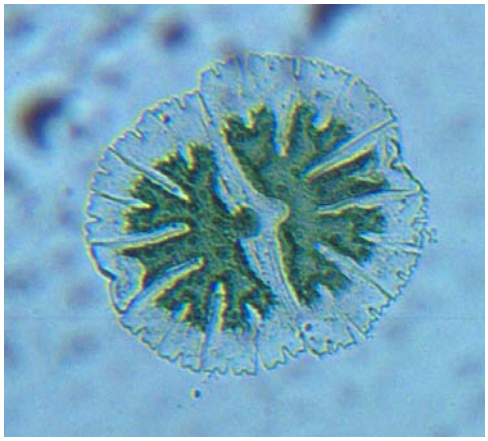


Desmids *Micrasterias*

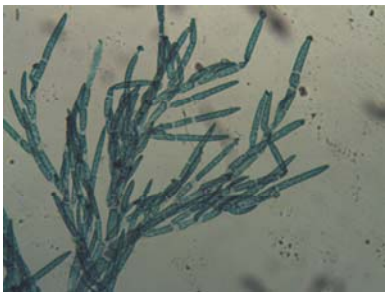
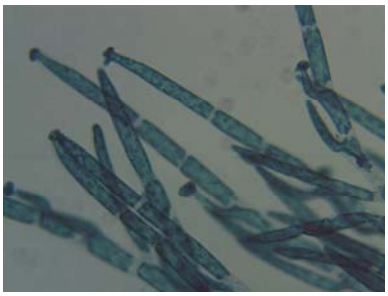




Sinus

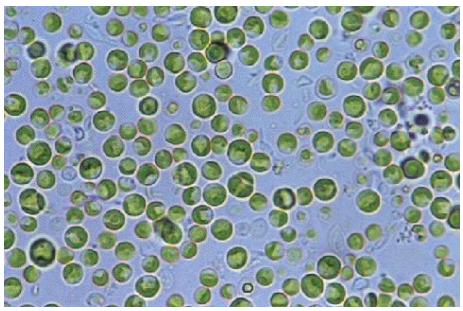
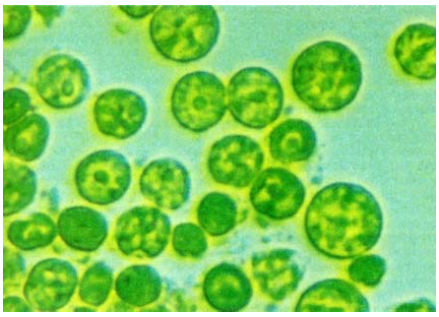
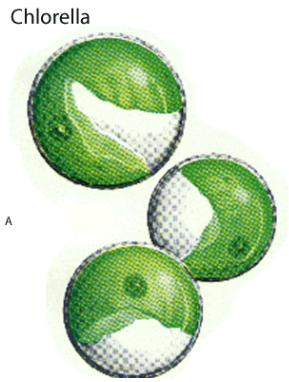


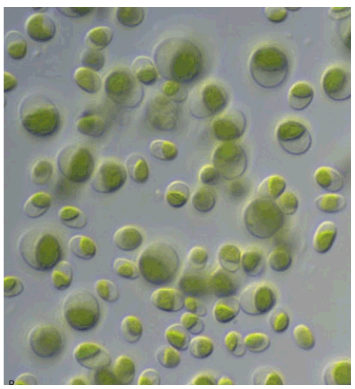
Cladophora

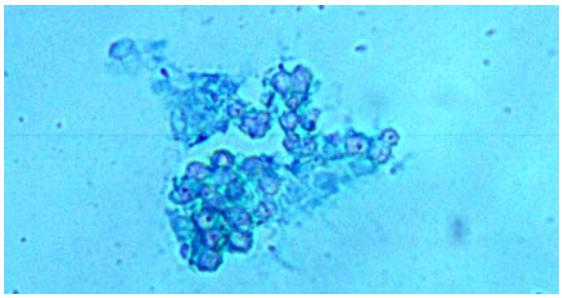



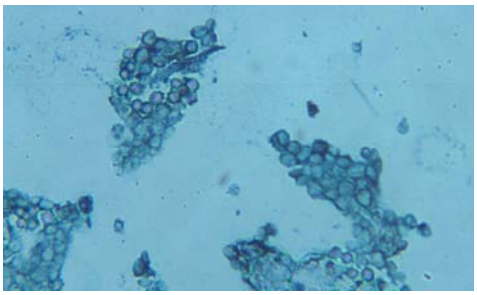

- *Cladophora* is an isomorphic with multinucleated cell.
- The gametes are biflagellated (sexual reproduction).
- The zoospores are quadriflagellated (asexual reproduction).
- The structure of the chloroplast varies with the age of the cell. In younger cells it is a continuous network, but in older ones it is largely peripheral and composed of irregular segments, in some of which pyrenoids are embedded.

Clorella

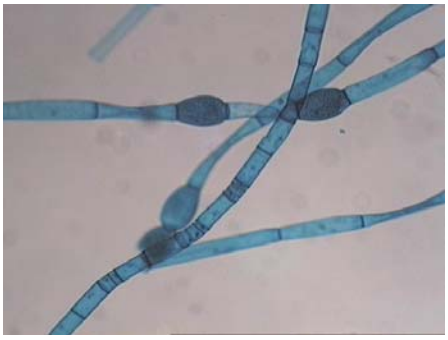


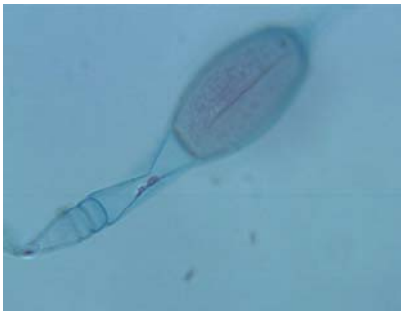


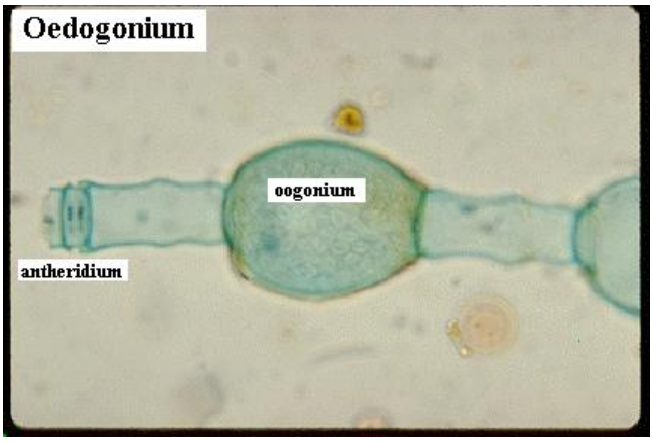


- The cells of most species are minute green spheres.
- The protoplast is composed of cuplike chloroplast, which may or may not contain a pyrenoid.
- The cytoplasm is colorless in which a minute nucleus is embedded in its center.
- A serious of bipartitions may occur, forming four or eight protoplasts endogenously.
- Delicate cell walls are then developed, and after they have begun to enlarge, they are liberated by rupture of the mother cell wall (asexual reproduction).
- Such asexual reproductive cells, which have no capacity for motility, are known as autospores.

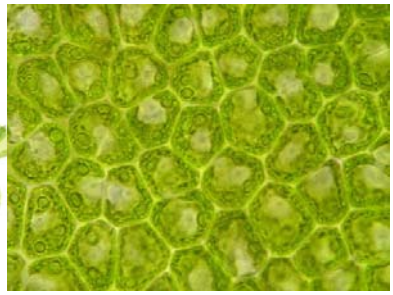
Oedogonium

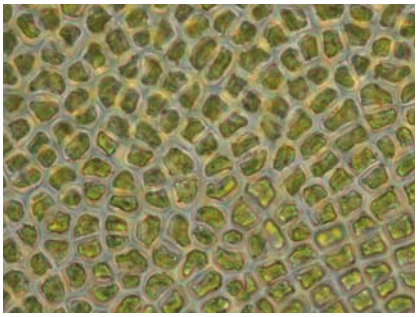






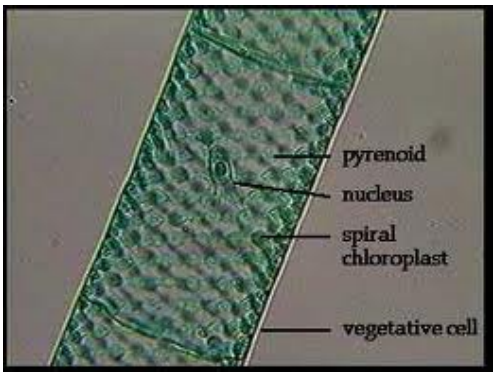
Ulva

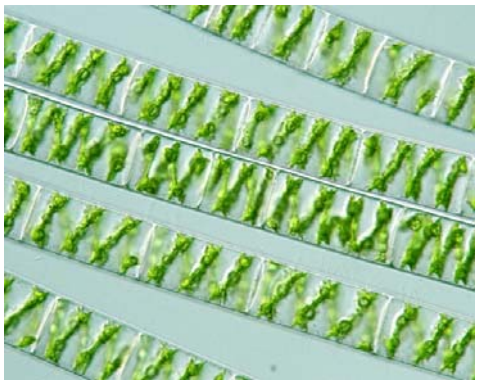




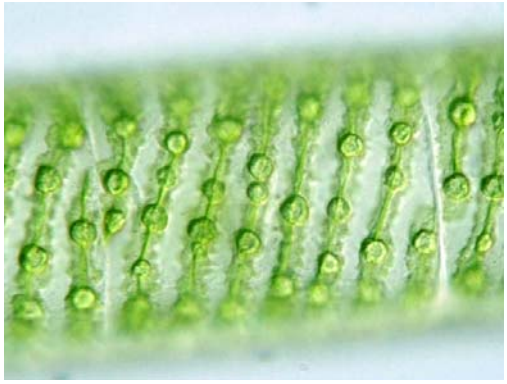
- *Ulva*, the sea lettuce is a green membranous alga.
- Grows attached to rocks, woodwork, and larger marine algae.
- The body is bladelike, often lobed and undulated, and anchored by a multicellular holdfast composed of cells with rhizoidal protuberances.
- The cell walls are thick so to withstand some desiccation when exposed at low tide.
- Each cell contains a single chloroplast with one or more pyrenoids.
- The cells of the blade are uninucleate, but those of the holdfast may have several nuclei in their rhizoidal processes.

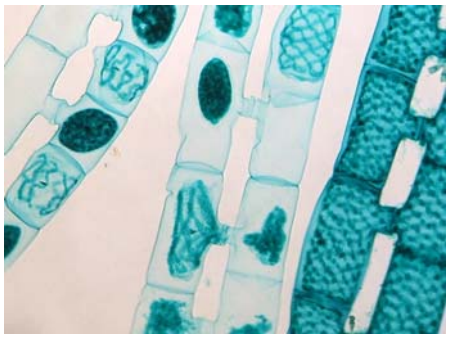
Spirogyra



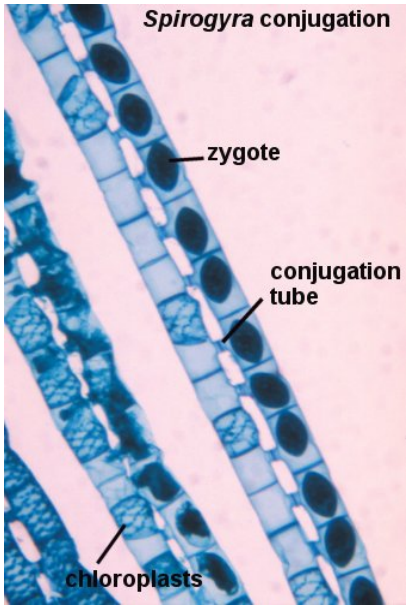


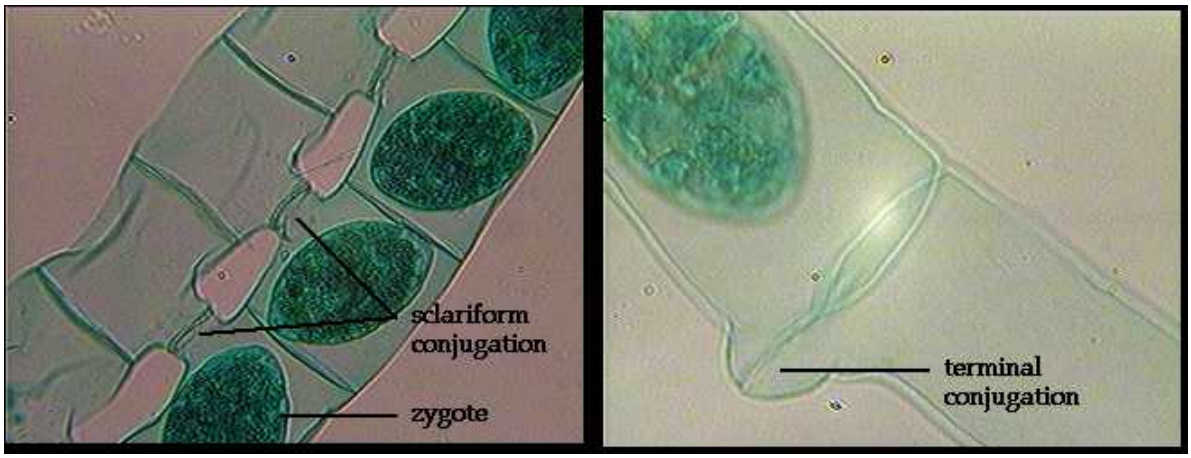
Vegetative filament





Scalariform conjugation





Types of reproduction in *Spirogyra*

1. Asexual reproduction (vegetative filament fragmentation).
2. Sexual conjugation

A- Lateral Conjugation-

1. It occurs in homothallic species.
2. Here two cells of a filament take part in gametic union.
3. Movement of gamete occurs through a passage formed either in partition wall or across the partition wall or two adjacent cells.
4. It does not look like a ladder.

B- Scalariform Conjugation-

1. It occurs in heterothallic species.
2. Here two cells of two different filaments take part in gametic union.
3. Movement of gamete occurs through a passage formed by the lateral walls of two filaments.
4. It looks like a ladder.

ALGAL PRODUCTION

The most important parameters regulating algal growth are nutrient quantity and quality, light, pH, salinity and temperature

Culture medium/nutrients: Concentrations of cells in phytoplankton cultures are generally higher than those found in nature. Algal cultures must therefore be enriched with nutrients Macronutrients include nitrate, phosphate (in an approximate ratio of 6:1), and silicate. Silicate is specifically used for the growth of diatoms which utilize this compound for production of an external shell. Micronutrients consist of various trace metals and the vitamins. Two enrichment media that have been used extensively and are suitable for the growth of most algae are the Walne medium and the Guillard's F/2 medium.

Light: As with all plants, micro-algae photosynthesize, i.e. they assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this reaction and in this regard intensity. Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture 5,000-10,000 is required for larger volumes). Light may be natural or supplied by fluorescent tubes. The duration of artificial illumination should be minimum 18 h of light per day, although cultivated phytoplankton develop normally under constant illumination.

pH: The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2-8.7.

Aeration/mixing: Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients and to improve gas exchange between the culture medium and the air

Temperature: The optimal temperature for growth between 20 and 24°C

Salinity : Marine phytoplankton are extremely tolerant to changes in salinity.

Algal Culture Media

In order to grow algae in the classroom you will need to make up some growth media. In their natural habitats algae obtain all the nutrients, minerals and vitamins they require from the water in which they live. To grow them in the lab you must provide them with all of these essential

the algae grow in theory culture condition should resemble the alga's natural environment as far as possible.

Algae cultures of limited volume (Batch culture)

In this method algal cells are allowed to grow and reproduce in a closed container. The batch culture consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density. In practice, algae are transferred to larger culture volumes prior to reaching the stationary phase. They have a finite amount of nutrient, and when that is exhausted, their growth stops and eventually they die. These types of cultures typically last for about one week. The most common culture system is the batch culture, due to its simplicity and low cost. This is a closed system in which there is no input or output of materials.

The photo below shows a typical batch culture set-up.

Limited volume of medium containing the necessary nutrient when inoculated with algae cells and then exposed to suitable conditions of light, temperature and aeration. Increase in cell number follows a characteristic course as:

Phases in the growth curve illustrated a typical algal batch culture

There are five phases of algal growth, lag phase, exponential growth phase, Declining growth ,stationary phases and death phase.

The Lag (induction) phase is the time where the alga is not reproduction, this lasts for about 4-6 days. This phase, during which little increase in cell density occurs.

After a while, the algae multiplies super-fast in a short period of time. This is called the **Exponential growth phase** during the second phase, the cell density increases.

Later, the algae reach a point where there is not enough space for growth and there are no more nutrients in the water so the algae stop reproducing and the growth rate are balanced, which results in a relatively constant cell density. This is called **the Stationary phase**. In the middle of this phase is the optimal time to harvest the algae.

Phase of **Declining growth** rate; cell division slows down when nutrients, light, pH, carbon dioxide light intensity, auto inhibition or other physical and chemical factors begin to limit growth.

If the algae are not harvested in the stationary phase, they will move to **the Death phase.** There is no more space and nutrients to grow so cell density decreases rapidly and the culture eventually collapses.

In practice, culture crashes can be caused by a variety of reasons, including the depletion of a nutrient, oxygen deficiency, overheating, pH disturbance, or contamination. The key to the success of algal production is maintaining all cultures in the exponential phase of growth

Continuous Culture

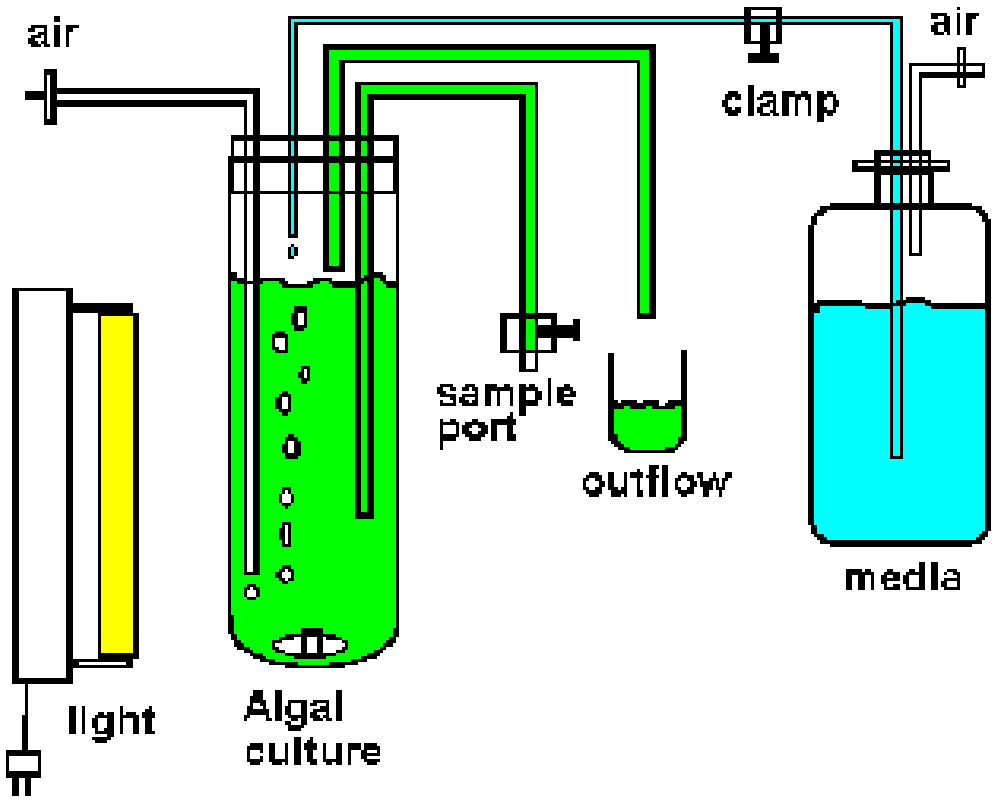
This method of culturing algae differs from the batch culture method in that fresh medium is added to the culture at a constant rate and old media (and some of the algae cells) is removed at the same rate. Two categories of continuous cultures can be distinguished:

Turbidostat culture, in which the algal concentration is kept at a preset level by diluting the culture with fresh medium by means of an automatic system.

Chemostat culture, in which a flow of fresh medium is introduced into the culture at a steady, predetermined rate. The latter adds a limiting vital nutrient (e.g. nitrate) at a fixed rate and in this way the growth

The diagram and photographs below show the parts of a continuous culture system.

First, fresh growth medium is stored in the large vessel. Air is pumped into the airspace in this medium vessel. This air pressure will push the medium through a tube which is connected to the culture vessel. By opening and closing the clamp on this medium line one can add medium to the culture vessel.



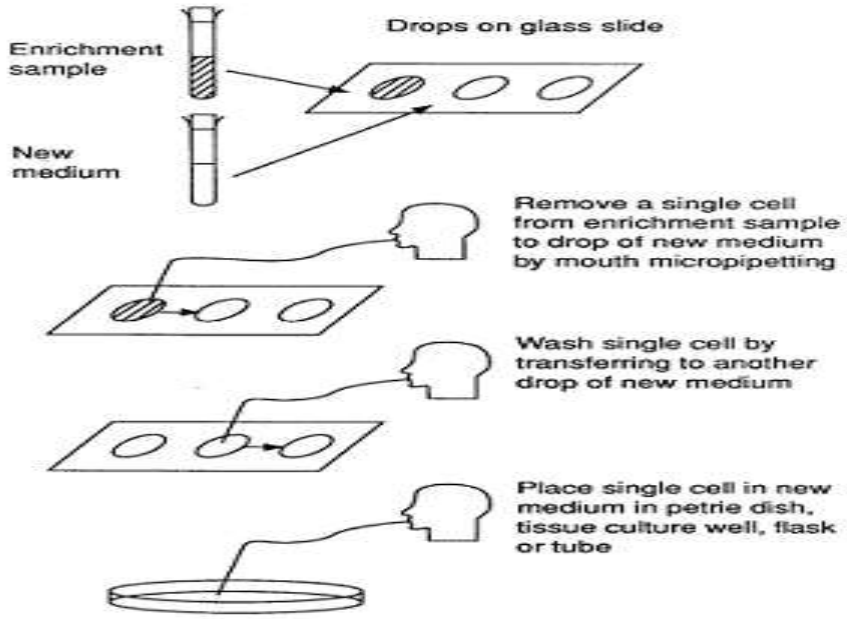
Air is also pumped into the culture vessel. This air passes down a long glass tube to the bottom of the culture and bubbles up. This serves to keep the culture well suspended as well as high in oxygen and CO2. The air flowing into the culture vessel flows out through an outflow tube. As fresh medium is added to the culture vessel the level of the liquid in the culture vessel rises. When that level reached the bottom of the outflow tube old medium and cells flow out of the culture vessel into a waste flask. There is one other glass tube in the culture vessel, the sample port. When you need a sample of cells from the culture vessel you open up the clamp on the sample port and medium and cells flow out. When you have enough you reclamp the sample port.

When choosing a culture medium the nature habitat of the species should be considered in order to determine its environmental requirements. Algae media refers to the solution or culture in which algae grow, and there are two major types of algae media, enrichment and artificial media. An enrichment medium is generally made by adding soil extracts to distilled or natural water or by simply adding chemical nutrients to seawater or

chemicals and doesn't include additions of soil extracts or natural lake or sea water. This artificial medium is mostly used under laboratory conditions to exacting standards, although unknown impurities can still be present in even the most carefully prepared artificial medium.

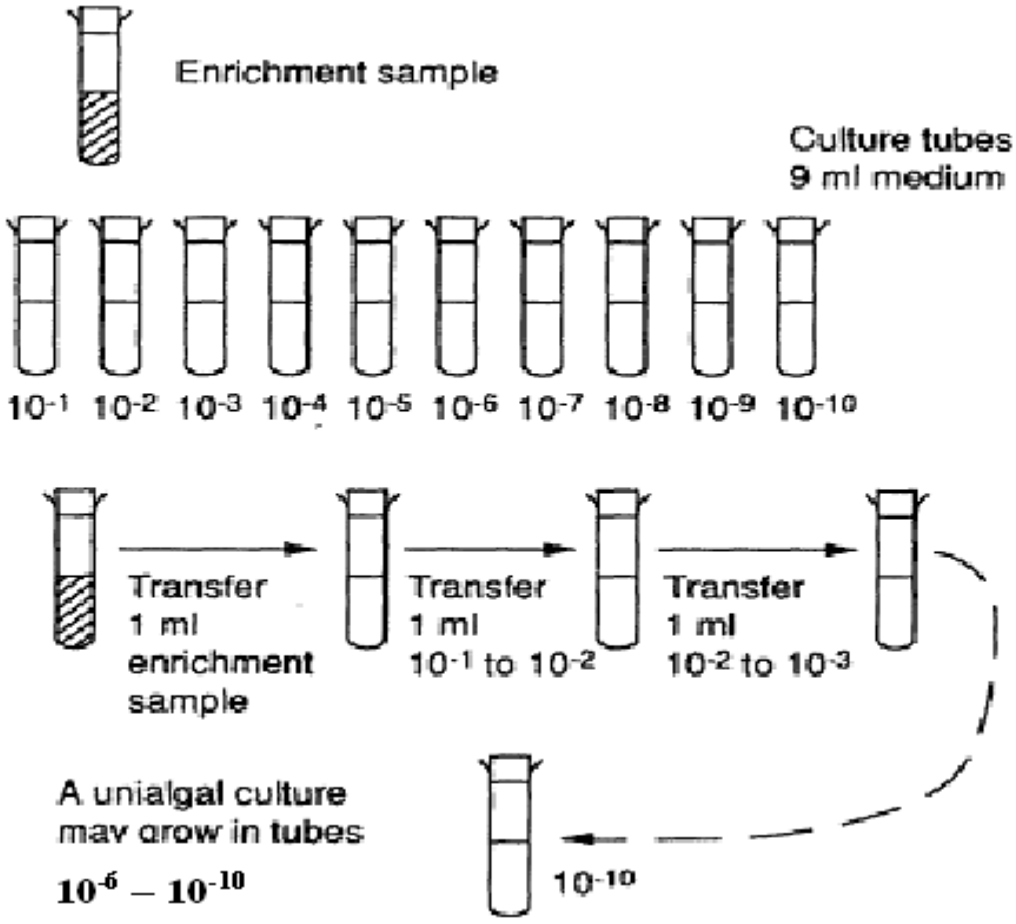
Microalgal Isolation Techniques

(a) Micromanipulation:



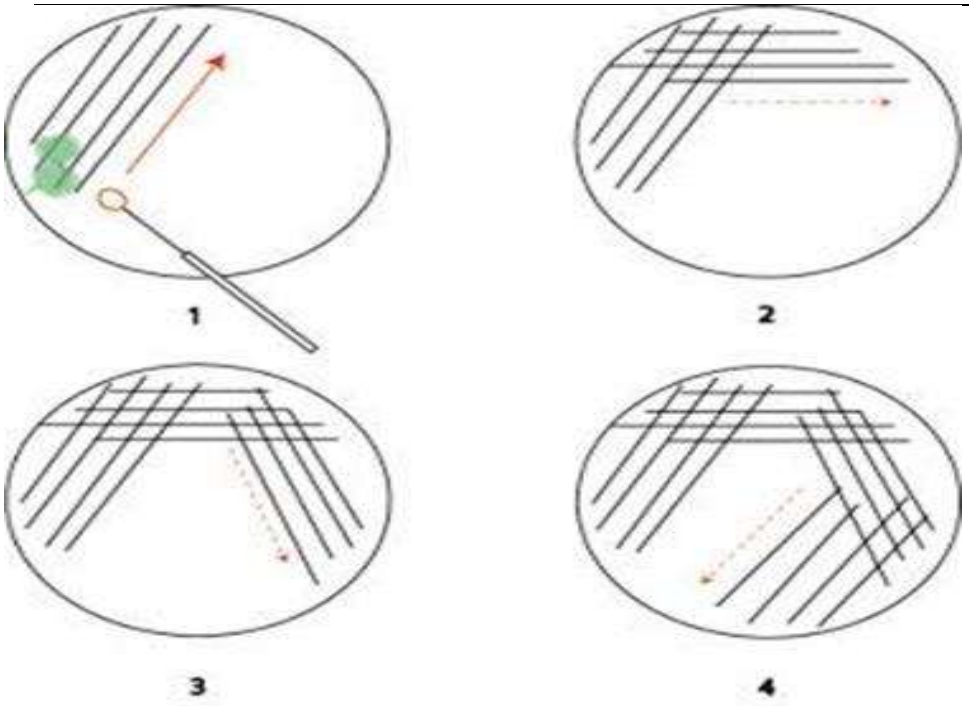
(b) Serial dilution:

dispense 9 ml of media into each of ten test tubes with sterile automatic dispenser. Label tubes 10^{-1} to 10^{-10} indicating dilution factor .Aseptically add 1 ml of enrichment sample to the first tube (10-1) and mix gently.Take 1 ml of this dilution and add to the next tube (10-2), mix gently. Incubate test-tubes under controlled temperature and light conditions



(c) Streak plating:

Prepare petri dishes containing growth medium solidified with 1-1.5% agar medium.Place 1—2 drops of mixed phytoplankton sample near the periphery of the agar. use the sterile loop to make parallel streaks of the suspension on the agar ·Remove a sample using a sterilized wire loop and place in a drop of sterile culture medium on a glass slide. Check microscopically that the desired species has been isolated and is unialgal . Repeat the streaking procedure. This second streaking reduces the possibility of bacterial contamination and of colonies containing more than one algal species.Transfer selected colonies to liquid or agar medium.



(d) Density centrifugation and Antibiotics and specific cell inhibitors

Indices of growth of algae:

in growing algae culture yield, dry weight, optical density of a suspension of algal cells and increase in cell number are used as a characteristic of increase of growth . Other indices of growth, such as accumulation of carbon, nitrogen, protein, or some products of cell metabolism (starch, acids) are used in growth measurement.

- **Yield as a growth indicator:** yield as an expression of organic production, is usually given in terms of fresh or dry weight of the organic mass produce over the period of the time per unite of volume or unit of area occupied by organism.

Determination of yield: Y=X1-X0/A(or V)

Where X1& X0 are quantitative expressions of the mass of cells at the beginning and at the end of the growth period and A (or V) the area or the volume occupied by population of microbial growth.

Algal Nutrition

(1) PHOTOTROPHIC: USING LIGHT TO PRODUCE CARBOHYDRATE . FROM H_2O and Co_2

(2) CHEMITROPHIC: employing inorganic substance

(3) HETEROTROPHIC: employing organic substance

(4) MIXOTROPHIC: autotrophic and heterotrophic

(5) Phagotrophic: which ingest organic and inorganic substance.

(6) Auxotrophic: is the inability of an organism to synthesize a particular organic compound required for its growth

Algal Nutrients: Sixteen chemical elements are known to be important to alga's growth and survival. The sixteen chemical elements are divided into two main groups: non-mineral and mineral.

- **Non-Mineral Nutrients:** The Non-Mineral Nutrients are hydrogen (H), oxygen (O), & carbon (C).These nutrients are found in the air and water .Algae use energy from the sun to change carbon dioxide (CO_2 - carbon and oxygen) and water (H_2O- hydrogen and oxygen) into starches and sugars. These starches and sugars are the alga's food.
- **The mineral nutrients**: are divided into two groups : macronutrients and micronutrients.Macronutrients can be broken into two more groups :

(1) The primary nutrients are nitrogen (N), phosphorus (P), and potassium (K). These major nutrients usually are lacking because algae use large amounts for their growth and survival .

(2) The secondary nutrients are calcium (Ca), magnesium (Mg), and sulfur (S).

(A) Macronutrients element:

(1) Phosphorus: is an essential part of the process of photosynthesis .Helps with the transformation of solar energy into chemical energy; proper plant maturation; withstanding stress.Effects rapid growth

(2) Potassium: algae require potassium ion as activator of enzymes helps in the building of protein, photosynthesis.

(3) Nitrogen: Nitrogen is a major component of proteins and amino acids.

(3) Calcium: required by most of algae for growth an essential part of plant cell wall structure

(4) Magnesium: is part of the chlorophyll in all green plants and essential for photosynthesis. It also helps activate many plant enzymes needed for growth

(5) Sulfur: Essential plant food for production of protein.

(B) Micronutrients element: Micronutrients are those elements essential for plant growth which are needed in only very small (micro) quantities.

The micronutrients are boron (B), copper (Cu), iron (Fe), chloride (Cl), manganese (Mn), molybdenum (Mo) and zinc (Zn). Providing micronutrients (as well as macronutrients) to growing plants.

Micronutrient element consider essential to all algae: An essential nutrient is a nutrient that the cell cannot synthesize on its own -- or not to an adequate amount

(1) Iron (Fe): iron required in biological oxidation and reduction reaction Essential for formation of chlorophyll.

(2) Manganese (Mn): Functions with enzyme systems involved in breakdown of carbohydrates, and nitrogen metabolism.

(3) Chloride (Cl): Aids plant metabolism.

(4) Molybdenum (Mo) Helps in the use of nitrogen

(5) Zinc (Zn) Essential for the transformation of carbohydrates.

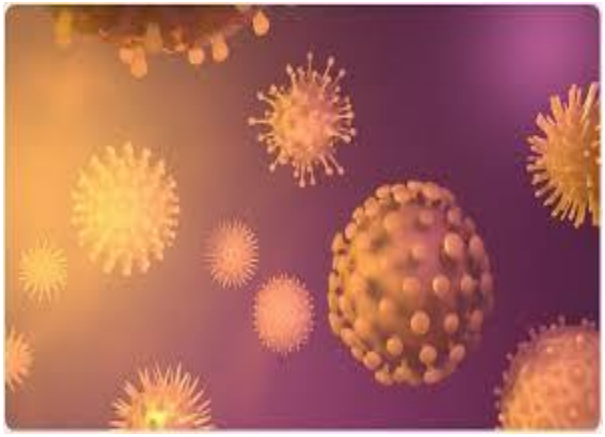
(6) Boron (B): Helps in the use of nutrients and regulates other nutrients . Aids production of sugar and carbohydrates .

(7) Copper (Cu): Important for reproductive growth.

South Valley University | Botany and microbiology Department | Faculty of Science

PRACTICAL VIROLOGY

4-th chemistry and Botany



Contents

Virology

Viruses:

They are entities' its genome is DNA or RNA single or double strand enveloped by a protein coat called capsid consists of structural units called capsomers.

Virus's classification:

The highest level of viral classification recognizes six major groups according to the nature of the genome:

1. Double stranded DNA viruses

   There are no plant viruses in this group. This group is defined to include only viruses that replicate without RNA intermediate, it is include those viruses with the largest known genomes about 400,000 base pairs and there is only one genome components, which may be linear or circular, well known viruses in this group include herpes and pox viruses.

2. Single strand DNA viruses:-

   There are two families of plant viruses in this group and both of these small circular genome components often with two or more segments.

3. Reverse-transcribing viruses:-

   These have dsDNA or ssRNA genomes and their replication includes the synthesis of DNA from RNA by the enzyme reverse transcriptase; many integrate into their host genomes. There is a single family of plant viruses in this group and this is characterized by a single component of circular dsDNA, the replication of which is *via* an RNA intermediate.

4. Double-stranded RNA (dsRNA):-

Some plant viruses and many of the mycoviruses are included in this group.

5. Negative sense single-stranded RNA (- ssRNA):-

   In this group, some or all of the genes are translated into protein from an RNA strand complementary to that of the genome (as packaged in the virus particle). There are some plant viruses in this group.

6. Positive sense single-stranded RNA (+ssRNA):

   The majority of plant viruses are included in this group.

   Within each of these groups, many different characteristics are used to classify the viruses into families, genera and species. Typically, a combination of characters is used and some of the most important are:-

Within each of these groups, many different characteristics are used to classify the viruses into families, genera and species. Typically, a combination of characters is used and some of the most important are:-

- Particle morphology: the shape and size of particles as seen under the electron microscope.
- Genome properties: this includes the number of genome components and the translation strategy. Where genome sequences have been determined, the relatedness of different sequences is often an important factor in discriminating between species.
- Biological properties: this may include the type of host and also the mode of transmission.

- Serological properties: the relatedness (or otherwise) of the virion protein(s).

Isolation and purification of plant viruses:

Isolation or purification of plant viruses is necessary to know about their structure and properties. By employing the method of purification ,a virus is finally obtained in its pure form as a colourless pellet in a test tube and may be used for various purposes following are steps involved in virus purification (isolation) :-

1. Infected leaves are homogenized in water or in phosphate, borate or citrate buffer in an electric grinder or in a motor with pestle.

2. Tissue homogenate is strained through a piece of muslin cloth or cheese cloth. Crude sap which comes out and contains virus is collected and then poured into centrifuge tube. The tube is spun at low –speed (3000-170000g). As a result, the crude sap differentiates into supernatant and a pellet. The pellet is discarded and the supernatant with virus is collected.

3. The supernatant with virus is poured into centrifuge tube .The tube is placed in fixed angle rotor of ultracentrifuge and spun at high speed (40000-150000) after the tube settles, the virus sediments and forms tiny pellet at the bottom of the tube and a supernatant over it. Supernatant is discarded and the pellet of virus is mixed with buffer and stirred with rod so that it resuspends in buffer.

4. Low and high speed centrifugation steps are repeated 2-3 times and the virus is purified by density gradient centrifugation, the most frequently used technique. A tier of layer of sucrose solutions of different concentrations (e.g., 10-40), and hence densities, is formed in the centrifuge tube ,layer at the

bottom being the most dense and one at the top of the least dense with layer of intermediate concentration .Virus suspensions is placed at top of the top most layer and centrifuged in swimming –bucket rotors at high speed ultracentrifuge .

5. When settled, virus partials move together as a band in gradient solution of sucrose. The virus band is collected as separate fraction through puncture at the bottom of the centrifuge tube .The virus fraction is placed in cellulose dialysis tubing and sucrose is removed by dialysis in buffer solution or water . Thus the virus is obtained in pure form.

External symptoms of plant viral diseases:

1- Chlolosis:-

These symptoms represent the area which becomes weakened in green colour due to either destruction or inhibition of chlorophyll formation subsequent to infection and the cells with chlorotic symptoms contain less chlorophyll thus appearing pale green or yellowish in colour.

2- Mosaic:-

These symptoms represent irregular intermingling of light green, yellow or white areas with the normal green colour of the plants or fruits forming a mosaic like pattern.

3- Mottling:-

It is an irregular pattern of indistinct light and dark areas. Mottling is considered equivalent to the mosaic by some virologists.

4- Vein chlorosis and vein clearing:-

The former represents the chlorosis restricted to the veins while the latter represents the translucence of the veins rather than being chlorotic or yellow. These two symptoms make the veins appear lighter in colour against dark background of normal green tissue.

5- Vein mosaic:-

These represent the chlorosis along the main vein with the lighter areas irregular in shape and distribution. Sometimes vein mosaic extends to adjacent areas in the form of irregular patterns.

6- Vein banding:-

In these symptoms the chlorotic area occur along the veins in a regular manner resulting in the presence of chlorotic and regular green coloured bands.

7- Necrosis:-

These are the common viral symptoms occurring in plants representing the death of the cells in localized areas .These areas of dead tissues are generally differentiated by the presence of dark brown border around them . Necrosis may remain localized but sometimes it spreads as long streaks producing systemic necrosis resulting in the death of the whole plant. One strain of TMV develops streaks on the stems and causes complete necrosis of leaves and fruits resulting in the death of the infected tomato plants.

8- Ring spots:-

These symptoms represent the localized circular spots formed by concentric rings of chlorotic and normal green tissue .ring spots are generally accompanied with necrosis. They may be presents singly or in groups developing concentrically on infected parts.

9- Enations:-

Enations are the out growths generally occurring on vein or midrib on the lower surface of the leaves.they may vary in number and shape .they may be small, large, papillate or spin like in shape .in some cases like pea enation mosaic virus the outgrowths develop between or adjacent to the veins on the lower surface of the leaves and look like leaves , funnels,wings,cups etc.

10- Leaf narrowing:-

In these symptoms the infected leaves generally become narrow due to reduced growth of laminar tissue. The veins and the midrib remain normal in growth, in the case where leaf narrowing is at extreme as represented by tmv infected tomato only the midriband veins are present and the laminar tissue is almost absent.

11- Leaf curling :-

These symptoms represent irregular and extensive wrinkling and furrowing of leaves due to reduced growth of veins in comparison to the growth of laminar tissue resulting in shrunken veins and raised up laminar tissue leading to the curling of the leaves.

Example: leaf curl of papaya, leaf of tomato etc.

12- Leaf rolling:-

In these symptoms the downward and upward rolling of leaves takes place and this convers their entire length example leaf roll of potato.

13- Stunting or dwarfing:-

In this case the infected plants show general retardation in all this organs resulting in stunting of plants. The morphology of the stunted plants remains normal. Example: chrysanthemum stunt virus.

14- Rosetting:-

Shortening of internodes due to reduced growth brings the leaves together at the tip of the branches giving rosette like appearance.

15- Tumors:-

These are large and irregular outgrowths developed due to abnormal increase in size and number of the cells .tumors generally occur on roots of some leguminous plants.tumors develop on roots of remix sp.infected with wound tumor virus.

16- Pollen abortion and pollen sterility:-

In some infected plants either the pollen may not be produced (tomato ring spot)or may remain sterile (quirking virus on datura sp.)

17- Premature abscission of leaves:-

This is acommon occurrence in many plant viral diseases.

18- Colour deviation:

* Chlorosis:

Appearance of light areas on the plant surface due to lack of plastid .chlorosis has two shapes either spotting (regular light areas) or motting (irregular light areas).

* Mosaic:

Areas with different colours ,larger than chlorosis colours ranged from white to deep green.

* Yellowish:

All the leaf becomes yellow due to destruction of plastid.

* Colour breaking: changes of flower colour.

19- Death of tissue:

*Necrosis: destroying of group of cells or tissues or whole of plant body.

*Local lesions: Death of certain tissues or cells.

*Streaks:

The dead part of the leaves appears as longitudinal lines ranged form (7-10) lines with brown colour.

20- Deformations or malformations:

*Leaf crul or rolling of leaves.

*Fly form shape of leaf blade.

*Enation: abnormal growth on the leaf blade.

*Blister: pits formed due to group of cells the pits are more green spots than other parts of leaf blade.

21- Vein clearing:

The region around the vein appears colourless or white.

Internal symptoms of plant viral diseases:

Instead of the external symptoms described earlier there are many symptoms developed endogenously with the virus infected plants they are called internal symptoms. Some important ones are being given in brief:

1- **Change in parenchymatous cell:**

Mainly mosaic viruses alter the structural and functional characteristics of various parenchymatous cells e.g.

a) Palisade parenchyma formation is poor. They also look like spongy parenchyma i.e. spherical
b) Cells of chlorotic areas remain smaller in size.
c) Intercellular spaces are reduced in size.
d) Hyperplasia may result in the infected cells.
e) Chlorophyll distruction may occur affecting the development of chloroplast and thus bringing various abnormalities in them.

2- **Changes in xylem:**

Viruses promote the formation of styluses and gummosis, tyloses the bladder like outgrowth. Block the xylem lumen. Gummosis represents the formation of gum like substances as a result of carbohydrate decomposition. These gums like substance sandty loses block the lumen of the xylem vessels stopping ascent of water and other inorganic substances. This results in finally wilting and even death of the host.

3- Change in phloem:

Necrosis is the ultimate fate of those phloem cells which are infected with viruses. These infections are however localized and mainly occur in the vicinity

of sieve elements. Sometimes hyperplasia has been observed before necrosis in infected phloem elements. Since phloem elements represent the food channel of the plant, any disturbance in it affects the food supply of the host or formation of callose in the sieve tube and companion cells.

4- Changes in cell and cell organelles

Following are some important changes that have been observed either in the cell organelles due to viral infections:

(a) Infected cell increase vacuole formation in their cytoplasm.

(b) The concentration of the cytoplasmic matrix becomes low.

(c) Ribosomes are reduced in number.

(d) There occur change in shape and size of nuclei of the infected cells.

(e) Abnormality may operate in number and morphology of mitochondria of infected cells. Mitochondria is the unit of energy deceasing the no. of it lead to decrease energy of plant.

(f) Meiosis and mitosis may also be affected in viral infected cells instead of above described internal symptoms, many amorphous and crystalline inclusions such as x-bodies, spindle or needle like crystals etc, have been reported within the cells infected by viruses. Such inclusions have never been observed in cells which are free from the concerned viral infection. On the lower surface of the leaves.

(g) Formation of vesicles of vacuoles inside plastid.

5- Destroying of cell membrane which is permeable membrane.

Examples for plant viral diseases:

Potato virus y (PVY)

Pvy is a member of the *Potyviridae* family of viruses.

Common symptoms:

Once infected, a potato plant can express symptoms in as few as 10 days, depending on the variety. Symptoms of PVY infection are variable and range from mild (foliar mottling, streaking and mosaic) to sever (leaf necrosis, leaf drop and stunting). The severity of the symptoms depends on the potato cultivar, environmental conditions and the strain of PVY infecting the plant.

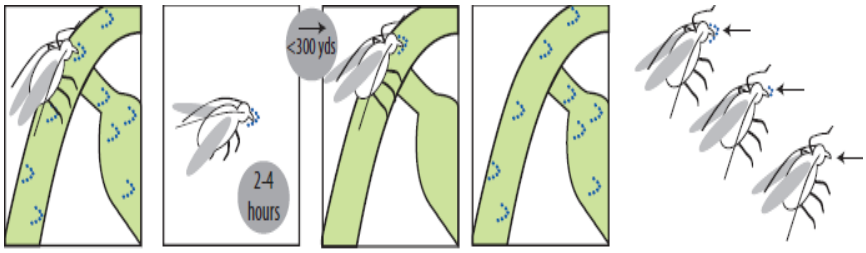
An example of how different PVY strains cause different levels of symptom severity. On the Pike variety, PVY^{N-WI} symptoms (top) are mild. PVY^{O} symptoms are more severe (bottom).

▲ **Figure 3.** A Yukon Gold tuber (top) when infected with certain strains of PVY is prone to develop potato tuber necrotic ringspot disease (bottom).

Usual means of spread:

PVY is spread from plant to plant by mechanical means or by aphids. Mechanical transmission generally occurs when an infected plant and an adjacent healthy plant are wounded by wind or human activity. The wounds of an infected plant leak sap that contains the virus and the wound of a nearby

healthy plant may take in some of that virus when the two plants touch. The more efficient and rapid form of transmission in fields involves aphid vectors. When an aphid feeds on a Pvy infected plant, virus particles adhere to the tips of its mouthparts. If the aphid then moves to a healthy plant and begins to feed, the virus particles are released and transferred to the healthy plant, leading to Pvy infection. After an aphid acquired the virus, it generally transmits it to uninfected plants for only a short period of time, usually less than two to four hours.



Non-PVY infected aphid feeds on plants infected with PVY. As the aphid feeds, PVY particles adhere to mouthparts.

The aphid moves to new plants, remaining infective for 2-4 hours, depending on how many plants it feeds on and for how long.

The infected aphid releases the virus into a new plant as it feeds.

The virus replicates within the new plant. Over time, this plant serves as a source of virus.

As the aphid continues to feed on uninfected plants, PVY is "cleaned" from its mouthparts.

Principal hosts:
Solanaceous crops including tomato, pepper, tobacco and potato.

Control:

Excluding sources of the virus from the planted crop (by using seed certified to be free of Pvy is one strategy to exclude the virus from the farm), reducing the attractiveness of the growing crop to migrating aphids and reducing the likelihood that infectious aphids will feeed on the crop.

Using seed certified to be free of PVY is one strategy to exclude the virus from the farm.

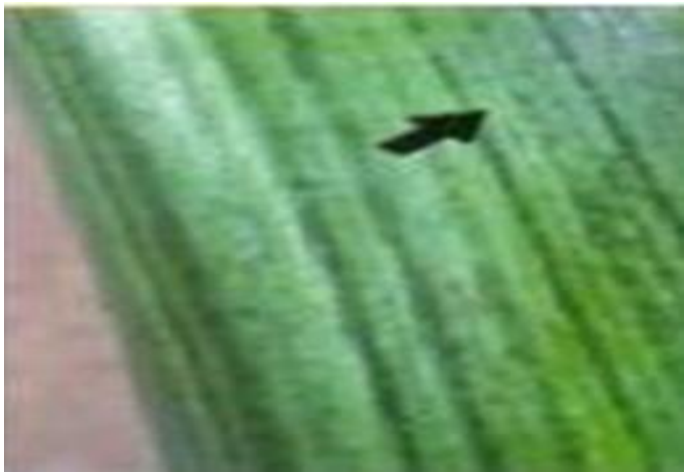
Banana bunchy top disease (rosetting)

What is banana bunchy top disease?

- The responsible for this disease is banana bunchy top virus.
- It is one of the most important and dangerous virus that infect banana.
- The virus becomes widely spread in presence of aphids.

Common symptoms:

- The initial symptoms are appearance of dark green lines on the down surface of midrib.



- The edges of the leaves become yellow then brown and become easy to break.
- As progress of infection the small leaves become crowded at the false tip of the brunches.
- Forming the rosette shape (rosetting).
- The leaves emerges difficulty from infected adult plants and the edges become wavy shape and narrow.
- Usually the infected plants become unproductive and if the plants product fruit the fruit will be disfigured.
- The next generation of the infected plants will be stunting and the leaves will be hard and erected and not flat and shorter than the leaves of normal plant.

Usual means of spread:

- It doesn't transmit mechanically by cellular sap.
- It transmit with banana aphid (*Pentalonia nigronervosea*).

Control:

- Plant uninfected seeds.
- Put a cup of kerosene inside the plant to avoid aphids and cut the plant from the middle.
- Take off the infected plants with roots (the full plant) and burn the location of the removed infected plants.
- Pour kerosene on the soil (the location of the removed infected plants) and leave the soil exposed to air for two weeks and put (CaO) inside the plant.

Banana bract mosaic virus

Banana bract mosaic virus (BBrMV) is also called banana bract mosaic and Kokkan disease. This disease was first found in the Philippines in 1979**.**

Description

Initial symptoms of banana bract mosaic virus include green or red-brown streaks or spindle shaped lesions on the leaf stalks and sometimes on the midribs of the new banana leaves. Chlorotic (pale yellow or yellow-white) streaks may also appear on the bunch stems and fingers. In severe cases the affected fruit can be rejected. If the outer leaves are removed from the banana plant stem; red-brown, spindle-shaped streaks can be seen on the exposed stem.

Life cycle

Banana bract mosaic virus infects the vegetative, flowering and fruiting stages of banana plants. The disease is transmitted by aphids which acquire the virus while feeding on infected plants. The virus only lives inside the aphids for a short period of time and is transmitted in a nonpersistent manner. Banana bract mosaic virus can be spread by four aphids that are present in Australia: banana aphid (*Pentalonia nigronervosa*), corn aphid (*Rhopaloshiphum maidis*) cotton or melon aphid (*Aphis gossypii*), cowpea aphid (*Aphis craccivora*). Double infections of banana bract mosaic virus and banana bunchy top virus (also transmitted by the banana aphid) can occur in banana plants.

Hosts

Banana bract mosaic virus infects cultivated and wild bananas (Musaceae plant family).

Spread

Banana bract mosaic virus is spread longer distances by infected banana planting material, including suckers, bits, corms and unindexed tissue culture plantlets.

Distribution

Banana bract mosaic virus is found in India, the Philippines, Sri Lanka, Thailand, Vietnam and Western Samoa.

Actions to minimise risks

Put in place biosecurity best practice actions to prevent entry, establishment and spread of pests and diseases: practise “Come clean, Go clean” ensure all staff and visitors are instructed in and adhere to your business management hygiene requirements source propagation material of a known high health status from reputable suppliers monitor your crop regularly keep records isolate banana plants or areas with suspect symptoms to prevent further spread

Figure 1 A dark, red-brown mosaic pattern on the banana flower bract is the main symptom of banana bract mosaic virus

Figure 2 Red-brown, spindle-shaped streaks on the exposed banana stem (when the outer leaves are removed) indicating banana bract mosaic virus

Tomato spotted wilt virus (TSWV):

Taxonomic position: Viruses: Bunyaviridae: *Tospovirus*

Symptoms

On tomatoes, plants show bronzing, curling, necrotic streaks and spots on the leaves. Dark-brown streaks also appear on leaf petioles, stems and growing tips. The plants are small and stunted as compared with healthy plants. The ripe fruit shows paler red or yellow areas on the skin. Sometimes, affected plants are killed by severe necrosis.

Usual means of spread:

- TSWV is spread by tiny insects called thrips, which pick up the virus by feeding on infected plants.
- If the virus and the thrips are present, the severity of the disease depends on the weather.

- TSWV is not seed-transmitted.

Principal host:

TSWV is polyphagous on a great number of mostly herbaceous hosts. *Capsicum annuum*, lettuces, tobacco, tomatoes and various ornamental crops are the main hosts.

Control

ž The presence of thrips in the crops should be monitored using yellow sticky cards. If the disease appears in a crop, infected plants should be rogued and destroyed immediately and the house treated with insecticide against thrips.

Sugarcane mosaic virus

List of symptoms/signs

- Leaves: abnormal colours, abnormal forms, abnormal patterns and necrotic areas
- Stems: discoloration of bark and stunting or rosetting
- Whole plant: dwarfing

The particular symptoms depend on the virus strain, the host cultivar and the environmental conditions, particularly temperature.

Prevention and control

Control of SCMV:

- Applying herbicides may be useful in maintaining mosaic-free seed plots of cane if the level of infection is lower than 5%.

- Use of mosaic-free seed cane is an effective control measure where inoculum pressures are not intense. Thermotherapy of planting material can result in some plants that are free of SCMV.

Control of Aphid Vectors

- Altering the times of planting and harvesting so that they do not coincide with high aphid vector populations and can reduce losses.
- Should not be grown near infected sugarcane crops.
- The use of insecticides failed to prevent aphid vectors from spreading SCMV.
- Aphids which transmit SCMV come from outside as well as inside the sugarcane crop, care should be given to reduce the build up of the vector species in the vicinity.

Cucumber mosaic virus {CMV }

- Cucumber mosaic virus (CMV) is one of the most common plant viruses and causes a wide range of symptoms, especially yellow mottling, distortion and stunting. Expect damage whenever susceptible plants are growing well in spring and summer.


Principle Hosts

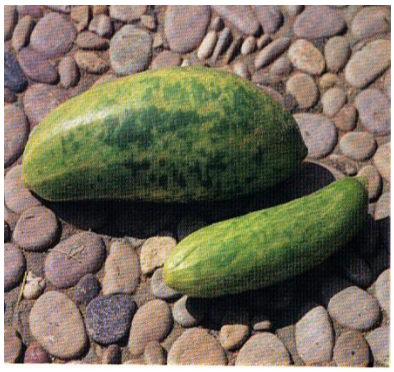
Apart from cucumbers and other cucurbits, it also attacks spinach ,lettuce and celery and many flowers, especially lilies.

Usually means of spread

- CMV is vectored by several aphid species which feed on a broad range of plants and this contributes to the very wide host range of this virus

- CMV is occasionally transmitted through seed in around 20 of plant species
- It can spread mecanically by a sap.
- It can also be transmitted in seeds and by the parasitic weeds, Cuscuta sp.

- It naturally spread in soil by zoospores of fungus.
- CMV is easily transmitted on garden tools and gardeners' fingers.



Cucumber: mosaic by cucumber mosaic virus (fruit symptoms) (Middle East)

Control

- **Chemical control: There are no chemical controls available to control virues. The use of insecticides to reduce aphid transmission is not practical.**
- **Non chemical: Avoid handling healthy plants after working with suspected infected ones until tools or hands have been washed with soapy water. Destroy suspect plants promptly to reduce the risk of transmission. Keep the garden weed free.**

Viruses' replication (Bacteriophage):

There are two methods for viral Replication:-

1. **lytic cycle.**
2. **Lysogenic cycle.**

Lytic cycle

- Bacteriophage is biologically extinct i.e. it can't activate singly but it must enter host cell.
- Lytic cycle called lysis cycle because bacteriophage lyse the cell wall of the bacterial cell and the bacterial cell die.

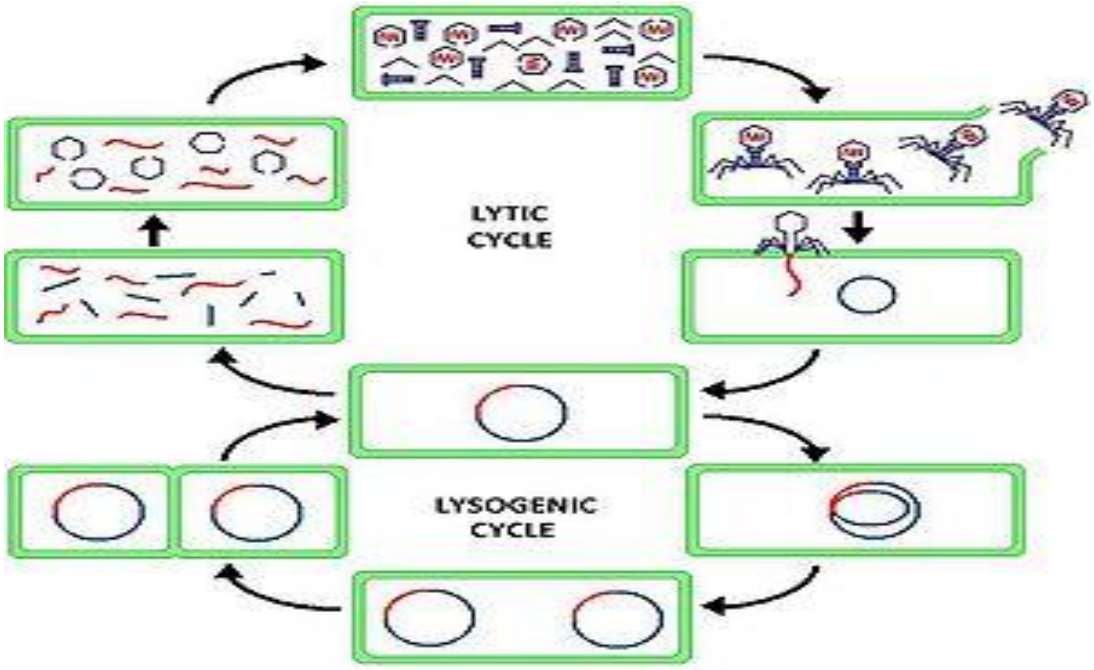
Phases of lytic cycle:-

- **Attachment phase:-**
  In this phase bacteriophage close from bacterial cell and by using plate core and fibers it begin to attach to bacterial cell wall. This called random collisions because if bacterial cell has a receptor. The virus will attack bacteria from it.
- **Penetration and injection of genome:-**
  In this phase bacteriophage begins to penetrate the wall of bacterial cell and injects its genome.
- **Multiplication phase:-**
  Also called copying phase. In this phase bacteriophage controls bacterial cell and dominates its activity and prevents it from division and use host machinery synthetic to make copies of its genome.
- **Maturation phase:-**

In this phase each bacteriophage enveloped by a protein coat and fibers and plate core begins to form.

- **Releasing phase:-**

  In this phase the unmatured bacteriophage matures and begins to lyse the wall of the bacterial cell and releases and repeats the cycle.



Lysogenic cycle

- Also called lysogeny.
- It is one of the two methods of viral replication.
- It begins with the same phases of the lytic cycle as attachment phase, penetration and injection of genome.

- It is characterized by integration of the bacteriophage nucleic acid as a part of the linear structure of the host bacterium genome.
- In this cycle the bacteriophage doesn't dominate bacterial cell and doesn't prevent it from division but it copies itself with the usual bacterial cell division.
- The newly integrated genetic material which called prophage can be transmitted to each subsequent cell division and bacterial cell continue in division and bacteriophage division continue with it.
- Prophages called temperate phages because they have moderate effect on the cells i.e. they don't cause death of the cell and it allows bacterial cell to survive for some period.
- Under unsuitable conditions or if bacteriophage undergo any stress or mutation or if it exposed any radiation such as U.V. radiation that cause releasing of prophages this occur through proliferation of phages via the lytic cycle.
- In case of lysogenic cycle the spread of viral DNA occurs through the usual prokaryotic reproduction.While in case of lytic cycle phages are spread through production of thousands of individual phages capable of surviving and infecting other bacterium.

Lysogenic conversion

In some interactions between lysogenic phages and bacteria, lysogenic conversion may occur; it is when temperate phages induce change in the phenotype of the bacterial cell.

Examples:-

1. **Corynebacterium diphtheria** produce the toxin of diphtheria only when it is infected with phage B.
2. **Vibrio cholera** is nontoxic strain also produce cholera toxin when infected with phage.

Vaccines

It is a biological preparation that improves the immunity to a particular disease.

Vaccine contains an agent resembles to a disease that are caused by microorganisms.

Vaccine made from weakened or killed forms of the microbes or its toxins.

The agent stimulate the body's immune system to recognize the agent as a foreign destroy it and remember it so that the immune system can more easily recognize and destroy any of these microorganisms that is later encounters.

There are several types of vaccines:

- **killed:**

  Some vaccines contained killed but previously virulent microorganisms that have been destroyed with chemicals, heat, radioactivity or antibiotics.

  Examples: influenza vaccine, cholera vaccine, hepatitis A vaccine

- **Attenuated**

  Some vaccine contain live attenuated microorganisms many of these are live viruses that have been cultivated under conditions that disable their virulent properties or which use closely related but less dangerous organisms to produce a broad immune response.

  Examples:

  Viral diseases like yellow fever, measles, rubella, bacterial disease like typhoid.

- **Toxoid:**

They are made from inactivated toxic compound that cause illness rather than the microorganisms.

Examples of toxoid based vaccines include tetanus and diphtheria.

- **Subunit:**

  Protein subunit rather than introducing inactivated or attenuated microorganisms to an immune system a fragment of it can create an immune response

  Examples:

  The subunit vaccine agent hepatitis B virus, which is composed only from the surface protein.

- **Conjugate:**

  Certain bacteria have polysaccharides outer coats that are poorly immunogenic.

  By linking these outer coats to proteins e.g. toxins that immune system can be lead to recognize the polysaccharides as it if were a protein antigen.

  Example:

  Haemophilus influenza type B vaccine.

Isolation of animal viruses

(1) Enrichment process: which occur by prepare nutrient broth which consists of 3 gm beef extract. 5 gm pepton.1000 ml distilled water.
(2) Take 90 ml of nutrient broth and add 10 ml of sewage water.
(3) Incubation process for 24 at 37◦c.

Observation

The virus attached to *E. coli* and released by high numbers.

The isolation occurs by filter unit: gives bacterial cell and viral suspension.

Determination the activity and availability of viruses:

Procedures:

- Prepare treptocase say agar with sterilization and keeps it at 45°c to still liquid.
- Prepare bacterial suspension.
- Add 1ml of bacterial suspension to sterilized Petri dish.
- Add 15 ml of treptocase say agar to every Petri dish under sterilized conditions.
- Let the media to become solid i.e. solidify.
- Take sterilized filter paper discs and immerse it in viral suspension using sterilized forcipes.
- Put filter discs with virus to the surface of media.
- Incubate at 37°c for 24h in incubator.

Observation:

An appearance of clear zone that means virus is active and make lytic cycle of bacteria.

Absence of clear zone this means that virus is not active and don't make lytic cycle for bacteria.

Your observation and comments:

Titration of virus activity:

The main target is to determine the activity of viruses.

There are two methods to determine the activity of viruses.

(1) In case of liquid media:
The test is called broth clearing assay.

(2) In case of solid media:
The test used is called plaque clearing assay.

In case of liquid media

Procedures:

- Make serial dilutions of viral suspension.
- Put 9 ml of n.b. in sterilized test tubes and make sterilization process.
- By using sterilized pipit add 1 ml of the bacterial suspension in all tubes.
- Incubate for 24h at 37°c in incubator.

Observation

We must detect the titre of virus activity which is the highest dilution and the lowest concentration at which clearing is occurring and turbidity is disappeared i.e. absence of turbidity.

The titre

It is defined as the highest dilution or the lowest conc. of viral suspension which causes complete inhibition of bacteria.

Or the disporical of the highest dilution which cause complete inhibition of bacterial growth.

Your observation and comments:

In case of solid media

Procedure:

- Prepare nutrient agar media and sterilize it.
- Keep it at 45◦c to make it semisolid.
- Add in every sterilize Petri dish 9 ml of nutrient broth agar.
- Make viral suspension and add 1 ml of it to every Petri dish.
- Add 1 ml of bacterial suspension to every dish.
- Incubate for 24 h at 37◦c in incubator.

Observation

Formation of plaque ranged from 30-300.

Lower than 30 plaque doesn't consider titre as it is non active.

Higher than 300 doesn't titre as it is highly active.

Titre=no. of plaque×1÷the highest dilutions.

Your observation and comments:

Antigen antibody reactions

- **Antigen:**

Any foreign substance which when introduced into an animal produces a specific immune response (antibodies, cell mediated immunity).

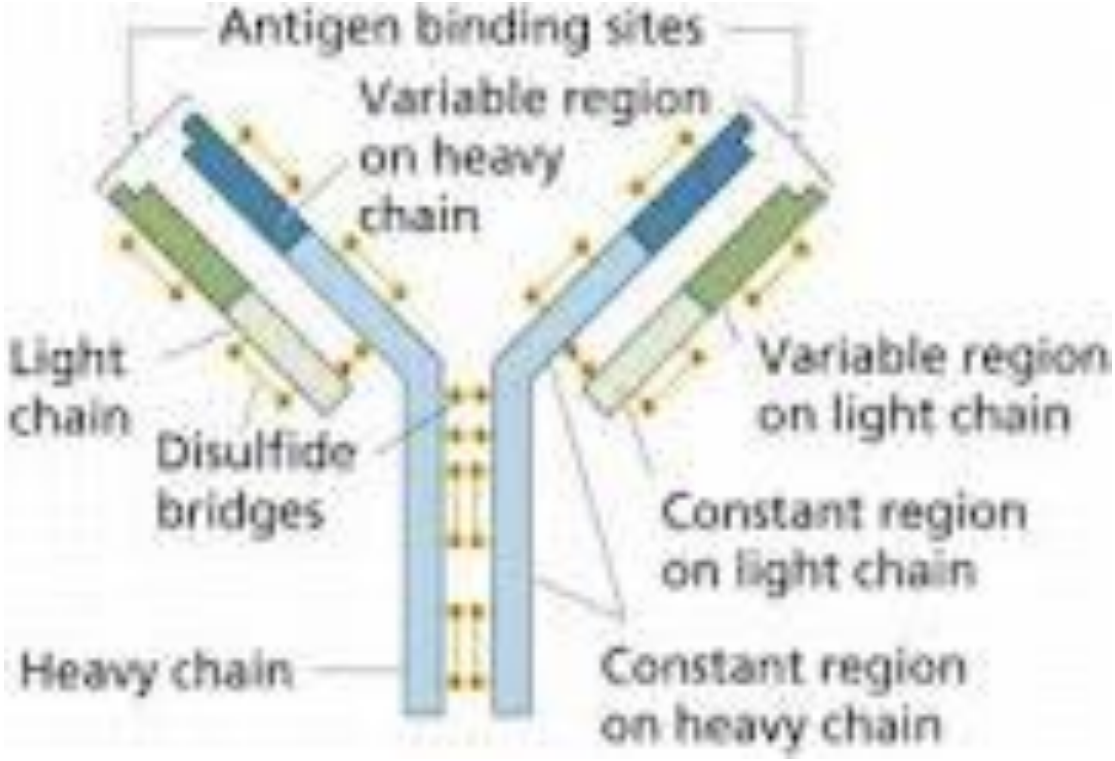
- **Antibody:**

It is a gamma globulin that appears in the serum, tissue fluids of animal after exposure to an antigen.

Types of antibodies:

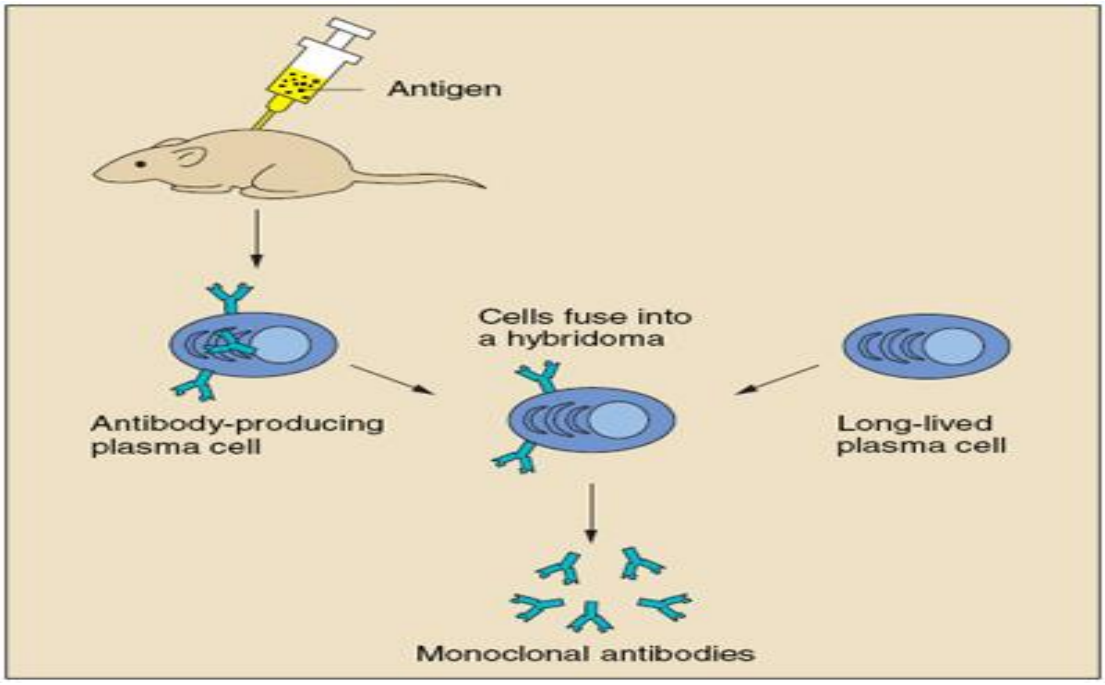
IgG, IgA, IgM, IgD, IgE

Structure of antibody


Antigen binding sites
Variable region on heavy chain
Light chain
Disulfide bridges
Variable region on light chain
Constant region on light chain
Heavy chain
Constant region on heavy chain

Monoclonal antibodies

They are made by aclone of cells that arouse from single cell.



Characters of antigen antibody reaction

1-Specific:

Sense antibody can combine only with the antigen which induced its formation.

2- The physical state of the antigen determines the observable result:

If antigen in the form of particles the resulting reaction is clumping of the particles or agglutination.

If in solution, the resulting reaction is precipitation.

3-The presence of electrolytes is essential for the reaction to occur as it decreases the repulsion forces between particles and facilitating anigen antibody reaction.

1- Zone phenomenon:

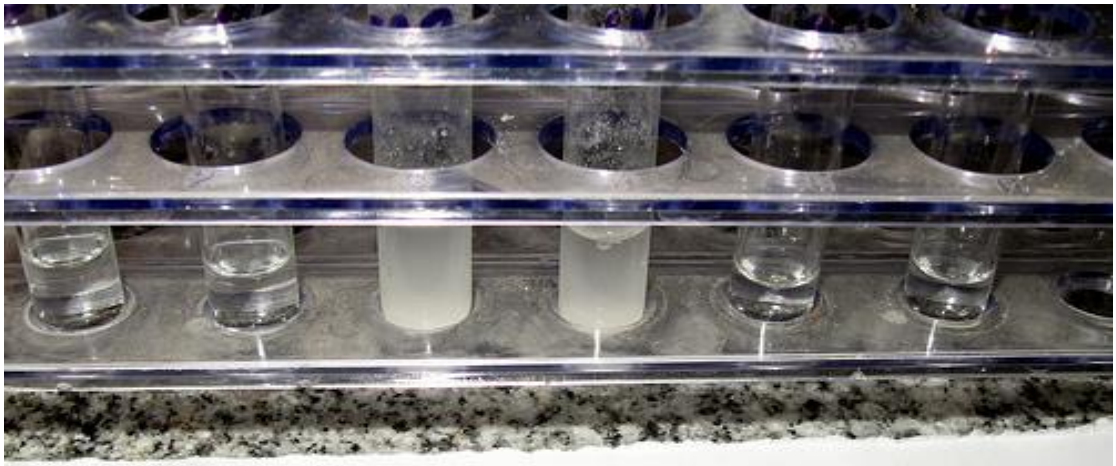
- In serological tests serum is prepared in a series of tubes to which constant amount of antigen is added for quantitative estimation of antibody and avoid zone phenomenon.

- In the first tubes antibody is present in excess→soluble complexes with antigen→no visible reaction.

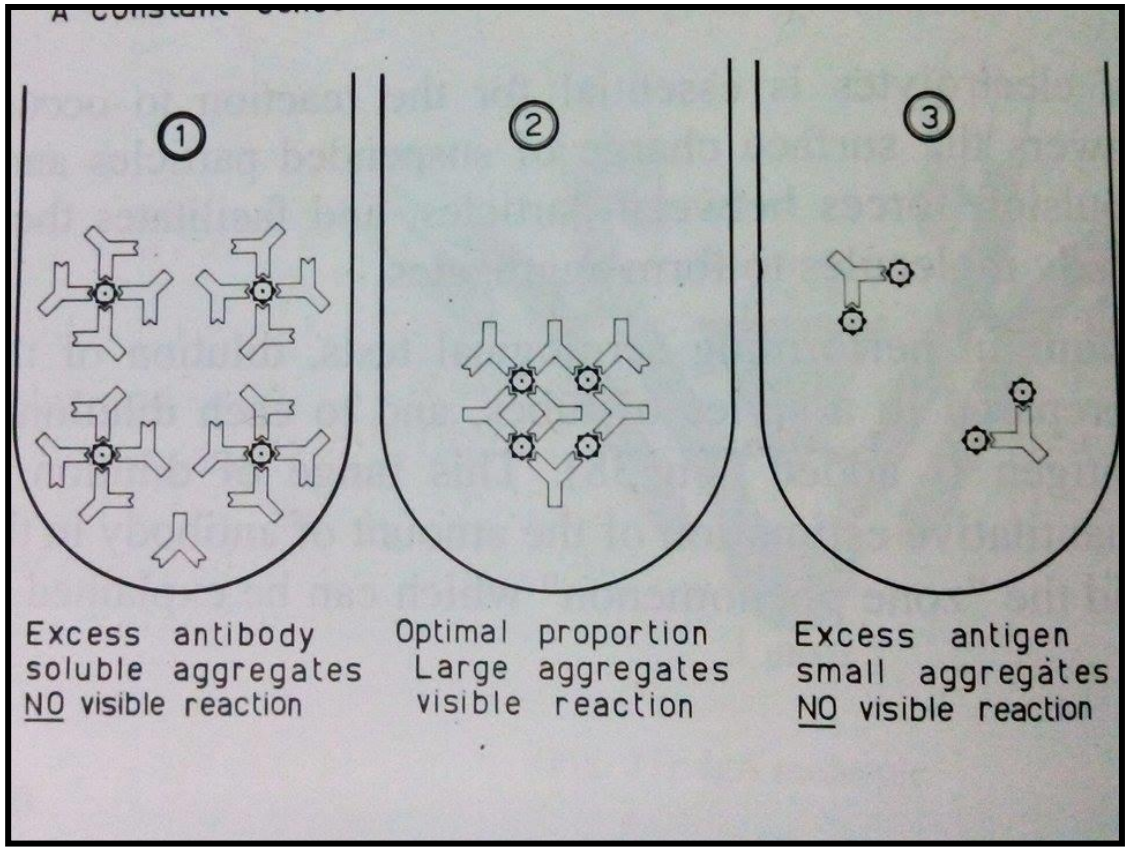
- In the far right tubes the antibody has been diluted beyond its capacity to bind free antigen→no large aggregates→no visible reaction.

- In between these two extremes antigen and antibody presnt at optimal concentrayion to each other→large aggregate is formed→visible reaction

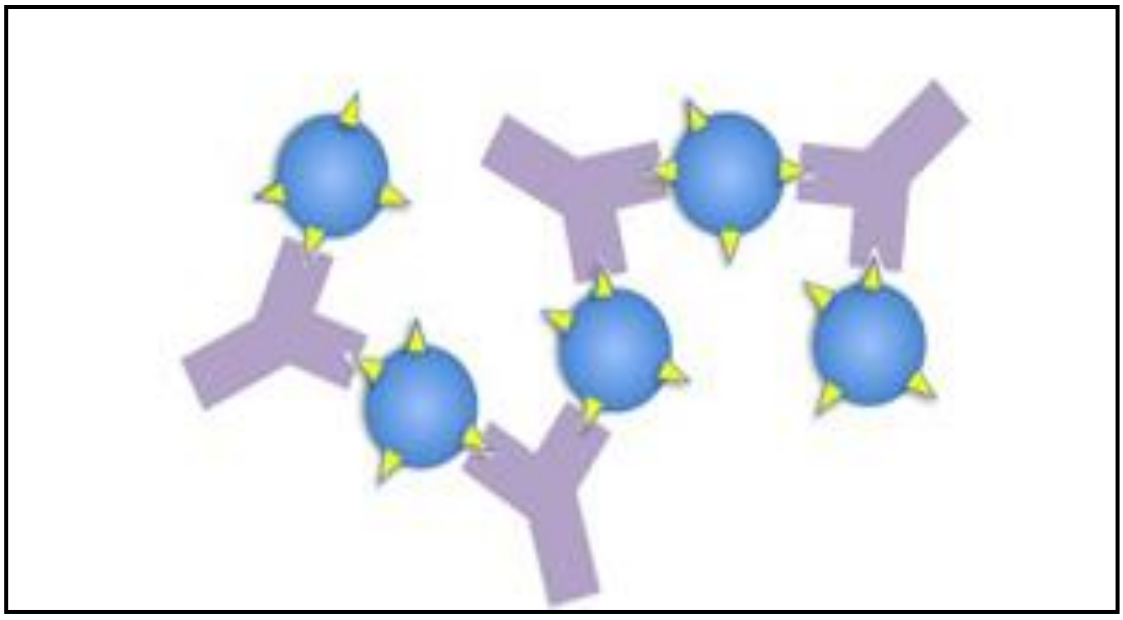
Zone phenomenon (cont.)



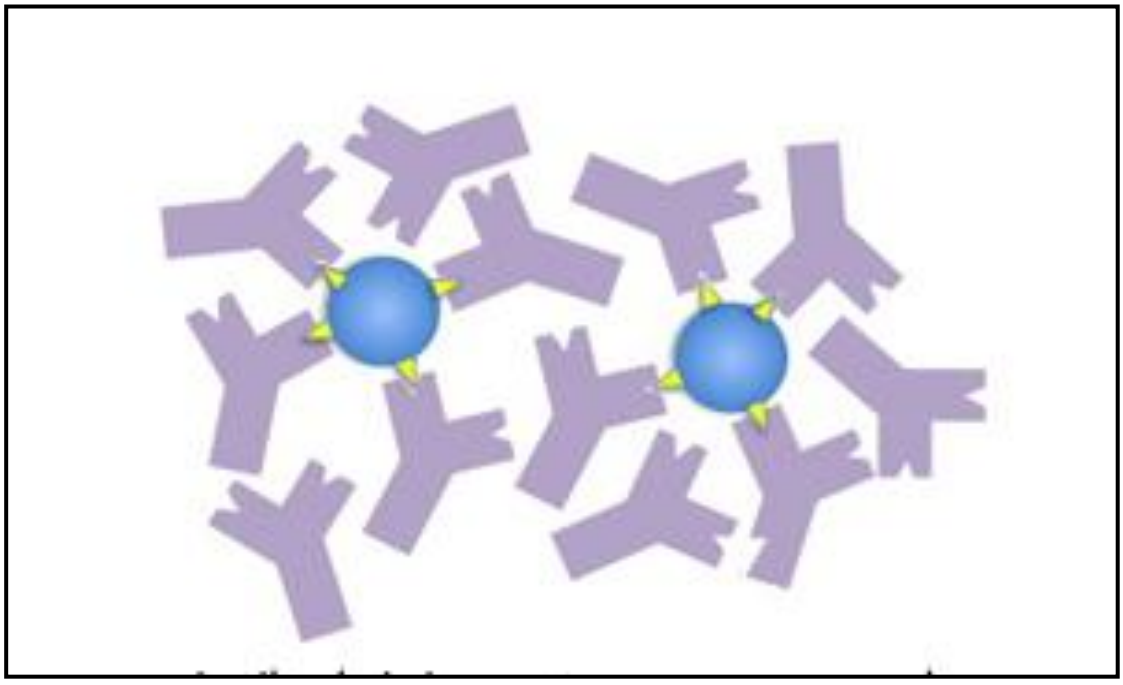
Zone phenomenon agglutination test seen in brucella



Diagramtic explanation of the zone phenomenon



Optimal proportion of antigen & antibody-large aggregates- visible reaction



Excess antibody- soluble aggregates – No visible reaction

Your observation and comments:

2- Agglutination

- Antigen in the form of particles e.g. microorganism, RBCs, latex particle

- When mixed with specific antisera particles become clumped.

Types of Agglutination:

- Direct agglutination.

- Antiglobulin agglutination test.

- Latex agglutination.

- Coagglutination (COA).

- Virus heamagglutination inhibition.

- Heterophile antibodies agglutination tests.

A- direct agglutination: has 2 forms

1- Slide agglutination:

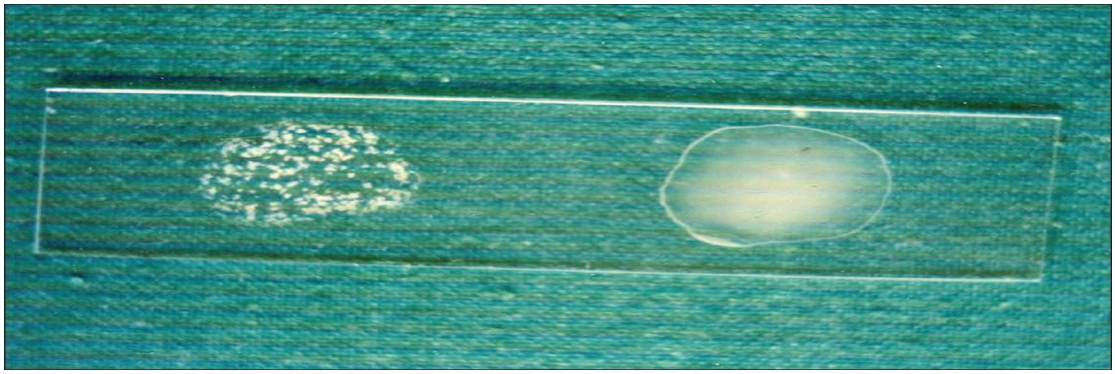
- 2drops of saline containing the unknown microorganism are placed on clean slide.

- known serum is added mixed well

- clumping occurs if serum is specific to the organism.

Application:

Blood grouping



Blood Group Testing

Usually restricted to the ABO and the Rhesus (D)

A) **ABO Blood Group**

- To determine the ABO type, red cells must be tested with anti-A and Anti-B and the serum/plasma tested with A and B red cells

- **Forward grouping** - identifies the **antigens** on the red cells
  - tests the recipient or donor red cells with anti-A and anti-B sera

eg Cells agglutinated only with anti-A serum are group A
Cells that do not agglutinate with anti-A or anti-B are group O

- **Reverse grouping** – identifies the presence of **antibodies** in the serum/plasma
  - confirms the reaction obtained by the forward grouping test.
  - tests the serum/plasma from the recipient or donor with group A red cells and group B red cells
    eg Agglutination with group B cells indicates the presence of
    anti-B in the plasma – Group A individual

B) **Rhesus Blood Group (Rh)**

Rhesus typing of red cells is determined by examining their reaction with anti-D serum.

There are no 'naturally- occurring' Rhesus antibodies, therefore reverse grouping is not performed.

Routine testing for other Rh antigens is not required.

Your observation and comments:

2- Tube agglutination

Quantitative test to determine amount of antibodies in the serum.

Steps:

- Serial dilution of the patient serum (1/10,1/20,1/40,1/80…..etc.) is done to which constant amount of known bacteria is added.

- Tubes incubated for 2-4hr. At 37^0c

- Examine for visible clumping

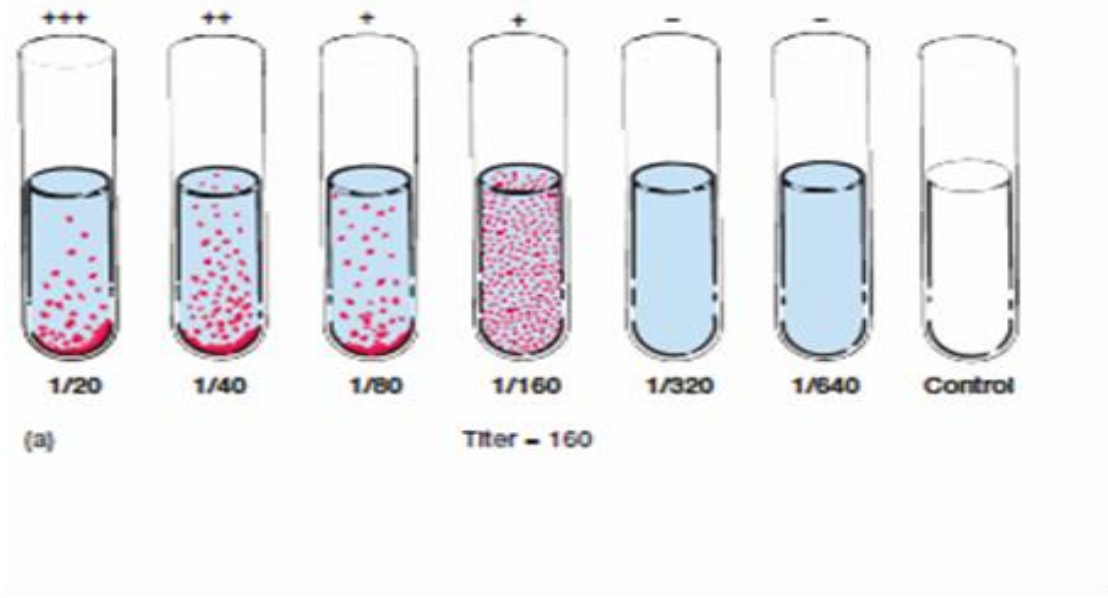
- Titre:

The hightest dilution that showing visible aggregate

- Titre measures the number of antibody units per unit volume e.g.1/320 means 320 units of antibody/ml of serum.

Application:

Widal test for diagnosis of Salmonella



Your observation and comments:

B-antiglobulin agglutination test=coomb's test.

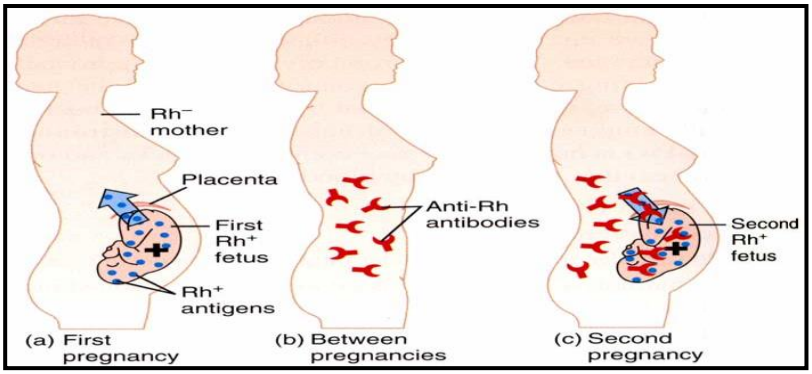
Application:

Used to determine the presence of Rh incompatibility which causes" *erythroblastosis foetalis*".

- red cells of Rh +ve foetus can induce Rh antibodies in his Rh –ve mother,these antibodies will pass through the placenta to the foetus of the next pregnancy causing heamolysis of his RBC's.

-anti-Rh antibodies are IgG incomplete antibodies(cannot bridge between 2 RBC's)so they can be detected by antiglobulin.

-there are 2 types of coomb's test:



Rh incompatibility which causes" *erythroblastosis foetalis*

1- Indirect coomb's test:

- Mother's serum, containing anti-Rh antibodies is mixed with Rh +ve RBC's(group O)

- Incubation at 37^0c for 30min-1hr.

- The mixture is centrifuged, deposit washed, antihuman globulin is added, tubes incubated

- The antihuman globulin causes agglutination by linking the incomplete antibodies together.

2-Direct coomb's test:

- It detects incomplete Rh antibodies coating the RBC's of newborn in *erythroblastosis foetalis.*

- *The antihuman* globulin is added directly to a washed suspension of the newborn RBC's, agglutination occurs.

- Both direct, indirect coomb's test are also used to detect autoimmune heamolytic anaemias.

3- Latex agglutination:

- Agglutination reaction in which inert particles e.g.latex are coated with various antigens or antibodies.

- These particles are aggregated in the presence of specific antibody or antigen.

Examples:

- Pregnancy test:

- The antigen is human chorionic gonadotrophic hormone (HCG) in the urine of pregnant female.

- The test is done by adding latex particles coated with anti-HCG to adrop of urine.

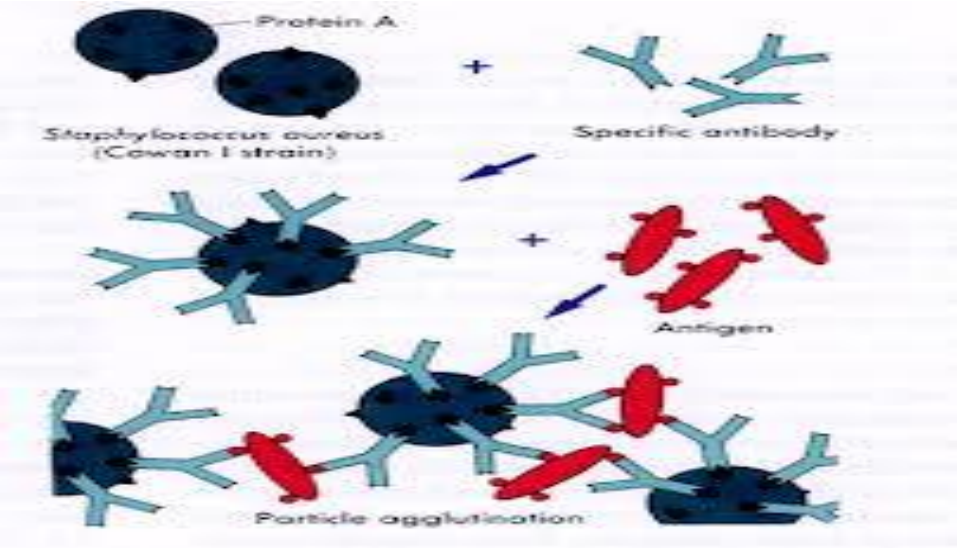
- Agglutination occurs if HCG is present.

Your observation and comments:

4 – Coagglutination (COA):

- *Staph. aureus* rich in protein A on there surface, can bind IgG non specifically through Fc region leaving Fab sites free.

- If staph. coated with antibody reacts with specific antigen →agglutination occurs.



Your observation and comments: