Plant (5)

Part I (Fungi)

3rd year students

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Book Contents

Chapter (I)

Introduction

Mycology: is the branch of science dealing with study of fungi "Greek, Mykes = mushrooms or fungus + Logos = subject. The term fungus (PL. Fungi) which originally means mushroom, but it is now applied to all types of fungi".

According to Alexopolus (1952), the fungus is defined as a nucleated achlorophyllous, thallophytes and usually filamentous, branched organisms which typically reproduce sexually and asexually and varies from bacteria by these previous features and the presence of cell wall surrounding the somatic cells split them from animal kingdom.

General characteristics of true fungi

All are eukaryotic: Possess membrane-bound nuclei (containing chromosomes) and a range of membrane-bound cytoplasmic organelles (e.g. mitochondria, vacuoles, endoplasmic reticulum).

1- Most are filamentous: Composed of individual microscopic filaments called hyphae, which exhibit apical growth and which branch to form a network of hyphae called a mycelium.

2- Some are unicellular e.g. yeasts.

3- Protoplasm of a hypha or cell is surrounded by a rigid wall composed primarily of chitin and glucans, although the walls of some species contain cellulose.
4- Many reproduce both sexually and asexually, both sexual and asexual reproduction often results in the production of spores.
5- Their nuclei are typically haploid and hyphal compartments are often multinucleate although the Oomycota and some yeast possess diploid nuclei.
6- All are achlorophyllous; they lack chlorophyll pigments and are incapable of photosynthesis.
7- All are chemoheterotrophic (chemo-organotrophic), they utilise pre-existing organic sources of carbon in their environment and the energy from chemical reactions to synthesise the organic compounds they require for growth and energy.
8- Possess characteristic range of storage compounds e.g. trehalose, glycogen, sugar alcohols and lipids.
9- May be free-living or may form intimate relationships with other organisms i.e. may be free-living, parasitic or mutualistic (symbiotic).

Fungal occurrence

- They are universal in their distribution. In fact, they found in almost every available substrate on earth (tropical, subtropical, Arctic ‟cold regions” and ....etc) where the organic material (living or dead) is present.
- Many fungi grow in our foodstuffs such as bread, Jams, fruits ...etc. Also, they are present in all the times in the air surrounded us.
- Most of them are terrestrial occur in the soil; produce non-motile cells ‟advanced forms” which mainly dispersed by wind, water, or animals.... etc.
- Some others are aquatic (primitive forms) they produce motile cell which live on the decaying organic matter and living organism found in water.

Fungal Nutrition

- All fungi are chemoheterotrophic (chemo-organotrophic) synthesising the organic compounds they need for growth and energy from pre-existing organic sources in their environment, using the energy from chemical reactions.
- Since their protoplasm is protected by a rigid wall, fungi must obtain their nutrients by the process of Absorption.

- Small molecules (e.g. simple sugars, amino acids) in solution can be absorbed directly across the fungal wall and plasma membrane.
- Larger, more complex molecules (e.g. polymers such as polysaccharides and proteins) must be first broken down into smaller molecules, which can then be absorbed. This degradation takes place outside the fungal cell or hypha and is achieved by enzymes which are either released through or are bound to the fungal wall. Because these enzymes act outside the cell they are called extracellular enzymes.
- Since water is essential for the diffusion of extracellular enzymes and nutrients across the fungal wall and plasma membrane, actively growing fungi are usually restricted to relatively moist (or humid) environments.

Obligate Saprophytes

They are colonizing dead organic material, and these are saprobes or saprophytes. However, most of fungi can exist on dead organic material. Fungi which live only on dead organic material and unable to attack living organisms are obligate saprobes such as *Rhizopus*, *Penicillium* and *Aspergillus* species.

Facultative saprophytes

Those which can live parasitically according to the prevailing conditions are known as facultative saprobes as *Rhizoctonia* and *Pythium* species if not found suitable host, they live as saprobes.

Obligate parasites

They are those fungi which pass their entire life on living organisms e.g. rust fungi, powdery mildews and downy mildews.

Facultative parasites

Those which can live saprobically according to the prevailing conditions are known as facultative parasites such as *Fusarium* species. In other meaning, this group of fungi when found suitable host turned to parasite form.

Symbiosis (Mutalisms)

There is another type of association in which the fungus enters in a partnership with another organism. The best examples of symbioses are mycorrhizae (myco = fungus and rhiza = root) and lichens.

There are two types of mycorrhizae:-

- **Ectotrophic- Mycorrhizae**

  In which are mainly live on the outer root surface for example Ascomycota and Basidiomycota with the forest trees root system eg: - Gymnosperms and Angiosperms.

- **Endotrophic- Endomycorrhzae**

  In which the fungal cells penetrate the roots and grow in their tissues, it is mainly belonging to the root system of herbaceous plants and some primitive fungi as Glomeromycetes

Lichens

They consist of a fungal cell almost (85%) related to sac fungi (Ascolichens) and rarely to basidiomycota (Basidiolichens) and an algal cell mainly belonged to green algae (chlorophyta) and sometimes to blue green algae (cyanobacteria).

The body is termed as the thallus, and its shapes differentiated to mainly three groups: Foliose, Fruticose and crustose.

Factors affect the growth of fungi

Temperature

Fungi can be grouped into broad categories, according to their temperature ranges for growth as follows:

1- Psychrophiles "cold-loving":

   They can grow at 0 °C, and some even as low as -10 °C, and their upper limit is often about 25 °C.

2- Mesophiles "room temperature":

They grow in moderate temperature range, minimum from about 10-20 °C, optimum 25-30 °C and maximum at 35 °C.

3- Thermophiles "heat-loving":

With an optimum growth temperature of 50 °C or more, a maximum of up to 70 °C or more, and minimum of about 40 °C.

4- Thermotolerants:

With optimum growth temperature of about 25 °C a maximum of up to 50 °C, and minimum of about 20 °C.

pH value

Fungi generally require an acidic medium for normal growth, with a pH of approximately 6.0. The optimum pH for growth of most of the fungi is 5.0 – 6.5; a few fungi grow much below pH 3.0 and a few others even above pH 9.0.

Light

Most fungi do not require light for their vegetative growth. However, it is needed by many species for sporulation and spore dispersal.

Oxygen supply

It is also an important factor for fungal growth. Most of the fungi are aerobic and stop growing in the absence of oxygen.

Water

Like other living organisms, is an essential requirement for all fungi.

What do fungi look like?

Many of us are familiar with the appearance of mushrooms and toadstools. But these structures are simply the large, macroscopic fruiting bodies produced by some groups of fungi. The actively growing and reproductive structures of most species are microscopic, and although most fungi are mycelial (filamentous), there are some exceptions to this growth form.

- Unicellular form (Yeasts)
- Mycelial (filamentous)
- Unicellular and primitively branched Chytrids
- Dimorphism

Unicellular form (Yeasts)

For example the yeasts, it is consists of a single, minute, oval or spherical cell. The cell wall is mainly made up only from glucan and chitin. It contain nucleus and nucleolus and mitocondria, and the main reserve food products are glycogen, oil, volutin and protein bodies.

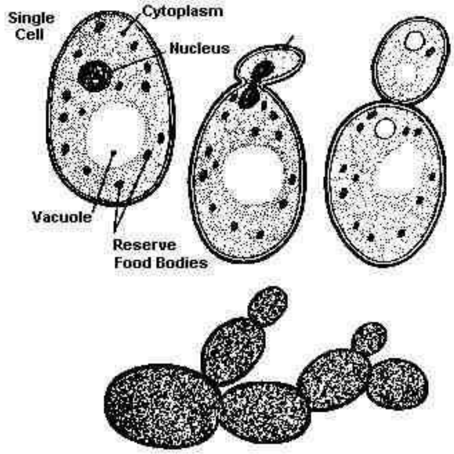


Figure (1): Unicellar form (Yeasts)

Mycelial (filamentous)

Most fungi are composed of microscopic filaments called hyphae, which branch to eventually form a network of hyphae, called a mycelium (colony). The mycelium extends over or through what ever substrate the fungus is using as a source of food. Each hypha is essentially a tube, containing protoplasm surrounded by a rigid wall composed from chitin.

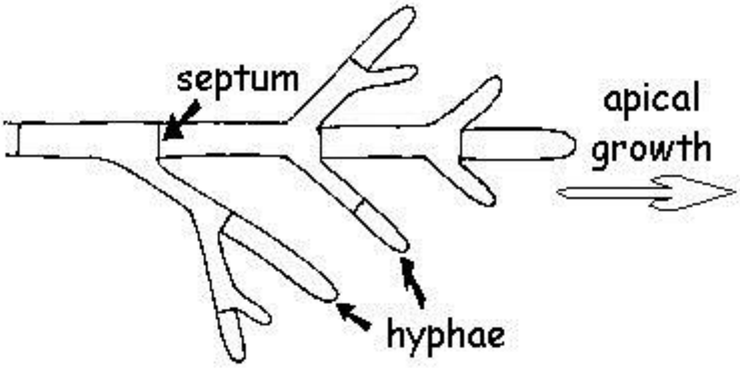


Figure (2): Mycelial form

1- **Aseptate hyphae**

In this case, protoplasm may form a continuous, interrupted mass running the length of the branching hyphae, in the other word, the nuclei are embedded in the cytoplasm and arranged regularly along the whole mass of the cytoplasm. The hyphae in this case is coenocyte

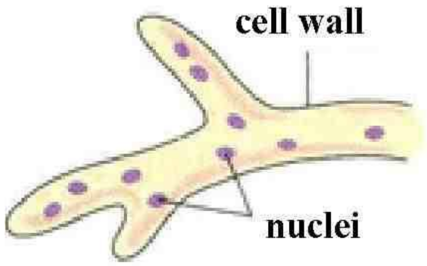


Figure (3): Aseptate hyphae

2- **Septate hyphae**

In this case the protoplasm may be interrupted at intervals by cross-walls called septa. Septa divide up hyphae into individual discrete cells or interconnected hyphal compartments.

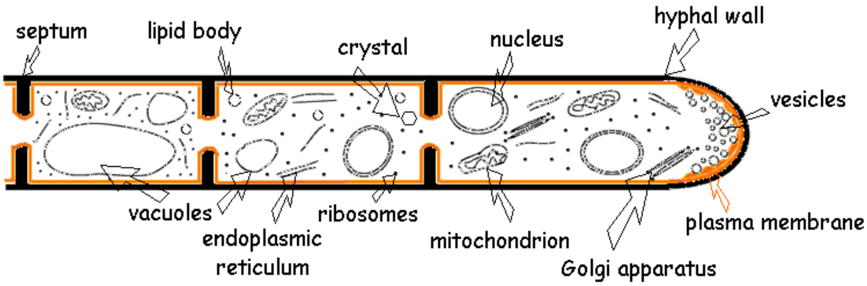


Figure (4): septate hyphae

Hyphae exhibit apical growth (i.e. they elongate at their tips) and, at least in theory, can grow indefinitely, provided that environmental conditions remain favourable for growth. Of course, their environment eventually limits or restricts their growth. Hyphae may initially develop from a germ-tube (a short, immature hypha) that emerges from a germinating spore. Spores are the microscopic dispersal or survival propagules produced by many species of fungi.

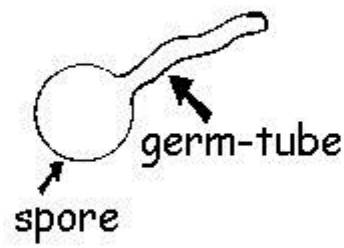


Figure (5): Germination of spore

3- Unicellular and primitively branched Chytrids (Chytridiomycota)

Fungi belonging to the Chytridiomycota exist as either single round cells (unicellular species) or primitively branched chains of cells. In either case, the fungus may be anchored to its substrate by structures called rhizoids.

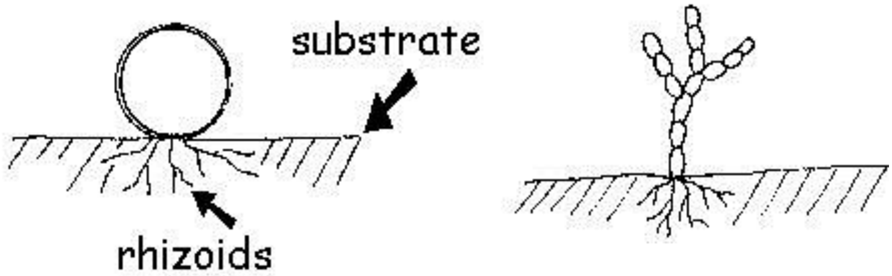


Figure (6): Unicellular and primitively branched Chytrids.

4- Dimorphism (i.e. existing in two forms)

Some fungi are capable of alternating between a mycelial growth form and a unicellular yeast phase. This change in growth form is often in response to some change in environmental conditions. This phenomenon is exhibited by several species of fungi that are pathogenic in humans, e.g. *Paracoccidioides brasiliensis*.

Structure of fungal hyphae

Each hypha is: essentially a tube - consisting of a rigid wall and containing protoplasm tapered at its tip, this is the region of active growth (i.e. the extension zone).

- Some fungi possess septa at regular intervals along the lengths of their hyphae, in others, cross-walls form only to isolate old or damaged regions of a hypha or to isolate reproductive structures.
- Some septa possess one of more pores, such septa divide up the hyphae into a series of interconnected hyphal compartments, rather than separate, discrete cells.
- The plasma membrane is closely associated with the hyphal wall and in some regions, may even be firmly attached to it making it difficult to plasmolyse hyphae.
- Each hyphal cell or compartment normally contains one or more nuclei. In species whose septa possess a large central pore, the number of nuclei within a hyphal compartment won't remain static because the nuclei are able to pass between adjacent compartments, via the central septal pore.
- Cytoplasm contains a well-developed endoplasmic reticulum and contains either many smaller vacuoles, or one large vacuole.

❖ Mitochondria and many food particles made up glycogen, oil droplets and lipids, other cell organelles include ribosomes, microbodies and crystals are also common.

❖ The growing tip is structurally and functionally very different from the rest of the hypha.

❖ There are no major organelles at the extreme tip, at the extreme tip there is an accumulation of membrane bound vesicles - the apical vesicular cluster (complex) (AVC) which plays an important role in apical growth.

❖ Vacuoles may be visible in sub-apical hyphal compartments although small at first, they grow larger and merge with one another; they store and recycle cellular metabolites, e.g. enzymes and nutrients.

❖ In the oldest parts of the hypha the protoplasm may breakdown completely, due either to autolysis (self-digestion) or in natural environments heterolysis (degradation due to the activities of other microorganisms).

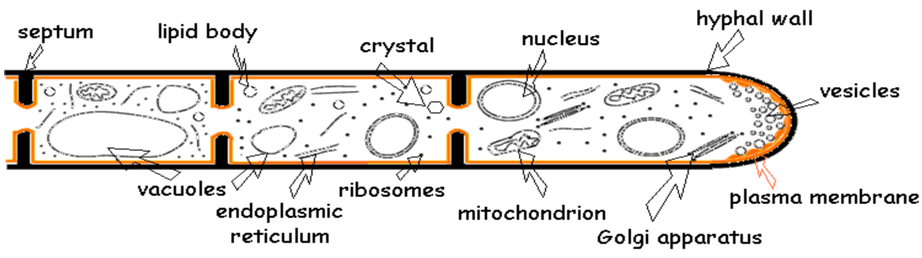


Figure (7): Fungal hyphae compostions

Hyphal aggregation

Many mycologists mentioned that mycelium in most of the true fungi gets organized into loosely, or compactly fungal tissue called plectenchyma which contains mainly two types as follows:

A- Prosenchyma

The hyphae remain arranged loosely but parallel to one another. This type is common in the fruiting bodies of some Asco and Basidiomycota and the main structure of the rhizomorphs

B- Pseudoparenchyma

The hyphae remain arranged compactly and therefore loose their individuality. The hyphal cells are oval or isodiametric. It is considered as the main structure of different fungal fruiting bodies.

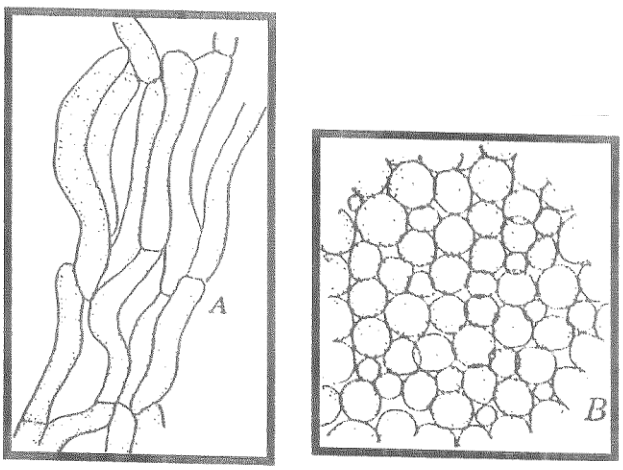


Figure (8): Prosenchyma (A) and Pseudoparenchyma (B).

Haustoria: (Haustor = drinker)

The mycelium of parasitic fungi may grow on the surface of the host and in this case, it is known as ectoparasites which they have special out growths to serve as hold fasts, or organs of attachment called **appressoria**. Haustorium may be knob-like, clavate or branched like a root system. Fungi that penetrate the host and develop their mycelia within the tissues called endoparasites and these mycelia may be restricted to small area as in leaf spots or may be distributed throughout the plant as in case of wilt diseases. The mycelium in this case is intercellular, lying between the cells, or intracellular lying within the cells.

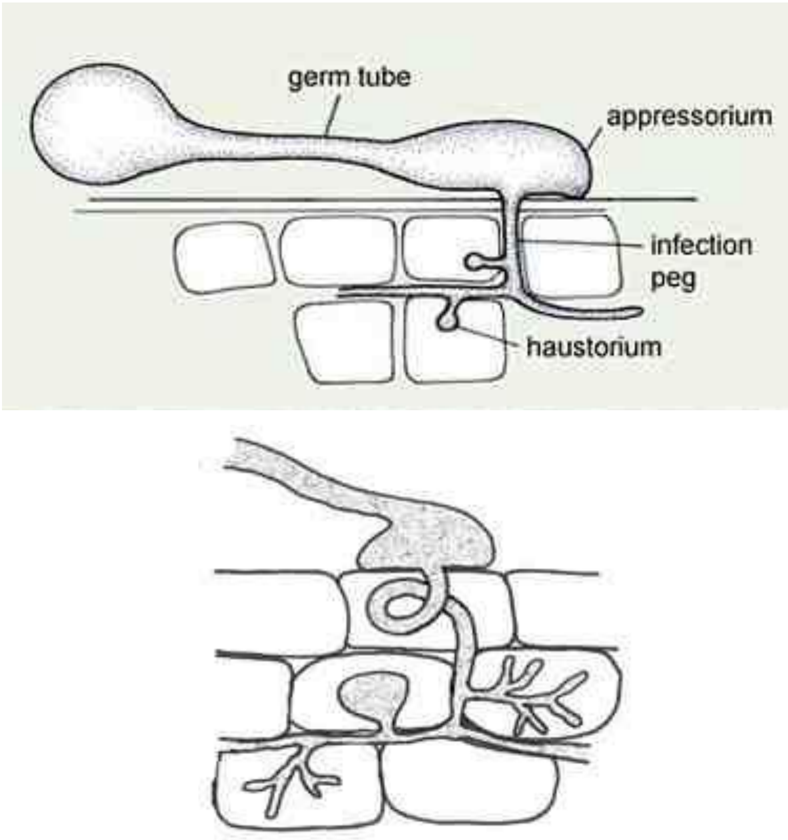


Figure (9): The formation of haustoria from fungal hyphae.

The cell wall of fungi

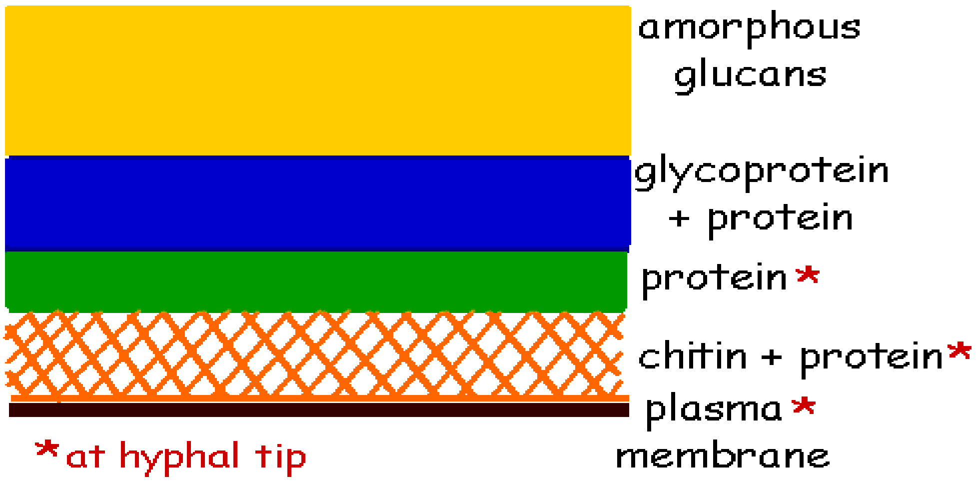
Functions:

- Protect the underlying protoplasm.
- Determines and maintains the shape of the fungal cell or hypha; if you remove the wall the resulting protoplast will always assume a spherical shape.
- Acts as an interface between the fungus and its environment.
- Acts as a binding site for some enzymes.
- Possesses antigenic properties which allow interactions with other organisms.

Chemical composition of the wall:

- Polymeric fibrils
  - Chitin
  - Cellulose (in the Oomycota)
- Amorphous matrix components
  - Glucans
  - Proteins
  - Lipids
  - Heteropolymers (mixed polymers) of mannose, galactose, fructose and xylose.

The types and amounts of these various components vary amongst different groups of fungi and may even vary during the life cycle of a single species.



Figue (10): The diagram above represents a section through the mature lateral wall of hyphae of *Neurospora crassa*.

- In general, the inner part of the wall consists of polymeric fibrils embedded in an amorphous matrix and this is covered by further layers of matrix material.
- At the hyphal tip the wall is thinner and simpler in structure, consisting of only two layers, the inner layer of fibrils embedded in protein and outer layer of mainly protein.

- Extra layers of wall material are deposited in the lateral walls behind the extending apex strengthening the wall as the hypha matures.
- In the oldest parts of the hyphae (and in many fungal spores) lipids and pigments may be deposited in the wall:
    - Lipids serve as a nutrient reserve and help prevent desiccation
    - Pigments, such as melanin, help protect the protoplast against the damaging effects of UV radiation

Growth of fungal hypha

We already know that the growing hyphal tip is structurally and functionally different from the rest of the hypha but, the hyphal tip (like the rest of the hypha) is surrounded by a wall although the wall may be thinner and simpler in structure than the mature lateral wall of the hypha further back.

The growth of a hypha is closely linked to the presence of vesicles which form the apical vesicular cluster (AVC) when a hypha stops growing, these vesicles disappear but, when growth of the hypha resumes, the vesicles reappear.

<u>The position of the vesicles</u> is linked to the direction of growth of a hypha: -

1) When a hypha is growing straight ahead, the vesicles are positioned in the center of the hyphal tip.
2) Movement of the vesicles to the left or right side of the hyphal tip is accompanied by a change in direction of growth of the hypha.

Vesicles of the AVC contain:

- Wall precursors - the sub-units or building blocks of the wall polymers - e.g. uridine diphosphate N-acetylglucosamine, the sub-unit of chitin.
- Wall lytic enzymes - which help breakdown and separate wall components - e.g. chitinase, glucanase.

- Wall synthase enzymes - which help assemble new wall components and so increase the size of the wall - e.g. chitin synthase, glucan synthase.

Two models have been proposed to explain the mechanisms of apical growth, they differ in whether or not wall lytic enzymes are necessary.

Model 1- involvement of wall lytic enzymes:

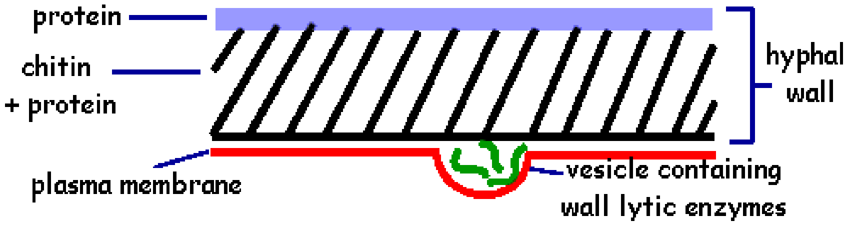
According to this model, if the hypha is going to be able to extend at its tip, there will have to be:

1) Some softening (lysis) of the existing wall, and
2) The synthesis and incorporation of new wall material.

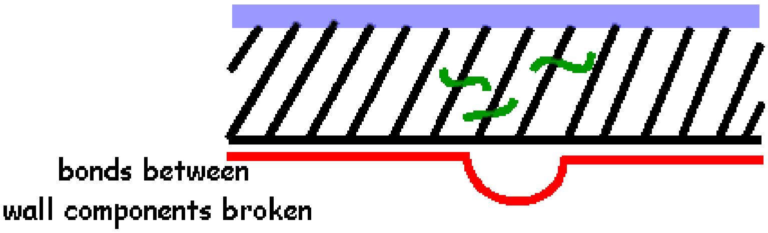
But these processes will have to be finely balanced. otherwise, the wall may become too weak or too rigid for further growth

The following series of diagrams helps illustrate what may happen:

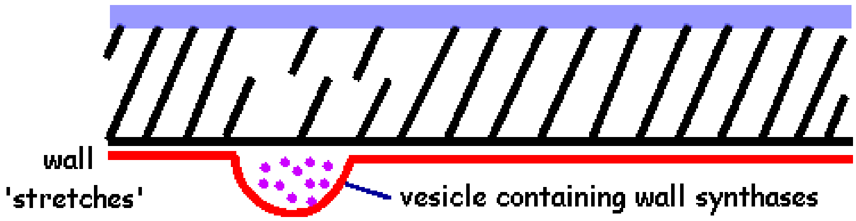
1- Vesicles containing lytic enzymes or wall precursors move through the cytoplasm towards the hyphal tip, where they fuse with the plasma membrane, releasing their contents into the wall.



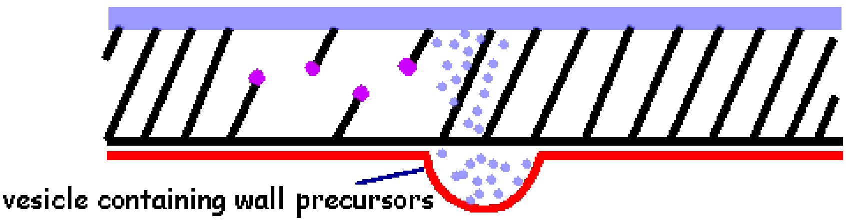
2- The lytic enzymes released into the wall attack the polymeric fibrils.



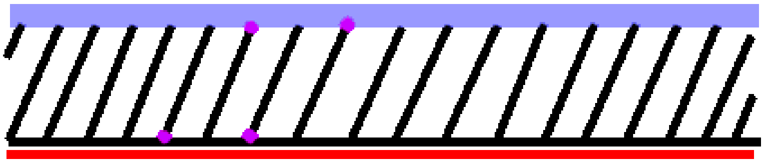
3- The weakened fibrils stretch out and become separated from one another due to the turgor pressure of the protoplasm.



4- Synthase enzymes and wall precursors build new fibrils and synthesize additional amorphous components of the wall.



5- The surface area of the hyphal wall increases. Fusion of the vesicles with the plasma membrane ensures that the former contribute to the increase in surface area of the latter.



<u>Model 2</u>- steady state:

According to this model the lytic enzymes are not involved in apical growth, because the newly formed wall at the extreme tip of the hypha is viscoelastic (essentially fluid).

So that as new wall components are added at the tip, the wall flows outwards and backwards. The wall then rigidifies progressively behind the tip by the formation of extra chemical bonds.

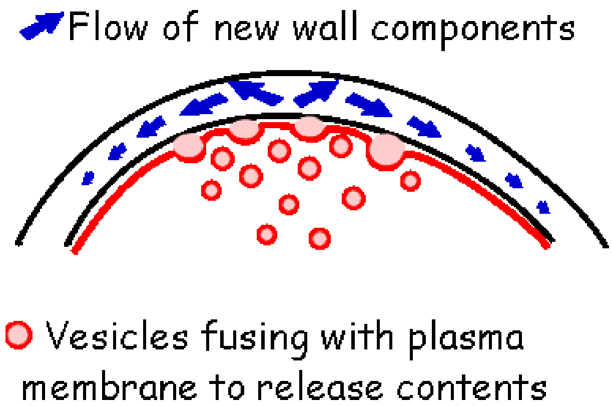


Figure (11): Model 2 for apical growth of fungal hyphae

Fungal septa

Septa (cross-walls) can be seen by light microscopy, but electron microscopy has revealed that several different types of septa exist among the major taxonomic groups of fungi.

Oomycota and Zygomycota

- In general, the hyphae of fungi belonging to these groups are not regularly septate (although there are some exceptions).
- But septa in the form of complete cross-walls are formed to isolate old or damaged regions of the mycelium or to separate reproductive structures from somatic hyphae.

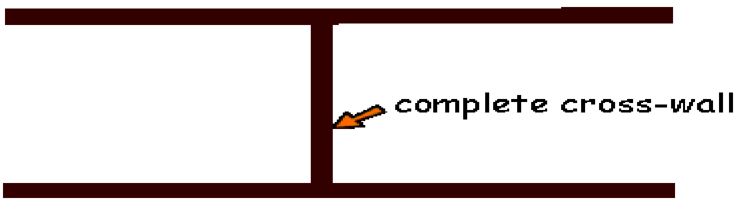


Figure (12): Septa Oomycota and Zygomycota fungi

Ascomycota and some mitosporic fungi

- Hyphae of fungi belonging to these groups possess perforated septa at regular intervals along their length.
- The septum consists of a simple plate with a relatively large central pore (50-500 nm diameter), this allows cytoplasmic streaming (the movement of organelles, incl. nuclei) between adjacent hyphal compartments.

- Cytoplasmic streaming enables sub-apical and intercalary (central) compartments of young hyphae to contribute towards growth of the hyphal tip - transporting nutrients and essential enzymes to the apex - so maximizing the capacity for somatic growth.
- Associated with each septum are spherical, membrane-bound organelles called woronin bodies that: -
    - Are composed of protein.
    - Remain close to the septal pore and tend not to be disturbed by the cytoplasmic streaming taking place.
    - Tend to be of the same or larger diameter than the septal pore and are, therefore, capable of blocking the pore.
    - Will block the septal pore if the adjacent hyphal compartment is damaged or ageing and becoming highly vacuolated.
- Not all fungi belonging to the Acomycota possess woronin bodies, some possess large hexagonal crystals of protein in the cytoplasm that can serve the same function, i.e. they can seal the septal pores of damaged or ageing hyphae.

Figure (13): Septa in Ascomycota and some mitosporic fungi

Some other mitosporic fungi

- Many mitosporic fungi possess septa with a single central pore, like that observed in the Ascomycota.
- But other mitosporic fungi may possess multi-perforate septa, e.g. the septa of *Geotrichum candidum* (illustrated below) possess characteristic micropores (approx. 9 nm diameters).
- The number of pores in each septum can vary up to a maximum of approx. 50.
- These micropores allow cytoplasmic continuity between adjacent hyphal compartments but are too small to allow cytoplasmic streaming to occur to the extent observed in fungi possessing larger septal pores.

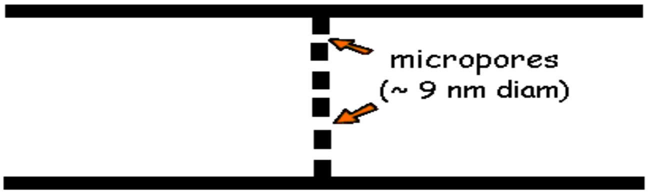


Figure (14): Septa in other mitosporic fungi

Basidiomycota:

- The most complex type of septum is found in fungi belonging to the Basidiomycota.
- Each septum is characterized by a swelling around the central pore (dolipore) and a hemispherical perforated cap (parenthosome) on either side of the pore.
- The perforated parenthosome allows cytoplasmic continuity but prevents the movement of major organelles.
- The plasma membrane lines both sides of the septum and the dolipore swelling, but the membrane of the parenthosome is derived from endoplasmic reticulum.

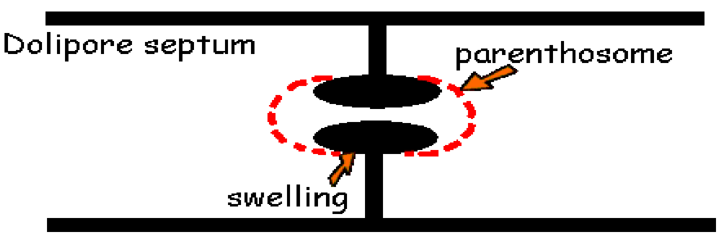


Figure (15): Septa in Basidiomycota fungi

Functions of septa

- Act as structural supports: The addition of plate-like cross-walls to what is essentially a long tube-like structure (hypha) will help stabilize it.
- Act as the first line of defence when part of a hypha is damaged
  - Large-pored septa that have woronin bodies or large proteinaceous crystals associated with them have the advantage that cytoplasmic streaming can occur between adjacent compartments.
  - But at the same time a mechanism exists for rapidly sealing the septal pore under conditions of stress (e.g. if the hypha is damaged) thereby helping protect the mycelium.
- Facilitate differentiation in fungi
  - Septa can isolate adjacent compartments from one another so that different biochemical and physiological processes can occur within them, these may result in differentiation of the hyphae into specialized structures, such as those associated with sporulation.
  - It's unlikely to be coincidental that the most complex and highly differentiated sporulating structures we see are those produced by fungi possessing the most complex types of septa, i.e. fungi belonging to the basidiomycota.

Importance of fungi

Fungi are important because they are:

- Agents of biodegradation and biodeterioration
- Responsible for most of plant diseases and several diseases of animals (including humans)
- Used in industrial fermentation processes
- Used in the commercial production of many biochemicals
- Cultured commercially to provide us with a direct source of food
- Used in bioremediation
- Beneficial in agriculture, horticulture and forestry.

Fungi are agents of biodegradation and biodeterioration:

Saprotrophic fungi utilise dead organic materials as sources of nutrients and are responsible for the biodegradation of organic materials in our environment, particularly plant materials in the form of leaf litter and other plant debris. Such fungi play a vital role in recycling essential elements, particularly carbon.

Fungi are very effective and efficient biodegraders because of the wide range of extracellular enzymes they produce, which are capable of degrading complex polymers, such as cellulose, proteins and lignins.

Unfortunately, their excellent biodegradative abilities mean that many saprotrophic fungi can contaminate our food sources or destroying many consumer goods we manufacture from natural raw organic materials.

For example, some saprotrophic fungi are particulalry dangerous contaminants of seeds and grains because they produce metabolites known as mycotoxins (fungal toxins). When ingested mycotoxins cause toxic or carcinogenic symptoms in humans and other animals. Some *Aspergillus* species produce a group of chemically related mycotoxins called aflatoxins.

A second example is provided by the 'dry rot' fungus, *Serpula lacrymans*, which attacks wood and can be a very costly, potentially dangerous and certainly most unwelcome visitor when it attacks timbers used in the construction of buildings (e.g. floor and wall joists or roof timbers).

Fungi are responsible for most of plant diseases and several diseases of animals (including humans):

For example, *Phytophthora infestans* is the causal agent of late blight disease in potatoes. The disease reached epidemic proportions across Europe in the mid 19th century and resulted in the Irish potato famine.

Some fungi are actively parasitic in humans and other animals, while others induce severe allergic reactions if their spores are inhaled - resulting in attacks of asthma or hay-fever.

Fungi are used in industrial fermentation processes:

Yeasts and mycelial fungi are used in a variety of industrial fermentation processes. For example, *Saccharomyces* species are used extensively in brewing beers and wines, as well as in bread making.

Fungi are also used in the commercial production of many biochemicals, e.g. *Aspergillus niger* is used in the large-scale commercial production of citric acid.

Many fungi provide us with a direct source of food:

Some yeasts and mycelial fungi are cultured on a large scale and then undergo further processing to provide various protein-rich food products for human or livestock consumption. For example, Quorn mycoprotein is produced commercially from the mycelial fungus *Fusarium venanatum*. The mycelium is harvested and processed to provide a protein rich meat substitute in a range of convenience foods.

Some species are cultivated for their edible fruiting bodies, e.g. the basidiocarps of *Agaricus bisporus*.

Fungi are used in bioremediation:

Some species of yeasts and mycelial fungi are used in processes aimed at reducing the concentrations and toxicity of waste materials, particularly from industrial processes, before those wastes are released into the environment, this process known as bioremediation. For example, *Aspergillus niger* is used to breakdown tannins in tannery effluents to less toxic compounds.

Some fungi prove highly beneficial in agriculture, horticulture and forestry:

For example, some species form symbiotic relationships with the roots of plants, known as mycorrhizas. Mycorrhizas significantly improve plant growth and vigour, resulting in increased yields in crop plants.

Other fungal species are used in the biological control of insect and nematode pests, weeds and pathogenic microorganisms. For example, the fungus *Beauvaria bassiana* is used to control several insect pests.

Growth of fungi

Fungal growth may be restricted or unrestricted. Unrestricted fungal growth occurs when the substrate contains an excess of all growth factors. During unrestricted growth, the total hyphal length and the number of tips of a mycelium increases indefinitely. In nature, unrestricted growth may only be possible within a short time due to unfavourable growth conditions. Restricted growth occurs when fungus grow on a solid substrate for example a drywall, eventually results in conditions such as nutrient depletion, change in pH at the center of the colony which are less favourable for growth than was initially the case. If there is no competition with other microorganisms, growth of the hyphae on the peripheral ring occurs at approximately maximum growth rate but growth proceeds at below maximum growth rate elsewhere in the colony, often falling to zero or near zero at the colony center, i.e., some parts of the fungus would be actively growing while others would not be growing at all or would be dead.

Phases of growth

A- Unicellular fungi

1- **lag phase**, during this phase unicellular fungi (yeasts) adapt themselves to growth conditions. During the lag phase of the fungal growth cycle, synthesis of DNA, enzymes and other molecules occurs. It is the period where the individual Cell are maturing and not yet able to divide. The length or shortness of this phase depend on many factors such as:
   a) Age and size of fungal inoculum
   b) The kind of the medium
   c) Environmental factors e.g temperature.

2- **Phase of accelerated growth** the cells start to divide and new protoplasm is formed from the nutrient found in the medium, and during this phase the time required for synthesis new cells decrease.

3- **Log phase** (sometimes called the logarithmic phase or the *exponential phase*) is a period characterized by cell doubling. The number of new yeast appearing per unit time is proportional to the present population. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line.

4- **Phase of decline acceleration**: During this phase the rate of growth is decreased because the time required for synthesis new cells increase.

5- **Stationary phase** is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is equal to the rate of cell death. The result is a “smooth,” horizontal linear part of the curve during the stationary phase.

6- **Death phase** (Decline phase). This could be due to lack of nutrients, a temperature which is too high or low, or the wrong living conditions.

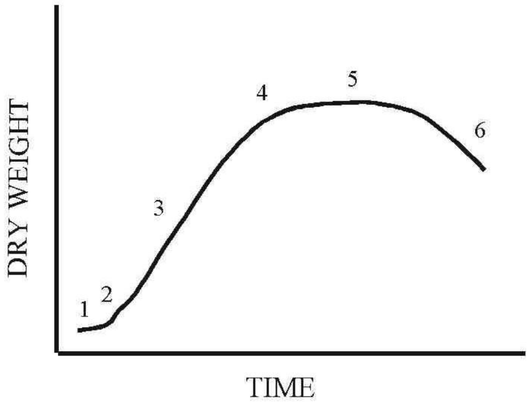


Figure (16): Growth phases of unicellular fungi

B- Filamentous Fungi

1- **Lag pase**

Once the growth conditions become favourable for the fungal propagules (i.e., viable spores or mycelial fragments) to germinate, new transport systems must be induced before growth commences. Thus, growth starts slowly and accelerates gradually. This phase is referred to as the lag phase.

2- **Exponential or log pase**

Exponential growth occurs only for a brief period as hyphae branches are initiated, and then the new hypha extends at a linear rate into un-colonized regions of substrate. The biomass of the growing fungus doubles per unit time. If the nutrients are in excess growth remains constant during the exponential phase.

3- **Stationary phase**

As soon as the nutrients are depleted or toxic metabolites are produced growth slows down or is completely stopped. The biomass increases gradually or remains constant. During the stationary phase, hyphal growth stops and, in some molds, cell differentiation occurs, resulting in spore formation. During this process nutrients are transferred from the vegetative mycelium to the developing spores. The spores are dispersed by air movement to other areas of the building where they can start new mold growth once the conditions for growth are favourable.

4- **Death pase**

During the death phase, the mycelium eventually dies off. The death phase is usually accompanied by breakdown of the mycelia through self-digestion. Some fungi form spores by fragmentation of the hyphae.

Reproduction in Fungi

Fungi reproduce asexually as well as sexually. The asexual cycles are repeatedly made during the season. In the asexual reproduction certain types of spores are formed without involving the fusion of nuclei or sex cells. In the sexual process is usually made once a year and it is only induced by specific unfavourable conditions which should be the fusion between two types of sex cells (gametes) take place.

Asexuall reproduction

It is the most important reproduction type in fungi. They are repeatedly made during the season, as it results in the production of uncountable units. This asexual reproduction process may take in one of the following ways:

A-Vegetatively:

1- Bifission “Fission yeast”

It is a process occur in unicellular forms which splitting of the unicellular somatic cells to two equal cells

2- Budding “budding yeast”

In budding, an outgrowth (bud) is developed from mature cell; the bud either separate from the parent cell and hehaves as a new individual or remain attached to the mother cell to produce chains of buds.

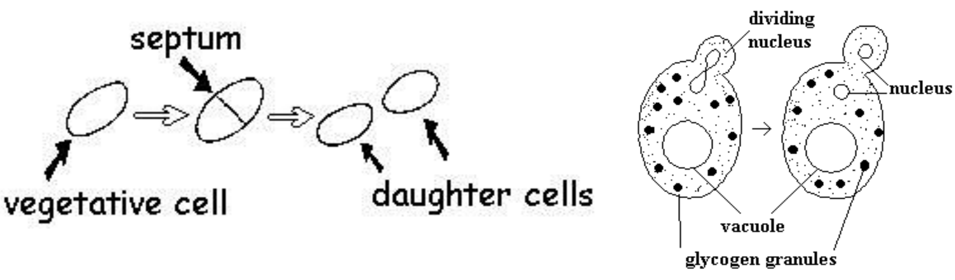


Figure (17): Bifission and budding in yeast

3- Fragmentation

In which the fungi are split into fragment, each fragment develops into mature, fully grown organism.

- **Arthrospres:** In which the hypha breaks up (at the favourable conditions) into separate thin walled units called arthrospores or Oidia (Greek= small egg).
- **Clamydosores:** Sometimes hyphal units (cell or compartment) are enlarges rounds up and develops a thickened, often pigmented wall, Contain dense cytoplasm and nutrient storage compounds. May be apical or intercalary (central) chlamydospores according their positions. Chlamydospres are formed during unfavourable or abnormal conditions

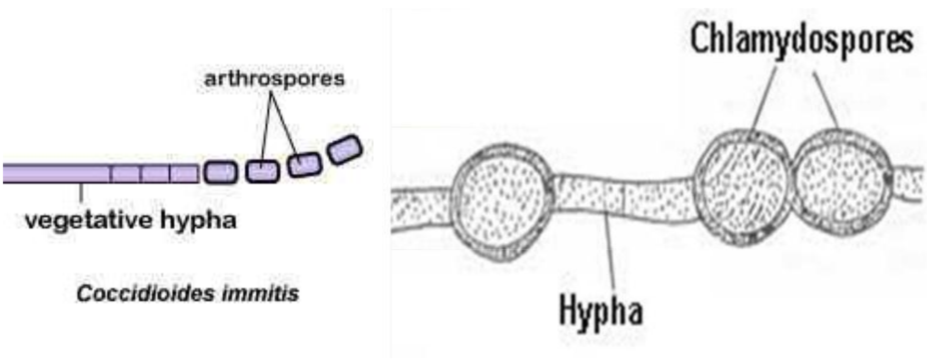


Figure (18): fragmentation and chlamydospore formation in filamentous fungi

B- Sporulation:

It is the most common methods in fungi, spores are minute units vary in color, size, and shape. On the basis of the variations in characters of spores is made, the most of systems of fungi classification. Some fungi are homospory (i.e. producing one type of spores), others are heterosporous (producing different types of spores) which may reach to four as in the case of *Puccinia.*

a- Endogenous spores

Are sporangiospores formed and contained within a sporangium and formed as a result of the cleavage of protoplasm around nuclei. Followed in some cases by formation of a wall around each nucleate portion of protoplasm. There are two types of endogenous spores: -

Zoospores (motile): Which formed in many lower fungi, especially aquatic form. Zoospores are uni or biflagellate which enclosed in zoosporangium.

- The protoplasm of zoospores is not surrounded by a wall, in some respects they resemble flagellate protozoa.
- Because zoospores are motile, the fungi that produce them will require water at some stage during their life cycle.
- Three different types of zoospore distinguish the Chytridiomycota, Hyphochytridiomycota and Oomycota.

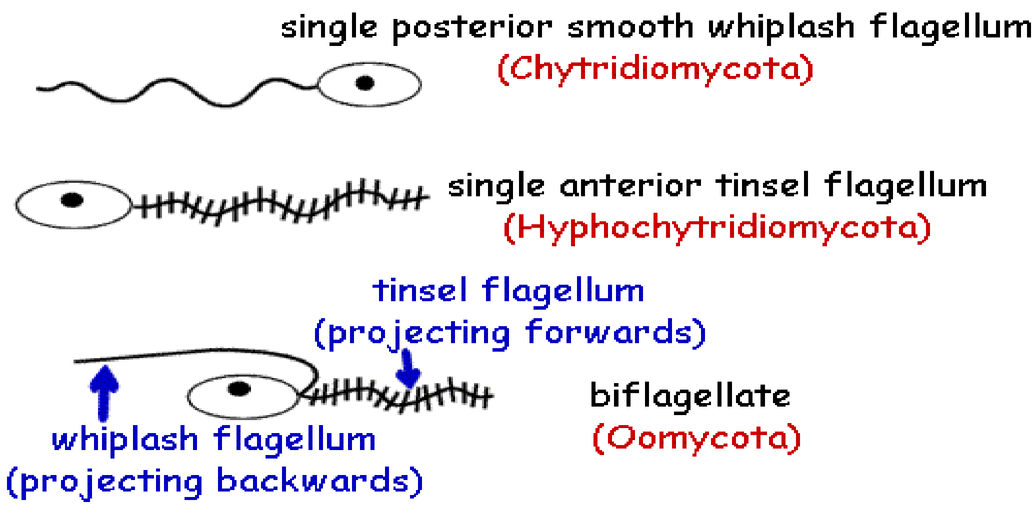


Figure (19): Different types of zoospore

Aplanospoes (non- motile or true sporangispores): Formed in sac like structure called sporangium. The protoplasm of sporangiospores is surrounded by cell wall.

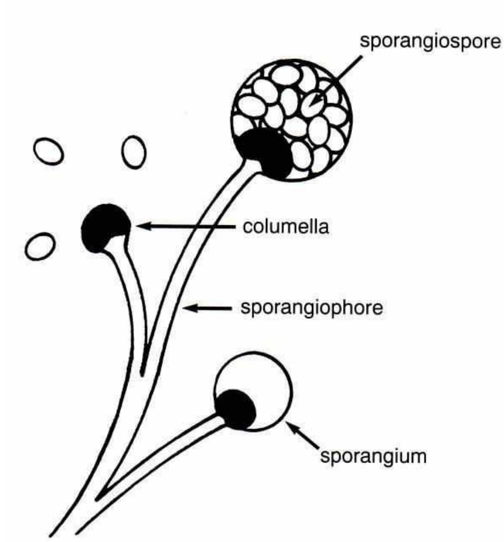


Figure (20): Aplanospores

b- Exogenous spores:

These spores called conidia (single = conidium), borne externally on specific locus of vegetative hyphae or specialized stalks or branch called conidiophores. Conidia are produce as solitary (single), in balls (clusters) or in chains either acrpetalae or basipetalae.

Conidia differ in shape (spherical, subspherical, ovate, pyriform, clavate, ……etc.), size, color (hyaline or pigmented), septation types (amero, didymo, phragmo, dictyospores).

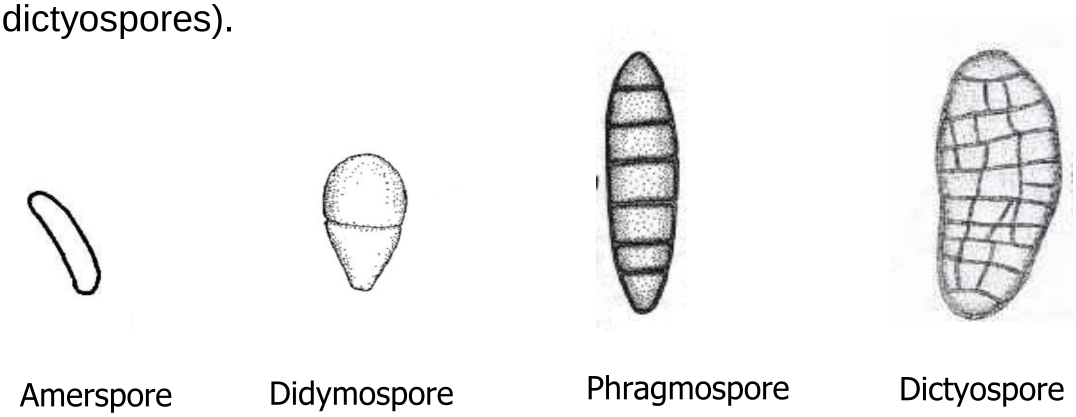
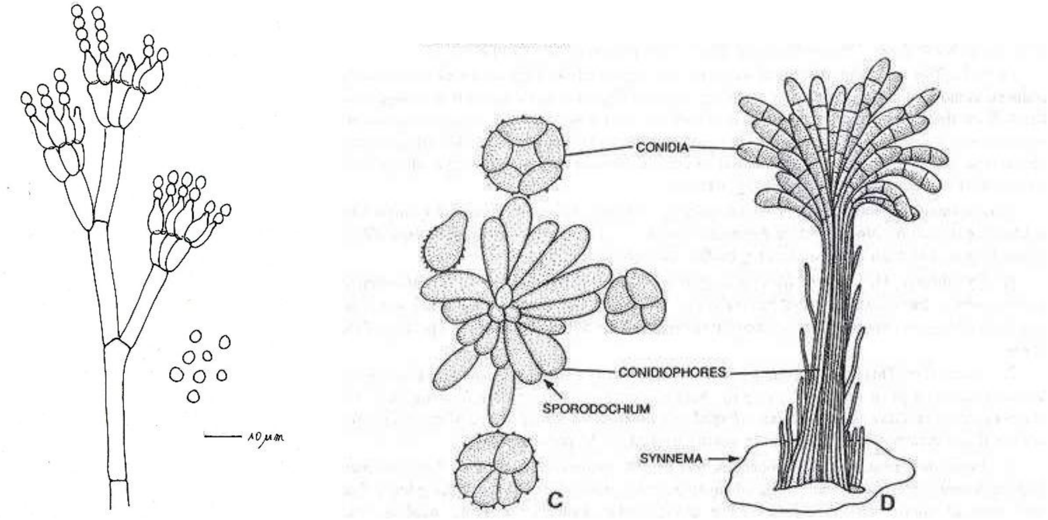


Figure (21): Different types of exogenous spores

Conidiophores

The conidia are produced directly on single conidiophores (the most common form) or as synnemata, or coremia special structures such long bundles of conidiophores or cushion-like sporodochium flattened bundles of conidiophores



Single conidiophore   Sporodochium   Synnenmata

Figure (22): Different shape of conidiophores

In some cases, conidiophores and conidia are developed within covered conidiomata (fruiting bodies). These fruiting bodies are consisting of pseudoparenchymatous structures and these mainly two types as follows:

Pycnidium: A flask-shaped structure with conidiophores developing from cells of the pycnidial wall. e.g. *Phoma* species.

Acervulus: In which, fungal hyphae aggregate and form a flat fertile layer of short conidiophores that produce many conidia. The pressure of accumulating conidia, and often of accessory of mucilage, eventually splits the host epidermis and allow the conidia to escape.

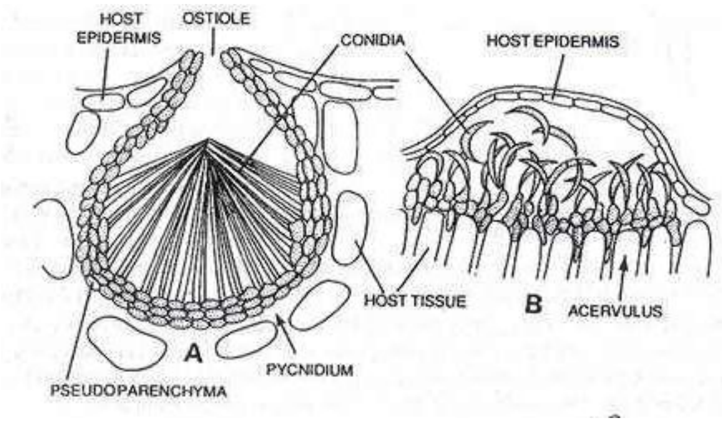


Figure (23): Pycnidium and Acervulus shape

Sexual Reproduction

The sexual process is usually made once/year and it is only induced by unfavourable conditions. Fungal sex organs known as gametangia

Stage of sexual reproduction

Usually sexual reproduction process should pass through three main different stages as follows:

1- Plasmogamy (Cytogamy)

It is union of two protoplasts of haploid nuclei (gametes) in one cell, takes place by means of the different methods. This phase may be very short as in case of yeasts or long time e.g. *Puccinia gramminis*.

2- Karyogamy

In which the two nuclei of gametes are fused together producing zygote (2n). This phase does not always take place immediately after plasmogamy but may be delayed.

3- Meiosis

In this stage, a reduction in the number of chromosomes of zygote (2n) take place, forming at the end haploid (n) sexual spores.

Types of sexual reproduction

The gametangia may be morphologically distinguished and these are heterogametangia. Sometimes the gametangia are in distinguishable and are, thus, known as isogametangia.

1- Iso or homogametangia

In which union or fusion take place between indistinguishable gametangia and gametes are called isogametes

2- Aniso or heterogametangia

In which union take place between one large (female) and one tiny (male) gametes. The gametes are morphologically distinguished.

3- Oogamy & Ascogamy

In some cases, these hetergametes are developing with sex organs. In oomycetes, the male gametangium called anthredium and the female gametangium oogonium which contains one ovum or more.

In case of Ascomycetes, the antheridium (male) and the ascogonium (female) have numerous male and female nuclei.

Chapter (II)

Classification of fungi

- The word classification may be defined as the scientific categoration of the organisms in a series group. Despite the existence of many varieties, biological strains and physiological or cultural races, the species is generally considered as the smallest group.
- More similar species are grouped together into a genus; similar genera are grouped into a family, families into order, orders into a class, similar classes into a division, and divisions into a kingdom. The latter is supposed to be the highest taxonomic rank.
- Generally, it is believed that there exist only two kingdoms i.e. plant and animal kingdoms, and all living organisms belong to one of the two great kingdoms.
- Barkley (1968) suggested a four-kingdom system. Whittaker (1969) suggested a five-kingdom system and raised fungi to the status of separate kingdom.
- The five-kingdom system based on the level of organization and mode nutrition of the living things provides fungi a status of an independent kingdom.
- Fungi are classified based on the characters of the sexual spores e.g.: oospores (Oomycota), zygospores

(Zygomycota), ascospores (Ascomycota), basidiospores (Basidiomycota).

- Deuteromycetes (fungi imperfecti) are mainly characterized by absence of sexual spores in their life cycles.
- Botanical nomenclature recommended the use of the following suffixes for the divisions of fungi:

Kingdom...mycetae
Divisions..mycota
Sub-divisions...mycotina
Calsses..mycetes
Subclasses..mycetidae
Orders ....ales
Family...aceae

- Each fungus, according to Linnaeus binomial system has two names a generic and species names. The generic name is always capitalized while the species name is not.

Classification systems

The kingdom of fungi is mainly divided into two subkingdoms:

A- Subkingdom: Gymnomycetae

In which the somatic phase is naked thallus (plasmodium) where as the reproductive cells are mainly with outer cell wall which includes the following common divisions:

Division: Myxomycota

Class: Myxomycetes (True slime moulds)

B- Subkingdom: Eumycetae

Division (1): Mastigomycota (motile form)

Subdivision (a): Haplomastigomycotina (uniflagellated forms)

Calss: Chytridiomycetes

Subdivision (b): Diplomastigomycotina (biflagellated forms)

Calss: Oomycetes

Division (2): Amastigomycota (immotile form)

Subdivision (a): Zygomycotina

Calss: Zygomycetes

Subdivision (b): Ascomycotina

Subdivision (c): Basidiomycotina

Subdivision (d): Deutermycotina

Division: Myxomycota

Division Myxomycota comprises the plasmodial slime molds. The slime molds are unicellular, colonial, and multicellular at different stages of their life cycles. They thrive in moist environments with bacteria, usually on decaying organic matter.

The plasmodium eats by extending pseudopodia, and, though large and branching, it is a continuous mass of cytoplasm with many nuclei. The thin tubes of the plasmodium optimize surface area to increase uptake of nutrients, oxygen, and water, and these are distributed by pulsing and sending streams of cytoplasm throughout the organism.

When food becomes scarce, the plasmodium differentiates into numerous reproductive structures, from which amoeboid or flagellated cells will emerge when the food supply decrease.

Myxomycota producing haploid spores which contain cell wall composed from cellulose.

Life cycle

Life cycle of slime moulds chracterized by alteration of generation in which appear haploid phase and diploid phase.

Life cycle of slime mold starts with the germination of a meiospore under favourable condition giving swarm cells and myxamoebae under wet condition. Fusion between two swarm cells or myxamoebae followed by karyogamy results in formation of zygote which is uninucleate, diploid and amoeboid in form. Then zygote germinate in size with successive divisions of the diploid parent nucleus forming multinucleate amoeboid mass of protoplasm called the young plasmodium. Favourable temperature, abundant moisture and food, these factors favor its growth, movement and reproduction.

Normally the plasmodium after attaining a certain size and stage of maturity enters the reproductive phase in which, the slime layer dries and the diploid proplast concentrates at a few points forming a mound like structure. The later grows into a stalked sporangium. The diploid protoplasm of the sporangium cleaves into numerous young spores each of which has a diploid nucleus. The diploid nuclei of the young spores undergo meioses to form meiospores (haploid nucleus). When mature meiospores are released, dispersed by wind and falling on sutitable substatum the meiospores germinates to release swarm cells or myxamoebae which fuse in pairs to form zygote.

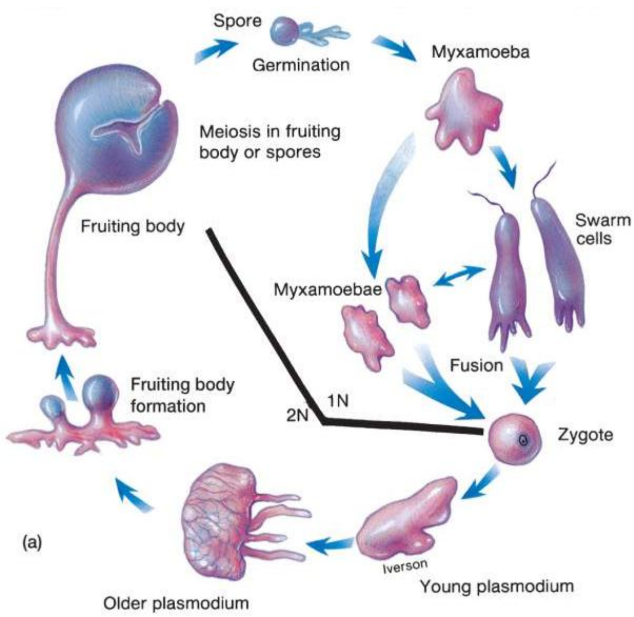


Figure (24): Life cycle of slime moulds

Division: Mastigomycota

This group of fungi consider primitive true fungi and characterized by formation of zoospores during life cycles and divided into two subgroups on the bases the number of zoospores flagella.

Haplomastigomycotina

The chief characteristic feature of this class is the production of uniflagellate reproductive cells (Zoospores). The single flagellum is of a whiplash type.

Allomyces

Division: Mastigomycota

Subdivision: Haplomastigomycotina

Class: Chytridiomycetes

Order: Blastocladiales

Family: Blastocladiaceae

Genus: *Allomyces* sp.

The genus of *Allomyces* was frist discovered in India at 1911. Subsequently it was found to be worldwide in tropical or warm country. It is saprophyte fungus found in soil, plant remains and decaying animals. The genus comprises 5 species.

Thallus structure: It is filamentous and attached to the substratum by delicate but well formed, branched rhizoidal hyphae constituting the rhizoidal system. From the latter arises a single slender hypha forming the lower trunk-like portion which subsequently undergoes several dichotomous branching to form dichotomously branched part of the mycelium on which the reproductive organs are borne terminally. The hyphae are thus multinucleate and coenocytic.

The vegetative thalli in *Allomyces* are two types, gametophyte (haploid) and sporophyte (diploid). In the vegetative phase the two are indistinguishable.

Towards maturity: -

1- The gametophyte bear more or less globose gametangia which are orange male gametangium alternating with colourless female gametangium. and sporothallus produce sporangia.
2- The sporophyte produce two types of sporangia:
   a. Colourless thin walled sporangia (Mitosporangium)
   b. Thick walled reddish Brown resting or resistance sporangia (Meiosporangium)

Life cycle

Allomyces in its life cycle exhibits distinct alteration of generations. One of these is the haploid (gametophyte) and other diploid (sporophyte).

At maturity the sporophyte produce two types of sporangia, thin walled mitosporangia and thick walled, pitted resting or resistant meisporangia. The mitosporangia produce diploid zoospores (mitozoospores) which after liberation grow to give diploide sporophyte (secondary sporophyte).

The meisporangium prior germination undergoes meiosis division forming meiozoospores which on germination produce an alternate plant in life cycle called gametophyte.

The gametophyte at maturity bearing orange male and colourless female gametangia which produce haploid male and female gametes. Afte liberation, fusion take place between male and female gamete forming zygote (2N) which germinate to form new sporophyte (2N).

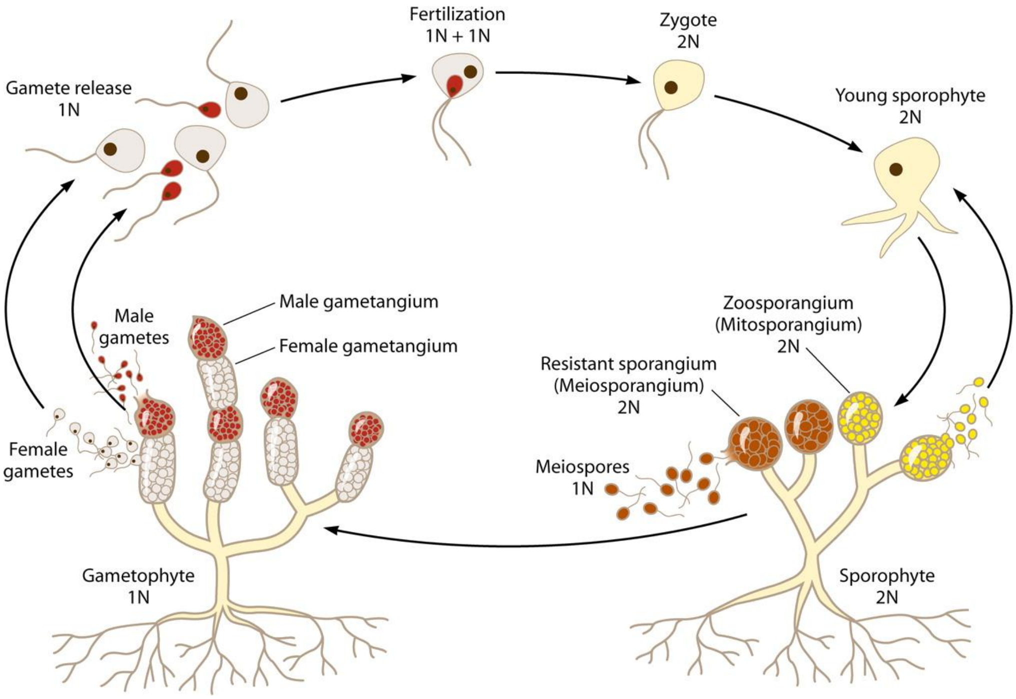


Figure (25): Life cycle of *Allomyces*

Diplomastigomycotina

The members of this class are chracterized by oogamous sexual reproduction by gametangial contact and these gametes are non-flagellated. The zoospores are biflagellated and may be pyriform or reniform in shape, one of the flagellum is of tinsel type and the other of whiplash type, the former directed forward and the the latter backward. The zoospores lack cell walls. The mycellium is extensive and coenocytic (lack septa) but the reproductive organs are separated by a basal septum. The hyphal Wall consists of β glucans and cellulose not chitin.

Phytophthora

Division: Mastigomycota

Subdivision: Diplomastigomycotina

Class: Oomycetes

Order: Peronosporales

Family: Pythiaceae

Genus: *Phytophthora* sp.

An terrestrial fungi, there are about 75 species of *phytophthora* some of them live as saprophytes but the majourity live on flowing plants attacking the aerial parts and causing leaf blight, canker and fruit rot diseases. One of the species *Phytophthora infestans* is great economic importance causes a serious potato disease known as potato blight or late blight of potato resulting in rot of tubers.

Asexual reproduction

The fungus survive in potato tubers in form dormant mycellium and when such tubers are used as seeds they grow intercellulary in host tissues and form haustoria to derive nutrition from adjacent cells.

Sporangiphores emerge from the lower surface of leaves through stomata bearing terminal, lemon shaped sporangia with distinct papilla.

1- Under low temperature (18-20 ºC) and high relative humidity (91-100%) sporangium content split forming zoospores, liberating zoospres swim in water for some times then come to rest. Each zoospore retracts its flagella and may encyst. The encysted zoospores then germinates forming short hypha called germ tube which grow to new fungus

2- Under high temperatura and low humidity: sporangium germinate (as conidia) directly to germ tube which produce new fungus.

Sexual reproduction

In *phytophthora* sexual reproduction is oogamous take place by forming antheridium and oogonium. Antheridium and oogonium arises as short lateral hypha from mycelium. The antheridium and oogonium of opposite mating types (A_1 and A_2) grow and curve towards each other. Eventually the tip of oogonium of one strain comes in contact with the young antheridium of the opposite mating strain, punctures and grows through it to emerge on the other side where it swells into globose structure. The antheridium now forms a collar-like structure surrounding the base of the oogonium. The oogonial wall swell at one point into the antheridium. This point is called the receptive spot. Then dissolves at this point through the resulting opening the antheridium pushes the fertilization tube, then the male nucleus transfere to fuse with female egg. Fertilized egg secretes a heavy wall around it and become oospores. Oospores nucleus divides repeatedly forming germ tube which grow either directly to new fungus or forming short hypha bearing sporangium contain large numbers of zoospores.

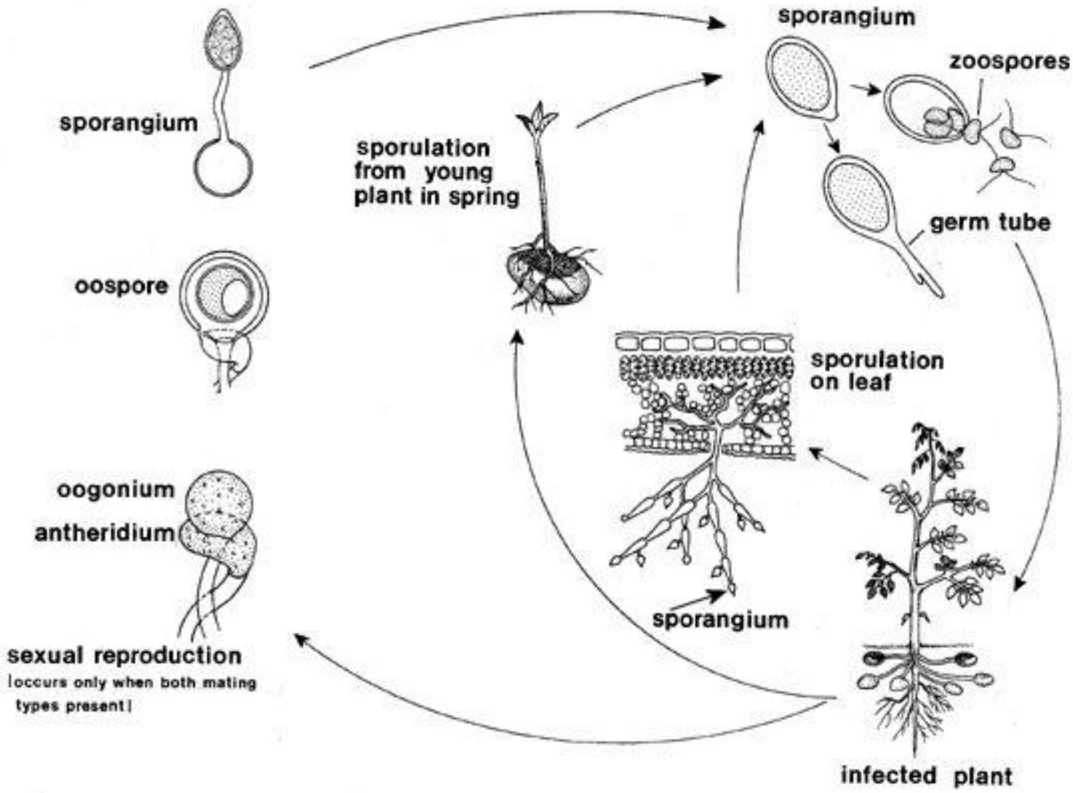


Figure (26): Life cycle of *phytophthora*

Division: Amastigomycota

Subdivision: Zygomycotina

The class of zygomycetes derives its name from thick-walled resting spores (zygospores) formed as result of complete fusion of two gametangia. It is comprises 450 species which are grouped under 70 genera. They all are terrestrial molds which show a wide range in thier habitate.

General features of zygomyctes

1- The hyphal walls are composed of chitosan.
2- The hyphae are coenocyte not contain cross wall (septa)
3- The motile cells are completely absent in the life cycle.
4- Asexual reproduction typically take place by means of non-motile sporangiospores commonly produced in large numbers within sporangia. Sometimes the entire sporangium functions as a single spore in the same manner as the conidium.
5- Chlamydospore formation is of frequent occurrence.
6- Sexual reproduction involves gametangial copulation.
7- The thick-walled sexually produced zygospore formed by the complete fusion of two gametangia is the resting structure.
8- The zygospore germinates to produce a hypha (promycelium) which bear a terminal sporangium.

Classification of zygomyctes. Traditionally the class comprises 3 orders: -

1- **Mucorales:** Chiefly saprophytes, some weak parasites on plants, a few edoparasites of vertebrates. Mycelium extensive, asexual reproduction by sporangiospores or rarely by conidia; zygospore wall may be formed by modification of gametangial walls.
2- **Entomophthorales:** Typically, parasitic on animals, rarely saprophytes, mycelium limited. Asexuall reproduction by sporangia turned to conidia or true conidia; gametangial wall not transformed to zygospore wall.
3- **Zoopagales**: Typically, parasite on animals, rarely saprophytes, mycelium present with complicated haustoria, Asexual reproduction by conidia; gametangial wall not transformed to zygospore wall.

***Rhizopus* sp.**

Division: Amastigomycota

Subdivision: Zygomycotina

Class: Zygomycetes

Order: Mucorales

Family: Mucoraceae

Genus: *Rhizopus* sp.

Occurrence: It is the commonest and the best known fungus found growing frequently on stale bread and thus is commonly called bread mold. It exclusively lives as saprophyte. Besides it occurs on damp decaying fruits, vegetables and other food material such as pickles, jams and jellies.

It consists of numerous, slender, freely branched filaments called hyphae. The hyphae are tangled and form a fluffy, White mass which makes up the thallus of the fungus. It is called the mycellium. It is cottony White during the vegetative phase, how ever, it son enters the reproductive phase, numerous black, pin head-like structures develop on the mycelium giving the mass a blackish appearance.

The thallus consists of three kinds of hyphae: -

1- Stolons. These haphae that grow horizontally over the surface of substratum. The stolons grow rapidly in all directions and cause further spread of the mycelium over the substratum.

2- Holdfast (Rhizoids). It is cluster of brown, slender, branched rooting hyphae which arise from the lower surface of the apparent node of each stolon and penétrate the starchy substanc of the substratum. The rooting or rhizoidal hyphae has two functions: they anchor the fungus to substratum and absorb wáter and nutrients from it for the entire thallus.

3- Sporangiophores. Just opposite each holdfast one or more vertically growing hyphae rise into the air. They are reproductive in function and bear sporangia singly and terminally. These negatively geotrophic, unbranched special hyphae are called the sporangiophores.

Asexal reproduction

During thr growing season fungus reproduces repeatedly by the formation of small, non motile spores (sporangispores). They reproduced in large numbers within round black structure known as sporangium. Sporangium produced by swelling of end of the sporangiospores forming knob-like vesicle. The vesicle grow in size due to flow into it the cytoplasm and nuclei then undergo to cleavage to form segment which round and secrete walls around them to become the sporangispores. After spores maturation columella strart to rise pushing spores to ward sporangium wall leading to sporangiospoes libetaion. Liberating spores under unsuitable condition remain viable for long periods when become suitable the germinate forming new fungus.

Sexal reproduction

Sexual reproduction take place by gametangial copulation. Under unsuitable condition each to hypha from different strains arranged and start to form zygophores. The zygophores begin to swell due to the follow of cytoplasm and nuclei into them. The swollen tips known as progametangia then trasverse seta (gametangial septum) appear in each progametangium splitting it into suspensor and small terminal cell called gametangium. When gametangia are mature the septum between them dissolves then fusion of cytoplasm take place followed by nuclei fusion forming zygote (2N) which surounded by thick wall and become dark in color forming zygospores.

Zygospores split from the suspensor and then enters to a resting period, after this period, zygospores undergo to meiosis division then germinate producing germ tube which form sporangiophore bearing sporangium containing sporangiospores. After spores maturation, spores liberate from sporangium and germinate to form new fungus.

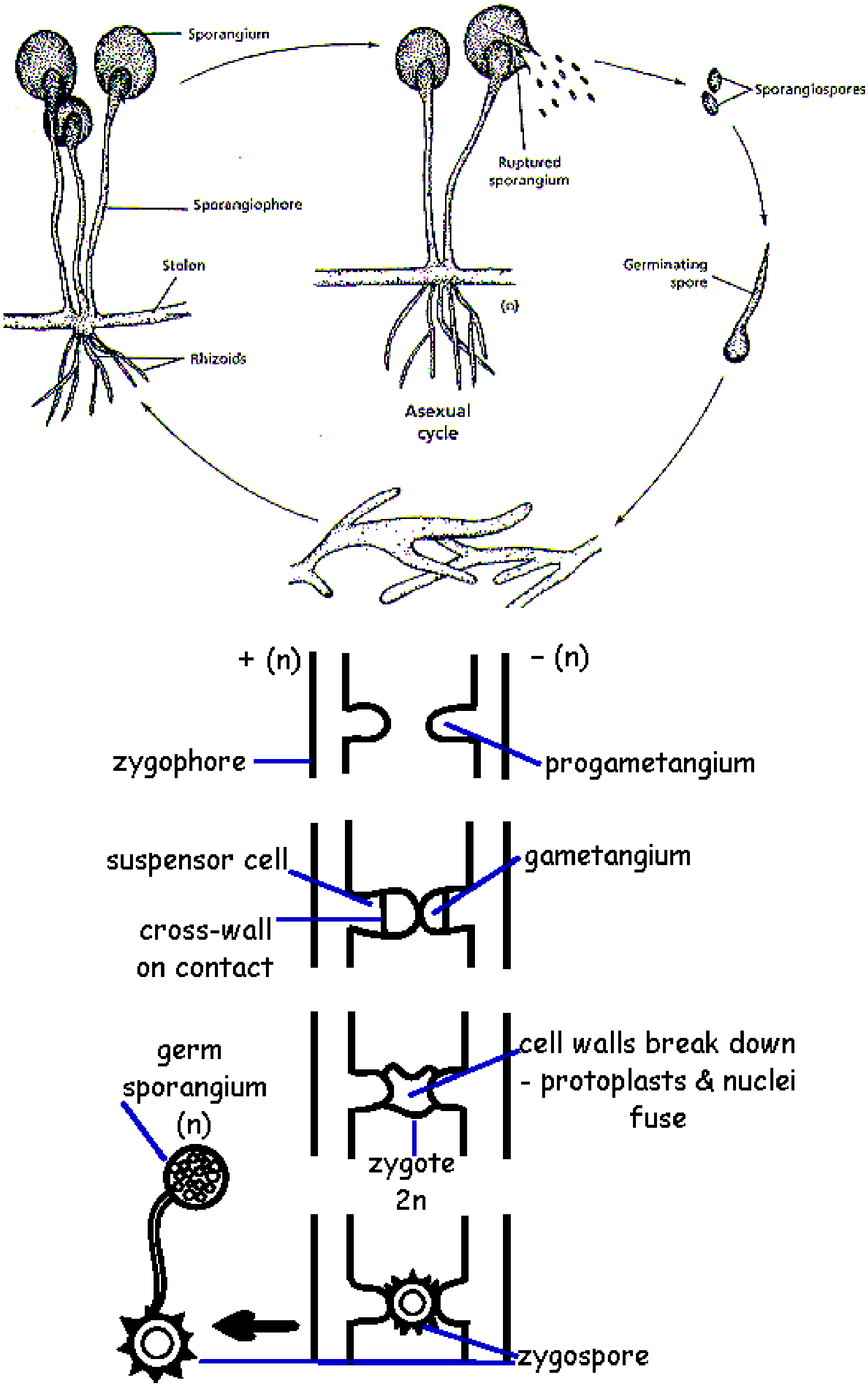


Figure (27): Asexual and sexual Life cycle of *Rhizopus*

Subdivision: Ascomycotina

It is a large group comprising fungi more complex in structure and mainly landforms which vary in form and grow in varied habitats.

General features: -

1- The somatic phase consists of a well-developed branched mycelium with regular septation of the hyphae. The cross wall has each a minute, central, simple pore.
2- There is complete absence of motile cells in life cycle.
3- Asexual reproduction take place by formation non-motile, exogenously produced spores called conidia. They are reproducing terminally on special, reproductive hyphae called conidiophores.
4- Sexual reproduction take place by formation ascospores which formed in special thin-walled sac like structure called ascus.
5- Asci in most species are grouped to form definite complex fruit bodies called ascocarps.

Development of Asci

Formation of ascus started by: -

1- Producing of ascogonium (Female organ) and antheridium (male organ).

2- With migration of male protoplast (cytoplasm + nuclei) each nucleus in the ascogonium becomes paired with one from the antheridium.
3- From dikaryons ascogonium arise papilla-like outgrowths forming ascogenous hyphae.
4- The apical compartment of a dakaryotic ascogenous hypha elongates and bends over to form a hook (Crozier).
5- The two compatible nuclei in the apical compartment then undergo mitosis simultaneously.
6- Two septa develop in such a way that the crozier becomes divided into three compartments, the tip and basal compartments are uninucleate; the middle compartment is binucleate and is called the ascus mother cell (since it is destined to become an ascus).
7- The nuclei in the ascus mother cell fuse to form a diploid nucleus, which then undergoes meiosis to form four haplid nuclei.
8- Each haploid nucleus then divides mitotically, resulting in eight haploid nuclei.
9- A portion of protoplasm surrounds each nucleus - this becomes enveloped by a wall and matures into an ascospore.

10- Meanwhile, another ascus mother cell will have been developing alongside the first.

11- In most (not all) fungi belonging to the ascomycota the asci don't occur singly; they form in groups surrounded by hyphae and are enclosed in fruiting bodies (ascocarps).

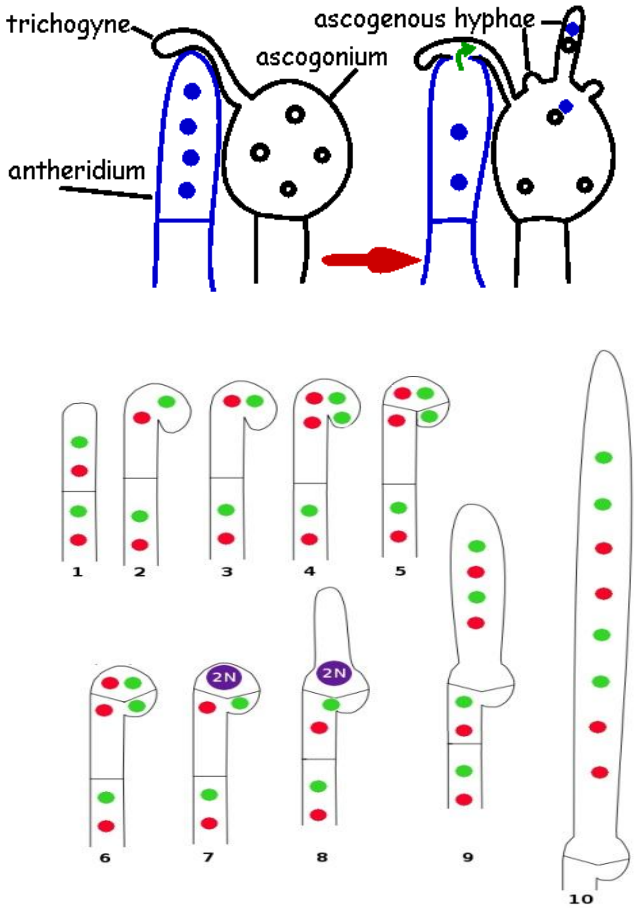


Figure (28): Development of asci

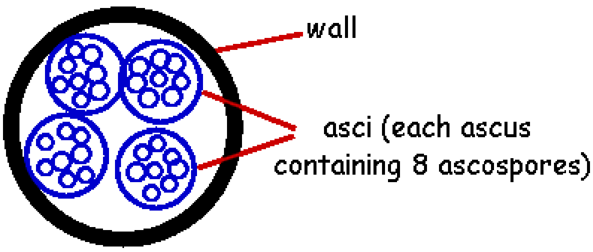
Use of ascocarps in the classification of fungi belonging to the Ascomycota:

1- Hemiascomycetes (includes yeasts)

- In this case the asci are not enclosed in an ascocarp.
- In which, the diploid cell (zygote) in yeast is transformed directly into an ascus containing four or eight ascospores.

2- Plectomycetes

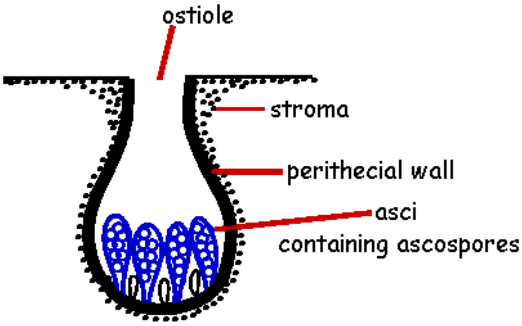
Fungi belonging to this group form **cleistothecia**. These are round, completely closed ascocarps, possessing no natural opening. The asci are arranged irregularly within them. When mature the cleistothecia burst open to release their asci and ascospores.



3- Pyrenomycetes

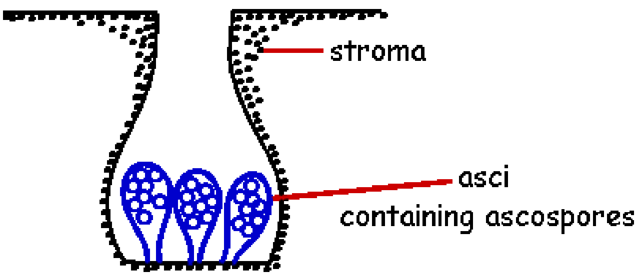
Fungi belonging to this group form **perithecia**. Perithecia are spherical or flask-shaped ascocarps. They open via a neck-like ostiole with a terminal pore through which the ascospores are liberated. The asci are arranged in an orderly layer at the base of the cavity.

Warning: do not confuse perithecia with pycnidia which contain conidia.



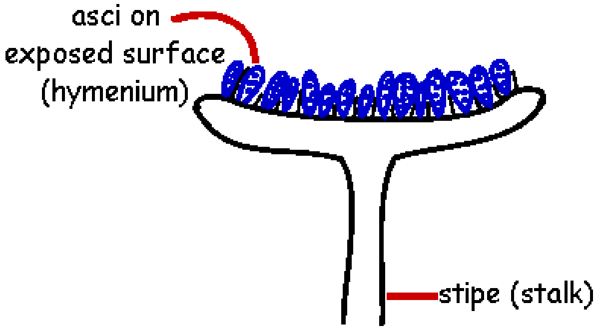
4- Loculoascomycetes

Fungi belonging to this group form **ascostromata** (or pseudothecia). Ascostromata resemble perithecia but in the former there is no wall surrounding the central region of the ascocarp, only a cavity within the mass of hyphal tissue (stroma) in which the asci are located.



5- Discomycetes

Fungi belonging to this group form **apothecia**. An apothecium is an open or cup-shaped ascocarp. The asci are arranged on the exposed surface (hymenium).



Yeasts

Division: Amastigomycota

Subdivision: Ascomycotina

Class: Ascomycetes

Subclass: Hemiascomycetidae

Order: Endomycetales

Family: Saccharomycetaceae

Genus: *Saccharomyces*

Mostly the yeasts are saprophytes. They are widespread in their distribution and like bacteria are found everywhere on the face of the earth usually in substrates containing sugar. The most common species is *Saccharomyces cerevisiae* which used in bread making and beer brewing.

Unlike other sac fungi the thallus is non-mycelial, it is not made up of hypha. It consists of single minute oval or spherical cell, has cell wall made up of two polysaccharides, namely glucans and mannan in combination with traces of protein, lipid and chitin, there is no cellulose.

Reproduction

Yeast reproduces both asexually as well as sexually.

Asexual Reproduction

Budding: Most of the yeasts multiply asexually by vegetative method called budding, under favourable conditions *Saccharomyces* reproduce by this method. At the time of budding, a small portion of the cell wall at or near one pole of the yeast cell softens and thin. The protoplast in this region, covered by the thin softened membrane, bulges out in the form of an outgrowth (protuberance). Outgrowth gradually increases in size and known as the bud. As the bud is forming, the nucleus of parent yeast cell divides forming two nuclei. The daughter nuclei migrate into enlarging bud. The bud grows, and the cytoplasm of the bud and mother cell remains continuous for sometimes. Eventually the opening between the two cells closes, then the bud separates from mother cell leaving a scar on both cells. In the presence of abundant food supply, the process of budding is quickened. It becomes so rapid that the buds often produce buds before separation from the mother cell. The process is repeated. In this way many buds are formed without being detached from one another. This result in the formation of branched or unbranched chains of cells constituting the pseudomycelium.

Fission: Vegetative reproduction by fission has also been reported but it occurs in some other yeast and not in *Saccharomyces*. They are called fission yeasts for this reason. The division is transverse. The mother cell elongates, and the nucleus divides into two. Meanwhile a ring-like ingrowth appears at the wall of the yeast cell in the middle. It grows in ward toward the centre of the cell. Finally, it stretches across the cell forming a complete partition called septum. The septum thickens and then splits into two layers, one for each daughter cell.

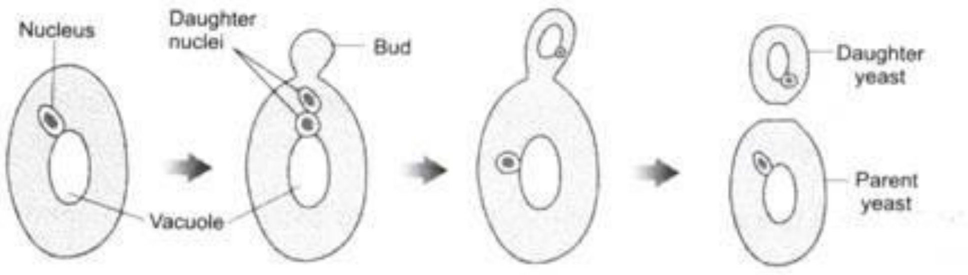


Figure (29): Budding in Yeast

Sexual reproduction

Sexual reproduction started by two haploid cells of the opposite mating types bend towards each other and fuse to form conjugation bridge. Fusion between the protoplast of the (+) and (–) strains takes place through the conjugation bridge called plasmogamy. Then karyogamy occur by fusion of two nuclei forming diploid nucleus (2N) called zygote. The zygote become spherical in shape and directly functions as ascus mother cell. Then the diploid nucleus of ascus mother cell undergoes meiosis forming four haploid nuclei which surrounded by cytoplasm to produce ascospores. Ascospores liberate from the ascus mother cell and germinate forming new yeast cell.

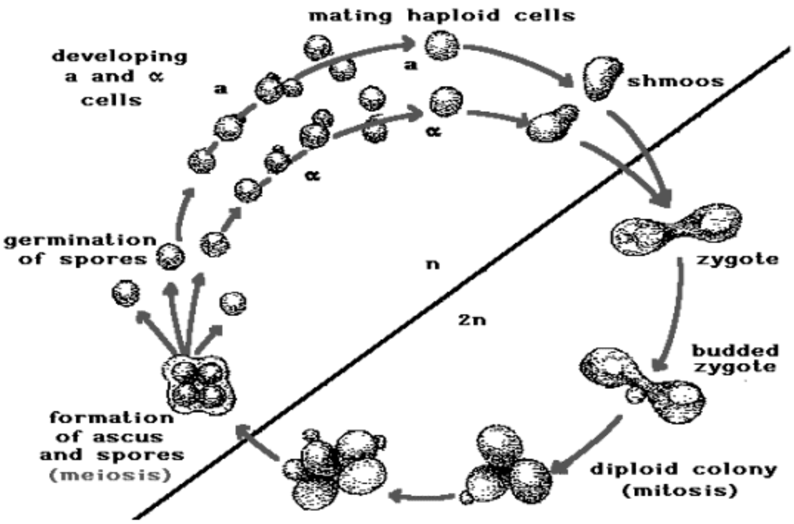


Figure (30): Life cycle of *Saccharomyces*

Genus *Aspergillus*

Division: Amastigmycota

Subdivision: Ascomycotina

Class: Ascomycetes

Subclass: Plectomycetidae

Order: Eurotiales (Aspergillales)

Family: Eurotiaceae (Aspergillaceae)

Genus: *Aspergillus*

- The genus is widely distributed fungus from the very cold region of arctic to the tropics. The name is derived from aspergillum, which means a special bruch resemblance to the conidial apparatus of *Aspergillus*.
- *Aspergillus* colonies are usually fast growing, some shades of green, blue, ... or other colour than green e.g. white, yellow, brown, to black and they mostly consist of erect conidiophores.
- *Aspergillus* produce antibiotics, mycotoxins and cause a lot of food spoilage.
- Spores of *Aspergillus* are carried by air, everywhere, and that is why of spoilage of organic materials.
- The successful colonization of numerous types of media, made by *Aspergillus* indicates that they are provided with an active enzyme system.

Asexual reproduction

- It takes place through the production of conidia. Conidiophore arises as aerial unbranched hypha. The cell from which the conidiophores emerge is known as foot cell.
- Conidiophores are produced singly and are non-septate, un-branched and each end with a head known as vesicle.
- Vesicle covered with either a single phialides (strigmata) or a layer of metulae which bear small phialides (uniserriate or biserriate). The vesicle, phialides, metulae (if present) and conidia form conidial head.
- Conidia born from phialides, are one-celled, smooth, or rough-walled, hyaline or pigmented and are basocatenate, forming long chains may be divergent (radiate), or aggregated in compact columns (columnar).
- Condia split from phialides and spread away, when find suitable substrate, it germinates forming new fungus

Sexual reproduction

The sexual reproduction is rare. *Aspergillus* is **homothallic**, a male (Antheridium) and female (Ascogonium) sex organs are developed close together on the same hypha or on separate nearby hyphae of the same mycelium. Both are elongate, multinucleate and generally coil around each other.

Ascogonium: A small, loosely coiled septate hyphal branch arise from vegetative hypha. It is differentiated into three parts. The terminal segment is generally the longest and single celled it contains up to 20 nuclei called trichogyne. It is functions as the **receptive part of the female organ**. The segment below trichogyne function as the female gametangium called ascogonium. Below ascogonium is the stalk consisting of few cells.

Fusion takes place between the antheridium and trichogyne. The tip of the antheridium arches over the apex of the trichogyne and fuse with it. The contents of antheridium then pass through the opening into the trichogyne.

The haploid male and female nuclei in the ascogonium come to lie in pairs (dikaryons). After pairing of the nuclei, ascogenous hyphae arise which differentiated to form ascus mother cell which produce ascus contain 8 ascospores. Ascospores after liberation germinate at suitable condition producing new fungus.

The ascocarp in *Aspergillus* is a small, rounded, yellow with smooth walls. Even at maturity it remains closed called cleistothecium or cleistocarp.

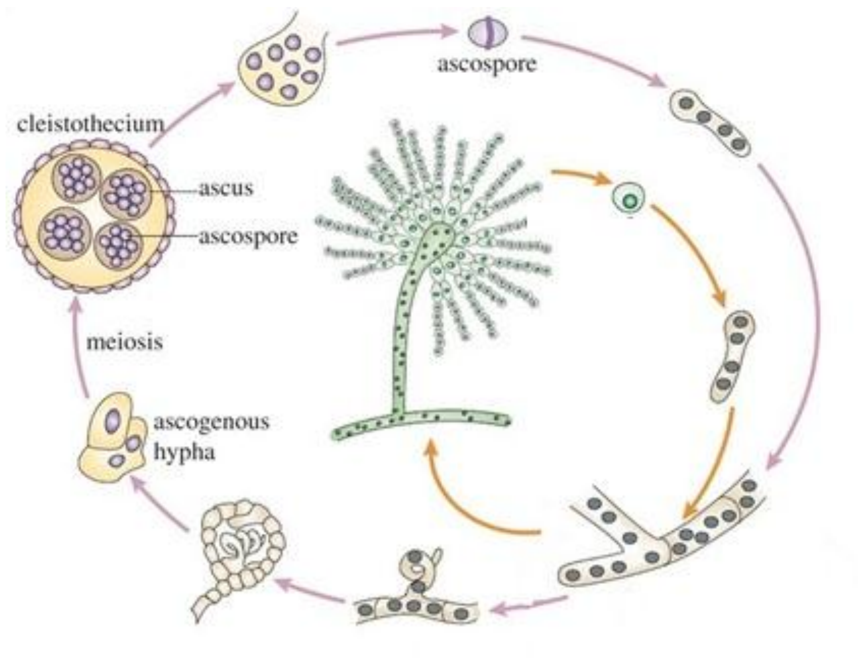


Figure (31): Life cycle of *Aspergillus*

Economic importance

1. *Aspergillus* causes rot of dates, decay of tobacco and cigars.
2. It spoils nuts, bread and other foodstuffs.
3. Some of *Aspergillus* produce mycotoxins such aflatoxin and ochratoxin.
4. They cause many diseases grouped under the name Aspergilloses also cause ear disease called otomycosis.
5. *Aspergillus* used for antibiotic production such as flavicin, aspergillin, geodin, patulin and ustin.

6. *Aspergillus gossypii* used for production of certain vitamins such as B 12.
7. Certain species used for production of many enzymes, lipids and several organic acids.

Subdivision: Basidiomycotina

It is the most advanced of all the fungal classes. It includes about 15.000 species with more than 500 genera. This big group of fungi includes both saprophytic and parasitic species. The saprophytic species live in moist, decaying wood, dung, wet dead leaves or humus of the soil.

As in ascomycetes motile structures are lacking in this class and the mycelium is septate. The basidiomycetes produce highly complicated fruiting bodies called the basidiocarps. The basidiocarps in the higher forms are visible portion of the fungus and they are concerned with production and bear reproductive structures called basidia

General Features of Basidiomycetes.

- The somatic phase consists of a well-developed, septate, filamentous mycelium which passes through two stages: -
  a) Primary mycelium. It is formed by germination of basidiospore and contain a single haploid (n) nucleus in each cell

b) Secondary or dikaryotic mycelium. It constitutes of the main food absorbing phase and consist of cells each containing two haploid nuclei (n+n). It is long lived and play prominent role in the life cycle also bearing basidia and basidiospores.

- Except in rusts and smuts the septal pores in basidiomycetes is complex. It is dolipore **parenthesome** type.
- The motile cells are absent in the life cycle.
- The clamp connections on the dikaryotic hyphae are of universal occurrence
- Asexual reproduction by spores plays a significant role in life cycle.
- The sex organs are lacking in the basidiomycetes
- Basidium is the reproductive organ of basidiomycetes in which both karyogamy and meiosis take place.
- Typically, the basidium bears four basidiospores.
- The basidiospores germinate to form primary mycelium.

Formation of basidiospores

Firstly, basidiospores germinate forming primary mycelium contain a single haploid (n) nucleus in each cell (monokaryon). Then take place interaction between two compatible primary mycelia forming secondary or dikaryon mycelium which consist of binucleate cells. During nuclear divisions of the dikaryotic cell special structure called the clamp connections are formed. These clamp connections ensure that the sister nuclei of the dikaryon at each division separate into daughter cells.

- Two haploid nuclei in an apical dikaryotic hyphal compartment (often within a basidiocarp) fuse to form a diploid nucleus.
- The diploid nucleus undergoes meiosis to yield four haploid nuclei.
- Four small outgrowths sterigmata begin to form at the top of the hyphal compartment and the tip of each sterigma begins to inflate.
- A fluid-filled vacuole develops near the base of the compartment and gradually enlarges as it enlarges it forces protoplasm into the inflated portions of the sterigmata.
- When each swelling at the tip of a sterigma has almost attained its full size, a nucleus passes into it.
- The uninucleate swelling at the the tip of each sterigma matures into a basidiospore.
- The compartment supporting the sterigmata and basidiospores is called a basidium.

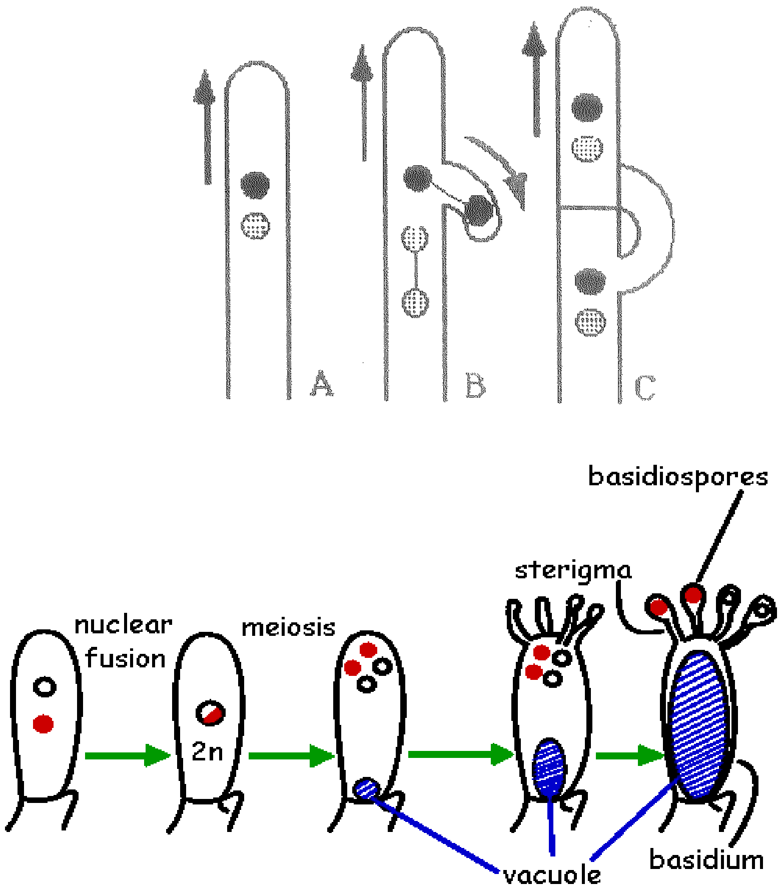


Figure (32): Formation of basidium

Classification of basidiomycetes

Classification of fungi belonging to the basidiomycota is based upon the presence or absence of fruiting bodies (basidiocarps) and the type of basidiocarp formed. Basidiocarps are amongst the most familiar of fungal structures, including toadstools, brackets and puff-balls. But the basidiomycota also contains many species that produce microscopic sporulating structures, i.e. micro-fungi.

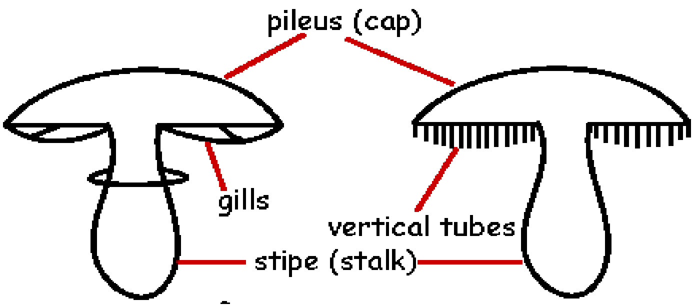
Classification of fungi belonging to the Basidiomycota:

1- Teliomycetes

In this group the basidia are not grouped together side by side in or on a basidiocarp (fruiting body). The uredinales and ustilaginales are two important orders of plant-pathogenic fungi belonging to the teliomycetes.

2- **Hymenomycetes**

The largest class in the basidiomycota. The basidia are arranged in a layer known as a hymenium that is fully exposed at maturity. The hymenium may cover the surface of gills, line vertical downward-facing pores, or cover an erect club or system of vertical branches or teeth.



3- Gasteromycetes

Includes fungi known as Puff-balls, Earth-stars and Birds' nest fungi. The spore-producing hymenium is not exposed at maturity. But these fungi have evolved a variety of mechanisms to ensure efficient spore liberation.

In other word: they include those basidial fungi having basidiospores produced within closed basidiocarp which will not open except after complete maturation of spores. Basidiocarp is either permanently subterranean or above ground.

Agaricus

Division: Amastigomycota

Subdivision: Basidiomycotina

Class: Basidiomycetes

Subclass: Holobasidiomycetes

Series: Hymenomycetes

Order: Agaricales

Family: Agaricaceae

Genus: *Agaricus*

Agaricus is a saprophyte and grow in the open fields, grass land and in soil in cellulose and lignin materials. It is also found growing in the decaying litter on forest floors or in the humus deposited at the surface and below the surface of the ground. It grows best in moist and is commonly seen during the rainy season. Some species are used for eaten by human such as *Agaricus campestris*, *A. rodmani*, *A. silvaticus* and *A. placomyces*.

Asexual reproduction

This type of reproduction is very rarely of occurrence. Take place by formation of chlamydiospores which germinate to form new mycelium

Sexual reproduction

The mushroom basidiocarp is typically composed of a stipe that elevates the basidiocarp above the substrate, a pileus and in some species a partial veil that encloses and protects the lamella as the basidia and basidiospores are developing. Sexual organs are absent. After germination of basidiospore give monokaryon mycelim, then dikaryon formation begins with the fusion of hyphal cells between compatible monokayons. The monokaryon stage of the Basidiomycotina is short-lived and fusion with a compatible monokaryon occurs soon after basidiospore germination. The dikaryon is the mycelium that produces the basidiocarp. The terminal cells develop into basida and are where karyogamy will take place to form the zygotes. The zygote is the only diploid stage in the life cycle. The zygote immediately undergoes meiosis to form four haploid nuclei, and the future basidiospores are formed as blown out structures, on the tips of sterigmata, of the basidium. The nuclei migrate into the blown-out areas which may then be properly referred to as basidiospores.

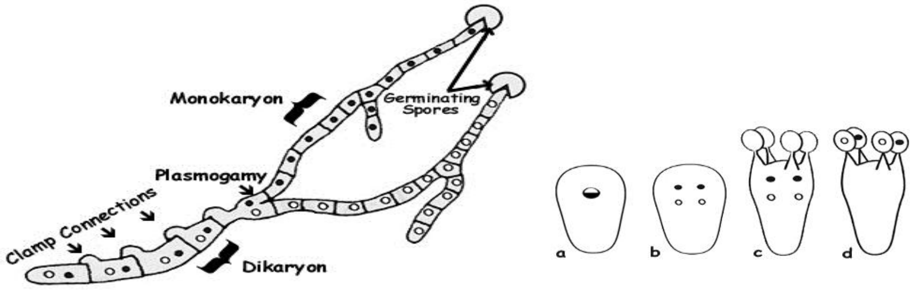


Figure (33): Life cycle of *Agaricus* sp.

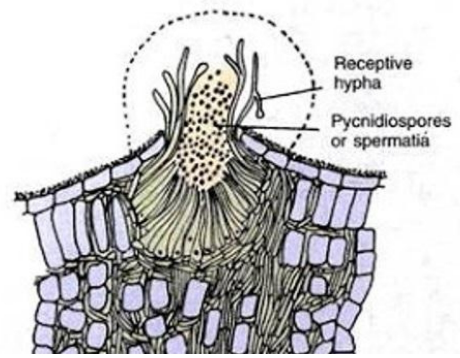
Puccinia graminis

It causes rust disease known as the black stem rust of wheat in all wheat growing areas of the world. It had been considered strictly obligate in its parasitism. Normally the fungal parasite passes a part of its life cycle constituting the dikaryophase on wheat and a part constituting the haplophase on barberry plant. The full life cycle is thus completed only when both hosts are present. The wheat plant called primary host and barberry is called secondary or alternate host.

Life cycle can summarize as following: -

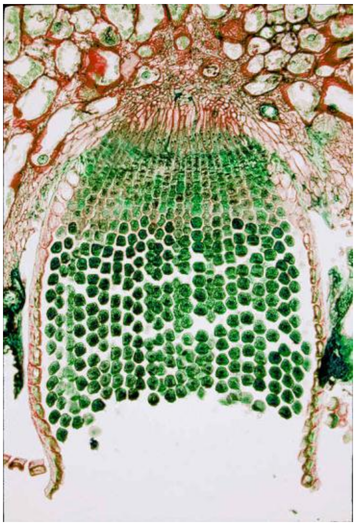
Spermogonium (Stage 0)

The spermogonium stage produces the sex organs in rusts. They are produced on the upper surface of the *Berberis* (barberry) leaf. Since the spermogonia are derived from basidiospores, they are of two mating types. They are flasked-shaped reproductive structure in which spore-like spermatia and specialized (receptive) hyphae are produced. Spermatia ooze out, from the neck, in a sweet-smelling nectar. The spermogonia are visited by flies which are attracted by the nectar secretions, and as they visit different spermagonia, spermatia of both mating types, adhere to their bodies and are transferred to receptive hyphae of the other mating types. This begins the dikaryon stage of the life cycle.



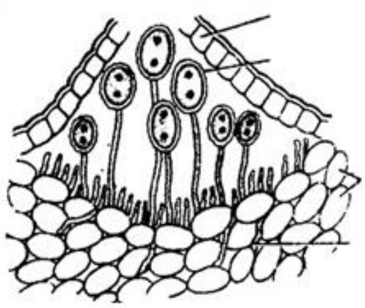
Aecium (Stage I)

The aecium stage is directly linked to the spermagonium stage. When spermatia are transfered to compatible receptive hyphae, this begins the dikaryotic stage of the life cycle and directly produces the aecium on the lower surface of the barberry leaf. The aecium is an upside-down, sac-shaped structure in which chains of aeciospores are formed. The aeciospores burst through the lower surface of the leaf and are dispersed by wind. Aeciospores are unicellular containing two nuclei.



Uredium (Stage II)

The aeciospores cannot reinfect the barberry host. Instead, infection can only occur on the primary host, *Triticum aestivum* (wheat), where a new dikaryotic infection occurs. When two hosts are required in the completion of a rust life cycle, the rust is said to be heteroecious. The wheat is said to be the primary host while barberry is said to be the alternate host. The dikaryons infect the wheat stems and leaves and will form uredia that contain orange-brown uredospores. This order is commonly called the rusts because of the orange-brown (rusty) colored pustules that form on the wheat plant after the uredospores have broken through the epidermal surface. The urediospores are comparable to conidia in that they will reinfect wheat plants and produce more uredia and uredospores. This stage begins during summer and continues until late summer in North America. Uredospores are unicellular containing two nuclei.



Telium (Stage III)

Towards the end of summer, the uredium begins to produce **teliospores**, a dark, thick-walled, two celled spores. Teliospores do not produce telia. It is the uredium that gradually becomes a telium by producing more and more teliospores. Because of the color of the teliospores, the telium is black. Following karyogamy, the teliospore overwinters. Meiosis takes place in each cell of the teliospore, in spring, and germinates to form the **promycelium** (=basidium). The promycelium becomes transversely septate, forming four cells. Each cell produces a sterigma and a basidiospore, and this now completes the life cycle.


Teliospores

Basidium (Stage IV)

Puccinia graminis is heterothallic and **basidia** produce **basidiospores** that are of two mating types, Basidiospores are capable of only infecting the leaves of *Berberis* sp*.* (barberry), the **alternative host** for this species**.** Species that require two hosts to complete their life cycles are said to be **heteroecious**. The cells of the **teliospore** germinates to produce a short germ tube that will develop into a basidium that is essentially transversely septate**.**

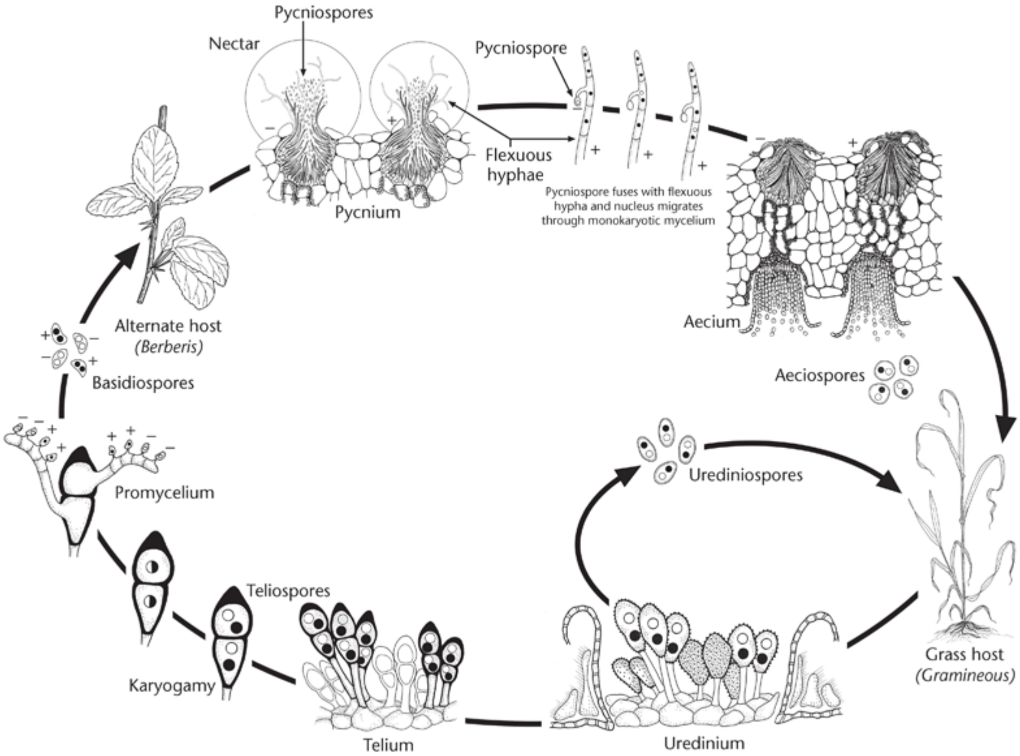


Figure (34): Life cycle of *Puccinia graminis*

Subdivision: Deuteromycotina (Fungi imperfecti)

This group of fungi is reproducing by asexual means usually by conidia, but lack perfect sexual stage. Deuteromyctes economically highly important, their number goes up to thousands. Many of them live as saprophytes and many more as parasites. The latter are the causative agents of diseases in plant and animals including man, some cause spoilage of stored products.

The somatic phase in the majority of these fungi consists only of the haploid mycelium, it is septate and profusely branched. Reproduction takes place chiefly by formation of conidia. Formation of oidia and chlamydospores has been also reported.

Conidia and conidial development

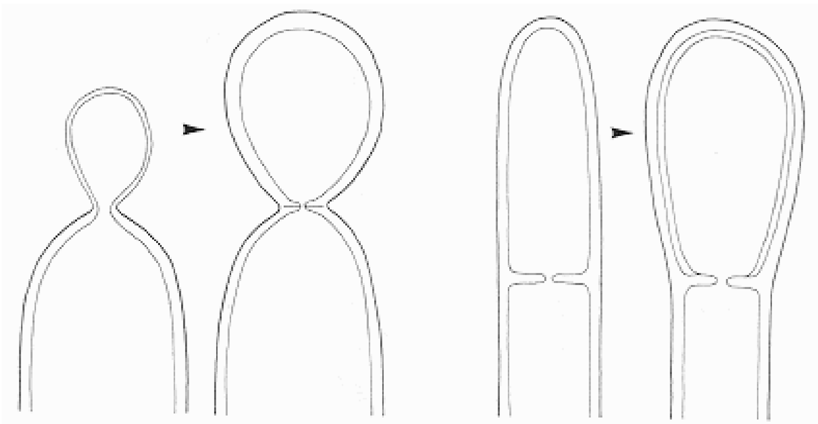
The conidia are exogenously produced spores. They are non-motile and are borne externally from growing hyphae called conidiophores. The apical region of conidiophore may produce a single conidium or a chain of conidia usually in basipetal succession.

Ellis (1971) has recognized two types of conidial developments

Blastic: in this type of conidial development marked enlargement of conidial initial take place before it is delimited by a septum. It may be of two types:

a. Holoblastic: both the inner and outer wall of conidiogenous cells takes part in the formation of conidia.
b. Enteroblastic: only the inner wall of conidiogenous cells takes part in the formation of conidia

Thallic: in this type of conidial development there is no enlargement of conidial initial. It takes place after the initial has been delimited by septum.



Blastic Thallic

Figure (35): Blastic and thallic conidial development

Genus: *Fusarium*

This genus includes many species and many forms within species. Many of these are saprophytic, some are facultative parasites and others are parasitic.

Mycelium: it is extensive. The hyphae are septate and branched. When young they may be colourless or with a tinge of pink, purple or yellow and become dark coloured at maturity.

Asexual reproduction

It takes place by the formation of three kinds of asexual spores, microconidia, macroconidia and chlamydospores. Sclerotia are also formed.

Micoconidia: they are very small conidia produced from the tips of simple or branched conidiophores. The conidiophores are distinguishable from the vegetative hyphae. The microconidia vary in shape from the rounded to oval. They are often held in small masses.

Macroconidia: they are large and multicellular. In form they are elongated, sickle-shaped or crescent-shaped. They are produced at the tips of simple or branched conidiophores which are assembled to form sporodochium.

Chlamydospores: they are rounded, oval, thick-walled cell formed in hyphae. They may be formed singly or in chains of two or more. They become separated from the parent hyphae after maturing and function as resting spores. Under suitable conditions, the chlamydospores germinate by means of germ tubes to form fresh mycelium.

Sclerotia: the mycelium often forms compact, resting bodies of thick-walled hyphae these called sclerotia. They function as storage organs and also serve as means of vegetative propagation.

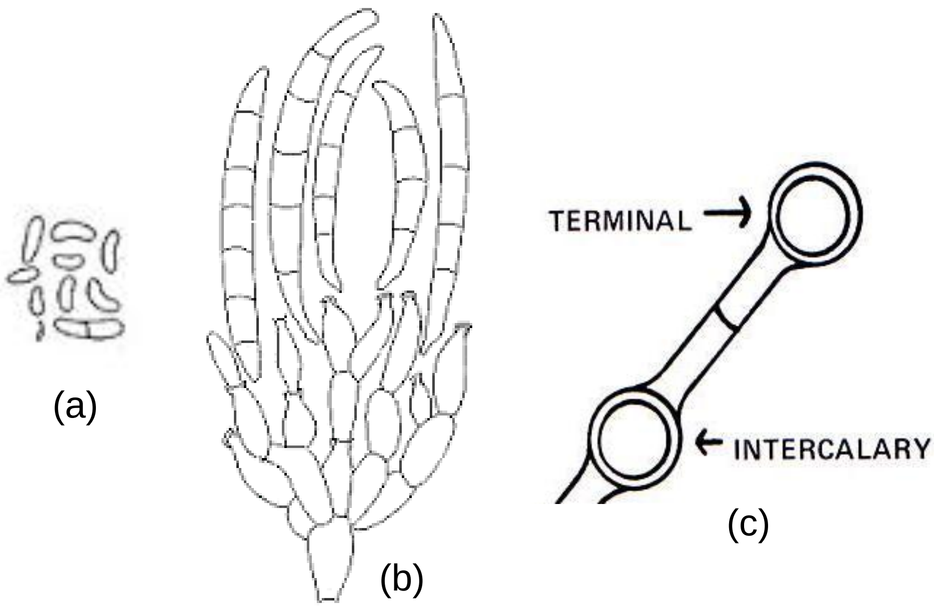


Figure (36): Microconidia (a), Macroconidia (b) and clamydospore (c) produced by *Fusarium*.

Genus: *Alternaria*

The genus *Alternaria* occurs universally. It has several species many of these grow as saprophytes on plant debris and dying plant parts and also in the soil. Several species are parasitic on plants. The important plant pathogens are *Alternaria alternata*, *A. solani*, *A. brassicicola* and *A. tenuis.*

Mycelium: it is not large and extensive but is short, septate, branched, light brown but becoming darker with age. The colony of *Alternaria* are woolly. The cells are multinucleate.

Reproduction:

Alternaria multiplies asexually by the method of sporulation. The characteristic asexual spores which are produced exogenously are the conidia. The conidia are produced at the tips of ordinary hyphae which are comparatively short and dark coloured. Special hyphae termed conidiophores are not recognisable. Each conidium originates as a bud from the terminal cell of a hypha. The conidia are large, dark coloured, several celled and beaked. The septa are transverse and vertical dividing the spore into cells, their number is not fixed. The conidia germinate readily in the presence of moisture and suitable temperature by putting out a number of germ tubes.

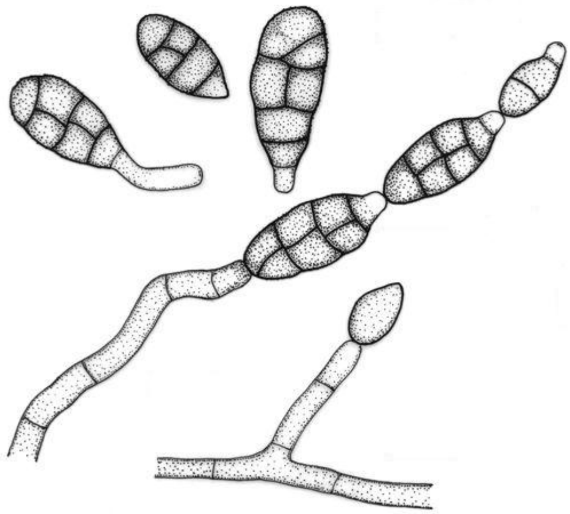
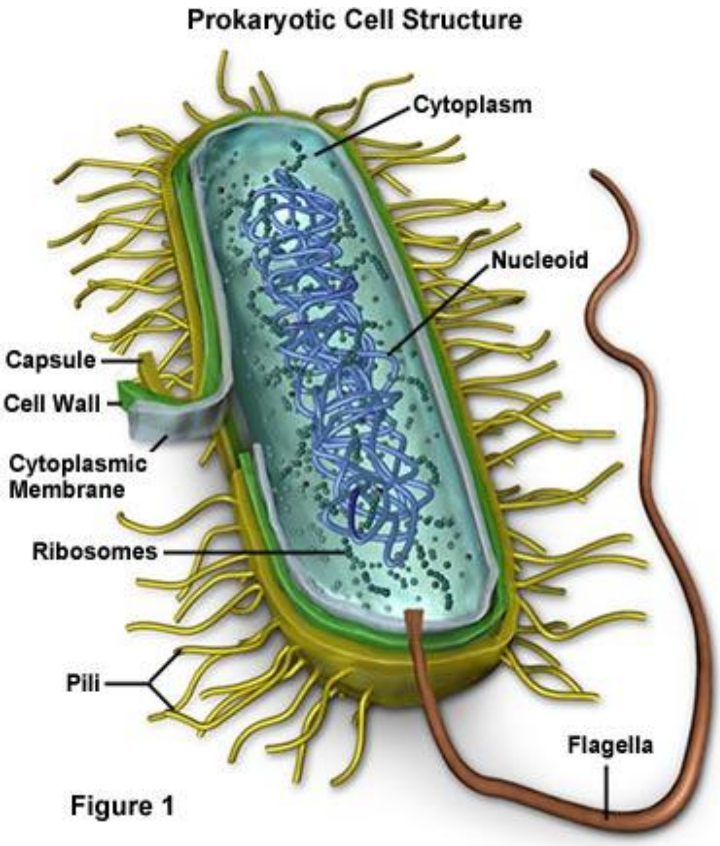


Figure (37): The shape of *Alternaria* conida.

Second Part

Bacteriology



BY

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Content

Introduction

Bacteriology is a one branch of Microbiology. Microbiology is the science that study microorganisms and derived this label from the three Latin word Micro means the precise size and the word bio means life, and the word "logy" means science, so the general meaning is minute biology

It is the third branch of the life sciences (plants and animals)

Many organisms are difficult to classify under these the two kingdoms because they have overlapping characteristics.

Euglena , for example, have plant qualities such as containing chlorophyll, which made scientists of the plant incorporates it into the plant kingdom, as it has animal qualities such as its ability to move which made zoologists place it within the animal kingdom.

TAXONOMY AND CLASSIFICATION OF PROCARYOTES

Haeckel (1866) was the first to create a natural Kingdom for the microorganisms, which had been discovered nearly two centuries before by van Leeuwenhoek. He placed all unicellular (microscopic) organisms in a new kingdom, **"Protista"**, separated from plants (**Plantae**) and animals (**Animalia**), which were multicellular (macroscopic) organisms. The development of the electron microscope in the 1950's revealed a fundamental dichotomy among Haeckel's **"Protista"**: some cells contained a membrane-enclosed nucleus, and some cells lacked this intracellular structure. The latter were temporarily shifted to a fourth kingdom, **Monera** (or **Moneres**), the procaryotes (also called **Procaryotae**). **Protista** remained as a kingdom of unicellular eucaryotic microorganisms. Whittaker refined the system into five kingdoms in 1967, by identifying the **Fungi** as a separate multicellular eucaryotic kingdom of organisms, distinguished by their absorptive mode of heterotrophic nutrition.

In the 1980's, Woese began phylogenetic analysis of all forms of cellular life based on comparative sequencing of the small subunit ribosomal RNA (ssrRNA) that is contained in all organisms. A new dichotomy was revealed, this time among the procaryotes: there existed two types of procaryotes, as fundamentally unrelated to one another as they are to eucaryotes. Thus, Woese defined the **three cellular Domains of life** as **Eucarya**, **Bacteria** and **Archaea**. Whittaker's Plant, Animal and Fungi kingdoms (all of the multicellular eucaryotes) are at the end of a very small branch of the tree of life, and all other branches lead to microorganisms, either procaryotes (Bacteria and Archaea), or protists (unicellular algae and protozoa), thus establishing clearly that microbial life is the predominant form of life on the planet.

Although the definitive difference between Woese's **Archaea** and **Bacteria** is based on fundamental differences in the nucleotide base sequence in the 16S ribosomal RNA, there are many biochemical and phenotypic differences between the two groups of procaryotes. (Table 1). The phylogenetic tree indicates that **Archaea** are more closely related to **Eucarya** than are **Bacteria**. This relatedness seems most evident in the similarities between transcription and translation in the **Archaea** and the **Eucarya**. However, it is also evident that the **Bacteria** have evolved into chloroplasts and mitochondria, so that these eucaryotic organelles derive their lineage from this group of procaryotes. Perhaps the biological success of eucaryotic cells springs from the evolutionary merger of the two procaryotic life forms.

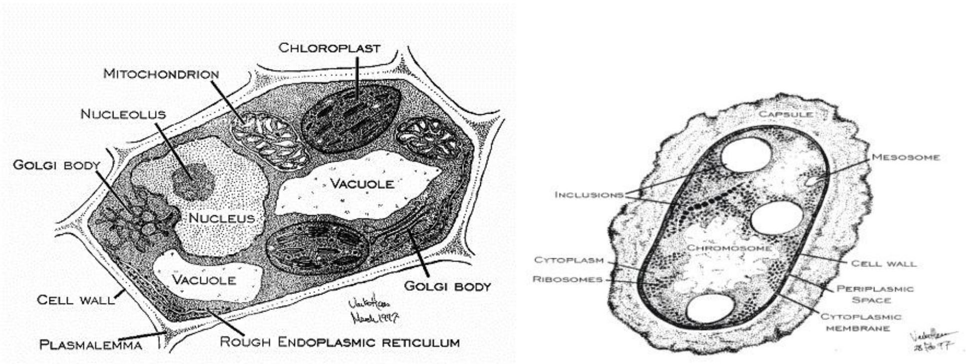


Figure 2 (Right) The structure of a typical procaryotic cell, in this case, a Gram-negative bacterium, compared with (Left) a typical eucaryotic cell (plant cell). The procaryote is about 1 micrometer in diameter and about the size of the eucaryotic chloroplast or mitochondrion.

Table 1. EUKARYOTIC VS. PROKARYOTIC

Eukaryotic cell	Prokaryotic cell
Chromosomes are enclosed inside the double- layered nuclear membrane	No nuclear membrane. Chromosomes are found in the cytoplasm
Chromosome structure is complex	Relatively simple
Cell wall, if present, may contain cellulose or chitin	Contains peptidoglycan
Presence of cell organelles like mitochondria, chloroplasts and Golgi apparatus	No cell organelles but their functions and enzymes are present
Ribosomes are of two types: a larger type in the cytoplasm and a smaller type in mitochondria and chloroplasts	Ribosomes are of one size only
Cell division by mitosis or meiosis	Binary fission
Flagella, if present, are of complex structure	Structure is relatively simple

IDENTIFICATION OF BACTERIA

The criteria used for microscopic identification of procaryotes include cell shape and grouping, Gram-stain reaction, and motility. Bacterial cells almost invariably take one of three forms: rod (**bacillus**), sphere (**coccus**), or spiral (**spirilla** and **spirochetes**). Rods that are curved are called **vibrios**. Fixed bacterial cells stain either Gram-positive (purple) or Gram-negative (pink); motility is easily determined by observing living specimens. Bacilli may occur singly or form chains of cells; cocci may form chains (**streptococci**) or grape-like clusters (**staphylococci**); spiral shape cells are almost always motile; cocci are almost never motile. This nomenclature ignores the **actinomycetes**, a prominent group of branched bacteria which occur in the soil. But they are easily recognized by their colonies and their microscopic appearance.

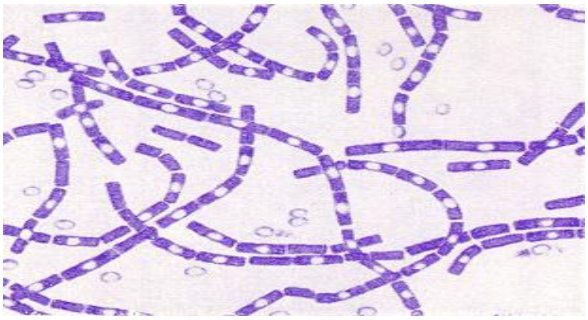


Figure 3. Gram stain of *Bacillus anthracis, the* cause of anthrax.

Such easily-made microscopic observations, combined with knowing the natural environment of the organism, are important aids to identify the group, if not the exact genus, of a bacterium - providing, of course, that one has an effective key. Such a key is **Bergey's Manual of Determinative Bacteriology**, the "field guide" to identification of the bacteria. Bergey's Manual describes affiliated groups of **Bacteria** and **Archaea** based on a few easily observed microscopic and physiologic characteristics. Further identification requires biochemical tests which will distinguish genera among families and species among genera. Strains within a single species are usually distinguished by genetic or immunological criteria.

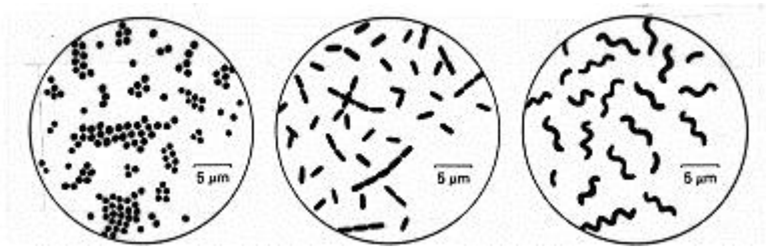


Figure 4. Size and fundamental shapes of procaryotes revealed by three genera of Bacteria (l to r): *Staphylococcus* (spheres), *Lactobacillus* (rods), and *Aquaspirillum* (spirals).

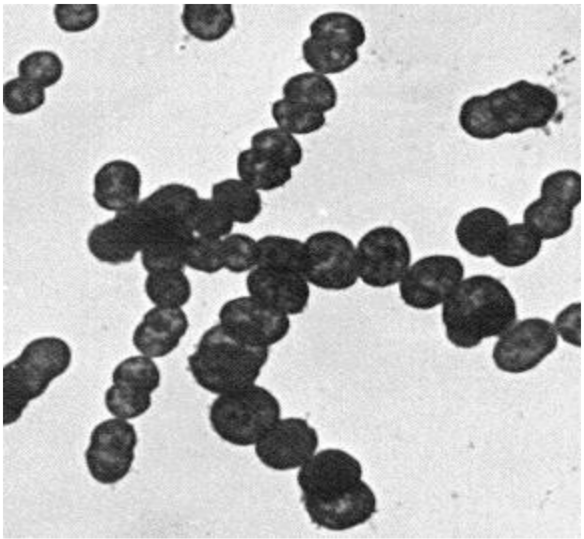


Figure 5. Chains of dividing streptococci. Electron micrograph of *Streptococcus pyogenes*.

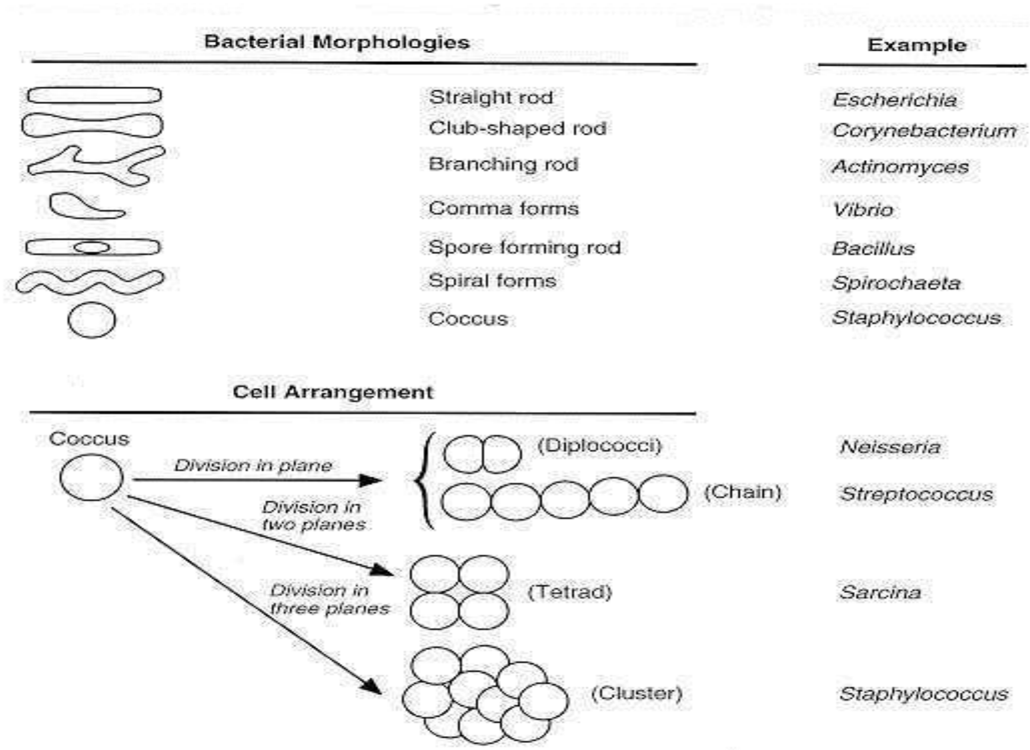


Figure 6. Different shapes and arrangements of bacterial cells, with examples.

STRUCTURE AND FUNCTION OF PROCARYOTIC CELLS

Procaryotes are unicellular organisms of relatively simple construction, especially if compared to eukaryotes. Whereas eukaryotic cells have a preponderance of organelles with separate cellular functions, procaryotes carry out all cellular functions as individual units. A procaryotic cell has five essential structural components: a **genome (DNA)**, **ribosomes**, **cell membrane**, **cell wall**, and some sort of **surface layer** which may or may not be an inherent part of the wall.

Other than enzymatic reactions, all the cellular reactions incidental to life can be traced back to the activities of these macromolecular structural components. Thus, functional aspects of procaryotic cells are related directly to the structure and organization of the macromolecules in their cell make-up, i.e., DNA, RNA, phospholipds, proteins and polysaccharides. Diversity within the primary structure of these molecules accounts for the diversity that exists among procaryotes.

At one time it was thought that bacteria were essentially "bags of enzymes" with no inherent cellular architecture. The development of the electron microscope, in the 1950s, revealed the distinct anatomical features of bacteria and confirmed the suspicion that they lacked a nuclear membrane. Structurally, a procaryotic cell (Figure 1) has three architectural regions: **appendages** (attachments to the cell surface) in the form of **flagella** and **pili (or fimbriae)**; a **cell envelope** consisting of a **capsule**, **cell wall** and **plasma membrane**; and a **cytoplasmic region** that contains the cell **genome** (**DNA**) and **ribosomes** and various sorts of **inclusions**. In this chapter, we will discuss the anatomical structures of procaryotic cells in relation to their adaptation, function and behavior in natural environments.

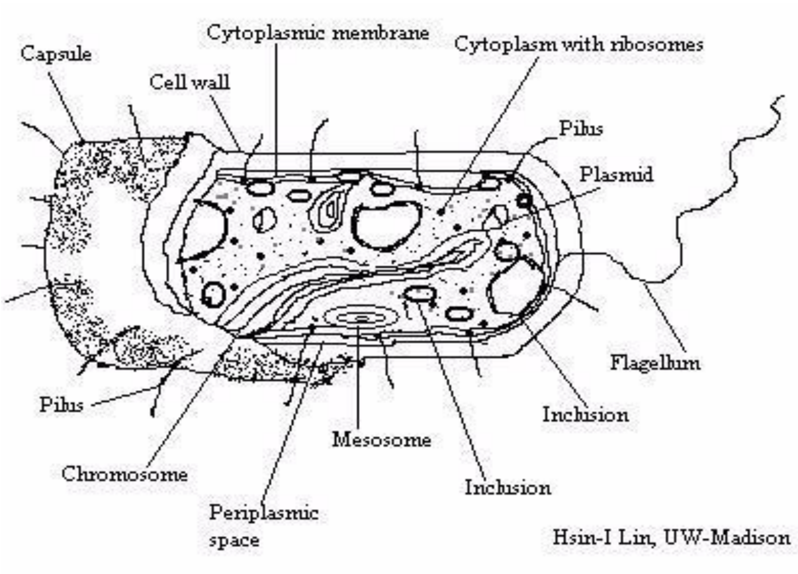


Figure 1. Schematic drawing of a typical bacterium.

Appendages

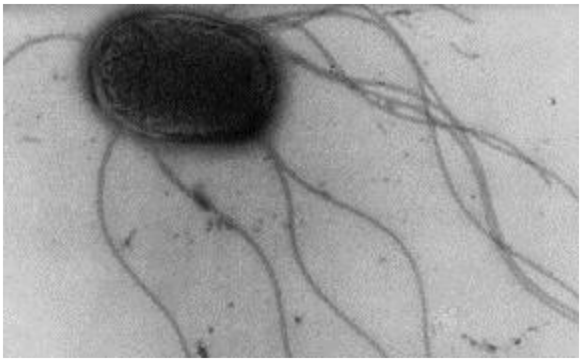


Figure 2. *Salmonella enteritidis* TEM about 10,000X. *Salmonella* is an enteric bacterium related to *E. coli.* The enterics are motile by means of peritriochous flagella.

Flagella

Flagella are filamentous protein structures attached to the cell surface that provide the swimming movement for most motile procaryotes.

Procaryotic flagella are much thinner than eukaryotic flagella. The diameter of a procaryotic flagellum is about 20 nanometers, well-below the resolving power of the light microscope. The flagellar filament is rotated by a motor apparatus in the plasma membrane allowing the cell to swim in fluid environments. Bacterial flagella are powered by proton motive force (chemiosmotic potential) established on the bacterial membrane, rather than ATP hydrolysis which powers eukaryotic flagella. About half of the bacilli and all of the spiral and curved bacteria are motile by means of flagella. Very few cocci are motile, which reflects their adaptation to dry environments and their lack of hydrodynamic design.

The ultrastructure of the flagellum of *E. coli* is illustrated in (Figure 3) below. About 50 genes are required for flagellar synthesis and function. The flagellar apparatus consists of several **distinct proteins**: a system of **rings** embedded in the cell envelope (the **basal body**), a **hook-like structure** near the cell surface, and the **flagellar filament**. The innermost rings, the **M** and **S** rings, located in the plasma membrane, comprise the motor apparatus. The outermost rings, the **P** and **L** rings, located in the periplasm and the outer membrane respectively, function as bushings to support the rod where it is joined to the hook of the filament on the cell surface. As the **M** ring turns, powered by an influx of protons, the rotary motion is transferred to the filament which turns to propel the bacterium.

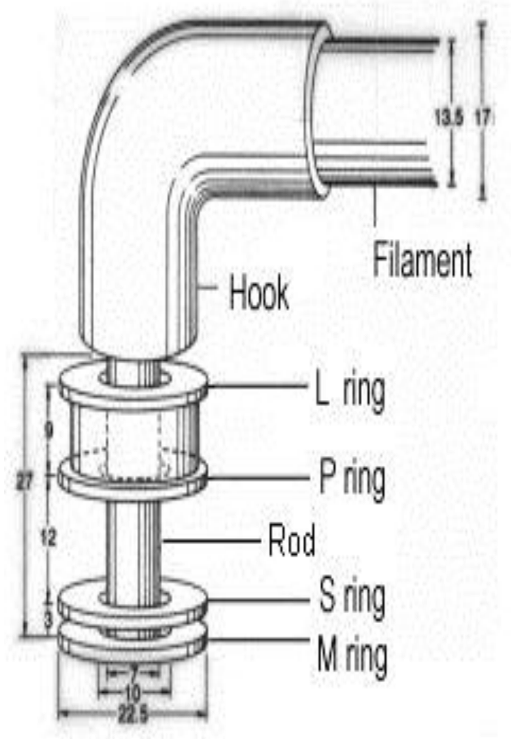


Figure 3. The ultrastructure of a bacterial flagellum. Measurements are in nanometers. The flagellum of *E. coli* consists of three parts, filament, hook and basal body, all composed of different proteins. The basal body and hook anchor the whip-like filament to the cell surface. The basal body consists of four ring-shaped proteins stacked like donuts around a central rod in the cell envelope. The inner rings, associated with the plasma membrane, are the flagellar powerhouse for activating the filament. The outer rings in the peptidoglycan and outer membrane are support rings or "bushings" for the rod. The filament rotates and contracts which propels and steers the cell during movement. Compare with Figure 21 below.

Flagella may be variously distributed over the surface of bacterial cells in distinguishing patterns, but basically flagella are either **polar** (one or more flagella arising from one or both poles of the cell) or **peritrichous** (lateral flagella distributed over the entire cell surface). Flagellar distribution is a genetically-distinct trait that is occasionally used to characterize or distinguish bacteria. For example, among Gram-negative rods, pseudomonads have polar flagella to distinguish them from enteric bacteria, which have peritrichous flagella.

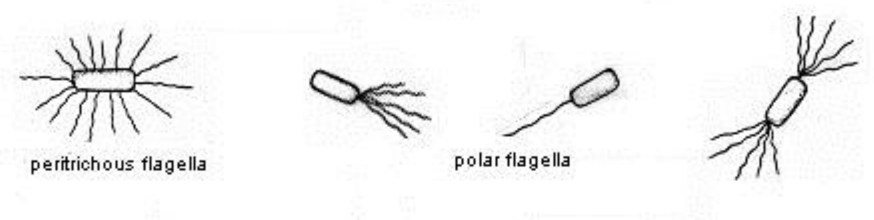


Figure 4. Different arrangements of bacterial flagella. Swimming motility, powered by flagella, occurs in half the bacilli and most of the spirilla. Flagellar arrangements, which can be determined by staining and microscopic observation, may be a clue to the identity of a bacterium. See Figure 5 below.

Flagella were proven to be organelles of bacterial motility by shearing them off (by mixing cells in a blender) and observing that the cells could no longer swim although they remained viable. As the flagella were regrown and reached a critical length, swimming movement was restored to the cells. The flagellar filament grows at its tip (by the deposition of new protein subunits) not at its base (like a hair).

Procaryotes are known to exhibit a variety of types of **tactic behavior**, i.e., the ability to move (swim) in response to environmental stimuli. For example, during **chemotaxis** a bacterium can sense the quality and quantity of certain chemicals in its environment and swim towards them (if they are useful nutrients) or away from them (if they are harmful substances). Other types of tactic response in procaryotes include **phototaxis**, **aerotaxis** and **magnetotaxis**. The occurrence of tactic behavior provides evidence for the ecological (survival) advantage of flagella in bacteria and other procaryotes.

Detecting Bacterial Motility

Since motility is a primary criterion for the diagnosis and identification of bacteria, several techniques have been developed to demonstrate bacterial motility, directly or indirectly.

1. **Flagellar stains** outline flagella and show their pattern of distribution. If a bacterium possesses flagella, it is presumed to be motile.

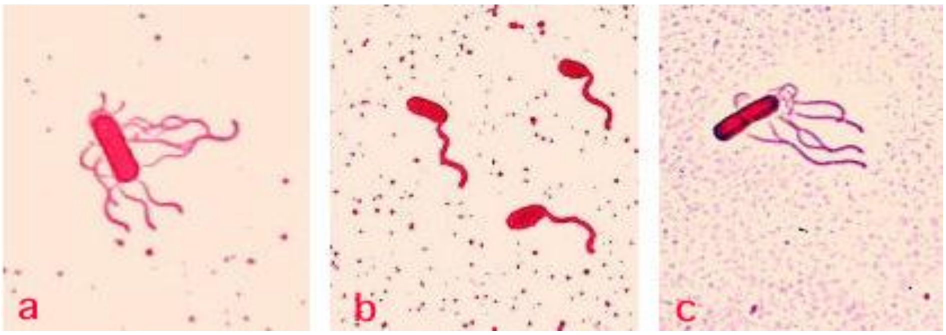


Figure 5. Flagellar stains of three bacteria a. *Bacillus cereus* b. *Vibrio cholerae* c. *Bacillus brevis*. Since the bacterial flagellum is below the resolving power of the light microscope, although bacteria can be seen swimming in a microscope field, the organelles of movenent cannot be detected. Staining techniques such as Leifson's method utilize dyes and other components that precipitate along the protein filament and hence increase its effective diameter. Flagellar distribution is occasionally used to differentiate between morphologically related bacteria. For example, among the Gram-negative motile rod-shaped bacteria, the enterics have peritrichous flagella while the pseudomonads have polar flagella.

2. **Motility test medium** demonstrates if cells can swim in a semisolid medium. A semisolid medium is inoculated with the bacteria in a

straight-line stab with a needle. After incubation, if turbidity (cloudiness) due to bacterial growth can be observed away from the line of the stab, it is evidence that the bacteria were able to swim through the medium.

OFF THE WALL. Julius Adler exploited this observation during his studies of chemotaxis in *E. coli.* He prepared a gradient of glucose by allowing the sugar to diffuse into a semisolid medium from a central point in the medium. This established a concentration gradient of glucose along the radius of diffusion. When *E. coli* cells were seeded in the medium at the lowest concentration of glucose (along the edge of the circle), they swam up the gradient towards a higher concentration (the center of the circle), exhibiting their chemotactic response to swim towards a useful nutrient. Later, Adler developed a tracking microscope that could record and film the track that *E. coli* takes as it swims towards a chemotactic attractant or away from a chemotactic repellent. This led to an understanding of the mechanisms of bacterial chemotaxis, first at a structural level, then at a biomolecular level.

3. **Direct microscopic observation** of living bacteria in a wet mount. One must look for transient movement of swimming bacteria. Most uincellular bacteria, because of their small size, will shake back and forth in a wet mount observed at 400X or 1000X. This is Brownian movement, due to random collisions between water molecules and bacterial cells. True motility is confirmed by observing the bacterium swim from one side of the microscope field to the other side.

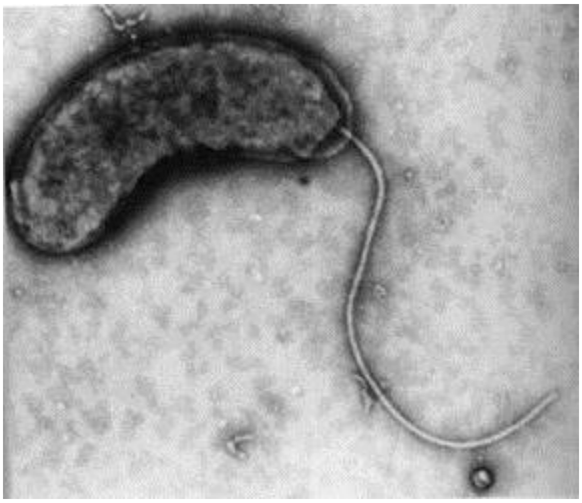


Figure 6. A *Desulfovibrio* species. TEM. About 15,000X. The bacterium is motile by means of a single polar flagellum. Of course, one can determine the presence of flagella by means of electron microscopy. Perhaps this is an alternative way to determine bacterial motility, if you happen to have an electron microscope.

Fimbriae

Fimbriae and **pili** are interchangeable terms used to designate short, hair-like structures on the surfaces of procaryotic cells. Like flagella, they are composed of protein. Fimbriae are shorter and stiffer than flagella, and slightly smaller in diameter. Generally, fimbriae have nothing to do with bacterial movement (there are exceptions, e.g. twitching movement on *Pseudomonas*). Fimbriae are very common in Gram-negative bacteria, but occur in some archaea and Gram-positive bacteria as well. Fimbriae are most often involved in adherence of bacteria to surfaces, substrates and other cells or tissues in nature. In *E. coli*, a specialized type of pilus, the **F or sex pilus**, mediates the transfer of DNA between mating bacteria during the process of

conjugation, but the function of the smaller, more numerous common pili is quite different.

Common pili (almost always called **fimbriae**) are usually involved in specific adherence (attachment) of procaryotes to surfaces in nature. In medical situations, they are major determinants of bacterial virulence because they allow pathogens to attach to (colonize) tissues and/or to resist attack by phagocytic white blood cells. For example, pathogenic *Neisseria gonorrhoeae* adheres specifically to the human cervical or urethral epithelium by means of its fimbriae; enterotoxigenic strains of *E. coli* adhere to the mucosal epithelium of the intestine by means of specific fimbriae; the M-protein and associated fimbriae of *Streptococcus pyogenes* are involved in adherence and to resistance to engulfment by phagocytes.

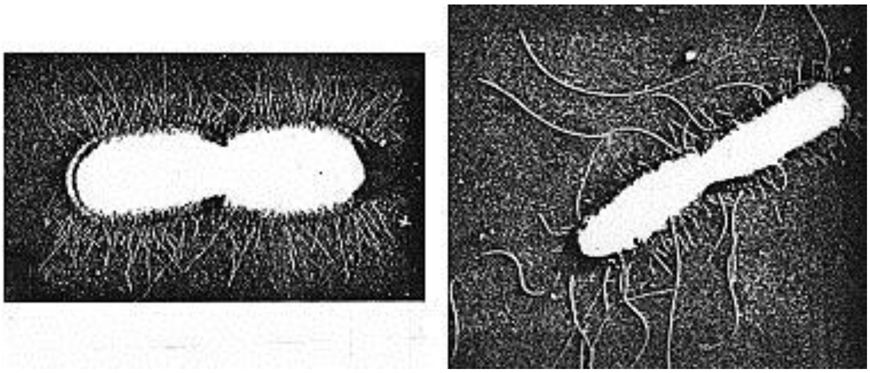


Figure 7. Fimbriae (common pili) and flagella on the surface of bacterial cells. Left: dividing *Shigella* enclosed in fimbriae. The structures are probably involved in the bacterium's ability to adhere to the intestinal surface. Right: dividing pair of *Salmonella* displaying both its peritrichous flagella and its fimbriae. The fimbriae are much shorter and slightly smaller in diameter than flagella. Both *Shigella* and *Salmonella* are enteric bacteria that cause different types of intestinal diarrheas. The bacteria can be differentiated by a motility test. *Salmonella* is motile; *Shigella* is nonmotile.

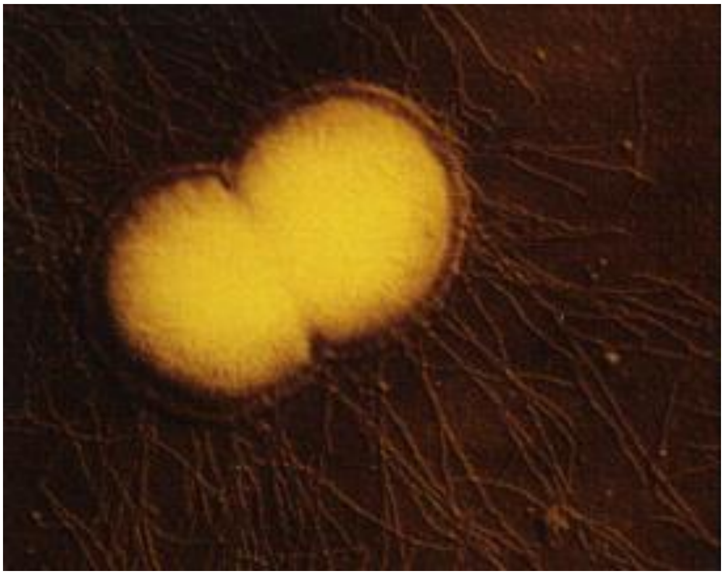


Figure 8. Fimbriae of *Neisseria gonorrhoeae* allow the bacterium to adhere to tissues. Electron micrograph.

The Cell Envelope

The **cell envelope** is a descriptive term for the several layers of material that envelope or enclose the protoplasm of the cell. The cell protoplasm (**cytoplasm**) is surrounded by the **plasma membrane**, a **cell wall** and a **capsule**. The cell wall itself is a layered structure in Gram-negative bacteria. All cells have a membrane, which is the essential and definitive characteristic of a "cell". Almost all procaryotes have a cell wall to prevent damage to the underlying **protoplast**. Outside the cell wall, foremost as a surface structure, may be a polysaccharide **capsule**, or at least a **glycocalyx**.

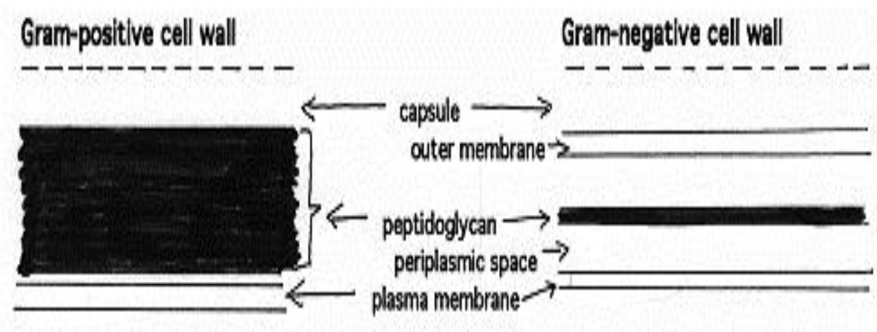


Figure 9. Profiles of the cell envelope the Gram-positive and Gram-negative bacteria. The Gram-positive wall is a uniformly thick layer external to the plasma membrane. It is composed mainly of peptidoglycan (murein). The Gram-negative wall appears thin and multilayered. It consists of a relatively thin peptidoglycan sheet between the plasma membrane and a phospholipid-lipopolysaccharide outer membrane. The space between the inner (plasma) and outer membranes (wherein the peptidoglycan resides) is called the periplasm.

Capsules

Most procaryotes contain some sort of a polysaccharide layer outside of the cell wall polymer. In a general sense, this layer is called a **capsule**. A **true capsule** is a discrete detectable layer of polysaccharides deposited outside the cell wall. A less discrete structure or matrix which embeds the cells is a called a **slime layer** or a **biofilm**. A type of capsule found in bacteria called a **glycocalyx** is a thin layer of tangled polysaccharide fibers which is almost always observed on the surface of cells growing in nature (as opposed to the laboratory).

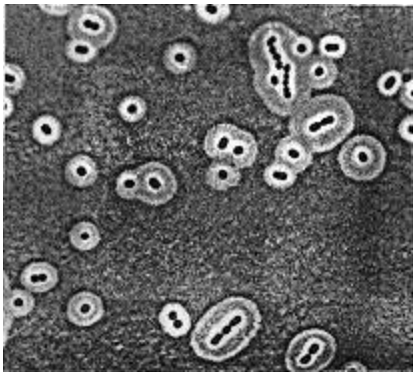


Figure 10. Bacterial capsules outlined by India ink viewed by light microscopy. This is a true capsule, a discrete layer of polysaccharide surrounding the cells. Sometimes bacterial cells are embedded more randomly in a polysaccharide matrix called a slime layer or biofilm. Polysaccharide films that may inevitably be present on the surfaces of bacterial cells, but which cannot be detected visually, are called glycocalyx.

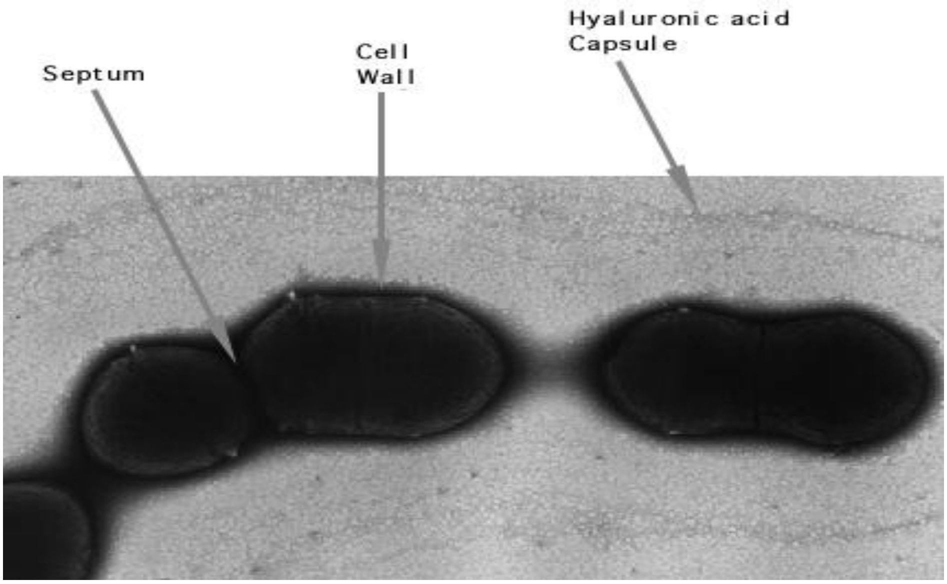


Figure 11. Negative stain of *Streptococcus pyogenes* viewed by transmission electron microscopy (28,000X). The halo around the chain of cells is the hyaluronic acid capsule that surrounds the exterior of the bacteria. The septa between dividing pairs of cells may also be seen. Electron micrograph of *Streptococcus pyogenes*.

Capsules are generally composed of polysaccharide; rarely they contain amino sugars or peptides.

Capsules have **several functions** and often have multiple functions in a particular organism. Like fimbriae, capsules, slime layers, and glycocalyx often **mediate adherence** of cells to surfaces. Capsules also **protect bacterial cells from engulfment** by predatory protozoa or white blood cells (phagocytes), or from attack by antimicrobial agents of plant or animal origin. Capsules in certain soil bacteria **protect cells from perennial effects of drying** or desiccation. Capsular materials (e.g. dextrans) may be overproduced when bacteria are fed sugars to become **reserves of carbohydrate** for subsequent metabolism.

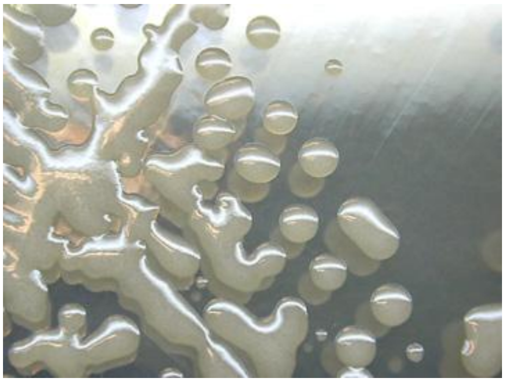


Figure 12. Colonies of *Bacillus anthracis*. The slimy or mucoid appearance of a bacterial colony is usually evidence of capsule production. In the case of *B. anthracis,* the capsule is composed of poly-D-glutamate. The capsule is an essential determinant of virulence to the bacterium. In the early stages of colonization and infection the capsule protects the bacteria from assaults by the immune and phagocytic systems.

Some bacteria produce slime materials to adhere and float themselves as colonial masses in their environments. Other bacteria produce slime materials to attach themselves to a surface or substrate. Bacteria may attach to surface, produce slime, divide and produce microcolonies within the slime layer, and construct a **biofilm**, which becomes an enriched and protected environment for themselves and other bacteria.

A classic example of biofilm construction in nature is the formation of **dental plaque** mediated by the oral bacterium, *Streptococcus mutans*. The bacteria adhere specifically to the pellicle of the tooth by means of a protein on the cell surface. The bacteria grow and synthesize a dextran capsule which binds them to the enamel and forms a biofilm some 300-500 cells in thickness. The bacteria are able to cleave sucrose (provided by the animal diet) into glucose plus fructose. The fructose is fermented as an energy source for bacterial growth. The glucose is polymerized into an extracellular dextran polymer that cements the bacteria to tooth enamel and becomes the matrix of dental plaque. The dextran slime can be depolymerized to glucose for use as a carbon source, resulting in production of lactic acid within the biofilm (plaque) that decalcifies the enamel and leads to dental caries or bacterial infection of the tooth.

Another important characteristic of capsules may be their ability to block some step in the phagocytic process and thereby prevent bacterial cells from being engulfed or destroyed by phagocytes. For example, the primary determinant of virulence of the pathogen *Streptococcus pneumoniae* is its polysaccharide capsule, which prevents ingestion of pneumococci by alveolar macrophages. *Bacillus anthracis* survives phagocytic engulfment because the lysosomal enzymes of the phagocyte cannot initiate an attack on the poly-D-glutamate capsule of the bacterium. Bacteria such as *Pseudomonas aeruginosa*, that construct a biofilm made of extracellular slime when colonizing tissues, are also resistant to phagocytes, which cannot penetrate the biofilm.

Cell Wall

Most procaryotes have a rigid **cell wall**. The cell wall is an essential structure that protects the cell protoplast from mechanical damage and from osmotic rupture or **lysis**. Procaryotes usually live in relatively dilute environments such that the accumulation of solutes inside the procaryotic cell cytoplasm greatly exceeds the total solute concentration in the outside environment. Thus, the osmotic pressure against the inside of the plasma membrane may be the equivalent of 10-25 atm. Since the membrane is a delicate, plastic structure, it must be restrained by an outside wall made of porous, rigid material that has high tensile strength. Such a material is **murein**, the ubiquitous component of bacterial cell walls.

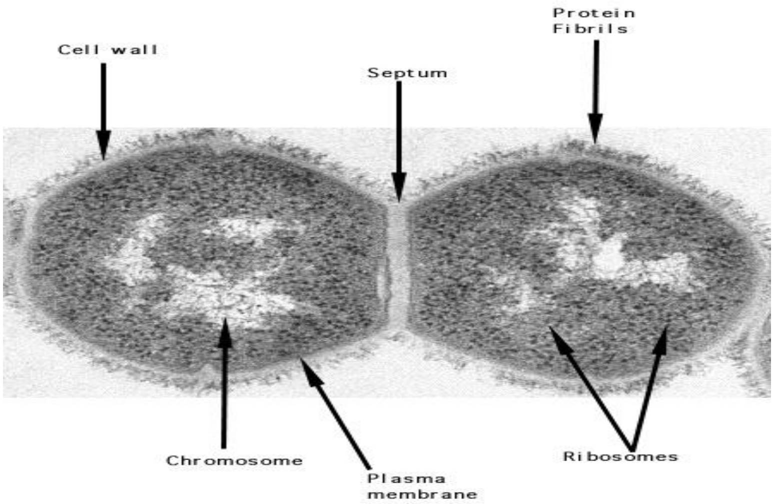


Figure 13. Electron micrograph of an ultra-thin section of a dividing pair of group A streptococci (20,000X). The cell surface fibrils, consisting primarily of M protein, are evident. The bacterial cell wall, to which the fibrils are attached, is also clearly seen as the light staining region between the fibrils and the dark staining cell interior. Cell division in progress is indicated by the new septum formed between the two cells and by the indentation of the cell wall near the cell equator. The streptococcal cell diameter is equal to approximately one micron. Electron micrograph of *Streptococcus pyogenes*.

The cell walls of bacteria deserve special attention for several reasons:

1. They are an essential structure for viability, as described above.
2. They are composed of unique components found nowhere else in nature.
3. They are one of the most important sites for attack by antibiotics.
4. They provide ligands for adherence and receptor sites for drugs or viruses.
5. They cause symptoms of disease in animals.
6. They provide for immunological distinction and immunological variation among strains of bacteria.

The cell walls of all **Bacteria** contain a unique type of **peptidoglycan** called **murein**. Peptidoglycan is a polymer of disaccharides (a glycan) cross-linked by short chains of amino acids (peptides), and many types of peptidoglycan exist. All **Bacterial** peptidoglycans contain **N-acetylmuramic acid**, which is the definitive component of **murein**. The cell walls of **Archaea** may be composed of protein, polysaccharides, or peptidoglycan-like molecules, but never do they contain murein. This feature distinguishes the **Bacteria** from the **Archaea**.

In the **Gram-positive Bacteria** (those that retain the purple crystal violet dye when subjected to the Gram-staining procedure) the cell wall is thick (15-80 nanometers), consisting of several layers of peptidoglycan. In the **Gram-negative Bacteria** (which do not retain the crystal violet) the cell wall is relatively thin (10 nanometers) and is composed of a single layer of peptidoglycan surrounded by a membranous structure called the **outer membrane**. The outer membrane of Gram-negative bacteria invariably contains a unique component, **lipopolysaccharide** (**LPS** or **endotoxin**), which is toxic to animals. In Gram-negative bacteria the outer membrane is usually thought of as part of the cell wall.

The Outer Membrane of Gram-negative Bacteria

Of special interest as a component of the Gram-negative cell wall is the **outer membrane**, a discrete bilayered structure on the outside of the peptidoglycan sheet (see Figure 16). For the bacterium, the outer membrane is first and foremost a permeability barrier, but primarily due to its lipopolysaccharide content, it possesses many interesting and important characteristics of Gram-negative bacteria. The outer membrane is a lipid bilayer intercalated with proteins, superficially resembling the plasma membrane. The inner face of the outer membrane is composed of phospholipids similar to the phosphoglycerides that compose the plasma membrane. The outer face of the outer membrane may contain some phospholipid, but mainly it is formed by a different type of amphiphilic molecule which is composed of lipopolysaccharide (LPS). Outer membrane proteins usually traverse the membrane and in one case, anchor the outer membrane to the underlying peptidoglycan sheet.

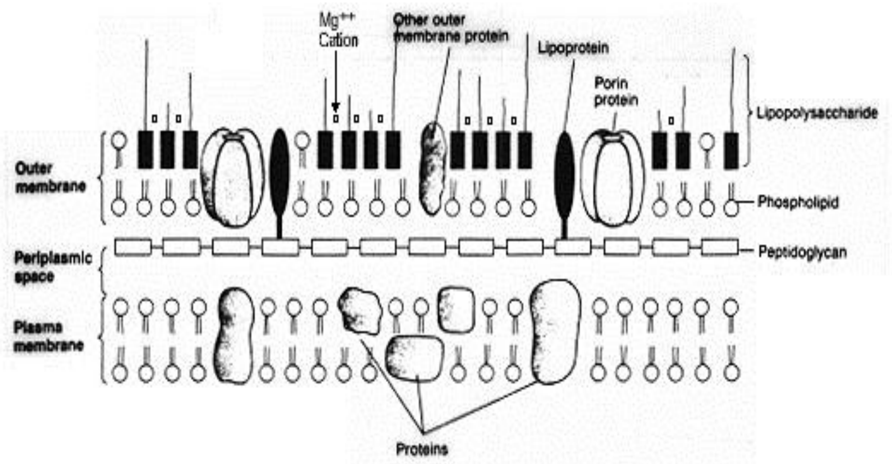


Figure 16. Schematic illustration of the outer membrane, cell wall and plasma membrane of a Gram-negative bacterium. Note the structure and arrangement of molecules that constitute the outer membrane.

The LPS molecule that constitutes the outer face of the outer membrane is composed of a hydrophobic region, called **Lipid A**, that is attached to a hydrophilic linear polysaccharide region, consisting of the **core polysaccharide** and **the O-specific polysaccharide**.

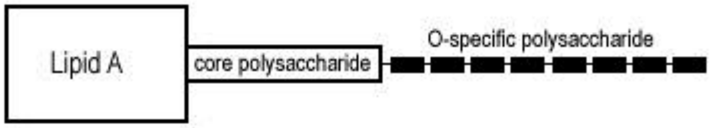


Figure 17. Structure of LPS

The Lipid A head of the molecule inserts into the interior of the membrane, and the polysaccharide tail of the molecule faces the aqueous environment. Where the tail of the molecule inserts into the head there is an accumulation of negative charges such that a magnesium cation is chelated between adjacent LPS molecules. This provides the lateral stability for the outer membrane, and explains why treatment of Gram-negative bacteria with a powerful chelating agent, such as EDTA, causes dispersion of LPS molecules.

Bacterial lipopolysaccharides are toxic to animals. When injected in small amounts LPS or **endotoxin** activates macrophages to produce pyrogens, activates the complement cascade causing inflammation, and activates blood factors resulting in intravascular coagulation and hemorrhage. Endotoxins may play a role in infection by any Gram-negative bacterium. The toxic component of endotoxin (LPS) is Lipid A. The O-specific polysaccharide may provide ligands for bacterial attachment and confer some resistance to phagocytosis. Variation in the exact sugar content of the O polysaccharide (also referred to as the O antigen) accounts for multiple antigenic types (serotypes) among Gram-negative bacterial pathogens. Therefore. even though Lipid A is the toxic component in LPS, the polysaccharides nonetheless contribute to virulence of Gram-negative bacteria.

Cell Wall-less Forms

A few bacteria are able to live or exist without a cell wall. The mycoplasmas are a group of bacteria that lack a cell wall. Mycoplasmas have sterol-like molecules incorporated into their membranes and they are usually inhabitants of osmotically-protected environments. *Mycoplasma pneumoniae* is the cause of primary atypical bacterial pneumonia, known in the vernacular as "walking pneumonia". For obvious reasons, penicillin is ineffective in treatment of this type of pneumonia. Sometimes, under the pressure of antibiotic therapy, pathogenic streptococci can revert to cell wall-less forms (called **spheroplasts**) and persist or survive in osmotically-protected tissues. When the antibiotic is withdrawn from therapy the organisms may regrow their cell walls and reinfect unprotected tissues.

The Plasma Membrane

The **plasma membrane**, also called the **cytoplasmic membrane**, is the most dynamic structure of a procaryotic cell. Its main function is a s a **selective permeability barrier** that regulates the passage of substances into and out of the cell. The plasma membrane is the definitive structure of a cell since it sequesters the molecules of life in a unit, separating it from the environment. The bacterial membrane allows passage of water and uncharged molecules up to mw of about 100 daltons, but does not allow passage of larger molecules or any charged substances except by means special membrane **transport processes** and **transport systems**.

Since procaryotes lack any intracellular organelles for processes such as respiration or photosynthesis or secretion, the plasma membrane subsumes these processes for the cell and consequently has a variety of functions in **energy generation**, and **biosynthesis**. For example, the **electron transport system** that couples **aerobic respiration** and **ATP synthesis** is found in the procaryotic membrane. The **photosynthetic chromophores** that harvest light energy for conversion into chemical energy are located in the membrane. Hence, the plasma membrane is the site of **oxidative phosphorylation** and **photophosphorylation** in procaryotes, analogous to the functions of mitochondria and chloroplasts in eukaryotic cells. Besides **transport proteins** that selectively mediate the passage of substances into and out of the cell, procaryotic membranes may contain **sensing proteins** that measure concentrations of molecules in the environment or **binding proteins** that translocate signals to genetic and metabolic machinery in the cytoplasm. Membranes also contain **enzymes** involved in many metabolic processes such as cell wall synthesis, septum formation, membrane synthesis, DNA replication, CO_2 fixation and ammonia oxidation. The predominant functions of bacterial membranes are listed in the table below.

Table 6. Functions of the procaryotic plasma membrane.

1. Osmotic or permeability barrier

2. Location of transport systems for specific solutes (nutrients and ions)

3. Energy generating functions, involving respiratory and photosynthetic electron transport systems, establishment of proton motive force, and transmembranous, ATP-synthesizing ATPase

4. Synthesis of membrane lipids (including lipopolysaccharide in Gram-negative cells)

5. Synthesis of murein (cell wall peptidoglycan)

6. Assembly and secretion of extracytoplasmic proteins

7. Coordination of DNA replication and segregation with septum formation and cell division

8. Chemotaxis (both motility per se and sensing functions)

9. Location of specialized enzyme system

Bacterial membranes are composed of 40 percent phospholipid and 60 percent protein. The phospholipids are amphoteric molecules with a polar hydrophilic glycerol "head" attached via an ester bond to two nonpolar hydrophobic fatty acid tails, which naturally form a bilayer in aqueous environments. Dispersed within the bilayer are various structural and enzymatic proteins which carry out most membrane functions. At one time, it was thought that the proteins were neatly organized along the inner and outer faces of the membrane and that this accounted for the double track appearance of the membrane in electron micrographs. However, it is now known that while some membrane proteins are located and function on one side or another of the membrane, most proteins are partly inserted into the membrane, or possibly even traverse the membrane as channels from the outside to the inside. It is possible that proteins can move laterally along a surface of the membrane, but it is thermodynamically unlikely that proteins can be rotated within a membrane, which discounts early theories of how transport systems might work. The arrangement of proteins and lipids to form a membrane is called the **fluid mosaic model**, and is illustrated in Figure 18.

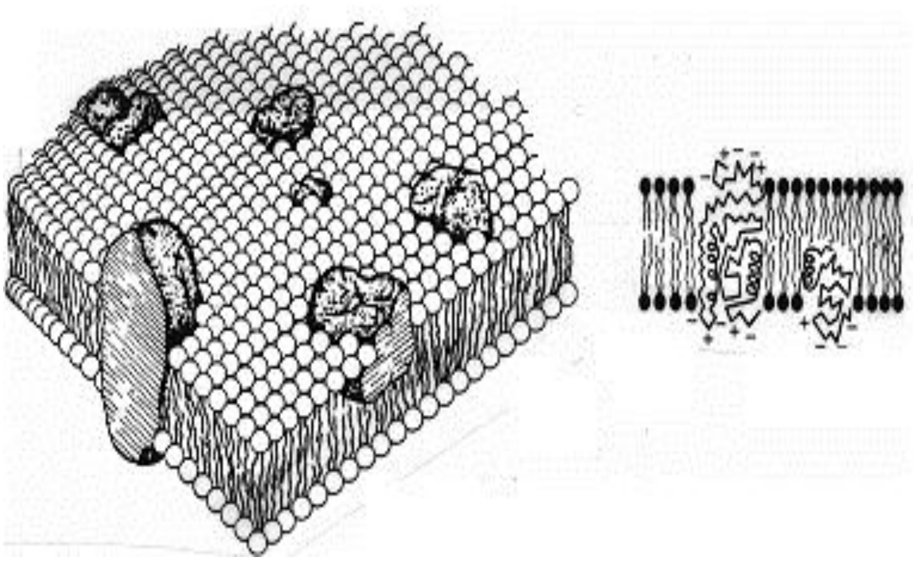


Figure 18. Fluid mosaic model of a biological membrane. In aqueous environments membrane phospholipids arrange themselves in such a way that they spontaneously form a fluid bilayer. Membrane proteins, which may be structural or functional, may be permanently or transiently associated with one side or the other of the membrane, or even be permanently built into the bilayer, while other proteins span the bilayer and may form transport channels through the membrane.

Transport Processes

The proteins that mediate the passage of solutes through membranes are referred to variously as **transport systems**, **carrier proteins**, **porters**, and **permeases**. Transport systems operate by one of three **transport processes** as described below in Figure 19. In a **uniport** process, a solute passes through the membrane unidirectionally. In **symport** processes (also called **cotransport**) two solutes must be transported in the same direction at the same time; in **antiport** processes (also called **exchange diffusion**), one solute is transported in one direction simultaneously as a second solute is transported in the opposite direction.

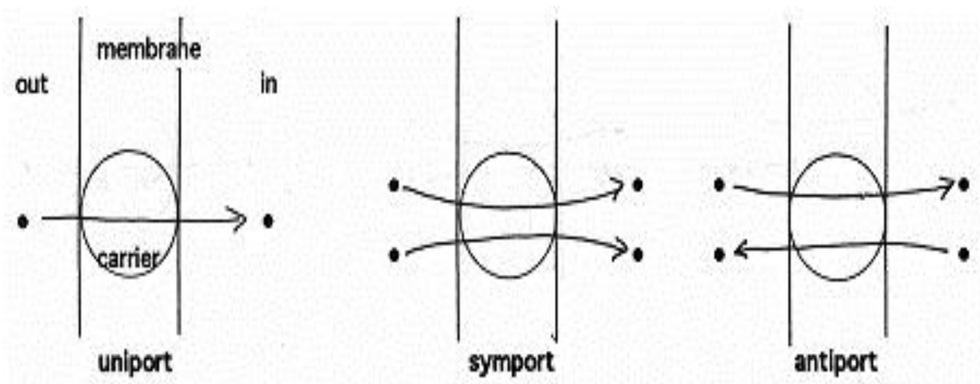


Figure 19. Transport processes in bacterial cells. Solutes enter or exit from bacterial cells by means of one of three processes: uniport, symport (also called cotransport) and antiport (also called exchange diffusion). Transport systems (Figure 20 below) operate by one or another of these processes.

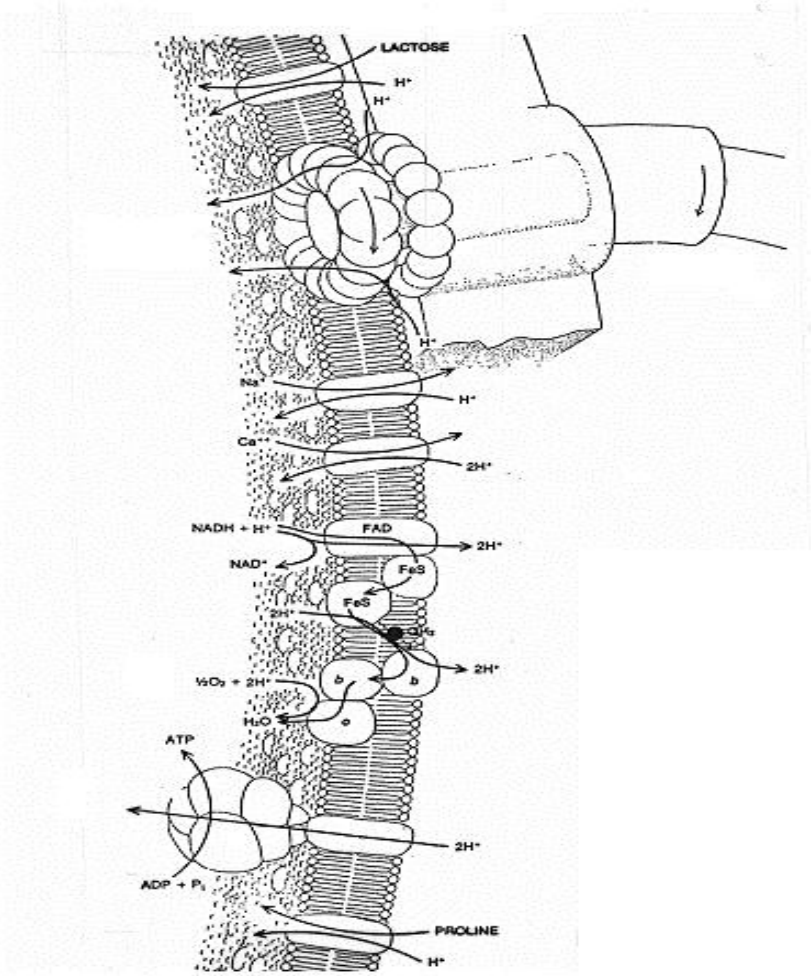


Figure 20. Schematic view of the plasma membrane of *Escherichia coli*. The S and M rings which constitute the flagellar motor are shown. The motor ring is imbedded in the phospholipid bilayer. It is powered by pmf to rotate the flagellar filament. The electron transport system is shown oxidizing NAD by removal of a pair of electrons, passing them through its sequence of carriers eventually to O2. ATPase is the transmembranous protein enzyme that utilizes protons from the outside to synthesize ATP on the inside of the membrane. Several other transmembranous proteins are transport systems which are operating by either symport or antiport processes.

The Cytoplasm

The cytoplasmic constituents of procaryotic cells invariably include the **procaryotic chromosome** and **ribosomes**. The chromosome is typically one large circular molecule of **DNA**, more or less free in the cytoplasm. Procaryotes sometimes possess smaller extrachromosomal pieces of DNA called **plasmids**. The total DNA content of a procaryote is referred to as the cell **genome**. During cell growth and division, the procaryotic chromosome is replicated in the usual semi-conservative fashion before for distribution to progeny cells. However, the eukaryotic processes of meiosis and mitosis are absent in procaryotes. Replication and segregation of procaryotic DNA is coordinated by the membrane, possibly by mesosomes.

The distinct granular appearance of procaryotic cytoplasm is due to the presence and distribution of **ribosomes**. The ribosomes of procaryotes are smaller than cytoplasmic ribosomes of eukaryotes. procaryotic ribosomes are 70S in size, being composed of 30S and 50S subunits. The 80S ribosomes of eukaryotes are made up of 40S and 60S subunits. Ribosomes are involved in the process of translation (protein synthesis), but some details of their activities differ in eukaryotes, Bacteria and Archaea. Protein synthesis using 70S ribosomes occurs in eukaryotic mitochondria and chloroplasts, and this is taken as a major line of evidence that these organelles are descended from procaryotes.

Inclusions

Often contained in the cytoplasm of procaryotic cells is one or another of some type of inclusion granule. **Inclusions** are distinct granules that may occupy a substantial part of the cytoplasm. Inclusion granules are usually reserve materials of some sort. For example, carbon and energy reserves may be stored as glycogen (a polymer of glucose) or as polybetahydroxybutyric acid (a type of fat) granules. Polyphosphate inclusions are reserves of PO_4 and possibly energy; elemental sulfur (sulfur globules) are stored by some phototrophic and some lithotrophic procaryotes as reserves of energy or electrons. Some inclusion bodies are actually membranous vesicles or intrusions into the cytoplasm which contain photosynthetic pigments or enzymes.

Table 10. Some inclusions in bacterial cells.

Cytoplasmic inclusions	Where found	Composition	Function
glycogen	many bacteria e.g. *E. coli*	polyglucose	reserve carbon and energy source
polybetahydroxyutyric acid (PHB)	many bacteria e.g. *Pseudomonas*	polymerized hydroxy butyrate	reserve carbon and energy source
polyphosphate (volutin granules)	many bacteria e.g. *Corynebacterium*	linear or cyclical polymers of PO4	reserve phosphate; possibly a reserve of high energy phosphate
sulfur globules	phototrophic purple and green sulfur bacteria and lithotrophic colorless sulfur bacteria	elemental sulfur	reserve of electrons (reducing source) in phototrophs; reserve energy source in lithotrophs
gas vesicles	aquatic bacteria especially cyanobacteria	protein hulls or shells inflated with gases	buoyancy (floatation) in the vertical water column

parasporal crystals	endospore-forming bacilli (genus *Bacillus*)	protein	unknown but toxic to certain insects
magnetosomes	certain aquatic bacteria	magnetite (iron oxide) Fe_3O_4	orienting and migrating along geo- magnetic field lines
carboxysomes	many autotrophic bacteria	enzymes for autotrophic CO_2 fixation	site of CO_2 fixation
phycobilisomes	cyanobacteria	phycobiliproteins	light-harvesting pigments
chlorosomes	Green bacteria	lipid and protein and bacteriochlorophyll	light-harvesting pigments and antennae

Endospores

A bacterial structure sometimes observed as an inclusion is actually a type of dormant cell called an **endospore**. Endospores are formed by a few groups of **Bacteria** as intracellular structures, but ultimately they are released as free endospores. Biologically, endospores are a fascinating type of cell. Endospores exhibit no signs of life, being described as **cryptobiotic**. They are highly resistant to environmental stresses such as high temperature (some endospores can be boiled for hours and retain their viability), irradiation, strong acids, disinfectants, etc. They are probably the most durable cell produced in nature. Although cryptobiotic, they retain viability indefinitely such that under appropriate environmental conditions, they germinate back into vegetative cells. Endospores are formed by vegetative cells in response to environmental signals that indicate a limiting factor for vegetative growth, such as exhaustion of an essential nutrient. They germinate and become vegetative cells when the environmental stress is relieved. Hence, endospore-formation is a mechanism of survival rather than a mechanism of reproduction.

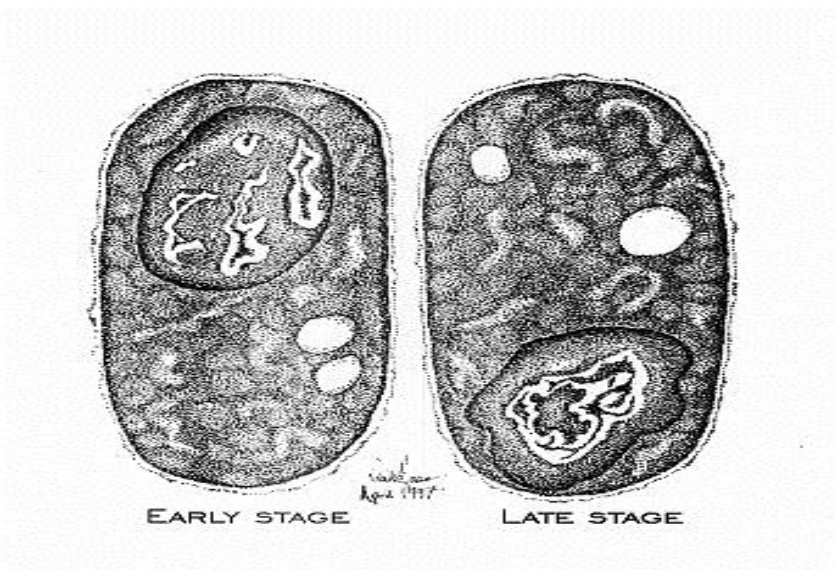


Figure 21. Early and late stages of endospore formation. Drawing by Vaike Haas, University of Wisconsin Madison. During endospore formation, a vegetative cell is converted to a heat-resistant spore. There are eight stages, O,I-VII, in the sporulation cycle of a *Bacillus* species, and the process takes about eight hours. During the early stages (Stage II,) one bacterial chromosome and a few ribosomes are partitioned off by the bacterial membrane to form a protoplast within the mother cell. By the late stages (Stage VI) the protoplast (now called a forespore) has developed a second membrane and several wall-like layers of material are deposited between the two membranes.

Table 11. Differences between endospores and vegetative cells.

Property	Vegetative cells	Endospores
Surface coats	Typical Gram-positive murein cell wall polymer	Thick spore coat, cortex, and peptidoglycan core wall
Microscopic appearance	Nonrefractile	Refractile
Calcium dipicolinic acid	Absent	Present in core
Cytoplasmic water activity	High	Very low
Enzymatic activity	Present	Absent
Macromolecular synthesis	Present	Absent
Heat resistance	Low	High
Resistance to chemicals and acids	Low	High
Radiation resistance	Low	High
Sensitivity to lysozyme	Sensitive	Resistant
Sensitivity to dyes and staining	Sensitive	Resistant

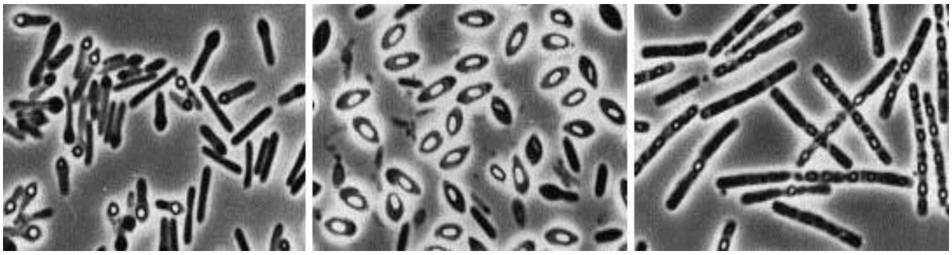


Figure 22. Bacterial endospores. Phase microscopy of sporulating bacteria demonstrates the refractility of endospores, as well as characteristic spore shapes and locations within the mother cell.

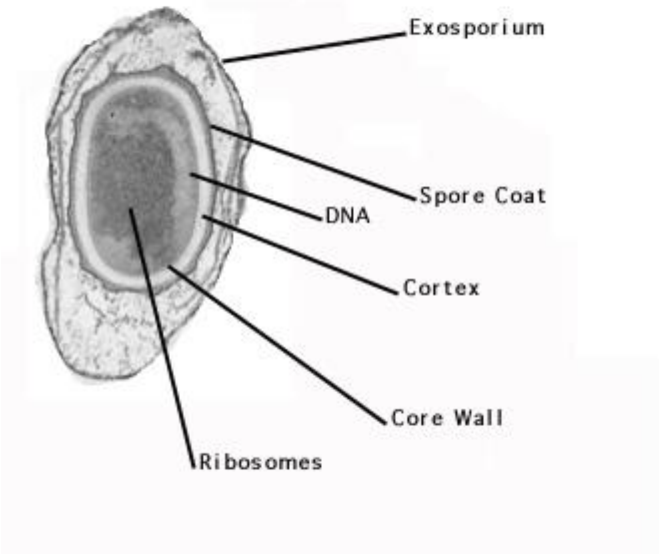


Figure 23. Electron micrograph of a bacterial endospore. The spore has a core wall of unique peptidoglycan surrounded by several layers, including the cortex, the spore coat and the exosporium. The dehydrated core contains the bacterial chromosome and a few ribosomes and enzymes to jump-start protein synthesis and metabolism during germination.

NUTRITION AND GROWTH OF BACTERIA

Nutritional Requirements of Cells

Every organism must find in its environment all of the substances required for energy generation and cellular biosynthesis. The chemicals and elements of this environment that are utilized for bacterial growth are referred to as **nutrients** or **nutritional requirements**. In the laboratory, bacteria are grown in **culture media** which are designed to provide all the essential nutrients in solution for bacterial growth.

The Major Elements

At an elementary level, the nutritional requirements of a bacterium such as *E. coli* are revealed by the cell's elemental composition, which consists of C, H, O, N, S. P, K, Mg, Fe, Ca, Mn, and traces of Zn, Co, Cu, and Mo. These elements are found in the form of water, inorganic ions, small molecules, and macromolecules which serve either a structural or functional role in the cells. The general physiological functions of the elements are outlined in Table 1 below.

Table 1. Major elements, their sources and functions in bacterial cells.

Element	% of dry weight	Source	Function
Carbon	50	organic compounds or CO_2	Main constituent of cellular material
Oxygen	20	H_2O, organic compounds, CO_2, and O_2	Constituent of cell material and cell water; O_2 is electron acceptor in aerobic respiration
Nitrogen	14	NH_3, NO_3, organic compounds, N_2	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	8	H_2O, organic compounds, H_2	Main constituent of organic compounds and cell water
Phosphorus	3	inorganic phosphates (PO_4)	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulfur	1	SO_4, H_2S, S^o, organic sulfur compounds	Constituent of cysteine, methionine, glutathione, several coenzymes
Potassium	1	Potassium salts	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	0.5	Magnesium salts	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	0.5	Calcium salts	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	0.2	Iron salts	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions

Trace Elements

Table 1 ignores the occurrence of trace elements in bacterial nutrition. **Trace elements** are metal ions required by certain cells in such small amounts that it is difficult to detect (measure) them, and it is not necessary to add them to culture media as nutrients. Trace elements are required in such small amounts that they are present as "contaminants" of the water or other media components. As metal ions, the trace elements usually act as cofactors for essential enzymatic reactions in the cell. One organism's trace element may be another's required element and vice-versa, but the usual cations that qualify as trace elements in bacterial nutrition are Mn, Co, Zn, Cu, and Mo.

Carbon and Energy Sources for Bacterial Growth

In order to grow in nature or in the laboratory, a bacterium must have an energy source, a source of carbon and other required nutrients, and a permissive range of physical conditions such as O_2 concentration, temperature, and pH. Sometimes bacteria are referred to as individuals or groups based on their patterns of growth under various chemical (nutritional) or physical conditions. For example, phototrophs are organisms that use light as an energy source; anaerobes are organisms that grow without oxygen; thermophiles are organisms that grow at high temperatures.

All living organisms require a source of energy. Organisms that use radiant energy (light) are called **phototrophs**. Organisms that use (oxidize) an organic form of carbon are called **heterotrophs** or

chemo(hetero)trophs. Organisms that oxidize inorganic compounds are called **lithotrophs**.

The carbon requirements of organisms must be met by organic carbon (a chemical compound with a carbon-hydrogen bond) or by CO_2. Organisms that use organic carbon are **heterotrophs** and organisms that use CO_2 as a sole source of carbon for growth are called **autotrophs**.

Thus, on the basis of carbon and energy sources for growth four major nutritional types of procaryotes may be defined (Table 2).

Table 2. Major nutritional types of procaryotes

Nutritional Type	Energy Source	Carbon Source	Examples
Photoautotrophs	Light	CO_2	Cyanobacteria, some Purple and Green Bacteria
Photoheterotrophs	Light	Organic compounds	Some Purple and Green Bacteria
Chemoautotrophs or Lithotrophs (Lithoautotrophs)	Inorganic compounds, e.g. H_2, NH_3, NO_2, H_2S	CO_2	A few Bacteria and many Archaea
Chemoheterotrophs or Heterotrophs	Organic compounds	Organic compounds	Most Bacteria, some Archaea

Almost all eukaryotes are either photoautotrophic (e.g. plants and algae) or heterotrophic (e.g. animals, protozoa, fungi). Lithotrophy is unique to procaryotes and photoheterotrophy, common in the Purple and Green Bacteria, occurs only in a very few eukaryotic algae. Phototrophy has not been found in the Archaea, except for nonphotosynthetic light-driven ATP synthesis in the extreme halophiles.

Growth Factors

This simplified scheme for use of carbon, either organic carbon or CO_2, ignores the possibility that an organism, whether it is an autotroph or a heterotroph, may require small amounts of certain organic compounds for growth because they are essential substances that the organism is unable to synthesize from available nutrients. Such compounds are called **growth factors**.

Growth factors are required in small amounts by cells because they fulfill specific roles in biosynthesis. The need for a growth factor results from either a blocked or missing metabolic pathway in the cells. Growth factors are organized into three categories.

1. **purines and pyrimidines**: required for synthesis of nucleic acids (DNA and RNA)

2. **amino acids**: required for the synthesis of proteins

3. **vitamins**: needed as coenzymes and functional groups of certain enzymes

Some bacteria (e.g *E. coli*) do not require any growth factors: they can synthesize all essential purines, pyrimidines, amino acids and vitamins, starting with their carbon source, as part of their own intermediary metabolism. Certain other bacteria (e.g. *Lactobacillus*) require purines, pyrimidines, vitamins and several amino acids in order to grow. These compounds must be added in advance to culture media that are used to grow these bacteria. The growth factors are not metabolized directly as sources of carbon or energy, rather they are assimilated by cells to fulfill their specific role in metabolism. Mutant strains of bacteria that require some growth factor not needed by the wild type (parent) strain are referred to as **auxotrophs**. Thus, a strain of *E. coli* that requires the amino acid tryptophan in order to grow would be called a tryptophan auxotroph and would be designated *E. coli* ***trp-.***

Some vitamins that are frequently required by certain bacteria as growth factors are listed in Table 3. The function(s) of these vitamins in essential enzymatic reactions gives a clue why, if the cell cannot make the vitamin, it must be provided exogenously in order for growth to occur.

Culture Media for the Growth of Bacteria

For any bacterium to be propagated for any purpose it is necessary to provide the appropriate biochemical and biophysical environment. The biochemical (nutritional) environment is made available as a **culture medium**, and depending upon the special needs of particular bacteria (as well as particular investigators) a large variety and types of culture media have been developed with different purposes and uses. Culture media are employed in the isolation and maintenance of pure cultures of bacteria and are also used for identification of bacteria according to their biochemical and physiological properties.

The manner in which bacteria are cultivated, and the purpose of culture media, varies widely. **Liquid media** are used for growth of pure batch cultures, while solidified media are used widely for the isolation of pure cultures, for estimating viable bacterial populations, and a variety of other purposes. The usual gelling agent for solid or **semisolid medium** is **agar**, a hydrocolloid derived from red algae. Agar is used because of its unique physical properties (it melts at 100 degrees and remains liquid until cooled to 40 degrees, the temperature at which it gels) and because it cannot be metabolized by most bacteria. Hence as a medium component it is relatively inert; it simply holds (gels) nutrients that are in aquaeous solution.

Types of Culture Media

Culture media may be classified into several categories depending on their composition or use. A **chemically-defined (synthetic) medium** is one in which the exact chemical composition is known. A **complex (undefined) medium** is one in which the exact chemical constitution of the medium is not known. Defined media are usually composed of pure biochemicals off the shelf; complex media usually contain complex materials of biological origin such as blood or milk or yeast extract or beef extract, the exact chemical composition of which is obviously undetermined. A defined medium is a **minimal medium** if it provides only the exact nutrients (including any growth factors) needed by the organism for growth. The use of defined minimal media requires the investigator to know the exact nutritional requirements of the organisms in question. Chemically-defined media are of value in studying the minimal nutritional requirements of microorganisms, for enrichment cultures, and for a wide variety of physiological studies. Complex media usually provide the full range of growth factors that may be required by an organism so they may be more handily used to cultivate unknown bacteria or bacteria whose nutritional requirement are complex (i.e., organisms that require a lot of growth factors, known or unknown).

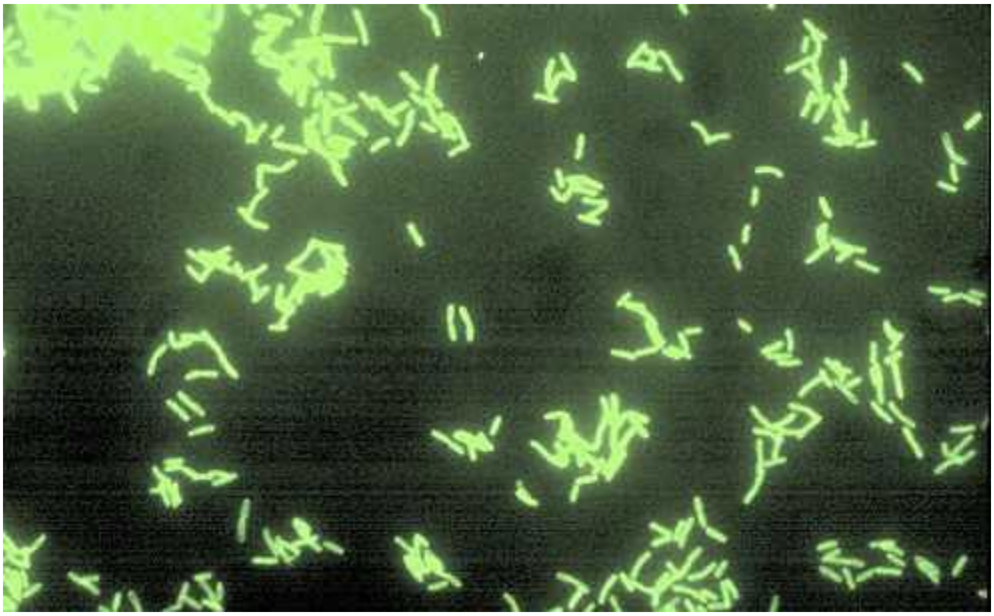


Figure 1. *Legionella pneumophila.* Direct fluorescent antibody (DFA) stain of a patient respiratory tract specimen. In spite of its natural ocurrence in water cooling towers and air conditioners, *Legionella* is a fastidious bacterium grown in the laboratory, which led to the long lag in identification of the first outbreak of Legionaire's disease in Philadelphia in 1977. Had fluoresent antibody to the bacterium

been available at that time, diagnosis could have been made as quickly as the time to prepare and view this slide.

Most pathogenic bacteria of animals, which have adapted themselves to growth in animal tissues, require complex media for their growth. Blood, serum and tissue extracts are frequently added to culture media for the cultivation of pathogens. Even so, for a few fastidious pathogens such as *Treponema pallidum*, the agent of syphilis, and *Mycobacterium leprae*, the cause of leprosy, artificial culture media and conditions have not been established. This fact thwarts the the ability to do basic research on these pathogens and the diseases that they cause.

Other concepts employed in the construction of culture media are the principles of selection and enrichment. A **selective medium** is one which has a component(s) added to it which will inhibit or prevent the growth of certain types or species of bacteria and/or promote the growth of desired species. One can also adjust the physical conditions of a culture medium, such as pH and temperature, to render it selective for organisms that are able to grow under these certain conditions.

A culture medium may also be a **differential medium** if allows the investigator to distinguish between different types of bacteria based on some observable trait in their pattern of growth on the medium. Thus a **selective, differential medium** for the isolation of *Staphylococcus aureus,* the most common bacterial pathogen of humans, contains a very high concentration of salt (which the staph will tolerate) that inhibits most other bacteria, mannitol as a source of fermentable sugar, and a pH indicator dye. From clinical specimens, only staph will grow.

S. aureus is differentiated from *S. epidermidis* (a nonpathogenic component of the normal flora) on the basis of its ability to ferment mannitol. Mannitol-fermenting colonies *(S. aureus)* produce acid which reacts with the indicator dye forming a colored halo around the colonies; mannitol non-fermenters (*S. epidermidis*) use other non-fermentative substrates in the medium for growth and do not form a halo around their colonies.

An enrichment medium employs a slightly different twist. An **enrichment medium** contains some component that permits the growth of specific types or species of bacteria, usually because they alone can utilize the component from their environment. However, an enrichment medium may have selective features. An enrichment medium for nonsymbiotic nitrogen-fixing bacteria omits a source of added nitrogen to the medium. The medium is inoculated with a potential source of these bacteria (e.g. a soil sample) and incubated in the atmosphere wherein the only source of nitrogen available is N_2. A selective enrichment medium for growth of the extreme halophile (*Halococcus*) contains nearly 25 percent salt [NaCl], which is required by the extreme halophile and which inhibits the growth of all other procaryotes.

Physical and Environmental Requirements for Microbial Growth

The procaryotes exist in nature under an enormous range of physical conditions such as O_2 concentration, Hydrogen ion concentration (pH) and temperature. The exclusion limits of life on the planet, with regard to environmental parameters, are always set by some microorganism, most often a procaryote, and frequently an Archaeon. Applied to all microorganisms is a vocabulary of terms used to describe their growth (ability to grow) within a range of physical conditions. A thermophile grows at high temperatures, an acidophile grows at low pH, an osmophile grows at high solute concentration, and so on. This nomenclature will be employed in this section to describe the response of the procaryotes to a variety of physical conditions.

The Effect of Oxygen

Oxygen is a universal component of cells and is always provided in large amounts by H_2O. However, procaryotes display a wide range of responses to molecular oxygen O_2 (Table 6).

Obligate aerobes require O_2 for growth; they use O_2 as a final electron acceptor in aerobic respiration.

Obligate anaerobes (occasionally called **aerophobes**) do not need or use O_2 as a nutrient. In fact, O_2 is a toxic substance, which either kills or inhibits their growth. Obligate anaerobic procaryotes may live by fermentation, anaerobic respiration, bacterial photosynthesis, or the novel process of methanogenesis.

Facultative anaerobes (or **facultative aerobes**) are organisms that can switch between aerobic and anaerobic types of metabolism. Under anaerobic conditions (no O_2) they grow by fermentation or anaerobic respiration, but in the presence of O_2 they switch to aerobic respiration.

Aerotolerant anaerobes are bacteria with an exclusively anaerobic (fermentative) type of metabolism but they are insensitive to the presence of O_2. They live by fermentation alone whether or not O_2 is present in their environment.

The response of an organism to O_2 in its environment depends upon the occurrence and distribution of various enzymes which react with O_2 and various oxygen radicals that are invariably generated by cells in the presence of O_2. All cells contain enzymes capable of reacting with O_2. For example, oxidations of flavoproteins by O_2 invariably result in the formation of H_2O_2 (peroxide) as one major product and small quantities of an even more toxic free radical, superoxide or $O_2^{\cdot -}$. Also, chlorophyll and other pigments in cells can react with O_2 in the presence of light and generate singlet oxygen, another radical form of oxygen which is a potent oxidizing agent in biological systems.

In aerobes and aerotolerant anaerobes the potential for lethal accumulation of superoxide is prevented by the enzyme superoxide dismutase (Figure 1). All organisms which can live in the presence of O_2 (whether or not they utilize it in their metabolism) contain superoxide dismutase. Nearly all organisms contain the enzyme catalase, which decomposes H_2O_2. Even though certain aerotolerant bacteria such as the lactic acid bacteria lack catalase, they decompose H_2O_2 by means of peroxidase enzymes which derive electrons from $NADH_2$ to reduce peroxide to H_2O. Obligate anaerobes lack superoxide dismutase and catalase and/or peroxidase, and therefore undergo lethal oxidations by various oxygen radicals when they are exposed to O_2. See Figure 2 below.

All photosynthetic (and some nonphotosynthetic) organisms are protected from lethal oxidations of singlet oxygen by their possession of carotenoid pigments which physically react with the singlet oxygen radical and lower it to its nontoxic "ground" (triplet) state. Carotenoids are said to "quench" singlet oxygen radicals.

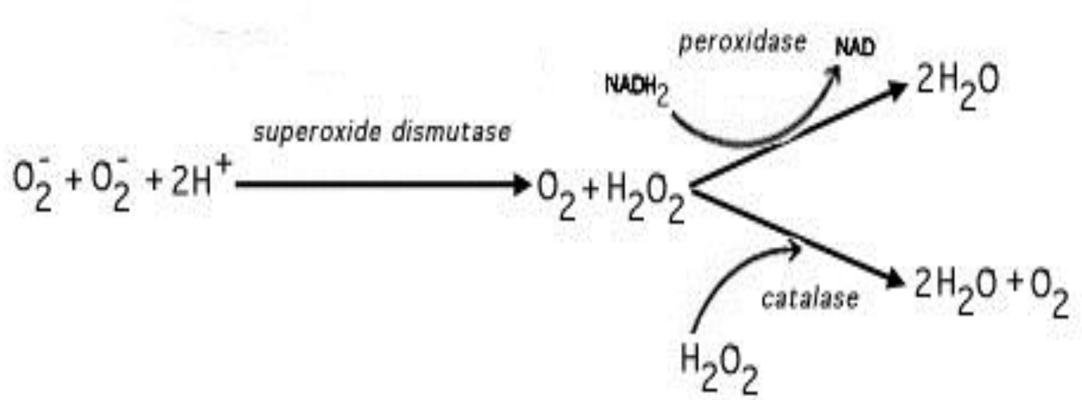


Figure 2. The action of superoxide dismutase, catalase and peroxidase. These enzymes detoxify oxygen radicals that are inevitably generated by living systems in the presence of O_2. The distribution of these enzymes in cells determines their ability to exist in the presence of O_2

The Effect of pH on Growth

The pH, or hydrogen ion concentration, $[H^+]$, of natural environments varies from about 0.5 in the most acidic soils to about 10.5 in the most alkaline lakes. Appreciating that pH is measured on a logarithmic scale, the $[H^+]$ of natural environments varies over a billion-fold and some microorganisms are living at the extremes, as well as every point between the extremes! Most free-living procaryotes can grow over a range of 3 pH units, about a thousand fold change in [H^+]. The range of pH over which an organism grows is defined by **three cardinal points**: the **minimum pH**, below which the organism cannot grow, the **maximum pH**, above which the organism cannot grow, and the **optimum pH**, at which the organism grows best. For most bacteria there is an orderly increase in growth rate between the minimum and the optimum and a corresponding orderly decrease in growth rate between the optimum and the maximum pH, reflecting the general effect of changing [H^+] on the rates of enzymatic reaction (Figure 3).

Microorganisms which grow at an optimum pH well below neutrality (7.0) are called **acidophiles**. Those which grow best at neutral pH are called **neutrophiles** and those that grow best under alkaline conditions are called **alkaliphiles**. Obligate acidophiles, such as some *Thiobacillus* species, actually require a low pH for growth since their membranes dissolve and the cells lyse at neutrality. Several genera of Archaea, including *Sulfolobus* and *Thermoplasma*, are obligate acidophiles. Among eukaryotes, many fungi are acidophiles, but the champion of growth at low pH is the eukaryotic alga *Cyanidium* which can grow at a pH of 0.

In the construction and use of culture media, one must always consider the optimum pH for growth of a desired organism and incorporate **buffers** in order to maintain the pH of the medium in the changing milieu of bacterial waste products that accumulate during growth. Many pathogenic bacteria exhibit a relatively narrow range of pH over which they will grow. Most diagnostic media for the growth and identification of human pathogens have a pH near 7.

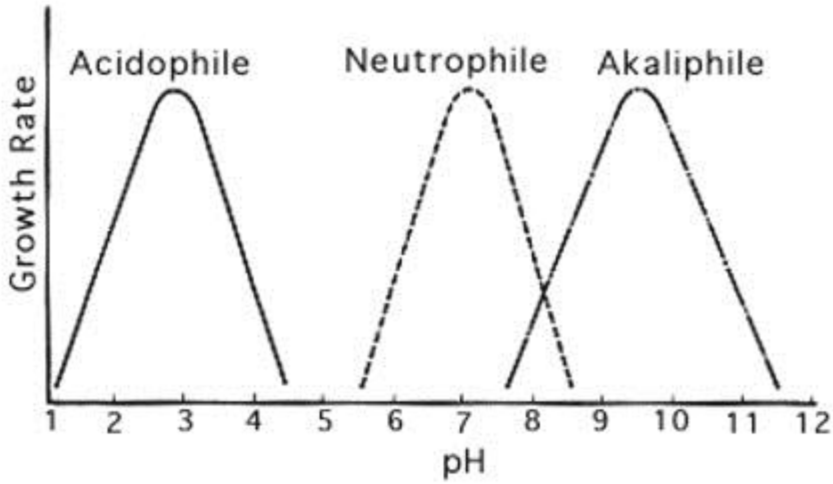


Figure 3. Growth rate vs pH for three environmental classes of procaryotes. Most free-living bacteria grow over a pH range of about three units. Note the symmetry of the curves below and above the optimum pH for growth.

The Effect of Temperature on Growth

Microorganisms have been found growing in virtually all environments where there is liquid water, regardless of its temperature. In 1966, Professor Thomas D. Brock, then at Indiana University, made the amazing discovery in boiling hot springs of Yellowstone National Park that bacteria were not just surviving there, they were growing and flourishing.

Subsequently, procaryotes have been detected growing around black smokers and hydrothermal vents in the deep sea at temperatures at least as high as 120 degrees. Microorganisms have been found growing at very low temperatures as well. In supercooled solutions of H_2O as low as -20 degrees, certain organisms can extract water for growth, and many forms of life flourish in the icy waters of the Antarctic, as well as household refrigerators, near 0 degrees.

A particular microorganism will exhibit a range of temperature over which it can grow, defined by three cardinal points in the same manner as pH (Figure 5, cf. Figure 3). Considering the total span of temperature where liquid water exists, the procaryotes may be subdivided into several subclasses on the basis of one or another of their cardinal points for growth. For example, organisms with an optimum temperature near 37 degrees (the body temperature of warm-blooded animals) are called **mesophiles**. Organisms with an optimum T between about 45 degrees and 70 degrees are **thermophiles**. Some Archaea with an optimum T of 80 degrees or higher and a maximum T as high as 115 degrees, are now referred to as **extreme thermophiles** or **hyperthermophiles**. The cold-loving organisms are **psychrophiles** defined by their ability to grow at 0 degrees. A variant of a psychrophile (which usually has an optimum T of 10-15 degrees) is a **psychrotroph**, which grows at 0 degrees but displays an optimum T in the mesophile range, nearer room temperature. Psychrotrophs are the scourge of food storage in refrigerators since they are invariably brought in from their mesophilic habitats and continue to grow in the refrigerated environment where they spoil the food. Of course, they grow slower at 2 degrees than at 25 degrees. Think how fast milk spoils on the counter top versus in the refrigerator.

Psychrophilic bacteria are adapted to their cool environment by having largely unsaturated fatty acids in their plasma membranes. Some psychrophiles, particularly those from the Antarctic have been found to contain polyunsaturated fatty acids, which generally do not occur in procaryotes. The degree of unsaturation of a fatty acid correlates with its solidification T or thermal transition stage (i.e., the temperature at which the lipid melts or solidifies); unsaturated fatty acids remain liquid at low T but are also denatured at moderate T; saturated fatty acids, as in the membranes of thermophilic bacteria, are stable at high temperatures, but they also solidify at relatively high T. Thus, saturated fatty acids (like butter) are solid at room temperature while unsaturated fatty acids (like safflower oil) remain liquid in the refrigerator. Whether fatty acids in a membrane are in a liquid or a solid phase affects the fluidity of the membrane, which directly affects its ability to function. Psychrophiles also have enzymes that continue to function, albeit at a reduced rate, at temperatures at or near 0 degrees. Usually, psychrophile proteins and/or membranes, which adapt them to low temperatures, do not function at the body temperatures of warm-blooded animals (37 degrees) so that they are unable to grow at even moderate temperatures.

Thermophiles are adapted to temperatures above 60 degrees in a variety of ways. Often thermophiles have a high G + C content in their DNA such that the melting point of the DNA (the temperature at which the strands of the double helix separate) is at least as high as the organism's maximum T for growth. But this is not always the case, and the correlation is far from perfect, so thermophile DNA must be stabilized in these cells by other means. The membrane fatty acids of thermophilic bacteria are highly saturated allowing their membranes to remain stable and functional at high temperatures. The membranes of hyperthermophiles, virtually all of which are Archaea, are not composed of fatty acids but of repeating subunits of the C5 compound, phytane, a branched, saturated, "isoprenoid" substance, which contributes heavily to the ability of these bacteria to live in superheated environments. The structural proteins (e.g. ribosomal proteins, transport proteins (permeases) and enzymes of thermophiles and hyperthermophiles are very heat stable compared with their mesophilic counterparts. The proteins are modified in a number of ways including dehydration and through slight changes in their primary structure, which accounts for their thermal stability.

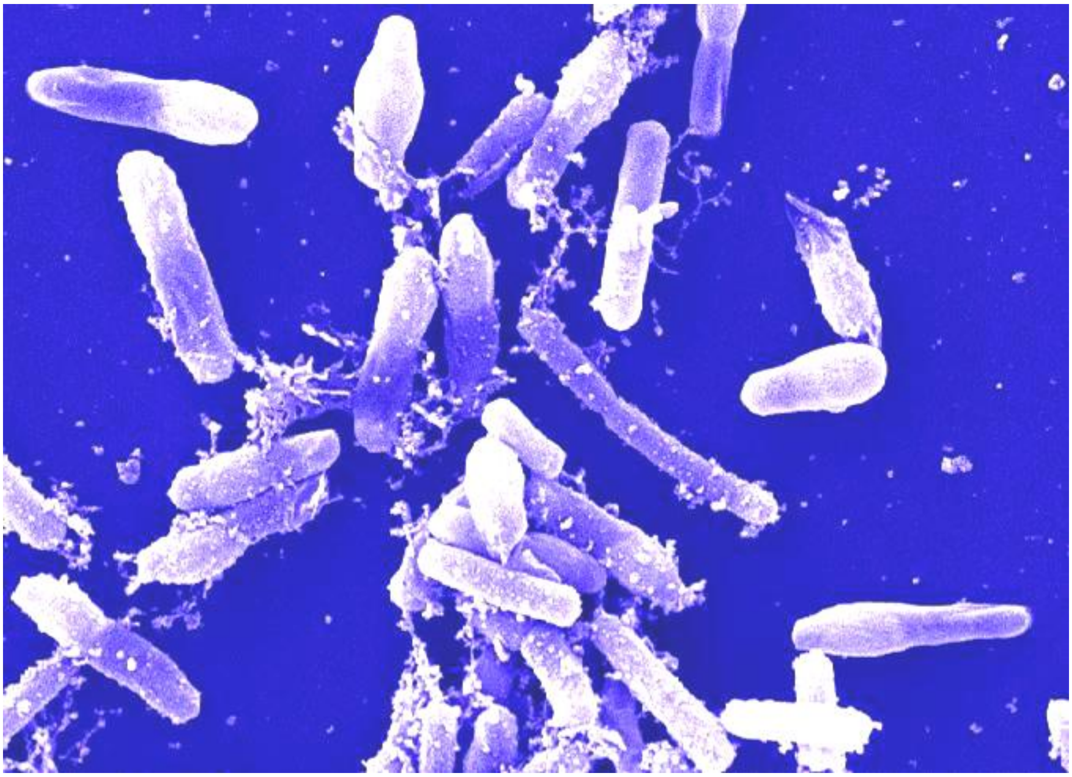


Figure 4. SEM of a thermophilic *Bacillus* species isolated from a compost pile at 55° C. The rods are 3-5 microns in length and 0.5 to 1 micron in width with terminal endospores in a slightly-swollen sporangium.

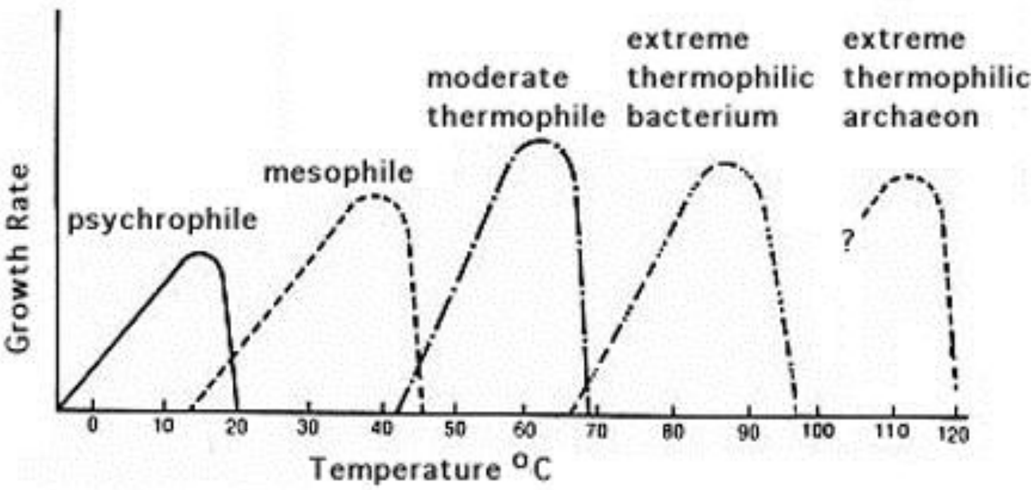


Figure 5. Growth rate vs temperature for five environmental classes of procaryotes. Most procaryotes will grow over a temperature range of about 30 degrees. The curves exhibit three cardinal points: minimum, optimum and maximum temperatures for growth. There is a steady increase in growth rate between the minimum and optimum temperatures, but slightly past the optimum a critical thermolabile cellular event occurs, and the growth rates plunge rapidly as the maximum T is approached. As expected and as predicted by T.D. Brock, life on earth, with regard to temperature, exists wherever water remains in a liquid state. Thus, psychrophiles grow in solution wherever water is supercooled below 0 degrees; and extreme thermophilic archaea (hyperthermophiles) have been identified growing near deep-sea thermal vents at temperatures up to 120 degrees. Theoretically, the bar can be pushed to even higher temperatures.

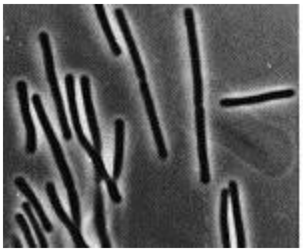
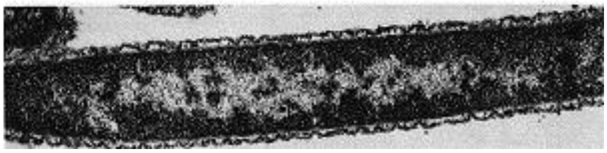
 

Figure 6. ***Thermus aquaticus,*** **the thermophilic bacterium that is the source of taq polymerase. L wet mount; R electron micrograph.**

Water Availability

Water is the solvent in which the molecules of life are dissolved, and the availability of water is therefore a critical factor that affects the growth of all cells. The availability of water for a cell depends upon its presence in the atmosphere (relative humidity) or its presence in solution or a substance (**water activity**). The water activity (A_w) of pure H_2O is 1.0 (100% water). Water activity is affected by the presence of solutes such as salts or sugars, that are dissolved in the water. The higher the solute concentration of a substance, the lower is the water activity and vice-versa. Microorganisms live over a range of A_w from 1.0 to 0.7. The A_w of human blood is 0.99; seawater = 0.98; maple syrup = 0.90; Great Salt Lake = 0.75. Water activities in agricultural soils range between 0.9 and 1.0.

The only common solute in nature that occurs over a wide concentration range is salt [NaCl], and some microorganisms are named based on their growth response to salt. Microorganisms that require some NaCl for growth are **halophiles**. **Mild halophiles** require 1-6% salt, **moderate halophiles** require 6-15% salt; **extreme**

halophiles that require 15-30% NaCl for growth are found among the archaea. Bacteria that are able to grow at moderate salt concentrations, even though they grow best in the absence of NaCl, are called **halotolerant**. Although halophiles are "osmophiles" (and halotolerant organisms are "osmotolerant") the term **osmophiles** is usually reserved for organisms that are able to live in environments high in sugar. Organisms which live in dry environments (made dry by lack of water) are called **xerophiles**.

The concept of lowering water activity in order to prevent bacterial growth is the basis for preservation of foods by drying (in sunlight or by evaporation) or by addition of high concentrations of salt or sugar.

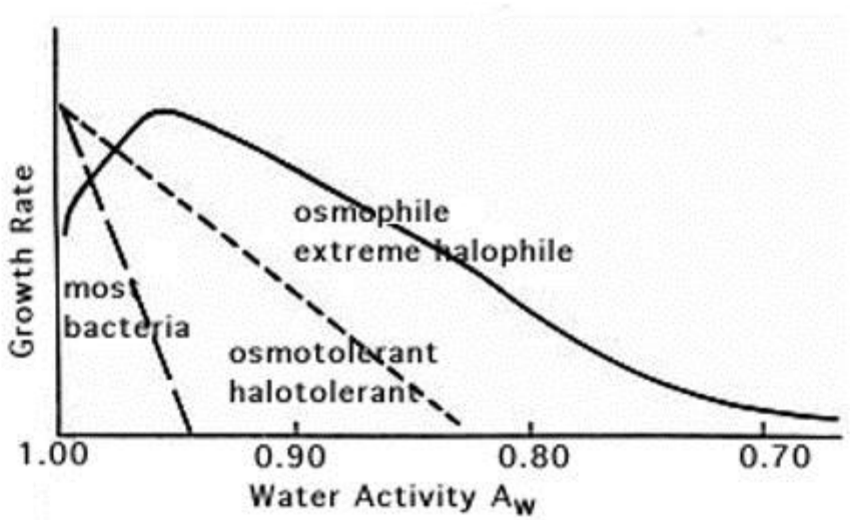


Figure 7. Growth rate vs osmolarity for different classes of procaryotes. Osmolarity is determined by solute concentration in the environment. Osmolarity is inversely related to water activity (A_w), which is more like a measure of the concentration of water (H_2O) in a solution. Increased solute concentration means increased osmolarity and decreased A_w. TFrom left to right the graph shows

the growth rate of a normal (nonhalophile) such as *E. coli* or *Pseudomonas,* the growth rate of a halotolerant bacterium such as *Staphylococcus aureus,* and the growth rate of an extreme halophile such as the archaean *Halococcus.*Note that a true halophile grows best at salt concentrations where most bacteria are inhibited.

GROWTH OF BACTERIAL POPULATIONS

Measurement of Bacterial Growth

Growth is an orderly increase in the quantity of cellular constituents. It depends upon the ability of the cell to form new protoplasm from nutrients available in the environment. In most bacteria, growth involves increase in cell mass and number of ribosomes, duplication of the bacterial chromosome, synthesis of new cell wall and plasma membrane, partitioning of the two chromosomes, septum formation, and cell division. This asexual process of reproduction is called **binary fission**.

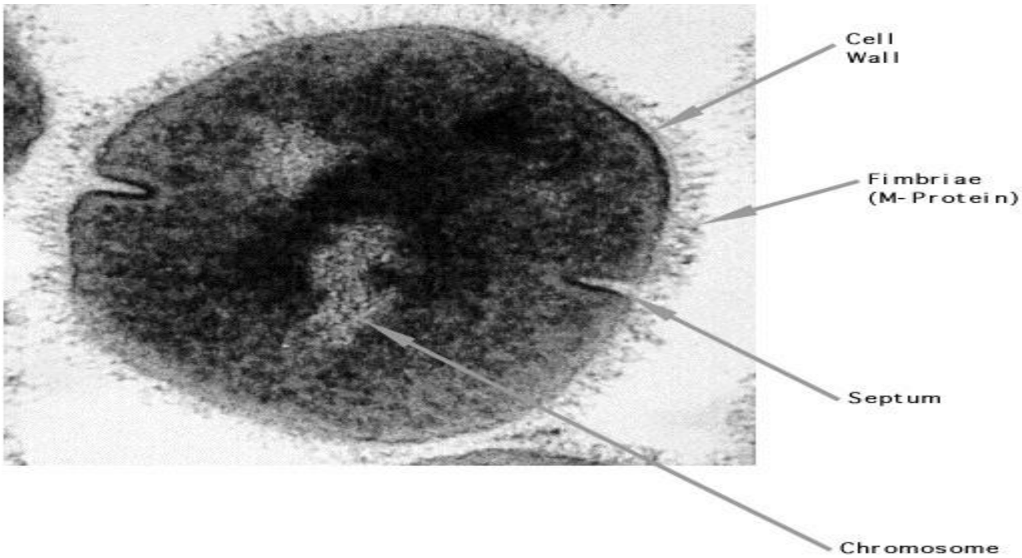


Figure 1. Bacterial growth by binary fission. Most bacteria reproduce by a relatively simple asexual process called binary fission: each cell increases in size and divides into two cells. During this process there is an orderly increase in cellular structures and components, replication and segregation of the bacterial DNA, and formation of a septum or cross wall which divides the cell into two progeny cells. The process is coordinated by the bacterial membrane perhaps by means of mesosomes. The DNA molecule is believed to be attached to a point on the membrane where it is replicated. The two DNA molecules remain attached at points side-by-side on the membrane while new membrane material is synthesized between the two points. This draws the DNA molecules in opposite directions while new cell wall and membrane are laid down as a septum between the two chromosomal compartments. When septum formation is complete the cell splits into two progeny cells. The time interval required for a bacterial cell to divide or for a population of bacterial cells to double is called the generation time. Generation times for bacterial species growing in nature may be as short as 15 minutes or as long as several days. Electron micrograph of *Streptococcus pyogenes*.

For unicellular organisms such as the bacteria, growth can be measured in terms of two different parameters: changes in **cell mass** and changes in **cell numbers**.

Methods for Measurement of Cell Mass

Methods for measurement of the cell mass involve both direct and indirect techniques.

1. Direct **physical measurement** of dry weight, wet weight, or volume of cells after centrifugation.

2. Direct **chemical measurement** of some chemical component of the cells such as total N, total protein, or total DNA content.

3. Indirect **measurement of chemical activity** such as rate of O_2 production or consumption, CO_2 production or consumption, etc.

4. **Turbidity measurements** employ a variety of instruments to determine the amount of light scattered by a suspension of cells. Particulate objects such as bacteria scatter light in proportion to their numbers. The turbidity or **optical density** of a suspension of cells is directly related to cell mass or cell number, after construction and calibration of a standard curve. The method is simple and nondestructive, but the sensitivity is limited to about 10^7 cells per ml for most bacteria.

Methods for Measurement of Cell Numbers

Measuring techniques involve direct counts, visually or instrumentally, and indirect viable cell counts.

1. **Direct microscopic counts** are possible using special slides known as counting chambers. Dead cells cannot be distinguished from living ones. Only dense suspensions can be counted (>10^7 cells per ml), but samples can be concentrated by centrifugation or filtration to increase sensitivity.

A variation of the direct microscopic count has been used to observe and measure growth of bacteria in natural environments. In order to detect and prove that thermophilic bacteria were growing in boiling hot springs, T.D. Brock immersed microscope slides in the springs and withdrew them periodically for microscopic observation. The bacteria in the boiling water attached to the glass slides naturally and grew as microcolonies on the surface.

2. **Electronic counting chambers** count numbers and measure size distribution of cells. For cells the size of bacteria the suspening medium must be very clean. Such elecronic devices are more often used to count eukaryotic cells such as blood cells.

3. **Indirect viable cell counts**, also called **plate counts**, involve plating out (spreading) a sample of a culture on a nutrient agar surface. The sample or cell suspension can be diluted in a nontoxic diluent (e.g. water or saline) before plating. If plated on a suitable medium, each

viable unit grows and forms a colony. Each colony that can be counted is called a **colony forming unit (cfu)** and the number of cfu's is related to the viable number of bacteria in the sample.

Advantages of the technique are its sensitivity (theoretically, a single cell can be detected), and it allows for inspection and positive identification of the organism counted. Disadvantages are (1) only living cells develop colonies that are counted; (2) clumps or chains of cells develop into a single colony; (3) colonies develop only from those organisms for which the cultural conditions are suitable for growth. The latter makes the technique virtually useless to characterize or count the **total number of bacteria** in complex microbial ecosystems such as soil or the animal rumen or gastrointestinal tract. Genetic probes can be used to demonstrate the diversity and relative abundance of procaryotes in such an environment, but many species identified by genetic techniques have so far proven unculturable.

The Bacterial Growth Curve

In the laboratory, under favorable conditions, a growing bacterial population doubles at regular intervals. Growth is by geometric progression: 1, 2, 4, 8, etc. or 2^0, 2^1, 2^2, 2^3.........2^n (where n = the number of generations). This is called **exponential growth**. In reality, exponential growth is only part of the bacterial life cycle, and not representative of the normal pattern of growth of bacteria in Nature.

When a fresh medium is inoculated with a given number of cells, and the population growth is monitored over a period of time, plotting the data will yield a **typical bacterial growth curve** (Figure 2).

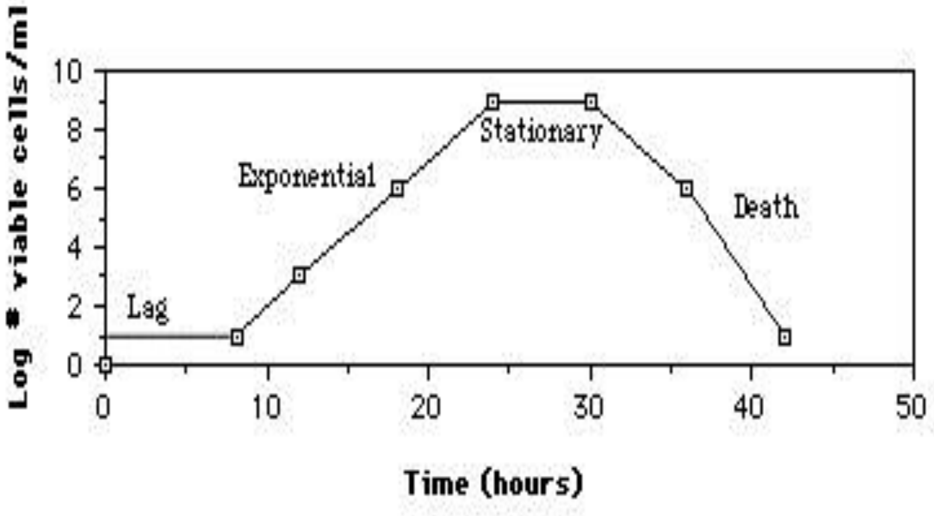


Figure 2. The typical bacterial growth curve. When bacteria are grown in a closed system (also called a batch culture), like a test tube, the population of cells almost always exhibits these growth dynamics: cells initially adjust to the new medium (lag phase) until they can start dividing regularly by the process of binary fission (exponential phase). When their growth becomes limited, the cells stop dividing (stationary phase), until eventually they show loss of viability (death phase). Note the parameters of the x and y axes. Growth is expressed as change in the number viable cells vs time. Generation times are calculated during the exponential phase of growth. Time measurements are in hours for bacteria with short generation times.

Four characteristic phases of the growth cycle are recognized.

1. **Lag Phase**. Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged. Although there is no apparent cell division occurring, the cells may be growing in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity.

The length of the lag phase is apparently dependent on a wide variety of factors including the size of the inoculum; time necessary to recover from physiacal damage or shock in the transfer; time required for synthesis of essential coenzymes or division factors; and time required for synthesis of new (inducible) enzymes that are necessary to metabolize the substrates present in the medium.

2. **Exponential (log) Phase**. The exponential phase of growth is a pattern of balanced growth wherein all the cells are dividing regularly by binary fission, and are growing by geometric progression. The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation. The rate of exponential growth of a bacterial culture is expressed as **generation time**, also the **doubling time** of the bacterial population. Generation time (G) is defined as the time (t) per generation (n = number of generations). Hence, $G=t/n$ is the equation from which calculations of generation time (below) derive.

3. **Stationary Phase**. Exponential growth cannot be continued forever in a **batch culture** (e.g. a closed system such as a test tube or flask).

Population growth is limited by one of three factors: 1. exhaustion of available nutrients; 2. accumulation of inhibitory metabolites or end products; 3. exhaustion of space, in this case called a lack of "biological space".

During the stationary phase, if viable cells are being counted, it cannot be determined whether some cells are dying and an equal number of cells are dividing, or the population of cells has simply stopped growing and dividing. The stationary phase, like the lag phase, is not necessarily a period of quiescence. Bacteria that produce **secondary metabolites**, such as antibiotics, do so during the stationary phase of the growth cycle (Secondary metabolites are defined as metabolites produced after the active stage of growth). It si during the stationary phase that spore-forming bacteria have to induce or unmask the activity of dozens of genes that may be involved in sporulation process.

4. **Death Phase**. If incubation continues after the population reaches stationary phase, a death phase follows, in which the viable cell population declines. (Note, if counting by turbidimetric measurements or microoscopic counts, the death phase cannot be observed.). During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase.

THE CONTROL OF MICROBIAL GROWTH

Introduction

The control of microbial growth is necessary in many practical situations, and significant advances in agriculture, medicine, and food science have been made through study of this area of microbiology.

"Control of growth", as used here, means to prevent growth of microorganisms. This control is affected in two basic ways: (1) by killing microorganisms or (2) by inhibiting the growth of microorganisms. Control of growth usually involves the use of physical or chemical agents which either kill or prevent the growth of microorganisms. Agents which kill cells are called **cidal** agents; agents which inhibit the growth of cells (without killing them) are referred to as **static** agents. Thus the term **bactericidal** refers to killing bacteria and **bacteriostatic** refers to inhibiting the growth of bacterial cells. A **bactericide** kills bacteria, a **fungicide** kills fungi, and so on.

Sterilization is the complete destruction or elimination of all viable organisms (in or on an object being sterilized). There are no degrees of sterilization: an object is either sterile or not. Sterilization procedures involve the use of heat, radiation or chemicals, or physical removal of cells.

Methods of Sterilization

Heat: most important and widely used. For sterilization always consider type of heat, time of application and temperature to ensure destruction of all microorganisms. Endospores of bacteria are considered the most thermoduric of all cells so their destruction guarantees sterility.

Incineration: burns organisms and physically destroys them. Used for needles , inoculating wires, glassware, etc. and objects not destroyed in the incineration process.

Boiling: 100^o for 30 minutes. Kills everything except some endospores (Actually, for the purposes of purifying drinking water 100^o for five minutes is probably adequate though there have been some reports that Giardia cysts can survive this process). To kill endospores, and therefore **sterilize** the solution, very long or **intermittent boiling** is required.

Autoclaving (steam under pressure or pressure cooker): 121^o for 15 minutes (15#/in^2 pressure). Good for sterilizing almost anything, but heat-labile substances will be denatured or destroyed.

Dry heat (hot air oven): 160^o/2hours or 170^o/1hour. Used for glassware, metal, and objects that won't melt.

The protocol and recommendations for the use of heat to control microbial growth

Irradiation: usually destroys or distorts nucleic acids. Ultraviolet light is usually used (commonly used to sterilize the surfaces of objects), although x-rays and microwaves are possibly useful.

Filtration: involvres the physical removal (exclusion) of all cells in a liquid or gas, especially important to sterilize solutions which would be denatured by heat (e.g. antibiotics, injectable drugs, amino acids, vitamins, etc.)

Chemical and gas: (formaldehyde, glutaraldehyde, ethylene oxide) toxic chemicals kill all forms of life in a specialized gas chamber.

Control of Microbial Growth by Physical Agents

Applications of Heat The lethal **temperature** varies in microorganisms. The **time** required to kill depends on the number of organisms, species, nature of the product being heated, pH, and temperature. Whenever heat is used to control microbial growth inevitably **both time and temperature are considered**.

Sterilization (boiling, autoclaving, hot air oven) kills all microorganisms with heat; commonly employed in canning, bottling, and other sterile packaging procedures.

Pasteurization is the use of mild heat to reduce the number of microorganisms in a product or food. In the case of pasteurization of milk the time and temperature depend on killing potential pathogens that are transmitted in milk, i.e., staphylococci, streptococci, Brucella abortus and Mycobacterium tuberculosis. For pasteurzation of milk: batch nethod: 63^{o}/30minutes; flash method: 71^{o}/15 seconds.

Low temperature (refrigeration and freezing): Most organisms grow very little or not at all at 0o. Store perishable foods at low temperatues to slow rate of growth and consequent spoilage (e.g. milk). Low temperatures are not bactericidal. Psychrotrophs, rather than true psychrophiles, are the usual cause of food spoilage in refrigerated foods.

Drying (removal of H_2O): Most microorganisms cannot grow at reduced water activity (Aw < 0.90). Often used to preserve foods (e.g. fruits, grains, etc.). Methods involve removal of water from product by heat, evaporation, freeze-drying, addition of salt or sugar.

Irradiation (microwave, UV, x-ray): destroys microorganisms as described under "sterilization". Many spoilage organisms are easily killed by irradiation. In some parts of Europe, fruits and vegetables are irradiated to increase their shelf life up to 500 percent. The practice has not been accepted in the U.S.

Control of microbial growth by chemical agents

Antimicrobial agents are chemicals that kill or inhibit the growth microorganisms. Antimicrobial agents include chemical preservatives and antiseptics, as well as drugs used in the treatment of infectious diseases of plants and animals. Antimicrobial agents may be of natural or synthetic origin, and they may have a static or cidal effect on microorganisms.

Types of antimicrobial agents

Antiseptics: microbicidal agents harmless enough to be applied to the skin and mucous membrane; should not be taken internaslly. Examples: mercurials, silver nitrate, iodine solution, alcohols, detergents.

Disinfectants: Agents that kill microorganisms, but not necessarily their spores,not safe for application to living tissues; they are used on inanimate objects such as tables, floors, utensils, etc. Examples: chlorine, hypochlorites, chlorine compounds, lye, copper sulfate, quaternary ammonium compounds.

Note: disinfectants and antiseptics are distinguished on the basis of whether they are safe for application to mucous membranes. Often, safety depends on the concentration of the compound. For example, sodium hypochlorite (chlorine), as added to water is safe for drinking, but "chlorox" (5% hypochlorite), an excellent disinfectant, is hardly safe to drink.

Preservatives: static agents used to inhibit the growth of microorganisms, most often in foods. If eaten they should be nontoxic. Examples; calcium propionate, sodium benzoate, formaldehyde, nitrate, sulfur dioxide. Table 3 is a list of common preservative and their uses.

Chemotherapeutic agents: antimicrobial agents of synthetic origin useful in the treatment of microbial or viral disease. Examples: sulfonilamides, isoniazid, ethambutol, AZT, chloramphenicol. Note that the microbiologist's definition of a chemotherapeutic agent requires that the agent be used for antimicrobial purposes and so excludes synthetic agents used for therapy against diseases that are not of microbial origin.

Antibiotics: antimicrobial agents produced by microorganisms that kill or inhibit other microorganisms. This is the microbiologist's definition. A more broadened definition of an antibiotic includes any chemical of natural origin (from any type of cell) which has the effect to kill or inhibit the growth of other types cells. Since most clinically-useful antibiotics are produced by microorganisms and are used to kill or inhibit infectious Bacteria, we will follow the classic definition.

Antibiotics are low molecular-weight (non-protein) molecules produced as secondary metabolites, mainly by microorganisms that live in the soil. Most of these microorganisms form some type of a spore or other dormant cell, and there is thought to be some relationship (besides temporal) between antibiotic production and the processes of sporulation. Among the molds, the notable antibiotic producers are Penicillium and Cephalosporium , which are the main source of the beta-lactam antibiotics (penicillin and its relatives). In the Bacteria, the Actinomycetes, notably Streptomyces species, produce a variety of types of antibiotics including the aminoglycosides (e.g. streptomycin), macrolides (e.g. erythromycin), and the tetracyclines. Endospore-forming Bacillus species produce polypeptide antibiotics such as polymyxin and bacitracin.

IMPORTANT GROUPS OF PROCARYOTES

BACTERIA

Phylogenetic analysis of the **Bacteria** has demonstrated the existence of at least 13 distinct groups, but many groups consist of members that are phenotypically and physiologically unrelated, and sometimes phylogenetically unrelated. The current edition of Bergey's Manual of Systematic Bacteriology (2001) recognizes 23 distinct phyla of Bacteria (Phylum is the highest taxon in a Domain), but there may still be great variation in phenotype among members. Below we discuss the major groups of Bacteria based on morphology, physiology, or ecology, and often use informal, but familiar, terms to identify them.

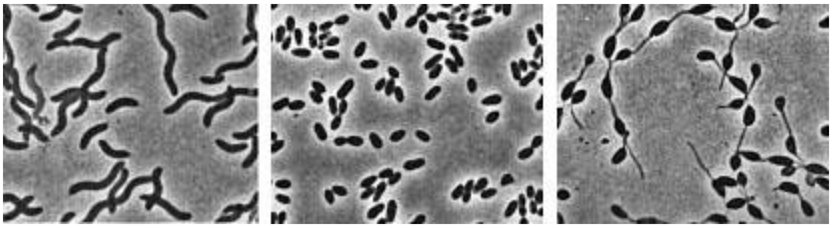
Photosynthetic purple and green bacteria. These bacteria conduct **anoxygenic photosynthesis**, also called **bacterial photosynthesis**. Bacterial photosynthesis differs from plant-type (oxygenic) photosynthesis in several ways. Bacterial photosynthesis does not produce O_2; in fact, it only occurs under anaerobic conditions. Bacterial photosynthesis utilizes a type of chlorophyll other than chlorophyll *a,* and only one photosystem, photosystem I. The electron donor for bacterial photosynthesis is never H_2O but may be H_2, H_2S or S^o, or certain organic compounds. The light-absorbing pigments of the purple and green bacteria consist of bacterial chlorophylls and carotenoids. Phycobilins, characteristic of the cyanobacteria, are not found. Many purple and green sulfur bacteria store elemental sulfur as a reserve material that can be further oxidized to SO_4 as a photosynthetic electron donor.

The **purple and green bacteria** may use H_2S during photosynthesis in the same manner that cyanobacteria or algae or plants use H_2O as an electron donor for autotrophic CO_2 reduction (the "dark reaction" of photosynthesis). Or they may utilize organic compounds as electron donors for photosynthesis. For example, *Rhodobacter* can use light as an energy source while oxidizing succinate or butyrate in order to obtain electrons for CO_2 fixation.

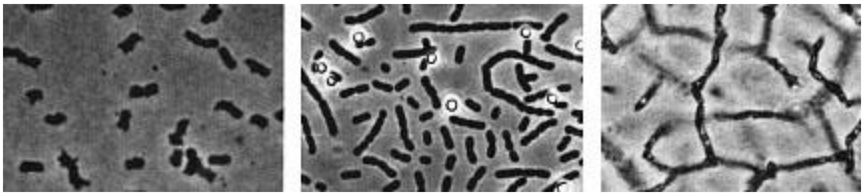
The bacterium that became an endosymbiont of eucaryotes and evolved into mitochondria is thought to be a relative of the purple nonsulfur bacteria. This conclusion is based on similar metabolic features of mitochondria and purple nonsulfur bacteria and on comparisons of the base sequences in their 16S rRNAs.

Figure 1. Photomicrographs (phase contrast and and ordinary illumination) of various photosynthetic bacteria. Magnifications are about 1400X. The purple and green bacteria exhibit a full range of procaryotic morphologies, as these photomicrographs illustrate. Diversity among their phylogenetic relationships is also noted.

A. Purple sulfur bacteria (L to R): *Chromatium vinosum, Thiospirillum jenense, Thiopedia rosea*.



B. Purple nonsulfur bacteria (L to R): *Rhodospirillum rubrum, Rhodobacter sphaeroides, Rhodomicrobium vannielii.* The purple nonsulfur bacteria are in the Alphaproteobacteria, which also includes *Rhizobium, Agrobacterium* and the Rickettsias. The latter bacteria represent a direct lineage to mitochondria.



C. Green sulfur bacteria (L to R): *Chlorobium limicola, Prosthecochloris aestuarii, Pelodictyon clathratiforme.* The Green sulfur bacteria represent a distinct phylogenetic lineage and cluster in their own phylum represented by *Chlorobium* .

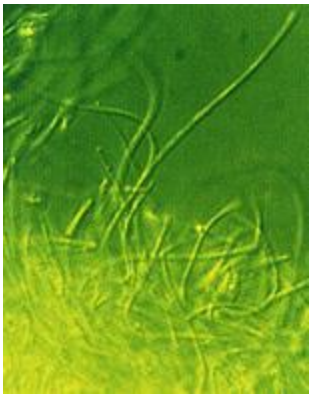


Figure 2. Green nonsulfur bacterium, *Chloroflexus* (T.D. Brock). *Chloroflexus* also represents a phylogenetically distinct group of green bacteria. *Chloroflexus* is a thermophilic, filamentous gliding bacterium.

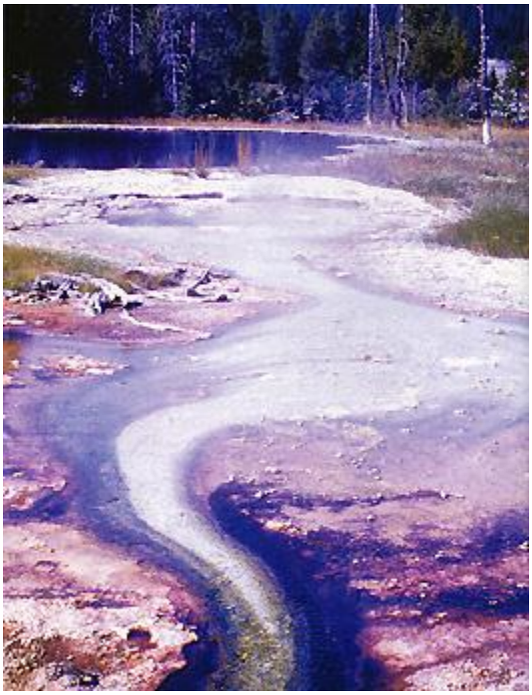


Figure 3. Photosynthetic procaryotes growing in a hot spring run-off channel (T.D. Brock). The white area of the channel is too hot for photosynthetic life, but as the water cools along a gradient, the colored phototrophic bacteria colonize and ultimately construct the colored microbial mats composed of a consortium of photosynthetic microorganisms.

Cyanobacteria. The cyanobacteria deserve special emphasis because of their great ecological importance in the global carbon, oxygen and nitrogen cycles, as well as their evolutionary significance in relationship to plants. Photosynthetic cyanobacteria have chlorophyll *a* and carotenoids in addition to some unusual accessory pigments named **phycobilins**. The blue pigment, **phycocyanin** and the red one, **phycoerythrin**, absorb wavelengths of light for photosynthesis that are missed by chlorophyll and the carotenoids. Within the cytoplasm of cyanobacteria are numerous layers of membranes, often parallel to one another. These membranes are photosynthetic thylakoids that resemble those found in chloroplasts, which, in fact, correspond in size to the entire cyanobacterial cell. The main storage product of the cyanobacteria is glycogen, and glycogen inclusions may be seen in the cytoplasm of the cells. Cyanobacteria are thought to have given rise to eucaryotic chloroplasts during the evolutionary events of endosymbiosis. In biochemical detail, cyanobacteria are especially similar to the chloroplasts of red algae (*Rhodophyta*).

Most cyanobacteria have a mucilaginous sheath, or coating, which is often deeply pigmented, particularly in species that occur in terrestrial habitats. The colors of the sheaths in different species include light gold, yellow, brown, red, green, blue, violet, and blue-black. It is these pigments that impart color to individual cells and colonies as well as to "blooms" of cyanobacteria in aquatic environments

Figure 4. Some common cyanobacteria L to R: *Oscillatoria,* a filamentous species common in fresh water and hot springs; *Nostoc,* a sheathed communal species; *Anabaena,* a nitrogen-fixing species. The small cell with an opaque surface (third from right) in the anabaena filament is a heterocyst, a specialized cell for nitrogen fixation. The large bright cell in the filament is a type of spore called an akinete; *Synechococcus,* a

unicelluar species in marine habitats and hot springs. *Synechococcus* is among the most important photosynthetic bacteria in the marine environment, estimated to account for about 25 percent of the primary production that occurs in typical marine habitats.

Although thousands of cyanobacteria have been observed, only about 200 species have been identified as distinct, free-living, nonsymbiotic procaryotes. Relative to other oxygenic phototrophs, cyanobacteria often grow under fairly extreme environmental conditions such as high temperature and salinity . They are the only oxygenic phototrophs present in many hot springs of the Yellowstone ecosystem; and in frigid lakes and oceans of Antarctica, they form luxuriant mats 2 to 4 centimeters thick in water beneath more than five meters of permanent ice. However, cyanobacteria are absent in acidic waters where their eucaryotic counterparts, the algae, may be abundant.

Layered chalk deposits called **stromatolites**, which exhibit a continuous geologic record covering 2.7 billion years, are produced when colonies of cyanobacteria bind calcium-rich sediments. Today, stromatolites are formed in only a few places, such as shallow pools in hot dry climates. The abundance of cyanobacteria in the fossil record is evidence of the early development of the cyanobacteria and their important role in elevating the level of free oxygen in the atmosphere of the early Earth.

Cyanobacteria often form filaments and may grow in large masses or "tufts" one meter or more in length. Some are unicellular, a few form branched filaments, and a few form irregular plates or irregular colonies. Cyanobacterial cells usually divide by binary fission, and the resulting progeny cells may separate to form new colonies. In addition, filaments may break into fragments, called **hormogonia**, which separate and develop into new colonies. As in other filamentous or colonial bacteria, the cells of cyanobacteria may joined by their walls or by mucilaginous sheaths, but each cell is an independent unit of life.

As true Bacteria, cyanobacteria contain peptidoglycan or murein in their cell walls. Most cyanobacteria have a Gram-negative type cell wall that consists of an outer membrane component, even though they may show a distant phylogenetic relationship with certain Gram-positive bacteria. Some of the filamentous cyanobacteria are motile by means gliding or rotating around a longitudinal axis. Short segments (hormogonia) may break off from a cyanobacterial colony and glide away from their parent colony at rates as rapid as 10 micrometers per second. The mechanism for this movement is unexplained but may be connected to the extrusion of slime (mucilage) through small pores in their cell wall, together with contractile waves in one of the surface layers of the wall.

Cyanobacteria are found in most aerobic environments where water and light are available for growth. Mainly they live in fresh water and marine habitats. Those inhabiting the surface layers of water are part of a complex microbial community called **plankton**. Planktonic cyanobacteria usually contain cytoplasmic inclusions called **gas vesicles** which are hollow protein structures filled with various gases. The vesicles can be inflated or deflated with gases allowing the organisms to maintain buoyancy and to float at certain levels in the water. Thus, the cyanobacteria can regulate their position in the water column to meet their optimal needs for photosynthesis, oxygen, and light-shielding. When numerous cyanobacteria become unable to regulate their gas vesicles properly (for example, because of extreme fluctuations of temperature or oxygen supply), they may float to the surface of a body of water and form visible "blooms". A planktonic species related to *Oscillatoria* gives rise to the redness (and the name) of the Red Sea.

The cyanobacteria have very few harmful effects on plants or animals. They may be a nuisance if they bloom in large numbers and then die and decay in bodies of fresh water that are used for drinking or recreational purposes. Many cyanobacteria are responsible for the earthy odors and flavors of fresh waters, including drinking waters, due to the production of compounds called **geosmins.** Some cyanobacteria that form blooms secrete poisonous substances that are toxic for animals that ingest large amounts of the contaminated water.

Many marine cyanobacteria occur in limestone (calcium carbonate) or lime-rich substrates, such as coral algae and the shells of mullosks. Some fresh water species, particularly those that grow in hot springs, often deposit thick layers of lime in their colonies.

Some cyanobacteria can fix nitrogen. In filamentous cyanobacteria, nitrogen fixation often occurs in **heterocysts**, which are specialized, enlarged cells, usually distributed along the length of a filament or at the end of a filament. Heterocysts have intercellular connections to adjacent vegetative cells, and there is continuous movement of the products of nitrogen fixation moving from heterocysts to vegetative cells, and the products of photosynthesis moving from vegetative cells to heterocysts. Heterocysts are low in phycobilin pigments and have only photosystem I. They lack the oxygen-evolving photosystem II. Furthermore, they are surrounded in a thickened, specialized glycolipid cell wall that slows the rate of diffusion of O_2 into the cell. Any O_2 that diffuses into the heterocyst is rapidly reduced by hydrogen, a byproduct of N_2 fixation, or is expelled through the wall of the heterocyst. The process of nitrogen fixation, specifically the enzyme nitrogenase, only functions in anaerobic conditions so the organism must maintain these oxygen-free compartments in order for N_2fixation to occur.

In addition to the heterocysts, some cyanobacteria form resistant spores called **akinetes** enlarged cells around which thickened outer walls develop. Akinetes are resistant to heat, freezing and drought (desiccation) and thus allow the cyanobacteria to survive unfavorable environmental conditions. They are functionally analogous to bacterial endospores, but they bear little resemblance and lack the extraordinary resistance properties of endospores.

A few cyanobacteria are symbionts of liverworts, ferns, cycads, flagellated protozoa, and algae, sometimes occurring as endosymbionts of the eucaryotic cells. In the case of the water fern, *Azolla,* the cyanobacterial endophyte (a species of *Anabaena)* fixes nitrogen that becomes available to the plant. In addition, it is often the case that the photosynthetic partners of **lichens** are cyanobacteria.

The planktonic cyanobacteria fix an enormous amount of CO_2 during photosynthesis, and as "primary producers" they are the basis of the food chain in marine environments. Their type of photosynthesis, which utilizes photosystem II, generates a substantial amount of oxygen present in the earth's atmosphere. Since many cyanobacteria can fix N_2 under certain conditions, they are one of the most significant free-living nitrogen-fixing procaryotes. Cyanobacteria carried out plant-type (oxygenic) photosynthesis for at least a billion and a half years before the emergence of plants, and cyanobacteria are believed to be the evolutionary forerunners of modern-day plant and algal chloroplasts. A group of phototrophic procaryotes, called **prochlorophytes** contain chlorophyll *a* and *b* but do **not** contain phycobilins. Prochlorophytes, therefore, resemble both cyanobacteria (because they are procaryotic and contain chlorophyll *a*) and the plant chloroplast (because they contain chlorophyll *b* instead of phycobilins). *Prochloron,*the first prochlorophyte discovered, is phenotypically very similar to certain plant chloroplasts and is the leading candidate for the type of bacterium that might have undergone endosymbiotic events that led to the development of the plant chloroplast.

Spirochetes are a phylogenetically distinct group of Bacteria which have a unique cell morphology and mode of motility. Spirochetes are very thin, flexible, spiral-shaped procaryotes that move by means of structures called **axial filaments** or **endoflagella**. The flagellar filaments are contained within a sheath between the cell wall peptidoglycan and an outer membrane. The filaments flex or rotate within their sheath which causes the cells to bend, flex and rotate during movement. Most spirochetes are free living (in muds and sediments), or live in associations with animals (e.g. in the oral cavity or GI tract). A few are pathogens of animals (e.g. leptospirosis in dogs, Syphilis in humans and Lyme disease in dogs and humans).

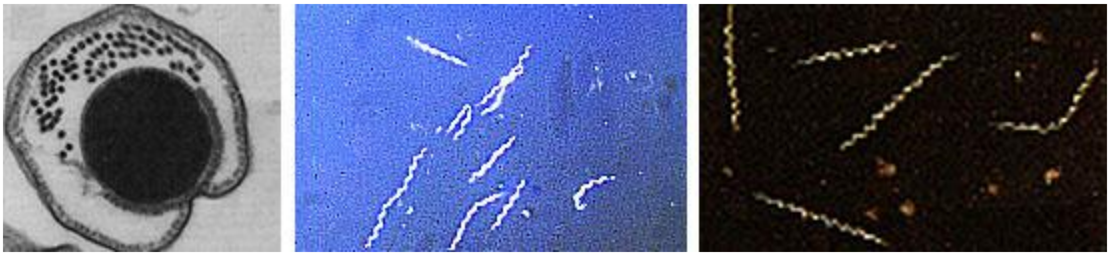


Figure 5. Spirochetes: A. Cross section of a spirochete showing the location of endoflagella between the inner membrane and outer sheath; B. *Borrelia burgdorferi,* the agent of Lyme disease; C. *Treponema pallidum,* the spirochete that causes syphilis.

Other Spiral-Shape and and Curved Bacteria. The main thing that unifies this group of bacteria is their spiral or vibrioid (curved) shape, although they are all classified among the Proteobacteria. Nontheless, in certain environments, their characteristic shape can instantly inform an observer of their identity. Bacteria referred to as "**spirilla**" are Gram-negative aerobic heterotrophic bacteria with a helical or spiral shape. Their metabolism is usually respiratory and never fermentative. Unlike spirochetes, they have a rigid cell wall and are motile by means of ordinary polar flagella. Spirilla are inhabitants of microaerophilic aquatic environments. Most spirilla require or prefer that oxygen in their environment be present in an amount that is well below atmospheric concentration. The *Rhodospirillaceae*are found in the Alpha group of

Proteobacteria; *Spirillaceae* and *Oceanospirillaceae* are Gammaproteobacteria.

As inhabitants of marine and fresh waters many spirilla are endowed with some interesting properties. *Magnetospirillum*contains **magnetosomes** and exhibits the property of **magnetotaxis** (movement in relationship to the magnetic field of the earth). *Oceanospirillum* lives in marine habitats and is able to grow at NaCl concentrations as high as 9 percent. *Azospirillum* is a nitrogen-fixing bacterium that enters into a mutualistic symbiosis with certain tropical grasses and grain crops. Spirilla are thought to play a significant role in recycling of organic matter, particularly in aquatic environments.

Two pathogens of humans are found among the spiril forms in the Epsilon group of Proteobacteria. *Campylobacter jejuni* is an important cause of bacterial diarrhea, especially in children. The bacterium is transmitted via contaminated food, usually undercooked poultry or shellfish, or untreated drinking water. *Helicobacter pylori* is able to colonize the gastric mucosal cells of humans, i.e., the lining of the stomach, and it has been well established as the cause of peptic ulcers.

Bacteria with a curved rod or comma shape are referred to as "**vibrios**". Like the spiral forms, vibrios are very common bacteria in aquatic environments. They are found among the Gammaproteobacteria and have structural and metabolic properties that overlap with both the enterics and the pseudomonads. In Bergey's Manual (2001) *Vibrionaceae* is a family on the level with *Enterobacteriaceae*. Vibrios are facultative like enterics, but they have polar flagella, are oxidase-positive, and dissimilate sugars in the same manner as the pseudomonads. In aquatic habitats they overlap with the *Pseudomonadaceae* in their ecology, although *Pseudomonas* species favor fresh water and vibrios prefer salt water. The genus *Vibrio* contains an important pathogen of humans, *Vibrio cholerae,* the cause of **Asiatic cholera**. Cholera is an intestinal disease with a pathology related to diarrheal diseases caused by the enteric bacteria.

Five species of marine vibrios exhibit the property of **bioluminescence**, the ability to emit light of a blue-green color. These bacteria may be found as saprophytes of dead fish or as symbionts of living fish and invertebrates in marine environments. Some grow in special organs of the fish and emit light for the benefit of the fish (to attract prey, or as a mating signal) in return for a protected habitat and supply of nutrients. The reaction leading to light emission, catalyzed by the enzyme **luciferase**, has been found to be the same in all procaryotes, and differs from light emission by eucaryotes such as the fire fly. Luciferase diverts electrons from the normal respiratory electron transport chain and causes formation of an excited peroxide that leads to emission of light.

The small vibrioid bacterium, *Bdellovibrio,*is a tiny curved rod that is a parasite of other Gram-negative bacteria, including *E. coli*. It preys on other bacteria by entering into the periplasmic space and obtaining nutrients from the cytoplasm of its host cell while undergoing an odd type of reproductive cycle. *Bdellovibrio*is a member of the Deltaproteobacteria.

The **Myxobacteria** are a group of **fruiting gliding bacteria** that comprise a unique order of Deltaproteobacteria. They exhibit a unique type of gliding motility. The vegetative cells move (glide) about together as a swarm, and then they aggregate together to form a multicellular fruiting body in which development and spore formation takes place. They exhibit the most complex behavioral patterns and life cycles of all known procaryotes. Myxobacteria are inhabitants of the soil. They have a eucaryotic counterpart in nature in the *Myxomycetes*, or slime molds, and the two types of organisms are an example of parallel or **convergent evolution**, having adopted similar life styles in the soil environment.

The vegetative cells of myxobacteria are typical Gram-negative rods that glide across a substrate such as a decaying leaf or piece of animal dung, or colonies of other bacteria. They obtain nutrients from the substrate as they glide across it and they secrete a slime track which other myxobacterial cells preferentially follow. If their nutrients become exhausted, the cells signal to one another to aggregate and form a swarm of myxobacteria which eventually differentiate into a multicellular **fruiting body** that contains **myxospores**, a type of dormant cell descended from a differentiated vegetative cell. In the case of *Stigmatella*, the myxospores are packed into secondary structures called **cysts**, which develop at the tips of the fruiting body (Figure 6). The bright-colored fruiting bodies of myxobacteria, containing millions of cells and spores, can often be seen with the unaided eye on dung pellets and decaying vegetation in the soil.

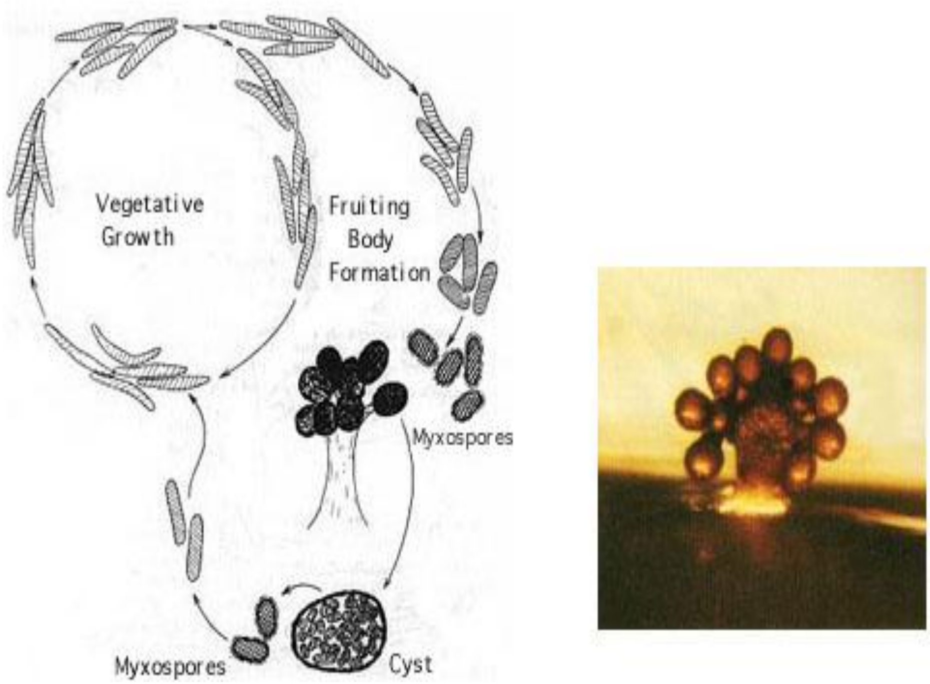


Figure 6. *Stigmatella aurantiaca*, a fruiting myxobacterium: L. Life Cycle R. Fruiting Body.

Lithotrophs. Lithotrophy, a type of metabolism that requires inorganic compounds as sources of energy. This metabolism is firmly established in both the Archaea and the Bacteria. The methanogens utilize H_2 as an energy source, and many extreme thermophiles use H_2S or elemental sulfur as a source of energy for growth. Lithotrophic Bacteria are typically Gram-negative species that utilize inorganic substrates including H_2, NH_3, NO_2, H_2S, S, Fe^{++}, and CO. Ecologically, the most important lithotrophic Bacteria are the **nitrifying bacteria**, *Nitrosomonas* and *Nitrobacter* that together convert NH_3 to NO_2, and NO_2 to NO_3, and the **colorless sulfur bacteria,** such as *Thiobacillus,* that oxidize H_2S to S and S to SO_4. Most lithotrophic bacteria are autotrophs, and in some cases, they may play an important role in primary production of organic material in nature. Lithotrophic metabolism does not extend to eucaryotes (unless a nucleated cell harbors lithotrophic endosymbiotic bacteria), and these bacteria are important in the biogeochemical cycles of the elements.

Figure 7. Lithotroph Habitats. A. Stream in Northern Wisconsin near Hayward is a good source of iron bacteria (John Lindquist). B. Bacteriologist J.C. Ensign of the University of Wisconsin observing growth of iron bacteria in a run-off channel from the Chocolate Pots along the Gibbon River, in Yellowstone National Park (K.Todar). C. An acid hot spring at the Norris Geyser Basin in Yellowstone is rich in iron and sulfur (T.D. Brock). D. A black smoker chimney in the deep sea emits iron sulfides at very high temperatures (270 to 380 degrees C).

Pseudomonads. "Pseudomonad" is an informal term for bacteria which morphologically and physiologically resemble members of the genus *Pseudomonas,* a very diverse group of Gram-negative rods with a strictly-respiratory mode of metabolism. The term is usually applied to bacteria in the genera *Pseudomonas,* and *Xanthomonas,* which are Alphaproteobacteria, and to plant and animal pathogens such as

Burkholderia, Ralstonia and *Acidovorax,* which are Betaproteobacteria. But many other related bacteria share their definitive characterictics, i.e., Gram-negative aerobic rods. The morphology and habitat of many pseudomonads sufficiently overlaps with the enterics (below) that microbiologists must quickly learn how to differentiate these two types of Gram-negative motile rods. Pseudomonads move by polar flagella; enterics such as *E. coli* swim by means of peritrichous flagella. Enterics ferment sugars such as glucose; pseudomonads generally do not ferment sugars. And most pseudomonads have an unusual cytochrome in their respiratory electron transport chain that can be detected in colonies by a colorimetric test called the **oxidase test**. Pseudomonads are typically oxidase- positive.

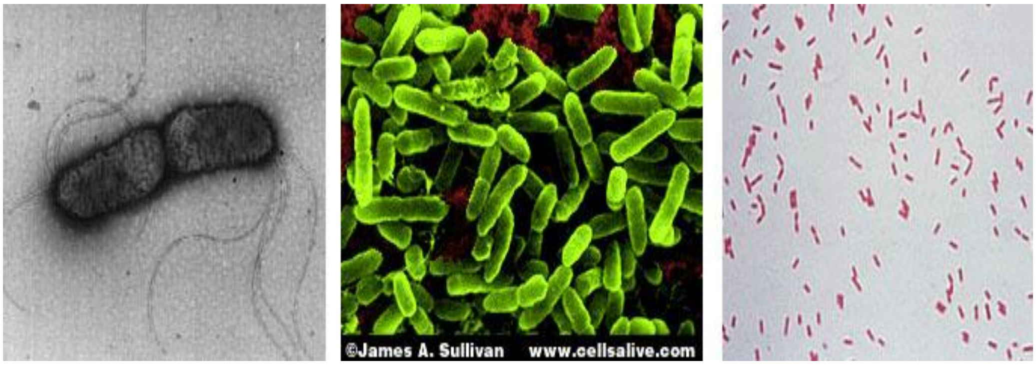


Figure 8. Profile of a pseudomonad: Gram-negative rods motile by polar flagella. A. Electron micrograph, negative stain. B. Scanning electron micrograph. C. Gram stain.

Most pseudomonads are free-living organisms in soil and water; they play an important role in decomposition, biodegradation, and the C and N cycles. The phrase "no naturally-occurring organic compound cannot be degraded by some microorganism" must have been coined to apply to members of the genus *Pseudomonas,* known for their ability to degrade hundreds of different organic compounds including insecticides, pesticides, herbicides, plastics, petroleum substances, hydrocarbons and other of the most refractory molecules in nature. However, they are usually unable to degrade biopolymers in their environment, such as cellulose and lignen, and their role in anaerobic decomposition is minimal.

There are about 150 species of *Pseudomonas*, but, especially among the plant pathogens, there are many strains and biovars among the species. These bacteria are frequently found as part of the normal flora of plants, but they are one of the most important bacterial pathogens of plants, as well. *Pseudomonas syringae* and *Xanthomonas* species cause a wide variety of plant diseases as discussed below. One strain of *Pseudomonas* that lives on the surfaces of plants can act as an "ice nucleus" which causes ice formation and inflicts frost damage on plants at one or two degrees *above* the conventional freezing temperature of water (0 degrees C). One *Pseudomonas* species is an important pathogen of humans, *Pseudomonas aeruginosa,* the quintessential opportunistic pathogen, which is a leading cause of hospital-acquired infections.

Among some interesting or important ecologic relatives of the pseudomonads are *Rhizobium*and *Bradyrhizobium,* species that fix nitrogen in association with leguminous plants, and related *Agrobacterium* species that cause tumors ("galls") in plants. These bacteria are discussed later in this article because of their special relationships with plants. Relatives of the pseudomonads also include the **methanotrophs** that can oxidize methane and other one-carbon compounds, the **azotobacters**, which are very prevalent free-living (nonsymbiotic) nitrogen-fixing bacteria.

Enterics. Enteric bacteria are Gram-negative rods with facultative anaerobic metabolism that live in the intestinal tracts of animals. This group consists of *Escherichia coli* and its relatives, the members of the family *Enterobacteriaceae.* Enteric bacteria are related phenotypically to several other genera of bacteria such as *Pseudomonas* and *Alcaligenes,* but are physiologically quite unrelated. Generally, a distinction can be made on the ability to ferment glucose: enteric bacteria all ferment glucose to acid end products while similar Gram-negative bacteria cannot ferment glucose. Because they are consistent members of the normal flora of humans, and because of their medical importance, an extremely large number of enteric bacteria have been isolated and characterized.

Escherichia coli is, of course, the type species of the enterics. *E. coli* is such a regular inhabitant of the intestine of humans that it is used by public health authorities as an indicator of fecal pollution of drinking water supplies, swimming beaches, foods, etc. *E. coli* is the most studied of all organisms in biology because of its occurrence, and the ease and speed of growing the bacteria in the laboratory. It has been used in hundreds of thousands of experiments in cell biology, physiology, and genetics, and was among the first cells for which the entire chromosomal DNA base sequence was determined. In spite of the knowledge gained about the molecular biology and physiology of *E. coli,* surprisingly little is known about its ecology, for example why it consistently associates with humans, how it helps its host, how it harms its host, etc. A few strains of *E. coli* are pathogenic (one is notorious, strain 0157:H7, that keeps turning up in raw hamburger headed for a fast-food restaurants). Pathogenic strains of *E. coli* cause **intestinal tract infections** (usually acute and uncomplicated, except in the very young), uncomplicated **urinary tract infections** and **neonatal meningitis.**

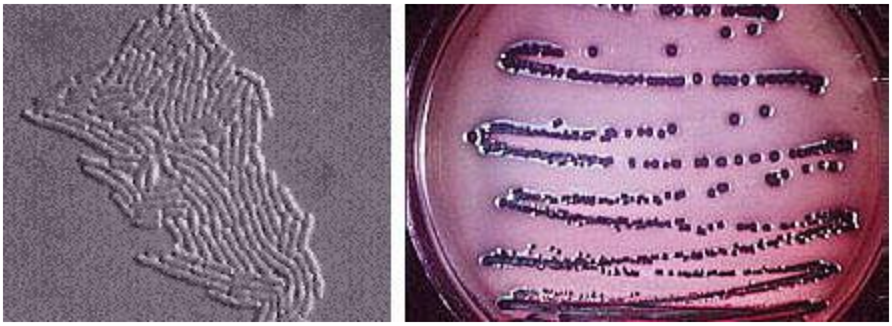


Figure 9. Left: *Escherichia coli* cells. Right: *E. coli* colonies on EMB Agar.

The enteric group also includes some other intestinal pathogens of humans such as *Shigella dysenteriae*, cause of **bacillary dysentery**, and *Salmonella typhimurium*, cause of **gastroenteritis**. *Salmonella typhi*, which infects via the intestinal route, causes **typhoid fever**. Some bacteria that don't have an intestinal habitat resemble *E. coli* in enough ways to warrant inclusion in the enteric group. This includes *Proteus,* a common saprophyte of decaying organic matter, *Yersinia pestis,* which causes **bubonic plague**, and *Erwinia,* an important pathogen of plants.

Gram-negative pathogens. The Gram negative bacteria that are important pathogens of humans are found sattered throughout the Proteobacteria. In the Alphaproteobacteria, one finds the Rickettsias, a group of obligate intracellular parasites which are the cause of **typhus** and **Rocky Mountain Spotted fever**. In the Beta group, the agents of **whooping cough (pertussis)** (*Bordetella pertussis),* gonorrhea (*Neisseria gonorrhoeae*), and meningococcal meningitis (*Neisseria meningitidis*) are found. Among the Gamma group, *Pseudomonas aeruginosa,* the enterics, and *Vibrio cholerae* have already been mentioned. Likewise, the agents of Legionaires' pneumonia (*Legionella pneumophilia),* and childhood meningitis *(Haemophilus influenzae)* are Gammaproteobacteria. *Campylobacter* and *Helicobacter* are Epsilonproteobacteria. Most of these bacteria are discussed elsewhere in this article and/or in separate chapters which deal with their pathogenicity for humans.

Nitrogen-fixing organisms. This is a diverse group of procaryotes, reaching into phylogenetically distinct groups of Archaea and Bacteria. Members are unified only on the basis of their metabolic ability to "fix" nitrogen. **Nitrogen fixation** is the reduction of N_2 (atmospheric nitrogen) to NH_3 (ammonia). It is a complicated enzymatic process mediated by the enzyme **nitrogenase**. Nitrogenase is found only in procaryotes and is second only to RUBP carboxylase (the enzyme responsible for CO_2 fixation) as the most abundant enzyme on Earth.

The conversion of nitrogen gas (which constitutes about 80 percent of the atmosphere) to ammonia introduces nitrogen into the biological nitrogen cycle. Living cells obtain their nitrogen in many forms, but usually from ammonia (NH_3) or nitrates (NO_3), and never from N_2. Nitrogenase extracts N_2 from the atmosphere and reduces it to NH_3 in a reaction that requires substantial reducing power (electrons) and energy (ATP). The NH_3 is immediately assimilated into amino acids and proteins by subsequent cellular reactions. Thus, nitrogen from the atmosphere is fixed into living (organic) material.

Although a widespread trait in procaryotes, nitrogen fixation occurs in only a few select genera. Outstanding among them are the symbiotic bacteria *Rhizobium* and *Bradyrhizobium* which form nodules on the roots of legumes. In this symbiosis the bacterium invades the root of the plant and fixes nitrogen which it shares with the plant. The plant provides a favorable habitat for the bacterium and supplies it with nutrients and energy for efficient nitrogen fixation. *Rhizobium* and *Bradyrhizobium* are Gram-negative aerobes related to the pseudomonads (above). An unrelated bacterium, an actinomycete (below), enters into a similar type of symbiosis with plants. The actinomycete, *Frankia,* forms nodules on the roots of several types of trees and shrubs, including alders (*Alnus*), wax myrtles (*Myrica*) and mountain lilacs (*Ceanothus*). They, too, fix nitrogen which is provided to their host in a useful form. This fact allows alder species to be "pioneer plants" (among the first to colonize) in newly-forming nitrogen-deficient soils. Still other bacteria live in regular symbiotic associations with plants on roots or leaves and fix nitrogen for their hosts, but they do not cause tissue hyperplasia or the formation of nodules.

Cyanobacteria are likewise very important in nitrogen fixation. Cyanobacteria provide fixed nitrogen, in addition to fixed carbon, for their symbiotic partners which make up lichens. This enhances the capacity for lichens to colonize bare areas where fixed nitrogen is in short supply. In some parts of Asia, rice can be grown in the same paddies continuously without the addition of fertilizers because of the presence of nitrogen fixing cyanobacteria. The cyanobacteria, especially *Anabaena*, occur in association with the small floating water fern *Azolla,* which forms masses on the paddies. Because of the nearly obligate association of *Azolla* with *Anabaena,* paddies covered with *Azolla* remain rich in fixed nitrogen.

In addition to symbiotic nitrogen-fixing bacteria, there are various free-living nitrogen-fixing procaryotes in both soil and aquatic habitats. Cyanobacteria may be able to fix nitrogen in virtually all habitats that they occupy. Clostridia and some methanogens fix nitrogen in anaerobic soils and sediments, including thermophilic environments. A common soil bacterium, *Azotobacter* is a vigorous nitrogen fixer, as is *Rhodospirillum*, a purple sulfur bacterium. Even *Klebsiella*, an enteric bacterium closely related to *E. coli,* fixes nitrogen. There is great scientific interest, of course, in knowing how one might move the genes for nitrogen fixation from a procaryote into a eucaryote such as corn or some other crop plant. The genetically engineered plant might lose its growth requirement for costly ammonium or nitrate fertilizers and grow in nitrogen deficient soils.

Besides nitrogen fixation, bacteria play other essential roles in the processes of the nitrogen cycle. For example, saprophytic bacteria, decompose proteins releasing NH_3 in the process of **ammoniafication**. NH_3 is oxidized by lithotrophic *Nitrosomonas* species to NO_2 which is subsequently oxidized by *Nitrobacter* to NO_3. The overall conversion of NH_3 to NO_3 is called **nitrification**. NO_3 can be assimilated by cells as a source of nitrogen (**assimilatory nitrate reduction**), or certain bacteria can reduce NO_3 during a process called **anaerobic respiration**, wherein nitrate is used in place of oxygen as a terminal electron acceptor for a process analogous to aerobic respiration. In the case of anaerobic respiration, NO_3 is first reduced to NO_2, which is subsequently reduced to N_2O or N_2 or NH_3 (all gases). This process is called **denitrification** and it occurs in anaerobic environments where nitrates are present. If denitrification occurs in crop soils it may not be beneficial to agriculture if it converts utilizable forms of nitrogen (as in nitrate fertilizers) to nitrogen gases that will be lost into the atmosphere. One rationale for tilling the soil is to keep it aerobic in order to discourage denitrification processes in *Pseudomonas* and *Bacillus* which are ubiquitous inhabitants.

The **pyogenic cocci** are spherical bacteria which cause various suppurative (pus-producing) infections in animals. Included are the Gram-positive cocci *Staphylococcus aureus, Streptococcus pyogenes* and *Streptococcus pneumoniae,* and the Gram-negative cocci, *Neisseria gonorrhoeae* and *N. meningitidis.* These bacteria are leading pathogens of humans. It is estimated that they produce at least a third of all the bacterial infections of humans, including strep throat, pneumonia, food poisoning, various skin diseases and severe types of septic shock, gonorrhea and meningitis. *Staphylococcus aureus* is arguably the most successful of all bacterial pathogens because it has a very wide range of virulence determinants (so it can produce a wide range of infections) and it often occurs as normal flora of humans (on skin, nasal membranes and the GI tract), which ensures that it is readily transmitted from one individual to another. In terms of their phylogeny, physiology and genetics, these genera of bacteria are quite unrelated to one another. They share a common ecology, however, as parasites of humans.

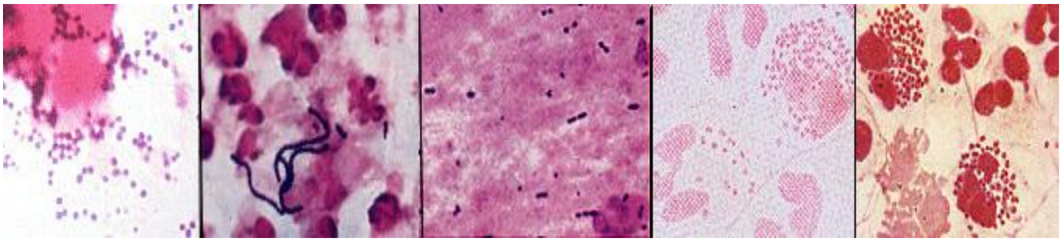


Figure 10. Gallery of pyogenic cocci, Gram stains of clinical specimens (pus), L to R: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*. The large cells with lobed nuclei are neutrophils. Pus is the outcome of the battle between phagocytes (neutrophils) and the invading cocci. As the bacteria are ingested and killed by the neutrophils, the neutrophils eventually lyse (rupture) and release their own components, plus the digested products of bacterial cells, which are the make-up of pus. As a defense against phagocytes the staphylococci and streptococci produce toxins that kill the neutrophils before they are able to ingest the bacteria. This contributes to the pus, and therefore these bacteria are "pyogenic" during their pathogenic invasions.

Two species of Staphylococcus live in association with humans: *Staphylococcus epidermidis* which lives normally on the skin and mucous membranes, and *Staphylococcus aureus* which may occur normally at various locales, but in particular on the nasal membranes (nares). *S. epidermidis* is rarely a pathogen and probably benefits its host by producing acids on the skin that retard the growth of dermatophytic fungi. *Staphylococcus aureus* always has the potential to cause disease and so is considered a pathogen. Different strains of *S. aureus* differ in the range of diseases they can cause, including boils and pimples, **wound infections**, **pneumonia, osteomyelitis**, **septicemia**, **food intoxication**, and **toxic shock syndrome**. *S. aureus* is the leading cause of **nosocomial (hospital-acquired) infections** by Gram-positive bacteria. Also, it is notoriously resistant to penicillin and many other antibiotics. Recently, a strain of *S. aureus* has been reported that is resistant to **EVERY** known antibiotic in clinical usage, which is a grim reminder that the clock is ticking on the lifetime of the usefulness of current antibiotics in treatment of infectious disease.

Streptococcus pypgenes, more specifically the **Beta-hemolytic Group A Streptococci**, like *S. aureus*, causes an array of suppurative diseases and toxinoses (diseases due to the production of a bacterial toxin), in addition to some autoimmune or allergic diseases. *S. pyogenes* is rarely found as normal flora (<1%), but it is the main streptococcal pathogen for man, most often causing tonsillitis or **strep throat**. Streptococci also invade the skin to cause localized infections and lesions, and produce toxins that cause **scarlet fever** and toxic shock. Sometimes, as a result of an acute streptococcal infection, anomalous immune responses are started that lead to diseases like **rheumatic fever** and **glomerulonephritis**, which are called **post-streptococcal sequelae**. Unlike the staphylococci, the streptococci have not developed widespread resistance to penicillin and the other beta lactam antibiotics, so that the beta lactams remain drugs of choice for the treatment of acute streptococcal infections.

Streptococcus pneumoniae is the most frequent cause of bacterial **lobar pneumonia** in humans. It is also a frequent cause of **otitis media** (infection of the middle ear) and **meningitis**. The bacterium colonizes the nasopharynx and from there gains access to the lung or to the eustachian tube. If the bacteria descend into the lung they can impede engulfment by alveolar macrophages if they possess a capsule which somehow prevents the engulfment process. Thus, encapsulated strains are able to invade the lung and are virulent (cause disease) and noncapsulated strains, which are readily removed by phagocytes, are nonvirulent.

The *Neisseriaceae* comprise a family of Gram-negative BetaProteobacteria with metabolic characteristics similar to pseudomonads. The neisseriae are small, Gram-negative cocci usually seen in pairs with flattened adjacent sides. Most neisseriae are normal flora or harmless commensals of mammals living on mucous membranes. In humans they are common residents of the throat and upper respiratory tract. Two species are primary pathogens of humans, *Neisseria gonorrhoeae* and *Neisseria meningitidis,* the bacterial causes of gonorrhea and meningicoccal meningitis.

Neisseria gonorrhoeae is the second leading cause of sexually-transmitted disease in the U.S., causing over three million cases of **gonorrhea** annually. Sometimes, in females, the disease may be unrecognized or asymptomatic such that an infected mother can give birth and unknowingly transmit the bacterium to the infant during its passage through the birth canal. The bacterium is able to colonize and infect the newborn eye resulting **neonatal ophthalmia**, which may produce blindness. For this reason (as well as to control Chlamydia which may also be present), an antimicrobial agent is usually added to the neonate eye at the time of birth.

Neisseria meningitidis is one bacterial cause of meningitis, an inflammation of the meninges of the brain and spinal cord. Other bacteria that cause meningitis include *Haemophilus influenzae*, *Staphylococcus aureus* and *Escherichia coli*. **Meningococcal meningitis** differs from other causes in that it is often responsible for epidemics of meningitis. It occurs most often in children aged 6 to 11 months, but it also occurs in older children and in adults. Meningococcal meningitis can be a rapidly fatal disease, and untreated meningitis has a mortality rate near 50 percent. However, early intervention with antibiotics is highly effective, and with treatment most individuals recover without permanent damage to the nervous system.

Lactic acid bacteria are Gram-positive, nonsporeforming rods and cocci which produce lactic acid as a sole or major end product of fermentation. They are important in the food industry as fermentation organisms in the production of cheese, yogurt, buttermilk, sour cream, pickles, sauerkraut, sausage and other foods. Important genera are *Streptococcus* and *Lactobacillus*. Some species are normal flora of the human body (found in the oral cavity, GI tract and vagina); some streptococci are pathogens of humans (see pyogenic cocci above). Certain oral lactic acid bacteria are responsible for the formation of dental plaque and the initiation of dental caries (cavities).

Endospore-forming bacteria produce a unique resting cell called an **endospore**. They are Gram-positive and usually rod-shaped, but there are exceptions. The two important genera are *Bacillus*, the members of which are aerobic sporeformers in the soils, and *Clostridium,* whose species are anaerobic sporeformers of soils, sediments and the intestinal tracts of animals.

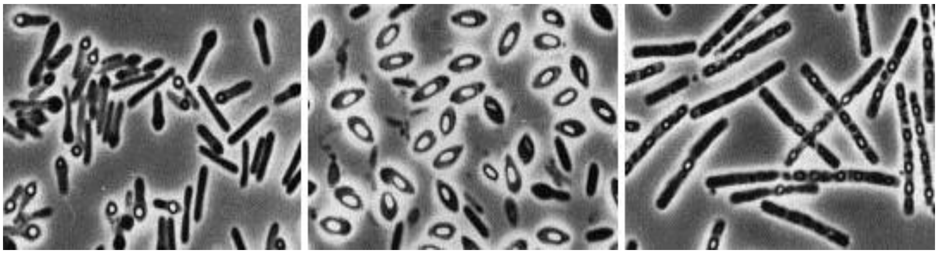


Figure 11. Endospore-forming bacilli (phase contrast illumination). Endospores are dehydrated, refractile cells appearing as points of bright light under phase microcsopy. Endospore-forming bacteria are characterized by the location (position) of the endospore in the mother cell (sporangium) before its release. The spore may be central, terminal or subterminal, and the sporangium may or may not be swollen to accomodate the spore.

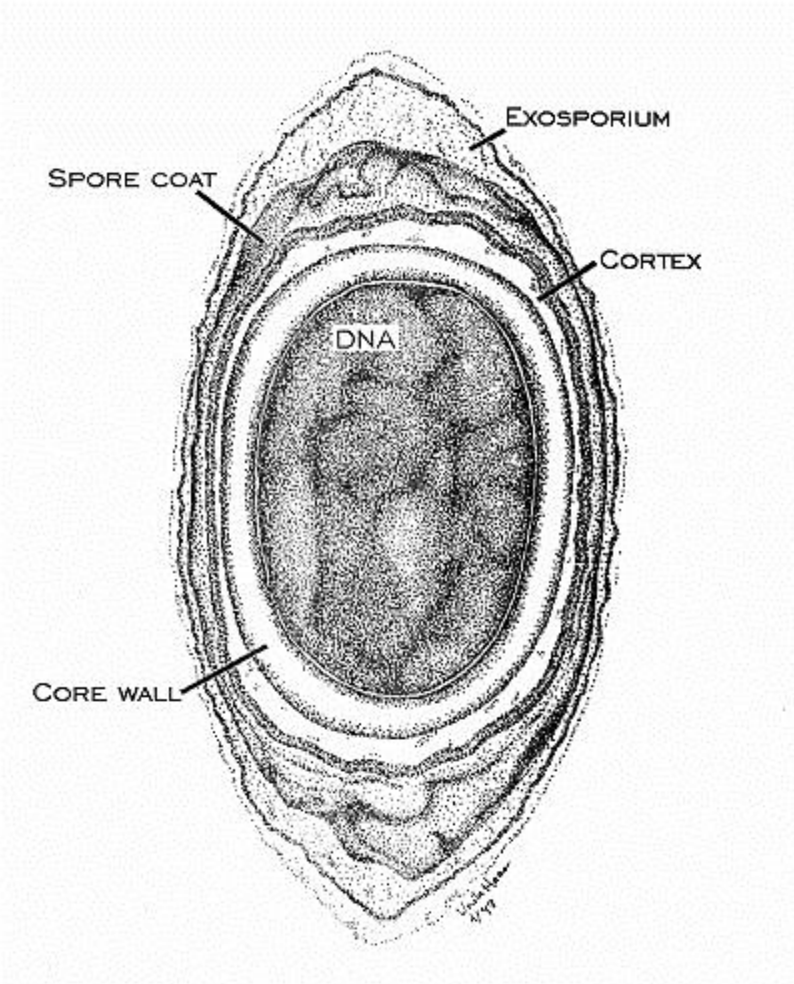


Figure 12. Anatomy of an endospore, cross section drawing by Viake Haas. Endospores differ from the vegetative cells that form them in a variety of ways. Several new surface layers develop outside the core (cell) wall, including the cortex and spore coat. The cytoplasm is dehydrated and contains only the cell genome and a few ribosomes and enzymes. The endospore is cryptobiotic (exhibits no signs of life) and is remarkably resistant to environmental stress such as heat (boiling), acid, irradiation, chemicals and disinfectants. Some endospores have remained dormant for 25 million years preserved in amber, only to be shaken back into life when extricated and introduced into a favorable environment.

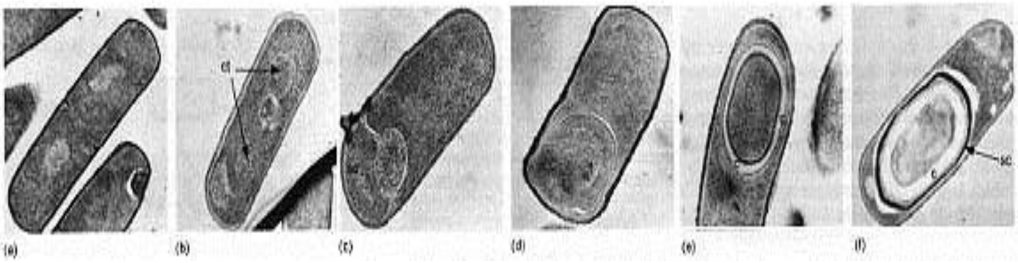


Figure 13. The sequential steps in the process of endospore formation in *Bacillus subtilis.*

Some sporeformers are pathogens of animals, usually due to the production of powerful toxins. *Bacillus anthracis* causes **anthrax**, a disease of domestic animals (cattle, sheep, etc.) which may be transmitted to humans. *Bacillus cereus* is becoming inceasingly recognized as an agent of food poisoning. *Clostridium botulinum* causes **botulism** a form of food-poisoning, and *Clostridium tetani* causes **tetanus**.

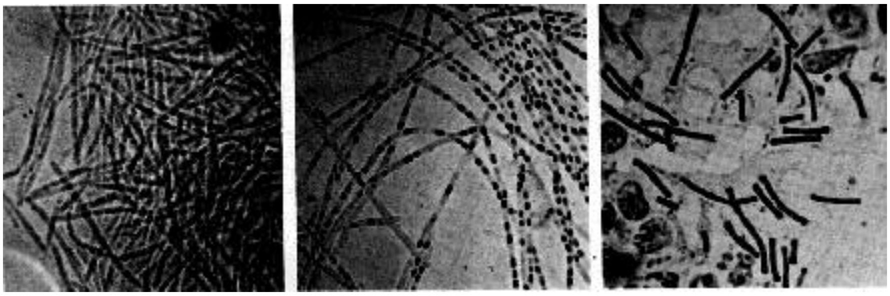


Figure 14. Robert Koch's original photomicrographs of *Bacillus anthracis.* In 1876, Koch established by careful microscopy that the bacterium was always present in the blood of animals that died of anthrax. He took a small amount of blood from such an animal and injected it into a healthy mouse, which subsequently became diseased and died. He took blood from that mouse and injected it into another healthy mouse. After repeating this several times he was able to recover the original anthrax organism from the dead mouse, demonstrating for the first time that a specific bacterium is the cause of a specific disease. In so doing, he established Koch's Postulates, which still today supply the microbiological standard to demonstrate that a specific microbe is the cause of a specific disease.

In association with the process of sporulation, some *Bacillus* species form a crystalline protein inclusion called **parasporal crystals**. The protein crystal and the spore (actually the spore coat) are toxic to lepidopteran insects (certain moths and caterpillars) if ingested. The crystals and spores of *Bacillus thuringiensis* are marketed as "Bt" a natural insecticide for use on garden or crop plants. Another species of *Bacillus*, *B. cereus*, produces an antibiotic that inhibits growth of *Phytophthera,*, a fungus that attacks alfalfa seedling roots causing a "damping off" disease. The bacteria, growing in association with the roots of the seedlings, can protect the plant from disease.

Also, apparently in association with the sporulation process, some *Bacillus* species produce clinically-useful antibiotics. *Bacillus* antibiotics such as polymyxin and bacitracin are usually polypeptide molecules that contain unusual amino acids.

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