UNIT – 1 HISTORY OF MICROBIOLOGY

i) Definition and concepts: -

Microbiology: - It is a science that deals with the study of microscopic forms of life (microorganisms). Study of microorganisms is called as Microbiology.

Microbiology: - It the study of microscopic organisms, derived its name from three Greek words: mikros (“small”), bios (“life”), and logos (“science”). It is the study of microorganisms which are very small and cannot be seen by unaided eye.

Microbiology: - It is a specialized area of biology that deals with living things ordinarily too small to be seen without magnification.

Such microscopic organisms are collectively referred to as **microorganisms, microbes**, or several other terms such as **germs or bugs** in reference to their role in infection and disease.

Microbiology: - It is a specialized area of biology (Greek. bios-life+logos-to study) that concerns with the study of microbes ordinarily too small to be seen without magnification.

Microbiology: - It often has been defined as the study of organisms and agents too small to be seen clearly by the unaided eye—that is, the study of microorganisms.

Because objects less than about one millimeter in diameter cannot be seen clearly and must be examined with a microscope, microbiology is concerned primarily with organisms and agents this small and smaller. However, some microorganisms, particularly some eucaryotic microbes, are visible without microscopes. For example, bread molds and filamentous algae are studied by microbiologists, yet are visible to the naked eye.

Microbiology: - Microbiology is the study of every aspect of microorganisms or microbes —Isolation, characterization and identification, growth control and sterilization, genetics, physiology, characteristics that may be harmful or beneficial, the ways they interact with the environment, the ways they interact with other organisms, and their uses in industry and agriculture.

Microbiology involves study in numerous areas involving cell structure, function, genetics, immunology, biochemistry, epidemiology, and ecology.

It is one of the largest and most complex of the biological sciences because it integrates subject matter from many diverse disciplines.

Microorganisms: - These are very small, generally less than 1 mm in size and not seen clearly with naked eye. For their observation an instrument called microscope is used.

Microorganisms: - Also called microbes, are organisms that require a microscope to be readily observed.

Microorganisms: - Also called Microbes, are minute living things that individually are usually too small to be seen with the unaided eye. The major groups include bacteria, fungi (yeasts and molds), protozoa, algae archaebacteria, actinomyctes, mycoplasma, and rickettisia. It also includes viruses, those noncellular entities sometimes regarded as straddling the border between life and nonlife.

Some microorganisms like mushrooms, filamentous algae and some molds (bread mold) are larger and visible to the naked eyes. Bacteria that are larger in size, *Epulopiscium (80 µm by 600 µm)* and *Thiomargarita (100 to 750 µm in diameter)* also have been discovered.

Microorganisms are present everywhere on the bodies of animals and humans, on plant surfaces, in the air, water, dust, soil, and even inside the intestinal canal of all insects, birds, animals and human beings.

In terms of numbers and range of distribution, microbes are the dominant organisms on earth. Microorganisms are essential to the operation of the earth's ecosystems, as photosynthesizers, decomposers, and recyclers.

Humans use the versatility of microbes to make improvements in industrial production, agriculture, medicine, and environmental protection. The beneficial qualities of microbes are in contrast to the many infectious diseases they cause.

Microorganisms are the oldest organisms, having evolved over the 4 billion years of earth's history to the modern varieties we now observe. Microbes are classified into groups according to evolutionary relationships, provided with standard scientific names, and identified by specific characteristics. Microorganisms can be classified by means of general categories called domains and cell types (procaryotes and eucaryotes).

Although microorganisms are ancient by many standards, microbiology itself is a comparatively new science. The existence of microorganisms was unknown until the discovery of Microscope. Microscope is an optical instrument which can magnify small objects which cannot be seen by naked eye. Microscopes were invented in the beginning of 17th century.

Microscope: - It is an instrument which magnifies (enlarges) the image of microorganisms (objects), so that we can able to see them clearly. There are two types of microscopes optical and electron microscopes.

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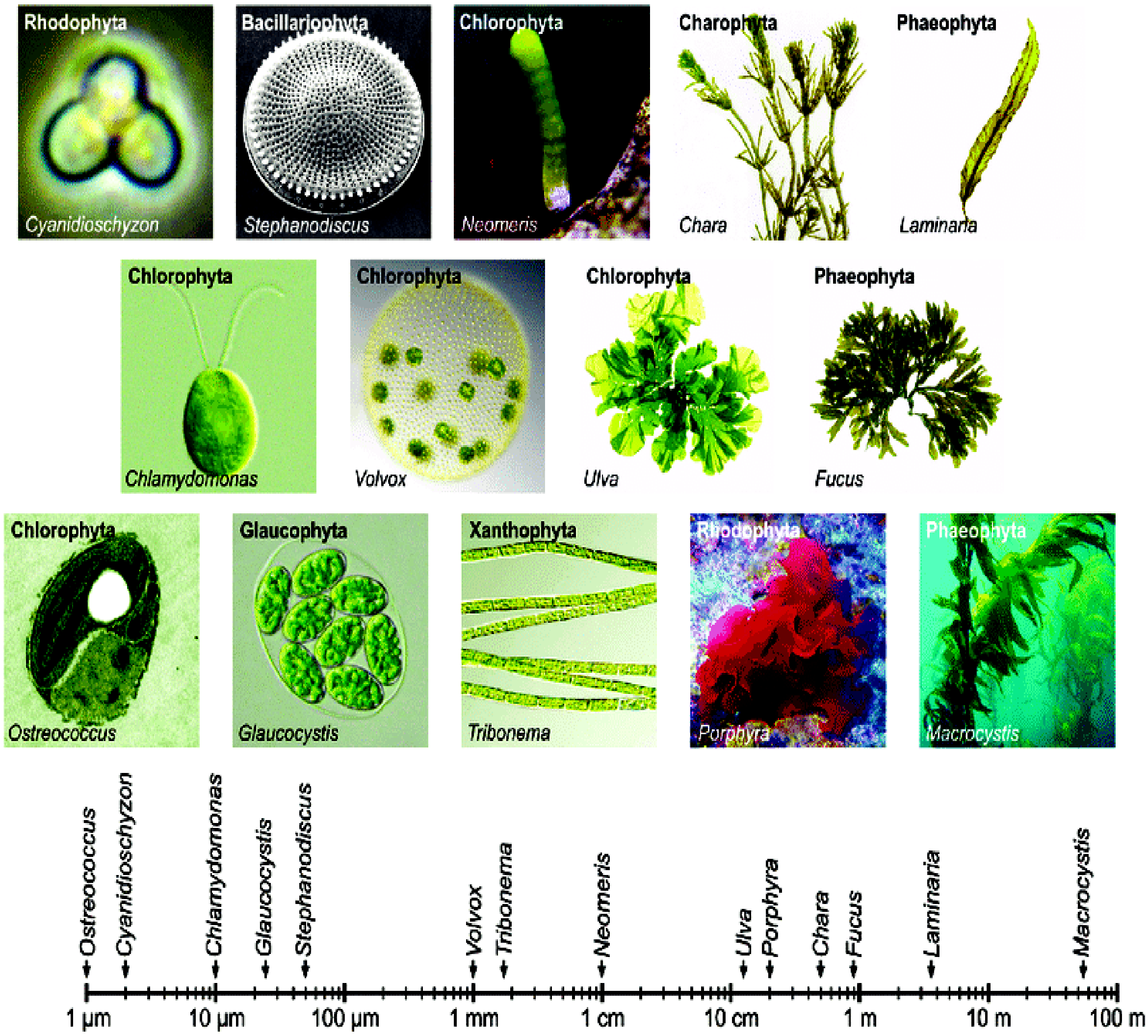
ii) Types of microorganisms: -

The major groups of microorganisms are –

1. Algae
2. Bacteria
3. Fungi (Molds & Yeasts)
4. Protozoa
5. Viruses
6. Mycoplasma
7. Actinomycetes (The Filamentous Bacteria)
8. Archaebacteria
9. Rickettsia

1. Algae: -

Algae (*singular:* **alga**) are unicellular or multicellular, photosynthetic, autotrophic and eukaryotic microorganisms. They produce oxygen. All algal cells contain photosynthetic pigment *chlorophyll* hence capable of photosynthesis. These are found most commonly in aquatic environments (water) or in damp soils. These multiply asexually or sexually e.g. ***Chlorophyta, Rhodophyta, Xanthophyta***.

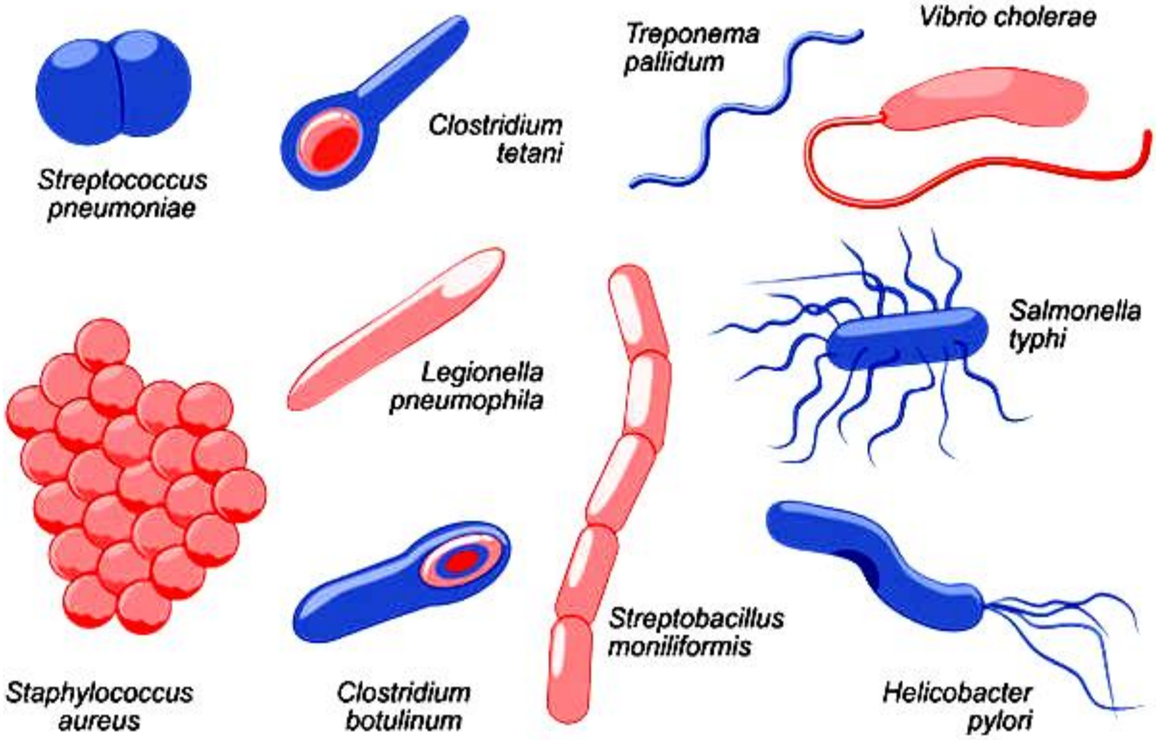


2. Bacteria: -

Bacteria (*singular*: **bacterium**) are a large group of single-celled, prokaryote microorganisms, typically a few micrometers (µm) in length. Bacteria have different types of shapes, like spheres, rods and spirals. Bacteria are found in every habitat on Earth, growing in soil, acidic hot springs, radioactive waste, water, and deep in the Earth's crust, as well as in organic matter and the live bodies of plants and animals. Bacteria are important in recycling nutrients, such as the fixation of nitrogen from the atmosphere and putrefaction, mineralization of C, S, P and micronutrients.

Bacteria were first observed by **Anton van Leeuwenhoek** in 1676, using a single-lens microscope of his own design. He called them **"animalcules"**. The name *bacterium* was introduced much later, by **Christian Gottfried Ehrenberg** in 1838.

Bacteria reproduce by binary fission. Few are beneficial, few are pathogenic and cause infectious diseases, including cholera, syphilis, anthrax, leprosy and plague.

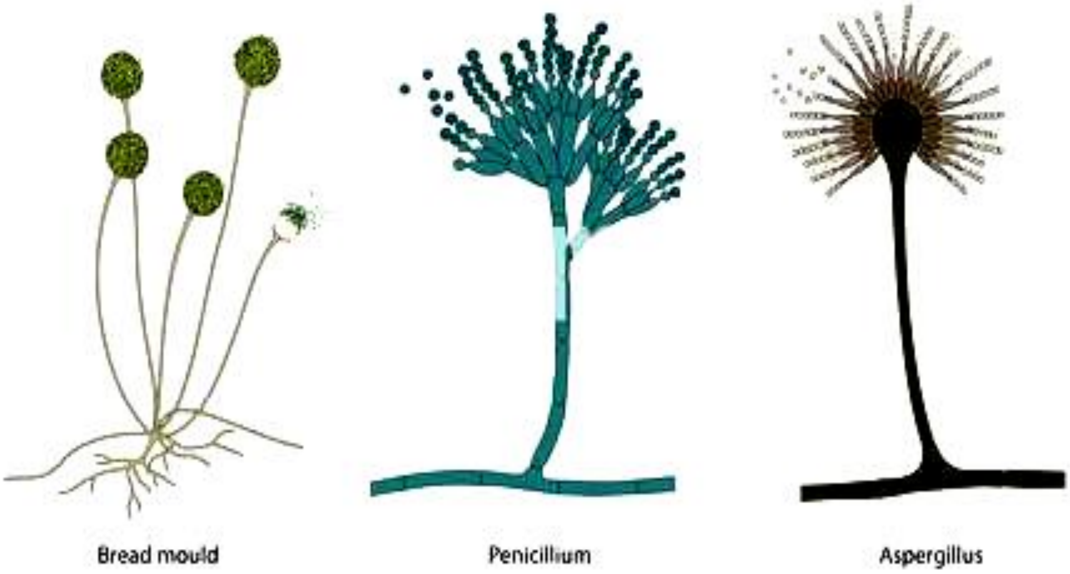


3. Fungi (molds, yeasts and mushrooms)

Fungi (*singular*: **fungus)** are a member of a large group of eukaryotic organisms that includes microorganisms such as ***yeasts and molds***, as well as the more familiar ***mushrooms***. These organisms are classified as a kingdom, **Fungi**, which are separate from plants, animals, and bacteria. Fungi do not contain chlorophylls and are heterotrophic organisms (non photosynthetic), requiring preformed organic compounds as energy sources. One major difference in fungi and plants is that cell walls of fungi contain **chitin**, while the cell walls of plants contain **cellulose**. Most fungi grow as hyphae, which are cylindrical, thread-like structures called as **molds**. Some species grow as single-celled oval structures called as **yeasts** and some as umbrella like structures called as **mushrooms**. **Mushrooms** are the fleshy, spore-bearing bodies of fungi, typically produced above ground on soil or on its food source, particularly at places where decaying matter is present and dogs perform urination. Hence mushrooms are called as umbrellas of dogs.

Fungi are found in soil, on dead matter, and as symbionts of plants, animals, or other fungi. Fungi play an important role in the decomposition of organic matter, in nutrient cycling and exchange. They reproduce sexually or asexually. Yeasts reproduce by budding or binary fission.

They are used in the production of antibiotics, enzymes, acids, alcohols, wine, beer, etc.

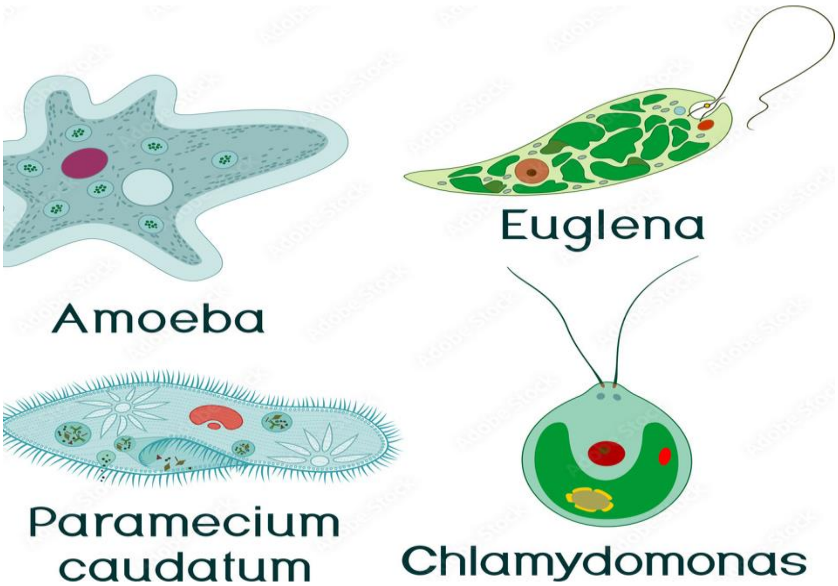


4. Protozoa: -

Protozoa (*singular:* **protozoan**) (from the Greek words *proto*, meaning "first", and *zoa*, meaning "animals") are group of eukaryotes, many of which are motile. Protozoa are unicellular organisms and are often called the animal-like protists because they depend entirely on other organisms for food.

They move with whip-like tails called **flagella**, hair-like structures called **cilia**, or foot-like structures called **pseudopodia**. Others do not move at all. Protozoa may absorb food via their cell membranes, some surround food and engulf it (phagocytosis in *amoeba)*, and some others have openings or "**mouth** pores" through which they take food.

Protozoa can reproduce by binary fission or multiple fission. Some protozoa reproduce sexually, some asexually, while some use a combination. **Amoebas, Paramecia, and Trypanosomes** are examples of animal-like Protists. Some protozoa are human parasites, causing diseases like Malaria, Amoebiasis, Giardiasis and Dysentery.



5. Viruses

Viruses are very small that can replicate only inside the living cells of organisms (obligate intracellular parasites). Viruses are not seen with a light microscope. For their observation, electron microscope is used. Viruses infect all types of organisms, from animals and plants to microorganisms. The shapes of viruses range from simple helical and icosahedral forms to more complex structures.

Virus particles (known as *virions*) consist of two or three parts:

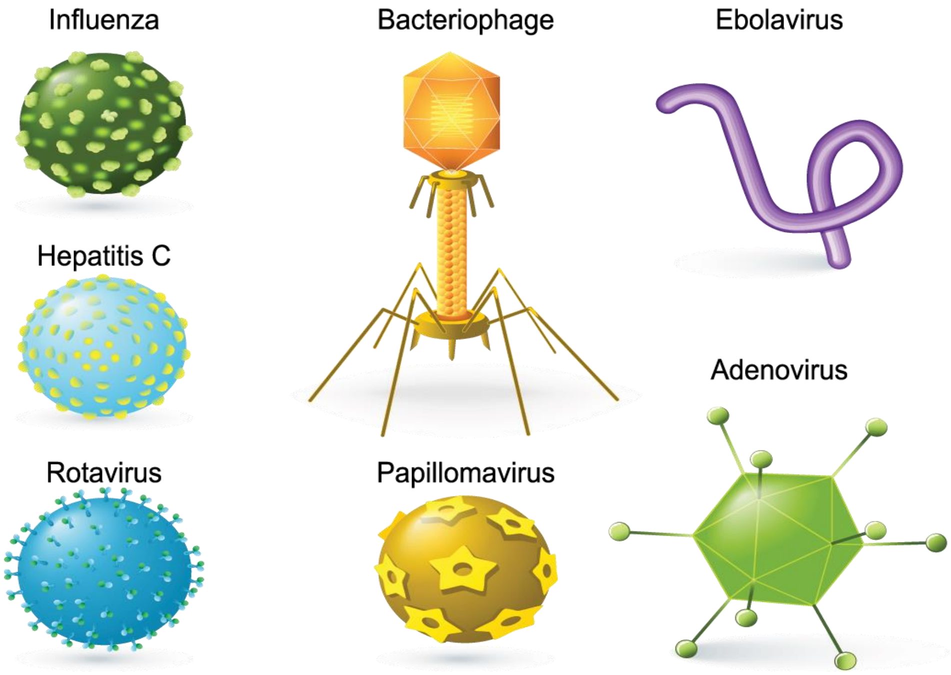
i) The genetic material made from either DNA or RNA,

ii) A protein coat that surrounds and protects genetic material

iii) In some viruses an envelope of lipids that surrounds the protein coat.

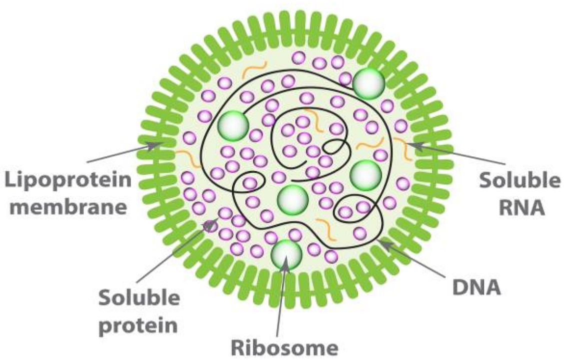
Viruses do not have a cellular structure and their own metabolism. They multiply in host cell only.

Examples of common human diseases caused by viruses include the common cold, influenza, chickenpox and cold sores. Many serious diseases such as AIDS, Polio, Swine flu, Jaundice, Rabbies, Chikungunya and SARS (*Severe Acute Respiratory Syndrome* by corona virus) are caused by viruses.



6. Mycoplasmas

Mycoplasmas are the smallest prokaryotic organisms with no cell wall that can grow in cell-free culture medium. They are found in man, animals, plants, insects, soil and sewage. Unicellular, prokaryotic, usually non motile and form fried egg shaped colonies. Highly pleomorphic, form varying with culture conditions. Under different microscopy, they appear small coccoid bodies, ring forms and fine filaments some of which are branched. Filtrable through bacterial filters.



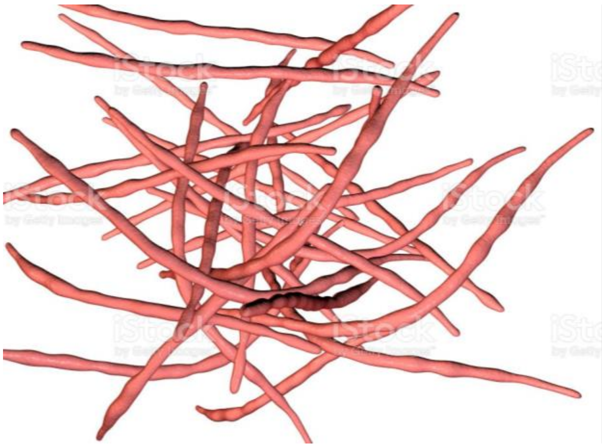
7. Actinomycetes (The Filamentous Bacteria)

The actinomycetes are a large group of aerobic, high G-C percentage Gram-positive filamentous bacteria that form branching filaments or hyphae and asexual spores.

These bacteria closely resemble fungi in overall morphology of branch forming a network of hyphae.

E. G. ***Actinomycetes, Mycobacteria, Frankia, Nacardia, Streptomycetes, Micromonospora.***

Streptomycetes are used for antibiotic production while Mycobacteria produce tuberculosis and leprosy.



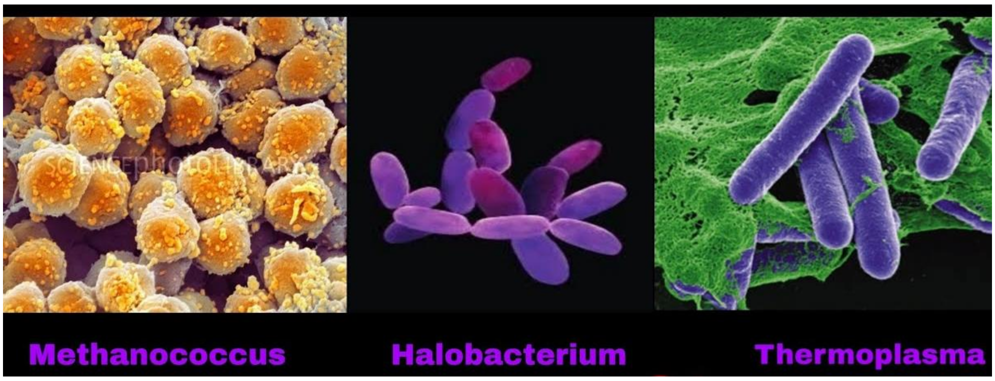
8. Archebacteria

Archaebacteria are described as being **obligate anaerobes**; that is, they can only live in areas without **oxygen**. Their oxygen-free environments, and the observations that habitats of Archaebacteria can frequently be harsh (so unfavorable that bacteria and eukaryotic organisms such as humans cannot survive).

This supports the view that Archaebacteria were ones of the first life forms to evolve on Earth. Archaebacteria, like all prokaryotes, have no membrane bound organelles. Archaebacteria have a cell wall that contains no peptidoglycan.

Archaebacteria can be divided into three groups.

a. **Methanogens (Methane producers)**
b. **Extreme halophiles (Salt loving)**
c. **Extreme thermophiles (Heat loving)**

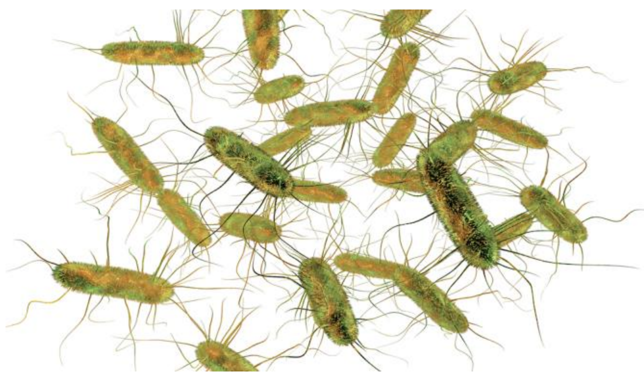


9. **Rickettsiae**

The rickettsiae (sing. rickettsia) measure about 0.3 - 0.5 µm in diameter and 0.3 -0.4 µm in length and usually appear as rods with rounded edges, a form known as the Coccobacillus. They also exist in alternate shapes and hence pleomorphic. They are hardly visible under the light microscope.

They have no flagella, pili, Capsules, or spores. The cell wall is chemically similar to that of Gram negative bacteria and the cytoplasm contains both DNA and RNA as well as many of the enzymes. Reproduction is by binary fission. They are obligate intracellular parasites.

Rickettsiae infect both humans and arthropods, the latter serving as vectors.



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The study of these microorganisms in specialization has following terms: -

i) Phycology; - Study of algae
ii) Bacteriology: - Study of bacteria
iii) Mycology: - Study of fungi
iv) Protozoology: - Study of protozoa
v) Virology: - Study of viruses

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i) Discovery of Microorganisms.

- **Rojar Bacon** (1220-1292): - He suggested that diseases might be produced by invisible living creatures.

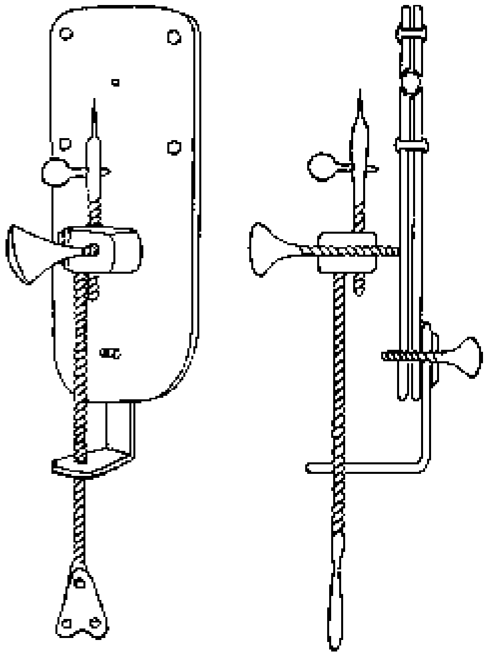
- **Kircher (1601-1680):** - referred 'worms', to these living creatures which are invisible to the naked eye found in decaying bodies, meat, milk & diarrhoeal secretions. Kircher was the first person to recognize the significance of microorganisms in disease.

- **Zacharias Janssen (1580-1632)**: - He discovered microscope. The discovery of microscope made possible to observe microorganisms.

- **Robert Hook (1665): -** One of the most important discoveries in the history of biology occurred in 1665 with the help of a relatively crude microscope. After observing a thin slice of cork, an Englishman, Robert Hooke, reported to the world that life's smallest structural units were "little boxes," or "cells," as he called them.

Using his improved version of a compound microscope (one that uses two sets of lenses), Hooke was able to see individual cells. Hooke's discovery marked the beginning of the cell theory—the theory that all living things are composed of cells. Subsequent investigations into the structure and functions of cells were based on this theory. Though Hooke's microscope was capable of showing large cells, he lacked the resolution that would have allowed him to see microbes clearly.

- **Anton van Leeuwenhoek of Delft, Netherlands (1632-1723)**: - He first time observed and gave accurate descriptions with diagrams of these living creatures or worms and called as ***animalcules***. He was a Merchant, a dealer in men's clothing and accessories. In his spare time he ground pieces of glasses into fine lenses, places them between two silver and brass plates wrapped up together as shown in figure.



Anton Van Leeuwenhoek prepared more than 500 optical lenses and constructed at least 250 microscopes of differing types, His microscopes were made of silver or copper frames, holding hand-ground lenses with magnification of 250 to 500 times. He observed hair, fibres, plant structures, crystals, an insect's eyes, and variety of fluids including blood and even scrapping from his teeth. He was the first to observe and describe small organisms, which he originally called as ***animalcules,*** now called as **micro-organisms**. Bacteria were first observed by **Anton van Leeuwenhoek** in 1676, using a single-lens microscope of his own design. All the main kinds of microorganisms that we know today – protozoa, algae, yeasts, bacteria were first described by him. Therefore he is called as **father of microbiology**. He observed different types of small organisms, their size, shape & movement etc & recorded on papers.

He gave this information to the Royal Society of London which was publishing scientific information at that time. He was very suspicious & secretive person and never shared the art of grinding lenses and constructing microscopes with anybody. Therefore microbiology developed slowly.

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2) Spontaneous generation theory Aristotle's view Charles Darwin's view

Spontaneous Generation (Abiogenesis):-

The spontaneous appearance of life from inanimate matter or origin of living things from nonliving things is called spontaneous generation or abiogenesis.

Biogenesis: - Origin of living things from living things only is called biogenesis.

Since at least 346 B.C. people believed that the life on earth appeared spontaneously from inanimate matter and **Goddess Gaea** (Greek) created peoples from inanimate objects.

Aristotle's view: (384–322 B.C.)

The theory of spontaneous generation, first comprehensively posited by Aristotle in his book "On the Generation of Animals" around 350 B.C., aims to explain the seemingly sudden emergence of organisms such as rats, flies and maggots within rotting meat and other decomposable items.

The theory suggests that organisms do not descend from other organisms or from a parent, and only require that certain conditions in their environment be fulfilled in order for creation to occur.

Aristotle theorized that non-living matter contained a "vital heat" called pneuma—the concept of a "breath of life" and translated later as "anima" meaning "soul" in Latin—and a combination of the four elements believed to make up all life: earth, air, fire and water.

He suggested that animals and plants could arise from earth and liquid, because there was "vital heat" within all air, there is air in water, and there is water in earth, meaning there is "vital heat" or "soul" within everything.

His explanation of the spontaneous generation was as follows:

"… living things form quickly whenever this air and vital heat are enclosed in anything. When they are so enclosed, the corporeal liquids being heated, there arises as it were a frothy bubble. Whether what is forming is to be more or less honorable in kind depends on the embracing of the psychical principle; this again depends on the medium in which the generation takes place and the material which is included."

Charles Darwin's view

When Charles Darwin published The Origin of Species 150 years ago, he deliberately avoided the subject of the origin of life. Darwin explained in "The Origin of Species" book in 1859 - All organic beings that have lived on Earth could be descended from some primordial form",

A comment in a notebook dating back to 1837, in which Darwin explains that "the intimate relationship between the vital phenomena with chemistry and its laws makes the idea of spontaneous generation conceivable",

Charles Darwin imagines a small, warm pool where the inanimate matter would arrange itself into evolutionary matter, aided by chemical components and sufficient sources of energy.

His opinion seems to have changed over time from his original remark in the 1861 3rd edition of The Origin of Species

«…it is no valid objection that science as yet throws no light on the far higher problem of the essence or origin of life», which he reiterated in a letter he mailed to his close friend Joseph Dalton Hooker on March 29, 1863, in which he wrote that

«…it is mere rubbish thinking, at present, of origin of life; one might as well think of origin of matter».

He stated that «it is often said that all the conditions for the first production of a living being are now present, which could ever have been present. But if (and oh what a big if) we could conceive in some warm little pond with all sort of ammonia and phosphoric salts,—light, heat, electricity present, that a protein compound was chemically formed, ready to undergo still more complex changes, at the present such matter would be instantly devoured, or absorbed, which would not have been the case before living creatures were formed

❖ Controversy (conflict) over spontaneous generation theory (contribution of scientists) / The Debate over Spontaneous Generation

After van Leeuwenhoek discovered the previously "invisible" world of microorganisms, the scientific community of the time became interested in the origins of these tiny living things. Until the second half of the nineteenth century, many scientists and philosophers believed that some forms of life could arise spontaneously from nonliving mailer; they called this hypothetical process spontaneous generation. Not much more than 100 years ago, people commonly believed that toads, snakes, and mice could be born of moist soil; that flies could emerge from manure; and that maggots, the larvae of flies, could arise from decaying corpses.

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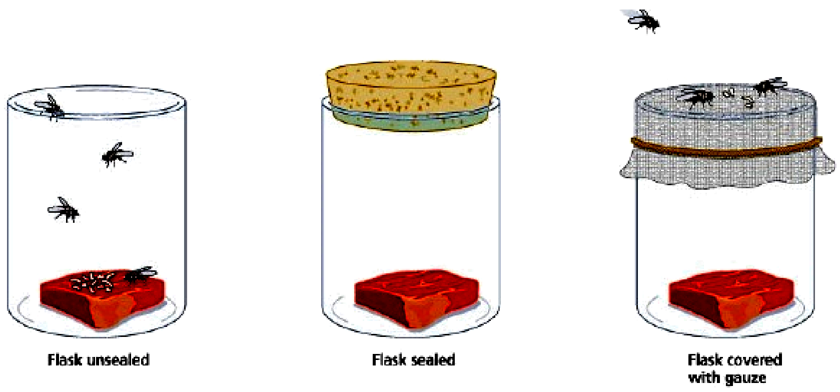
Vergil:

He gave directions for the artificial breeding of bees. In 17th century people had accepted that maggots could be produced by exposing meat to air and rats from rags (A piece of cloth used for cleaning, washing, or dusting). But some scientists doubted this and performed the experiments.

Francesco Redi: (1626–1697)

He was an Italian physician; he opposed spontaneous generation by performing experiments in 1665. He carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously. Redi placed meat in three containers. One was uncovered, a second was covered with paper, and the third was covered with fine gauze to prevent flies to enter into flask. Flies laid their eggs on the uncovered meat and maggots developed. The other two pieces of meat did not produce maggots spontaneously. However, flies were attracted to the gauze-covered container and laid their eggs on the gauze; these eggs produced maggots.

Thus the generation of maggots by decaying meat resulted from the presence of fly eggs, and meat did not spontaneously generate maggots as previously believed.



A) Flies entered the jar that was open to air and landed on meat where they laid their eggs that developed into maggots.
B) Jar sealed with cheesecloth flies could not enter, and maggots did not appear.

Anton Van Leeuwenhoek's discovery of microorganisms renewed the controversy. Some proposed that microorganisms are formed by spontaneous generation and larger organisms are not formed by spontaneous generation. They pointed out that microorganisms are formed from boiled extracts of hay or meat after some time.

John Needham (1713–1781):

He supported the spontaneous generation. John Needham's studies consisted of tightly corked flasks containing boiled mutton broth & periodically observing them for cloudiness as an indication of microbial growth. Some of the containers remained clear, while most of them become cloudy or turbid. Upon examining few drops of fluid under microscope, he found microorganisms.

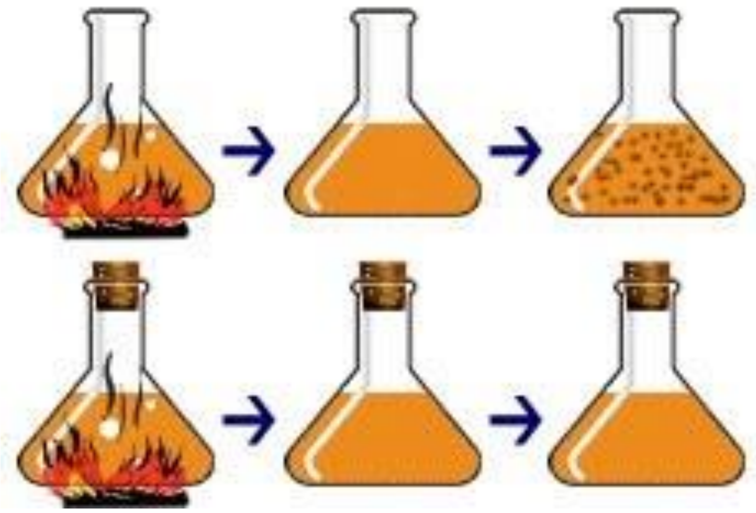
Since boiling was known to destroy microorganisms, Needham justified that his experiments clearly demonstrated the existence of spontaneous generation. He postulated that the organic matter in his flask possessed a vital or vegetative force, which could give the properties of life on the nonliving elements present.

Lazzaro spallanzani:

This Italian naturalist in 1767 refused Needham's work and opposed spontaneous generation. He boiled the flasks for larger time periods and sealed them shut by melting the glass in the neck. His control flasks were boiled only briefly and plugged with porous corks. Control flasks showed the microbes; where as experimental flasks were free of any life.

Needham however argued the prolonged heating had killed that life force. Spallenzani responded to this argument by taking heated closed flask and breaking the seal allowing exposure to air. Within a short time, the contents of these flasks became turbid, thus showing that the long heated organic matter still was capable of supporting life.

But after the discovery of oxygen by Joseph Priestley and its importance to life, the arguments for spontaneous generation began once again. Spallenzani's findings were criticized from the stand point that sufficient air was not present in his sealed flasks or air is not allowed to enter in the flask to support microbial growth.



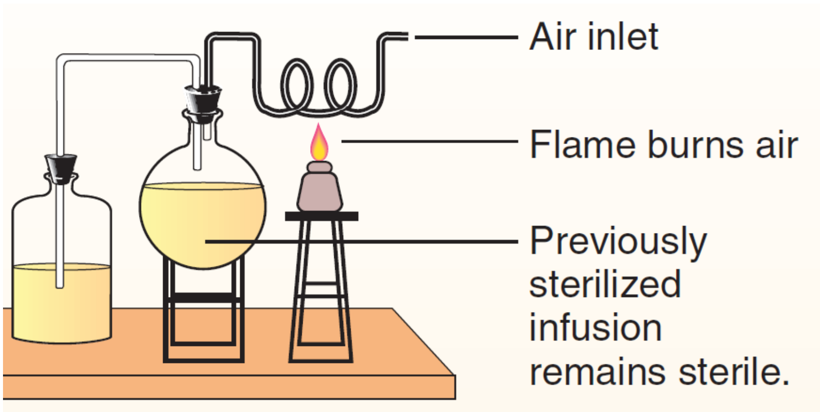
Schwann and Schulze:

They opposed spontaneous generation. Additional experiments now were necessary to show that bacterial growth in nutrient containing flasks was brought about through an exposure to air containing these organisms and that the result was not a case of spontaneous generation.

Schwann passed air in a flask through red-hot tube containing heated nutrient broth and in another flask unheated air is passed containing heated nutrient broth.

Schulze passed the air in one flask through strong chemical agent H_2SO_4 or KOH. In another flask untreated air is passed containing heated nutrient broth.

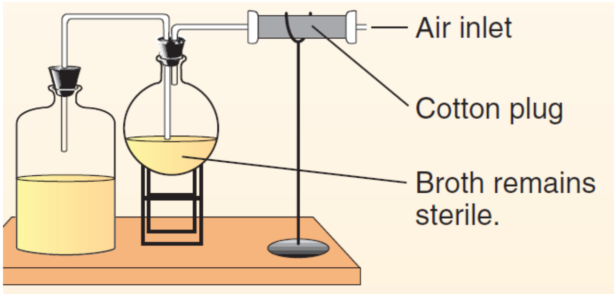
In both experiments, microorganisms did not appear in the flask in which heated air and air passed through chemical agents was allowed to enter. While microorganisms were appeared in the flasks in which untreated air was allowed to enter.



But still some scientists raised objection against the experiments of Schwann & Schulze that due to passing of air through red hot tube and strong chemical, destroyed all possible life rendering force or the composition of air is changed hence air would not support the growth.

Schroder and Dusch:

They opposed spontaneous generation. These scientists performed more convincing experiments by passing air through sterile cotton into a flask containing heated medium and another flask air is not passed through cotton. Flask, which received filtered air showed no sign of growth, while flask, which received unfiltered air, showed presence of growth.

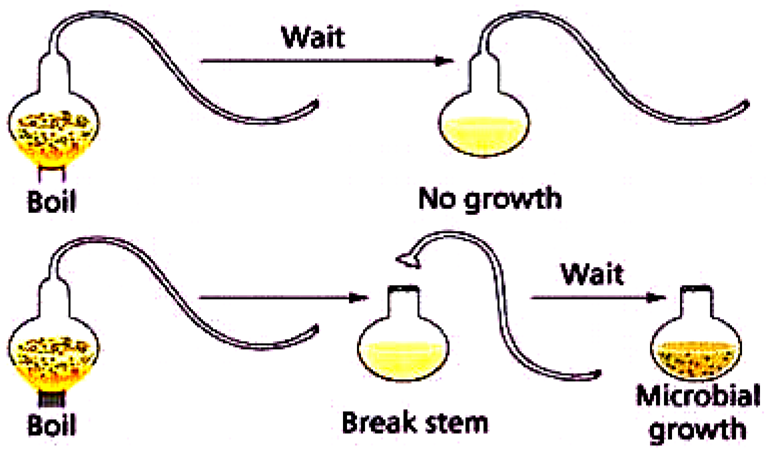


These studies confirmed that the treatment of air with chemicals or with heat, in reality was unrelated to the development of growth in nutrient broth. Moreover these findings demonstrated not only the sensitivities of microorganisms in air to chemicals and heat, but they could be removed from air by filtration through cotton wool.

Louis Pasteur (1822 to 1895):

He ended completely the idea of spontaneous generation. He demonstrated the presence of bacteria in cotton wool used for filtration in the experiment of Scrodder and Dusch showing that these organisms were trapped in the cotton. He added a piece of cotton used for filtration to sterile meat broth, soon microorganisms appeared. Thus he confirmed how microorganisms gained access to and also how they could be prevented from entering into nutrients.

At last he performed a beautiful experiment that ended completely the idea of spontaneous generation. He took a flask with long swan neck or gooseneck opening. The nutrient broth is heated into a flask and untreated; unfiltered air is allowed to pass in or out. No growth is observed, because of length and bend of the flask neck, microorganisms present in air could not reach the nutrient broth in the flask. However if the top of the flask was broken off or if a flask was tilted so that the heated liquid nutrient ran into an exposed part of the neck and then returned, microbes soon appeared in the fluid.

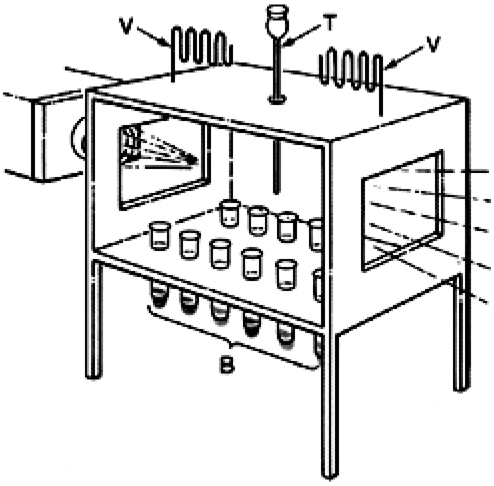


John Tyndall:

During his studies on optical properties of atmospheric dust, he observed that a beam of light passing through air lacking dust particles could not be seen, but a beam of light passing through air containing dust particles was readily visible.

Aware of Pasteur's conclusion regarding the presence of microorganisms on dust, Tyndall devised a system to determine if air lacking dust particles contained microorganisms. He built a specialized culture chamber; equipped with vents through which bacteria could not enter, lateral windows and the test tubes in the racks.

In addition, the sides of this box were coated with glycerol in order to trap the dust particles in the chamber. When the chamber was found to be optically devoid of floating matter, as determined by shining of a beam of light through its lateral windows, the test tubes were filled with a broth medium, which was then sterilized by placing the bottoms of these tubes in a pan of boiling water.



Tyndall observed that the broth remained sterile even though it was in direct contact with air of the chamber. When dust-containing air was introduced, microbial growth appeared after a short incubation period. Thus with this specialized chamber and technique Tyndall demonstrated that bacterial life occurred in sterile broth only after it was introduced from an outside source. Thus spontaneous generation theory completely ended.

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ii) Recognition of Microbial role in disease (Germ theory of diseases)

Although Fracastoro of Verona and few others had suggested that invisible organisms might be responsible for diseases, most people believed that diseases were caused due to

→ Supernatural forces
→ Poisonous vapours (miasmas)
→ Imbalance between the four humors thought to be present in the body

e.g. Blood, Phlegm (bedka, thunki, sputum), Choler (yellow bile, pitta) and Melancholy (depression / nervousness)

Support for germ theory of diseases:

1. Agostino Bassi: -

First showed microorganisms could cause disease. He demonstrated that a silkworm disease was due to fungus infection. He also suggested that many diseases were due to microbial infections.

2. M.J. Berkeley (1845): -

He proved that the great potato blight of Ireland was also caused by fungus.

3. Louis Pasteur: -

During the investigation of pebrine disease of silkworm, he showed that it was due to protozoan parasite. Disease was controlled by raising caterpillars from eggs produced by healthy moths.

Pasteur tackled the problem of anthrax disease of cattle, sheep and some times human beings. He grew the microbes in the laboratory flasks after isolating them from the blood of the animals that had died of the disease.

4. Joseph Lister (Indirect evidence for germ theory of diseases): -

Indirect evidence that microorganisms were agents of human disease came from the work of the English surgeon Joseph Lister on the prevention of wound infections. Lister developed a system of antiseptic surgery designed to prevent microorganisms from entering wounds. Instruments were heat sterilized and phenol was used on surgical dressings and at times sprayed over the surgical area. The approach was remarkably successful. It also provided strong indirect evidence for the role of microorganisms in disease because phenol, which killed bacteria, also prevented wound infections.

5. Robert Koch (1843 – 1910) / Koch's postulates (Direct evidence for the germ theory of disease): -

The first direct demonstration of role of bacteria in causing disease came from the study of anthrax disease by the German physician Robert Koch. Koch studied the relation between *Bacillus anthracis* bacteria and anthrax disease. Anthrax disease is a disease of cattle, sheep and some times human beings.

Koch injected healthy mice with material from diseased animals & the mice became ill. A piece of spleen from infected mice containing the anthrax bacillus is incubated in serum. The bacilli grew, reproduced and produced spores. When the isolated bacilli or spores were injected into healthy mice, anthrax disease developed.

His criteria for proving the causal relationship between microorganisms and specific disease are known as **Koch's postulates** and can be summarized as follows –

1. A specific organism can always be found in association with a given disease.
2. The organism can be isolated and grown in pure culture in laboratory.
3. The pure culture must produce the same disease when inoculated into a susceptible healthy host.
4. The same microorganisms must be isolated again from the inoculated diseased host.

Koch developed media suitable for growing bacteria. He isolated the bacillus causing tuberculosis.

He perfected many bacteriological techniques and known as "Father of Practical Bacteriology". He is also called as Father of Modern Science of Tuberculosis.

- **Koch's "Direct Stimulation" Theory**

The first theory offered to account for the increased yield obtained from soils treated with an antiseptic was the "direct stimulation" theory advanced by Koch in 1899.

He considered carbon bisulphide to have a direct stimulating effect on the plants themselves. He later found ether to have a similar effect. In experiments dealing with the addition of ether to the soil Koch found that the increased yield was pronounced on the first crop, whereas the residual effect was slight, as with carbon bisulphide the beneficial effect increases with the amount of application. He further found that soils sterilized with heat produced better crops when treated with carbon bisulphide than when not so treated and concludes that the effect of the antiseptic, therefore, cannot be due to its effect on bacteria.

The theory of Koch has been supported by Fred who fertilized soil with an abundant supply of sodium nitrate and found that in every case in which carbon bisulphide was added the growth and yield of crop were much superior to those in the corresponding pots not treated with that substance. He concludes that as there was no lack of plant-food and other conditions were favourable to plant growth, the effect of the antiseptic must have been directly upon the plant.

There is ample evidence to prove that many of these antiseptics in dilute solutions stimulate the plants directly, yet there is no evidence which will substantiate the claim that this is the only or even the principal influence on the plant and soil.

6. Charls Chamberland (Discovery of Viruses & their role in diseases):-

Discovery of Viruses & their role in diseases was made possible when Charles Chamberland constructed a porcelain bacteriological filter. The first viral pathogen to be studied was Tobacco Mosaic Virus.

Later on most of the microbial pathogens were isolated. The list is given in the following table.

Sr. No.	Name of the disease	Causative agent
	BACTERIAL DISEASES	
1	Anthrax	*Bacillus anthracis*
2	Gonorrhea (Garmi)	*Neisseria gonorrhoeae*
3	Typhoid fever (Vishamajwar)	*Salmonella typhi*
4	Tuberculosis (Kshaya)	*Mycobacterium tuberculosis*
5	Cholera (Pataki)	*Vibrio cholarae*
6	Diptheria (Ghatasarpa)	*Corynebacterium diptheriae*
7	Tetanus (Dhanurvat)	*Clostridium tetani*
8	Pneumonia (Havare)	*Diplococcus pneumoniae*
9	Meningitis (Menducha tap)	*Neisseria meningitidis*
10	Plague	*Yersinia pestis*
11	Botulism (Anna Vish Badha)	*Clostridium botulinum*
12	Dysentry (Hagvan)	*Shigella dysentriae*
13	Syphilis (Garmi)	*Treponema pallidum*
14	Whooping cough (Dangya Khokla)	*Bordetella pertusis*
15	Pus in wound (Jakhamet Pu)	*Staphylococcus aureus*
	PROTOZOAL DISEASES	
1	Malaria (Hivtap)	*Plasmodium vivax*
2	Amoebic dysentery (Shembyukta Hagvan)	*Entamoeba histolytica*
3	White discharge in females (Angavar Pandhare Jane)	Different protozoa
	FUNGAL DISEASES	
1	Candidiasis (Kharuj)	*Candida albicans*

2	Dermatophytosis (Gachakarna)	Fungal species
	VIRAL DISEASES	
1	Polio (Hat pay Lule padane)	Polio virus
2	Flu	Influenza
3	Rabbies (Pisalne)	Rabbies virus
4	Jaundice (Kavil / Kamin)	Hepatitis virus
5	Measles (Govar/ Kanjanya)	Measles virus
6	Mumps (Galfugi)	Mumps virus
7	AIDS	Human Immuno Deficiency Virus
8	Swine Flu	H_1N_1
9	Chikungunya	Chikungunya virus
10	Ebola (internal bleeding)	Ebola virus

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iii) Aseptic surgery: -

The introduction of anaesthetics into surgery and obstetrics during the 1840s contributed greatly to the development of efficient surgical techniques. Due to this it became easy for surgeons to perform complex and lengthy operations, which previously were not feasible to undertake. Unfortunately the incidences of wound infections from surgical procedures increased and quite often result in the death of the patient.

The young English surgeon Joseph Lister faced this problem and he decided to prevent wound infections. Impressed with Pasteur's studies, Lister reasoned that the basis of surgical infection (sepsis) might be microbial nature. Consequently he devised a series of procedures designed to prevent the access of microorganisms to wounds. This system came to be known as **Antiseptic or Aseptic Surgery.** It included →

→ The heat sterilization of instruments.
→ The application of carbolic acid (phenol) to wounds by means of dressings.

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iv) Discovery of microbial effects on organic and inorganic matter. (Transformation of organic and inorganic matter)

Some scientists demonstrated that microorganisms can serve as specific agents for large scale chemical transformation. They studied microbial involvement in Carbon, Nitrogen and Sulphur cycles taking place in soil. Two of the pioneers **Winogradsky and Beijerinck** made many contributions in soil microbiology. They discovered the bacteria, which can oxidise iron, sulphur, and ammonia to obtain energy and many bacteria that could incorporate CO_2 into organic matter like photosynthetic organisms. They isolated anaerobic nitrogen fixing bacteria and studied the decomposition of cellulose.

They isolated aerobic **nitrogen fixing** bacterium Azotobactor, a root nodule bacterium Rhizobium and sulphate reducing bacteria. They developed enrichment culture technique and the use of selective media, which have been a great importance in microbiology.

Microorganisms accomplish major element transformations (mineralization and immobilization) in soil. They convert the complex organic nutrients into simpler inorganic compounds (mineralization) so that plants can make use of the nutrients and these microbes absorb the simpler minerals (immobilization) and prevent them from leaching out and thus conserve the essential nutrients in the soil so that when they die and become a part of the organic matter, these essential nutrients are once again mineralized by the microorganisms for plant use. Thus the soil fertility that is created by the microbes is also conserved by the same.

The major transformations by the microbes are: **carbon, nitrogen. Phosphorus, sulphur, iron, manganese, potassium and trace elements**. Apart from the soil based transformations, bacteria like Azotobacter, Azospirillum are able to convert the atmospheric gaseous nitrogen to reduced inorganic nitrogen for direct assimilation by plant roots thus limiting the use of chemical nitrogenous fertilizers. Besides, some microbes like Rhizobium, Bradyrhizobium, etc. form symbiotic association with the roots of leguminous plants where they form root nodules and fix atmospheric nitrogen directly in the nodule.

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v) Recognition of role of microorganisms in fermentation

Louis Pasteur was interested in knowing why the local wines in the France were turning sour. The popular belief was that fermentation of grape juice to wine was a natural chemical process, involving the breakdown of protein albumin. However Pasteur observed some oval yeast cells and some rods in wine. He believed that, the yeasts played major role in fermentation.

In a series of classic experiments, Pasteur first showed that alcohol would be produced from grape by yeast mixtures. Using heat, he destroyed the yeasts, were up on alcohol failed to appear in grape juice. However when yeasts were returned to the flasks, fermentation took place and wine was formed. More over if care were taken to eliminate the bacteria, the wine would not become sour.

In 1857 Pasteur showed that lactic acid fermentation is caused by living organisms. In 1860 he demonstrated that bacteria cause souring in milk, a process formerly thought to be merely a chemical change, and his work in identifying the role of microorganisms in food spoilage led to the process of pasteurization. In 1877 he defined fermentation as "Life without air," (anaerobic life) and showed specific types of microorganisms caused specific types of fermentations and specific end products.

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vi) Pure culture concept

Microorganisms occur in nature in extremely large populations made up of many different species (mixed culture). In order to study characteristics of particular species, it is necessary to isolate it from all other species. Laboratory procedures have been developed; those make it possible to isolate microorganisms representing each species and grow each of the species separately.

Pure culture: - The growth mass of cells of the same species is called pure culture.

Pure cultures of bacteria were first obtained by Joseph Lister in 1878 using serial dilution technique in liquid media, with a specially constructed syringe. After incubation bacteria in the container were of a single kind all derived from single parent cell.

During Koch's studies on bacterial diseases, it became necessary to isolate suspected bacterial pathogens. At first, Koch cultured bacteria on the sterile surfaces of cut, boiled potatoes. This was unsatisfactory because bacteria would not always grow well on potatoes. He then tried to solidify regular liquid medium by adding gelatin. Separate bacterial colonies developed on the surface. Despite of advantages, gelatin was not an ideal solidifying agent because it was digested by many bacteria and melted when the temperature rises above 28^{o} C. A better alternative was provided by **Fannie Hesse, a wife of Walter Hesse**, one of Koch's assistants. She suggested the use of agar as a solidifying agent. She had been using agar successfully to make jellies for some time.

Agar is a sulfacted polymer composed mainly of D- galactose, 3 - 6 unhydro – L – galactose and D- glucuronic acid. It is usually extracted from red algae (***Geledium cornium***). It is well suited as a solidifying agent because after it has been melted in boiling water, it can be cooled to about 40 to 42 ^{0} C before hardening and will not melt again until the temperature rises to about 80 to 90^{0} C. It is also an excellent hardening agent because most microorganisms cannot degrade it.

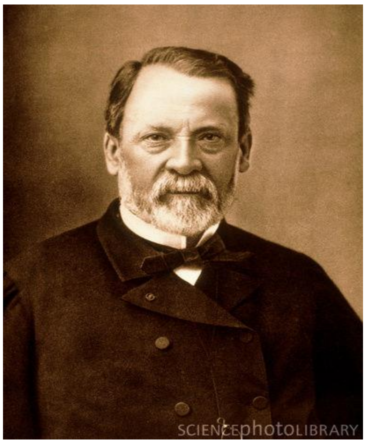
One of Koch's assistants **Richard Petri** developed a **Petri Dish,** a container for solid culture media. These developments made possible the isolation of pure cultures that contained only one type of bacterium, and directly stimulated progress in all areas of bacteriology. Thus development of liquefiable solid culture medium was a fundamental importance. The pure cultures are very important to the development of science of microbiology because by using pure culture technique, the microorganisms responsible for many infections, fermentations, nitrogen fixation and other activities were isolated and identified.

❖ Noble laureates and their contribution

1) Anton van Leeuwenhoek (1632-1723)

Antony van Leeuwenhoek was born on October 24, 1632 in Delft, Holland (now Netherlands). In **1674**, he made first observation of microorganisms and was the **first person to observe and accurately describe and measure bacteria and protozoa**, termed by him, as **"animalcules"** which he thought were tiny animals. In **1677**, he became the **first person to describe spermatozoa and was one of the earliest to describe red blood corpuscles**. In 1680, he was elected a fellow of the Royal Society of London, and with Isaac Newton and Robert Boyle, he became one of the first famous men of his time. He died on August 30, 1723 at the age of 90. Because of his extraordinary contribution to microbiology, he is considered as the **Father of Microbiology**.

2) Louis Pasteur (1822–1895)



Louis Pasteur, a French microbiologist, was born on December 27, 1822 in Dole, France. He studied at the French school, the *Ecole Normale Superieure*. In 1848, he achieved distinction in organic chemistry for his discovery that tartaric acid, a four carbon organic compound forms two different types of crystals. Using a microscope, Pasteur successfully separated the crystals and developed a skill that would aid his later studies of microorganisms. In 1854, at the young age of 32, he was appointed Professor of Chemistry at the University of Lille in northern France. He died in 1895, at the age of 73.

Notable Contributions

1857 – Lactic acid fermentation is due to a microorganism
1860 – Yeasts are involved in alcoholic fermentation
1861 – Disproved the theory of spontaneous generation

1861 – Introduction of the terms aerobic and anaerobic for yeasts. Production of more alcohol in the absence of oxygen during sugar fermentation- **The Pasteur Effect**

1862 – Proposed germ theory of disease

1867– Pasteur devised the process of destroying bacteria known as **pasteurization.**

1881 – Development of anthrax vaccine. Resolved Pebrine problem of silkworms.

1885 – Development of a special vaccine for rabies (the **Pasteur treatment**)

Due to his contribution he is called as **Father of Modern Microbiology.**

3) Robert Koch (1843–1910)

Robert Koch was born in Hanover, Germany in 1843. For his contributions on tuberculosis, Robert Koch was awarded the 1905 Nobel Prize for Physiology or Medicine. He died in the year 1910 at the age of 67.

Notable contributions

1876 – Koch demonstrated that anthrax is caused by *Bacillus anthracis.*

1877 – Developed methods for staining bacteria, photographing and preparing permanent visual records on slides.

1881 – Koch developed solid culture media and the methods for studying bacteria in pure cultures.

1882 – Isolated the bacterium—*Mycobacterium tuberculosis*—that causes tuberculosis.
1882 – Use of agar as a support medium for solid culture in Koch's lab by Hesse.
1883 – Isolation of *Vibrio cholerae*, the cause of cholera.
1883 – Verification of the germ theory of disease by relating a specific organism to the specific disease.
1884 – Koch put forth his postulates—known as Koch's postulates.

4) Joseph Lister (1827–1912)

Joseph Lister was born in 1827. He developed a system of antiseptic surgery by using phenol and sterile equipments designed to prevent microorganisms from entering wounds in 1867. In 1878, Lister studied the lactic acid fermentation of milk and demonstrated the *specific cause of milk souring.* He also developed a method for isolating a pure culture of a bacterium, named as *Bacterium lactis.* Because of his notable contribution-first introduction of principles of sterile surgery in medical practice, which was so far reaching in its effects—Lister will always be known as the **Father of antiseptic surgery.** He died at the age of 85 in the year 1912.

5) Edward Jenner (1749–1823)

Edward Jenner, born in 1749, was an English physician from Berkeley, Gloucestershire, England. As a scientist who was the pioneer of smallpox vaccine, the world's first vaccine. He is often called "**Father of Immunology**", and his work is said to have "saved more lives than the work of any other human". He was a member of the Royal Society. Jenner was named in the BBC's list of the 100 Greatest Britons. His great gift to mankind was his **vaccine for smallpox** (characterized by production of skin lesions called pox (pocks), caused by *Variola*, belonging to the category of pox viruses). Jenner's discovery, **that a less pathogenic agent could confer protection against a more pathogenic one**, is especially remarkable in view of the fact that microscopy was still in its infancy and the nature of the virus was not known. **The modern era of vaccines and vaccination, thus began in 1798 with Edward Jenner's use of cowpox as a vaccine against smallpox.**