Chapter 1 Lecture Notes: The History and Scope of Microbiology

I. What is microbiology?

A. Microbiology is the study of organisms and agents that are generally too small to be seen clearly by the unaided eye. These organisms include viruses, bacteria, algae, fungi, and protozoa.

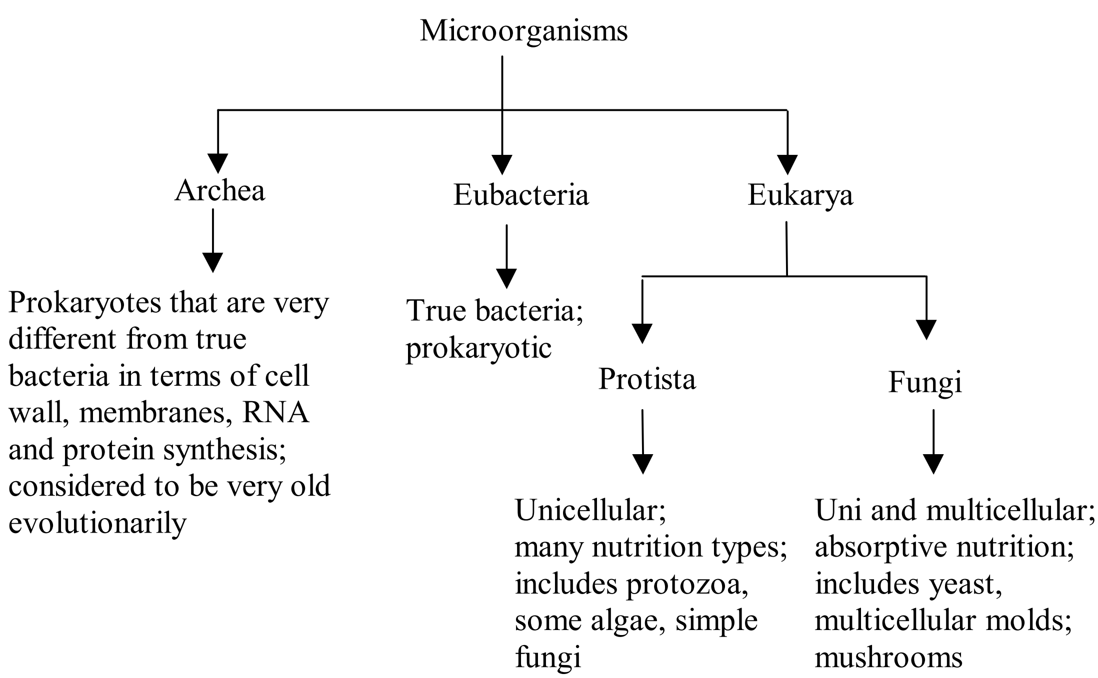
B. Microbiology can be applied or basic.

C. Microbiology is linked to many other scientific disciplines including biochemistry, cell biology, evolution, ecology.

D. Subdisciplines (both applied and basic research)

1. General microbiology: broad range of microbiological questions
2. Medical microbiology: microbes that cause human disease
3. Public health and epidemiology: Studies and controls transmission, frequency, and distribution of disease
4. Immunology: the immune system
5. Agricultural microbiology: impact of microbes on agriculture
6. Microbial ecology: relationships between microbes and their habitats
7. Food microbiology: Prevention of food borne disease; microbes that make food and drink
8. Industrial microbiology: commercial use of microbes to produce products
9. Biotechnology: manipulation of organisms to form useful products

II. What are microbes?



III. The scope and relevance of microbes

A. L. Pasteur "The role of the infinitely small in nature is infinitely large."
B. 1st living organisms
C. Live in every possible environment
D. More numerous than macroorganisms
E. Constitute the largest component of biomass
F. Fundamental to the ecosystem
G. Have changed the course of history in obvious and not so obvious ways

IV. History

A. Discovering the "organisms"
   1. 1676: A. Leeuwenhoek – first to observe and describe microbes accurately
   2. 1884: C. Chamberland – constructed a bacterial filter that allowed the identification of viruses
   3. 1898: Loeffler and Frosch – identified filterable infectious agent as cause of foot-and-mouth disease in cattle
   4. 1898-1900: M. Beijerinck – identified tobacco mosaic virus
   5. 1982: S. Prusiner – described prions (infectious protein that causes a particular normal protein to alter its shape and become a prion)

B. Disproving spontaneous generation (that living organisms could develop from nonliving matter)
   1. 1688: F. Redi – first to challenge theory of spontaneous generation by showing that if raw meat was protected from flies, the formation of maggots was prevented
   2. 1748: R. Needham – supported spontaneous generation of microbes by showing that even after boiling mutton broth and pouring into sealed containers, growth of microbes occurred
   3. 1776: L. Spallanzani - challenged spontaneous generation as it pertained to microbes by showing that sealed containers that were boiled do not produce microbes
   4. 1861: L. Pasteur – rigorously disproved spontaneous generation
      a) filtered air → showed that air contained microbial organisms
      b) constructed flasks with curved neck that allowed air into the flasks while dust, etc. remained in the neck → placed broth into the flasks and boiled → showed that no microbial growth resulted unless flasks were tipped to allow the broth into the neck

C. The germ theory of disease
   1. Previously, people thought that disease was punishment for an individual's crimes, due to poisonous vapors, and/or an imbalance of the "four humors".
   2. First proponents of the idea that invisible organisms caused disease were Lucretius (B.C.) and Fracastoro (1546)
   3. 1835: A. Bassi showed that silkworm disease was due to a fungus.

4. 1867: J. Lister showed that antiseptic surgical procedures reduced the frequency of wound infections.
5. 1876/1884: R. Koch definitively proved that *Bacillus anthracis* caused the disease anthrax in cows and *Mycobacterium tuberculosis* caused the disease tuberculosis using Koch's postulates.
    a) The suspected pathogen should be present in ALL cases of the disease and NOT present in healthy animals.
    b) The suspected pathogen should be grown *in vitro* in pure culture.
    c) Cells from a pure culture of the putative pathogen should cause disease in healthy animals.
    d) The putative pathogen should be reisolated from the infected animal.

D. Preventing disease by vaccination
1. E. Jenner inoculated people with cowpox to protect against smallpox.
2. 1885 – Pasteur developed the rabies vaccine.
3. 1890: von Behring and Kitasato produced antibodies to purified toxins to protect against diphtheria and tetanus.
4. 1884: E. Metchnikoff described phagocytosis of bacteria.

E. Discovering the effect of microbes on organic and inorganic matter
1. 1856: Pasteur described lactic acid fermentation; contributions to wine industry.
2. 1887-1900: S. Winogradsky and M. Beijerinck studied soil microbes and their role in the biochemical cycles of sulfur, carbon, nitrogen

V. Recent history of microbiology – the 20th century

A. Infectious diseases: The etiological agent of most infectious diseases has been ascertained. Current research focuses on understanding the molecular mechanisms by which disease is caused.

B. Chemotherapy: Discovery of antibiotics; antibiotic resistance

C. Immunology develops as a science.

D. Physiology and biochemistry: Using microbes as a model, many physiological and biochemical processes have been elucidated.

E. Genetics: Many of the advances in molecular genetics were made using bacteria as models. A few of the many:
1. 1941: Beadle and Tatum – 1 gene = 1 enzyme
2. 1943: Luria and Delbruck – mutations are spontaneous in nature
3. 1944: Avery, MacLeod, and McCarty – DNA is the genetic material
4. 1961: Jacob and Monod – the operon and gene regulation

F. Molecular biology: Many of the advances in molecular biology were made using bacteria as models. A few of the many:
1. 1970: Restriction enzymes discovered
2. 1979: Insulin synthesized using recombinant techniques
3. 1990: Gene therapy trials begin
4. 1995: The nucleotide sequence of the first free-living organism (*Haemophilus influenzea*) published

What Microbiologists?

Microbiologists study microorganisms such as bacteria, viruses, algae, fungi, and some types of parasites. They try to understand how these organisms live, grow, and interact with their environments.

Duties of Microbiologists

Microbiologists typically do the following:

- Plan and conduct complex research projects, such as improving sterilization procedures or developing new drugs to combat infectious diseases
- Perform laboratory experiments that are used in the diagnosis and treatment of illnesses
- Supervise the work of biological technicians and other workers and evaluate the accuracy of their results
- Isolate and maintain cultures of bacteria or other microorganisms for study
- Identify and classify microorganisms found in specimens collected from humans, plants, animals, or the environment
- Monitor the effect of microorganisms on plants, animals, other microorganisms, or the environment
- Review literature and the findings of other researchers and attend conferences
- Prepare technical reports, publish research papers, and make recommendations based on their research findings
- Present research findings to scientists, nonscientist executives, engineers, other colleagues, and the public

Many microbiologists work in research and development conducting basic research or applied research. The aim of basic research is to increase scientific knowledge. An example is growing strains of bacteria in various conditions to learn how they react to those conditions. Other microbiologists conduct applied research and develop new products to solve particular problems. For example, microbiologists may aid in the development of genetically engineered crops, better biofuels, or new vaccines.

Microbiologists use computers and a wide variety of sophisticated laboratory instruments to do their experiments. Electron microscopes are used to study bacteria, and advanced computer software is used to analyze the growth of microorganisms found in samples.

It is increasingly common for microbiologists to work on teams with technicians and scientists in other fields, because many scientific research projects involve multiple disciplines. Microbiologists may work with medical scientists or molecular biologists while researching new drugs, or they may work in medical diagnostic laboratories alongside physicians and nurses to help prevent, treat, and cure diseases.

The following are examples of types of microbiologists:
Bacteriologists study the growth, development, and other properties of bacteria, including the positive and negative effects that bacteria have on plants, animals, and humans.

Clinical microbiologists perform a wide range of clinical laboratory tests on specimens collected from plants, humans, and animals to aid in detection of disease. Clinical and medical microbiologists whose work involves directly researching human health may be classified as medical scientists.

Environmental microbiologists study how microorganisms interact with the environment and each other. They may study the use of microbes to clean up areas contaminated by heavy metals or study how microbes could aid crop growth.

Industrial microbiologists study and solve problems related to industrial production processes. They may examine microbial growth found in the pipes of a chemical factory, monitor the impact industrial waste has on the local ecosystem, or oversee the microbial activities used in cheese production to ensure quality.

Mycologists study the properties of fungi such as yeast and mold. They also study the ways fungi can be used to benefit society (for example, in food or the environment) and the risks fungi may pose.

Parasitologists study the life cycle of parasites, the parasite-host relationship, and how parasites adapt to different environments. They may investigate the outbreak and control of parasitic diseases such as malaria.

Public health microbiologists examine specimens to track, control, and prevent communicable diseases and other health hazards. They typically provide laboratory services for local health departments and community health programs.

Virologists study the structure, development, and other properties of viruses and any effects viruses have on infected organisms.

Important Qualities for Microbiologists
Communication skills. Microbiologists should be able to effectively communicate their research processes and findings so that knowledge may be applied correctly.
Detail oriented. Microbiologists must be able to conduct scientific experiments and analyses with accuracy and precision.

Interpersonal skills. Microbiologists typically work on research teams and thus must work well with others toward a common goal. Many also lead research teams and must be able to motivate and direct other team members.

Logical-thinking skills. Microbiologists draw conclusions from experimental results through sound reasoning and judgment.

Math skills. Microbiologists regularly use complex mathematical equations and formulas in their work. Therefore, they need a broad understanding of math, including calculus and statistics.

Observation skills. Microbiologists must constantly monitor their experiments. They need to keep a complete, accurate record of their work, noting conditions, procedures, and results.

Perseverance. Microbiological research involves substantial trial and error, and microbiologists must not become discouraged in their work.

Problem-solving skills. Microbiologists use scientific experiments and analysis to find solutions to complex scientific problems.
Time-management skills. Microbiologists usually need to meet deadlines when conducting research and laboratory tests. They must be able to manage time and prioritize tasks efficiently while maintaining their quality of work.

Advancement for Microbiologists

Microbiologists typically receive greater responsibility and independence in their work as they gain experience. They also gain greater responsibility through certification and higher education. Ph.D. microbiologists usually lead research teams and control the direction and content of projects.

HISTORY OF MICROBIOLOGY: SPONTANEOUS GENERATION THEORY

Microbiology 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.

Microbial World

Microorganisms are everywhere. Almost every natural surface is colonized by microbes (including our skin). Some microorganisms can live quite happily in boiling hot springs, whereas others form complex microbial communities in frozen sea ice.

Most microorganisms are harmless to humans. You swallow millions of microbes every day with no ill effects. In fact, we are dependent on microbes to help us digest our food and to protect our bodies from pathogens. Microbes also keep the biosphere running by carrying out essential functions such as decomposition of dead animals and plants.

Microbes are the dominant form of life on planet Earth. More than half the biomass on Earth consists of microorganisms, whereas animals constitute only 15% of the mass of living organisms on Earth.

This Microbiology course deals with

- How and where they live
- Their structure
- How they derive food and energy
- Functions of soil micro flora
- Role in nutrient transformation
- Relation with plant
- Importance in Industries

The microorganisms can be divided into two distinct groups based on the nucleus structure:

Prokaryotes – The organism lacking true nucleus (membrane enclosed chromosome and nucleolus) and other organelles like mitochondria, golgi body, entoplasmic reticulum etc. are referred as Prokaryotes. (Ex : Bacteria, archaea)

Eukaryotes - The organism possessing membrane enclosed nucleus and other cell organelles are referred as Eukaryotes (Ex : algae, fungi, protozoa)
The microorganisms were divided into 6 distinct groups based on the phylogenic, morphological and physiological characters.

The major groups of microorganisms are

1. Bacteria are phylogenetically related group of unicellular prokaryotic organisms distinct from archeae
2. Archaea is phylogenetically related group of prokaryotes which are primitive and distinct from bacteria
3. Fungi are group of eukaryotic organisms lacking chlorophyll. They range in size and shape from single celled yeast to multicellular mushrooms.
4. Algae refer the group of eukaryotic organisms with chlorophyll. They range in size and shape from single celled algae (Ex: *Chlorella*) to complex cellular structured plant like algae (Ex. Kelp)
5. Protozoa are group of eukaryotic organisms lack of cell wall. The morphology, nutrition and physiology is different from other groups
6. Viruses are group of non-cellular organisms, parasite or pathogen to plant, animals and other microorganisms. They are too small and cab be visualized only under electron microscopes

History of Microbiology in brief

Obviously human have had to deal with microbes even before the recorded history. The first record of human using comes from ancient tablets from mid east.

Babylonians were using yeast to make beer over 8000 years ago and acetic acid bacteria to make vinegar over 6000 years ago.

About 5000 years ago, Persia (Now Iran) region recorded the wine making.

The Romans had God for that were specific for microorganisms. The roman God of mold and mildew was "*Robigus*" and "*Robigo"* which means crop rust. (Rust is one of the plant disease caused by fungus). God Robigus was very much feared because of crop lost.

About 2000 years ago, Romans proposed that diseases were caused by tiny animals. But, fundamentalist religions had a strong hold over the progress.

The real microbiology history starts from 1600s, when people began to make crude lenses and microscopes.

HIGHLIGHTS IN THE HISTORY OF MICROBIOLOGY

Effects of Disease on Civilization

- Infectious diseases have played major roles in shaping human history
- Bubonic Plague epidemic of mid 1300's, the "Great Plague", reduced population of western Europe by 25%. Plague bacterium was carried by fleas, spread from China via trade routes and poor hygiene. As fleas became established in rat populations in Western Europe, disease became major crisis.
- Smallpox and other infectious diseases introduced by European explorers to the Americas in 1500's were responsible for decimating Native American populations. Example: In the century after Hernan Cortez's arrival in Mexico, the Aztec population declined from about 20 million to about 1.6 million, mainly because of disease.
- Infectious diseases have killed more soldiers than battles in all wars up to WW II. Example: in U. S. Civil war, 93,000 Union soldiers died in direct combat; 210,000 died as a result of infections.
- Until late 1800's, no one had proved that infectious diseases were caused by specific microbes, so the possibility of prevention or treatment had no sound empirical base.

Brueghel: The Triumph of Death (1560)

Discovery of Microbes

- To see microbes, you need a microscope. The first microscope was invented by Antony van Leeuwenhoek (1632-1723), a Dutch businessman.
- Leeuwenhoek took up lens grinding to make magnifiying glasses so he could examine fine weave of fabrics. In testing his lenses, he discovered many small

Antony van Leeuwenhoek

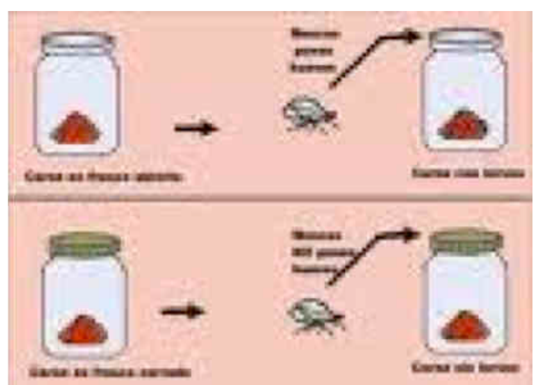
creatures he called "animalcules" in samples such as pond water. His best lenses could magnify 300-500X.

- Leeuwenhoek microscopes were crude, relied on a single lens held in a metal plate.
- Leeuwenhoek described many previously unseen life forms, including different forms of bacteria, mold spores, etc. Leeuwenhoek reported discoveries to Royal Society from 1670's on, firmly established existence of microbes. Nevertheless, the significance of this discovery was not apparent for almost 200 years.

Origin of Life Controversy

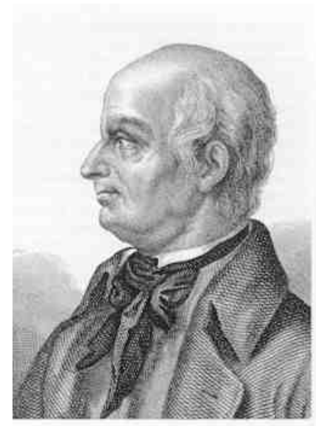
- Where did microbes come from? Many believed they arose from simple materials by process of spontaneous generation. This notion had been posited by Aristotle (382-322 B.C.) and other Greek philosophers to explain decay and appearance of animals such as flies and frogs, and was widely held as common sense even in 1700's and 1800's.

- **Francisco Redi (1626-1697)** demonstrated that flies did not arise spontaneously from rotting meat by simple experiment. If jar of meat was covered by fine muslin, maggots did not arise. However, the simpler life forms discovered by Leeuwenhoek lacked visible complexity, and most people still believed these could arise spontaneously.



- **John Needham (1731-1781),** a Scottish clergyman and naturalist, showed that mirobes grew in soups exposed to air. Claimed existence of a "life force" present in inorganic matter that could cause spontaneous generation. One of his more convincing demonstrations was to boil some soup (briefly), pour into clean flasks with cork lids, and show that microbes would soon arise.

- **Lazzaro Spallanzani (1729-1799)** claimed Needham's organisms came from heat-resistant microbes. If flasks were boiled long enough (1-2 h), nothing grew. But Needham countered that prolonged heating destroyed the "life force".



- **Louis Pasteur (1822-1895)** was passionate believer that life only originated from previous life, developed several experiments that finally deflated claims for spontaneous generation. Pasteur filtered air through cotton to trap airborne materials, then dissolved the cotton and examined the particulate matter under a microscope; many bacteria and spores of other life forms such as molds were present. Since most skeptics kept arguing that overheating killed the life force present in air, Pasteur developed and ingenious experiment using a swan neck flask that allowed fresh air to remain in contact with boiled materials. The long passageway prevented airborne microbes from reaching the nutrient liquid, without impeding access to air. One of Pasteur's flasks is still sterile after 100+ years of being exposed to the air (Pasteur Institute, Paris).

Louis Pasteur

Spontaneous Generation theory

From earliest times, people had believed in **spontaneous generation**—that living organisms could develop from nonliving matter. Even the great Aristotle (384–322 B.C.) thought some of the simpler invertebrates could arise by spontaneous generation. This view finally was challenged by the Italian physician Francesco Redi (1626–1697), who 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 a fine gauze that would exclude flies. 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. Similar experiments by others helped discredit the theory for larger organisms.

Leeuwenhoek's discovery of microorganisms renewed the controversy. Some proposed that microorganisms arose by spontaneous generation even though larger organisms did not. They pointed out that boiled extracts of hay or meat would give rise to microorganisms after sitting for a while. In 1748 the English priest John Needham (1713–1781) reported the results of his experiments on spontaneous generation. Needham boiled mutton broth and then tightly stoppered the flasks. Eventually many of the flasks became cloudy and contained microorganisms. He thought organic matter contained a vital force that could confer the properties of life on nonliving matter. A few years later the Italian priest and naturalist Lazzaro Spallanzani (1729–1799) improved on Needham's experimental design by first sealing glass flasks that contained water and seeds. If the sealed flasks were placed in boiling water for 3/4 of an hour, no growth took place as long as the flasks remained sealed. He proposed that air carried germs to the culture medium, but also commented that the external air might be required for growth of animals already in the medium. The supporters of spontaneous generation maintained that heating the air in sealed flasks destroyed its ability to support life. Several investigators attempted to counter such arguments. Theodore Schwann (1810–1882) allowed air to enter a flask containing a sterile nutrient solution after the air had passed through a red-hot tube. The flask remained sterile. Subsequently Georg Friedrich Schroder and Theodor von Dusch allowed air to enter a flask of heat-sterilized medium after it had passed through sterile cotton wool. No growth occurred in the medium even though the air had not been heated. Despite these experiments the French naturalist Felix Pouchet claimed in 1859 to have carried out experiments conclusively proving that microbial growth could occur without air contamination.

This claim provoked Louis Pasteur (1822–1895) to settle the matter once and for all. Pasteur first filtered air through cotton and found that objects resembling plant spores had been trapped. If a piece of the cotton was placed in sterile medium after air had been filtered through it, microbial growth appeared. Next he placed nutrient solutions in flasks, heated their necks in a flame, and drew them out into a variety of curves, while keeping the ends of the necks open to the atmosphere .Pasteur then boiled the solutions for a few minutes and allowed them to cool. No growth took place even though the contents of the flasks were exposed to the air. Pasteur pointed out that no growth occurred because dust and germs had been trapped on the walls of the curved necks. If the necks were broken, growth commenced immediately. Pasteur had not only resolved the controversy by 1861 but also had shown how to keep solutions sterile. The English physicist John Tyndall (1820–1893) dealt a final blow to spontaneous generation in 1877 by demonstrating that dust did indeed carry germs and that if dust was absent, broth remained sterile even if directly exposed to air. During the course of his studies, Tyndall provided evidence for the existence of exceptionally heat-resistant forms of bacteria. Working independently, the German botanist Ferdinand Cohn (1828–1898) discovered the existence of heat-resistant bacterial endospores

The Spontaneous Generation Experiment: Pasteur's swan neck flasks used in his experiments on the spontaneous generation of microorganisms.

2. Disproval of Spontaneous Generation theory

At that time, the age old idea of "Spontaneous Generation theory" was the dominant one. The idea that organism originate directly from non-living matter. (Life from non-living) also called as abiogenesis (a – not; bio – life; genesis – origin).

Eg : Maggots were developed spontaneously via recombination of matters in rotting materials. (ex meat)

Microbiology starts with the disproval of SG theory.

Louis Pasteur (1822 – 1895) and disproval of Spontaneous generation theory

He performed “gooseneck experiment”. The nutrient of flask was heated and the untreated – unfiltered air could pass in or out, but the germs settled in the gooseneck and no microbes were observed in the nutrient solution.

His concept of Germs theory of disease (means germs are responsible for the disease not the inert mater) ends the SG theory.

Contributions of Louis Pasteur (1822 – 1895)

- Disproved the SG theory
- Discovered that fermenting fruit to alcohol by microbes – From now the Fermentation started
- Sorted different microbes giving different taste of wine.
- He selected a particular strain (Yeast) for high quality wine.
- He developed a method to remove the undesired microbes from juice without affecting its quality. Heating the juice at 62.8°C for half-an hour did the job. This technique is called as Pasteurization, which is commonly used in the field of milk industry.
- He discovered that parasites (protozoa) causing pebrine disease of silk worm. He suggested that disease free caterpillars can eliminate the disease.
- He isolated the anthrax causing bacilli from the bloods of cattle, sheep and human being.
- He also demonstrated the virulence (ability of microbe to cause disease) of bacteria
- He developed vaccine (a killed or attenuated microbe to induce the immunity) against rabbis from the brains and spinal cord of rabbit

John Tyndall (1820 -1893)

- Proved that dust carries the germs and if no dust in the air, the sterile broth remained free of microbial growth for indefinite period.
- He also developed a sterilization method “Tyndallization”, referred as intermittent or fractional sterilization. The subsequent cooling and heating by steam for 3 days will remove the germs and their spores.

Martinus Willium Beijerinck (1851 – 1931)

- Developed the enrichment technique to isolate various group of bacteria.
- Isolated sulphur reducing bacteria and sulphur oxidizing bacteria from soil

- Isolated free-living nitrogen fixing bacterium, *Azotobacter* from soil,
- Root nodulating bacterium, *Rhizobium*, *Lactobacillus*, green algae were identified by him
- He confirmed the Tobacco mosaic virus causes disease and it incorporated in the host plant to reproduce.

Sergei Winogradsky (1856 – 1953)

The following are the contributions of Winogradsky to soil microbiology.

- Microorganisms involved in N cycle, C cycle, S cycle
- Nitrification process in soil
- Autotrophic nutrition of bacteria
- Chemolithotrophic nutrition of soil bacteria
- Discovered anaerobic nitrogen fixing bacterium *Clostridium pasteurianum*

Walther Hesse & Fannie E. Hesse (1883)

They used agar instead of gelatin for preparation of media. Agar goes to solution at 100°C and solidifies at 45°C. Till now this was not replaced by any other substance.

Joseph Lister (1878)

Developed Pure culture technique. Pure culture referred as the growth of mass of cells of same species in a vessel. He developed the pure cultures of bacteria using serial dilution technique.

He also discovered that carbolic acid to disinfect the surgical equipments and dressings leads the reduction of post-operational deaths/infections.

Alexander Fleming (1928) identified *Penicillium notatum* inhibiting *Staphylococcus aureus* and identified the antibiotic Penicillin

- 1929-Discovered antibiotic penicillin –important milestone in medical microbiology
- Found that natural substances having antimicrobial activity-Saliva,Nasal mucous
- Worked on Staphylococcus aureus,-inhibition of growth-due to Penicillin
- Florey &Chain-isolated Penicillin in pure culture.

Selman A Waksman, 1945 identified *Streptomycin* antibiotic from soil bacterium. He also coined the term antibiotics (referring a chemical substance of microbial origin which is in small quantity exert antimicrobial activity.

- 1927- Wrote the book on Principles of soil Microbiology
- In 1939 Waksman and his colleagues undertook a systematic effort to identify soil organisms producing soluble substances that might be useful in the control of infectious diseases, what are now known as antibiotics
- Within a decade ten antibiotics were isolated and characterized,
- three of them with important clinical applications
- actinomycin in 1940, streptomycin in 1944, and neomycin in 1949.
- Eighteen antibiotics were discovered under his general direction.

GERM THEORY OF DISEASE

Introduction

Bacteria are mostly unicellular organisms that lack chlorophyll and are among the smallest living things on earth—only viruses are smaller. Multiplying rapidly under favorable conditions, bacteria can aggregate into colonies of millions or even billions of organisms within a space as small as a drop of water.The Dutch merchant and amateur scientist Anton van Leeuwenhoek was the first to observe bacteria and other microorganisms. Using single-lens microscopes of his own design, he described bacteria and other microorganisms (calling them "animacules") in a series of letters to the Royal Society of London between 1674 and 1723.

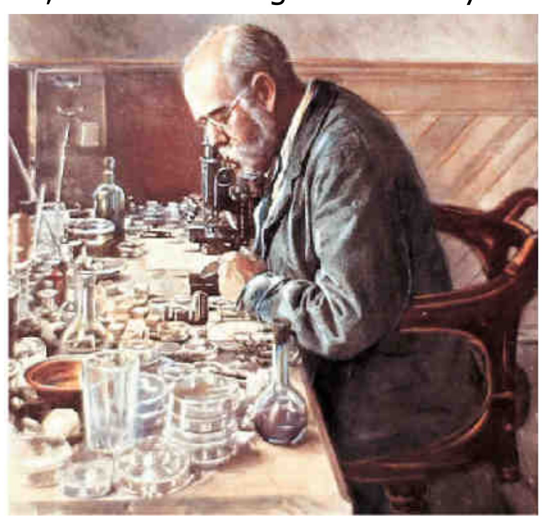
Bacteria are classified as prokaryotes. Broadly, this taxonomic ranking reflects the fact that the genetic material of bacteria is contained in a single, circular chain of deoxyribonucleic acid (DNA) that is not enclosed within a nuclear membrane. The word prokaryote is derived from Greek meaning "prenucleus." Moreover, the DNA of prokaryotes is not associated with the special chromosome proteins called histones, which are found in higher organisms. In addition, prokaryotic cells lack other membrane-bounded organelles, such as mitochondria. Prokaryotes belong to the kingdom Monera. Some scientists have proposed splitting this designation into the kingdoms Eubacteria and Archaebacteria. Eubacteria, or true bacteria, consist of more common species, while Archaebacteria (with the prefix archae—meaning ancient) represent strange bacteria that inhabit very hostile environments. Scientists believe these bacteria are most closely related to the bacteria which lived when the earth was very young. Examples of archaebacteria are those bacteria which currently live in extremely salty environments or extremely hot environments, like geothermal vents of the ocean floor

Microbes are organisms that we need a microscope to see. The lower limit of our eye's resolution is about 0.1 to 0.2 mm or 100 - 200 um. Most microbes range in size from about 0.2 um to the 200 um upper limit, although some fruiting bodies of fungi can become much larger. Microbes include the bacteria, algae, fungi, and protozoa. In this lecture we will discuss mostly the bacteria and the fungi.

Bacteria are found everywhere in water, soil, and even air. These small prokaryotic cells, typically from 0.2 to 1 um in length, are capable of living in boiling water, frozen ground, acid volcanoes, and at the bottom of the ocean. They can reproduce by doubling with a generation time of 20 minutes, or survive for centuries in a resting stage. In natural waters (lakes, streams, oceans) their generation time is around 1 day. In soils they live in a film of water around plant roots or other particles, and their activity is dependent on the temperature and the amount of available moisture. In general, bacteria are found in concentrations of 106 cells/mL of water in surface waters, and 109 cells/mL of soil in soils and sediments.

Robert Koch (1843 -1910): The Father of Microbial Techniques

Robert Koch, a German Physician, is well known to the world of microbiology for this significant contributions especially in the area of microbial techniques. He introduced analine dyes for staining bacteria; used agar-agar and gelatin to prepare solid culture media; stressed the need for pure culture to study microbes in details; confirmed germ theory of disease, and laid down Koch's postulates to test the pathogenesity of causative agents. He also discovered the casual organisms of anthrax disease of cattle (*Bacillus anthracis*) and tuberculosis (*Mycobacterium tuberculosis*).



Robert Koch was particularly concerned with this problem and, at first, he cultured bacteria on solid fruits and vegetables such as slices of boiled potato but many bacteria did not grow on such substrates. Then he perceived that it would be far better if a well-tried liquid medium could be solidified with some clear substance. Koch (1881) tried gelatin as a solidifying agent and succeeded in developing solid culture media, but gelatin, the first solidifying agent used, had serious disadvantage of becoming liquid above 28-30°C which is below the optimum temperature for the growth of human disease producing bacteria.

However, Koch replaced gelatin by agar in 1883-84 on the recommendation of F.E. Hesse, a German housewife, who had gained experience with the characteristics of agar in the process of making jelly. Agar is still frequently used as solidifying agent in microbiological laboratories. The development of solid culture media to grow pure culture was of fundamental importance and may be considered one of the Koch's greatest contributions.

Besides developing solid culture media using gelatin and agar, Koch also evolved methods to placed microbes on glass slides and colour them with analine dyes (stains) so that the individual cells could be seen more clearly under the microscope.

KOCH’S POSTULATES

1. The microorganism must be present in every case of the disease but absent from healthy organisms.
2. The suspected microorganism must be isolated and grown in a pure culture.
3. The same disease must result when the isolated microorganism is inoculated into a healthy host.
4. The same microorganism must be isolated again from the diseased host.

"One microbe, one disease"

• Robert Koch (1843-1910) was the first to rigorously demonstrate that a specific disease was caused by a specific microorganism.

• Koch worked on anthrax, a disease mainly of animals. Koch noticed that cattle that died of anthrax all seemed to have a certain rod-shaped bacterium in blood, not found in healthy animals. Koch was able to isolate the bacterium in pure culture, put it back into healthy cows, and reproduce the disease.

• Koch's Postulates: a logical way to identify the microbe causing a disease

A specific microbe must be present in all disease cases

Microbe must be cultivated outside host in a pure culture

When pure culture of microbe is inoculated into healthy hosts, disease symptoms identical to those of initial host must be reproduced

Microbe can be isolated again in pure culture from this experimentally inoculated host.

• Initial attempts to isolate microbes used sliced potatoes or nutrient media containing gelatin -- not ideal media. Then Fannie Hesse (wife of lab worker) suggested agar, a gelling agent used in cooking. Agar rapidly became the standard gelling agent for microbial isolation because it is relatively inert (only some marine microbes have enzymes to digest agar). Agar only melts at high temperatures (100^{o}C); once melted, it remains liquid until about 45^{o}C, at which point it gels.

• Koch's success at identifying anthrax with bacterium Bacillus anthracis led both Koch and Pasteur to identify the causes of many diseases -- cholera, tuberculosis, plague, etc. -- over the next few decades (late 1880's) -- the "Golden Age of Microbiology" (~ 1870-1920). Note that many microbiologists would regard the present as a new "Golden Age", since the development of molecular biological techniques, PCR, molecular phylogeny, and other developments have revealed many new insights and opened a world of new research directions and ways of understanding microbes.

DEFINITION AND SCOPE OF MICROBIOLOGY

Microbiology is a branch of science that deals with the structure, function, classes and economic importance of microorganisms. Microbiology is one of the exciting; ever developing field of science with greater scopes as the microbes play a major role in our day to day life. This chapter introduces the subject of microbiology to the undergraduate beginners who have little knowledge about this subject.

1.1. DEFINITION OF MICROBIOLOGY

Microbiology is defined as the study of organisms and agents that are too small to be seen clearly by the unaided eye. To be more simple, microbiology is the **study of microorganisms** which are the living organisms of microscopic size. **Microorganisms** are the living organisms that are less than 1 millimeter in diameter which cannot be seen by our naked eye. Microorganisms can be viewed through microscopes and they can exist as single cells or clusters. Microorganisms include the cellular organisms like **bacteria, fungi, algae and protozoa**. **Viruses** are also included as one of the microorganism but they are acellular.

1.2. DIFFERENCE BETWEEN MICROBIAL CELL AND PLANT/ANIMAL CELL

S. No	Microbial cell	Plant/Animal cell
(a)	A microbial cell can **live alone**	Plant or animal cell exist only as part of organisms
(b)	Growth, energy generation and reproduction by a microbial cell are **independent**	Plant or animal cell depend on other cells for all processes

1.3. OCCURRENCE OF MICROORGANISMS

One of the interesting things about the microorganism is that, they **occur everywhere**, even in the atmosphere, water and soil. Almost all natural surfaces are colonized by microbes. Some microorganisms are even adapted to live comfortably in boiling hot springs and frozen sea ice. Microbes are the **dominant form of life** in the universe. More than 50 per cent of the biomass on earth consists of microorganisms compared to animals which constitute only 15 per cent of the mass of living organisms on earth. Majority of the microorganisms are not dangerous to humans. In fact, microbes help to digest our food and protect our bodies from pathogens. Additionally, they are considered as **beneficial** ones as they keep the biosphere running by carrying out essential functions such as decomposition of dead animals and plants, nutrient cycling which enhances the soil health and crop productivity.

1.4. MEMBERS OF THE MICROBIAL WORLD

Based on the structure of nucleus, fundamentally two types of cells exist. They are

i. Prokaryotes and

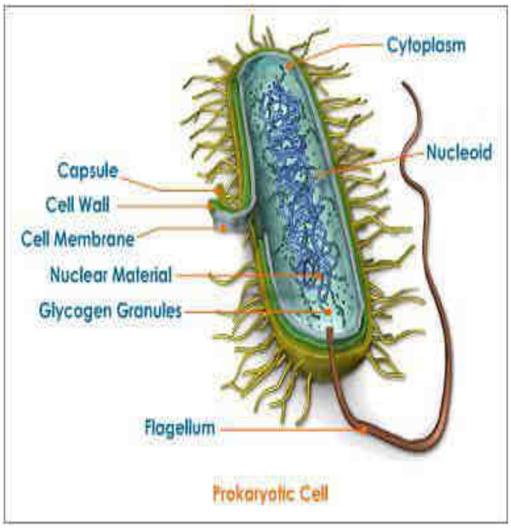
ii. Eukaryotes

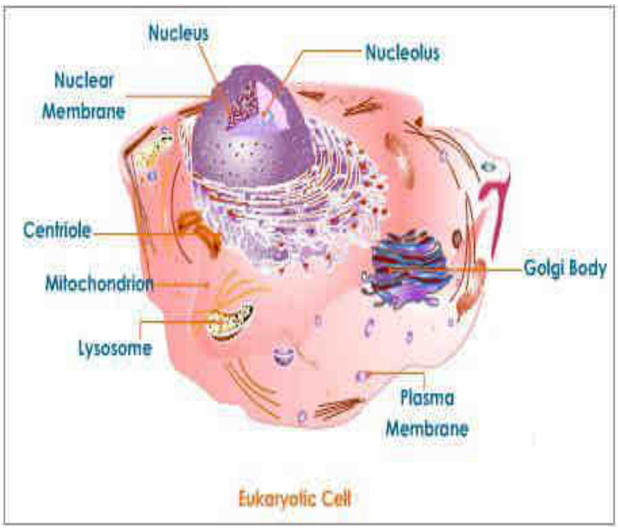
PROKARYOTIC CELLS

Prokayote is a Greek word, *pro* - before and *karyon* - nut or kernel. Prokaryotes are the organism with a primordial nucleus. They have a much simpler morphology than eukaryotic cells and **lack** a true membrane bound nucleus and cell organelles like mitochondria, golgi bodies, endoplasmic reticulum, etc. All **bacteria** and **archaea** are prokaryotic.

EUKARYOTIC CELLS

Eukaryote is a Greek word, *eu* - true and *karyon* - nut or kernel. Eukaryotes **posses** a membrane enclosed nucleus and cell organelles. They are more complex morphologically and are usually larger than prokaryotes. **Algae, fungi, protozoa, higher plants** and **animals** are eukaryotic.





1.5. DIFFERENCE BETWEEN PROKARYOTES AND EUKARYOTES

	Characteristics	Prokaryotes	Eukaryotes
i.	***Cell size***	Generally 1 to 10 μm in linear dimension	Generally 5 to 100 μm in linear dimension
ii.	***Cell division***	Binary fission	Mitosis
iii.	***Cellular organism***	Unicellular	Mostly multicellular with differentiation of many types
iv.	***Cell wall***	Complex structure with peptidoglycan layer, protein and lipids	Absent or composed of cellulose or chitin
v.	***Plasma membrane***	Present, no sterols except in mycoplasma	Present, contain sterols
vi.	***Metabolism***	Anaerobic or aerobic	Aerobic
vii.	***DNA***	Circular DNA in cytoplasm	Very long, linear DNA molecule bounded by nuclear envelope
viii.	***Membrane bound***	Absent	Present

	nucleus and nucleoli		
ix.	***Extra chromosomal DNA (Plasmid)***	Present	Absent
x.	***Histones***	Absent	Present
xi.	***RNA and protein***	RNA and protein synthesized in same compartment	RNA synthesized and processed in nucleus; proteins synthesized in cytoplasm
xii.	***Membrane bound organelles***	Absent	Present (Nucleus, mitochondria, chloroplast, endoplasmic reticulum, etc)
xiii.	***Ribosomes***	70S type	80S type
xiv.	***Lysosomes***	Absent	Present
xv.	***Locomotion***	Rotating flagella and gliding movement	Undulating flagella and cilia and amoeboid movement
xvi.	***Flagella***	Consists of two protein building blocks	Consists of multiple microtubules
xvii.	***Pili***	Present	Absent
xviii.	***Glyocalyx***	Present as a slime layer or capsule	Present in some cells that lacks cell wall
xix.	***Site for cellular respiration***	Cell membrane	Mitochondria
xx.	***Sexual reproduction***	Conjugation	Meiosis
xxi.	***Examples***	Bacteria and Archaea	Fungi, Algae and Protozoa

1.6. MICROBIAL GROUPS

Based on the morphological, phylogenetic and physiological characteristics, microorganisms are divided into six distinct groups, they are as follows

1) Bacteria
2) Archaea
3) Fungi
4) Protozoa
5) Algae
6) Viruses

1) **BACTERIA** are **prokaryotes** that are usually single celled organisms. They multiply by binary fission and reproduces asexually. They are the most **dominant** group of microorganisms in soil, water and air. Some bacteria even live in environment that has extreme temperatures, pH or salinity. Many of them play more **beneficial roles** in

nutrient cycling, decomposition of organic matter, production of commercial industrial products like vitamins, antibiotics, etc. Wherein, some of them cause diseases and food spoilage. Ex: *Bacillus, Pseudomonas.*

2) **ARCHAEA** are phylogenetically related **prokaryotes** that are distinguished from bacteria by many features, most notably their unique ribosomal RNA sequences. Many archaea are found in **extreme environments**. Some have unusual metabolic characteristics, such as the **methanogens**, which generate methane gas. Ex: *Methanobacterium.*

3) **ALGAE** are **eukaryotes** that **contain chlorophyll** and are capable of performing photosynthesis. Algae are found most commonly in **aquatic environments**. They reproduce either sexually or asexually. Mostly they are used as food supplements. They are mainly used in the preparation of agar. Ex: *Spirulina, Gelidium.*

4) **FUNGI** are **eukaryotes**. Next to bacteria, they are the most dominant organism in the soil. In general, fungi range in size and shape from single-celled microscopic yeasts to gaint multicellular mushrooms. They possess filamentous **mycelium** composed of individual **hyphae** and reproduce either sexually or asexually by fission, budding or by means of spores borne on fruiting structures. **Unicellular fungi** like **yeast** are involved in the production of alcoholic beverages like wine and beer. **Multicellular fungi** like **molds** are useful for industrial production of antibiotics like penicillin. Ex: *Mucor, Rhizopus*.

5) **PROTOZOA** are unicellular **eukaryotes** that are usually **motile** and **lack cell wall**. Many free living protozoa function as the **primary hunters** and **grazers** of the microbial world. They can be found in many different environments and some are normal inhabitants of the intestinal tracts of animals, where they aid in digestion of complex materials such as cellulose. Some of them are parasitic and can cause diseases. Ex: *Amoeba, Paramecium*.

6) **VIRUSES** are **acellular** (non cellular) organisms that are too small and can be visualized only using electron microscopes. All are **obligate parasites** that require a living cell for reproduction. They are **pathogenic** to plants, animals and humans. At most of the cases they cause human diseases. Ex: Cauliflower mosaic virus, Cucumber mosaic virus.

1.7. SCOPE OF MICROBIOLOGY

Currently, we are in the era of Microbiology. Microorganisms are recognized as the **basic research tools** as they help to understand the chemical and physical basis of life as they are the dominant group of living organisms in the biosphere and are actively involved in our day to day activities. Microbiology primarily paves way to analyze the biochemical and genetic **background of living things**. Moreover as microbes are the excellent **models** for understanding the cell functions and as they play important role in the field of medicine, agriculture and industry that assures human welfare, microbiology is considered as one of the vital branch of science with utmost promising scopes. Microbiology is not just one small subject to be explored. It has nearly six major branches. They are as follows,

1. **Agricultural Microbiology** deals with soil nutrient cycling by microbes, microbial decomposition of organic wastes, plant associated microbes that enhance soil fertility, etc.
2. **Food Microbiology** covers information about the microbes involved in food spoilage, food borne diseases, commercial food products prepared using microbes, etc.
3. **Industrial Microbiology** explores the utility of microbes in the production of antibiotics, enzymes, alcoholic beverages, fermented food products, etc.
4. **Medical Microbiology** deals with the studies related to the microbes that causes diseases, their diagnostic and preventive measures, drug designing, etc.
5. **Aquatic Microbiology** deals with water purification and biological degradation of wastes in aquatic ecosystems by microbes.
6. **Aero Microbiology** talks about the microorganisms prevalent in air, their abundance and beneficial or harmful issues.
7. **Exomicrobiology** is all about the exploration of life in outer space.
8. **Geochemical Microbiology** analyses the microbial life and their contribution in coal, oil and gas formation areas.

As each branch of microbiology have got their own specialization that contribute to the development of science and technology, always microbiology are crowned as innovate, ever green branch of biology that have wider scopes for the emerging scientists to be explored. We are living in the world of microbes without which life won't be trouble-free and comfy.

LETURE 2 &3 Biogenesis and a biogenesis theory. Contributions by Antonie Van Leeuwenhoek, Louis Pasteur, Contributions of John Tyndall, Joseph Lister, Edward Jenner, Robert Koch, Alexander Fleming and Waksman. Germ theory of fermentation and disease

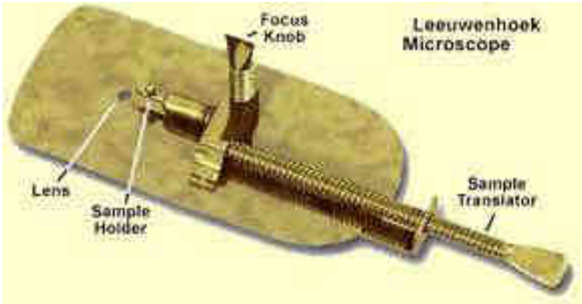
The field of microbiology developed further and gained its importance after the fascinating discoveries later than 1600's by the discovery of microscopes by pioneer scientists. The important discoveries that contributed much to the discipline of microbiology is the conflict over the 'Theory of Spontaneous Generation' followed by 'Koch's Postulates' that completely changed the view of microorganisms. This chapter gives a vivid outlook on the contributions of many pioneers like Pasteur, Koch, etc.

2.1. ROBERT HOOKE (1635 – 1700)

Hooke was the first person to discover the **cell** (honey comb like structures) from the cross sections of a cork. He noticed some microscopic fungi too. He also developed simple microscopes of 30x magnification and observed few microorganisms.

2.2. ANTONY VAN LEEUWENHOEK (1632 – 1723)

Leeuwenhoek is a famous person who is always praised as the **Father of Microbiology**. He was a Dutch merchant and his hobby was making lenses and microscopes. His microscopes were simple microscopes composed of double convex glass lenses held between two silver plates that could magnify 50 to 300 times. He was the first to describe the protozoa and bacteria. He observed some bacteria from plagues of his own teeth. He named them as **animalcules**.



Leeuwenhoek and his microscope that was developed first

2.3. THE THEORY OF SPONTANEOUS GENERATION (ABIOGENESIS)

After the discovery of microorganisms by Leeuwenhoek, scientists began investigations about the origin of microbes. Since organic matter decomposes quickly outside the living body, it was assumed that microorganisms were arising by spontaneous generation. **Francesco Redi** (1626), supported spontaneous generation theory. He boiled the meat and covered the mouth of the flask with wire gauze. The flies attracted due to the odour of meat, laid eggs on the wire gauze, that later developed into maggots. Thus he established that origin of maggots was from meat and not from fly. Additionally, **John Needham** (1749), an Irish priest, observed the appearance of microorganisms in putrefying meat and interpreted this as spontaneous generation.

2.3.1. LA ZARO SPALLANZANI (1729-1799) - THEORY OF BIOGENESIS

Spallanzani, an Italian priest, boiled beef broth for an hour, sealed the flasks and observed no appearance of microorganisms and disproved the theory of spontaneous generation or abiotic origin of life and proposed the **theory of biogenesis**. He said that every form of life takes its origin from their parents, germ cells or seeds. This theory of biogenesis was later proved and supported by Louis Pasteur.

2.3.2. LOUIS PASTEUR (1822-1895)

He was a Professor of Chemistry at the University of Lille, France. He is considered as "Pioneer of Microbiology", as his contribution led to the development of Microbiology as a separate scientific discipline.

He proved the theory of "Biogenesis" and **disproved the "Theory of spontaneous generation"** (Abiogenesis), experimentally by using swan-necked flasks. Pasteur passed the untreated and unfiltered air in to boiled nutrient broth, germs settled in the goose neck and no microbes appeared in the solution. Thus he disproved that living organisms appear from non living matter. He also worked on souring of wine and beer and found that this alcohol spoilage is due to the growth of undesirable organisms, while the desirable microorganisms produce alcohol by a chemical process called "**Fermentation**". He showed that wine did not spoil, if it is heated to 50-60°C for a few minutes. This method is called **"Pasteurization"**, now widely used in dairy units, to kill pathogenic microorganisms in milk.

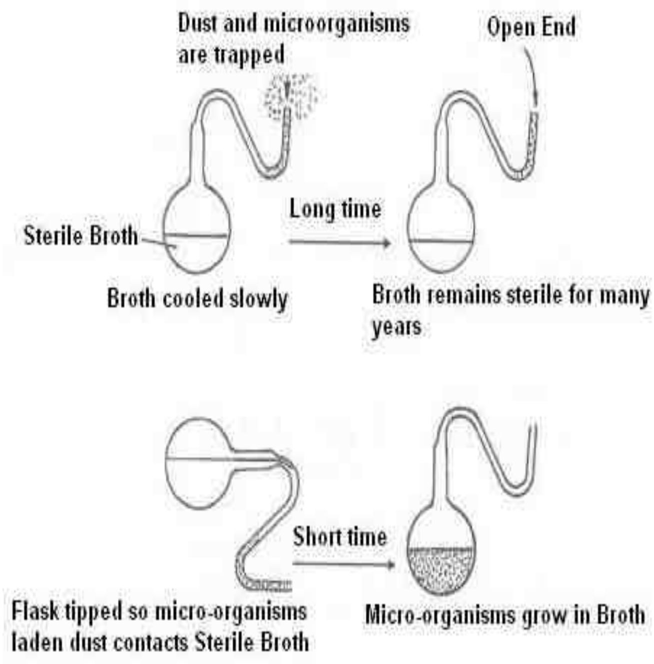
He is a founder of **"Germ theory of disease"** as he visualized that diseases are caused by microorganisms. In course of his research, he discovered the importance of sterilization and discovered steam sterilizer, autoclave and hot air oven. He also established the importance of cotton wool plugs for protection of culture media from aerial contamination. He differentiated between aerobic and anaerobic bacteria and coined the term "**anaerobic**" to refer to the organisms that do not require oxygen for growth.

He worked on "Pebrine", a silk-worm disease caused by a protozoan and showed that infection can be controlled by choosing worms free from the parasite for breeding.

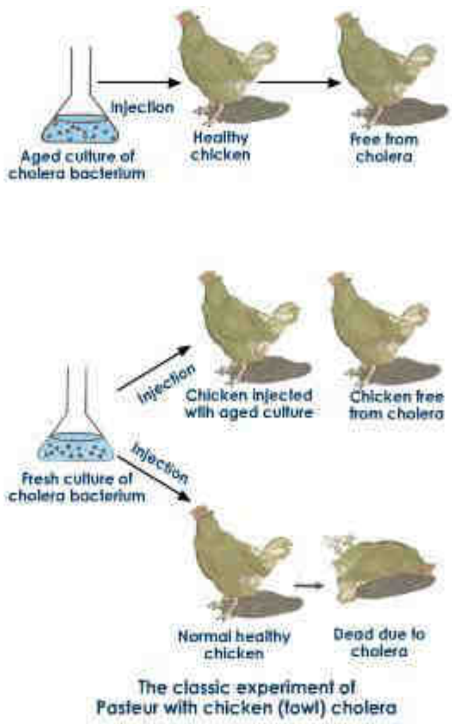
He developed the process of **"attenuation"** during his work on "chicken cholera" in fowls. He found that cultures which had been stored in the laboratory for sometime would not kill the animals as fresh cultures did. This attenuation is now used in protective vaccination against diseases.

Pasteur showed that the anthrax disease in cattle and sheep is caused by a bacterium. He cultivated anthrax organisms in sterile yeast water, and showed that these cultures can produce disease when inoculated in to healthy animals. He developed a live attenuated **anthrax vaccine**, by incubation at 40-42°C, which proved to be useful in protecting animals against anthrax. He also worked on swine erysipelas.

Pasteur developed a **vaccine against rabies** (Hydrophobia), which made a greatest impact in medicine. He obtained the causative agent of rabies by serial intracerebral passage in rabbits and the vaccine was prepared by drying pieces of spinal cord. He tested with a boy named Joseph Meister and he saved his life. In 1888, Pasteur institute was established for mass antirabic treatment. Pasteur gave the general term "**Vaccine**" (Vacca=cow) in honour of Jenner's cow pox vaccine, to various materials used to induce active immunity.



Pasteur's goose neck flask experiment



Pasteur's study on immunization

2.3.3. JOHN TYNDALL (1820-1893)

He designed a special chamber to free the dust in the air and kept the sterile broth in the chamber. No microbial growth was observed when a sterilized broth was kept in the chamber. Thus, he proved that dust in the air carried the germs and this is the source for the growth of microorganisms and not the spontaneous generation. He also developed a sterilization method called **tyndallization**. Tyndallization is otherwise called as the intermittent or fractional sterilization. In case of tyndallization, subsequent heating and cooling by steam for 3 days will remove the germs and their spores. Heating at 100°C kills the vegetative cells. The spore forms are killed on subsequent heating upon germination of spores.

2.4. ROBERT KOCH (1843-1912)/KOCH'S POSTULATES

He was a German country Doctor who later became the Professor of hygiene and Director of institute of infective diseases at Berlin. He perfected many bacteriological techniques and known as "**Father of Practical Bacteriology**".

He discovered rod shaped organisms in the blood of animals that died of anthrax. He experimentally obtained the anthrax organisms in pure culture on a depression slide by inoculation of infected blood into the aqueous humour of a bullock's eye. He observed multiplication of bacteria and spore formation. He injected these spores into mice and reproduced the disease. He found that in certain conditions, the anthrax bacillus forms spores, that can survive on earth for years. He passed anthrax bacilli, from the blood of an infected animal, from one mouse to another through twenty generations, and found that they bred true. He worked out its life-history.

He introduced staining techniques. He prepared dried bacterial films (Smears) on glass slides and stained them with aniline dyes for producing a better contrast under microscope. He discovered tubercle bacillus (*Mycobacterium tuberculosis*) which is popularly called as **Koch's bacillus**. He injected tubercle bacilli into laboratory animals and reproduced the disease, satisfying all Koch's postulates.

He discovered *Vibrio cholerae*, the causative agent of cholera disease. He developed pure culture techniques by introducing solid media. The use of agar-agar obtained from dried sea weeds (*Gelidium Sp.*) in the preparation of solid bacteriological media was first suggested by Frau Hesse, the wife of Koch' student. This agar-agar is totally inert with no nutritive value, solidifies at 45°C and melts at 90°C, and was found to be most suitable solidifying agent in the preparation of culture media. Koch isolated bacteria in pure cultures on these solid media. It revolutionized bacteriology.

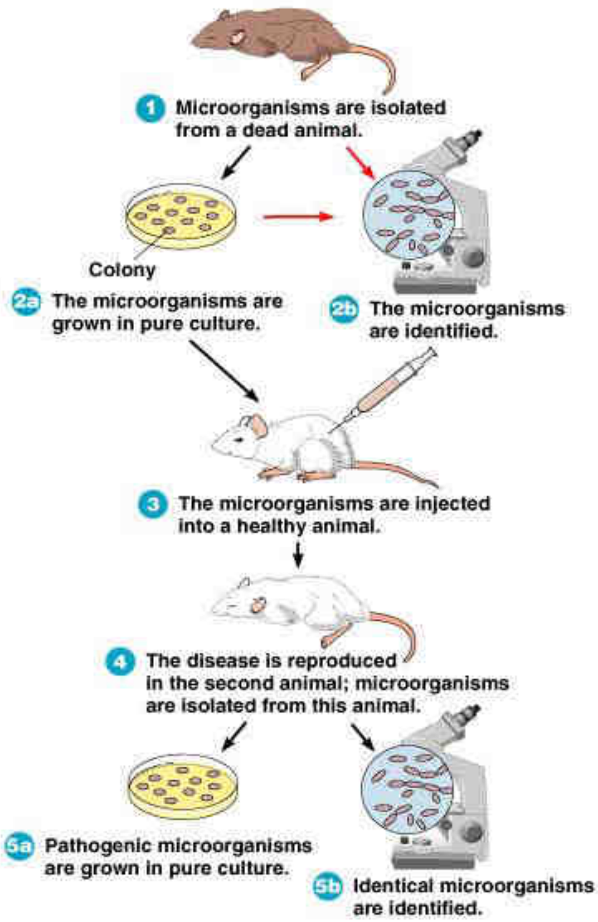
He discovered "Old **Tuberculin**". Koch noted that when tubercle bacilli or its protein extract was injected into a Guinea-pig already infected with the bacillus, an exaggerated reaction took place and the reaction remains localized. This is popularly called **"Koch Phenomenon"** and it is a demonstration of cell mediated immunity. The tuberculin test is based on Koch's phenomenon. He erroneously thought that protein extracted from tubercle bacilli, called "Old tuberculin", could be used in the treatment of tuberculosis. Koch did a series of experiments to fulfill the criteria laid by his teacher Henle to establish the causative role between a particular microorganism and a particular disease. They are popularly known as **Koch's postulates** (Henle-Koch's Postulates). They are:

1. A specific organism should be found constantly in association with the disease.
2. The organism should be isolated and grown in a pure culture in the laboratory.

3. The pure culture when inoculated into a healthy susceptible animal should produce symptoms/ lesions of the same disease.

4. From the inoculated animal, the microorganism should be isolated in pure culture.

5. An additional criterion introduced is that specific antibodies to the causative organism should be demonstrable in patient's serum.



Kotch's postulate

2.5. EDWARD JENNER (1749-1823)

Jenner was an English country physician, who discovered a safe and efficient vaccination against small pox which ultimately led to the eradication of small pox (**Variola**). Jenner observed that dairy workers, exposed to occupational cowpox infection were immune to small pox. He proved experimentally that resistance to small pox can be induced by injecting cow pox material (**Vaccinia**) from disease pustules in to man (in 1796). He tested his vaccine with a small boy named James Philipp.

Pasteur gave the general term "**Vaccine**" (Vacca = cow) in honour of Jenner's cow pox vaccine, to various materials used to induce active immunity. Jenner published his findings in 1798 in a pamphlet "*An inquiry into the cause and effect of variole vaccine*".

2.6. JOSEPH LISTER (1827-1912)

He is popularly known as **"Father of antiseptic surgery".** He was a professor of surgey at University of Glasgow and Edinburg and later at King's College, London. He was deeply interested in the prevention of post-operative sepsis. He was attracted by Pasteur's germ theory of disease and concluded that sepsis or wound infection may be due to microbial growth, derived from the atmosphere.

He successfully prevented post-operative sepsis by introducing antiseptic techniques. He chose **carbolic acid** (Phenol) and used as spray on the wound or during surgery. He applied dressings soaked in carbolic acid on wounds. As a result, there was a marked reduction of post-operative sepsis, wound inflammation and suppuration. It saved millions of lives from the jaws of death due to wound infections.

Lister's antiseptic surgery later led to the development of aseptic surgery. He suffered much criticism but never lose courage and followed his own ideas and revolutionized the science of surgery by introducing antiseptic system in 1867.

2.7. IWANOWSKY (1892)

Dmitri Iwanowsky, a Russian botanist, occupies a pivotal position in the history of virology. In 1866, Adeolf E. Meyer, a Dutch agricultural chemist described a disease of tobacco called "Mosaic" and showed that the disease could be transmitted to healthy plants through the sap of the diseased plant.

Iwanowsky (1892) demonstrated that this disease was caused by an agent which could pass through the filter, which withholds bacteria. He obtained the sap from infected leaves and passed it through a bacterial filter, called chamberland candle filter, which retained all bacteria and the filtered sap still retained infectivity when applied to healthy leaves.

Beijerinck (1898), a Dutch Microbiologist, showed that this infectious agent could diffuse through an agar gel and that it was a non-corpuscular "*Contagion vivum fluidum*" which he called "**Virus**".

Stanley (1935), a British Mycologist was able to obtain the infectious agent of tobacco mosaic in a crystalline form.

2.8. METCHNIKOFF (1845-1916)

Elie Metchnikoff, the Russian-French biologist, discovered the phenomenon of phagocytosis, the cellular concept of immunity. In Italy, where he had gone on a research visit, he studied the transparent larvae of starfish and noticed some of their cells could engulf and digest foreign protein particles. These cell eaters are called "**Phagocytes**".

He continued his work on phagocytic action, at Pasteur Institure, Paris and found that in human blood a large proportion of the leucocytes (White blood cells) are phagocytic and attack invading bacteria.

This, in turn, results in increased numbers of leucocytes in the infected areas followed by the inflamed area becoming hot, red, swelled and painful due to dead phagocytes forming pus. He spent his last two decades on the study of human aging, since he believed that phagocytes eventually begin to digest the host cells aided by the effects of intestinal bacteria and that effectively combating them would increase the life span of human being.

2.9. SELMAN A WAKSMAN (1945)

He is an American microbiologist. He isolated *Thiobacillus thiooxidans* which is an important discovery before he identified **Streptomycin** antibiotic from soil bacterium. In 1939 Waksman and his colleagues undertook a systematic effort to identify soil organisms producing soluble substances that might be useful in the control of infectious diseases, what are now known as **antibiotics**. Within a decade ten antibiotics were isolated and characterized. Three of them with important clinical applications - actinomycin in 1940, streptomycin in 1944, and neomycin in 1949. Eighteen antibiotics were discovered under his general direction.

2.10. ALEXANDER FLEMMING (1881-1955)

He was an English scientist worked at St. Mary's hospital in London. Flemming was associated with two major discoveries - **lysozyme** and **penicillin**. In 1922, he discovered lysozyme by demonstrating that the nasal secretion has the power of dissolving or lysing certain kinds of bacteria. Subsequently, he showed that lysozyme was present in many tissues of the body.

In 1929, Flemming made an accidental discovery that the fungus *Penicillium notatum* produces an antibacterial substance which he called penicillin. Flemming was culturing Staphylococci in Petridishes and some of his cultures were contaminated with a mold, subsquently indetified as *Penicillium notatum*.

Around the mold colony, there were clear zones, where Staphylococci disappeared. Flemming attributed this to the production of an antibacterial substance by the mold. Flemming cultured the fungus *Penicilium notatum* in broth cultures, filtered the fungal mat and obtained the penicillin in soluble form in the culture filtrate.

In 1940, Howard Florey and Ernst Chain demonstrated its antibacterial action *in vivo*. Working in U.S.A., they were able to produce large quantities of penicillin in pure form. In 1945, Flemming, Florey and Chain shared the nobel prize in physiology and medicine for the discovery of penicillin.

2.11. PAUL EHRLICH (1854-1915)

He was a German Bacteriologist, who pioneered the technique of **chemotheraphy** in medicine. From his discovery that certain tissues have a specific affinity, he reasoned that organisms causing diseases could be selectively killed with chemical drugs. This led him to produce "arsphenamine" (an arsenic compound), the first synthetic drug, which destroyed the syphilis microbe in the body.

Ehrlich observed that organic arsenicals killed trypanosomes in an infected animal, but, if smaller doses were administered, the trypanosomes acquired tolerance to the drug. Therefore, he aimed at "*therapia magna sterilans*" *i.e.,* the introduction into the blood of a single dose of chemotherapeutic agent sufficient to kill the parasite. He also observed that drug would undergo certain changes in the body after it would produce the desired action.

2.12. MARTINUS W. BEIJERINCK, (1851-1931)

He developed the enrichment culture technique, simultaneously with Sergey Winogradsky, which permits the isolation of highly specialized microorganisms. Beijerinck cultivated and isolated *Bacillus radicicola (*later named as *Rhizobium leguminosarum)*, a bacteria that fixes free nitrogen and causes the formation of nodules on the roots of Legumes. He also characterized *Azobacter* as nitrogen-fixing, and isolated the new genus, *Aerobacter*. Isolated **sulphur reducing bacteria and sulphur oxidizing bacteria** from soil**.** In studying tobacco mosaic disease, he concluded that the filterable pathogen was a *contagium vivum fluidum*, a term coined to convey his concept of a living infectious agent in a fluid (noncellular) form.

2.13. SERGEY WINOGRADSKY (1856 – 1953)

He developed **Enrichment Culture Technique** for the isolation of *Beggiatoa* sp. He explained the chemoautotrophic nature of bacteria and initially called them as "anorgoxydants." He also identified the process of Nitrification; isolated the nitrifying bacteria *Nitrobacter* and isolated *Azotobacter chroococcum* and proved its nitrogen fixing capacity. Due to his varied contributions in soil microbiology he is considered as the **"Father of Soil Microbiology".** Additionally, he identified the Chemolithotrophic nutrition of soil bacteria and also discovered anaerobic nitrogen fixing bacterium *Clostridium pasteurianum*.