

# Milestones in microbiology

- Microbiology is the study of living organisms of microscopic size, which includes bacteria, fungi, algae, protozoa and viruses.
- It aims to study their form, structure, reproduction, physiology, metabolism and classification.
- Microorganisms are closely associated with health and welfare of human beings. Some are beneficial while others are harmful.
- The science of microbiology did not start until the science of microbiology did not start until the invention of microscope in 16<sup>th</sup> century.

# Three periods of development

- I) Pre 1865 period where there was slow accumulation of facts about bacteria and accumulation of facts about bacteria and existence of microbes.
- II) The period between 1865 and 1882, also called as Golden Era where the pioneering works of Louis Pasteur ,Robert Koch, Lister etc. made the most important discoveries and laid the foundation of microbiology.
- III) The post 1882 period till date, the modern period which has experienced rapid developments in this field due to accumulation of huge knowledge (**Modern Era/Post Golden era**).

# Discovery of Microscope

- The ancient Greeks and Romans knew the use of glass and quartz lenses.
- The earliest record of use of magnifying lens goes back to Conrad Gesner (1558), a Swiss biologist who published his work on structure of foraminiferans (protozoans).
- It was Zacharias Janssen, a Dutch astronomer who added one more lens to the telescope and provided the first prototype of the present day compound microscope.

- Galileo also constructed a microscope around 1610 which was used for study of compound eye of insects.
- However the first person to report seeing microbes under the microscope was an Englishman, Robert Hooke.
- He saw cellular structures of plants and fungi in 1665 using a crude microscope and discovered living things are made of cells.
- However he was unable to observe bacteria .

# Discovery of Microbes

- The existence of microbes was suspected centuries before their discovery.
- The Roman philosopher Lucretius (98 –55 B.C.) suggested the existence of invisible B.C.) suggested the existence of invisible disease causing organisms.
- This suggestion was again made by Roger Bacon (1220 –1292), physician Girolamo Fracastoro of Verona (1478 –1553) and Anton van Plencizin 1762.
- Kircher (1601 –1680 ) said that “worms” invisible to naked eye are present in decaying meat ,milk and diarrheal secretions.

- The first person to observe and describe micro organisms accurately was Anton van Leeuwenhoek.
- Antony van Leeuwenhoek (1632 –1723) was a habedascher (cloth merchant ) who lived in Delft ,Holland.
- His hobby was grinding lenses and making microscopes.
- During his lifetime he made more than 250 microscopes consisting of home ground lenses mounted in brass and silver plates.
- His microscope could magnify 270 X which allowed him to see micro organisms.

- He observed seeds, embryo of plants and small invertebrate animals.
- He discovered the existence of spermatozoa and red blood cells.
- His greatest discovery was on June 16, 1675 when he saw bacteria, fungi and many protozoa in rain water.
- He called them “animalcules” or little animals.
- He recorded his observation in a series of letters addressed to Henry Oldenberg, Secretary of the Royal Society, England. ,Secretary of the Royal Society, England.
- Most of his letters were published in the Proceedings of the Royal Society.
- For his contributions he is honoured as Father of Microbiology.

# Conflict over spontaneous generation

- After Leeuwenhoek's discovery of microbes, scientists began to wonder about their origin.
- It was believed that the Greek Goddess Gaea could create people from stones.
- Even Aristotle (384 –322 B.C.): taught that animals originate spontaneously from soil, plants and inanimate things.
- The belief in the spontaneous formation of living beings from non-living matter is known as Doctrine of spontaneous generation or abiogenesis.
- This doctrine was accepted without question till renaissance.
- Some scientists did not accept this doctrine and believed in Biogenesis i.e. “ Life arises from living things”.



# Mouse Generation experiment

- Van Helmont (1577 –1664) believed in spontaneous generation and devised an experiment for generation of mice from decaying wheat bran and soiled linen.
- It decaying wheat bran and soiled linen.
- It was also an accepted fact, that maggots could be produced by exposing meat to warmth and air.
- This was challenged by Italian physician Francesco Redi (1626-1697).
- He carried out a series of experiments on decaying meat.
- He placed meat in three decaying meat.
- One was uncovered, second was covered with paper and third was covered with fine gauze that would exclude flies.

- Flies laid their eggs on uncovered meat and maggots developed.
- Maggots did not develop in the other two containers as they were covered.
- However, flies were attracted to the gauze covered container and laid their eggs on the gauze ;these eggs produced flies.
- Thus he proved that maggots donot generate spontaneously from meat but by eggs which are laid by flies.
- Some scientist proposed that boiled infusions of meat or hay would give rise to microorganisms after some time.

- Louis Joblot (1645 –1723) the French scientist, performed experiments with hay infusion in 1710.
- He divided boiled hay infusion into two heat-treated containers, one was open and the other was closed.
- Growth was open and the other was closed.
- Growth appeared in open vessel and broth in closed vessel remained sterile.
- Thus his results proved biogenesis.

- In 1748 English Priest John Needham carried out similar experiments to prove spontaneous generation.
- He boiled mutton broth and then tightly stoppered the flask.
- Eventually microorganisms developed in the flask and the broth became cloudy. Developed in the flask and the broth became cloudy. He stated that organic matter had a vital force that confers the property of life on non living matter.
- This result may be due to insufficient heating, which failed to kill heat resistant endospores.

- In 1765, Italian priest and naturalist Lazzaro Spallanzani improved Needham's experiment and showed that heating infusions for a longer time prevents the growth of organisms.
- He also showed that air carries micro organisms and if the flask with infusions are sealed hermetically ,they remain sterile for a very long time.
- In the late eighteenth century Priestly, Cavendish and Lavoiser discovered oxygen, which was soon recognized to be essential for life of animals.
- Thus the proponents of spontaneous generation commented that hermetic sealing recommended by Spallanzani excluded oxygen required for microbial growth.

- Schulz(1836) passed air through sulphuric acid or potassium hydroxide in a flask containing preheated meat infusion.
- The infusion remained sterile.
- Similarly Theoder Schwann(1837) passed air through a red-hot glass tube in a flask containing preheated meat infusion.
- No microbial growth occurred in the infusion.
- Georg Friedrich Schroder & Theoder von Dusch passed air through sterile cotton plug in a flask containing heat sterilized medium.
- This was No growth occurred in the medium.
- This was the first time that cotton plugs were used to prevent the entry on microorganisms in sterile medium

- Despite these experiments the French naturalist Felix Pouchet claimed in 1859, that he had performed experiments that proved that, microbial growth could occur without air contamination.
- This provoked Louis Pasteur and he decided to settle the matter once and for all.
- Pasteur first filtered air through cotton and found that plant spore like bodies were trapped in it.
- He then placed that cotton in sterile medium and found microbial growth occurs in that sterile medium.
- Secondly he took sterile nutrient solutions in S – shaped flasks.
- The swan necked flasks were kept open at one end and he allowed unheated, unfiltered air to pass through it.
- No growth occurred in the flask because the dust particles and germs were trapped on the walls of the curved necks.

- If the necks were broken, growth occurred immediately.
- Pasteur published his work in 1861 as Memoir on the organized bodies which exist in the atmosphere and resolved the controversy.
- The English physicist John Tyndall dealt a final blow to spontaneous generation in 1877 by demonstrating that dust indeed carries germs and if dust were absent, broth remains sterile even if exposed to air.
- .He performed a series of experiments in which he discovered presence of heat resistant he discovered presence of heat resistant spores of bacteria in hay infusion.
- .He developed a method of sterilisation by discontinuous heating ,later called tyndallisation which kills bacteria and all heat resisting spores.



# Germ Theory of Fermentation

- Conversion of carbohydrates to acid/alcohol is called as fermentation ,
- while degradation of proteins to produce off smelling amines is called as putrefaction.
- In 1837 Cagniard-Latour, Theoder Schwann and F.Kutzing independently proposed that micro organism (yeast) is responsible for the physiological conversion of sugar to alcohol in fermentation.
- This theory was bitterly attacked by leading chemist who believed that fermentation and putrefaction are purely chemical processes.
- Pasteur performed a series of experiments and convinced the scientific world that fermentative processes are a result of microbial activity.
- The involvement of microorganisms with fermentation is popularly known as Germ theory of fermentation.

# Contributions of Louis Pasteur

- Louis Pasteur was a French scientist born in Dole
- on 27 December 1822.
- on 27 December 1822.
- He was a chemistry student
- but he contributed largely to Biology
- He disproved spontaneous generation by his experiments using swan necked flask.
- He proved that fermentation is carried out by microorganisms.
- He observed that souring of wine occurs due to production of lactic acid from sugar. He also observed that different bacteria (Lactic Acid Bacteria ) and not yeast produced this lactic acid. (Lactic Acid Bacteria ) and not yeast produced this lactic acid.
- He showed that souring of wine can be prevented by holding at temperature between 50 to 60 °C for some time. This technique is now known as pasteurization, which is used for milk (63 °C for 30 min).

- He found that acid wines, ropy wines and bitter wines were a result of microbial growth.
- He discovered the existence of microbes in the absence of oxygen ,i.e “anaerobes”while studying butyric acid fermentaion.
- He described fermentation as life without air.
- He introduced vaccine for anthrax, rabies and chicken cholera.
- **Germ Theory of Disease:**
- Pasteur showed that spoilage of beer and wine was due to microbes. He referred to spoilage as “diseases of beer and wine”.
- A.Bassishowed in 1836 that fungi cause silkworm disease.
- Davaine showed that in anthrax, rods are present in diseased animals but absent in healthy ones.
- Robert Koch, a German Doctor, confirmed the germ theory of disease in 1876.He performed a series of experiments using anthrax bacilli and put forward a hypothesis, known as Koch Postulates.

- The microorganism must be present in every case of the disease
- The microorganism must be isolated from the diseased host and grown in pure culture.
- The microorganism must be able to produce the same disease when injected in a susceptible host.
- The microorganism must be recoverable from the experimentally infected host.

### **Surgical Antisepsis**

British surgeon Joseph Lister reasoned that surgical sepsis might result from microbial infection of tissues exposed during operation.

- He developed the method of sterilization of surgical instruments, use of disinfectant dressing and instruments, use of disinfectant dressing and conduct of surgery under spray of disinfectant .
- He succeeded in reducing airborne infections and thus preventing surgical asepsis.

## **Pure culture Technique**

A pure culture is one that contains only a single kind of microorganism.

Brefeld did the pioneering work on pure cultures.

He introduced cultivation of fungi on solid media and also added gelatin to liquid media.

- Robert Koch performed a number of experiments and devised streak plate technique for isolation of pure cultures.
- The spread plate technique was also developed in his laboratory by Loeffler and Gaffky.
- The first solidifying agent used by Koch was gelatin.
- A better alternative for gelatin was suggested by Fannie Hesse, wife of Walther Hesse, one of Koch's assistant.
- She suggested use of agar as solidifying agent.
- Agar is a polymer extracted from red algae *Gelidium* and it melts at 100 0C.

- Richard Petri, Koch's assistant developed the petri dish used for solid media.
- Koch used meat infusions in culture media.
- Nutrient broth & nutrient agar was an outcome of Koch's experiments.
- In 1880 Koch used these techniques to isolate bacillus that caused tuberculosis.
- There followed a golden era of 30 –40 years where major bacterial pathogens were discovered.
- **Discovery of viruses:**
- In 1880 Pasteur discovered that rabies is caused by agents other than bacteria.
- Russian scientist Dimitri Iwanowsky discovered viruses in 1892.
- He found that a filtrate of a plant extract infected with tobacco mosaic virus was fully infectious when applied to healthy plants.