

# **Microbiology: Introduction, History, Branches, Principles and Applications**

## **1. Introduction to Microbiology:**

The study of organisms, invisible by the naked eyes is called as Microbiology. It is concerned with the study of morphology, physiology and other activities of micro-organisms.

### **Medical Microbiology is:**

1. The study of microbes that infect humans.
2. About the diseases they Cause.
3. Diagnosis of the diseases.
4. Prevention and treatment of diseases.
5. It also deals with the response of the human host to microbes and other antigens.

Microbiology is the close study of living organisms of microscopic size, which includes bacteria, fungi, algae, viruses, Protozoans and Parasites. It is concerned with their morphology physiology and classification.

6. They are closely associated with the health and welfare of human beings. Some are beneficial and some are pathogenic for man. They are involved in making of yogurt, cheese, paneer, wine etc. and they can also cause diseases.
7. Most micro-organism are unicellular i.e. all life processes are performed by a single cell and some other are multicellular.
8. Some microorganism called anaerobes and are capable of carrying out their vital functions in the absence of free Oxygen called aerobes.
9. The introduction of microbiology is made sure with the discovery of the microorganism. Those have some beneficial and harmful interaction with human being. The credit of discovery of, simple microscope goes to Antonyvan Leeuwenhoek, he gave them a name animalcules.

## **2. History of Ancient Microbiology:**

Ancient man recognized many of the factors involved in disease. The fact that people who recovered from a particular disease were immune to that disease was probably recognized many different times in many places. Often these survivors were expected to nurse the ill. Greek and Roman physicians routinely prescribed diet and exercise as a treatment for ills.

The first person to report seeing microbes under the microscope was an Englishman, Robert Hooke. Working with a crude compound microscope he saw the cellular structure of plants around 1665. He also saw fungi which he drew. However, because his lenses were of poor quality he was apparently unable to “see” bacteria.

Antonyvan Leeuwenhoek was a man born before his time. Although not the first to discover the microscope or to use magnifying lens, he was the first to see and describe bacteria.

Being meticulous, he developed his lens grinding to an art and in the process tested them by seeing how much detail he could observe with a given lens. One can guess that he chanced to look at a sample of pond water or other source rich in microbes and was amazed to see distinct, uniquely shaped organisms going, apparently purposefully, about their lives in a tiny microcosm. He made numerous microscopes from silver and gold and viewed everything.

His best lens could magnify 300-500 folds which allowed him to see microscopic algae and protozoa and larger bacteria. He clearly had excellent eyesight because he accurately drew pictures of microbes that were at the limit of the magnification of his lens. He used only single lens and not the compound lens of the true microscopes we employ today; which makes his observations all the more amazing.

He wrote of his observations to the Royal Society of London in 1676 and included numerous drawings. He astonished everyone by claiming that many of the tiny things he saw with his lens were alive because he saw them swimming purposefully about.

Robert Hooke was the first person to propose the cellular forms of life.

Many scientists and trendy high society people visited him to view his "little animalcules". He was a superior observer and an excellent scientist except for the crucial flaw of not allowing others to copy his techniques and verify his results. Because of this and the failure of people to relate these tiny microbes with disease, it was another 200 years before the science of Microbiology really took off.

In the 1800s people (mainly the poor) began to use hospitals. Hospitals also became centers of physician training. In 1841 (30 years before the germ theory of disease was established) young doctor Ignaz Semmelweis was hired to run a maternity ward in a Vienna hospital.

There were two birthing wards in his preview, one run by midwives and the other by doctors. Semmelweis noticed that the death rate among mothers in the doctor's ward ran as high as 18% from the blood infection (of a streptococcus or strep) known as child bed fever or puerperal sepsis, whereas in the midwife ward the death rate was much lower.

He saw a friend die from Puerperal Sepsis after cutting himself during an autopsy of a patient who had died of Puerperal Sepsis. He reasoned that there was an invisible agent that caused both deaths and that one could transfer it from the autopsy room to the birthing rooms and thus infect the mothers during birthing.

Acting on this assumption, Semmelweis instituted sanitary measures which included having the doctors wash their hands in disinfectant and change from lab coats dripping with pus and blood from the autopsy room to clean lab coats before examining patients or assisting in a birth. The death rate of the mothers dropped by 2/3 in his ward.

### **3. Branches of Microbiology:**

**Some of the established branches are as follows:**

#### **1. Industrial Microbiology:**

It encompasses the uses of a variety of microbes in industrial processes. Initially they were being used for industrial fermentation and waste water treatment. As today industry is linked to biotechnology, several new industrial applications have been found for a variety of microbes.

It is sometimes also studied as microbial biotechnology and is the application of scientific and engineering principles to the processing of materials by microorganisms (such as bacteria, fungi, algae, protozoa and viruses) or plant and animal cells to create useful products or services. Areas of industrial microbiology include quality assurance for the food, pharmaceutical, and chemical industries.

#### **2. Medical Microbiology:**

This branch of microbiology deals with the scientific study of pathogenic microbes, the diseases they cause, their mode of survival in environment and their hosts (including life-cycle); their diagnosis, prevention and treatment.

In fact, as early as Varo and Columella in the first century BC had postulated that the diseases were caused by invisible beings (animalia minuta). Von Plenciz (1762) had put forth the idea that each disease was caused by a separate agent.

It covers a variety of topics where microbes are responsible for causing diseases of skin and eye infections, pneumonia (by bacteria), several sexually transmitted diseases (STD's), minor arthropod diseases, gastrointestinal infections including infections from drinking cow milk harbouring certain pathogenic bacteria and their remedies etc.

#### **3. Agricultural Microbiology:**

This branch deals with microbes having an impact on agriculture and food chains. Both, the harmful (microbes causing plant diseases) as well as useful microbes (e.g., N<sub>2</sub> fixing microbes, use of microbes in bio-fertilizers etc.) are studied under this branch.

Certain ruminants also carry a mixture of complex bacteria that enable the animal to extract sufficient nutrient from a diet of grasses. Future research in microbial ecology will help to determine in preserving a balance in microbial communities that favour agriculture.

#### **4. Environmental Microbiology:**

In the late 1800's, and early 19th century Sergei Winogradsky, a Russian Mineralogist, pioneered the field of microbial autotrophs, and initiated the field of Environmental Microbiology. This branch includes the study of composition and physiology of microbial communities of the environment.

It also deals with the activities of microbial entities, their interactions among themselves and with macroorganisms. Adhesion, biofilm formation, global element cycles, biogeochemical processes and microbial life in extremes of environment or unexplored environs all fall in its preview.

### **5. Food and Dairy Microbiology:**

As the microorganisms are ubiquitous (present almost everywhere) food and milk are no exceptions. Hence the microbes are studied from the viewpoint that they (e.g., Bacteria, Yeasts, molds etc.) can either act as spoilage microorganisms or pathogenic microorganisms.

And thus how they can cause spoilage, prevent spoilage through fermentation or can be the cause of human illness, all comes under the realm of this branch of microbiology. It is a thrust area of microbiology these days, as more and more food items are being packaged (including milk and its products) for later use.

### **6. Biotechnology:**

The UN convention on Biological Diversity defined Biotechnology as: any technological application that uses biological systems, living organisms, or derivatives thereof to make or modify products or processes for specific use. Bio-engineering, including recombinant genetic technology of the 21<sup>st</sup> century is the science upon which all biotechnological applications are based.

It combines disciplines like genetics, molecular biology, biochemistry, food sciences, mechanical engineering, chemical engineering, microbiology, cell biology and all are interrelated to electronics, information technology and robotics.

### **7. Bacteriology:**

The current science of bacteriology includes the study of both domains of prokaryotic cells (the Bacteria, and Eucarya). But recently due to out-break of molecular techniques applied to phylogeny of life, another group of prokaryotes was defined and informally named, "archaeobacteria (it has now been renamed as Archaea) and included in the study of bacteriology".

### **8. Virology:**

It is the study of viruses, complexes of nucleic acids and proteins that have the capacity for replication in animal, plant or bacterial cells. To replicate, the viruses use their genomes (DNA or RNA) or of the host cells and cause changes in cells, particularly its antigenicity and may cause several diseases in plants and animals is all covered under this branch.

### **9. Soil Microbiology:**

This branch deals with the biota that inhabits the soil and the processes they mediate. As the soil is a complex environment, colonized by an immense variety of microorganisms, the soil microbiology focuses on soil viruses, bacteria, actinomycetes, fungi and protozoa, but traditionally it has also included investigations of soil animals such as nematodes, mites and other arthropods.

Modern soil microbiology represents an integration of microbiology with the concepts of soil science, chemistry and ecology to understand the functions of microorganism in the soil environment.

### **10. Sewage Microbiology:**

This branch deals with the study of microbial flora of various types of sewage. The sewage may, depending upon source, can contain harmless (*E. coli* and other coli forms) to potential pathogens including enterococci, *Vibrio cholerae*, *Salmonella typhi*, etc. This branch studies their qualitative as well as quantitative details and ways to combat them following various treatment processes.

### **11. Mycology:**

It deals with the study of various fungi. Fungi are eukaryotic organisms and around 300 species are shown to be pathogenic for man. It studies their morphology, taxonomy, biosystematics, distribution, propagation, and several mycotic diseases they cause including hypersensitivity, mycotoxicoses, mycetismus and other infections and their remedies.

### **12. Phycology:**

It is a sub-discipline of botany, and deals with the scientific study of algae. As many species are single celled and microscopic (e.g. Phytoplankton and micro algae); yet others are multicellular (some growing) very large as seaweeds such as Kelp and Sargassum they are also studied in microbiology.

It also covers aspects like cyanobacteria (blue-green algae) and other microscopic forms occurring as symbionts in lichens.

### **13. Protozoology:**

Earlier much in use, this branch is the study of protozoa (motile and heterotrophic) protists. Protozoa-despite their small size and unicellularness offer complex and unique biological features. They also serve as experimental models in a variety of cellular, molecular, biochemical and ecological researches.

One of the applied sub-branches of this old branch is medical protozoology (covering protozoa infecting humans). It covers life-cycles, morphological features, host-parasite interactions, geographical distributions, reservoir hosts, method of transmission and control, pathology, immunological aspects, diagnosis and remedies are all included in it.

### **14. Aquatic Microbiology:**

It covers the study of microorganisms and their activities in natural water. As the natural waters include lakes, ponds, streams, rivers, estuaries and oceans, it initially started covering all of them.

**But due to the growth of the subject several other branches have also been recognized and are as follows:**

(a) Marine microbiology

(b) Estuarine microbiology

(c) Groundwater microbiology, and

(d) Deep-sub-surface microbiology

The aquatic microbiology deals with the variability of aquatic habitat and rapid changes in characteristics and associated microbial component. The microbial activity and biomass measurement studies are performed to follow microbial functions in water ecosystems. Also the balance between N, P, O and H is studied in lakes, eutrophic systems, and streams etc.

### **15. Marine Microbiology:**

As marine environment is the largest part of the biosphere, being about 97-98% of all the water on earth, efforts are being made to study seas, especially deep seas (and their microbial functioning), as 75% of the ocean is below 1000 m depth (and is constantly cold at about 3°C on an average).

The oceanic explorations like Challenger expedition and Galathea expedition were among the initial efforts to critically explore microbial aspects of the deep seas and the nature of psychrophilic bacteria. Barotolerant bacteria are among the unique fauna of deep oceans.

As the most of the earlier work on seas and oceans remains confined to the near-shore and estuarine marine environments, the interest is growing in the off-shore and pelagic ocean microbiology.

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#### **4. Spontaneous Generation of Microbiology:**

The mystery of life has puzzled and confounded humans since the first human began to contemplate his world. The religions of ancient societies were built around the seasons, the sun and the renewal of life as these were so clearly tied to survival; both of the human species through birth and death, and of the individual in the attainment of sustenance.

Spontaneous generation or the idea that life routinely arises from non-life was a common sense explanation of the miracle of life. As science and the scientific method grew with the slow accumulation of knowledge, observant individuals began to consider the origin of life more deeply. Simple observations convinced many people that all the larger animals and plants produced life from previous life.

Despite this, the mass of humans clung to the comfortable idea of spontaneous generation further; religions saw it as a convenient way to demonstrate the hand of God operating continuously in the world. Some individuals, such as J.B. van Helmont even described how one could make mice from grain, a jar and dirty rags by putting them together in a dark place for a few weeks and soon mice would appear in the jar.

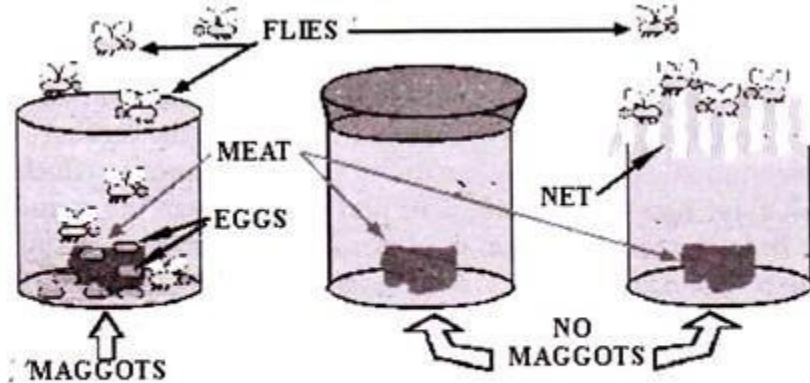
Other, more perceptive individuals, like Francesco Redi tested the common idea that maggots arise via spontaneous generation on rotting meat. He placed a piece of meat in three jars, one he left open, one he corked tightly and the third he covered with a fine mesh gauze. Maggots only appeared in the open container, no matter how long he left the jars.

Redi's experiment was important because of its eloquent simplicity. Anyone could repeat it and obtain the same clear results. Nevertheless, many people clung fiercely to the idea of spontaneous generation, while others designed experiments to test it. In every case the results of the majority of these experiments indicated that spontaneous generation did not occur.

The intellectual ferment this controversy stirred up gradually evolved into the scientific method as the various antagonists questioned each other's assumptions and, more importantly, their experimental design. These arguments forced the designing of better experiments and eventually

persuaded all but the most recalcitrant believers to discard spontaneous generation as an explanation for all higher life forms.

Then, in the 1800's the refinement of the microscope, through which people could see tiny life forms that they assumed were simple, gave spontaneous generation proponents new life.



**Fig. 1.2: "Redi's experiment proving maggots are not spontaneously produced in rotten" meat.**

Again, flawed common sense led reasonable people astray. As the existence of microscopic life was accepted, the assumption was that such life must be simple compared to higher, more complex life. The reasoning that followed this erroneous assumption was that since the microbes were small they must be simple & it followed that they were formed by spontaneous generation; hence God was still at work creating micro- life.

A number of scientists performed elementary experiments in which they treated soups and broth's, which left unheated, would team with microbes after a few days, with heat to destroy any life present in them and asked the question – "Would new life arise in these sterile soups"? Spallanzani boiled "soup" in glass containers and melted the glass closed.

The observation that nothing subsequently grew in this "heated" soup suggested that spontaneous generation didn't work. His detractors rightly criticized his experiments, proposing that since air is necessary for life and since he had sealed the flask to air, obviously no life could develop.

Others boiled soups and microbes grew, thus apparently supporting spontaneous generation. But again the preponderance of data suggested that spontaneous generation did not even apply to the "simple" microbes.

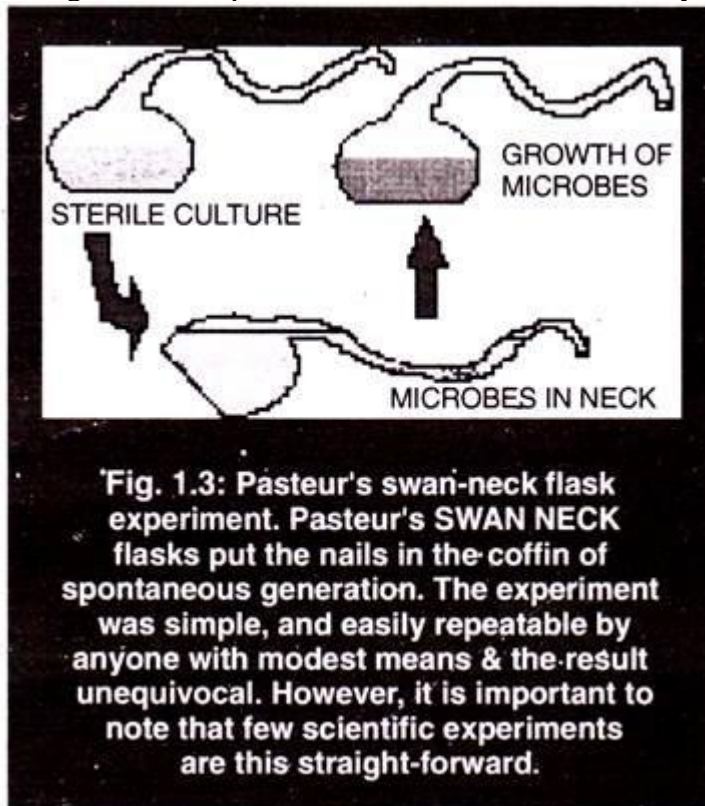
In 1859 one of the fathers of modern microbiology, Louis Pasteur decided to settle the question of spontaneous generation once and for all. A genius at devising definitive experiments, Pasteur first drew the necks of glass flasks out so that they remained open to the air, but were bent so that air could only enter by a curved path. He then added broth and boiled it to destroy contaminating microbes.



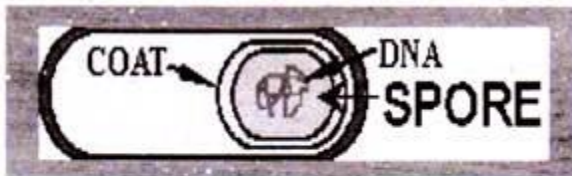
These flasks were then incubated and observed for months. He reasoned that the microbes in the air that could contaminate the sterile broth would be trapped on the sides of the thin glass necks before they reached the sterile broth if spontaneous generation didn't occur no growth should take place.

This is exactly what happened, the flasks remained sterile indefinitely, and until Pasteur tipped the sterile broth up into the curved neck where he predicted the airborne organisms would have settled. After doing this the broths always grew microbes.

These experiments ended the spontaneous generation controversy because this experiment was so elegant and simple, and the results so clear, that anyone could repeat them.



Later, an earlier problem, in which occasional heated broths did not remain sterile, was explained with the discovery of the heat resistant bacterial spores, some of which could survive several hours of boiling without being killed.



**Fig. 1.4: Spore structure**

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## **5. Principles of Microbiology:**

Pasteur discovered many of the basic principles of microbiology and, along with Robert Koch, laid the foundation for the science of microbiology. In 1857 Napoleon III was having trouble with his sailors mutinying because their wine was spoiling after only a few weeks at sea.

He could distinguish between the contaminants that caused the spoilage and even predict the taste of the wine solely from his microscopic observations. He then reasoned that if one was to heat the wine to a point where its flavor was unaffected, but the harmful microbes were killed it wouldn't spoil.

As we are aware this process, today known as pasteurization, worked exactly the way he predicted and is the foundation of the modern treatment of bottled liquids to prevent their spoilage. It is important to realize that pasteurization is not the same as sterilization.

Pasteurization only kills organisms that may spoil the product, but it allows many microbes to survive, whereas sterilization kills all the living organisms in the treated material. Pasteur also realized that the yeast that was present in all the wine produced the alcohol in wine.

### **Robert Koch:**

In the late 1870s a country physician, R. Koch became interested in anthrax, a common disease of both the farmers and their animals in his rural practice. Using a microscope, Koch saw a large bacterium in the blood of anthrax victims. He reasoned that it might be the agent of the disease.

Using a closet at home as his lab and developing basic microbiological techniques as he proceeded, Koch painstakingly teased out the anthrax bacterium and purified it. He then inoculated the purified bacteria into healthy animals and produced the classical clinical disease.

When he examined the blood of the inoculated animals he was able to re-isolate the same bacterium. He repeated the isolation, infection and disease cycle until he was certain he had found the agent of anthrax. Because it was such an important commercial disease and because his techniques could be easily duplicated, others quickly verified his findings and Koch became famous.

He soon had his own institute (like Pasteur) and other discoveries soon followed. Koch attracted other bright scientists and together they (along with Pasteur's group) developed the basic techniques of microbiology labs we still use today.

These include the sterile culture techniques, pure culture techniques, the use of petri plates, inoculation needles, solid medium, the use of agar and gelatin to produce a solid surface, the Gram stain and other staining procedures. In addition Koch discovered the etiological agents of cholera, and tuberculosis. His studies, in combination of those of Pasteur's, established the germ theory of disease.

**His procedure for defining the agent of any disease, called Koch's postulates, consists of the following 4 steps:**

First, isolate the suspected agent from a disease victim.

Second, grow the agent in pure culture.

Third, infect a healthy host and show that the organism produces the classical clinical disease.

Fourth, isolate the "same" organism from the new victim.

**Microbial Serendipity:**

Fanny Angelina Eilshemius used AGAR-AGAR, a complex polysaccharide extracted from seaweed, to keep them solid in hot weather. Agar – Agar had been used as a gelling agent in ASIA for centuries.

**The following characteristics of Agar – Agar make it almost perfect for the growth of microbes on solid medium:**

(a) Non-toxic to most microbes.

(b) Only melts at 100°C, but solidifies at about 45°C (a temperature most bacteria can survive).

(c) Nontoxic to other forms of life.

(d) Stable to sterilization temperatures.

(e) Physiologically inert as very few bacteria have the enzymes for digesting it.

**Edward Jenner:**

Smallpox was one of the greatest scourges of mankind. For thousands of years it swept through human populations, killing up to 40% of its victims and leaving many of the survivors horribly scarred for life; their faces covered with deep red pits. The ancient Chinese recognized that those who recovered from a case of "the pox" were immune to smallpox.

Through a series of serendipitous events, Jenner was led to the discovery of immunization and to the eventual elimination of the scourge of smallpox from the earth. As a young man he had lived in the country and had been told by a milkmaid that "She never had to worry about catching smallpox because she had "cowpox", a mild chronic disease of cows that milk maids usually contracted as a rash on their hands".

By 1796 he became convinced that the story was true so he inoculated an 8yr. old boy with cowpox and 8 weeks later inoculated the same boy with the pus from a smallpox lesion. The boy showed no effects and Jenner repeated the experiment.

As word of his results spread, others began to test it and by 1803 it was an established medical procedure in England. Shortly thereafter Ben Franklin encouraged American doctors to adapt this technique in view of the dangers inherent in the older technique.

**The Magic Bullet:**

Paul Ehrlich worked in Koch's lab where he learned to study bacteria. While considering the phenomenon of differential staining of different bacteria and of different components of

eukaryotic cells, he speculated that if a dye chemical could bind to one cell and not another or to one substance within a cell and not others, perhaps you could find chemicals that would selectively kill certain pathogens without harming the surrounding host cells; this would act like a magic bullet selectively killing the villain and sparing the innocent victim.

He embarked on a search for a magic bullet to cure syphilis, which in the late 19th century was a scourge as terrible as AIDS is today. In the final stages of syphilis, a sexually transmitted disease or STD, its victim suffered horribly and eventually died insane as the brain was destroyed by the infection.

Over many years he tested 100s of chemicals and finally in 1910 he found one, he named Salvarsan or Compound 606, that killed the syphilis organisms without killing the host (usually). This discovery laid the ground for the discovery of antibiotics and other chemotherapeutic agents.

### **History:**

1. Varo and Columella is the first century BC postulated that diseases were caused by invisible being, inhaled or ingested.
2. Fracastorius of Verona (1546) proposed contagious agent as a source of infection.
3. Von Plenciz (1762) suggested that each disease was caused by a separate agent.
4. Kircher (1659) reported minute worms in the blood of Plague victims.
5. Basic credit goes to Antony Van Leeuwenhock after the development of Microorganism (1683) He made accurate description of various types of bacteria and communicated them to the royal society of London, Hook termed that creature as animalcule.
6. After two centuries Augustino Bassi (1835), discovered pathogenic microorganism. Davaine and Pollender (1850) observed anthrax bacilli in the blood of animals dying of the disease.
7. Louis Pasteur (1822-95) established that fermentation was the result of microbial activity.
8. Needham, an Irish priest (1745) published the theory of Spontaneous generation (Abiogenesis)
9. Spallanzani opposed the theory of spontaneous generation and proved that all forms of life arose only from their likes; He also introduced the methods of sterilization.
10. Robert Koch (1843-1910) perfected bacteriological techniques, studied on culture and life cycle of anthrax. He discovered Tuberculosis bacilli in (1882).
11. Koch's postulate – According to his thesis, a microorganism can be accepted as the causative agent of infectious diseases only if the following conditions are satisfied.
  - (a) The Bacterium should be constantly associated with the lesions of the diseases.
  - (b) It should be possible to isolate the bacterium in pure culture from the lesion.
  - (c) Inoculation of such pure culture into suitable Laboratory animals should reproduce the lesions of the disease.

(d) It should be possible to re-isolate the bacterium in pure culture from the 'lesions produced in the experimental animals.

12. Existence of ultramicroscopic microbes was proved by Ivanovsky (1892), reproduced mosaic disease in the tobacco plant.

13. Biejerinck (1898) term those ultramicroscopic microbes as Virus (1898)

14. Viral infection could lead to malignancy was first put up by Ellerman and Bang in 1908.

15. Pasteur developed vaccines against cholera, anthrax, rabies. Metchnikoff (1883) discovered the phenomena of Phagocytosis.

16. Fleming (1929) made the discovery of fungus penicillium.

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## **6. Applications of Microbiology:**

**The major fields of applied microbiology are described below:**

### **1. Microbes in Food and Dairy Industries:**

#### **(i) Molds:**

Food microbiology not only includes the study of those microbes which provide food due to their high protein value (such as yeast), but on the other hand, those microbes also which use our food supply as a source of nutrient for their growth and result in deterioration of the food by increasing their numbers, utilizing nutrients, producing enzymatic changes, and contributing off flavours by means of break down products.

Microorganisms, such as molds (*Mucor*, *Rhizopus*, *Botrytis*, *Aspergillus*, *Penicillium* etc.) lead to deterioration of food. Special molds are useful in the manufacture of certain foods or ingredients of foods.

Some cheese are mold ripened e.g. blue, Roquefort, camembert etc., molds are also used in production of oriental foods, e.g. soy sauce, miso, sonji, etc., used as food or feed and are involved in making enzymes such as amylase for bread making or citric acid used in soft drinks. Some molds are harmful (*Aspergillus flavus*) and some molds (*A. parasiticus*) produce toxic metabolites (mycotoxins).

#### **(ii) Yeasts:**

Yeast refers to those fungi which are generally not filamentous but unicellular and oval or spherical, reproduce by budding or fission, and may be useful or harmful in food.

Yeast fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar and surface ripened cheese and yeasts are grown for enzymes and for food. Yeasts are undesirable when spoil fruit juices, syrups, molasses, jam, pickles wine, beer and other foods.

Example of some of the genera is *Saccharomyces*, *Schizosaccharomyces*, *Candida*, *Kluyveromyces*, *Zygosaccharomyces*, *Pichia*, *Hansenula*, *Debaromyces*, *Hanseniaspora*, etc.

### **(iii) Bacteria:**

Bacteria in a food may be of special significance. Pigmented bacteria cause changes in colour on the surfaces of foods, form film over the surfaces of liquid food, etc. which result in undesirable cloudiness or sediment. Some genera, such as *Acetobacter* oxidises ethyl alcohol to acetic acid, *Aeromonas*, a facultative anaerobe also pathogenic not only to human beings but to fish, frogs and other mammals.

*Alcaligenes* as the name indicates produce an alkaline reaction in the medium for growth, causes ropiness in milk, and gives slimy growth on cottage cheese. *Bacillus coagulans*, a proteolytic species, curdle milk.

*Bacillus purimilus* is recommended as test organism in sterility testing. *B. stearothermophilus* is used for testing procedures involving steam sterilization. *B. subtilis* var. *niger* (ATCC 9372) is recommended for ethylene oxide sterilization testing. *Brochothrix* can spoil a wide variety of meats and their products. The genus *Microbacterium lacticum* used in production of vitamins while *Micrococcus luteus* and *M. rosens* help in meat-curing brines.

*Lactobacillus viridescens* causes greening of sausage. *Proteus* is involved in the spoilage of meats, seafood, and eggs. Its presence in food suspect as a cause of food poisoning. *Pseudomonas* can cause food spoilage. The greenish fluorescence is developed due to pyoverdine formation and white, cream-coloured reddish, brown, or even black colours are formed due to *P. nigrifaciens*.

Different species such as *Clostridium thermosaccharolyticum*, saccharolytic obligate thermophile, causes gaseous spoilage of canned vegetables. Putrefaction i.e. deterioration of food under anaerobic condition takes place due to proteolytic action of *C. putrefaciens* while *C. perfringens* causes violent disruption of the curd in milk. *Desulfotomaculum* is responsible for sulfide stinker spoilage in canned food.

*Erwinia carotovora* is associated with the bacterial rot in vegetables (carrot, etc.). *Flavobacterium* involved in the spoilage of shellfish, poultry, eggs, butter and milk, while *Halo bacterium* causes discolouration in salted fish. *Lactobacillus* ferments sugars with the production of lactic acid, but also resulting in the deterioration of wine or beer.

They synthesize most of the vitamins they require. *Leuconostoc cremoris*, ferments citric acid of milk and also stimulate lactic streptococci so called 'lactic starter' for butter milk, butter and cheese. *L. mesenteroides* produces high sugar concentration in syrups, ice-cream mixes, etc. which is a desirable characteristic.

*Pediococcus* is salt tolerant acid producer and psychrophilic in nature. *Pediococci* have been found growing during the fermentation of brined vegetables and have been found responsible for the spoilage of alcoholic beverages, e.g. beer.

*Photo bacterium* causes phosphorescence of meats and fish, while *Propionibacterium freudeureichii* ferments the lactates to produce the gas that helps, forms the holes or eyes and also contributes flavours in cheese.

*Salmonella*, enteric pathogens, may grow in foods and cause food infections. Usually they are only transported by foods. *Staphylococcus aureus* gives yellow to orange growth and some produce an enterotoxin which causes food poisoning. *Streptococci* are homo fermentative; *S. thermophilus*, a coccus important in cheese made by cooking the curd at high temperature and in certain cases fermented milk such as yoghurt, and *S. boris* comes from cow manure and saliva.

*S.lacti*, an important dairy bacterium used as starter for cheese, cultured butter milk and some types of butter along with *Leuconostoc spp.*, *S.lactis* often causes souring of raw milk while *S.faecalis* and *S.faecium* are commonly found in raw foods. *Vibrio cholerae* and *V. vulnificus* both are pathogenic to human beings and associated with the sea food.

*Shigella sonnei* and *S. dysenteriae* are the bacteria found in macaroni salads, beans, potato etc. and cause bacillary dysentery (*Shigellosis*) in human beings. *Yersinia pestis* causes food-borne diseases and also is causal organism of plague in humans and in rats (this bacteria caused disease outbreak in Gujarat in 1994-95).

## **2. Microbes in the Production of Industrial Products:**

Enzymes, amino acids, vitamins, antibiotics, organic acids and alcohols are commercially produced by microorganisms. Some of them are used by microbes during their growth and

therefore, are called primary microbial products or primary metabolites such as enzymes, amino acids and vitamins, while some are not used by the cell for their growth such as antibiotics, alcohol and organic acids.

These are called secondary microbial products or metabolites. Commercially important microbial enzymes are usually extracellular and marketed in crude form. Lipases are supplemented in detergents such as surf and proteases are also used in detergents and in leather and food industries; pectinases used in clarifying fruit juices and penicillin acylases in the production of semi synthetic penicillin.

Amylases are used in the preparation of starch hydro lysates which in turn is used in various product formations such as beer, vinegar, etc. Various small quantities of other enzymes of microbial origin such as asparaginase is used against leukemia (blood cancer) and lymphomas (cancer) which acts upon high percentage of the amino acid asparagin present in cancer cells.

Microbes produce some important amino acids such as glutamic acid, lysine and methionine. The microbes produce only L-isomers of the amino acids. Monosodium glutamate (MSG) imparts flavours, while lysine and methionine use essential amino acids which are obtained in human beings by their diets.

On the other hand, citric acid, a Krebs cycle (TCA cycle) intermediate is produced mainly by *Aspergillus niger* (Koji) and used in preservation of food by microorganisms and as animal feed additives since they stimulate growth.