# Chapter-I

#### **Brief History of Microbiology**

Microbiology has had a long, rich history, initially centered in the causes of infectious diseases but now including practical applications of the science. Many individuals have made significant contributions to the development of microbiology.

**Early history of microbiology:** Historians are unsure who made the first observations of microorganisms, but the microscope was available during the mid-1600s, and an English scientist named **Robert Hooke** made key observations. He is reputed to have observed strands of fungi among the specimens of cells he viewed. In the 1670s and the decades thereafter, a Dutch merchant named **Anton van Leeuwenhoek** made careful observations of microscopic organisms, which he called **animalcules.** Until his death in 1723, van Leeuwenhoek revealed the microscopic world to scientists of the day and is regarded as one of the first to provide accurate descriptions of protozoa, fungi, and bacteria.

After van Leeuwenhoek died, the study of microbiology did not develop rapidly because microscopes were rare and the interest in microorganisms was not high. In those years, scientists debated the theory of **spontaneous generation**, which stated that microorganisms arise from lifeless matter such as beef broth. This theory was disputed by **Francesco Redi**, who showed that fly maggots do not arise from decaying meat (as others believed) if the meat is covered to prevent the entry of flies. An English cleric named **John Needham** advanced spontaneous generation, but **Lazzaro Spallanzani** disputed the theory by showing that boiled broth would not give rise to microscopic forms of life.

Louis Pasteur and the germ theory. Louis Pasteur worked in the middle and late 1800s. He performed numerous experiments to discover why wine and dairy products became sour, and he found that bacteria were to blame. Pasteur called attention to the importance of microorganisms in everyday life and stirred scientists to think that if bacteria could make the wine "sick," then perhaps they could cause human illness.

Pasteur had to disprove spontaneous generation to sustain his theory, and he therefore devised a series of **swan-necked flasks** filled with broth. He left the flasks of broth open to the air, but the flasks had a curve in the neck so that microorganisms would fall into the neck, not the broth. The flasks did not become contaminated (as he predicted they would not), and Pasteur's experiments put to rest the notion of spontaneous generation. His work also encouraged the belief that microorganisms were in the air and could cause disease. Pasteur postulated the **germ theory of disease**, which states that microorganisms are the causes of infectious disease.



Pasteur's attempts to prove the germ theory were unsuccessful. However, the German scientist **Robert Koch** provided the proof by cultivating anthrax bacteria apart from any other type of organism. He then injected pure cultures of the bacilli into mice and showed that the bacilli invariably caused anthrax. The procedures used by Koch came to be known as **Koch's postulates** (Figure 1). They provided a set of principles whereby other microorganisms could be related to other diseases.

The development of microbiology. In the late 1800s and for the first decade of the 1900s, scientists seized the opportunity to further develop the germ theory of disease as enunciated by Pasteur and proved by Koch. There emerged a **Golden Age** of **Microbiology** during which many agents of different infectious diseases were identified. Many of the etiologic agents of microbial disease were discovered during that period, leading to the ability to halt epidemics by interrupting the spread of microorganisms.

Despite the advances in microbiology, it was rarely possible to render life-saving therapy to an infected patient. Then, after World War II, the **antibiotics** were introduced to medicine. The incidence of pneumonia, tuberculosis, meningitis, syphilis, and many other diseases declined with the use of antibiotics.

Work with viruses could not be effectively performed until instruments were developed to help scientists see these disease agents. In the 1940s, the **electron microscope** was developed and perfected. In that decade, cultivation methods for viruses were also introduced, and the knowledge of viruses developed rapidly. With the development of vaccines in the 1950s and 1960s, such viral diseases as polio, measles, mumps, and rubella came under control.

Starting from 1970s - production of first **industrial concentrated cultures**, frozen or freeze dried cultures, for the direct inoculation of processed milk, improving the regularity of production processes.

**Modern microbiology.** Modern microbiology reaches into many fields of human endeavor, including the development of pharmaceutical products, the use of qualitycontrol methods in food and dairy product production, the control of disease-causing microorganisms in consumable waters, and the industrial applications of microorganisms. Microorganisms are used to produce vitamins, amino acids, enzymes, and growth supplements. They manufacture many foods, including fermented dairy products (sour cream, yogurt, and buttermilk), as well as other fermented foods such as pickles, sauerkraut, breads, and alcoholic beverages.

One of the major areas of applied microbiology is **biotechnology**. In this discipline, microorganisms are used as living factories to produce pharmaceuticals that otherwise could not be manufactured. These substances include the human hormone insulin, the antiviral substance interferon, numerous blood-clotting factors and clot dissolving enzymes, and a number of vaccines. Bacteria can be reengineered to increase plant resistance to insects and frost, and biotechnology will represent a major application of microorganisms in the next century.

#### Germ theory of disease



Scanning electron microscope image of Vibrio cholerae. This is the bacteria that causes cholera

The **germ theory of disease** refers to the discovery in the late 19th century that some infectious diseases are caused by microorganisms, small organisms too small to see without magnification, that invade the host. The theory supplanted earlier explanations for disease such as miasma theory.

### **Miasma theory**



A representation of the cholera epidemic of the 19th century depicts the spread of the disease in the form of poisonous air

**Spontaneous generation** — also called **abiogenesis**— is the belief that organisms can appear from nonliving materials like water, air and dead flesh. In the **fourth century BC**, the Greek philosopher Aristotle included this process in his list of methods of reproduction, along with sexual and asexual reproduction, and budding. This belief continued to be accepted during the Middle Ages and beyond. For example, people believed that maggots could appear from decaying meat, and snakes could be born from horse hairs left in stagnant water.

The **miasma theory** of disease transmission (the other name of spontaneous generation) held that diseases such as cholera, chlamydia or the Black Death were caused by a *miasma* ( $\mu \quad \mu$ , ancient Greek: "pollution"), a noxious form of "bad air". The theory held that the origin of these epidemic diseases was a miasma, emanating from rotting organic matter.<sup>[1]</sup> Miasma was considered to be a poisonous vapor or mist filled with particles from decomposed matter (miasmata) that caused illnesses. The miasmatic position was that diseases were the product of environmental factors such as contaminated water, foul air, and poor hygienic conditions. Such infection was not passed between individuals but would affect individuals within the locale that gave rise to such vapors. It was identifiable by its foul smell.

This was the predominant theory of disease transmission before the germ theory of disease took hold in the 19th century.

### **Development: Pre-19th century**

Girolamo Fracastoro proposed in 1546 that epidemic diseases are caused by transferable seed-like entities that transmit infection by direct or indirect contact, or even without contact over long distances.

Italian physician Francesco Redi in 1668 provided early evidence against spontaneous generation. He also noticed that the maggots in meatloaf and egg appear from accessible by flies from outside source like air. From this he concluded that spontaneous generation is not a plausible theory.

Microorganisms were first directly observed by Anton van Leeuwenhoek, who was an early pioneer in microbiology. Building on Leeuwenhoek's work, physician Nicolas Andry argued in 1700 that microorganisms he called "worms" were responsible for smallpox and other diseases.

Several other workers had also contributed ideas and prove that diseases are caused by microorganisms and their infections, not caused by spontaneous generation methods.

#### **Louis Pasteur**



Louis Pasteur in his laboratory, painting by A. Edelfeldt in 1885

The more formal experiments on the relationship between germ and disease were conducted by Louis Pasteur between 1860 and 1864. He discovered the pathology of the puerperal fever and the pyogenic vibrio in the blood, and suggest using boric acid to kill these microorganisms before and after confinement.

Louis Pasteur further demonstrated between 1860 and 1864 that **fermentation** and the growth of microorganisms in nutrient broths did not proceed by spontaneous generation.

Pasteur discovered that another serious disease of silkworms, pebrine, was caused by a small microscopic organism now known as Nosema *bombycis* (1870). Pasteur saved the silk industry in France by developing a method to screen silkworms eggs for those that are not infected, a method that is still used today to control this and other silkworm diseases.

Thus, Louis Pasteur, a French chemist in the 1800's, made many contributions to microbiology. This included a greater understanding of fermentation by microbes; the development of the germ theory of disease; and the creation of a technique for destroying microbes in perishable fluids, such as milk. The last one was named after him — **pasteurization**. He also ran a series of experiments in 1860 to show that organisms could not appear out of nonliving materials.

#### **Fermentation History**

The word *fermentation* is derived from the Latin verb **fervere**, which means *to boil* (same root as effervescence). It is thought to have been first used in the late fourteenth century in alchemy, but only in a broad sense. It was not used in the modern scientific sense until around 1600.

The first solid evidence of the living nature of yeast appeared between 1837 and 1838 when three publications appeared by C. Cagniard de la Tour, T. Swann, and F. Kuetzing, each of whom independently concluded as a result of microscopic investigations that yeast is a living organism that reproduces by budding. The word *yeast*, it should be noted, is cognate with the Sanskrit word meaning *boiling*. It is perhaps because wine, beer, and bread were each basic foods in Europe that most of the early studies on fermentation were done on yeasts, with which they were made. Soon, bacteria were also discovered; the term was first used in English in the late 1840s, but it did not come into general use until the 1870s, and then largely in connection with the new germ theory of disease.

Louis Pasteur (1822–1895), during the 1850s and 1860s, showed that fermentation is initiated by living organisms in a series of investigations. In 1857, Pasteur showed that lactic acid fermentation is caused by living organisms. In 1860, he demonstrated that bacteria cause souring in milk, a process formerly thought to be merely a chemical change, and his work in identifying the role of microorganisms in food spoilage led to the process of pasteurization. In 1877, working to improve the French brewing industry, Pasteur published his famous paper on fermentation, "*Etudes sur la Bière*", which was translated into English in 1879 as "*Studies on Fermentation*". He defined fermentation (incorrectly) as "*Life without air*", but correctly showed that specific types of microorganisms cause specific types of fermentations and specific end-products.

Although showing fermentation to be the result of the action of living microorganisms was a breakthrough, it did not explain the basic nature of the fermentation process, or prove that it is caused by the microorganisms that appear to be always present. Many scientists, including Pasteur, had unsuccessfully attempted to

extract the fermentation enzyme from yeast. Success came in 1897 when the German chemist Eduard Buechner ground up yeast, extracted a juice from them, then found to his amazement that this "*dead*" liquid would ferment a sugar solution, forming carbon dioxide and alcohol much like living yeasts. The "*unorganized ferments*" behaved just like the organized ones. From that time on, the term enzyme came to be applied to all ferments. It was then understood that fermentation is caused by enzymes that are produced by microorganisms. In 1907, Buechner won the Nobel Prize in chemistry for his work.

Advances in microbiology and fermentation technology have continued steadily up until the present. For example, in the late 1970s, it was discovered that microorganisms could be mutated with physical and chemical treatments to be higheryielding, faster-growing, tolerant of less oxygen, and able to use a more concentrated medium. Strain selection and hybridization developed as well, affecting most modern food fermentations.

Fermentation is a form of anaerobic digestion that generates ATP by the oxidation of certain organic compounds, such as carbohydrates. Fermentation uses an endogenous, organic electron acceptor. In contrast, respiration is where electrons are donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain. Fermentation is important in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of ATP (adenosine triphosphate) by glycolysis. During fermentation, pyruvate is metabolized to various compounds. Homolactic fermentation is the production of lactic acid from pyruvate; alcoholic fermentation is the production of lactic acid as well as other acids and alcohols. Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption (a phenomenon known as the Crabtree effect). The antibiotic activity of hops also inhibits aerobic metabolism in yeast.

Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, lactose, and hydrogen. However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone. Yeast carries out fermentation in the production of ethanol in beers, wines, and other alcoholic drinks, along with the production of large quantities of carbon dioxide. Fermentation occurs in mammalian muscle during periods of intense exercise where oxygen supply becomes limited, resulting in the creation of lactic acid.

- Fermentation (biochemistry), a metabolic process whereby electrons released from nutrients are ultimately transferred to molecules obtained from the breakdown of those same nutrients
  - Ethanol fermentation, the production of ethanol for use in food, alcoholic beverage, fuel and industry
- Fermentation (food), the process of converting sugar to carbon dioxide and alcohol with yeast
- Fermentation (wine), the process of fermentation used in wine-making
  - Lactic acid fermentation, the biological process by which sugars such as glucose, fructose, and sucrose, are converted into cellular energy and the metabolic byproduct lactate
  - Industrial fermentation, the breakdown and re-assembly of biochemicals for industry, often in aerobic growth conditions
  - Fermentative hydrogen production, the fermentative conversion of organic substrate to biohydrogen manifested by a diverse group of bacteria
- *Fermentation (oxidation)*, the term used in the tea industry in tea processing for the aerobic treatment of tea leaves to break down certain unwanted chemicals and modify others to develop the flavor of the tea.

### **Robert Koch**

Robert Koch is known for developing four basic criteria (known as Koch's Postulates, 1882) for demonstrating, in a scientifically sound manner, that a disease is caused by a particular organism. These postulates grew out of his seminal work with the anthrax using purified cultures of the pathogen that had been isolated from diseased animals.

Koch's postulates were developed in the 19th century as general guidelines to identify pathogens that could be isolated with the techniques of the day. Even in Koch's time, it was recognized that some infectious agents were clearly responsible for disease even though they did not fulfill all of the postulates. Attempts to rigidly apply Koch's postulates to the diagnosis of viral diseases in the late 19th century, at a time when viruses could not be seen or isolated in culture, may have impeded the early development of the field of virology. Currently, a number of infectious agents are accepted as the cause of disease despite their not fulfilling all of Koch's postulates. Therefore, while Koch's postulates retain historical importance and continue to inform the approach to microbiologic diagnosis, fulfillment of all four postulates is not required to demonstrate causality.

Koch's postulates have also influenced scientists who examine microbial pathogenesis from a molecular point of view. In the 1980s, a molecular version of Koch's postulates was developed to guide the identification of microbial genes encoding virulence factors.

### Koch's postulates are the following:

- 1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
- 2. The microorganism must be isolated from a diseased organism and grown in pure culture.
- 3. The cultured microorganism should cause disease when introduced into a healthy organism.
- 4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.



**Figure 1:** The steps of Koch's postulates used to relate a specific microorganism to a specific disease. (a) Microorganisms are observed in a sick animal and (b) cultivated in the lab. (c) The organisms are injected into a healthy animal, and (d) the animal develops the disease. (e) The organisms are observed in the sick animal and (f) reisolated in the lab.

However, Koch abandoned the universalist requirement for the following reasons

 The *first postulate*: Asymptomatic or subclinical infection carriers are now known to be a common feature of many infectious diseases, especially viruses such as polio, herpes simplex, HIV, and hepatitis C. As a specific example, all doctors and virologists agree that poliovirus causes paralysis in just a few infected subjects.

- The second postulate: Certain microorganisms or entities that cannot be grown in pure culture, such as prions responsible for Creutzfeldt–Jakob disease (a neurological brain disease).
- The *third postulate*: Not all organisms exposed to an infectious agent will acquire the infection. Noninfection may be due to such factors as general health and proper immune functioning; acquired immunity from previous exposure or vaccination; or genetic immunity, as with the resistance to malaria conferred by possessing at least one sickle cell allele.

### Public Hygiene & Microbes

With major epidemics occurring in Europe throughout the eighteenth and nineteenth centuries, public hygiene emerged as an important concept. Governments noticed a connection between poor living conditions and outbreaks of diseases such as cholera, smallpox and typhoid. In response, they instituted various sanitation measures: garbage and human waste collection, and increased ventilation in buildings. The measures, however, failed to identify the role of microbes in these diseases.

In 1848, Ignaz Semmelweis a German physician, made a connection between hygiene and the prevention of microbial disease. He noticed that medical students could spread disease from infected dead bodies during autopsies to pregnant women that they later examined. To prevent that, he required students to wash their hands with chlorinated lime water. The number of women that contracted the disease dropped greatly, although not all doctors accepted this new technique. The use of hand washing — along with the sterilization of medical tools — continues to be used in hospitals to prevent the spread of infectious diseases.

| Physical     |                         |               |                      |                                      | Chemical             |
|--------------|-------------------------|---------------|----------------------|--------------------------------------|----------------------|
| Heat         |                         | Radiation     |                      | Filtration                           |                      |
| Dry<br>heat  | Moist heat              | lonizing      | Non-ionizing         |                                      |                      |
| Hot air      | Steam under<br>pressure | Gamma<br>rays | Ultra-violet<br>rays | Sieving<br>through micro-<br>filters | Acid                 |
| Infra<br>red | Tyndalization           |               |                      |                                      | Alcohol              |
|              | Pasteurization          |               |                      |                                      | Formaldehyde         |
|              | Hot oil bath            |               |                      |                                      | Phenol               |
|              |                         |               |                      |                                      | Ethylene<br>oxide    |
|              |                         |               |                      |                                      | Mercuric<br>chloride |
|              |                         |               |                      |                                      | Hypochlorite         |

#### Methods used to destroy microorganisms

**Infrared radiation**: is electromagnetic radiation (EMR) with longer wavelengths (upto 1050 nm) than those of visible light, and is therefore generally invisible to the human eye, sterilize the object without attaining high temperature using carbon lamp, bacteria on the surface only are killed.

**Tyndallization**: Nmaed after the scientist John Tyndall. Tyndallization consists of heating the substance to boiling point (or just a little bellow BP) and holding it there for 15 min, 3 days in succession, thus it kills the bacterial spores.

#### Application /scope of Microbiology

In spite of their size, microbes are extremely useful creatures. They have many applications within the world of medicine and beyond. Medical microbiology looks at how microbes cause disease, as well as the development of treatments. Industrial microbiology involves the use of microbes for processes such as fermentation and wastewater treatment. Food and agricultural microbiology explores how microbes affect the growing and production of food.

Microbes are also used in genetic engineering, which is the changing of an organism's DNA to alter how it looks or functions. This activity falls under the specialty of microbial biotechnology. This field includes medical applications, as well as agricultural and energy uses. For example, plants and animals can be altered at their genetic level to be resistant to disease, or to produce more meat or nutrients.

Microbial Food Cultures are live bacteria, yeasts or moulds used in food production. Microbial Food Cultures carry out fermentation process in foodstuffs. Used by humans since the Neolithic period (around 10000 years BC) fermentation helps to preserve perishable foods and to improve their nutritional and organoleptic qualities (relating to the senses, taste, sight, smell, touch). Today, fermented food represents between one quarter and one third of food consumed in Central Europe. More than 260 different species of Microbial Food Cultures are identified and described for their beneficial use in fermented food products from all over the world. This shows the great importance of the use of Microbial Food Cultures.

The scientific rationale of the function of microbes in fermentation has started to be built following the discoveries of Louis Pasteur in the second half of 19th century. Since then, thanks to extensive scientific studies Microbial Food Cultures, traditionally used in food fermentation are being characterized (taxonomically, physiologically, biochemically and genetically). It allows better understanding and improving of traditional food processing and opens up new fields of applications.

### Contribution of scientists in field of microbiology

- 1654: Live microorganisms observed through magnifying lenses (simple microscope 200-300 x magnification), describing them as "animalcules", stating that they could be found in rainwater and in the "material" scraped from his teeth. The first time made the authentic drawings of microorganisms (protozoa, bacteria, fungi). (Antoni van Leeuwenhoek)
- 1665: Used a compound microscope to observe individual cells, the beginning of the Cell Theory, i.e. all living things are composed of cells (**Robert Hooke**)
- 1735: Binomial nomenclature for organisms [Carl Linnaeus (Latinised as Carolus Linnaeus)]
- 1796: First scientific Small pox vaccination using cowpox (Edward Jenner)
- 1850: Advocated washing hands to stop the spread of disease (Ignaz Semmelweis)
- 1857: Fermentation (Louis Pasteur)
- 1861: Disproved spontaneous generation (Louis Pasteur)
- 1862: Supported Germ Theory of Disease (Louis Pasteur)
- 1864: Pasteurisation (Louis Pasteur)
- 1867: Practiced antiseptic/aseptic surgery (Joseph Lister)
- 1876: First proof of Germ Theory of Disease with B. anthracis discovery (Robert Koch)
- 1880: Immunisation techniques (Louis Pasteur)
- 1881: Pure culture- Growth of Bacteria on solid media (Robert Koch)
- 1882: Agar (solid) media (Hess)
- 1882: Outlined Kochs postulates (Robert Koch)
- 1882: Developed acid-fast Stain (Paul Ehrlich)
- 1882: Mycobacterium tuberculosis, for human tuberculosis (Robert Koch)
- 1884: Developed Gram Staining procedure (Christian Gram)
- 1885: First Rabies vaccination (Louis Pasteur)
- 1887: Invented Petri Dish (R.J. Petri)
- 1892: Discovered viruses (Dmitri losifovich lvanovski)
- 1899: Recognized viral dependence on cells for reproduction (Martinus Beijerinck)
- 1900: Proved mosquitoes carried the yellow fever agent (Walter Reed)
- 1910: Discovered cure for syphilis (Paul Ehrlich)
- 1918: Conn developed "Direct soil examination" technique for studying soil microorganisms.
- 1928: Discovered Penicillin (Alexander Fleming)
- 1977: Developed a method to sequence DNA (W. Gilbert & F. Sanger)
- 1983: Polymerase Chain Reaction invented (Kary Mullis)
- 1995: First microbial genomic sequence published **(***H. influenzae***)** (The Institute for Genomic Research: TIGR, California founded by J. Craig Venter)

# Contribution of scientists in field of Soil microbiology

**J. B. Boussingault (1838)** showed that leguminous plants can fix atmospheric nitrogen and increase nitrogen content in the soil.

**J. Von Liebig (1856)** showed that nitrates were formed in soil due to addition of nitrogenous fertilizers in soil.

**S. N. Winogradsky (1890)** discovered the autotrophic mode of life among bacteria and established the microbiological transformation of nitrogen and sulphur. Isolated for the first time nitrifying bacteria and demonstrated role of these bacteria in nitrification, further he demonstrated that free-living *Clostridium pasteuriamum* could fix atmospheric nitrogen (1893). Therefore, he is considered as "**Father of soil microbiology**".

W. B. Leismaan (1858) and M. S. Woronin (1866) demonstrated that root nodules in legumes were formed by a specific group of bacteria.

**Jodin (1862, France)** gave the first experimental evidence of elemental nitrogen fixation by microorganisms.

**R. Warington (1878)** showed that nitrification in soil was a microbial process.

**B.** Frank i) discovered (1880) an actinomycetes "Frankia" (Actinorhizal symbiosis) inducing root nodules in non-legumes tress of genera *Alnus sp* and *Casurina* growing in temperate forests, ii) coined (1885) the term " Mycorrhiza" to denote association of certain fungal symbionts with plant roots (Mycorrhiza-A symbiotic association between a fungus and roots of higher plants. Renamed the genus Bacillus as Rhizobium (1889).

**H. Hellriegel and H. Wilfarth (1886)** showed that the growth of non-legume plant was directly proportional to the amount of nitrogen supplied, whereas, in legumes there was no relationship between the quantity of nitrogen supplied and extent of plant growth. They also suggested that bacteria in the root nodules of legumes accumulate atmospheric nitrogen and made it available to plants. Showed that a mutually beneficial association exists between bacteria (*Rhizobia*) and legume root and legumes could utilize atmospheric nitrogen (1988).

**M. W. Beijerinck (1888)** isolated root nodule bacteria in pure culture from nodules in legumes and named them as *Bacillus radicola* Considered as **father of "Microbial ecology"**. He was the first Director of the Delft School of microbiology (Netherland).

**M.W. Beijerinck and S.N. Winogradsky (1890)** developed the enrichment culture technique for isolation of soil organisms, proved independently that transformation of nitrogen in nature is largely due to the activities of various groups of soil microorganisms (1891). Therefore, they are considered as "Pioneer's in soil bacteriology".

**S. N. Winogadsky (1891)** demonstrated the role of bacteria in nitrification and further in fill 1983 demonstrated that free living *Clostridium pasteurianum* could fix atmospheric nitrogen.

**Omeliansky** (1902) found the anaerobic degradation of cellulose by soil bacteria.

**J. G. Lipman and P. E. Brown (1903, USA**) studied ammonification of organic nitrogenous substances by soil microorganisms and developed the **Tumbler or Beaker** for studying different types of transformation in soil.

**Hiltner (Germany, 1904)** coined the term **"Rhizosphere"** to denote that region of soil which is subjected to the influence of plant roots. Rhizosphere is the region where soil and plant roots make contact.

**Russel and Hutchinson** (1909, England), proved the importance of protozoa controlling/ maintaining bacterial population and their activity in soil.

**Conn (1918)** developed "**Direct soil examination**" technique for studying soil microorganisms.

Rayner (192I) and Melin (1927) carried out the intensive study on Mycorrhiza.

# Applied fields of Microbiology

- Medical microbiology: The study of the pathogenic microbes and the role of microbes in human illness. Includes the study of microbial pathogenesis and epidemiology and is related to the study of disease pathology and immunology.
- **Pharmaceutical microbiology**: The study of microorganisms that are related to the production of antibiotics, enzymes, vitamins, vaccines, and other pharmaceutical products and that cause pharmaceutical contamination and spoil.
- **Industrial microbiology:** The exploitation of microbes for use in industrial processes. Examples include industrial fermentation and wastewater treatment. Closely linked to the biotechnology industry. This field also includes brewing, an important application of microbiology.
- **Microbial biotechnology:** The manipulation of microorganisms at the genetic and molecular level to generate useful products.
- Food microbiology and Dairy microbiology: The study of microorganisms causing food spoilage and foodborne illness. Using microorganisms to produce foods, for example by fermentation.
- **Agricultural microbiology:** The study of agriculturally relevant microorganisms. This field can be further classified into the following:
  - **Plant microbiology and Plant pathology:** The study of the interactions between microorganisms and plants and plant pathogens.
  - Soil microbiology: The study of those microorganisms that are found in soil.
- Veterinary microbiology: The study of the role in microbes in veterinary medicine or animal taxonomy.
- Environmental microbiology: The study of the function and diversity of microbes in their natural environments. This involves the characterization of key bacterial habitats such as the rhizosphere and phyllosphere, soil and groundwater ecosystems, open

oceans or extreme environments (extremophiles). This field includes other branches of microbiology such as:

- Microbial ecology
- o Microbially-mediated nutrient cycling
- Geomicrobiology
- Microbial diversity
- o Bioremediation
- Water microbiology (or Aquatic microbiology): The study of those microorganisms that are found in water.
- Aeromicrobiology (or Air microbiology): The study of airborne microorganisms.