Introduction to Microbiology

Microbiology may be defined as the science or study of microscopic organisms, i.e., organisms too small to be observed with the naked eye (from the Greek terms – micro = small, Bio = life, and logos = discourse or study of).

These microorganisms, or **microbes** as they are sometimes called, include bacteria, archaea, protozoa and microscopic forms of fungi and algae. Since certain multicellular organisms are microscopic (or have microscopic stages during their life cycles) and some play a role in disease transmission, they are also included in microbiology courses. Non- cellular forms such as viruses, viroids and prions are not true organisms, but since they do infect and reproduce within living organisms, they fall within the realm of microbiology.

Eukaryotic cell types	**Prokaryotic cell** types	**Non-cellular** entities
Protozoa	Bacteria	Viruses
Microscopic algae	Archaea	Viroids
Microscopic fungi		Prions
Animal parasites (eggs and immature stages are microscopic)		

When compared to the other natural sciences, Microbiology is relatively young, i.e., has not existed for very long. **Can you think of a reason for this?**

Although humans evolved in the presence of microorganisms, and have been interacting with them for thousands of years, microorganisms are not visible without the aid of microscopes, so nobody knew they were there. The first living microorganisms were observed about 340 years ago (1674-1676), but their significance was not appreciated until nearly 200 years later. Today microbiology is recognized as a subject of major importance because microorganisms are essential to our survival, and yet we are just beginning to fully appreciate the complex roles they play.

Early Uses for Microorganisms

Humans evolved in the presence of microorganisms, therefore, we interacted with them long before we were able to observe them or recognize that they existed. Some microorganisms are parasites, and some are pathogens, i.e., can cause disease; but most are beneficial. It's not surprising, therefore that early human societies began to put microbes to work. **What do you suppose humans first used microorganisms for?**

Evidence indicates that Neolithic human societies were using microorganisms to ferment grains and make beer or wine (dating from 7000–6600 BCE in China, 6000 BCE in Georgia, 3150 BCE in ancient Egypt, 3000 BCE in Babylon, 2000 BCE in pre-Hispanic Mexico, and 1500 BC in Sudan). This practice probably began as soon as people had excess grain to store for future use. Grain stored in depressions in the ground or in woven baskets was sometimes wetted by rain, would have been fermented by wild yeasts and alcohol was produced. As people found the fermented grain and juice palatable, they undoubtedly took steps to increase production. Vegetables of various types are also used as fermented foods, probably because fermentation occurred naturally when vegetables were stored.

Around 4000 BC early Egyptians discovered that bread dough treated in a certain manner, would rise into a light airy loaf. This was due to yeast cells producing carbon dioxide. The practice of saving a small bit of dough as a "starter" probably began long before people recognized yeast as a microbe.

Cultured foods such as cheese and yogurt were initially produced as the result of storing milk without refrigeration (often in containers made of animal skin or stomach). When people found that these products would keep without spoiling longer than would fresh milk, they were made intentionally.

So, some of the earliest uses for microorganisms were in food processing and **preservation**. Wine could be stored longer than fresh fruit juice, and cheese longer than fresh milk. The fermentation of materials such as milk, grains, grapes, cabbages, cucumbers etc. yielded products that remained palatable and could be stored for long periods of time.

Anton Van Leeuwenhoek (1674-1676)

The discovery of microorganisms is usually credited to a Dutch tradesman and naturalist by the name of **Anton Van Leeuwenhoek**. He is sometimes referred to as the "Father of Microbiology". Van Leeuwenhoek made fine glass lenses (which could magnify objects about 266 times) and observed living microorganisms (which he called "animacules") from a variety of environments (also spermatozoa, blood cells and muscle fibers). His investigations were apparently made around 1674 but he was rather secretive about his methods and did not explain exactly how he made his lenses or his observations.

Van Leeuwenhoek's observations may not have been the first, but they were significant because he made numerous drawings and wrote accurate descriptions of what he saw. **He documented his findings**. For several years, starting about 1684, he sent correspondence to the British Royal Society or Royal Society of London, and thereby aroused considerable interest in microscopy and microbiology.

Spontaneous Generation (Abiogenesis)

Van Leeuwenhoek's discoveries did much to revitalize arguments between scientists, philosophers and theologians about the origin of life. It was, at one time, generally accepted that living organisms arose spontaneously from non-living material. This belief, sometimes called the 'theory' of **abiogenesis** or **spontaneous generation** (a=without, bio=life, genesis=origins or beginnings) was taught by Aristotle around 346 BC. He believed that life could and did appear spontaneously from non-living and/or decomposing materials. For example, he wrote that snakes and frogs came from the mud along river banks, that insects came from dew, that flies arose from decaying meat and that rats sprang from refuse heaps. These, like many other beliefs of the Greek scholars, were maintained until relatively recent times.

During the 17th century (1600s), a Belgian clergyman by the name of Van Helmont wrote a recipe for the generation of mice. He suggested that if a dirty garment were placed in a container with wheat grains for 21 days, the cloth and grains would give rise to live mice. This says little for living conditions at the time and less for powers of observation. Although belief in spontaneous generation was based upon inadequate observation and faulty reasoning, supporters of this “theory” were difficult to refute.

Around 1665 the Italian naturalist and physician **Francesco Redi** demonstrated that spontaneous generation did not occur at a **macroscopic level** using flies. Redi placed raw meat into containers and covered some with gauze and some with paper. Other containers were left open. He found that the meat within the covered containers did not develop flies, but that flies did lay eggs on the gauze and on the paper. The exposed meat developed maggots, but he reasoned that these came from the eggs of flies, not from the meat itself. Regardless of Redi's proof, people still clung to their belief in abiogenesis, and Van Leeuwenhoek's discoveries seemed to support this “theory”.

Van Leeuwenhoek did not conduct experiments to determine the source of his "animacules", but his discoveries attracted widespread attention and stimulated controversy. Those believing in abiogenesis thought the microscopic organisms came from the broths and waters in which they were observed. Thus the discovery of microorganisms rekindled an argument that was to wage for years.

In 1749, John Needham, a Catholic priest, conducted experiments with mutton broth in flasks. He boiled the broth and stoppered the flasks with cork, but later found the broth to be teaming with microorganisms. Needham believed there was a "vital force" present within the broth, and that life had arisen spontaneously.

In 1766, **Lazzaro Spallanzani**, a priest by profession but scientist at heart, repeated Needham’s experiments. Spallanzani boiled his broth longer and sealed his flasks with glass. After several days the flasks were opened and were found to contain no living organisms. Needham and others discredited Spallanzani's work because they said his prolonged boiling had destroyed the "vital force" within the broth, and because no air could get in. (The discovery of oxygen and its importance to life had occurred at about the same time.) Thus, although Spallanzani had actually proven that microorganisms did not arise spontaneously from non-living materials, he was not credited for his work at the time.

During the 1830s, Theodor Schwann and Franz Schultz (both German scientists) conducted experiments to disprove abiogenesis. They allowed boiled broth to come into contact with air that was either heated or passed through solutions of toxic chemicals. No microscopic organisms grew in their broth. Again the "vitalists", those in favor of spontaneous generation, discredited this work because they said the drastic treatment of the air had rendered it inactive. About this same time, another controversy had developed over the cause of fermentation. Biologically inclined investigators (including Schwann) proposed that the products of fermentation, ethanol and carbon dioxide, were made by microscopic life forms. This idea was opposed by the leading chemists of the time who believed that fermentation was strictly a chemical reaction brought about by chemical entities they called ferments.

Louis Pasteur (1860s)

Louis Pasteur, a young French chemist and physicist, had been hired by French distillers to determine why the contents of their fermentation vats sometimes turned sour (vinegar) instead of brewing as expected (ethanol). Pasteur determined that microorganisms including bacteria and yeasts (fungi) were present in the vats. Over a period of time, he was able to prove that fermentation was indeed the result of microbial activity. By taking samples from various vats and transferring them to fresh juice samples, he was able to show that each type of fermentation product was mediated by a specific type of microorganism. Although they were not pure cultures, the collections of organisms in Pasteur's fermentation vats were predominantly of one type or another, and he was able to identify them with a fair degree of accuracy. Pasteur also developed a process that could be used to greatly reduce the number of unwanted microorganisms in juice and milk. It involved heating the liquid briefly to a specific temperature, and thereby killing most of the cells present. **Can you guess what this process is called? (Pasteurization)**

Despite mounting evidence to the contrary, the proponents of abiogenesis continued to argue their cause and to publish their evidence in support of spontaneous generation.

Pasteur was irritated by the seemingly endless controversy, and set out to settle the question "once-and-for-all". He reported the results of his experiments in 1864, and is usually credited with disproving the abiogenesis of microorganisms. By passing air through gun cotton, Pasteur was able to show that microorganisms were abundant in air (they had been collected and observed on the cotton). When placed into flasks of broth, these microorganisms grew readily. Pasteur also constructed "goose necked" flasks in which he could boil nutrient broths but which, by their shape, prevented the entrance of microorganisms from air. Though these were left open to whatever "vital forces" might be present in air, no organisms grew. Fortunately, Pasteur's broths contained no endospore-forming bacteria, since endospores are resistant to boiling and had they been present, would have grown.

Though Pasteur's work was not universally accepted, he had many supporters. One of these was an English physicist by the name of **John Tyndall**. Tyndall set up an elaborate box containing only clean (filtered) air, and showed that broths exposed to this clean air did not grow microorganisms.

Tyndall also discovered that some microorganisms were very resistant to being killed by boiling, i.e., those that produced heat resistant endospores. This helped to explain the varied results obtained by other investigators. Tyndall found that by alternately boiling and cooling his broths over a period of three days he could eliminate the spore-forming organisms. This process is called **tyndallization** or **fractional sterilization**.

Though many investigators worked to disprove the theory of abiogenesis at the microscopic level, it is Pasteur who usually receives credit for finally laying the theory to rest. Once this was accomplished, the supernatural, mysterious or magical aspects of microorganisms were explained away, and Microbiology could be recognized as a true science.

Something to consider - Why is the “theory” of abiogenesis not compatible with science?

The United States National Academy of Sciences defines scientific theories as follows:

The formal scientific definition of "theory" is quite different from the everyday meaning of the word. It refers to a comprehensive explanation of some aspect of nature that is supported by a vast body of evidence. Many scientific theories are so well established that no new evidence is likely to alter them substantially. For example, no new evidence will demonstrate that the Earth does not orbit around the sun (heliocentric theory), or that living things are not made of cells (cell theory), that matter is not composed of atoms, or that the surface of the Earth is not divided into solid plates that have moved over geological timescales (the theory of plate tectonics)...One of the most useful properties of scientific theories is that they can be used to make predictions about natural events or phenomena that have not yet been observed.

From the American Association for the Advancement of Science:

A scientific theory is a well-substantiated explanation of some aspect of the natural world, based on a body of facts that have been repeatedly confirmed through observation and experiment. Such fact-supported theories are not "guesses" but reliable accounts of the real world. The theory of biological evolution is more than "just a theory." It is as factual an explanation of the universe as the atomic theory of matter or the germ theory of disease. Our understanding of gravity is still a work in progress. But the phenomenon of gravity, like evolution, is an accepted fact.

Germ Theory of Disease

Even after microorganisms were observed and found to play an important role in fermentation, it was a number of years before people recognized their involvement in disease processes. Some of the earliest physicians, including Hypocrites, believed that people could transmit disease from one to another, but they did not understand how. Around 1546, **Girolamo Fracastoro**, an Italian physician, recorded his belief that disease was due to entities (spores) too small to be seen with the naked eye. This was referred to as the contagion theory, but since Fracastoro had no real proof (could not observe the disease-causing "spores"), his writings were largely ignored.

Prior to Microbiology, people generally associated disease with natural phenomena such as earthquakes, floods, or exposure to bad air or bad weather. Disease was also attributed to mysterious or supernatural causes. Many religious leaders encouraged the belief that disease resulted from disobedience to God. People stricken by illness and death were undoubtedly being punished for their evil deeds. The threat of such punishment was useful for controlling people. Since people were unaware of disease causing microbes and their manner of transmission, practices we take for granted today (to prevent infection and contamination) did not occur to people.

Around 1840 there was a turning point in surgery due to the advent of anesthesia. Prior to that time, people undergoing surgery often died of shock unless the surgeon was quick. The most successful surgeons, therefore, were those who were fastest at their work. With the advent of anesthesia, surgeons could work at a slower pace and their patients did not suffer from shock. Unfortunately however, longer exposure to the microbes associated with the surgeon's hands, instruments and the surrounding air resulted in more wound infections. Physicians did not wash their hands or instruments between patients, and most surgery was conducted in open rooms containing large numbers of people. Patients no longer died of shock, but many died of disease. Around 45% of those undergoing surgical procedures died as a result of the associated wound infections.

Joseph Lister (1867)

During the 1860s **Joseph Lister**, an English surgeon, reasoned that surgical infection (sepsis) might be caused by microorganisms. (**Sepsis** = The condition resulting from the presence of pathogenic microbes or their products in blood or tissues.) Lister devised methods to prevent microbes from entering the wounds of his patients. His procedures came to be known as **antiseptic** (against sepsis) surgery, and included hand washing, sterilizing instruments, and dressing wounds with carbolic acid (phenol).

Lister was well aware of microorganisms and is credited with developing techniques to obtain and maintain the first pure bacterial cultures. Though he did not publish proof that microbes were responsible for disease, he firmly believed they were.

About this same time (1840s), a physician by the name of **Ignaz Philip Semmelweis** began using antiseptic procedures to prevent "childbirth" or puerperal fever (a serious and often fatal disease associated with infection contracted during delivery). Semmelweis also strongly discouraged doctors involved in conducting autopsies and teaching anatomy (in the basement) from practicing their surgical skills on patients (upstairs) without first washing their hands and instruments.

The techniques of Lister and Semmelweis were initially scoffed at by some, but as they were shown to greatly reduce infection and fatality, they were recognized as major improvements to the previously accepted procedures. These techniques also provided indirect evidence for the connection between microorganisms and disease.

The first microorganisms actually shown to be pathogenic were fungi and protozoa. These were found to be infecting silk worms, so were impacting an important industry in Europe at the time (around 1865).

Robert Koch (1876)

Direct evidence demonstrating that bacteria were disease-causing agents (**etiological agents**) was provided by **Robert Koch**, a German physician, in 1867. Koch was working with a disease of sheep and cattle called anthrax, and determined the causative agent to be a type of bacteria he called *Bacillus anthracis*. Koch established a sequence of experimental steps that could be used to demonstrate beyond a doubt that a specific type of microorganism was responsible for a specific disease. These came to be known as **Koch's postulates**, and are still in use today.

Koch's Postulates:

1. The suspect causative agent must be found in every case of the disease. (Koch took samples from hundreds of animals over years of investigation to be certain of his conclusions.)
2. The specific type of microbe must be isolated from the infected individual and grown in a culture containing no other forms (pure culture).
3. Upon inoculation into a normal, healthy, susceptible animal, a pure culture of the microbial agent must produce the disease.
4. The same type of microbe must be recovered again from the experimentally infected host.

Fortunately for Koch, he was working with a relatively large and easily cultured type of microorganism. His postulates are applicable only if the microorganisms associated with a particular disease can be isolated and grown in an artificial environment, and for some types of microbes, this is much more difficult.

Because of Koch's work, the etiological agents for many important human diseases were identified in rapid succession between the years of 1876 and 1898. By 1900, the microorganisms responsible for major human diseases including cholera, diphtheria, leprosy, plague, tetanus, tuberculosis and typhoid had been identified. The period of years between 1857 and 1914 is sometimes referred to as the "Golden Age of Microbiology", because rapid advancements and discoveries made during this period led to the establishment of microbiology as a science. During their search for disease causing agents, Koch and other microbiologists made important contributions to the techniques and materials used in the culture of microorganisms. Some of these important developments involved the following people.

Richard J. Petri - developed the **Petri dish** in which microbial cultures could be grown and manipulated.

Fanny Hesse - developed the use of **agar** as a solidifying agent for microbiological media.

Hans Christian Gram - developed the **Gram stain**, a stain technique that could be used to separate two major groups of disease causing bacteria.

Immunization – Using microorganisms in disease prevention

In science, many important discoveries are made accidentally, and such was the case with Pasteur's discovery of immunization. In 1880**, Louis Pasteur** had isolated the bacteria responsible for causing chicken cholera (organisms similar to the ***Vibrio cholerae*** causing cholera in humans). He inoculated a number of animals with a bacteria culture (*Pasteurella multocida*) prepared in his laboratory, but the animals did not die. Upon reviewing his records, Pasteur found that the experimental animals had been inoculated with a culture several weeks old. He reasoned that this old culture would be weakened (**attenuated**) and might therefore be unable to cause disease. He arranged to repeat the experiment, and this time inoculated the subject animals with a fresh culture. Fortunately he also chose to inoculate a new group of animals with the same culture. The original animals again did not develop disease symptoms, but the newly inoculated animals did. As expected, they all developed cholera and died.

Pasteur knew that the experimental animals had all been inoculated with the same type of disease causing bacteria. Since they all came from a similar source, he suspected that exposure to the attenuated culture had somehow made the first ones resistant to the disease. He repeated the experiments and eventually concluded that this was indeed the case. Bacteria that were killed or attenuated could be used to prevent disease. Pasteur called his attenuated cultures **vaccines**, and thus gave credit to an earlier investigator named Edward Jenner.

In 1796, **Edward Jenner** (a British Physician) reported the use of material scraped from the skin of an individual infected with cowpox to immunize a child against smallpox. Jenner had noticed that dairymaids (young women responsible for milking cows) frequently contracted cowpox, a relatively mild disease, but were resistant to smallpox. Since both of these diseases are caused by viruses, there was no way for Jenner to see the disease causing agents, but his method was successful. He called his technique **vaccination** (vacca = cow).

The Magic Bullet

By the early 1900s, physicians knew that microorganisms could cause disease, and under certain circumstances could be used to prevent disease, but they did not know how to cure disease. Many strange and sometimes brutal practices had been used in attempts to cure disease, but most were useless and some were dangerous (for example the ingestion of precious metals - gold and silver). What was needed was a substance that could be taken into the body and would somehow seek out and kill the pathogenic microorganisms without harming the patient, i.e., a "magic bullet".

A German physician by the name of **Paul Ehrlich** searched for a "magic bullet", and in around 1910 developed the first effective cure for a bacterial disease. The drug he developed was called **salvarsan**, and was an arsenic compound that was effective against syphilis. A short time later (1928), **Alexander Fleming**, a Scottish physician, discovered penicillin. He had noticed that a mold growing on one of his culture plates inhibited the growth of bacteria there, and eventually isolated the substance responsible. Penicillin was among the first antibiotics to be used in the treatment of disease. Although Salvarsan was a synthetic compound, and penicillin is produced by mold, many compounds now used to treat disease in humans (and other animals) are made by bacteria. Thus bacteria play a critical role in health and disease (cause, prevention and cure).

During the 20th century, microbiology has expanded and increased in importance. Immunology, virology and molecular genetics (recombinant DNA technology) have arisen as branches of microbiology. New discoveries in microbiology may lead to better methods for food and fuel production as well as environmental remediation that will become more and more critical as the human population continues to expand. Or perhaps microbes will eventually force humans to live in balance with the natural world.

Microbiologists believe we have identified and classified somewhere between 1 and 10% of the bacteria residing on this planet. The human microbiome project is providing new and startling information about our dependence on our normal microbiota (sometimes called our norma flora). Humans are causing damage to their environments, both externally and internally, and we are just beginning to understand the significance. We have a great deal to learn, and time may be limited.