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MY MICROBLOG

JV'n SHIVI SAXENA

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Chapter-1
Alexander Fleming

Introduction

Alexander Fleming, fully Sir Fleming , (born Transfiguration , 1881, Lochfield Farm, Darvel, Ayrshire, Scotland—died March 11, 1955, London, England), Scottish bacteriologist best known for his discovery of penicillin. Fleming had a genius for technical ingenuity and original observation. His work on wound infection and lysozyme, an antibacterial enzyme found in tears and saliva, guaranteed him an area within the history of bacteriology. But it had been his discovery of penicillin in 1928, which started the antibiotic revolution that sealed his lasting reputation. Fleming was recognized for that achievement in 1945, when he received the Nobel Prize for Physiology or Medicine, alongside Australian pathologist Howard Walter Florey and German-born British biochemist Ernst Boris Chain, both of whom isolated and purified penicillin.

Education and Early Career

Fleming was the seventh of eight children of a Scottish hill farmer (third of 4 children from the farmer's second wife). His country upbringing in southwestern Scotland sharpened his capacities for observation and appreciation of the wildlife at an early age. He began his elementary schooling at Loudoun Moor then moved on to a bigger school at Darvel before enrolling in Kilmarnock Academy in 1894. In 1895 he moved to London to measure together with his elder brother Thomas (who worked as an oculist) and completed his basic education at Regent Street Polytechnic.
After working as a London clerk, Fleming began his medical studies at St. Mary's Hospital school of medicine in 1901, funded by a scholarship and a legacy from his uncle. There he won the 1908 trophy as top medico at the University of London. Initially he planned to become a surgeon, but a short lived position within the laboratories of the Inoculation Department at St. Mary's Hospital convinced him that his future lay within the new field of bacteriology. There he came under the influence of bacteriologist and immunologist Sir Almroth Edward Wright, whose ideas of vaccine therapy appeared to offer a revolutionary direction in medical treatment.Between 1909 and 1914 Fleming established a successful private practice as a venereologist, and in 1915 he married Sarah Marion McElroy, an Irish nurse. Fleming's son, Robert, born in 1924, followed his father into medicine. Fleming was one among the primary doctors in Britain to administer arsphenamine (Salvarsan), a drug effective against syphilis that was discovered by German scientist Ehrlich in 1910. During war I, Fleming had a commission within the Royal Army Medical Corps and worked as a bacteriologist studying wound infections during a laboratory that Wright had found out during a hospital housed during a casino in Boulogne, France. There he demonstrated that the utilization of

strong antiseptics on wounds did more harm than good and recommended that the injuries simply be kept clean with a light saline . Fleming returned to St. Mary's after the war and was promoted to assistant director of the Inoculation Department. Years later, in 1946, he succeeded Wright as principal of the department, which was renamed the Wright-Fleming Institute.
In November 1921 Fleming discovered lysozyme, an enzyme present in body fluids like saliva and tears that features a mild antiseptic effect. That was the primary of his major discoveries. It happened when he had a chilly and a drop of his nasal mucus fell onto a culture plate of bacteria. Realizing that his mucus may need an impact on bacterial growth, he mixed the mucus into the culture and a couple of weeks later saw signs of the bacteria's having been dissolved. Fleming's study of lysozyme, which he considered his best work as a scientist, was a big contribution to the understanding of how the body fights infection. Unfortunately, lysozyme had no effect on the most-pathogenic bacteria.

Discovery of Penicillin

On September 3, 1928, shortly after his appointment as professor of bacteriology, Fleming noticed that a culture plate of Staphylococcus aureus he had been performing on had become contaminated by a fungus. A mold, later identified as Penicillium notatum (now classified as P. chrysogenum), had inhibited the expansion of the bacteria. He initially called the substance "mould juice" then "penicillin," after the mold that produced it. Fleming decided to research further, because he thought that he had found an enzyme stronger than lysozyme. In fact, it had been not an enzyme but an antibiotic—one of the primary to be discovered. By the time Fleming had established that, he was curious about penicillin for itself. considerably the lone researcher with an eye fixed for the weird , Fleming had the liberty to pursue anything that interested him. Although that approach was ideal for taking advantage of an opportunity observation, the therapeutic development of penicillin required multidisciplinary teamwork. Fleming, working with two young researchers, did not stabilize and purify penicillin. However, he did means that penicillin had clinical potential, both as a topical antiseptic and as an inject able antibiotic, if it might be isolated and purified. Penicillin eventually came into use during war II because the results of the work of a team of scientists led by Florey at the University of Oxford. Though Florey, his coworker Ernst Chain, and Fleming shared the 1945 Nobel prize , their relationship was clouded due to the difficulty of who should gain the foremost credit for penicillin. Fleming's role was emphasized by the press due to the romance of his chance discovery and his greater willingness to talk to journalists. Fleming was knighted in 1944. In 1949 his first wife, who had changed her name to Sareen, died. In 1953, two years before his death, Fleming married Greek microbiologist Amalia Coutsouris-Voureka, who had been involved within the Greek resistance movement during war II and had been Fleming's colleague since 1946, when she enrolled at St. Mary's Hospital on a scholarship. For the last decade of his life, Fleming was feted universally for his discovery of penicillin and acted as a world ambassador for medicine and science. Initially a shy uncommunicative man and a poor lecturer, he blossomed under the eye he received, becoming one among the world's best-known scientists.

Chapter-2
Antonie van Leeuwenhoek

INTRODUCTION

Antonie van Leeuwenhoek, (born october 24, 1632, Delft, Netherlands—died August 26, 1723, Delft), Dutch microscopist who was the primary to watch bacteria and protozoa. His researches on lower animals refuted the doctrine of abiogenesis , and his observations helped lay the foundations for the sciences of bacteriology and protozoology.

Early Life and Career

At a young age, Leeuwenhoek lost his biological father. His mother later married painter Jacob Jansz Molijn. When his stepfather died in 1648, Leeuwenhoek was sent to Amsterdam to become an apprentice to a linen draper. Returning to Delft when he was 20, he established himself as a draper and haberdasher. He was married in 1654 to a draper's daughter. By the time of her death, in 1666, the couple had five children, just one of whom survived childhood. Leeuwenhoek remarried in 1671; his second wife died in 1694.
In 1660 Leeuwenhoek obtained an edge as chamberlain to the sheriffs of Delft. His income was thus secure, and it had been thereafter that he began to devote much of his time to his hobby of grinding lenses and using them to review tiny objects.

Discovery of Microscopic Life

Leeuwenhoek made microscopes consisting of one high-quality lens of very short focal length; at the time, such simple microscopes were preferable to the light microscope, which increased the matter of aberration. Although Leeuwenhoek's studies lacked the organization of formal research project, his powers of careful observation enabled him to form discoveries of fundamental importance. In 1674 he likely observed protozoa for the primary time and a number of other years' later bacteria. Those "very little animalcules" he was ready to isolate from different sources, like rainwater, pond and ground water, and therefore the human mouth and intestine. He also calculated their sizes. In 1677 he described for the primary time the spermatozoa from insects, dogs, and man, though Stephen Hamm probably was a co discoverer. Leeuwenhoek studied the

structure of the optic lens, striations in muscles, the mouthparts of insects, and therefore the spectrum line of plants and discovered parthenogenesis in aphids. In 1680 he noticed that yeasts contains minute globular particles. He extended Marcello Malpighi's demonstration in 1660 of the blood capillaries by giving the primary accurate description of red blood cells. In his observations on rotifers in 1702, Leeuwenhoek remarked that altogether falling rain, carried from gutters into water-butts, animalcules are to be found; which altogether sorts of water, standing within the outdoors, animalcules can happen. For these animalcules are often carried over by the wind, alongside the bits of dust floating within the air.

The Royal Society and Later Discoveries

A friend of Leeuwenhoek put him in-tuned with the Royal Society of England, to which he communicated by means of informal letters from 1673 until 1723 most of his discoveries and to which he was elected a fellow in 1680. His discoveries were for the foremost part made public within the society's Philosophical Transactions. The primary representation of bacteria is to be found during a drawing by Leeuwenhoek therein publication in 1683.
His researches on the life histories of varied low sorts of animal life were con to the doctrine that they might be produced spontaneously or bred from corruption. Thus, he showed that the weevils of granaries (in his time commonly alleged to be bred from wheat also as in it) are really grubs hatched from eggs deposited by winged insects. His letter on the flea, during which he not only described its structure but traced out the entire history of its metamorphosis, is of great interest, not such a lot for the exactness of his observations as for an illustration of his opposition to the abiogenesis of the many lower organisms, like "this minute and despised creature." Some theorists asserted that the flea was produced from sand, others from dust or the likes of , but Leeuwenhoek proved that it bred within the regular way of winged insects.Leeuwenhoek carefully studied the history of the ant and was the primary to point out that what had been commonly reputed to be ants' eggs were really their pupae, containing the right insect nearly ready for emergence, which truth eggs were much smaller and gave origin to maggots, or larvae. He argued that the ocean mussel and other shellfish weren't generated out of sand found at the seashore or mud within the beds of rivers at low tide but from spawn, by the regular course of generation. He maintained an equivalent to be true of the freshwater clam , whose embryos he examined so carefully that he was ready to observe how they were consumed by "animalcules," many of which, consistent with his description, must have included ciliates in conjugation, flagellates, and therefore the Vorticella. Similarly, he investigated the generation of eels, which were at that point alleged to be produced from dew without the standard process of generation. The dramatic nature of his discoveries made him famous, and he was visited by many notables—including Peter I (the Great) of Russia, James II of England, and Frederick II (the Great) of Prussia.Leeuwenhoek carefully studied the history of the ant and was the primary to point out that what had been commonly reputed to be ants' eggs were really their pupae, containing the right insect nearly ready for emergence, which truth eggs were much smaller and gave origin to maggots, or larvae. He argued that the ocean mussel and other shellfish weren't generated out of sand found at the seashore or mud within the beds of rivers at low tide but from spawn, by the regular course of generation. He maintained an equivalent to be

true of the freshwater clam , whose embryos he examined so carefully that he was ready to observe how they were consumed by “animalcules,” many of which, consistent with his description, must have included ciliates in conjugation, flagellates, and therefore the Vorticella. Similarly, he investigated the generation of eels, which were at that point alleged to be produced from dew without the standard process of generation. The dramatic nature of his discoveries made him famous, and he was visited by many notables—including Peter I (the Great) of Russia, James II of England, and Frederick II (the Great) of Prussia.Methods of Microscopy Leeuwenhoek’s methods of microscopy, which he kept secret, remain something of a mystery. During his lifetime he ground quite 500 lenses, most of which were very small—some no larger than a pinhead—and usually mounted them between two thin brass plates, riveted together. an outsized sample of these lenses, bequeathed to the Royal Society , were found to possess magnifying powers within the range of fifty to, at the foremost , 300 times. so as to watch phenomena as small as bacteria, Leeuwenhoek must have employed some sort of oblique illumination, or other technique, for enhancing the effectiveness of the lens, but this method he wouldn't reveal. Leeuwenhoek continued his work almost to the top of his long lifetime of 90 years.

Contributions to Scientific Literature

Leeuwenhoek’s contributions to the Philosophical Transactions amounted to 375 and people to the Memoirs of the Paris Academy of Sciences to 27. Two collections of his works appeared during his life, one in Dutch (1685–1718) and therefore the other in Latin (1715–22); a variety was translated by Samuel Hoole, The Select Works of A. van Leeuwenhoek (1798–1807).

Fast Facts: Anton van Leeuwenhoek
Known For: Improvements to the microscope, discovery of bacteria, discovery of sperm, descriptions of all manner of microscopic cell structures (plant and animal), yeasts, molds, and more
Also Known As: Antonie Van Leeuwenhoek, Antony Van Leeuwenhoek
Born: Oct. 24, 1632 in Delft, Holland
Died: Aug. 30, 1723 in in Delft, Holland
Education: Only basic education
Published Works: " Arcana naturœ detecta," 1695, a set of his letters sent to the Royal Society of London, translated into Latin for the scientific community
Awards: Member of the Royal Society of London
Spouse(s): Barbara de Mey (m.1654–1666), Cornelia Swalmius (m. 1671–1694)
Children: Maria
Notable Quote: "My work...was not pursued so as to realize the praise I now enjoy, but chiefly from a craving after knowledge."

Chapter- 3
Louis Pasteur

Introduction-:

Louis Pasteur, (born December 27, 1822, Dole, France—died September 28, 1895, Saint-Cloud), French chemist and microbiologist who was one among the foremost important founders of medical microbiology. Pasteur's contributions to science, technology, and medicine are nearly without precedent. He pioneered the study of molecular asymmetry; discovered that microorganisms cause fermentation and disease; originated the method of pasteurization; saved the beer, wine, and silk industries in France; and developed vaccines against anthrax and rabies. Pasteur's academic positions were numerous, and his scientific accomplishments earned him France's highest decoration, the Legion of Honour, also as election to the Académie des Sciences and lots of other distinctions. Today there are some 30 institutes and a powerful number of hospitals, schools, buildings, and streets that bear his name—a set of honours bestowed on few scientists.

Early Education

Pasteur's father, Jean-Joseph Pasteur, was a tanner and a sergeant major decorated with the Legion of Honour during the Napoleonic Wars . This fact probably instilled within the younger Pasteur the strong patriotism that later was a defining element of his character. Pasteur was a mean student in his early years, but he was gifted in drawing and painting. His pastels and portraits of his parents and friends, made when he was 15, were later kept within the museum of the Pasteur Institute in Paris. After attending grade school in Arbois, where his family had moved, and lyceum in nearby Besançon, he earned his Bachelor of Arts degree (1840) and bachelor of science degree (1842) at the Royal College of Besançon.

Research Career

In 1843 Pasteur was admitted to the École Normale Supérieure (a teachers' college in Paris), where he attended lectures by French chemist Jean-Baptiste-André Dumas and have become Dumas's teaching assistant. Pasteur obtained his master of science degree in 1845 then acquired a complicated degree in physical sciences. He later earned his doctorate in sciences in 1847. Pasteur was appointed professor of physics at the Dijon Lycée (secondary school) in 1848 but shortly thereafter accepted an edge as professor of chemistry at the University of Strasbourg. On May 29,

1849, he married Marie Laurent, the daughter of the rector of the university. The couple had five children; however, only two survived childhood.

Molecular asymmetry

Soon after graduating from the École Normale Supérieure, Pasteur became puzzled by the invention of the German chemist Eilhardt Mitscherlich, who had shown that tartrates and paratartrates behaved differently toward polarized light: tartrates rotated the plane of polarized light, whereas paratartrates didn't . This was unusual because the compounds displayed identical chemical properties. Pasteur noted that the tartrate crystals exhibited asymmetric forms that corresponded to their optical asymmetry. He made the surprising observation that crystalline paratartrate consisted of a mix of crystals during a right-handed configuration. However, when these crystals were separated manually, he found that they exhibited right and left asymmetry. In other words, a balanced mixture of both right and left crystals was optically inactive. Thus, Pasteur discovered the existence of molecular asymmetry, the inspiration of stereochemistry, because it was revealed by optical activity. Over the course of subsequent 10 years, Pasteur further investigated the power of organic substances to rotate the plane of polarized light. He also studied the connection that existed between crystal structure and molecular configuration. His studies convinced him that asymmetry was one among the elemental characteristics of living matter.

Germ theory of fermentation

In 1854 Pasteur was appointed professor of chemistry and dean of the science faculty at the University of Lille. While performing at Lille, he was asked to assist solve problems associated with alcohol production at an area distillery, and thus he began a series of studies on alcoholic fermentation. His work on these problems led to his involvement in tackling a spread of other practical and economic problems involving fermentation. His efforts proved successful in unraveling most of those problems, and new theoretical implications emerged from his work. Pasteur investigated a broad range of aspects of fermentation, including the assembly of compounds like carboxylic acid that are liable for the souring of milk. He also studied butanoic acid fermentation. In 1857 Pasteur left Lille and returned to Paris, having been appointed manager and director of scientific studies at the École Normale Supérieure. that very same year he presented experimental evidence for the participation of living organisms altogether fermentative processes and showed that a selected organism was related to each particular fermentation. This evidence gave rise to the scientific theory of fermentation.

Pasteur Effect

The realization that specific organisms were involved in fermentation was further supported by Pasteur's studies of butanoic acid fermentation. These studies led Pasteur to the unexpected discovery that the fermentation process might be arrested by passing air (that is, oxygen) through the fermenting fluid, a process known today because the Pasteur effect. He concluded that this was thanks to the presence of a life-form that would function only within the absence of oxygen. This led to his introduction of the terms aerobic and anaerobic to designate organisms that sleep in the

presence or absence of oxygen, respectively. He further proposed that the phenomena occurring during putrefaction were thanks to specific germs that function under anaerobic conditions.

Pasteurization

Pasteur readily applied his knowledge of microbes and fermentation to the wine and beer industries in France, effectively saving the industries from collapse thanks to problems related to production and with contamination that occurred during export. In 1863, at the request of the emperor of France, Napoleon III , Pasteur studied wine contamination and showed it to be caused by microbes. to stop contamination, Pasteur used an easy procedure: he heated the wine to 50–60 °C (120–140 °F), a process now known universally as pasteurization. Today pasteurization is seldom used for wines that enjoy aging, since it kills the organisms that contribute to the aging process, but it's applied to several foods and beverages, particularly milk.
Following Pasteur's success with wine, he focused his studies on beer. By developing practical techniques for the control of beer fermentation, he was ready to provide a rational methodology for the brewing industry. He also devised a way for the manufacturing of beer that prevented deterioration of the merchandise during long periods of transport on ships.

Spontaneous generation

Fermentation and putrefaction were often perceived as being spontaneous phenomena, a perception stemming from the traditional belief that life could generate spontaneously. During the 18th century the talk was pursued by English naturalist and Roman Catholic divine John Turberville Needham and therefore the French naturalist Georges-Louis Leclerc, count de Buffon. While both supported the thought of abiogenesis , Italian abbot and physiologist Spallanzani maintained that life could never spontaneously generate from dead matter. In 1859, the year English naturalist Darwin published his On the Origin of Species; Pasteur decided to settle this dispute. He was convinced that his scientific theory couldn't be firmly substantiated as long as belief in abiogenesis persisted. Pasteur attacked the matter by employing a simple procedure . He showed that beef stock might be sterilized by boiling it during a "swan-neck" flask, which features a long bending neck that traps dust particles and other contaminants before they reach the body of the flask. However, if the broth was boiled and therefore the neck of the flask was broken off following boiling, the broth, being reexposed to air, eventually became cloudy, indicating microbial contamination. These experiments proved that there was no abiogenesis , since the boiled broth, if never reexposed to air, remained sterile. This not only settled the philosophical problem of the origin of life at the time but also placed on land the new science of bacteriology, which relied on proven techniques of sterilization and aseptic manipulation.

Work with silkworms

In 1862 Pasteur was elected to the Académie des Sciences, and therefore the following year he was appointed professor of geology, physics, and chemistry at the École des Beaux-Arts (School of Fine Arts). Shortly after this, Pasteur turned his attention to France's silkworm crisis. within the middle of the 19th century, a mysterious disease had attacked French silkworm nurseries. Silkworm eggs could not be produced in France, and that they couldn't be imported from other countries, since the disease had spread everywhere Europe and had invaded the Caucasus region of Eurasia, also as China and Japan. By 1865 the silkworm industry was almost completely ruined in France and, to a lesser extent, within the remainder of western Europe. Pasteur knew virtually nothing about

silkworms, but, upon the request of his former mentor Dumas, Pasteur took charge of the matter , accepting the challenge and seizing the chance to find out more about infectious diseases. He soon became an expert silkworm breeder and identified the organisms that caused the silkworm disease. After five years of research, he succeeded in saving the silk industry through a way that enabled the preservation of healthy silkworm eggs and prevented their contamination by the disease-causing organisms. Within a few of years, this method was recognized throughout Europe; it's still used today in silk-producing countries.
In 1867 Pasteur resigned from his administrative duties at the École Normale Supérieure and was appointed professor of chemistry at the Sorbonne, a university in Paris. Although he was partially paralyzed (left hemiplegia) in 1868, he continued his research. For Pasteur, the study of silkworms constituted an initiation into the matter of infectious diseases, and it had been then that he first became conscious of the complexities of infectious processes. Accustomed as he was to the constancy and accuracy of laboratory procedures, he was puzzled by the variability of animal life, which he had come to acknowledge through his observation that individual silkworms differed in their response to disease counting on physiological and environmental factors. By investigating these problems, Pasteur developed certain practices of epidemiology that served him well a couple of years later when he addressed animal and human diseases.

Vaccine development

In the early 1870s Pasteur had already acquired considerable renown and respect in France, and in 1873 he was elected as an associate member of the Académie de Médecine. Nonetheless, the medical establishment was reluctant to simply accept his scientific theory of disease, primarily because it originated from a chemist. However, during subsequent decade, Pasteur developed the general principle of vaccination and contributed to the inspiration of immunology.

Pasteur's first important discovery within the study of vaccination came in 1879 and anxious a disease called chicken cholera. (Today the bacteria that cause the disease are classified within the genus Pasteurella.) Pasteur said, "Chance only favours the prepared mind," and it had been chance observation through which he discovered that cultures of chicken cholera lost their pathogenicity and retained "attenuated" pathogenic characteristics over the course of the many generations. He inoculated chickens with the attenuated form and demonstrated that the chickens were immune to the fully virulent strain. From then on, Pasteur directed all his experimental work toward the matter of immunization and applied this principle to several other diseases.
Pasteur began investigating anthrax in 1879. At that point an anthrax epidemic in France and in another parts of Europe had killed an outsized number of sheep, and therefore the disease was attacking humans also . German physician Koch announced the isolation of the anthrax bacillus, which Pasteur confirmed. Koch and Pasteur independently provided definitive experimental evidence that the anthrax bacillus was indeed liable for the infection. This firmly established the scientific theory of disease, which then emerged because the fundamental concept underlying medical microbiology.

Pasteur wanted to use the principle of vaccination to anthrax. He prepared attenuated cultures of the bacillus after determining the conditions that led to the organism's loss of virulence. Within the spring of 1881 he obtained support, mostly from farmers, to conduct a large-scale public experiment of anthrax immunization. The experiment happened in Pouilly-le-Fort, located on the southern outskirts of Paris. Pasteur immunized 70 livestock , and therefore the experiment was an entire success. The vaccination procedure involved two inoculations at intervals of 12 days with

vaccines of various potencies. One vaccine, from a low-virulence culture, was given to half the sheep and was followed by a second vaccine from a more virulent culture than the primary. Fortnight after these initial inoculations, both the vaccinated and control sheep were inoculated with a virulent strain of anthrax. Within a couple of days all the control sheep died, whereas all the vaccinated animals survived. This convinced many of us that Pasteur's work was indeed valid. Following the success of the anthrax vaccination experiment, Pasteur focused on the microbial origins of disease. His investigations of animals infected by pathogenic microbes and his studies of the microbial mechanisms that cause harmful physiological effects in animals made him a pioneer within the field of infectious pathology. it's often said that English surgeon Jenner discovered vaccination which Pasteur invented vaccines. Indeed, almost 90 years after Jenner initiated immunization against smallpox, Pasteur developed another vaccine—the first vaccine against rabies. He had decided to attack the matter of rabies in 1882, the year of his acceptance into the Académie Française. Rabies was a dreaded and horrible disease that had fascinated popular imagination for hundreds of years due to its mysterious origin and therefore the fear it generated. Conquering it might be Pasteur's final endeavour.
Pasteur suspected that the agent that caused rabies was a microbe (the agent was later discovered to be an epidemic , a nonliving entity). it had been too small to be seen under Pasteur's microscope, then experimentation with the disease demanded the event of entirely new methodologies. Pasteur chose to conduct his experiments using rabbits and transmitted the infective agent from animal to animal by intracerebral inoculations until he obtained a stable preparation. so as to attenuate the invisible agent, he desiccated the spinal cords of infected animals until the preparation became almost nonvirulent. He realized later that, rather than creating an attenuated sort of the agent, his treatment had actually neutralized it. (Pasteur perceived the neutralizing effect as a killing effect on the agent, since he suspected that the agent was a living organism.) Thus, rather unknowingly, he had produced, rather than attenuated live microorganisms, a neutralized agent and opened the way for the event of a second class of vaccines, referred to as inactivated vaccines.
On July 6, 1885, Pasteur vaccinated Joseph Meister, a nine-year-old boy who had been bitten by a rabid dog. The vaccine was so successful that it brought immediate glory and fame to Pasteur. Many other bite victims throughout the planet were subsequently saved by Pasteur's vaccine, and therefore the era of medicine had begun. a world fund-raising drive was launched to create the Pasteur Institute in Paris, the inauguration of which happened on November 14, 1888.

Implications of Pasteur's Work

The theoretical implications and practical importance of Pasteur's work were immense. Pasteur once said, "There are not any such things as pure and applied science; there are only science and therefore the application of science." Thus, once he established the theoretical basis of a given process, he investigated ways to further develop industrial applications. (As a result, he deposited variety of patents.)
However, Pasteur didn't have enough time to explore all the sensible aspects of his numerous theories. one among the foremost important theoretical implications of his later research, which emerged from his attenuation procedure for vaccines, is that the concept that virulence isn't a

continuing attribute but a variable property—a property which will be lost and later recovered. Virulence might be decreased, but Pasteur suspected that it might be increased also . He believed that increased virulence was what gave rise to epidemics. In Pasteur , Free Lance of Science (1950), American microbiologist René Dubos quoted Pasteur:

Thus, virulence appears during a new light which can be disturbing for the longer term of humanity unless nature, in its long evolution, has already had the occasions to supply all possible contagious diseases—a impossible assumption.

What is a microorganism that's innocuous to man or to a given animal species? It's a living being which doesn't possess the capacity to multiply in our body or within the body of the animal. But nothing proves that if an equivalent microorganism should chance to return into contact with another of the thousands of animal species within the Creation, it'd invade it and render it sick. Its virulence might increase by repeated passages through that species, and might eventually affect man or domesticated animals. Thus could be caused replacement virulence and new contagions. i'm much inclined to believe that such mechanisms would explain how smallpox, syphilis, plague, yellow jack , etc. have happen within the course of your time , and the way certain great epidemics appear once during a while.

Pasteur was the primary to acknowledge variability in virulence. Today this idea remains relevant to the study of communicable disease, especially with reference to understanding the emergence of diseases like bovine spongiform encephalopathy (BSE), severe acute respiratory syndrome (SARS), and purchased immunodeficiency syndrome (AIDS).

After Pasteur's 70th birthday, which was acknowledged by an outsized but solemn celebration at the Sorbonne that was attended by several prominent scientists, including British surgeon Lister , Pasteur's health continued to deteriorate. His paralysis worsened, and he died on September 28, 1895. He was buried within the cathedral of Notre-Dame de Paris, but his remains were transferred to a Neo-Byzantine crypt at the Pasteur Institute in 1896.

During Pasteur's career, he touched on many problems, but an easy description of his achievements doesn't do justice to the intensity and fullness of his life. He never accepted defeat, and he always tried to convince skeptics, though his impatience and intolerance were notorious when he believed that truth was on his side. Throughout his life he was an immensely effective observer and readily integrated relevant observations into conceptual schemes.

Chapter-4
Karl landstainer

Lived 1868 — 1943.
Karl Landsteiner revolutionized medicine when, in 1900-1901, he identified three major human blood types: A, B, and O, which led to safe blood transfusions and many lives saved. He also suggested the utilization of blood types to help in police enquiries. Thirty years later, he was awarded the Nobel prize in Physiology or Medicine for his discovery of the ABO blood type system. Working with Erwin Popper, Landsteiner proved polio is an communicable disease spread by an epidemic and, with Viktor Mucha, he showed that dark-field microscopy might be wont to diagnose syphilis.
Later in his career, Landsteiner and his colleagues made another life-saving scientific breakthrough once they discovered the Rh factor in blood.

Beginnings
Karl Landsteiner, the sole child during a prosperous Jewish family, was born on Flag Day, 1868 in Baden bei Wien, a spa town 16 miles south of Austria's capital city, Vienna.
Karl's father was Leopold Landsteiner, a doctor of law and editor-in-chief of Die Presse, a top quality newspaper. Leopold died when Karl was seven.Karl was very on the brink of his mother, Fanny, née Hess. Following her death in 1908, Karl, who was by then 40 years old, had her cast taken and, rather morbidly, kept it on his bedroom wall for the remainder of his life.From the age of 12, Karl was educated at the State Grammar School in Linz, a city 115 miles west of Vienna.
He entered the University of Vienna's school of medicine in 1885, age 17. In 1891, just before his twenty-third birthday he obtained his degree: Doctor of internal medicine.The year before he graduated, Landsteiner and his mother converted from Judaism to Romanism , responding perhaps to rising levels of anti-Semitism in Vienna at the time.

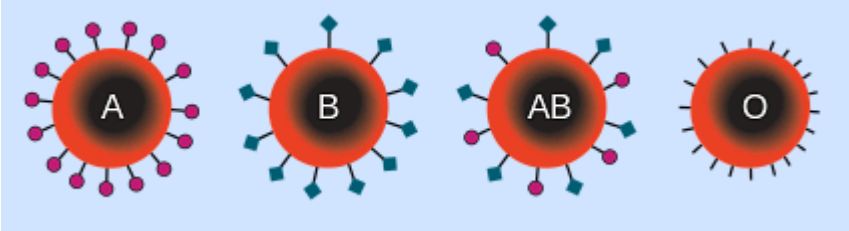
Postdoctoral Work
While working for his medical degree, Landsteiner became fascinated by chemistry. Instead of pursue a career as a practicing physician; he decided to figure in research. He spent several years working in Germany and Switzerland learning state-of-the-art laboratory techniques with a number of the most important names in chemistry, including Emil Fischer.He kept in-tuned with medicine by working within the University of Vienna's surgical clinic.

Discovery of Blood Groups

On January 1, 1896, age 27, Landsteiner was appointed assistant in Vienna's Institute of Hygiene. Working there, he developed a passion for immunology and particularly the immune reaction of serum, the straw liquid that carries all the substances in blood round the body.

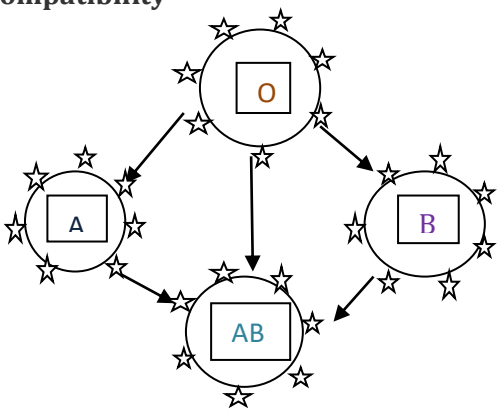
The following year he transferred to the University of Vienna's Pathology and Anatomy department. He continued pursuing his immunology and serum work with enormous energy, performing thousands of autopsies and publishing 75 research papers – over 50 of those papers concerned serology, the study of body fluids like serum.In 1900, age 32, Landsteiner studied the consequences of blending red blood cells from one person with serum from another. He found the mixtures often produced clumping of the red cells – or hemagglutination in medical jargon. Other scientists believed hemagglutination was a disease-linked response, but Landsteiner's blood cells and serum were taken from healthy people.In Landsteiner's experiments some mixes of blood and serum led to no hemagglutination, while others did. He deduced the existence of three blood groups, which he called A, B, and C. Today these are referred to as A, B, and O. In 1902, Landsteiner's colleagues followed advice he gave them and identified a fourth type – the AB group.Landsteiner found that mixing blood from people of an equivalent blood type resulted in no clumping. Mixing blood from people with different blood groups could end in hemagglutination, which he identified as an immune reaction. Previously, scientists had abandoned using blood transfusions because they might cause severe illnesses or death. Landsteiner showed blood transfusions failed because incompatible blood groups were mixed.

Blood Groups



The four major blood groups. The red cells have different antigens protruding from their cell membranes. Antigens are molecules that produce an immune reaction. In red blood cells the antigens are sugars or proteins. When the system detects an unfamiliar antigen it attacks it – this will happen if someone receives a transfusion of an incompatible blood type.

Blood Group Compatibility



Antigens determine that blood is often transfused safely between people with an equivalent blood type. Moreover, O-group blood is safe for everybody, while A-group and B-group are safe for people with AB blood. AB-group blood is merely safe for people with AB blood.Dr. Reuben Ottenberg administered the primary clinical transfusion supported Landsteiner's research in 1907 at Mt. Sinai Hospital, New York.Blood transfusions were wont to save wounded soldiers' lives during war 1. Transfusions became practical after several different scientists in 1914 discovered that sodium

citrate stabilizes blood for several days, allowing it to be stored. In 1917, Captain Oswald Robertson of the U.S. Army, attached to British Army on the Western Front, began the world's first bank.

Fighting Crime
In 1903, together with his colleague Max Richter, Landsteiner showed how a blood type might be determined from a sample of dried blood, and suggested this might be utilized in crime-fighting to narrow the list of possible suspects.

Syphilis
The sexually transmitted disease syphilis provided Landsteiner together with his next major breakthrough. In 1905, he was the primary scientist to get the means to infect monkeys with syphilis, opening the door to further research. the subsequent year, working with Viktor Mucha, Landsteiner found dark-field microscopy might be wont to diagnose syphilis by detecting the bacterium that causes the disease, Treponema pallidum.

Polio

In 1908, Landsteiner was appointed chief of pathology at Vienna's Wilhelmina Hospital. Within the same year, working together with his assistant Erwin Popper, he proved that poliomyelitis is an communicable disease, successfully infecting monkeys with it, and identifying the disease-agent as an epidemic.

Haptens – Landsteiner's most vital Work
In 1919, age 53, Landsteiner left Vienna for Holland's capital city The Hague. There, at the Catholic St. Joannes de Deo hospital, he administered what he considered his best and most vital work. He said that nearly anyone could have discovered the blood type system – it had been just good luck that it fell to him. However, his discovery in 1921 of haptens was different. He believed few others could have done it at the time.Landsteiner gave the name haptens to very small molecules that, alone, produce no immune reaction during a body. However, if they're attached to an outsized carrier like a protein, they provoke an immune reaction.Landsteiner pioneered the utilization of synthetic haptens, adding small molecules to proteins to impress immune responses. In doing so, he made a useful contribution to system chemistry research

More Blood Groups
In the spring of 1923, Landsteiner arrived together with his wife and son in ny City. He had been offered and accepted full membership – the very best rank – at the Rockefeller Institute for Medical Research.In 1927, working with Philip Levine, Landsteiner discovered new blood groups: M, N, and P.

The Rh factor
In 1937, with Alexander S. Wiener, Landsteiner discovered the Rh factor, now called the rhesus factor . They published their add 1940.Compared with the ABO blood group system blood system, the Rh system has different antigens on the surfaces of red blood cells.Most people's blood is Rh-positive blood type . If an Rh-negative blood type mother is pregnant with an Rh-positive blood type baby, the mother's system may attack the growing baby. The identification and understanding

of the Rhesus factor led to methods preventing the mother producing antibodies against the baby in her womb.Identification of the rhesus factor also explained the very fact that blood transfusions between people with ABO compatible groups sometimes led to problems. After the Rh factor's discovery a plus or sign was added to a person's ABO blood type. For instance, people whose blood was once classed as A, are now classed as A+ or A-.

Honors

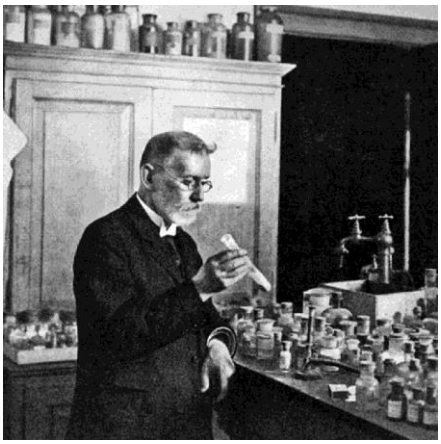
Landsteiner received variety of highly prestigious awards and honors including:

- 1911 – Chevalier of the French Legion of Honor.
- 1926 – Aronson Prize for achievements in microbiology and immunology.
- 1930 – Nobel prize in Physiology or Medicine.
- 1930 – Ehrlich Medal.
- 1932 – Elected member, National Academy of Sciences.
- 1933 – Dutch Red Cross Medal.
- 1938 – Cameron Prize.
- 1941 – Elected Honorary foreign member, Royal Society.
- 1946 – (Posthumously) Albert Lasker Clinical Medical Research Award.

Personal Details and therefore the End

In 1916, age 48, Landsteiner married 36-year-old Leopoldine Helene Wlasto, a member of the Orthodox Church. Before they married she converted to her husband's Roman Catholic faith.
In 1917, Leopoldine gave birth to their only child, Ernst Karl, who became a surgeon. Ernst was born in Vienna during war 1, a time when people were going hungry. Worried for his family's health, Landsteiner bought a goat to supply them with milk and personally went foraging for herbs; Landsteiner and his family were living within the village of Purkersdorf about 10 miles from the middle of Vienna, which Landsteiner believed was a far better environment for a family.
In 1929, six years after they arrived in America, Landsteiner and his family became americans .
In 1937, Landsteiner sued an American publisher for including him within the book Who's Who in American Jewry, arguing that he wasn't a Jew, having converted to Romanism almost five decades previously. Landsteiner said, "…it is going to be detrimental to me to stress publicly the faith of my ancestors."He lost the lawsuit.
Landsteiner was a superb pianist and a secret devotee of detective novels – he was embarrassed about his, because he felt someone together with his background should read more cultured literature.Landsteiner became an Emeritus Member of the Rockefeller Institute in 1939, but refused to retire, and continued working with great energy. Latterly, his wife Leopoldine was diagnosed with thyroid cancer and Landsteiner worked all the harder, checking out how to save lots of her life.
Karl Landsteiner died age 75 of coronary failure on June 26, 1943. Two days earlier, working in his Rockefeller Institute laboratory, he had suffered an attack. His wife died on Christmas six months later. Survived by their son Ernst, they were buried side by side within the Prospect Hill Cemetery, Nantucket, Massachusetts.

Chapter-5
Paul Eharlich



Paul Ehrlich, (born March 14, 1854, Strehlen, Silesia, Prussia died Aug. 20, 1915, Bad Homburg vor der Höhe, Ger.), German scientist known for his pioneering add hematology, immunology, and chemotherapy and for his discovery of the primary effective treatment for syphilis. He received jointly with Metchnikoff the Nobel Prize for Physiology or Medicine in 1908.

Early Life

Ehrlich was born into a Jewish family prominent in business and industry. Although he lacked formal training in experimental chemistry and applied bacteriology, he was introduced by his mother's cousin, the pathologist Carl Weigert, to the technique of staining cells with chemical dyes, a procedure wont to view cells under the microscope. As a medico at several universities, including Breslau, Strasbourg, Freiburg, and Leipzig, Ehrlich continued to experiment with cellular staining. The selective action of those dyes on differing types of cells suggested to Ehrlich that chemical reactions were occurring in cells which these reactions formed the idea of cellular processes. From this concept he reasoned that chemical agents might be wont to heal diseased cells or to destroy infectious agents, a theory that revolutionized medical diagnostics and therapeutics.

After receiving his medical degree from the University of Leipzig in 1878, Ehrlich was offered an edge as head physician at the distinguished Charité Hospital in Berlin. There he developed a replacement staining technique to spot the tuberculosis bacillus (a bacterium) that had been discovered by the German bacteriologist Koch. Ehrlich also differentiated the various sorts of blood cells of the body and thereby laid the inspiration for the sector of hematology.

While developing new methods for the staining of live tissue, Ehrlich discovered the uses of methylthionine chloride within the treatment of nervous disorders. In other diagnostic advances, he traced a selected reaction within the urine of typhoid patients, tested various medications for reducing or removing fever, and made valuable suggestions for the treatment of eye diseases. Of the 37 scientific contributions that he published between 1879 and 1885, Ehrlich considered the last because the most important: Das Sauerstoff-Bedürfniss des Organismus (1885; "The Requirement of the Organism for Oxygen"). In it he established that oxygen consumption varies with differing types of tissue which these variations constitute a measure of the intensity of important cell processes.

In 1883 Ehrlich married Hedwig Pinkus, with whom he had two daughters

Immunity and therefore the Side-Chain Theory

A bout with tuberculosis forced Ehrlich to interrupt his work and seek a cure in Egypt. When he returned to Berlin in 1889, the disease had been permanently arrested. After working for a few

time during a tiny and primitive private laboratory, he transferred to Koch's Institute for Infectious Diseases, where he targeting the matter of immunity. little or no was known at the time about the precise manner during which bacteria cause disease, and even less was known about the body's defenses against infection or how these immune defenses might be enhanced. The hypothesis Ehrlich developed to elucidate immunological phenomena was the side-chain theory, which described how antibodies—the protective proteins produced by the immune system—are formed and the way they react with other substances. Delivered to the Royal Society in 1900, this theory was supported an understanding of the way during which a cell was thought to soak up and assimilate nutrients. Ehrlich postulated that every cell has on its surface a series of side chains, or receptors, that function by attaching to certain food molecules. While all sides chain interacts with a selected nutrient—in an equivalent manner as a key fits into a lock—it can also interact with other molecules, like disease-causing toxins (antigens) produced by an infective agent . When a toxin binds to a side chain, the interaction is irreversible and blocks subsequent binding and uptake of nutrients. The body then tries to overwhelm the obstruction by producing an excellent number of replacement side chains—so many who they can't fit on the surface of the cell and instead are secreted into the circulation. consistent with Ehrlich's theory, these circulating side chains are the antibodies, which are all gauged to and ready to neutralize the disease-causing toxin then remain within the circulation, thus immunizing the individual against subsequent invasions by the infective agent .

This much-debated hypothesis, although ultimately proven to be incorrect in many particulars, had a profound influence on Ehrlich's later work and on the work of his successors. Thus Ehrlich was ready to show experimentally that rabbits subjected to a slow and measured increase of toxic matter were ready to survive 5,000 times the fatal dose. Within the end, he established precise quantitative patterns of immunity. These findings assumed great importance in 1890, when he met Emil von Behring, who had succeeded in creating an antitoxin against diphtheria. Behring had tried to organize a serum that would be utilized in clinical practice, but it had been only by adopting Ehrlich's technique of using the blood of live horses that the preparation of a serum of optimum antitoxic effectiveness became possible. Ehrlich developed how of measuring the effectiveness of serums that was soon adopted everywhere the planet for the standardization of diphtheria serum. He also demonstrated, in 1892, that antibodies are passed in breast milk from mother to newborn. On the idea of those achievements, Ehrlich was made director of a government-supported institute near Berlin, which was transferred to Frankfurt am Main in 1899 because the Royal Institute for Experimental Therapy. No restrictions of any kind were placed upon the direction of his research. While this corresponded to Ehrlich's own talents and inclinations, it didn't please Behring, who endeavoured to possess his colleague concentrate on immunology and serum therapy. The strained relationship between the 2 men was exacerbated by personality differences. Ehrlich, utterly indifferent to monetary rewards, had no ambition to become an industrialist like Behring; he was content to hold out his research.

He had by then recognized the restrictions of serum therapy. Many infectious disorders, especially those caused by protozoa instead of bacteria, did not answer serum treatment. the popularity of this fact marks the birth of chemotherapy. Ehrlich started experimenting with the identification and synthesis of drugs , not necessarily found in nature, that would kill parasites or inhibit their growth without damaging the organism. He began with trypanosomes, a species of protozoa that he unsuccessfully attempted to regulate by means of pitch dyes. There followed compounds of arsenic and benzene; other compounds proved to be too toxic. Rather than declaring himself vanquished by these difficulties, Ehrlich turned his attention to the spirochete Treponema pallidum, the causal organism of syphilis.Syphilis Studies

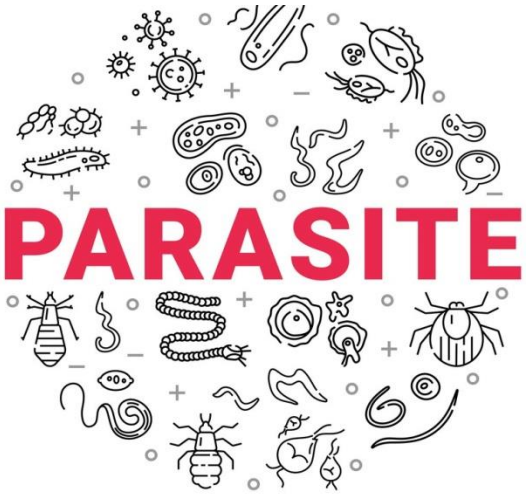
Ehrlich had at this time several institutes at his disposal as well as sizable research funds. He also had a staff of highly competent collaborators; in fact, his colleague Hata Sahachirō contributed

much to his eventual success in combating syphilis. His preparation 606, later called Salvarsan, was extraordinarily effective and harmless despite its large arsenic content. The first tests, announced in the spring of 1910, proved to be surprisingly successful in the treatment of a whole spectrum of diseases; in the case of yaws, a tropical disease akin to syphilis, a single injection was sufficient. It seemed as if a "magic bullet," to use a favourite expression of Ehrlich's, had been found.

The devastation wrought by syphilis provoked worldwide demand for a new weapon against the disease. Ehrlich, however, would not yet release his discovery for general use, believing as he did that the usual few hundred clinical tests did not suffice in the case of an arsenic preparation, the injection of which required special precautions. In an unheard-of transaction, the manufacturer with whom Ehrlich had collaborated closely, Farbwerke-Hoechst, released a total of 65,000 units gratis to physicians all over the globe. Although harmful side effects remained nominal in number, some envious competitors did not hesitate to attack Ehrlich. The most libelous among them was given a jail sentence.

The greatest distinction bestowed on Ehrlich by the Prussian state was the title "Wirklicher Geheimer Rat," or Privy Councillor, with the predicate of "Exzellenz." Along with numerous other honours, Ehrlich was presented with honorary doctorates by the Universities of Oxford, Chicago, and Athens and an honorary citizenship by Frankfurt am Main, where the institute he founded still bears his name. Having suffered a first stroke in December 1914, Ehrlich succumbed to a second stroke in August of the following year. In its obituary the London *Times* acknowledged Ehrlich's achievement in opening new doors into the unknown, saying, "The whole world is in his debt."

Chapter-6
Introduction in Parasitology



Definition of Parasitology

Parasitology is a discipline handling the biology of animal parasites, ecology of parasitism with emphasis on parasite--host and parasite--environmental interactions. Human parasitology or Medical parasitology is restricted in studying those parasites that are of importance in medicine Protozoology, Helminthology and Entomology

Parasitology is typically within the scope of preventive medicine and therefore the foundation of clinical parasitic diseases.

Why can we study parasitology?

1. Parasites provide unique samples of biological phenomena not found in free-living organisms
•Medical importance
•Veterinary importance
•Economic importance
Medical Importance of Parasites

2. Humans are hosts to over 100 species of parasites.
•Many of those parasites are causative agents of major public health problems of the planet.

Medical Importance of Parasites

Ten major tropical diseases (UNDP/World bank/TDR, 2000)
•Malaria
•Shistosomaiasis
•Filariasis(Lymphatic filariasis and Onchocerciasis)
•Leishmaniasis
•Trypanosomiasis (African trypanosomiasis and changes disease)
•Leprosy

•Tuberculosis
•Dengue fever

Major human parasites (WHO 1999)
•Estimated World Prevalence of the main Parasitic Infection of Human:
–Malaria 300-500 million
–Schistosomiasis 200 million
–Lymphatic filariasis 120 million
–Onchocerciasis 85 million
–Leishmaniasis 12 million
–Trypanosoma cruzi(South America) 18 million
–Ascaris infection 1300 million
–Hookworm infection 1300 million
–Amoebiasis 60 million
–Trichuriasis 900 million
–Gardiasis 200 million

What sorts of living organisms are parasitic?

Parasites occur in two of the five kingdoms of living organisms.
What are the 5 kingdoms?

KINGDOM ANIMALIA contains 32 phyla.Parasites of importance are concentrated in 3 phyla.

•PHYLUM PLATYHELMINTHES –Class Trematoda; Cestoda
•PHYLUM NEMATODA –Class Nematoda
•PHYLUM ARTHROPODA –Class Insecta……

KINGDOM PROTISTA-contains the single-celled protozoans.

Evolution of parasitism:
Understanding start with basic concept of symbiosis
•Symbiosis was first coined by the German de Bary in 1879 -to mean "living together". it had been originally coined to ask all cases where dissimilar organisms or species (e.g., heterogenetic associations) live together in an intimate association

Interactions of Symbionts
In order to facilitate our understanding of symbiosis, 3 subordinate categories of symbiotic relationships are indicated. They are: commensalism (including phoresis), mutualism, and parasitism.
Symbiosis (cont.)

1. Mutualism

•this occurs when each member of the association benefits the opposite

•the mutualsare metabolically hooked in to each other. Sometimes, one cannot survive within the absence of the opposite

eg. The flagellate cannot survive outside the termite

2. Commensalism

•Commensalism means ―eating at an equivalent table‖ and in many commensalisticrelationships one organism (the commensal) is feeding on food that wasn't consumed by the host

•Commensalism occurs when one member of the associating pair, usually the smaller, receives all the benefit and therefore the other member is neither benefited nor harmed

•To carry --phoresis

•Example: Remora fish related to sharks feeds on leftover food

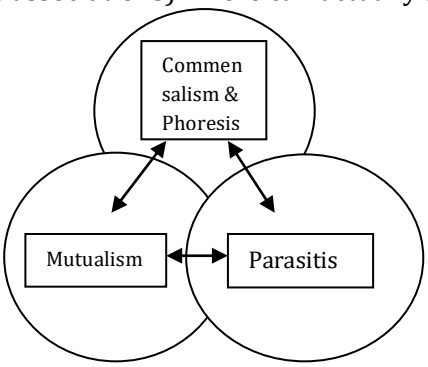
3. Parasitism

•A parasitos(para: beside; sitos: grain or food): Original meaning from the Greek may be a relationship during which "one eats at another's table or lives at another's expense."

•Parasitismis a relationship during which one among the participants, the parasite, either harms its host (the part that got harmed) or in some sense lives at the expense of the host.

The true nature of parasitism involves an ecological relationship between the parasite and its host. A parasite is metabolically hooked in to its host.

•The categories of symbiosis are man-made constructs introduced primarily for convenience (they allow us to categorize natural symbiosis associations). There can actually be overlap between various categories.



Overlap between the major categories f symbiosis

Kinds of Parasites

•An organism that doesn't absolutely depend upon the parasitic way of life, but is capable of adapting thereto if placed in such a relationship is understood as a facultative parasite

•If an organism is totally hooked in to the host during a segment or all of its life cycle the parasite is understood as an obligatory parasite

•Parasites that live within the body of their host (intestinal tract, liver, etc.) are called endoparasites

•Parasites that are attached to the outer surfaces of their hosts are called ectoparasites

Chapter-7
Introduction in Mycology

- The term "mycology" springs from Greek word "mykes" meaning mushroom. Therefore mycology is that the study of fungi.
- The ability of fungi to invade plant and tissue was observed in early 19th century but the primary documented
- Animal infection by any fungus was made by Bassi, who in 1835 studied the muscardine disease of silkworm and proved the that the infection was caused by a fungus Beauveria bassiana.
- In 1910 Raymond Sabouraud published his book Les Teignes, which was a comprehensive study of dermatophytic fungi. he's also considered father of medical mycology.

Importance of fungi: Fungi inhabit almost every niche within the environment and humans are exposed to those organisms in various fields of life.

Beneficial Effects of Fungi:

1. Decomposition - nutrient and carbon recycling.
2. Biosynthetic factories. The fermentation property is employed for the economic production of alcohols, fats, citric,oxalic and gluconic acids.
3. Important sources of antibiotics, like Penicillin.
4. Model organisms for biochemical and genetic studies. Eg: Neurospora crassa
5. Saccharomyces cerviciae is extensively utilized in recombinant deoxyribonucleic acid technology, which incorporates the hepatitis B Vaccine.
6. Some fungi are edible (mushrooms).
7. Yeasts provide nutritional supplements like vitamins and cofactors.
8. Penicillium is employed to flavour Roquefort and Camembert cheeses.
9. Ergot produced by ergot contains medically important alkaloids that help in inducing uterine contractions, controlling bleeding and treating migraine.
10. Fungi (Leptolegnia caudate and Aphanomyces laevis) are wont to trap mosquito larvae in paddy fields and thus help in malaria control

Harmful Effects of Fungi:

1. Destruction of food, lumber, paper, and cloth.
2. Animal and human diseases, including allergies.
3. Toxins produced by poisonous mushrooms and within food (Mycetism and

Mycotoxicosis).
4. Plant diseases.
5. Spoilage of agriculture produce like vegetables and cereals within the godown.
6. Damage the products like magnetic tapes and disks, glass lenses, marble statues, bones and wax.

General properties of fungi:

1. they're eukaryotic; cells contain membrane bound cell organelles including nuclei, mitochondria, Golgi body, endoplasmic reticulum, lysosomes etc. They also exhibit mitosis.
2. Have ergosterols in their membranes and possesses 80S ribosomes.
3. Have a rigid cell membrane and are therefore non-motile, a feature that separates them from animals. All fungi possess cell membrane made from chitin.
4. Are chemoheterotrophs (require organic compounds for both carbon and energy sources) and fungi lack chlorophyll and are therefore not autotrophic.
5. Fungi are osmiotrophic; they obtain their nutrients by absorption.
6. They obtain nutrients as saprophytes (live off of decaying matter) or as parasites (live off of living matter).
7. All fungi require water and oxygen and there are not any obligate anaerobes.
8. Typically reproduce asexually and/or sexually by producing spores.
9. They grow either reproductively by budding or non-reproductively by hyphal tip elongation.
10. Food storage is usually within the sort of lipids and glycogen.

Classification of fungi:

Fungi were initially classified with plants and were a topic of interest for botanists; hence the influence of botany is often seen on their classification. In 1969 R.H Whittaker classified all living organisms into five kingdoms namely Monera, Protista, Fungi, Plantae and Animalia. Traditionally the classification proceeds during this fashion: Kingdom - Subkingdom - Phyla/phylum - Subphyla - Class - Order - Family - Genus- Species This classification is just too complicated to be dealt here. There are alternate and more practical approaches, one supported amphimixis and therefore the other supported morphology of the thallus (vegetative structure).

Based on Sexual reproduction:

1. Zygomycetes: which produce through production of zygospores.
2. Ascomycetes: which produce endogenous spores called ascospores in cells called asci.
3. Basidiomycetes: which produce exogenous spores called basidiospores in cells called basidia.
4. Deuteromycetes (Fungi imperfecti): fungi that aren't known to supply any sexual spores (ascospores or basidiospores). this is often a heterogeneous group of fungi where no amphimixis has yet been demonstrated.

Based on Morphology:
1. Moulds (Molds): Filamentous fungi Eg: Aspergillus sps, Trichophyton rubrum
2. Yeasts: Single celled cells that bud Eg: Cryptococcus neoformans, Saccharomyces cerviciae
3. Yeast like: almost like yeasts but produce pseudohyphae Eg: Candida albicans
4. Dimorphic: Fungi existing in two different morphological forms at two different environmental conditions. They exist as yeasts in tissue and in vitro at 37oC and as moulds in their natural habitat and in vitro at temperature. Eg: Histoplasma capsulatum, Blastomyces dermatidis, Paracoccidiodes

brasiliensis, Coccidioides immitis

Morphology of fungi:

Fungi exist in two fundamental forms; the filamentous (hyphal) and single celled budding forms (yeast). But, for the classification sake they're studied as moulds, yeasts, yeast like and dimorphic fungi. All fungi have typical eukaryotic morphology. They need rigid cell membrane composed of chitin, which can be layered with mannans, glucans and other polysaccharides in association with polypeptides. Some lower fungi possess cellulose in their cell membrane. Some fungi like Cryptococcus and yeast sort of Histoplasma capsulatum possess polysaccharide capsules that help them to evade phagocytosis. Inner to the cell membrane is that the cell wall that's a typical bi-layered membrane additionally to the presence of sterols. Fungal membranes possess ergosterol in contrast to cholesterol found in mammalian cells. The cytoplasm consists of varied organelles like mitochondria, Golgi body, ribosomes, endoplasmic reticulum, lysosomes, microtubules and a membrane enclosed nucleus. a singular property of nuclear membrane is that it persists throughout the metaphase of mitosis unlike in plant and animal cells where it dissolves and re-forms. The nucleus possesses paired chromosomes.

Moulds:

The thallus of mould is formed of hyphae, which are cylindrical tube like structures that elongates by growth at tips. A mass of hyphae is understood as mycelium. it's the hypha that's liable for the filamentous nature of mould. The hyphae could also be branched or unbranched. they'll be septate or aseptate. Hyphae usually have cross walls that divide them into numerous cells. These cross walls, called septa have small pores through which cytoplasm is continuous throughout the hyphae. Therefore all hyphal fungi tend to be coenocytic (multinucleate). With exception of zygomycetes (Rhizopus, Mucor), all moulds are septate. Non-septate hyphae are considered to be more primitive because if a hyphal strand is broken the whole strand dies. When a septate hyphal strand is broken , the pores between adjacent compartments are often plugged, thus preventing death of the entire hyphal strand. Mycelium is of three kinds:

1. Vegetative mycelium is people who penetrates the surface of the medium and absorbs nutrients.
2. Aerial mycelium are people who grow above the agar surface
3. Fertile mycelium is aerial hyphae that bear reproductive structures like conidia or sporangia.

Since hypha is that the structural unit of mould, the mycelium imparts colour, texture and topography to the colony. Those fungi that possess melanin pigments in their cell membrane are called phaeoid or dematiaceous and their colonies are coloured grey, black or olive. Examples are species of Bipolaris, Cladosporium, Exophiala, Fonsecaea, Phialophora and Wangiella Those hyphae that do not possess any pigment in their cell membrane are called hyaline. Hyphae may have some specialized structure or appearance that aid in identification. a number of these are:

a) Spiral hyphae: These are spirally coiled hyphae commonly seen in Trichophyton mentagrophytes.

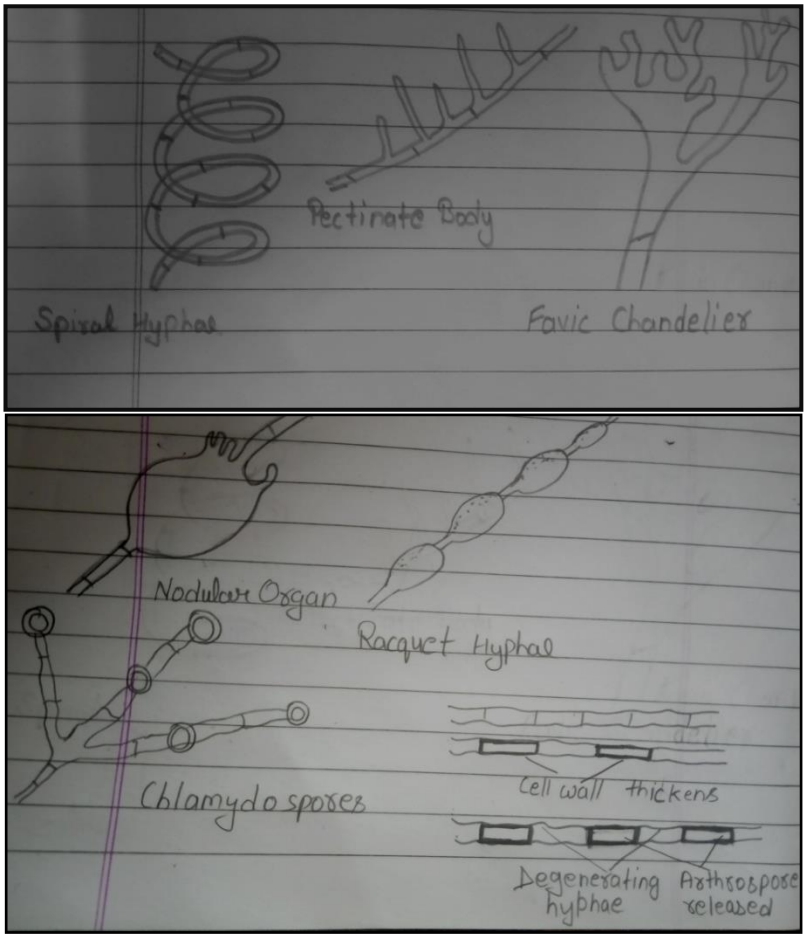
b) Pectinate body: These are short, unilateral projections from the hyphae that resemble a broken comb.Commonly seen in Microsporum audouinii.

c) Favic chandelier: These are the group of hyphal tips that collectively resemble a chandelier or the antlers of the deer (antler hyphae). They occur in Trichophyton schoenleinii and Trichophyton violaceum.

d) Nodular organ: this is often an enlargement within the mycelium that consists of closely twisted hyphae. Often seen in Trichophyton mentagrophytes and Microsporum canis.

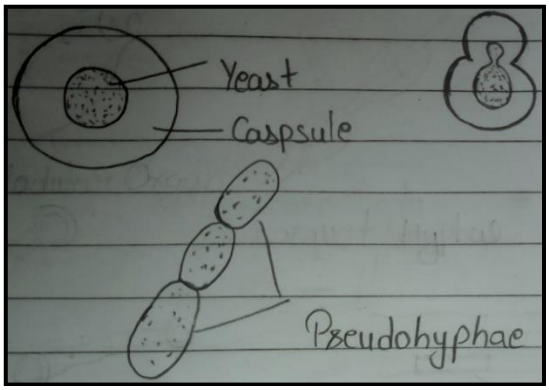
e) Racquet hyphae: there's regular enlargement of 1 end of every segment with the opposing end

remaining thin. Seen in Epidermophyton floccosum, Trichophyton mentagrophytes.
f) Rhizoides: These are the basis like structures seen in portions of vegetative hyphae in some members of
zygomycetes.
g) There are structures within the hyphae, which arise out of modification of one cell and transform into thick walled resting cells. Chlamydospore (or chlamydoconidia), which are produced by Trichophyton schoenleinii and Trichophyton verrucosum are thick walled cells that are larger than other cells and arranged singly or in groups. In some fungi like Trichosporon beigeilli and Coccidioides immitis some alternating cells become thick walled and subsequently the intervening cells disintegrate leaving arthrospores (or arthroconidia).



Yeasts:
Yeasts are unicellular spherical to ellipsoid cells. They reproduce by budding, which end in blastospore (blastoconidia) formation. In some cases, because the cells buds the buds fail to detach and elongate thus forming a sequence of elongated hyphae like filament called pseudohyphae. This property is seen in Candia albicans. An equivalent species even have the power to supply true hypha, which is seen as germ tube. The difference between the 2 is that there's a constriction in psueudohyphae at the purpose of budding, while the germ tube has no constriction. Some yeast like Cryptococcus and therefore the yeast sort of Blastomyces dermatatidis produce polysaccharide
capsule. Capsules are often demonstrated by negative staining methods using India ink or Nigrosin.

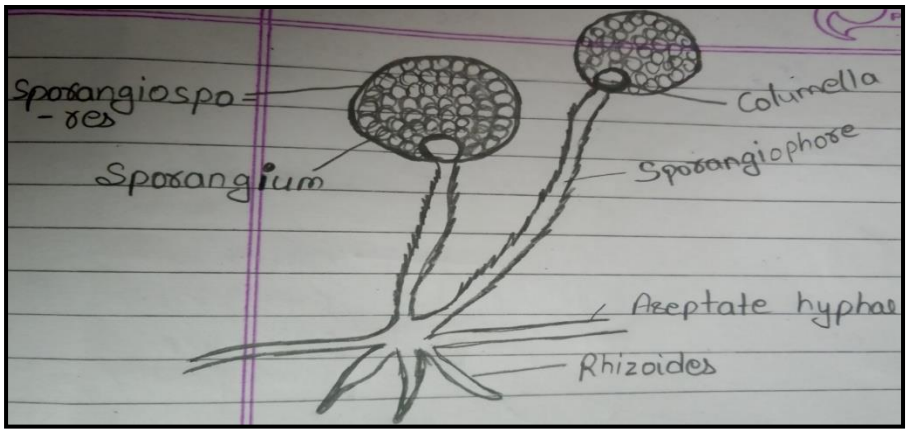
The capsule itself is often stained by Meyer Mucicarmine stain. Some yeasts are pigmented. Rhodotorula sps produces pink colonies thanks to carotenoid pigments while some yeasts like Phaeoannellomyces werneckii and Piedraia hortae are dematiaceous, producing brown to olivaceous colonies. True yeasts like Saccharomyces cerviciae don't produce pseudohyphae. Yeast-like fungi could also be basidiomycetes, like Cryptococcus neoformans or ascomycetes like Candida albicans .



Reproduction in fungi:

Fungi reproduce by asexual, sexual and parasexual means. agamogenesis is that the commonest mode in most fungi with fungi participating in sexual mode only under certain circumstances. The shape of fungus undergoing agamogenesis is understood as anamorph (or imperfect stage) and when an equivalent fungus is undergoing amphimixis , the shape is claimed to be teleomorph (or perfect stage). The entire fungus, including both the forms is referred as holomorph.
(Taxonomically, the teleomorph or the holomorph is employed, but practically it's more convenient to use the anamorph.)

Asexual reproduction:
Asexual propagules are termed either spores or conidia counting on their mode of production. Asexual spores are produced following mitosis where as sexual spores are produced following meiosis. The asexual spores of zygomycetes, which are referred to as sporangiospores form within sac like structure referred to as sporangia. The sporangiospores result from the mitotic cleavage of cytoplasm within the sporangium. The sporangia are borne on special hyphae called sporangiophore. This endogenous process of spore formation within a sac is understood as sporogenesis. Conidia arise either by budding off conidiogenous hyphae or by differentiation of preformed hyphae. These develop following mitosis of a parent nucleus and are formed in any manner except involving cytoplasmic cleavage. This exogenous process is understood as conidiogenesis, a process that happens both in yeasts and moulds. Conidia are borne on specialised structures called conidiophore. Conidia production could also be blastic or thallic. In blastic development the conidium begins to enlarge and a septum is made . Here the conidium originates from a part of parent. In thallic mode of development the conidium is differentiated by a septum before its differentiation. Thus the conidium results from the conversion of entire parent cell into the conidium.
The cell that provides rise to a conidium is named a conidiogenous cell. Conidiophores are specialised hyphae that bear conidia or conidiogenous cells. In many cases conidiogenous cells are referred as phialides.



Sexual Reproduction:

Sexual propagules are produced by the fusion of two nuclei that then generally undergo meiosis. The primary step in sexual methods of reproduction involves plasmogamy (cytoplasmic fusion of two cells). The second step is karyogamy (fusion of two compatible nuclei), leading to production of diploid or zygote nucleus. this is often followed by genetic recombination and meiosis. The resulting four haploid spores are said to be sexual spores, e.g. zygospores, ascospores and basidiospores. If a sexual spore is produced only by fusion of a nucleus of 1 mating type with a nucleus of another mating type (+ and - strains), the fungus is claimed to be heterothallic. In contrast, homothallic moulds produce sexual spores following the fusion of two nuclei from an equivalent strain. For amphimixis to occur, two compatible isolates are required. Zygospores, which are the sexual spores of zygomycetes are round, thick walled reproductive structures that result from the union of two gamentagia. Ascomycetes produce sexual spores called ascospores during a special sac like cell referred to as ascus. In basidiomycetes the basidiospores are released from basidium, which is that the terminal cell of hyphae.

Parasexual reproduction:

Parasexual reproduction, first seen in Aspergillus is understood to occur in basidiomycetes, ascomycetes and deuteromycetes. The method involves genetic recombination without the need of specific sexual structures.

Importance of Spores:
A. Biological
1) Allows for dissemination
2) Allows for copy
3) Allows the fungus to maneuver to new food source.
4) Allows fungus to survive periods of adversity.
5) Means of introducing new genetic combinations into a population

B. Practical
1) Rapid identification (also helps with classification)

2) Source of inocula for human infection
3) Source of inocula for contamination

ZYGOMYCETES

Commonly referred to as bread moulds, these are fast growing, terrestrial, largely saprophytic fungi. Hyphae are coenocytic and mostly aseptate. Asexual spores include chlamydoconidia, conidia and sporangiospores. Sporangiophores could also be simple or branched. amphimixis involves producing a thick-walled sexual resting spore called a zygospore. Medically important orders and genera include:

1. Entomophthorales: Conidiobolus and Basidiobolus are involved in subcutaneous zygomycosis
2. Mucorales: Rhizopus, Mucor, Rhizomucor, Absidia and Cunninghamella are involved in subcutaneous and systemic zygomycosis (formerly called Mucormycosis)

BASIDIOMYCETES

They exist as saprobes and parasites of plants. Hyphae are dikaryotic and may often be distinguished by the presence of clamp connections over the septa. amphimixis is by the formation of exogenous
basidiospores, typically four, on a basidium. Occasional species produce conidia but most are sterile.
Genera of medical importance include:

1. Teleomorph of Cryptococcus neoformans, which is Filobasidiella neoformans
2. Agents of basidiomycosis like Coprinus and Schizophyllium
3. Food poisoning by Aminita, Lepiota, Coprinus and Psilocybe etc.

ASCOMYCETES

They exist as saprophytes and parasites of plants. Hyphae are septate with simple septal pores. agamogenesis is by conidia. amphimixis is by the formation of endogenous ascospores, typically eight, in an ascus. Medically important genera include the:
1. Teleomorphs of known pathogenic fungi e.g. Arthroderma (of Trichophyton and Microsporum), Ajellomyces dermatitidis (of Blastomyces dermatitidis), Pseudallescheria boydii (of Scedosporium apiospermum)
2. Agents of mycetoma, like Leptosphaeria
3. Agents of black piedra, like Piedraia hortae.

DEUTEROMYCETES

Deuteromycetes also are referred to as Deuteromycota due to absence of sexually reproducing forms (teleomorph or perfect stage). As their teleomorph still be discovered, they might be classified among the previous categories, until then this remains a man-made and heterogeneous group.
There are three classes of Deuteromycota .

1. Blastomycetes: These include asexual budding sorts of Cryptococcus, Candida, Torulopsis and Rhodotorula. counting on the presence of melanin in their cell walls, they'll be non-dematiaceous or dematiaceous.
2. Hyphomycetes: a category of mycelial moulds which reproduce asexually by conidia on hyphae. Hyphae are septate. This class contains the bulk of medically important fungi. Dematiaceous hyphomycetes are those conidial fungi that produce dark brown, green-black, or black colonies and are the causative agents of phaeohyphomycosis. Hyaline hyphomycetes include those conidial fungi, which aren't darkly pigmented; colonies could also be colourless or brightly coloured. These include the agents of hyalohyphomycosis, aspergillosis, dermatophytosis and therefore the dimorphic pathogens, like Histoplasma capsulatum.
3. Coelomycetes: These produce acervuli, which are tightly bound mats of hyphae on which conidia are produced.

- **Pathogenesis of fungal diseases (Mycoses):**

Most fungi are saprophytic or parasitic to plants and are adapted to their natural environment. Infection in humans may be an accident, occurring only conditions are favourable. apart from few fungi like the dimorphic fungi that cause systemic mycoses and dermatophytes, which are primary pathogens, the remainder are only opportunistic pathogens. physical body may be a hostile environment and offers great resistance to fungal invasion. Most fungi are saprophytic and their enzymatic pathways function more efficiently at the redox potential of non-living substrates than at the relatively more reduced state of living metabolizing tissue. Some fungi like Candida and Malasezzia have adapted to human environment and exist as commensals. The complex interplay between fungal virulence factors and host defence factors will determine if a mycosis will cause a disease. Infection depends on inoculum size and therefore the general immunity of the host.

Fungal Pathogenicity (virulence factors):

• Ability to stick to host cells by way of cell membrane glycoproteins
• Production capsules allowing them to resist phagocytosis
• Production of a cytokine called GM-CSF by Candida albicans that suppress the assembly of complement.
• Ability to accumulate iron from red blood cells as in Candida albicans
• Ability to wreck host by secreting enzymes like keratinase, elastase, collagenase
• Ability to resist killing by phagocytes as in dimorphic fungi
• Ability to secrete mycotoxins
• Having a singular enzymatic capacity
• Exhibiting thermal dimorphism
• Ability to dam the cell-mediated immune defences of the host.
• Surface hydrophobicity

Host defence factors:

• Physical barriers, like skin and mucus membranes
• The carboxylic acid content of the skin

• The pH of the skin, mucosal surfaces and body fluids
• Epithelial cell turnover
• Normal flora
• Chemical barriers, like secretions, serum factors
• Most fungi are mesophilic and can't grow at 37oC.
• Natural Effector Cells (polymorphonuclear leucocytes) and therefore the Professional Phagocytes (monocytes and macrophages)

Factors predisposing to fungal infections:

• Prolonged antibiotic therapy
• Underlying disease (HIV infection, cancer, diabetes, etc.)
• Age
• Surgical procedures
• Immunosuppressive drugs
• Irradiation therapy
• Indwelling catheters
• Obesity
• Drug addiction
• Transplants
• Occupation

Immunity to fungal infections:

Mechanism of immunity to fungal infections are often innate or acquired. The non-specific immunity includes the physical barriers offered by skin and mucus membranes along side their secretions and normal flora. The pH, blood heat and serum factors along side phagocytic cells play a crucial part in providing non-specific immunity. albeit body mounts both humoral and cell mediated immunity, it's the latter that's the mainstay of host defence.

Cell mediated immunity:
Immunity is provided non-specifically be effector cells (polymorphonuclear leucocytes) and professional phagocytes (monocytes and macrophages) and specifically by T lymphocytes. The phagocytes are vital in defence against Candia, Aspergillus and Zygomycetes as is evidenced by their severity in granulomatous diseases, myeloperoxidase deficiency and cytotoxic chemotherapy. Expression of T-cell-mediated immunity to fungi includes:
• delayed-type hypersensitivity
• contact allergy
• chronic granulomatous reactions

Humoral immunity:

Even though antibodies are produced against many fungi, their role in protection isn't very clear. However, antibodies help in clearing fungal pathogens through opsonisation, which is vital against Candida and Cryptococcus. Another component of humoral immunity is that the complement, which may act as opsonins and should even cause damage to their cells through complement activation. Antibodies are important to fungal serodiagnosis.

Hypersensitivity:

As results of dermatophyte infection some fungus-free skin lesions of variable morphology occur elsewhere on the body, which are thought to result from hypersensitivity to the fungus. These reactions are called "id reaction". These reactions also are seen n Candida infections. An inflamed boggy lesion of the scalp called the kerion may result from a robust immune response to the dermatophyte. Granulomas thanks to intracellular fungi represent delayed hypersensivities. Many fungi are significant allergens to humans, the allergens being spores, conidia, hyphae and other fungal products. On inhalation they'll produce allergic pulmonary diseases like allergic bronchopulmonary aspergillosis, thresher's lung , maple bark stripper's lung, asthma etc, which can be Type I or III hypersensitivity.

Fungal Diseases (Mycoses):

Mycoses are often conveniently studied as:

1. Superficial mycoses

I. Superficial phaeohyphomycosis
II. Tinea versicolor
III. Black piedra
IV. White piedra

2. Cutaneous mycoses

I. Dermatophytosis
II. Dermatomycosis

3. Subcutaneous mycoses

a. Chromoblastomycosis
b. Rhinosporidiasis
c. Mycetoma
d. Sporotrichosis
e. subcutaneous phaeohyphomycosis
f. Lobomycosis

4. Systemic (deep) mycoses

I. Blastomycosis
II. Histoplasmosis
III. Coccidioidomycosis
IV. Paracoccidioidomycosis

5. Opportunistic mycoses

I. Candidiasis
II. Cryptococcosis
III. Aspergillosis

6. Other mycoses

I. Otomycosis

II. Occulomycosis

7. Fungal allergies

8. Mycetism and mycotoxicosis

Laboratory diagnosis of mycoses:

Specimen collection: specimen collection depends on the location affected. Different specimens include hair, skin scrapings, nail clippings, sputum, blood, CSF, urine, corneal scraping, discharge or pus from lesions and biopsy.
• All specimens must be transported to the laboratory with none delay to stop bacterial overgrowth. Just in case of delay specimens except skin specimen, blood and CSF could also be refrigerated for a brief period.
• Infected hairs could also be plucked using forceps. Those hairs that fluoresce under Wood's lamp could also be selectively plucked. Hairs could also be collected in sterilized paper envelopes.
• Surface of the skin must be disinfected with spirit before specimen collection. The advancing fringe of the lesion is scraped with the assistance of a blunt forceps and picked up in sterilized paper envelopes.
• Discoloured or hyperkeratotic areas of nail could also be scraped or diseased manicure could also be collected in sterilized paper envelopes.
• Specimens from mucus membranes (oral) must be collected by gentle scraping and transported to laboratory in sterile tube containing saline. Swabs could also be collected from vagina.
• Corneal scrapings could also be collected employing a fine needle and inoculated at bedside.
• Pus could also be collected by aspiration; use of cotton swabs may give false positive microscopic results.
• Clean catch urine could also be collected during a sterile wide-mouthed container.
• Biopsy specimens must be transported in saline.

In certain cases, pus or exudates must be searched for presence of granules.

Microscopy: Microscopy is employed to watch clinical specimens for the presence of fungal elements or to spot the fungus following culture. Within the latter case, lactophenol cotton blue is stain of choice, which stains the fungal elements blue. Interrogation of clinical specimens might be stained or unstained.
• Wet mount: Candida could also be observed in urine wet mounts
• 10-20% KOH mounts: Several specimens are subjected to KOH mount for interrogation. The fabric is mixed with 20% KOH on a slide and a canopy slip is placed. The slide is then gently heated by passing through the flame 2-3 times. The slide is observed on cooling. KOH serves to digest the protein debris and clears keratinised tissue and increases the visibility. Addition of Dimethyl sulphoxide (DMSO) permits rapid clearing within the absence of warmth.
• Calcofluor white: this is often a fluorescein , which binds selectively to chitin of the fungal cell membrane . The specimen then is often observed under fluorescent microscope.
• India Ink: Capsules of Cryptococcus neoformans are often demonstrated by this negative staining technique.
• Periodic Acid-Schiff (PAS) stain: On staining by this stain, fungal elements appear bright

magenta coloured while the background stains green. It's useful in staining tissue specimens.
• Giemsa's stain: it's particularly useful within the detection of Histoplamsa capsulatum within the bone marrow smears.
• Haematoxylin and Eosin (H&E) stain: Useful for staining tissue sections.
• Gomori's methenamine nitrate (GMS) stain: Outlines of the fungi are black, internal parts stain pink black while the background stains light green. Candida and Aspergillus could also be missed in H&E stained sections, therefore GMS stained sections are essential for tissue pathology.
• Gridley's stain: It stains hyphae and yeasts dark blue-pink, tissues deep blue and background yellow.
• Meyer mucicarmine stain: Capsules of C. neoformans and inner walls of Rhinosporidium seeberi's sporangium are stained pink.
• Gram's method : Candida is best demonstrated in clinical specimen by Gram stain.
• Masson-Fontana stain is useful in staining phaeoid (dematiaceous) fungi in tissue.
• Immunofluorescence: antibody labelled with fluorescent dyes is often wont to detect several fungi within the clinical specimens.

Culture: one among the foremost common media wont to culture fungi in laboratory is Sabouraud's Dextrose Agar (SDA). It consists of peptone, dextrose and agar. High concentration of sugar and a coffee pH (4.5-5.5) prevents growth of most bacteria and makes it selective for fungi. Emmon's modification of SDA contains 2% dextrose and has pH of 6.8. Other basal media to grow fungi include Potato Dextrose Agar, Malt Extract Agar etc. Most fungi are ready to grow at temperature while few pathogenic fungi (e.g, Cryptococcus, dimorphic fungi) can grow at 37oC. Saprophytic fungi grow much quickly than pathogenic fungi (e.g, dermatophytes). In such situations the saprophytic fungi are often inhibited by the addition of cycloheximide (actidione) to the SDA. Addition of antibiotics like Chloramphenicol, Gentamicin or Streptomycin to SDA serves to inhibit bacterial multiplication. An example of SDA with cycloheximide
and Chloramphenicol is Mycosel agar. Other specialized media used for various fungi include:
• Brain Heart Infusion Agar general isolation of fungi and conversion of dimorphic fungi.
• Inhibitory Mould Agar, an isolation medium with Chloramphenicol to suppress most bacteria.
• Caffeic Acid Agar and Birdseed Agar for isolation of Cryptococcus neoformans.
• Corn Meal Agar: Enhances production of chlamydospores in Candida albicans and formation of conidia in fungi.
• Trichophyton Agars: Used for selective identification of Trichophyton species.

- Dermatophyte Test Medium: Used for recovery of dermatophytes from clinical specimens.

- Sabhi Medium: Isolation of ***Histoplasma capsulatum*.**
- 'CHROM agar Candida' is useful in identification of Candida species.

Conversion of mould to yeast phase must be demonstrated in vitro for identification of dimorphic fungi. Since some fungi grow slowly cultures shouldn't be discarded for 4-6 weeks. Fungi are identified on the idea of colony morphology (including pigmentation) and microscopic observation by tease-mount preparation or slide culture technique.

Serology: Detection of anti-fungal antibody is useful in diagnosis of sub-cutaneous and systemic

mycoses, prognosis and response to anti-fungal drugs. Different serologic techniques that are used include agglutination, immunodiffusion, counter-immunoelectrophoresis, immune response test, immunofluorescence, RIA and ELISA.

Antigen detection: it's particularly useful within the diagnosis of cryptococcal meningitis from CSF specimens. The test is performed by Latex Agglutination or immunodiffusion tests. It's also helpful within the detection of Aspergillus and Candida antigens in systemic infections.

Skin tests: Delayed hypersensitivity reactions to fungal antigens are often demonstrated by skin tests. A positive skin doesn't necessarily indicate a lively infection; it only indicates sensitization of the individual. Hence, its value is in epidemiological studies than diagnosis. These tests could also be performed in Histoplasmosis, Candidiasis, Sporotrichosis, Coccidioidomycosis, Blastomycosis, Paracoccidiodomycosis and dermatophytosis.

Molecular techniques: Newer techniques like DNA hybridization, PCR are useful in diagnosis of mycoses during a shorter period also as detect those fungi that are difficult or dangerous to cultivate in vitro.

Chapter-8
Prokaryotic Cells

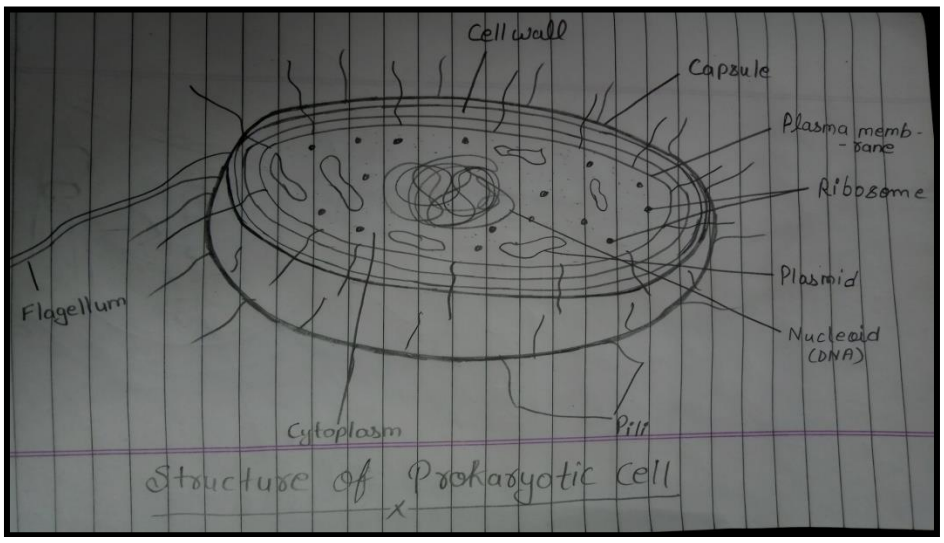
All living things are made from cells, and cells are the littlest units which will be alive. Life on Earth is assessed into five kingdoms, and that they each have their own characteristic quite cell. However the most important division is between the cells of the Prokaryote kingdom (the bacteria) and people of the opposite four kingdoms (Animals, Plants, Fungi and Protoctista), which are all eukaryotic cells. Prokaryotic cells are smaller and simpler than eukaryotic cells, and don't have a nucleus.

Prokaryote = "before carrier bag" i.e. without a nucleus
Eukaryote = "good carrier bag" i.e. with a nucleus

We'll examine these two sorts of cell intimately, supported structures seen in electron micrographs (= photos crazy an electron microscope). These show the individual organelles inside a cell.

Components of Prokaryotic Cells

The prokaryotic cells have four main components:
Plasma Membrane- it's an outer protective covering of phospholipid molecules which separates the cell from the encompassing environment.
Cytoplasm- it's a jelly-like substance present inside the cell. All the cell organelles are suspended in it.
DNA- it's the genetic material of the cell. All the prokaryotes possess a circular DNA. It directs what proteins the cell creates. It also regulates the actions of the cell.
Ribosomes- Protein synthesis occurs here.
Some prokaryotic cells possess cilia and flagella which help in locomotion.



Reproduction in Prokaryotes

A prokaryote reproduces in two ways:
• Asexually by binary fission
• Sexually by conjugation

Binary Fission

1. The DNA of an organism replicates and therefore the new copies attach to the cell wall.
2. The cell membrane starts increasing in size and starts moving inwards.
3. A cell membrane is then formed between each DNA, dividing the cell into two daughter cells.

Recombination

In this process, genes from one bacteria are transferred to the genome of other bacteria. It takes place in three ways-conjugation, transformation, and transduction.

• Conjugation is that the process during which genes are transferred between two bacteria through a protein tube structure called a pilus.

• Transformation is that the mode of amphimixis during which the DNA from the environment is taken by the bacterial cell and incorporated in its DNA.

• Transduction is that the process during which the genetic material is transferred into the bacterial cell with the assistance of viruses. Bacteriophages are the virus that initiates the method.

Examples of Prokaryotic Cells

The samples of the prokaryotic cells are mentioned below:

Bacterial Cells

- These are unicellular organisms found everywhere on earth from soil to the physical body.
- They have different shapes and structures.
- The cell membrane consists of peptidoglycan that gives structure to the cell membrane.
- Bacteria have some unique structures like pili, flagella and capsule.
- They also possess extra chromosomal DNA referred to as plasmids.
- They have the power to make tough, dormant structures referred to as endospores that helps them to survive under unfavourable conditions. The endospores become active when the conditions are favourable again.

Archaeal Cells

- Archae bacteria are unicellular organisms almost like bacteria in shape and size.
- They are found in extreme environments like hot springs and other places like soil, marshes, and even inside humans.
- They have a cell membrane and flagella. The cell membrane of archaea doesn't contain peptidoglycan.
- The membranes of the archaea have different lipids with a totally different stereochemistry. Just like bacteria, archaea have one circular chromosome.
- They also possess plasmids. For more information on Prokaryotic Cells, its definition, structure, characteristics and examples, keep visiting BYJU'S Biology website or download BYJU'S app for further reference.

Difference between eukaryotes and prokaryotes cell-:

Sr. No.	Prokayotic Cells	Eukaryotic cells
1	Small cells (< 5 μm)	Larger cells (> 10 μm)
2	Always unicellular	Often multicellular
3	No nucleus or any membrane-bound organelles, such as mitochondria	Always have nucleus and other membrane- bound organelles
4	DNA is circular, without proteins	DNA is linear and associated with proteins to form chromatin
5	Ribosomes are small (70S)	Ribosomes are large (80S)
6	No cytoskeleton	Always has a cytoskeleton
7	Motility by rigid rotating flagellum (made of flagellin)	Motility by flexible waving cilia or flagellae (made of tubulin)
8	Cell division is by binary fission	Cell division is by mitosis or meiosis
9	Reproduction is always asexual	Reproduction is asexual or sexual
10	Huge variety of metabolic pathways	Common metabolic pathways

Chapter-9
Bacterial Capsule

Capsule (also referred to as K antigen) may be a major virulence factor of bacteria, e.g. all of the principal pathogens which cause pneumonia and meningitis, including Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, Klebsiella pneumoniae, Escherichia coli , and B streptococci have polysaccharide capsules on their surface. Nonencapsulated mutants of those organisms are a virulent.

Capsule may be a

- Gelatinous layer covering the whole bacterium
- Composed of polysaccharide (i.e. poly: many, saccharide: sugar). These polymers are composed of repeating oligosaccharide units of two to four monosaccharides.
- Capsule is found immediately exterior to the murein (peptidoglycan) layer of gram-positive bacteria and therefore the outer membrane (Lipopolysaccharide layer) of gram-negative bacteria.
- The sugar components of polysaccharide vary within the species of bacteria, which determines their serologic types. Example: Streptococcus pneumoniae has 84 different serologic types discovered thus far.

Importance of Bacterial Capsule

1. Virulence determinants: Capsules are anti-phagocytic. They limit the power of phagocytes to engulf the bacteria. The graceful nature and charge of the capsule prevents the phagocyte from adhering to and engulfing the bacterial cell. If a pathogenic bacteria lose capsule (by mutation), they won't be ready to cause disease (i.e. loses disease causing capacity).
2. Saving engulfed bacteria from the action of neutrophil: Bacterial capsule prevents the direct access of lysosome contents with the bacterial cell, preventing their killing.
3. Prevention of complement-mediated bacterial cell lysis.
4. Protection of anaerobes from oxygen toxicity
5. Identification of bacteria
a) Using specific antiserum against capsular polysaccharide. E.g. quellung
b) Colony characteristics in culture media: Bacteria with capsules form smooth (S) colonies while those without capsules form rough (R) colonies. A given bacterial species may undergo a phenomenon called S-R variation whereby the cell loses the power to make a capsule. Some capsules are very large and absorb water; bacteria with this sort of capsule (e.g., Klebsiella pneumoniae) form mucoid (M) colonies.
6. Development of Vaccines: Capsular polysaccharides are used because the antigens in certain vaccines. For examples:
• Polyvalent (23 serotypes) polysaccharide vaccine of Streptococcus pneumoniae capsule.
• Polyvalent (4 serotypes) vaccine of Neisseria meningitidis capsule.
• A monovalent vaccine made from capsular material from Haemophilus influenzae.

7. Initiation of infection: Capsules helps the organism to stick to host cells.The capsule also facilitates and maintains bacterial colonization of biologic (e.g. teeth) and inanimate (e.g. prosthetic heart valves) surfaces through formation of biofilms.
8. Receptors for Bacteriophages.

Examples of Capsulated bacteria/yeasts:

Mneomonics to recollect capsulated bacteria– Some Killers Have Pretty Nice Capsule

1. Streptococcus pneumoniae
2. Klebsiella pneumoniae
3. Haemophilus influenzae
4. Pseudomonas aeruginosa
5. Neisseria meningitides
6. Cryptococcus neoformans

Compositions of capsules of capsulated organisms

Capsulated Bacteria-:

Klebsiella pneumoniae	Polysaccharide
Pseudomonas aeruginosa	Polysaccharide
Streptococcus pneumoniae (Pneumococcus)	Polysaccharide
Neisseria meningitidis (Meningococcus)	Polysaccharide
Bacteriodes fragilis	Polysaccharide
Bacillus anthracis	Polypeptide (glutamate)
Streptococcus pyogenes (some strain)	Hyaluronic acid

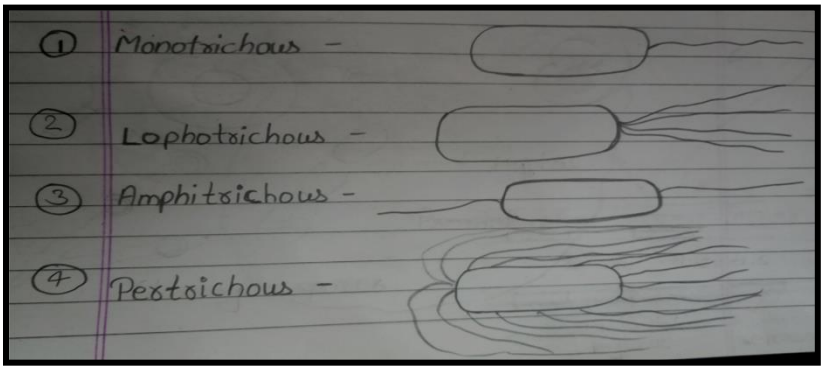
Capsulated Fungus-:

Cryptococcus neoformans	Polysaccharide

Chapter-10
Flagella

Flagella are the complex filamentous cytoplasmic structure protruding through cell membrane. These are unbranched, long, thread like structures, mostly composed of the protein flagellin, intricately embedded within the cell envelope. They're about 12-30 nm in diameter and 5-16 µm long . they're liable for the bacterial motility. Motility plays a crucial role in survival and therefore the ability of certain bacteria to cause disease.

Types and samples of Flagella



There are 4 sorts of flagellar distribution on bacteria-

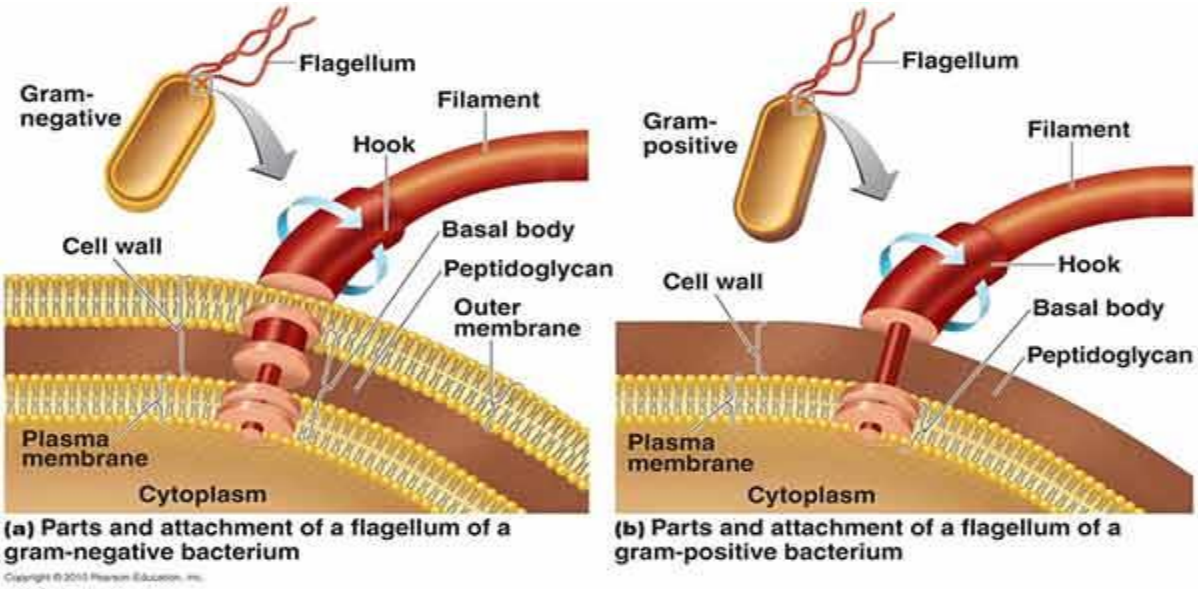
1. Monotrichous
– Single polar flagellum
– Example: Vibrio cholerae

2. Amphitrichous
– Single flagellum on each side
– Example: Alkaligens faecalis

3. Lophotrichous
– Tufts of flagella at one or each side
– Example: Spirillum

4. Peritrichous
– Numerous falgella everywhere the bacterial body
– Example: typhoid bacillus

Parts of Flagella
Each flagellum consists of three distinct parts- Filament, Hook and Basal Body.
• The filament lies external to the cell.
• Hook is embedded within the cell envelope.
• Basal Body is attached to the cytoplasmic membrane by ring-like structures.



Functions of Flagella

- Movements
- Sensation
- Signal transduction
- Adhesion
- For cells anchored during a tissue, just like the epithelial cells lining our air passages, this moves liquid over the surface of the cell (e.g., driving particle-laden mucus toward the throat).
- Flagella are generally accepted as being important virulence factors

Principle of Flagella staining

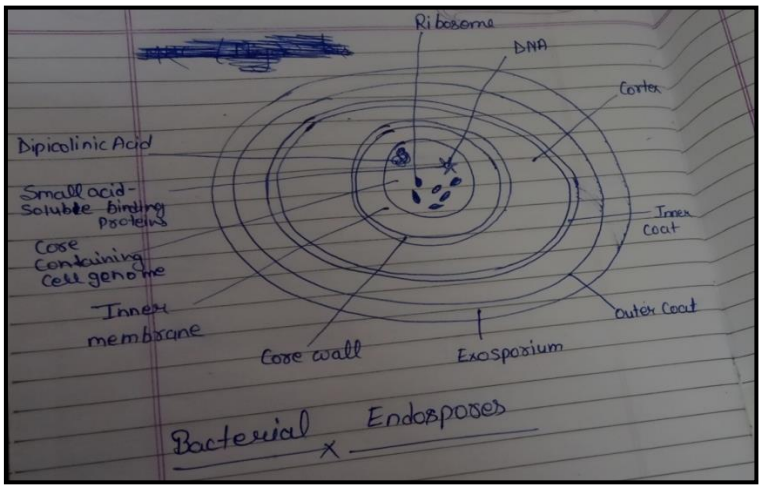
A wet mount technique for staining bacterial flagella is straightforward and is beneficial when the amount and arrangement of flagella are critical in identifying species of motile bacteria.

Procedure of Flagella Staining-:

1. Grow the organisms to be stained at temperature on agar for 16 to 24 hours.
2. Add alittle drop of water to a slide .
3. Dip a sterile inoculating loop into sterile water
4. Touch the loopful of water to the colony margin briefly (this allows motile cells to swim into the droplet of water).
5. Touch the loopful of motile cells to the drop of water on the slide.
6. Cover the faintly turbid drop of water on the slide with a canopy slip. a correct wet mount has only enough liquid to fill the space under a canopy slip. Small air spaces round the edge are preferable.
7. Examine the slide immediately under 40x for motile cells.
8. If motile cells are seen, leave the slide at temperature for five to 10 minutes.
9. Apply 2 drops of RYU flagella stain gently on the sting of the duvet slip. The stain will flow by capillarity and blend with the cell suspension.
10. After 5 to 10 minutes at temperature, examine the cells for flagella.
11. Cells with flagella could also be observed at 100 x.

Chapter-11
Bacterial Spore

- Spore is metabolically dormant structure produced during unfavorable condition by the method called sporulation.
- Sporulation occur during late log phase or early stationary phase
- Under favorable condition spores germinate to offer somatic cell .
- Size: 0.2 µm



Spore are immune to nutrition starvation, temperature, extreme pH, antibiotics etc
Structure of endospore:
An endospore has following layers
1. Exosporium
2. Spore coat
3. Cortex
4. Core

Exosporium:
• It is that the outermost layer made from protein that encloses spore coat.
• In some bacterial spore, exosporium is formed from polysaccharide and lipid.
Spore coat:
• It is thick double layered covering that encloses cortex
• Spore coat consists of spore specific protein, mainly contains cysteine and hydrophobic amino acids. thanks to presence of those aminoacids,spore are immune to adverse condition .

Cortex:
• Inside the Spore coat, there's cortex made from loosely arranged peptidoglycan layer.
• Inner layer: comprises about 20% of peptidoglycan, it's tightly arranged
• Outer layer: it's loosely arranged, it are often hydrolysed during spore germination.it comprises alalnine (55%), tetra-peptide (15%) and muramic lactum (30%).

Core:
• It is that the innermost a part of spore
• It is additionally referred to as spore protoplast
• Core consists of core wall, cytoplasmic membrane, cytoplasm, nucleoid, ribosomes and other cellular materials.

• Core contains (10-25%) water so, the cytoplasm is gel like
• It contains high amount of calcium and dipicolinic acid within the sort of calcium dipicolinate (10-15% by dry weight).
• Core also contains high percentage of small acid soluble protein (SASP).
• SASP is synthesized during sporulation and it binds to DNA in core and protect it from potential damage caused by UV radiation, desiccation and drying.
• In addition, SASPs also provides nutrition and energy for spore germination.

Types of bacterial spore

1. Endospore:

• It is produced within the bacterial cell.
• Bacteria producing endospore are: Bacillus, Clostridium, Sporosarcina etc

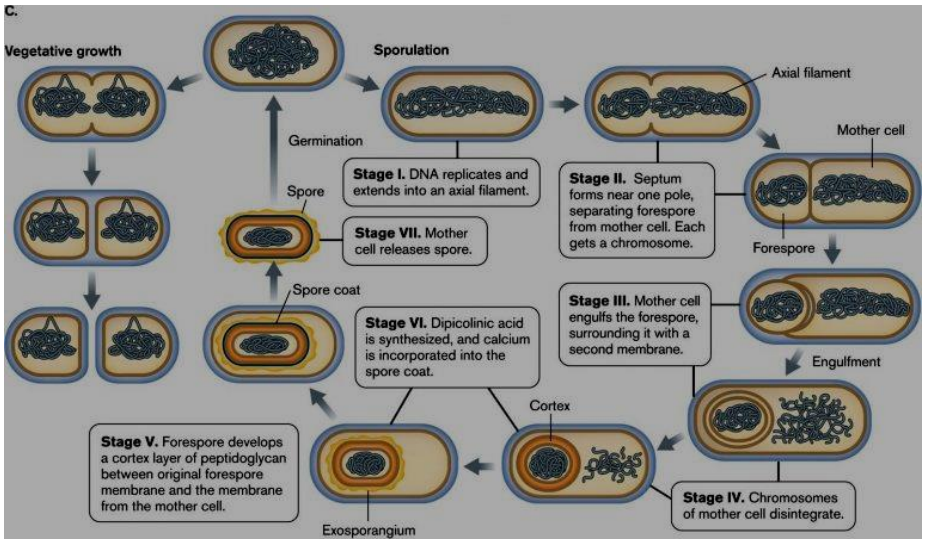
2. Exospore:

• It is produced outside the cell
• Bacteria producing exospore: Methylosinus

Stages of Sporulation:

Sporulation

• During unfavorable condition, somatic cell converts into spore by the method referred to as sporulation
• Sporulation are often divided into several stages. In Bacillus subtilis , entire process of sporulation takes 8 hours to finish from stage 0 to stage VII



Stage 0

• Normal conditions of a somatic cell

Stage I: Axial filament formation stage

• In this stage bacterial chromosome become thread like referred to as axial filament
• Axial filaments attached to cytoplasmic membrane by mesosome
• Elongation of cell take places
• PHBA is that the reserved food material in Bacillus spp is employed in sporulation.

Stage II: forespore formation
• Asymmetric cellular division occurs
• Cell membrane forms septum near one end which encloses alittle portion of DNA forming forespore

Stage III: engulfment of forespore
• Mother cell wall grow round the forespore engulfing it.
• Fore spore now has two membrane layer

Stage IV: synthesis of exosporium
• Chromosome of cell disintegrates
• Exosporium synthesis occurs
• Forespore starts Forming primodial cortex between two membrane.
• Dehydration of cell

Stage V: synthesis of dipicolonic acid
• Production of SASPs and dipicolinic acid occurs
• Incorporation of calcium ions with dipicolonic acid occur forming calcium dipicolonate
• Further dehydration of cytoplasm
• Formation of coat layer

Stage VI: maturation
• Maturation of endospore

Stage VII: release of endospore

• Cell lysis and release of endospore
Spore germination
• Endospore remains dormant for years. But under favorable conditions each endospore germinates to offer rise to a somatic cell.

Spore germination involves 3 processes.

1. Activation
2. Germination
3. Outgrowth

Activation of endospore:
• The germination of bacterial spore don't occur even when the environment is favorable unless it's first activated.
• At first the Spore coat must be damaged by heating for several minutes.
Germination:
• The activated spore initiates germination after binding of effector molecules.
• Binding of effectors molecules activates autolysis that destroys peptidoglycan of cortex.
• After destruction of peptidoglycan, water is haunted and calcium dipicolinic acid is released.
Outgrowth:
• After uptake of water swelling of spore occurs.
• Along with swelling, synthesis of DNA, RNA and proteins also occurs.
• A small reproductive cell emerges out after breaking the Spore coat and begins to grow into somatic cell.

Chapter-12
Sterilization and Disinfection

Sterilization is the process of destroying or physically removing all forms of microbial life including vegetative cells, spores and viruses from a surface, a medium or an article. The principal reasons for controlling microorganisms are:

- To prevent transmission of disease and infection
- To prevent contamination by undesirable microorganisms
- To prevent deterioration and spoilage of materials by microorganisms

The various agents used in sterilization can be grouped into physical and chemical agents

Physical Agents (Physical Methods)

Sunlight

Direct sunlight has an active germicidal effect due to the combined effect of the ultraviolet radiation and heat. This is a natural method of sterilization.

Drying

Moisture is essential for the growth of bacteria. Drying in air has therefore a deleterious effect on many bacteria. But spores are unaffected by drying. Hence this is very unreliable method.

Heat

Heat has a killing effect on microorganisms and is one of the most popular reliable methods to destroy. Microorganisms have minimum, optimum and maximum growth temperatures. Temperature below the minimum usually produces static (inhibition of metabolism).

Temperature above the maximum, generally kill microorganisms. This is because biochemical changes in the cells organic molecules result in its death. These changes arise from alterations in enzyme molecules or chemical break down of structural molecules especially in the cell membranes.

Heat also drives off water and this loss of water may be lethal to the organisms.
The killing rate of heat may be expressed as a function of time and temperature. Each microbial species has a Thermal Death Time (TDT). It's the minimum time required to kill a population of microorganism in a microbial suspension at a given temperature and under defined condition. Each species also has a Thermal Death Point (TDP), the temperature at which it dies in a given time.

In determining the time and temperature for microbial destruction with heat, the following factors are considered.

- ❖ Type of organism to be killed
- ❖ Type of material to the treated
- ❖ Presence of organic matter
- ❖ Acidic or basic nature of the material

Nature of heat:

Dry heat

Many objects are best sterilized in the absence of water by dry heat sterilization; killing by dry heat is due to protein denaturation, oxidative damage and toxic effect of elevated levels of electrolytes.

Methods of Dry Heat

Flaming

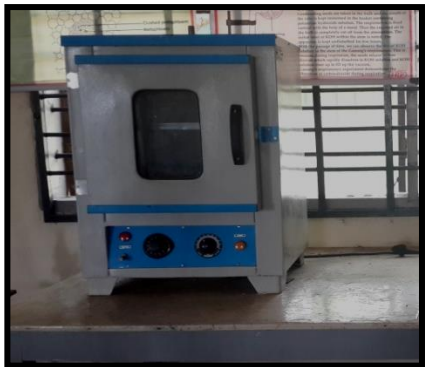
- Inoculating loops and points of forceps may be heated in the Bunsen flame, till they are red- hot. Articles such as mouth of the culture tubes, cotton wool plugs, glass slides etc. are passed over the flame without allowing it to become red hot.

Incineration

- This is an excellent method for rapidly destroying, animal carcasses, pathological material and disposables.

Hot Air Oven

This is the most widely adopted method of sterilization by dry heat. The hot air oven utilizes radiating dry heat for sterilization. This type of energy does not penetrate materials easily and thus, long periods of exposure to high temperature are necessary. For example, at a temperature of 160°C, a period of two hours is required for the destruction of bacterial spores. Hot air oven is used to sterilize glassware, forceps, scissors, scalpels, glass syringes, liquid paraffin, dusting powder etc. A holding period of 160°C for an hour is used. The oven is usually heated by electricity, with heating elements in the wall of the chamber and it must be filled with a fan to ensure even distribution of hot air and elimination of air pockets. The materials should be arranged in a manner which allows free circulation of hot air in between the objects. It should not be over-loaded. Glass wares should be perfectly dry before being placed in the oven. Test tubes, flasks etc. should be wrapped in craft paper. Oven must be allowed to cool slowly for about 2 hours before the door is opened, since the glasswares may get cracked by sudden or uneven cooling.



Hot Air Oven

Control:

The spores of a non – toxigenic strain of Clostridium tetani are used as a microbiological test of dry heat efficiency. Paper stripes impregnated with 106 spores are placed in envelop and inserted into suitable packs. After sterilization is over, the strips are removed and inoculated into thioglycollate or cooked meat media and incubated for sterility test under strict anaerobic conditions for five days at 37°C.

Moist heat

Moist heat kills microorganisms by coagulating their proteins and is much more rapid and effective than dry heat because water molecules conduct heat better than air. Lower temperature and less time of exposure are therefore required than for dry heat. Moist heat readily kills viruses, bacteria, fungi etc.

- Temperature below 100°C
- Pasteurization of milk

a) For pasteurization of milk, there are two methods

- **Holding Method or Low Temperature Holding Method (LTH)**

In this method, the milk is exposed to a temperature of 63°C (145°F) for 30 minutes in appropriately designed equipment. This is followed by sudden cooling to 13°C or below.

- **Flash Process or High Temperature Short Time (HTST)**

In this method, the milk is exposed to a temperature of 72°C for 15 seconds in the equipment. This is followed by sudden cooling to 13°C or below. The finished product should be stored at a low temperature to retard growth of microorganisms and pasteurization removes the pathogenic bacteria in milk. By these processes all non-sporing pathogens such as mycobacteria, salmonellae and brucella are destroyed 'Coxiella bumetic' is relatively heat resistant and may survive the holder method.

- **Vaccine bath**

It's used for killing non-sporing bacteria which may be present in vaccine. In vaccine bath, the vaccine is treated with moist heat for one hour at 60°C.

Serum containing coagulable proteins can be sterilized by heating for one hour at 56°C in a water bath for several successive days.

b) Temperature at 100°C

- **Boiling**

Most of the vegetative forms of bacteria, fungi etc. are killed almost immediately at 90-100°C, but sporing bacteria required considerable periods of boiling. Boiling water is not considered as a sterilizing agent because destruction of bacterial spores and inactivation of viruses cannot always be assured. Under ordinary circumstances, most species of microbes can be killed within 10 minutes. However, spores of bacteria and

fungi, protozoa cysts and large concentrations of Hepatitis A viruses requires up to 30 minutes exposure or more because inadequate information exists on the heat tolerance of many microorganisms, boiling water is not reliable for sterilization purpose especially the sterilization of instruments and for surgical procedures.
In cases where boiling is considered adequate, the material should be immersed in water and boiled for a period of 10-30 minutes. The lid of the sterilizer should not be opened during that period. Addition of little acid, alkali or washing soda will increase the efficiency of the process.

- **Steam under atmospheric pressure (100°C)**

Steam under atmospheric pressure is used to sterilize culture media which may decompose if subjected to higher temperature. A Koch or Arnold sterilizer is an instrument that generates free floating steam.
The container and the medium are simultaneously sterilized and evaporation from the medium is prevented one exposure of 90 minutes usually ensures complete sterilization of the medium. This is an inexpensive method.

- **Sterilization above 100°C (steam under pressure)**

Heat in the form of saturated steam under pressure is the most practical and dependable agent for sterilization. Steam under pressure provides temperature above those obtainable by boiling. Moreover, it has advantages of rapid heating, penetration and moisture in abundance, which facilitates the coagulation of the protein of microorganisms, resulting in complete destruction of all forms of microbial life, including bacterial endospores. It is important to note that the sterilizing agent is the moist heat not the pressure. The laboratory apparatus designed to use steam under regulated pressure is called an autoclave. It is essentially a double jacketed steam chamber equipped with devices which permit the chamber to be filled with saturated steam and maintained at a designed temperature and pressure for any period of time
The articles to be sterilized are placed in the sterilizing chamber and steam is maintained in the steam jacket into the sterilizing chamber, cool air is forced out and a special valve increases the pressure to 15 pounds/square inch above normal atmospheric pressure. The temperature rises to 121.5°C and the superheated water molecules rapidly conduct heat into microorganisms and will be killed. The time for destruction of the most resistant bacterial spore is reduced to 15 minutes. For denser objects, up to 30 minutes of exposure may be required.
Autoclave is essential equipment in every microbiology laboratory. It's used to sterilize many media, solutions, discarded cultures, glass wares, metal wares etc.

- **Filtration**

Filtration is the process of removal of microorganisms from liquid or gases using a mechanical device known as filter. This is an excellent way to reduce the microbial population in solution of thermo labile materials such as sera, antibiotic solutions, intravenous solutions, carbohydrates solutions used in the preparation of culture media etc. As fluid passes through the filter, microorganisms are trapped in the pores of the filtering material. The solution that drips through the filter is collected in a previously sterilized container. Porosity, electric charges of the filter, electric charge carried by the organisms, nature of the fluid being filtered etc. can influence efficiency of filtration.

Types of filters:
Seltz Filter,
Berkefeld Filter,
Membrane Filter,
High Efficiency Particulate Air (HEPA) filter

- **Irradiation**

The process of exposing organisms to anyone of the radiation such as UV-rays, X-rays, gamma rays etc. is known as irradiation. Irradiation is an effective method of sterilization. Two types of radiations are used for sterilization.

- **Non ionizing radiation**

UV radiation
Infrared radiation
Ionizing radiation
X rays
Gamma rays

Chemical Sterilization

- **Disinfectant:**

• Are chemical materials used for sterilization but are toxic to the human tissues and cells.

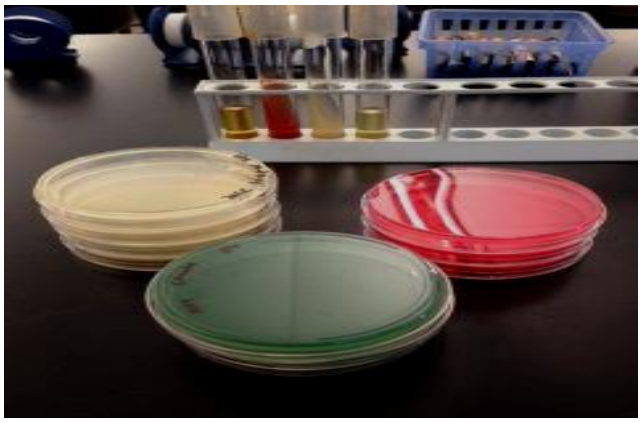
- **Antiseptics:**

• Are chemicals for sterilization but not toxic to the human body e.g. "mouth gargles".

- **Examples of disinfectant and antiseptics**

There are a number of chemicals that can act as disinfectants or antiseptics. These include:
• Phenol and its derivatives e.g. Dettol.
• Halogens e.g. Chlorine, Tincture iodine.
• Alcohols e.g. ethyl alcohol.
• Aldehydes e.g. glutaraldehyde (Cidex), Formalin.
• Quaternary Ammonium Compounds (Cationic detergents).

Chapter-13
Culture Medium



Culture media contain nutrients and physical growth parameters necessary for microbial growth. All microorganisms cannot grow in a single culture medium and in fact many can't grow in any known culture medium.

Organisms that cannot grow in artificial culture medium are known as obligate parasites. Mycobacterium leprae, rickettsias, Chlamydias, and Treponema pallidum are obligate parasites. Bacterial culture media can be classified on the basis of composition, consistency and purpose.

Classification of bacterial culture media on the basis of consistency

Solid medium

Solid medium contains agar at a concentration of 1.5-2.0% or some other, mostly inert solidifying agent. Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways (e.g. as colonies_or in streaks). Solid medium is useful for isolating bacteria or for determining the colony characteristics of the isolate.

Semisolid media

Semisolid media are prepared with agar at concentrations of 0.5% or less. They have soft custard like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility.

Liquid (Broth) medium

These media contains specific amounts of nutrients but don't have trace of gelling agents such as gelatin or agar. Broth medium serves various purposes such as propagation of large number of organisms, fermentation studies, and various other tests. e.g. sugar fermentation tests, MR-VR broth.

Classification of culture media on the basis of composition

Synthetic or chemically defined medium

A chemically defined medium is one prepared from purified ingredients and therefore its exact composition is known. Synthetic medium may be simple or complex depending up on the supplement incorporated in it. A simple non-synthetic medium is capable of meeting the nutrient

requirements of organisms requiring relatively few growth factors where as complex non-synthetic medium support the growth of more fastidious microorganisms.

Non synthetic or chemically undefined medium

Non-synthetic medium contains at least one component that is neither purified nor completely characterized nor even completely consistent from batch to batch. Often these are partially digested proteins from various organism sources. Nutrient broth, for example, is derived from cultures of yeasts.

Classification of Bacterial Culture media on the basis of purpose/ functional use/ application

Many special purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria. To meet these needs, numerous media are available.

General purpose media/ Basic media
Basal media are basically simple media that supports most non-fastidious bacteria.
Peptone water, nutrient broth and nutrient agar (NA) are considered as basal medium.
These media are generally used for the primary isolation of microorganisms.

Enriched medium:
Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes enriched media.
Enriched media are used to grow nutritionally exacting bacteria.
Blood agar, chocolate agar, Loeffler's serum slope etc are few of the enriched media.
Blood agar is prepared by adding 5-10% (by volume) blood to a blood agar base.
Chocolate agar is also known as heated blood agar or lysed blood agar.

Chocholate Agar & Blood agar

Selective and enrichment media

Selective and enrichment media are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen of interest. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

Principle: Differential growth suppression Selective medium is designed to suppress the growth of some microorganisms while allowing the growth of others. Selective medium are agar based (solid) medium so that individual colonies may be isolated.

Examples of selective media include:

Thayer Martin Agar used to recover Neisseria gonorrhoeae contains antibiotics; vancomycin, colistin and nystatin.
Mannitol Salt Agar and Salt Milk Agar used to recover S.aureus contain 10% NaCl.
Potassium tellurite medium used to recover C.diphtheriae contains 0.04% potassium tellurite.
MacConkey's Agar used for Enterobacteriaceae members contains bile salt that inhibits most gram positive bacteria.
Pseudosel Agar (Cetrimide Agar) used to recover P. aeruginosa contains cetrimide (antiseptic agent).
Crystal Violet Blood Agar used to recover S. pyogenes contains 0.0002% crystal violet.
Lowenstein Jensen Medium used to recover M.tuberculosis is made selective by incorporating malachite green.
Wilson and Blair's Agar for recovering S. typhi is rendered selective by the addition of dye brilliant green.

Selective media such as TCBS Agar used for isolating V. cholerae from fecal specimens have elevated pH (8.5-8.6), which inhibits most other bacteria.

MacConkey's Agar

Enrichment culture medium

Enrichment medium is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium. Unlike selective media, enrichment culture is typically used as broth medium. Enrichment media are liquid media that also serves to inhibit commensals in the clinical specimen. Selenite F broth, tetrathionate broth and alkaline peptone water (APW) are used to recover pathogens from fecal specimens.

Differential/ indicator medium: differential appearance:
Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.
Examples of differential media include:

Mannitol salts agar (mannitol fermentation = yellow)
Blood agar (various kinds of hemolysis i.e. α, β and γ hemolysis)
Mac Conkey agar (lactose fermenters, pink colonies whereas non- lactose fermenter produces pale or colorless colonies.
TCBS (Vibrio cholerae produces yellow colonies due to fermentation of sucrose)

Transport media: Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors.
Cary Blair transport medium and Venkatraman Ramakrishnan (VR) medium are used to transport feces from suspected cholera patients.
Sach's buffered glycerol saline is used to transport feces from patients suspected to be suffering from bacillary dysentery.
Pike's medium is used to transport streptococci from throat specimens.

Anaerobic media: Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation –reduction potential and extra nutrients.

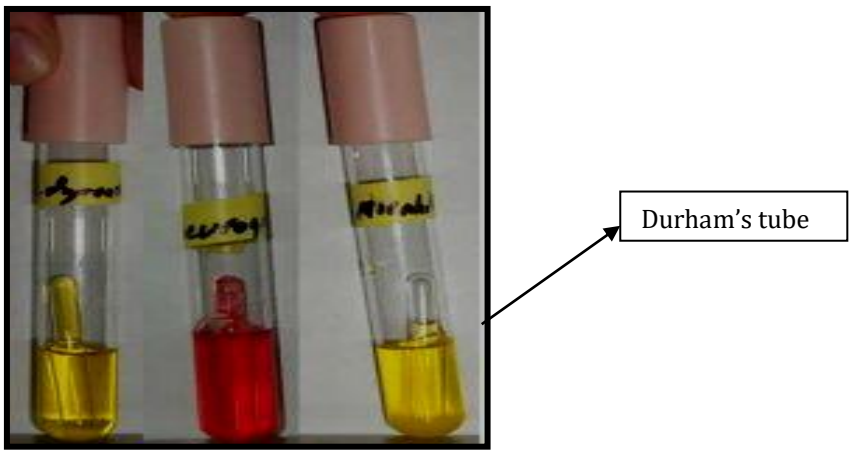
Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Such media may also have to be reduced by physical or chemical means. Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings can render a medium reduced. Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.

Robertson Cooked Meat (RCM) medium that is commonly used to grow Clostridium spps contains a 2.5 cm column of bullock heart meat and 15 ml of nutrient broth. Thioglycollate broth contains sodium thioglycollate, glucose, cystine, yeast extract and casein hydrolysate.
Methylene blue or resazurin is an oxidation-reduction potential indicator that is incorporated in the medium. Under reduced condition, methylene blue is colorless.

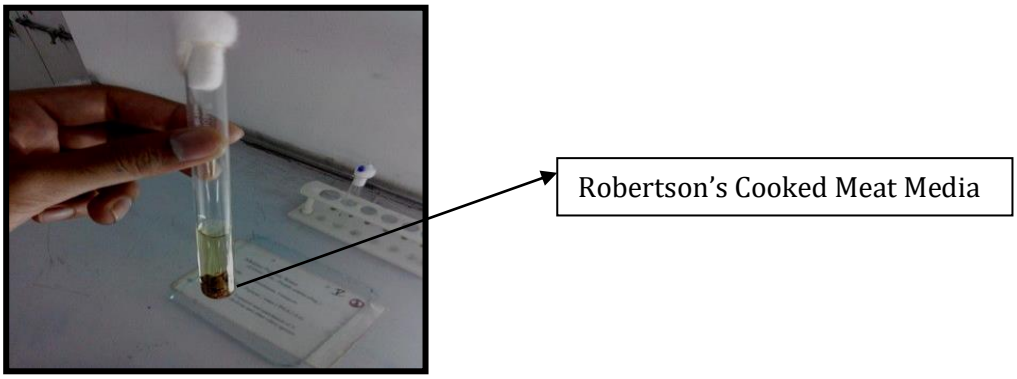
Assay media-: These media are used for the assay of vitamins, amino acids and antibiotics. E.g. antibiotic assay media are used for determining antibiotic potency by the microbiological assay technique.

Indicator Media
These media contain an indicator which changes colour when a bacterium grows in them. eg. Incorporation of sulphite in Wilson and Blair medium Salmonella typhi reduces sulphite to sulphide in Wilson and Blair medium and the colonies of S. typhi have a black metallic sheen.

Sugar Media
The usual sugar media consist of 1% sugar concerned. In peptone water along with appropriate indicator, a small tube (Durham's tube) is kept inverted in sugar tube to detect gas production.



Anaerobic Media
These media are used to grow anaerobic organisms. eg. Robertson's Cooked Meat Media.



Chapter-14
Plating Techniques

The common plating techniques employed in microbiology are Streak Plate Method, Spread Plate Method and Pour Plate Method.

- **Streak Plate Method-:**

Streak plate technique is used for the isolation into pure culture of the organisms (mostly bacteria), from mixed population. The inoculum is streaked over the agar surface in such a way that it "thins out" the bacteria. Some individual bacterial cells are separated and well spaced from each other. As the original sample is diluted by streaking it over successive quadrants, the number of organism's decreases. Usually by the third or fourth quadrant only a few organisms are transferred which will give discrete colony forming units (CFUs).

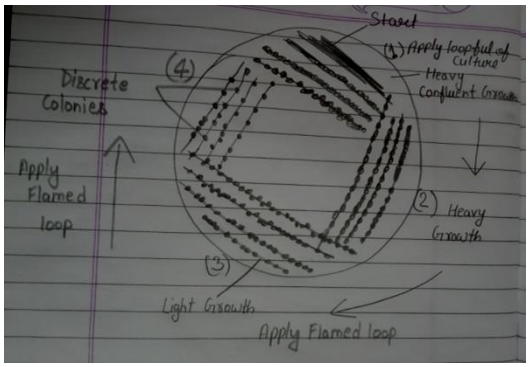
Principle

The sample/inoculum is diluted by streaking it across the surface of the agar plate. While streaking in successive areas of the plate, the inoculum is diluted to the point where there is only one bacterial cell deposited every few millimeters on the surface of the agar plate. When these lone bacterial cells divide and give rise to thousands and thousands of new bacterial cells, an isolated colony is formed.

Pure cultures can be obtained by picking well isolated colonies and re-streaking these on fresh agar plates.

Materials required:

- A source of bacteria (stock culture, previously streaked agar plate or any other inoculum)
- Inoculation loop,
- A striker/lighter
- Bunsen burner,
- Lysol (10%v/v)
- Agar plate (Nutrient agar or any other agar medium)
- Paper towels



Streak Plate Method

Procedure

1. Sterilize the inoculating loop in the bunsen burner by putting the loop into the flame until it is red hot. Allow it to cool.
2. Pick an isolated colony from the agar plate culture and spread it over the first

quadrant (approximately 1/4 of the plate) using close parallel streaks or Insert your loop into the tube/culture bottle and remove some inoculum. You don't need a huge chunk.

3. Immediately streak the inoculating loop very gently over a quarter of the plate using a back and forth motion
4. Flame the loop again and allow it to cool. Going back to the edge of area 1 that you just streaked, extend the streaks into the second quarter of the plate (area 2).
5. Flame the loop again and allow it to cool. Going back to the area that you just streaked (area 2), extend the streaks into the third quarter of the plate (area 3).
6. Flame the loop again and allow it to cool. Going back to the area that you just streaked (area 3), extend the streaks into the center fourth of the plate (area 4).
7. Flame your loop once more.

Results

Streaked plate is incubated at **37°C** for 24 hours. Examine the colonies grown in the plate carefully. All colonies should have the same general appearance. If there is more than one type of colony, each type should be streaked again on a separate plate to obtain a pure culture.

- **Spread Plate Method**

Spread plate technique is a method employed to plate a liquid sample for the purpose of isolating or counting the bacteria present in that sample. A perfect spread plate technique will results visible and isolated colonies of bacteria that are evenly distributed in the plate and are countable. The technique is most commonly applied for microbial testing of foods or any other samples or to isolate and identify variety of microbial flora present in the environmental samples e.g. soil.

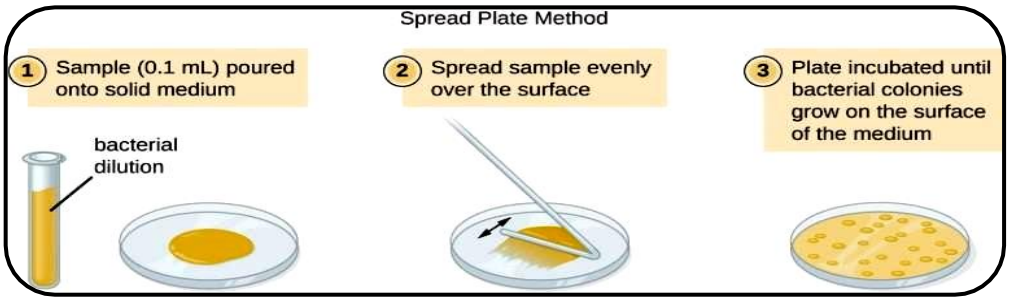
Requirements:

1. Glassware's: screw capped test tubes, sterile pipettes, glass spreader
2. Medium: Plate count agar

Procedure for Spread Plate Technique:

A: Serial Dilution

1. Prepare a series of at least 6 test tubes containing 9 ml of sterile distilled water.
2. Using a sterile pipette, add 1ml of sample in the first tube of the set. Label it as 10^{-1}
3. Mix the contents well by swirling the tube upside down few times.
4. From the first tube, take 1ml of the sample and transfer to second tube. Label it as 10^{-2}.
5. Repeat the procedure with all the remaining tubes labeling them until 10^{-6}.



B: Plating

1. Pipette out 0.1 ml from the appropriate desired dilution series onto the center of the surface of an agar plate.
2. Dip the L-shaped glass spreader *(hockey stick)* into alcohol.
3. Flame the glass spreader over a bunsen burner.
4. Spread the sample evenly over the surface of agar using the sterile glass spreader, carefully rotating the Petri dish underneath at an angle of 45^{o}at the same time.
5. Incubate the plate at 37°C for 24 hours.
6. Calculate the conlony forming units (CFU) value of the sample. Once you count the colonies, multiply by the appropriate dilution factor to determine the number of CFU/mL in the original sample.

Calculation of result:

CFU/ml = (no. of colonies x dilution factor) / volume of culture plate
For example, suppose the plate of the 10^6 dilutions yielded a count of 130 colonies. Then, the number of bacteria in 1 ml of the original sample can be calculated as follows:
Bacteria/ml = (130) x (10^6) = 1.3 × 10^8 or 130,000,000.

- **Pour Plate Method**

Pour plate method is usually the method of choice for counting the number of colony-forming bacteria present in a liquid specimen. In this method, fixed amount of inoculum (generally 1 ml) from a broth/sample is placed in the center of sterile Petri dish using a sterile pipette. Molten cooled agar (approx. 15mL) is then poured into the Petri dish containing the inoculum and mixed well. After the solidification of the agar, the plate is inverted and incubated at 37°C for 24-48 hours.
Microorganisms will grow both on the surface and within the medium. Colonies that grow within the medium generally are small in size and may be confluent; the few that grow on the agar surface are of the same size and appearance as those on a streak plate. Each (both large and small) colony is carefully counted (using magnifying colony counter if needed). Each colony represents a "colony forming unit" (CFU).

The number of microorganisms present in the particular test sample is determined using the formula:

CFU/mL= CFU * dilution factor * 1/aliquot

For accurate counts, the optimum count should be within the range of 30-300 colonies/plate. To insure a countable plate a series of dilutions should be plated.

The pour plate method of counting bacteria is more precise than the **streak plate method**, but, on the average, it will give a lower count as heat sensitive microorganisms may die when they come contact with hot, molten agar medium.

Materials and Equipments

1. Test sample
2. Plate Count Agar (PCA) or Nutrient Agar
3. Hot water bath 45°C

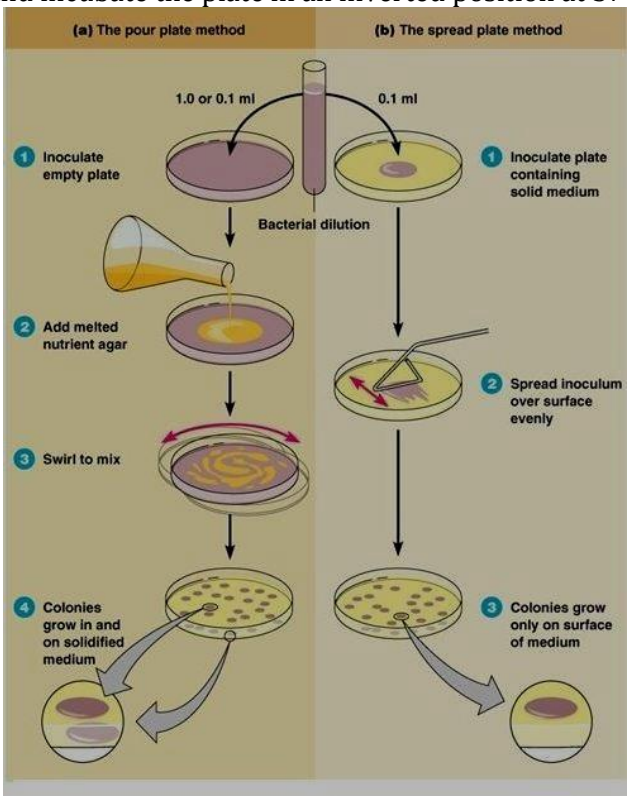
4. Sterile Petri dishes
5. Flame
6. Colony counter with magnifying glass
7. Sterile capped 16*150 mm test tubes
8. Pipettes of various sizes (e.g. 01, 1.0 and 2.0 mL)

Procedure of Pour plate technique

1. Prepare the dilution of the test sample expected to contain between 30-300 CFU/mL. *(Follow serial dilution technique)*
2. Inoculate labeled empty petri dish with specified mL (0.1 or 1.0 mL) of diluted specimen

Pouring the molten agar and incubation

1. Collect one bottle of sterile molten agar *(containing 15 mL of melted Plate Count Agar or any other standard culture media)* from the water bath (45°C).
2. Hold the bottle in the right hand; remove the cap with the little finger of the left hand.
3. Flame the neck of the bottle.
4. Lift the lid of the Petri dish slightly with the left hand and pour the sterile molten agar into the Petri dish and replace the the lid.
5. Flame the neck of the bottle and replace the cap.
6. Gently rotate the dish to mix the culture and the medium thoroughly and to ensure that the medium covers the plate evenly. Do not slip the agar over the edge of the petri dish.
7. Allow the agar to completely gel without disturbing it, it will take approximately 10 minutes.
8. Seal and incubate the plate in an inverted position at 37°C for 24-48 hours.



Results:

After 24-48 hours, count all the colonies. A magnifying colony counter can aid in counting small embedded colonies.
Calculate CFU/mL using the formula: CFU/mL= CFU * dilution factor * 1/aliquot (the volume of diluted specimen (aliquot) is either 0.1 or 1.0 mL)

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