SATHYABAMA UNIVERSITY **FACULTY OF BIO AND CHEMICAL ENGINEERING**

SBT1103	MICROBIOLOGY	L	T	P	Credits	Total Marks
		3	0	0	3	100

COURSE OBJECTIVES

- To enable students to learn about the principles of Microbiology to emphasize structure and biochemical aspects of various micro organisms.
- To know the control and preventive measures of microbial infections and environmental pollutions.

UNIT 1 INTRODUCTION TO MICROBIOLOGY 11 Hrs.

Introduction, History and scope of microbiology, Contributions of Leewenhoek, Pasteur, Koch, Jenner and Fleming, Microbial classification: Classical and Current systems, Methods of identifying microbes.

Basics of Microscopy, Staining: simple, differential (Gram staining, Acid fast staining), special staining (flagella,capsule,endospore)

UNIT 2 MICROBIAL STRUCTURE AND REPRODUCTION 9 Hrs.

Morphology and Reproduction: Bacteria - General structure and forms, Reproduction methods - Fission, budding and sporulation, Virus - TMV, HIV & T4 bacteriophage - lytic, lysogenic cycle, Fungi - Fungal morphology - Mycelial and yeast forms - sexual and asexual Reproduction, Actinomycete

UNIT 3 MICROBIAL GROWTH AND PHYSIOLOGY 7 Hrs.

Microbial Growth and Nutrition, Types of media - Based on Consistency, Nutritional components, Funtional uses and application, Microbial types based on nutrition, Growth of microbes in culture - Pure culture techniques, Batch & Continous - Growth curve - Enumeration methods , Types of fungal growth media. Aerobic and Anaerobic metabolism of sugars, mixed acid fermentation.

UNIT 4 CONTROL OF MICROORGANISMS 9 Hrs

Definitions of frequently used terms - Pattern or Rate of Microbial Death, Physical methods of Microbial Control: Heat (Moist & Dry), Low temperature, Filtration, High pressure, Desiccation, Osmotic pressure, Radiation. Chemical methods of Microbial Control: Liquids - Alcholos, Aldehydes, Phenolics, Halogens - Heavy metals, Surface active agents & Dyes, Gases - Formaldehyde, Ethylene Oxide, Plasma - Physico-chemical methods - Chemotherapeutic agents - Evaluation of effectiveness of antimicrobial agents. Difference between cleaning - sanitizing - sterilizing agents. Moist heat sterilization: D, Z and F Values and significance.

UNIT 5 APPLICATIONS OF MICROBIOLOGY 9 Hrs.

Microbial ecology: Microbe-Microbe interaction - Mutualism, Commensalism, Altruism, Microbe - host interactions - Colonization and Infection- Causes and Transmission of Infectious Diseases, Emerging and re-emerging infectious diseases - Mechanism and examples, Multidrug resistance - MRSA, Diagnostic Microbiology, Childhood and adult vaccinations - MMR, Polio, Rabies etc, bioterrorism agents, Biofilm - Quorum sensing,

Max. 45 Hours.

TEXT / REFERENCE BOOKS

1. Pelczar, Jr E.C.S Chan and noel R.Krieg, Microbiology, 5th edition Tata McGrawHill -2006
2. Joanne M. Willey, Linda Sherwood, Christopher J. Woolverton, Prescott's Microbiology, 8th Edition, McGraw-Hill Higher Education, 2008
3. Jawetz, Melnick and Adelberg's Medical Microbiology . McGraw-Hill Medical, 2007
4. University of South Carolina School of Medicine (http://pathmicro.med.sc.edu/book/bact-sta.htm)

END SEMESTER EXAMINATION QUESTION PAPER PATTERN

Max Marks : 80 **Exam Duration : 3 Hrs.**

PART A : 10 questions of 2 marks each - No choice **20 Marks**

PART B : 2 questions from each unit of internal choice; each carrying 12 marks **60 Marks**

UNIT – I - Introduction to Microbiology

Microbiology (from Greek μῑκρος, *mīkros*, "small"; βίος, *bios*, "life"; and -λογία, -*logia*) is the study of microscopic organisms, those being unicellular (single cell), multicellular (cell colony), or acellular (lacking cells).[1] Microbiology encompasses numerous sub-disciplines including virology, mycology, parasitology, and bacteriology.

Eukaryotic micro-organisms possess membrane-bound cell organelles and include fungi and protists, whereas prokaryoticorganisms—which all are microorganisms—are conventionally classified as lacking membrane-bound organelles and includeeubacteria and archaebacteria. Microbiologists traditionally relied on culture, staining, and microscopy. However, less than 1% of the microorganisms present in common environments can be cultured in isolation using current means.[2]Microbiologists often rely on extraction or detection of nucleic acid, either DNA or RNA sequences.

Viruses have been variably classified as organisms,[3] as they have been considered either as very simple microorganisms or very complex molecules. Prions, never considered microorganisms, have been investigated by virologists, however, as the clinical effects traced to them were originally presumed due to chronic viral infections, and virologists took search—discovering "infectious proteins".

As an application of microbiology, medical microbiology is often introduced with medical principles of immunology as *microbiology and immunology*. Otherwise, microbiology, virology, and immunology as basic sciences have greatly exceeded the medical variants, applied sciences.[4][5][6]

Branches

The branches of microbiology can be classified into pure and applied sciences.[7] Microbiology can be also classified based on taxonomy, in the cases of bacteriology, mycology, protozoology, and phycology. There is considerable overlap between the specific branches of microbiology with each other and with other disciplines, and certain aspects of these branches can extend beyond the traditional scope of microbiology.

Pure microbiology

Taxonomic arrangement

- Bacteriology: The study of bacteria.
- Mycology: The study of fungi.
- Protozoology: The study of protozoa.
- Phycology/algology: The study of algae.
- Parasitology: The study of parasites.
- Immunology: The study of the immune system.
- Virology: The study of viruses.
- Nematology: The study of nematodes.

- Microbial cytology: The study of microscopic and submicroscopic details of microorganisms.
- Microbial physiology: The study of how the microbial cell functions biochemically. Includes the study of microbial growth, microbial metabolism and microbial cell structure.
- Microbial ecology: The relationship between microorganisms and their environment.
- Microbial genetics: The study of how genes are organized and regulated in microbes in relation to their cellular functions. Closely related to the field of molecular biology.
- Cellular microbiology: A discipline bridging microbiology and cell biology.
- Evolutionary microbiology: The study of the evolution of microbes. This field can be subdivided into:
  - Microbial taxonomy: The naming and classification of microorganisms.
  - Microbial systematic: The study of the diversity and genetic relationship of microorganisms.
- Generation microbiology: The study of those microorganisms that have the same characters as their parents.
- Systems microbiology: A discipline bridging systems biology and microbiology.
- Molecular microbiology: The study of the molecular principles of the physiological processes in microorganisms.

Other

- Nano microbiology: The study of those organisms on nano level.
- Exo microbiology (or Astro microbiology): The study of microorganisms in outer space (see: List of microorganisms tested in outer space)
- Biological agent: The study of those microorganisms which are being used in weapon industries.

Applied microbiology

- Medical microbiology: The study of the pathogenic microbes and the role of microbes in human illness. Includes the study of microbial pathogenesis andepidemiology and is related to the study of disease pathology and immunology. This area of microbiology also covers the study of human microbiota, cancer, and the tumor microenvironment.
- Pharmaceutical microbiology: The study of microorganisms that are related to the production of antibiotics, enzymes, vitamins,vaccines, and other pharmaceutical products and that cause pharmaceutical contamination and spoil.
- Industrial microbiology: The exploitation of microbes for use in industrial processes. Examples include industrial fermentation and wastewater treatment. Closely linked to the biotechnology industry. This field also includes brewing, an important application of microbiology.
- Microbial biotechnology: The manipulation of microorganisms at the genetic and molecular level to generate useful products.
- Food microbiology: The study of microorganisms causing food spoilage and foodborne illness. Using microorganisms to produce foods, for example by fermentation.
- Agricultural microbiology: The study of agriculturally relevant microorganisms. This field can be further classified into the following:

  - Plant microbiology and Plant pathology: The study of the interactions between microorganisms and plants and plant pathogens.
  - Soil microbiology: The study of those microorganisms that are found in soil.
- Veterinary microbiology: The study of the role of microbes in veterinary medicine or animal taxonomy.
- Environmental microbiology: The study of the function and diversity of microbes in their natural environments. This involves the characterization of key bacterial habitats such as the rhizosphere and phyllosphere, soil and groundwater ecosystems, open oceans or extreme environments (extremophiles). This field includes other branches of microbiology such as:
  - Microbial ecology
  - Microbially mediated nutrient cycling
  - Geomicrobiology
  - Microbial diversity
  - Bioremediation
- Water microbiology (or Aquatic microbiology): The study of those microorganisms that are found in water.
- Aeromicrobiology (or Air microbiology): The study of airborne microorganisms.

Benefits

Fermenting tanks with yeast being used to brew beer

While some fear microbes due to the association of some microbes with various human illnesses, many microbes are also responsible for numerous beneficial processes such as industrial fermentation (e.g. the production of alcohol, vinegar anddairy products), antibiotic production and as vehicles for cloning in more complex organisms such as plants. Scientists have also exploited their knowledge of microbes to produce biotechnologically important enzymes such as Taq polymerase,reporter genes for use in other genetic systems and novel molecular biology techniques such as the yeast two-hybrid system.

Bacteria can be used for the industrial production of amino acids. *Corynebacterium glutamicum* is one of the most important bacterial species with an annual production of more than two million tons of amino acids, mainly L-glutamate and L-lysine.[8]

A variety of biopolymers, such as polysaccharides, polyesters, and polyamides, are produced by microorganisms. Microorganisms are used for the biotechnological production of biopolymers with tailored properties suitable for high-value medical application such as tissue engineering and drug delivery. Microorganisms are used for the biosynthesis

of xanthan,alginate, cellulose, cyanophycin, poly(gamma-glutamic acid), levan, hyaluronic acid, organic acids, oligosaccharides and polysaccharide, and polyhydroxyalkanoates.[9]

Microorganisms are beneficial for microbial biodegradation or bioremediation of domestic, agricultural and industrial wastes and subsurface pollution in soils, sediments and marine environments. The ability of each microorganism to degrade toxic waste depends on the nature of each contaminant. Since sites typically have multiple pollutant types, the most effective approach to microbial biodegradation is to use a mixture of bacterial and fungal species and strains, each specific to thebiodegradation of one or more types of contaminants.[10]

Symbiotic microbial communities are known to confer various benefits to their human and animal hosts health including aiding digestion, production of beneficial vitamins and amino acids, and suppression of pathogenic microbes. Some benefit may be conferred by consuming fermented foods, probiotics (bacteria potentially beneficial to the digestive system) and/or prebiotics (substances consumed to promote the growth of probiotic microorganisms).[11][12] The ways the microbiome influences human and animal health, as well as methods to influence the microbiome are active areas of research.[13]

Research has suggested that microorganisms could be useful in the treatment of cancer. Various strains of non-pathogenic clostridia can infiltrate and replicate within solid tumors. Clostridial vectors can be safely administered and their potential to deliver therapeutic proteins has been demonstrated in a variety of preclinical models.[14]

Scope of microbiology

There is vast scope in the field of microbiology due to the advancement in the field of science and technology. The scope in this field is immense due to the involvement of microbiology in many fields like medicine, pharmacy, diary, industry, clinical research, water industry, agriculture, chemical technology and nanotechnology. The study of microbiology contributes greatly to the understanding of life through enhancements and intervention of microorganisms. There is an increase in demand for microbiologists in India and globally. A microbiologist can innovate new diagnostic kits, discover new drugs, teach, research, etc.

Since the microbes are living, it follows that microbiology deals with a group of particular life forms and it comes under the broad domain of biology which includes the study of all aspects of living beings including man.

Where can we fit in microbes in the hierarchy of living beings? Traditionally living beings are divided into plants and animals. But members of microbes can be accommodated in both plants (fungi) and animals (protozoa) and some cannot be accommodated in either plants or animals as they share the characters of both. (For instance *Euglena*was a disputed property till recently between botanists and zoologists.)

In one of the earlier attempts to resolve this problem, Haeckel (1866) a German Zoologist suggested that there should be a third kingdom besides Plantae (plants) and Animalia (animals) to include all the microorganisms. He gave the name *Protista* to this kingdom to include all unicellular microorganisms that are neither plants nor animals.

Haeckel's classification raised some questions like how to distinguish a fungus from a bacterium or from an alga. The discovery in late 1940s of the prokaryotic and eukaryotic nature of the cells rendered the three kingdom classification unsatisfactory.

A recent and comprehensive classification proposed by R.H. Whittaker (1969) has five kingdoms of living beings

Kingdom Monera

Kingdom Protista

Kingdom Fungi

Kingdom Animalia

Kingdom Plantae

Microorganisms include three of the (Monera, Protista and Fungi) five kingdoms mentioned above. At present it is agreed that within the preview of microbiology, five major groups of microorganisms-viruses, bacteria, fungi, algae and protozoa are dealt with.

As it will be evident from the above discussion, the scope of microbiology extends to both eukaryotic as well as prokaryotic microbes. While discussing the scope of microbiology it should be evident to us that it does not deal with merely the enumeration of structural diversity or classification but extends to all aspects of microbial life. Microbiology is concerned with their form, structure, reproduction, physiology, metabolism classification and most important their economic importance. In other words, what the microbes *can do* and *should not be allowed to do* (some times) as for as human beings are concerned is one of the vital aspects of microbiology on which rests human destiny.

History

Ancient

The existence of microorganisms was hypothesized for many centuries before their actual discovery. The existence of unseen microbiological life was postulated byJainism which is based on Mahavira's teachings as early as 6th century BCE.[15] Paul Dundas notes that Mahavira asserted existence of unseen microbiological creatures living in earth, water, air and fire.[16] Jain scriptures also describe nigodas which are sub-microscopic creatures living in large clusters and having a very short life and are said to pervade each and every part of the universe, even in tissues of plants and flesh of animals.[17]

TIMELINE

MICROBIOLOGY'S 50 **MOST SIGNIFICANT EVENTS** 1875–1995

1875 - Ferdinand J. Cohn contributes to the founding of the science of bacteriology. He publishes an early classification of bacteria using the genus name Bacillus for the first time.

1876 - Robert Koch publishes a paper on his work with anthrax, pointing explicitly to a bacterium as the cause of this disease. This validates the germ theory of disease. His work on anthrax was presented and his papers on the subject were published under the auspices of Ferdinand Cohn.

1878 - Joseph Lister publishes his study of lactic fermentation of milk, demonstrating the specific cause of milk souring. His research is conducted using the first method developed for isolating a pure culture of a bacterium, which he names *Bacterium lactis.*

1880 - Louis Pasteur develops a method of attenuating a virulent pathogen the agent of chicken cholera, so it would immunize and not cause disease. This is the conceptual breakthrough for establishing protection against disease by the inoculation of a weakened strain of the causative agent. Pasteur uses the word "attenuated" to mean weakened. As Pasteur acknowledged, the concept came from Edward Jenner's earlier success at smallpox vaccination.

1881 - Robert Koch struggles with the disadvantages of using liquid media for certain experiments. He seeks out alternatives, and first uses an aseptically cut slice of a potato as a solid culture medium. He also turns to gelatin, which is added to culture media; the resulting mixture is poured onto flat glass plates and allowed to gel. The plate technique is used to isolate pure cultures of bacteria from colonies growing on the surface of the plate.

1882 - Ilya Ilich Metchnikoff demonstrates that certain body cells move to damaged areas of the body where they consume bacteria and other foreign particles. He calls the process phagocytosis. He proposes a theory of cellular immunity. With Paul Ehrlich, Metchnikoff is awarded the Nobel Prize in Medicine or Physiology in 1908.

1884

Robert Koch puts forth a set of postulates, or standards of proof, involving the tubercle bacillus. Koch's postulates are published in *The Etiology of Tuberculosis*, in which he demonstrated three major facts: 1) the presence of the tubercule bacillus (as proved by staining) in tubercular lesions of various organs of humans and animals, 2) the cultivation of the organisms in pure culture on blood serum, and 3) the production of tuberculosis at will by its inoculation into guinea pigs. Koch was awarded the Nobel Prize in Medicine or Physiology in 1905.

1885 - Louis Pasteur oversees injections of the child Joseph Meister with "aged" spinal cord allegedly infected with rabies virus. Pasteur uses the term "virus" meaning poison, but has no idea of the nature of the causitive organism. Although the treatment is successful, the experiment itself is an ethical violation of research standards. Pasteur knew he was giving the child successively more dangerous portions.

1889 - Martinus Beijerinck uses enrichment culture, minus nitrogenous compounds, to obtain a pure culture of the root nodule bacterium *Rhizobium,* demonstrating that enrichment culture creates the conditions for optimal growth of a desired bacterium.

1890 - Emil von Behring and Shibasaburo Kitasato working together in Berlin in 1890 announce the discovery of diphtheria antitoxin serum, the first rational approach to therapy of infectious diseases. They inject a sublethal dose of diphtheria filtrate into animals and produce a serum that is specifically capable of neutralizing the toxin. They then inject the antitoxin serum into an uninfected animal to prevent a subsequent infection. Behring was awarded the Nobel Prize in Medicine or Physiology in 1901.

1890 - Sergei Winogradsky succeeds in isolating nitrifying bacteria from soil. During the period 1890-1891, Winogradsky performs the definitive work on the organisms responsible for the process of nitrification in nature.

1891 - Paul Ehrlich proposes that antibodies are responsible for immunity. He shows that antibodies form against the plant toxins ricin and abrin. With Metchnikoff, Ehrlich is jointly awarded the Nobel Prize in Medicine or Physiology in 1908.

1892 - Dmitri Ivanowski publishes the first evidence of the filterability of a pathogenic agent, the virus of tobacco mosaic disease, launching the field of virology. He passes the agent through candle filters that retain bacteria, but he isn't sure that the agent is a unique organism.

1893 - Theobald Smith and **F.L. Kilbourne** establish that ticks carry *Babesia microti,* which causes babesiosis in animals and humans. This is the first account of a zoonotic disease and also the foundation of all later work on the animal host and the arthropod vector.

1899 - Martinus Beijerinck recognizes "soluble" living microbes, a term he applies to the discovery of tobacco mosaic virus. He demonstrates that juice pressed from tobacco leaves that had been filtered free of bacteria retains the ability to cause disease in plants even after repeated dilutions. He calls the disease agent "contagium vivum fluidium" or contagious living fluid.

1911 - Francis Peyton Rous discovers a virus that can cause cancer in chickens. In 1909, a farmer brought Rous a hen that had a breast tumor. Rous performed an autopsy, extracted tumor cells and injected them into other hens, which

subsequently developed tumors. This is the first experimental proof of an infectious etiologic agent of cancer. Rous is awarded the Nobel Prize in Medicine or Physiology in 1966.

1912 - Paul Ehrlich announces the discovery of an effective cure (Salvarsan) for syphilis, the first specific chemotherapeutic agent for a bacterial disease. Ehrlich was seeking an arsenic derivative and finally the 606th compound worked. He brought news of the treatment to London, where Alexander Fleming became one of the few physicians to administer it.

1915 - Frederick Twort announces the first discovery of bacteriophages, or bacteria-infecting viruses. Twort's discovery was something of an accident. He had spent several years growing viruses and noticed that the bacteria infecting his plates became transparent, indicating that they had been lysed or broken open and destroyed. Felix d'Herrelle independently describes bacterial viruses and coins the term "bacteriophage."

1926 - Albert Jan Kluyver and **Hendrick Jean Louis Donker** propose a universal model for metabolic events in cells based on a transfer of hydrogen atoms. The model applies to aerobic and anaerobic organisms.

1928 - Frederick Griffith discovers transformation in bacteria and establishes the foundation of molecular genetics. He shows that injecting mice with a mixture of live, avirulent, rough *Streptococcus pneumoniae* Type I and heat-killed, virulent smooth *S. pneumoniae* Type II, leads to the death of the mice. Live, virulent, smooth *S. pneumoniae* Type II are isolated from the dead mice

1929 - Alexander Fleming publishes the first paper describing penicillin and its effect on gram-positive microorganisms. This finding is unique since it is a rare example of bacterial lysis and not just microbial antagonism brought on by the mold *Penicillium*. Fleming kept his cultures 2-3 weeks before discarding them. When he looked at one set he noticed that the bacteria seemed to be dissolving and the mold was contaminating the culture. When penicillin is finally produced in major quantities in the 1940s, its power and availability effectively launch the "Antibiotics Era," a major revolution in public health and medicine. With Florey and Chain, Fleming is awarded the Nobel Prize in Medicine or Physiology in 1945.

1931 - C. B. van Niel shows that photosynthetic bacteria use reduced compounds as electron donors without producing oxygen. Sulfur bacteria use H2S as a source of electrons for the fixation of carbon dioxide. He posits that plants use water as a source and release oxygen.

1935 - Gerhard J. Domagk uses a chemically synthesized anti-metabolite, Prontosil, to kill *Streptococcus* in mice. One of the first patients to be treated with Protonsil is Domagk's daughter who has a streptococcal infection that is unresponsive to other treatments. Near death, she is injected with large quantities of Protonsil and makes a dramatic recovery. Domagk is awarded the Nobel Prize in Medicine or Physiology in 1939.

1935 - Wendell Stanley crystallizes tobacco mosaic virus and shows that it remains infectious. However, he does not recognize that the infectious material is nucleic acid and not protein. Together with Northrop and Sumner, Stanley is awarded the Nobel Prize in Chemistry in 1946.

1941 - George Beadle and **Edward Tatum** jointly publish a paper on their experiments using the fungus *Neurospora crassa* to establish that particular genes are expressed through the action of correspondingly specific enzymes. The first gene to be identified controlled the synthesis of an enzyme in a series that led to generation of niacin. This report is the genesis of the "one gene-one enzyme" concept. With Lederberg, Beadle and Tatum are awarded the Nobel Prize in Medicine or Physiology in 1958.

1943 - Salvador Luria and **Max Delbruck** provide a statistical demonstration that inheritance in bacteria follows Darwinian principles. Particular mutants, such as viral resistance, occur randomly in bacterial populations, even in the absence of the virus. More important, they occur in small numbers in some populations and in large numbers in other cultures. With Hershey, Delbruck and Luria are awarded the Nobel Prize in Medicine or Physiology in 1969.

1944 - Oswald Avery, **Colin MacLeod**, and **Maclyn McCarty** show that DNA is the transforming material in cells. They demonstrate that the transformation of *Streptococcus pneumoniae* from an avirulent type to a virulent type is the result of the transfer of DNA from dead smooth organisms to live rough ones. They also show that the transforming principle is destroyed by pancreatic deoxyribonuclease —an enzyme that hydrolyzes DNA —but is not affected by pancreatic ribonuclease or enzymes that destroy proteins.

1944 - Albert Schatz, **E. Bugie** and **Selman Waksman** discover streptomycin, soon to be used against tuberculosis. Streptomycin has the same specific antibiotic effect against gram-negative microorganisms as penicillin does on gram-positive ones. Waksman is awarded the Nobel Prize in Medicine or Physiology in 1952.

1946 - Joshua Lederberg and **Edward L. Tatum** publish the first paper on a type of bacterial mating called conjugation. The proof is based on the generation of daughter cells able to grow in media that cannot support growth of either of the parent cells. Their experiments showed that this type of gene exchange requires direct contact between bacteria. At the time Lederberg began studying with Tatum, scientists believed that bacteria reproduced asexually, but from the work of Beadle and Tatum, Lederberg knew that fungi reproduced sexually and he suspected that bacteria did as well.

1949 - Microbiologist **John Franklin Enders**, virologist **Thomas H. Weller** and physician **Frederick Chapman Robbins** together develop a technique to grow poliovirus in test tube cultures of human tissues. This approach gave virologists a practical tool for the isolation and study of viruses. Enders, Weller and Robbins were awarded the Nobel Prize in Medicine or Physiology in 1954.

1952 - Joshua Lederberg and **Norton Zinder** report on transduction, or transfer of genetic information to cells by viruses. They show that a phage of *Salmonella typhimurium* can carry DNA from one bacterium to another.

1952 - Alfred Hershey and **Martha Chase** suggest that only DNA is needed for viral replication. Using radioactive isotopes 35S to track protein and 32P to track DNA, they show that progeny T2 bacteriophage isolated from lysed bacterial cells have the labeled nucleic acid. Further, most of the labeled protein doesn't enter the cells but remains attached to the bacterial cell membrane.

1953 - Francis Crick and **Maurice Wilkins**, together with James Watson, describe the double-helix structure of DNA. The chemical structure is based on X-ray crystallography of DNA done by Rosalind Franklin. Crick, Wilkins and Watson are awarded the Nobel Prize in Medicine or Physiology in 1962.

1959 - Peter Mitchell proposes the chemiosmotic theory, in which a molecular process is coupled to the transport of protons across a biological membrane. He argues that this principle explains ATP synthesis, solute accumulations or expulsions, and cell movement (flagellar rotation). Mitchell is awarded the Nobel Prize in Chemistry in 1978.

1960 - Francois Jacob, **David Perrin**, **Carmen Sanchez** and **Jacques Monod** propose the operon concept for control of bacteria gene action. Jacob and Monod later propose that a protein repressor blocks RNA synthesis of a specific set of genes, the *lac* operon, unless an inducer, lactose, binds to the repressor. With Lwoff, Jacob and Monod are awarded the Nobel Prize in Medicine or Physiology in 1965.

1961 - Marshall Nirenberg and **J.H. Matthaei** observe that a synthetic polynucleotide, poly U, directs the synthesis of a polypeptide composed only of phenylalanine. They conclude that the nucleotide base triplet UUU must code for phenylalanine. This is the start of successful efforts to decipher the genetic code. With Robert Holley and Har Gobind Khorana, Nirenberg is awarded the Nobel Prize in Medicine or Physiology in 1968.

1961 - Sydney Brenner, **Francois Jacob** and **Matthew Meselson** use phage-infected bacteria to show that ribosomes are the site of protein synthesis and confirm the existence of messenger RNA. They demonstrate that infection of *Escherichia coli* by phage T4 stops cell synthesis of host RNA and leads to T4 RNA synthesis. The T4 RNA attaches to cellular ribosomes and directs protein synthesis.

1964 - Charles Yanofsky and coworkers define the relationship between the order of mutatable sites in the gene coding for the *Escherichia coli* enzyme tryptophan synthetase and the corresponding amino acid replacements in the enzyme. It worked well for tyrptophan synthetase because the enzyme has two subunits, one of which could be mutated. The missense mutants in the alpha subunit could be mapped and related to the genetic fine structure of the gene. The property of correlating a mutation with an amino acid replacement is called colinearity.

1970 - Howard Temin and **David Baltimore** independently discover the enzyme reverse transcriptase in RNA viruses. Reverse transcriptase uses RNA as a template to synthesize a single-stranded DNA complement. This process establishes a pathway for genetic information flow from RNA to DNA. With Dulbecco, Baltimore and Temin are awarded the Nobel Prize in Medicine or Physiology in 1975.

1973 - Stanley Cohen, **Annie Chang**, **Robert Helling** and **Herbert Boyer** show that extrachromosomal bits of DNA called plasmids act as vectors for maintaining cloned genes in bacteria. They show that if DNA is broken into fragments and combined with plasmid DNA, such recombinant DNA molecules will reproduce if inserted into bacterial cells. The discovery is a major breakthrough for genetic engineering, allowing for such advances as gene cloning and the modification of genes

1975 - Georg Kohler and **Cesar Milstein** physically fuse mouse lymphocytes with neoplastic mouse plasma cells to yield hybrid cells called hybridomas that can produce specific antibodies and survive indefinitely in tissue culture. This approach offers a limitless supply of monoclonal antibodies. Monoclonal antibodies permit the generation of diagnostic tests that are highly specific. They also function as probes to study cell function. With Jerne, Kohler and Milstein are awarded the Nobel Prize in Medicine or Physiology in 1984.

1977 - Carl Woese uses ribosomal RNA analysis to recognize a third form of life, the *Archaea,* whose genetic makeup is distinct from but related to both *Bacteria* and *Eucarya.*

1977 - Walter Gilbert and **Fred Sanger** independently develop methods to determine the exact sequence of DNA. Gilbert uses the technique to determine the sequence of the operon of a bacterial genome. Sanger and colleagues use the technique to determine the sequence of all 5,375 nucleotides of the bacteriophage phi-X174, the first complete determination of the genome of an organism. With Paul Berg, Gilbert and Sanger are awarded the Nobel Prize in Chemistry in 1980.

1979 - Smallpox (*variola*) is declared officially eliminated, the last naturally occurring case having been seen in 1977 in Somalia. Small quantities remain held under tightly controlled conditions in the U.S. and former U.S.S.R. Smallpox is the only microbial disease to ever have been deliberately eradicated.

1982 - Stanley Prusiner finds evidence that disease can be caused by a class of infectious proteins he call prions. These abnormal proteins cause scrapie, a fatal neurodegenerative disease of sheep. Prusiner is awarded the Nobel Prize in Medicine or Physiology in 1997.
1983 - Luc Montagnier and **Robert Gallo** announce their discovery of the immunodeficiency virus (HIV) believed to cause AIDS.
1986 - Kary Mullis uses a heat stable enzyme from *Thermus aquaticus* to establish polymerase chain reaction technology. PCR is used to amplify target DNA many-fold. Mullis is awarded the Nobel Prize in Chemistry in 1993.
1995 - Craig Venter, **Hamilton Smith**, **Claire Fraser** and colleagues at TIGR elucidate the first complete genome sequence of a microorganism: *Haemophilus influenza.*

Antonie van Leeuwenhoek

Antonie Philips van Leeuwenhoek - October 24, 1632 – August 26, 1723) was a Dutch tradesman and scientist. He is commonly known as "the Father of Microbiology", and considered to be the first microbiologist. He is best known for his work on the improvement of the microscope and for his contributions towards the establishment of microbiology.

Raised in Delft, Netherlands, Van Leeuwenhoek worked as a draper in his youth, and founded his own shop in 1654. He made a name for himself in municipal politics, and eventually developed an interest in lens making. Using his handcrafted microscopes, he was the first to observe and describe microorganisms, which he originally referred to as *animalcules*. Most of the "animalcules" are now referred to as unicellular organisms though he observed multi cellular organisms in pond water. He was also the first to document microscopic observations of muscle fibers, bacteria, spermatozoa, and blood flow in capillaries (small blood vessels). Van Leeuwenhoek did not author any books; his discoveries came to light through correspondence with the Royal Society, which published his letters.

Early life and career

Antonie van Leeuwenhoek was born in Delft, Dutch Republic, on October 24, 1632. On 4 November he was baptized as *Thonis*. His father, Philips Antonisz van Leeuwenhoek, was a basket maker who died when Antonie was only five years old. His mother, Margaretha (Bel van den Berch), came from a well-to-do brewer's family, and remarried Jacob Jansz Molijn, a painter. Van Leeuwenhoek married Barbara de Mey in July 1654, with whom he would have one surviving daughter, Maria (four other children died in infancy). That same year he returned to Delft, where he would live and study for the rest of his life. He opened a draper's shop, which he ran throughout the 1650s. His wife died in 1666, and in 1671 Van Leeuwenhoek remarried, to Cornelia Swalmius, with whom he had no children.[6] His status in Delft had grown throughout the years. In 1660 he received a lucrative job as chamberlain for the Delft sheriffs' assembly chamber in the City Hall, a position which he would hold for almost 40 years. In 1669 he was appointed as a land surveyor by the Court of Holland; at some time he combined it with another municipal job, being the official "wine-gauger" of Delft and in charge of the city's wine imports[7] and (wine) taxation..

Microscopic study

While running his draper's shop, Van Leeuwenhoek wanted to see the quality of the thread, better than the then-current magnifying lenses available. Therefore, he began to develop an interest in lensmaking, although few records exist of his early activity. Van Leeuwenhoek's interest in microscopes and a familiarity with glass processing led to one of the most significant, and simultaneously well-hidden, technical insights in the history of science. By placing the middle of a small rod of soda lime glass in a hot flame, Van Leeuwenhoek could pull the hot section apart to create two long whiskers of glass. Then, by reinserting the end of one whisker into the flame, he could create a very small, high-quality glass sphere. These spheres became the lenses of his microscopes, with the smallest spheres providing the highest magnifications.

Recognition by the Royal Society

After developing his method for creating powerful lenses and applying them to the study of the microscopic world, Van Leeuwenhoek introduced his work to his friend, the prominent Dutch physician Reinier de Graaf. When the Royal Society in London published the groundbreaking work of an Italian lensmaker in their journal *Philosophical Transactions of the Royal Society*, De Graaf wrote to the journal's editor Henry Oldenburg with a ringing endorsement of Van

Leeuwenhoek's microscopes which, he claimed, "far surpass those which we have hitherto seen". In response the Society published in 1673 a letter from Van Leeuwenhoek, which included his microscopic observations on mold, bees, and lice.[9]

Antonie van Leeuwenhoek was elected to the Royal Society in February 1680 on the nomination of William Croone, a then-prominent physician.[note 3] Van Leeuwenhoek was "taken aback" by the nomination, which he considered a high honor, although he did not attend the induction ceremony in London, nor did he ever attend a Royal Society meeting.[16]

Scientific fame

By the end of the 17th century, Van Leeuwenhoek had a virtual monopoly on microscopic study and discovery. His contemporary Robert Hooke, an early microscope pioneer, bemoaned that the field had come to rest entirely on one man's shoulders.[17] He made about 200 microscopes with different magnification.

On this occasion Van Leeuwenhoek presented the Tsar an "eel-viewer", so Peter could study the blood circulation, whenever he wanted.

Techniques and discoveries

Antonie van Leeuwenhoek made more than 500 optical lenses. He also created at least 25 single-lens microscopes, of differing types, of which only nine survived. These microscopes were made of silver or copper frames, holding hand-made lenses. Those that have survived are capable of magnification up to 275 times. It is suspected that Van Leeuwenhoek possessed some microscopes that could magnify up to 500 times. Although he has been widely regarded as a dilettante or amateur, his scientific research was of remarkably high quality.[20]

The single-lens microscopes of Van Leeuwenhoek were relatively small devices, the biggest being about 5 cm long.[21] They are used by placing the lens very close in front of the eye, while looking in direction of the sun. The other side of the microscope had a pin, where the sample was attached in order to stay close to the lens. There were also three screws that allowed to move the pin, and the sample, along three axes: one axis to change the focus, and the two other axes to navigate through the sample.

Van Leeuwenhoek maintained throughout his life that there are aspects of microscope construction "*which I only keep for myself*", in particular his most

critical secret of how he made the lenses. For many years no-one was able to reconstruct Van Leeuwenhoek's design techniques. However, in 1957 C.L. Stong used thin glass thread fusing instead of polishing, and successfully created some working samples of a Van Leeuwenhoek design microscope.[22] Such a method was also discovered independently by A. Mosolov and A. Belkin at the Russian Novosibirsk State Medical Institute.[23]

Van Leeuwenhoek used samples and measurements to estimate numbers of microorganisms in units of water.[24][25] He also made good use of the huge lead provided by his method. He studied a broad range of microscopic phenomena, and shared the resulting observations freely with groups such as the British Royal Society.[26] Such work firmly established his place in history as one of the first and most important explorers of the microscopic world. Antonie van Leeuwenhoek was one of the first people to observe cells, much like Robert Hooke.

Van Leeuwenhoek's main discoveries are:

- the infusoria (protists in modern zoological classification), in 1674
- the bacteria, (e.g., large Selenomonads from the human mouth), in 1676[27]
- the vacuole of the cell.
- the spermatozoa in 1677.
- the banded pattern of muscular fibers, in 1682.[note 4]

In 1687 Van Leeuwenhoek reported his research on the coffee bean. He roasted the bean, cut it into slices and saw a spongeous interior. The bean was pressed, and an oil appeared. He boiled the coffee with rain water twice, set it aside.[28]

Van Leeuwenhoek's discovery that smaller organisms procreate similarly to larger organisms challenged the contemporary belief, generally held by the 17th century scientific community, that such organisms generated spontaneously. The position of the Church on the exact nature of the spontaneous generation of smaller organisms was ambivalent.[*citation needed*]

Death and legacy

By the end of his life, Van Leeuwenhoek had written approximately 560 letters to the Royal Society The last few contained a precise description of his own illness. He suffered from a rare disease, an uncontrolled movement of the midriff, which is now named *Van Leeuwenhoek's disease*.[35] He died at the age of 90, on August 26, 1723 and was buried four days later in the Oude Kerk (Delft).

Louis Pasteur

Louis Pasteur (/ˈluːi pæˈstɜːr/, French: [lwi pastœʁ]; December 27, 1822 – September 28, 1895) was a French chemist and microbiologist renowned for his discoveries of the principles of vaccination, microbial fermentation and pasteurization. He is best known to the general public for his invention of the technique of treating milk and wine to stop bacterial contamination, a process now called pasteurization. He is regarded as one of the three main founders of bacteriology, together with Ferdinand Cohn and Robert Koch, and is popularly known as the "father of microbiology".[3][4][5]

Pasteur was responsible for disproving the doctrine of spontaneous generation. He performed experiments that showed that without contamination, microorganisms could not develop.

Although Pasteur was not the first to propose the germ theory, Pasteur also made significant discoveries in chemistry, most notably on the molecular basis for the asymmetry of certain crystals and racemization. Early in his career, his investigation of Tartaric acid resulted in the first resolution of what we now call optical isomers. His work led the way to our current understanding of a fundamental principal in the structure of organic compounds.

He was the director of the Pasteur Institute, established in 1887, till his death, and his body lies beneath the institute in a vault covered in depictions of his accomplishments in Byzantine mosaics.[8]

Education and early life

Louis Pasteur was born on December 27, 1822, in Dole, Jura, France, to a Catholic family of a poor tanner. He was the third child of Jean-Joseph Pasteur and Jeanne-Etiennette Roqui. In 1827, the family moved to Arbois, where he entered primary school in 1831. He was an average student in his early years, and not particularly academic, as his interests were fishing and sketching. His pastels and portraits of

his parents and friends, made when he was 15, were later kept in the museum of the Pasteur Institute in Paris. In 1838, he left for Paris to join the Institution Barbet, but became homesick and returned in November. In 1839, he entered the Collège Royal de Besançon and earned his *baccalauréat* (BA) degree in 1840. He was appointed teaching assistant at the Besançon college while continuing a degree science course with special mathematics. He failed his first examination in 1841. He managed to pass the *baccalauréat scientifique* (general science) degree in 1842 from Dijon but with a poor grade in chemistry. After one failed attempt for the entrance test for the *École Normale Supérieure* in Paris in 1842, he succeeded in 1844. In 1845 he received the *licencié ès sciences* (Bachelor of Science) degree.

After serving briefly as professor of physics at the Dijon Lycée in 1848, he became professor of chemistry at the University of Strasbourg, where he met and courted Marie Laurent, daughter of the university's rector in 1849. They were married on May 29, 1849, and together had five children, only two of whom survived to adulthood; the other three died of typhoid. These personal tragedies were his motivations for curing infectious diseases.[3][11]

Career

Pasteur was appointed to the Chair of Chemistry in the faculty of sciences of the University of Strasbourg in 1848. In 1854, he was named dean of the new faculty of sciences at Lille University,)

In 1857, he moved to Paris as the director of scientific studies at the *École Normale Supérieure* where he took control from 1858 to 1867 and introduced a series of reforms to improve the standard of scientific work. In 1862, he was appointed professor of geology, physics, and chemistry at the *École nationale supérieure des Beaux-Arts*, the position which held until his resignation in 1867. In Paris, he established the Pasteur Institute in 1887, in which he was its director for the rest of his life.[4][5][11]

Fermentation and germ theory of diseases

Pasteur demonstrated that fermentation is caused by the growth of micro-organisms, and the emergent growth of bacteria in nutrient broths is due not to spontaneous generation, but rather to biogenesis (*Omne vivum ex vivo* "all life from life"). He was motivated to investigate the matter while working at Lille. In 1856 a local wine manufacturer, M. Bigot, the father of his student, sought for his advice on the problems of making beetroot alcohol and souring after long storage.[21] In 1857 he developed his ideas stating that: "I intend to establish that, just as there is

an alcoholic ferment, the yeast of beer, which is found everywhere that sugar is decomposed into alcohol and carbonic acid, so also there is a particular ferment, a lactic yeast, always present when sugar becomes lactic acid."[22] According to his son-in-law, Pasteur presented his experiment on sour milk titled "Latate Fermentation" in August 1857 before the Société des Sciences de Lille. (But according to a memoire subsequently published, it was dated November 30, 1857).[23][24] It was published in full form in 1858.[25][26][27] He demonstrated that yeast was responsible for fermentation to produce alcohol from sugar, and that air (oxygen) was not required. He also demonstrated that fermentation could also produce lactic acid (due to bacterial contamination), which make wines sour. This is regarded as the foundation of Pasteur's fermentation experiment and disprove of spontaneous generation of life.

Pasteur's research also showed that the growth of micro-organisms was responsible for spoiling beverages, such as beer, wine and milk. With this established, he invented a process in which liquids such as milk were heated to a temperature between 60 and 100 °C.[28] This killed most bacteria and moulds already present within them. Pasteur and Claude Bernard completed the first test on April 20, 1862.[7] Pasteur patented the process, to fight the "diseases" of wine, in 1865.[28] The method became known as pasteurization, and was soon applied to beer and milk.[29]

Beverage contamination led Pasteur to the idea that micro-organisms infecting animals and humans cause disease. He proposed preventing the entry of micro-organisms into the human body, leading Joseph Lister to develop antiseptic methods in surgery. Lister's work in turn inspired Joseph Lawrence to develop his own alcohol-based antiseptic, which he named in tribute Listerine.[30]

In 1865, two parasitic diseases called *pébrine* and *flacherie* were killing great numbers of silkworms at Alais (now Alès). Pasteur worked several years proving that these diseases were caused by a microbe attacking silkworm eggs, and that eliminating the microbe in silkworm nurseries would eradicate the disease.[7]

Spontaneous generation

Following his fermentation experiments, Pasteur demonstrated that the skin of grapes was the natural source of yeasts, and that sterilized grapes and grape juice never fermented. He drew grape juice from under the skin with sterilzed needles, and also covered grapes with sterilized cloth. Both experiments could not produce wine in sterilized containers. His findings and ideas were against the prevailing

notion of spontaneous generation. He received a particularly stern criticism from Félix Archimède Pouchet, who was director of the Rouen Museum of Natural History. To settle the debate between the eminent scientists, the French Academy of Sciences offered Alhumbert Prize carrying 2,500 francs to whoever could experimentally demonstrate for or against the doctrine.[31][32][33]

To prove himself correct, Pasteur exposed boiled broths to air in swan-neck flasks that contained a filter to prevent all particles from passing through to the growth medium, and even in flasks with no filter at all, with air being admitted via a long tortuous tube that would not allow dust particles to pass. Nothing grew in the broths unless the flasks were broken open, showing that the living organisms that grew in such broths came from outside, as spores on dust, rather than spontaneously generated within the broth. This was one of the last and most important experiments disproving the theory of spontaneous generation for which Pasteur won the Alhumbert Prize in 1862. He concluded that:[34][35]

Never will the doctrine of spontaneous generation recover from the mortal blow of this simple experiment. There is no known circumstance in which it can be confirmed that microscopic beings came into the world without germs, without parents similar to themselves.

Immunology and vaccination

Pasteur's later work on diseases included work on chicken cholera. During this work, a culture of the responsible bacteria had spoiled and failed to induce the disease in some chickens he was infecting with the disease. Upon reusing these healthy chickens, Pasteur discovered he could not infect them, even with fresh bacteria; the weakened bacteria had caused the chickens to become immune to the disease, though they had caused only mild symptoms.[3][7]

His assistant, Charles Chamberland (of French origin), had been instructed to inoculate the chickens after Pasteur went on holiday. Chamberland failed to do this, but instead went on holiday himself. On his return, the month-old cultures made the chickens unwell, but instead of the infections being fatal, as they usually were, the chickens recovered completely. Chamberland assumed an error had been made, and wanted to discard the apparently faulty culture when Pasteur stopped him. Pasteur guessed the recovered animals now might be immune to the disease, as were the animals at Eure-et-Loir that had recovered from anthrax.[36]

In the 1870s, he applied this immunization method to anthrax, which affected cattle, and aroused interest in combating other diseases.

Pasteur publicly claimed he had made the anthrax vaccine by exposing the bacilli to oxygen. His laboratory notebooks, now in the Bibliothèque Nationale in Paris, in fact show that he used the method of rival Jean-Joseph-Henri Toussaint, a Toulouse veterinary surgeon, to create the anthrax vaccine.[20][37] This method used the oxidizing agent potassium dichromate. Pasteur's oxygen method did eventually produce a vaccine but only after he had been awarded a patent on the production of an anthrax vaccine.

The notion of a weak form of a disease causing immunity to the virulent version was not new; this had been known for a long time for smallpox. Inoculation with smallpox (Variolation) was known to result in far less scarring, and greatly reduced mortality, in comparison with the naturally acquired disease. Edward Jenner had also discovered vaccination using cowpox (Vaccinia) to give cross-immunity to smallpox in 1796, and by Pasteur's time this had generally replaced the use of actual smallpox (Variola) material in inoculation. The difference between smallpox vaccination and anthrax or chicken cholera vaccination was that the weakened form of the latter two disease organisms had been "generated artificially", so a naturally weak form of the disease organism did not need to be found. This discovery revolutionized work in infectious diseases, and Pasteur gave these artificially weakened diseases the generic name of "vaccines", in honour of Jenner's discovery. Pasteur produced the first vaccine for rabies by growing the virus in rabbits, and then weakening it by drying the affected nerve tissue.[38]

The rabies vaccine was initially created by Emile Roux, a French doctor and a colleague of Pasteur who had been working with a killed vaccine produced by desiccating the spinal cords of infected rabbits. The vaccine had been tested in 50 dogs before its first human trial.[39][40] This vaccine was first used on 9-year old Joseph Meister, on July 6, 1885, after the boy was badly mauled by a rabid dog.[20][38] This was done at some personal risk for Pasteur, since he was not a licensed physician and could have faced prosecution for treating the boy. After consulting with colleagues, he decided to go ahead with the treatment. Three months later he examined Meister and found that he was in good health.[41] Pasteur was hailed as a hero and the legal matter was not pursued. The treatment's success laid the foundations for the manufacture of many other vaccines. The first of the Pasteur Institutes was also built on the basis of this achievement.[20]

Legal risk was not the only kind Pasteur undertook. In *The Story of San Michele*, Axel Munthe writes of the rabies vaccine research:

Pasteur himself was absolutely fearless. Anxious to secure a sample of saliva straight from the jaws of a rabid dog, I once saw him with the glass tube held

between his lips draw a few drops of the deadly saliva from the mouth of a rabid bull-dog, held on the table by two assistants, their hands protected by leather gloves.

Because of his study in germs, Pasteur encouraged doctors to sanitize their hands and equipment before surgery. Prior to this, few doctors or their assistants practiced these procedures.

Fermentation

He regarded himself as the first to show the role of microorganisms in fermentation.[48] Pasteur started his experiments only in 1857 and published his findings in 1858 (April issue of *Comptes Rendus Chimie*, Béchamp's paper appeared in January issue), which, as Béchamp noted, did not bring any novel idea or experiments that earlier works had not shown. Particularly on the spontaneous generation because Pasteur in his 1858 paper explicitly stated that the lactic acid bacteria (he named them "lactic yeasts"), which caused wine souring, "takes birth spontaneously, as easily as beer yeast every time that the conditions are favourable." This statement directly implied that Pasteur did believe in spontaneous generation. He condemned the ideas of Pasteur as "'the greatest scientific silliness of the age".[22] However, Béchamp was on the losing side, as the *BMJ* obituary remarked: His name was associated with bygone controversies as to priority which it would be unprofitable to recall.[51] Pasteur and Béchamp believed that fermentation was exclusively cellular activity, that is, it was only due to living cells. But later extraction of enzymes such as invertase by Marcelin Barthelot in 1860 showed that it was simply an enzymatic reaction.[52]

Anthrax vaccine

Pasteur had given a misleading account of the preparation of the anthrax vaccine used in the experiment at Pouilly-le-Fort.[9] The fact is that Pasteur publicly claimed his success in developing anthrax vaccine in 1881.[41] However, his admirer-turned-rival Toussaint was the one who developed the first vaccine. Toussaint isolated the Gram-negative bacteria *cholera des poules* (later named – to add irony – *Pasteurella* in honour of Pasteur) in 1879 and gave samples to Pasteur who used for his own works. In 1880 with his publishing on July 12 at the French Academy of Sciences, Toussaint presented his successful result with an attenuated vaccine against anthrax in dogs and sheep.[53] Pasteur purely on grounds of jealousy contested the discovery by publicly displaying his vaccination method in Pouilly-le-Fort on 5 May 1881. The promotional experiment was a success and helped Pasteur sell his products, getting all the benefits and glory.[54][55][56]

Experimental ethics

Pasteur experiments are often cited as against medical ethics, especially on his vaccination of Meister. Firstly, he did not have any experience in medical practice, and more importantly, a medical license. This is often cited as a serious threat to his professional and personal reputation.[57][58]

Awards and honours

Pasteur was awarded the prize of 1,500 francs in 1853 by the Pharmaceutical Society for the synthesis of racemic acid. In 1856 the Royal Society of London presented him the Rumford Medal for his discovery of the nature of racemic acid and its relations to polarized light, and the Copley Medal in 1874 for his work on fermentation.

Pasteur Institute

The Pasteur Institute was established by Pasteur to perpetuate his commitment to basic research and its practical applications.

Death

Pasteur was frequently stricken by strokes beginning in 1868, and the one in 1894 severely impaired his health. Failing to fully recover, he died in 1895, near Paris.[20] He was given a state funeral and was buried in the Cathedral of Notre Dame, but his remains were reinterred in a crypt in the Pasteur Institute in Paris, where the crypt is engraved with his life-saving work.

Robert Koch

Robert Heinrich Herman Koch (/ˈkɔːx/;[3] German: [ˈkɔχ]; 11 December 1843 – 27 May 1910) was a celebrated German physician and pioneering microbiologist. As the founder of modern bacteriology, he is known for his role in identifying the specific causative agents of tuberculosis, cholera, and anthrax and for giving experimental support for the concept of infectious disease.[4] In addition to his trail-blazing studies on these diseases, Koch created and improved laboratory technologies and techniques in the field of microbiology, and made key discoveries in public health.[5] His research led to the creation of Koch's postulates, a series of four generalized principles linking specific microorganisms to specific diseases that remain today the "gold standard" in medical microbiology.[5] As a result of his groundbreaking research on tuberculosis, Koch received the Nobel Prize in Physiology or Medicine in 1905.[5]

Personal life

Robert Koch was born in Clausthal, Hanover, Germany, on 11 December 1843, to Hermann Koch and Mathilde Julie Henriette Biewand.[6] Koch excelled in academics from an early age. Before entering school in 1848, he had taught himself how to read and write.[4] He graduated from high school in 1862, having excelled in science and maths.[4] At the age of 19, Koch entered the University of Göttingen, studying natural science.[7] However, after three semesters, Koch decided to change his area of study to medicine, as he aspired to be a physician.[4] During his fifth semester of medical school, Jacob Henle, an anatomist who had published a theory of contagion in 1840, asked him to participate in his research project on uterine nerve structure.[4] In his sixth semester, Koch began to conduct research at the Physiological Institute, where he studied Succinic acid secretion.[4] This would eventually form the basis of his dissertation.[5] In January 1866, Koch graduated from medical school, earning honors of the highest distinction.[4] In July 1867, Koch married Emma Adolfine Josephine Fraatz, and the two had a daughter, Gertrude, in 1868.[5] After his graduation in 1866, he worked as a surgeon in the Franco-Prussian War, and following his service, worked as a physician in Wollstein, Posen.[7] Koch's marriage to Emma Fraatz ended in 1893, and later that same year, he married actress Hedwig Freiberg.[5] From 1885 to 1890, he served as an administrator and professor at Berlin University.[4] Koch suffered a heart attack on 9 April 1910, and never made a complete recovery.[4] On 27 May, only three days after giving a lecture on his tuberculosis research at the Prussian Academy of Sciences, Robert Koch died in Baden-Baden at the age of 66.[7] Following his death, the Institute named its establishment after him in his honour.[4]

Research contributions

Anthrax

Robert Koch is widely known for his work with anthrax, discovering the causative agent of the fatal disease to be *Bacillus anthracis*.[8] Koch discovered the formation in anthrax bacteria of spores that could remain dormant under specific conditions.[7] However, under optimal conditions, the spores were activated and caused disease.[7] To determine this causative agent, he dry-fixed bacterial cultures onto glass slides, used dyes to stain the cultures, and observed them through a microscope.[4] Koch's work with anthrax is notable in that he was the first to link a specific microorganism with a specific disease, rejecting the idea of spontaneous generation and supporting the germ theory of disease.[8]

Koch's four postulates

Koch accepted a position as government advisor with the Imperial Department of Health in 1880.[9] During his time as government advisor, he published a report in which he stated the importance of pure cultures in isolating disease-causing organisms and explained the necessary steps to obtain these cultures, methods which are summarized in Koch's four postulates.[10] Koch's discovery of the causative agent of anthrax led to the formation of a generic set of postulates which can be used in the determination of the cause of most infectious diseases.[8] These postulates, which not only outlined a method for linking cause and effect of an infectious disease but also established the significance of laboratory culture of infectious agents, are listed here:[8]

1. The organism must always be present, in every case of the disease.
2. The organism must be isolated from a host containing the disease and grown in pure culture.
3. Samples of the organism taken from pure culture must cause the same disease when inoculated into a healthy, susceptible animal in the laboratory.
4. The organism must be isolated from the inoculated animal and must be identified as the same original organism first isolated from the originally diseased host.

Isolating pure culture on solid media

Koch began conducting research on microorganisms in a laboratory connected to his patient examination room.[7] Koch's early research in this laboratory proved to yield one of his major contributions to the field of microbiology, as it was there that he developed the technique of growing bacteria. Koch's second postulate calls for the isolation and growth of a selected pathogen in pure laboratory culture.[11] In an attempt to grow bacteria, Koch began to use solid nutrients such as potato slices.[11] Through these initial experiments, Koch observed individual colonies of identical, pure cells.[11] Coming to the conclusion that potato slices were not suitable media for all organisms, Koch later began to use nutrient solutions with gelatin.[11] However, he soon realized that gelatin, like potato slices, was not the optimal medium for bacterial growth, as it did not remain solid at 37 °C, the ideal temperature for growth of most human pathogens.[11] As suggested to him by Walther and Fanny Hesse, Koch began to utilize agar to grow and isolate pure cultures, as this polysaccharide remains solid at 37 °C, is not degraded by most bacteria, and results in a transparent medium.[11][12]

Cholera

Koch next turned his attention to cholera, and began to conduct research in Egypt in the hopes of isolating the causative agent of the disease.[7] However, he was not able to complete the task before the epidemic in Egypt ended, and subsequently traveled to India to continue with the study.[4] In India, Koch was indeed able to determine the causative agent of cholera, isolating *Vibrio cholerae*.[4][13] The bacterium had originally been isolated in 1854 by Italian anatomist Filippo Pacini,[14] but its exact nature and his results were not widely known.

Tuberculosis

During his time as the government advisor with the Imperial Department of Health in Berlin in the 1880s, Robert Koch became interested in tuberculosis research.[4] At the time, it was widely believed that tuberculosis was an inherited disease.[4] However, Koch was convinced that the disease was caused by a bacterium and was infectious, and tested his four postulates using guinea pigs.[4] Through these experiments, he found that his experiments with tuberculosis satisfied all four of his postulates.[4] In 1882, he published his findings on tuberculosis, in which he reported the causative agent of the disease to be the slow-growing *Mycobacterium tuberculosis*.[11] His work with this disease won Koch the Nobel Prize in Physiology and Medicine in 1905.[4] Additionally, Koch's research on tuberculosis, along with his studies on tropical diseases, won him the Prussian Order Pour le Merite in 1906 and the Robert Koch medal, established to honour the greatest living physicians, in 1908.[4]

Awards and honours

In addition to being awarded a Nobel Prize, Koch was elected a Foreign Member of the Royal Society (ForMemRS) in 1897.[2] His microbial postulates are named in his honour, Koch's postulates.

Edward Jenner

Edward Jenner, FRS (/ˈdʒɛnər/; 17 May 1749 – 26 January 1823) was an English physician and scientist who was the pioneer of smallpox vaccine, the world's first vaccine.[1][2] He is often called "the father of immunology", and his work is said to have "saved more lives than the work of any other human".[3][4][5]

He was also the first person to describe the brood parasitism of the cuckoo.

Early life

Edward Anthony Jenner was born on 17 May 1749[6] (6 May Old Style) in Berkeley, Gloucestershire, as the eighth of nine children. His father, the Reverend Stephen Jenner, was the vicar of Berkeley, so Jenner received a strong basic education.[6]

He went to school in Wotton-under-Edge and Cirencester.[6] During this time, he was inoculated for smallpox, which had a lifelong effect upon his general health.[6] At the age of 14, he was apprenticed for seven years to Mr Daniel Ludlow, a surgeon of Chipping Sodbury, South Gloucestershire, where he gained most of the experience needed to become a surgeon himself.[6]

In 1770, Jenner became apprenticed in surgery and anatomy under surgeon John Hunter and others at St George's Hospital.[7] William Osler records that Hunter gave Jenner William Harvey's advice, very famous in medical circles (and characteristic of the Age of Enlightenment), "Don't think; try."[8] Hunter remained in correspondence with Jenner over natural history and proposed him for the Royal Society. Returning to his native countryside by 1773, Jenner became a successful family doctor and surgeon, practising on dedicated premises at Berkeley.

Jenner and others formed the Fleece Medical Society or Gloucestershire Medical Society, so called because it met in the parlour of the Fleece Inn, Rodborough (in Gloucestershire), meeting to dine together and read papers on medical subjects. Jenner contributed papers on angina pectoris, ophthalmia, and cardiac valvular disease and commented on cowpox. He also belonged to a similar society which met in Alveston, near Bristol.[9]

He became a master mason 30 December 1802, in Lodge of Faith and Friendship #449. From 1812–1813, he served as worshipful master of Royal Berkeley Lodge of Faith and Friendship.[10]

Zoology

Jenner was elected fellow of the Royal Society in 1788, following his publication of a careful study of the previously misunderstood life of the nested cuckoo, a study that combined observation, experiment, and dissection.

Marriage and human medicine

Jenner married Catharine Kingscote (died 1815 from tuberculosis) in March 1788. He might have met her while he and other fellows were experimenting with balloons. Jenner's trial balloon descended into Kingscote Park, Gloucestershire, owned by Anthony Kingscote, one of whose daughters was Catharine.[15]

He earned his MD from the University of St Andrews in 1792. He is credited with advancing the understanding of angina pectoris.[16] In his correspondence with Heberden, he wrote, "How much the heart must suffer from the coronary arteries not being able to perform their functions."

Invention of the vaccine

Inoculation was already a standard practice, but involved serious risks. In 1721, Lady Mary Wortley Montagu had imported variolation to Britain after having observed it in Istanbul, where her husband was the British ambassador. Voltaire, writing of this, estimates that at this time 60% of the population caught smallpox and 20% of the population died of it.[17] Voltaire also states that the Circassians used the inoculation from times immemorial, and the custom may have been borrowed by the Turks from the Circassians.[18]

Jenner's Hypothesis:

The initial source of infection was a disease of horses, called "the grease", which was transferred to cattle by farm workers, transformed, and then manifested as cowpox.

Noting the common observation that milkmaids were generally immune to smallpox, Jenner postulated that the pus in the blisters that milkmaids received from cowpox (a disease similar to smallpox, but much less virulent) protected them from smallpox.

On 14 May 1796, Jenner tested his hypothesis by inoculating James Phipps, an eight-year-old boy who was the son of Jenner's gardener. He scraped pus from cowpox blisters on the hands of Sarah Nelmes, a milkmaid who had caught cowpox from a cow called Blossom,[23] whose hide now hangs on the wall of the St George's medical school library (now in Tooting). Phipps was the 17th case described in Jenner's first paper[24] on vaccination.

Jenner inoculated Phipps in both arms that day, subsequently producing in Phipps a fever and some uneasiness, but no full-blown infection. Later, he injected Phipps with variolous material, the routine method of immunization at that time. No disease followed. The boy was later challenged with variolous material and again showed no sign of infection.

Donald Hopkins has written, "Jenner's unique contribution was not that he inoculated a few persons with cowpox, but that he then proved [by subsequent challenges] that they were immune to smallpox. Moreover, he demonstrated that the protective cowpox pus could be effectively inoculated from person to person, not just directly from cattle.[25] Jenner successfully tested his hypothesis on 23 additional subjects.

Jenner continued his research and reported it to the Royal Society, which did not publish the initial paper. After revisions and further investigations, he published his findings on the 23 cases. Some of his conclusions were correct, some erroneous; modern microbiological and microscopic methods would make his studies easier to reproduce. The medical establishment, cautious then as now, deliberated at length over his findings before accepting them. Eventually, vaccination was accepted, and in 1840, the British government banned variolation – the use of smallpox to induce immunity – and provided vaccination using cowpox free of charge. (See Vaccination acts). The success of his discovery soon spread around Europe and, for example, was used *en masse* in the Spanish Balmis Expedition,[26] a three-year-long mission to the Americas, the Philippines, Macao, China, and Saint Helena Island led by Dr. Francisco Javier de Balmis with the aim of giving thousands the smallpox vaccine. The expedition was successful, and Jenner wrote, "I don't imagine the annals of history furnish an example of philanthropy so noble, so extensive as this."

Jenner's continuing work on vaccination prevented him from continuing his ordinary medical practice. He was supported by his colleagues and the King in petitioning Parliament, and was granted £10,000 in 1802 for his work on vaccination. In 1807, he was granted another £20,000 after the Royal College of Physicians had confirmed the widespread efficacy of vaccination.

In 1803 in London, he became president of the **Jennerian Society**, concerned with promoting vaccination to eradicate smallpox. The Jennerian ceased operations in 1809. In 1808, with government aid, the National Vaccine Establishment was founded, but Jenner felt dishonoured by the men selected to run it and resigned his directorship.[27] Jenner became a member of the Medical and Chirurgical Society on its founding in 1805 and presented a number of papers there. The society is now the Royal Society of Medicine. He was elected a foreign honorary member of the American Academy of Arts and Sciences in 1802.[28] In 1806, Jenner was elected a foreign member of the Royal Swedish Academy of Sciences.

Returning to London in 1811, Jenner observed a significant number of cases of smallpox after vaccination. He found that in these cases the severity of the illness was notably diminished by previous vaccination. In 1821, he was appointed physician extraordinary to King George IV, a great national honour, and was also made mayor of Berkeley and justice of the peace. He continued to investigate natural history, and in 1823, the last year of his life, he presented his "Observations on the Migration of Birds" to the Royal Society.

Jenner was found in a state of apoplexy on 25 January 1823, with his right side paralysed. He never fully recovered and eventually died of an apparent stroke, his second, on 26 January 1823, aged 73. He was buried in the Jenner family vault at the Church of St. Mary's, Berkeley, Gloucestershire.[29]

Edward Jenner was survived by one son and one daughter, his elder son having died of tuberculosis at the age of 21.

Legacy

In 1979, the World Health Organization declared smallpox an eradicated disease.[33] This was the result of coordinated public health efforts by many people, but vaccination was an essential component.

Alexander Fleming

Born	6 August 1872 Lochfield, East Ayrshire, Scotland
Died	11 March 1955 (aged 73) London, England
Citizenship	British
Fields	Bacteriology, immunology
Alma mater	• Royal Polytechnic Institution • St Mary's Hospital Medical School • Imperial College London
Known for	Discovery of penicillin

Sir Alexander Fleming FRS FRSE FRCS[1] (6 August 1881 – 11 March 1958) was a Scottish biologist, pharmacologist and botanist. His best-known discoveries are the enzyme lysozyme in 1923 and the antibiotic substance benzylpenicillin (Penicillin G) from the mould *Penicillium notatum* in 1928, for which he shared the Nobel Prize in Physiology or Medicine in 1945 with Howard Florey and Ernst Boris Chain.[2][3][4][5][6][7] He wrote many articles on bacteriology, immunology, and chemotherapy.

Early life and education

Born on 6 August 1881 at Lochfield farm near Darvel, in Ayrshire, Scotland, Alexander was the third of the four children of farmer Hugh Fleming (1816–1888) from his second marriage to Grace Stirling Morton (1848–1928), the daughter of a neighbouring farmer. Hugh Fleming had four surviving children from his first marriage. He was 59 at the time of his second marriage, and died when Alexander (known as Alex) was seven.

Fleming went to Loudoun Moor School and Darvel School, and earned a two-year scholarship to Kilmarnock Academy before moving to London, where he attended the Royal Polytechnic Institution.[8] After working in a shipping office for four years, the twenty-year-old Fleming inherited some money from an uncle, John Fleming. His elder brother, Tom, was already a physician and suggested to him

that he should follow the same career, and so in 1903, the younger Alexander enrolled at St Mary's Hospital Medical School in Paddington; he qualified with an MBBS degree from the school with distinction in 1906.

Fleming had been a private in the London Scottish Regiment of the Volunteer Force since 1900,[2] and had been a member of the rifle club at the medical school. The captain of the club, wishing to retain Fleming in the team suggested that he join the research department at St Mary's, where he became assistant bacteriologist to Sir Almroth Wright, a pioneer in vaccine therapy and immunology. In 1908, he gained a BSc degree with Gold Medal in Bacteriology, and became a lecturer at St Mary's until 1914. Fleming served throughout World War I as a captain in the Royal Army Medical Corps, and was Mentioned in Dispatches. He and many of his colleagues worked in battlefield hospitals at the Western Front in France. In 1918 he returned to St Mary's Hospital, where he was elected Professor of Bacteriology of the University of London in 1928. In 1951 he was elected the Rector of the University of Edinburgh for a term of 3 years.

Research

Following World War I, Fleming actively searched for anti-bacterial agents, having witnessed the death of many soldiers from sepsis resulting from infected wounds. Antiseptics killed the patients' immunological defences more effectively than they killed the invading bacteria. In an article he submitted for the medical journal *The Lancet* during World War I, Fleming described an ingenious experiment, which he was able to conduct as a result of his own glass blowing skills, in which he explained why antiseptics were killing more soldiers than infection itself during World War I. Antiseptics worked well on the surface, but deep wounds tended to shelter anaerobic bacteria from the antiseptic agent, and antiseptics seemed to remove beneficial agents produced that protected the patients At St Mary’s Hospital Fleming continued his investigations into antibacterial substances. Testing the nasal secretions from a patient with a heavy cold, he found that nasal mucus had an inhibitory effect on bacterial growth.[10] This was the first recorded discovery of lysozyme, an enzyme present in many secretions including tears, saliva, human milk as well as mucus. Lysozyme degrades the bonds in bacterial peptidoglycan cell walls, particularly in Gram-positive organisms. Unfortunately, lysozyme had little therapeutic potential.

Accidental discovery

"When I woke up just after dawn on September 28, 1928, I certainly didn't plan to revolutionise all medicine by discovering the world's first antibiotic, or bacteria killer," Fleming would later say, "But I suppose that was exactly what I did."[11]

By 1927, Fleming had been investigating the properties of staphylococci. He was already well-known from his earlier work, and had developed a reputation as a brilliant researcher, but his laboratory was often untidy. On 3 September 1928, Fleming returned to his laboratory having spent August on holiday with his family. Before leaving, he had stacked all his cultures of staphylococci on a bench in a corner of his laboratory. On returning, Fleming noticed that one culture was contaminated with a fungus, and that the colonies of staphylococci immediately surrounding the fungus had been destroyed, whereas other staphylococci colonies farther away were normal, famously remarking *"That's funny"*.[12] Fleming showed the contaminated culture to his former assistant Merlin Price, who reminded him, "That's how you discovered lysozyme."[13] Fleming grew the mould in a pure culture and found that it produced a substance that killed a number of disease-causing bacteria. He identified the mould as being from the *Penicillium* genus, and, after some months of calling it *"mould juice"*, named the substance it released *penicillin* on 7 March 1929.[14] The laboratory in which Fleming discovered and tested penicillin is preserved as the Alexander Fleming Laboratory Museum in St. Mary's Hospital, Paddington.

He investigated its positive anti-bacterial effect on many organisms, and noticed that it affected bacteria such as staphylococci and many other Gram-positive pathogens that cause scarlet fever, pneumonia, meningitis and diphtheria, but not typhoid fever or paratyphoid fever, which are caused by Gram-negative bacteria, for which he was seeking a cure at the time. It also affected *Neisseria gonorrhoeae*, which causes gonorrhoea although this bacterium is Gram-negative.

Fleming published his discovery in 1929, in the British *Journal of Experimental Pathology*,[15] but little attention was paid to his article. Fleming continued his investigations, but found that cultivating *penicillium* was quite difficult, and that after having grown the mould, it was even more difficult to isolate the antibiotic agent. Fleming's impression was that because of the problem of producing it in quantity, and because its action appeared to be rather slow, penicillin would not be important in treating infection. Fleming also became convinced that penicillin would not last long enough in the human body (*in vivo*) to kill bacteria effectively. Many clinical tests were inconclusive, probably because it had been used as a surface antiseptic. They started mass production after the bombing of Pearl Harbor.

By D-Day in 1944, enough penicillin had been produced to treat all the wounded with the Allied forces.

Purification and stabilisation

In Oxford, Ernst Boris Chain and Edward Abraham discovered how to isolate and concentrate penicillin. Abraham was the first to propose the correct structure of penicillin.[17][18] Shortly after the team published its first results in 1940, Fleming telephoned Howard Florey, Chain's head of department, to say that he would be visiting within the next few days. When Chain heard that Fleming was coming, he remarked *"Good God! I thought he was dead."*

Norman Heatley suggested transferring the active ingredient of penicillin back into water by changing its acidity. This produced enough of the drug to begin testing on animals. There were many more people involved in the Oxford team, and at one point the entire Dunn School was involved in its production.

After the team had developed a method of purifying penicillin to an effective first stable form in 1940, several clinical trials ensued, and their amazing success inspired the team to develop methods for mass production and mass distribution in 1945.

Fleming was modest about his part in the development of penicillin, describing his fame as the *"Fleming Myth"* and he praised Florey and Chain for transforming the laboratory curiosity into a practical drug. Fleming was the first to discover the properties of the active substance, giving him the privilege of naming it: penicillin. He also kept, grew, and distributed the original mould for twelve years, and continued until 1940 to try to get help from any chemist who had enough skill to make penicillin. But Sir Henry Harris said in 1998: "Without Fleming, no Chain; without Chain, no Florey; without Florey, no Heatley; without Heatley, no penicillin."[19]

Antibiotics

Fleming's accidental discovery and isolation of penicillin in September 1928 marks the start of modern antibiotics. Before that, several scientists had published or pointed out that mould or *penicillium sp.* were able to inhibit bacterial growth, and even to cure bacterial infections in animals. Ernest Duchesne in 1897 in his thesis "Contribution to the study of vital competition in micro-organisms: antagonism between moulds and microbes",[20] or also Clodomiro Picado Twight whose work at Institut Pasteur in 1923 on the inhibiting action of fungi of the "Penicillin sp"

genre in the growth of staphylococci drew little interest from the direction of the Institut at the time. Fleming was the first to push these studies further by isolating the penicillin, and by being motivated enough to promote his discovery at a larger scale. Fleming also discovered very early that bacteria developed antibiotic resistance whenever too little penicillin was used or when it was used for too short a period. Almroth Wright had predicted antibiotic resistance even before it was noticed during experiments. Fleming cautioned about the use of penicillin in his many speeches around the world. He cautioned not to use penicillin unless there was a properly diagnosed reason for it to be used, and that if it were used, never to use too little, or for too short a period, since these are the circumstances under which bacterial resistance to antibiotics develops.

Personal life

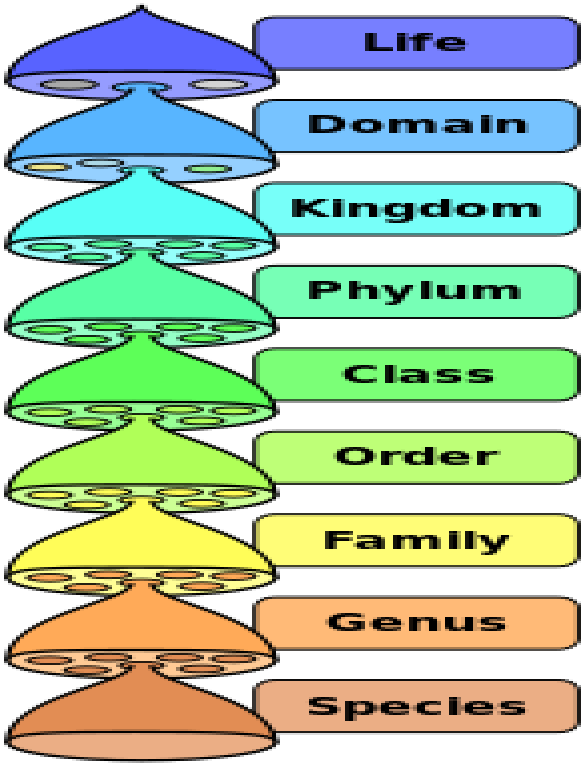
On 24 December 1915, Fleming married a trained nurse, Sarah Marion McElroy of Killala, County Mayo, Ireland. Their only child, Robert Fleming, (1924 - 2 July 2015) became a general medical practitioner. Robert married Kathleen, a trained radiographer on 10 September 1955 and had two children: Andrew (b. 1956) and Sarah (b. 1959).

Fleming has two great grandsons (James and Christopher) and one great granddaughter (Claire). After Sarah's death in 1949, Fleming married Dr. Amalia Koutsouri-Vourekas, a Greek colleague at St. Mary's, on 9 April 1953; she died in 1986.[*citation needed*]

Death

On 11 March 1955, Fleming died at his home in London of a heart attack. He was buried in St Paul's Cathedral

Classification of microorganisms



In biology, **kingdom** (Latin: ***regnum***, pl. ***regna***) is a taxonomic rank, which is either the highest rank or in the more recent three-domain system, the rank below domain. Kingdoms are divided into smaller groups called phyla (in zoology) or divisions in botany.

Definition and associated terms

When Carl Linnaeus introduced the rank-based system of nomenclature into biology, the highest rank was given the name "kingdom" and was followed by four other main or principal ranks.[1] Later two further main ranks were introduced, making the sequence kingdom, phylum or division, class, order, family, genus and species.[2] In the 1960s a rank was introduced above kingdom, namely domain (or empire), so that kingdom is no longer the highest rank.

Systems of classification

Historically, the number of kingdoms in widely accepted classifications has grown from two to six. However, phylogenetic research from about 2000 onwards does not support any of the traditional systems[*citation needed*].

Classical classification

An initial dichotomy: Two kingdoms

The classification of living things into animals and plants is an ancient one. Aristotle (384–322 BC) classified animal species in his work *The History of Animals*, and his pupil Theophrastus (c. 371–c. 287 BC) wrote a parallel work on plants (*Historia Plantarum* (The History of Plants)).[4]

Carolus Linnaeus (1707–1778) laid the foundations for modern biological nomenclature, now regulated by the Nomenclature Codes. He distinguished two kingdoms of living things: *Regnum Animale* ('animal kingdom') for animals and *Regnum Vegetabile* ('vegetable kingdom') for plants. (Linnaeus also included minerals, placing them in a third kingdom, *Regnum Lapideum*.) Linnaeus divided each kingdom into classes, later grouped into phyla for animals and divisions for plants.

life
- Regnum Vegetabile
- Regnum Animalia

An increasing number of kingdoms

Three kingdoms

In 1674, Antonie van Leeuwenhoek, often called the "father of microscopy", sent the Royal Society of London a copy of his first observations of microscopic single-celled organisms. Until then, the existence of such microscopic organisms was entirely unknown. In 1866, following earlier proposals by Richard Owen and Ernst Haeckel proposed a third kingdom of life. Haeckel revised the content of this kingdom a number of times before settling on a division based on whether organisms were unicellular (Protista) or multicellular (animals and plants).[5]

life
- Kingdom Protista
- Kingdom Plantae
- Kingdom Animalia

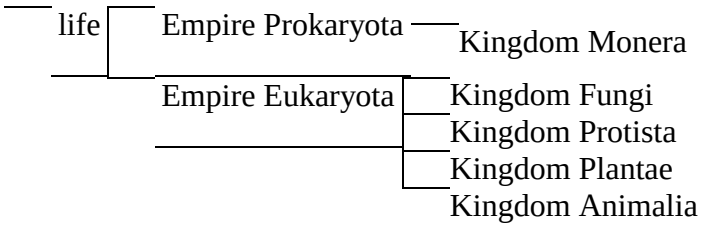
Four kingdoms

The development of microscopy, and the electron microscope in particular, revealed an important distinction between those unicellular organisms whose cells do not have a distinct nucleus, prokaryotes, and those unicellular and multicellular organisms whose cells do have a distinct nucleus, eukaryotes. In 1938, Herbert F. Copeland proposed a four-kingdom classification, moving the two prokaryotic groups, bacteria and "blue-green algae", into a separate Kingdom Monera.[5]

life
- Kingdom Monera (prokaryotes, i.e. bacteria and "blue-green algae")
- Kingdom Protista (single-celled eukaryotes)
- Kingdom Plantae
- Kingdom Animalia

Five kingdoms (Whittaker system)

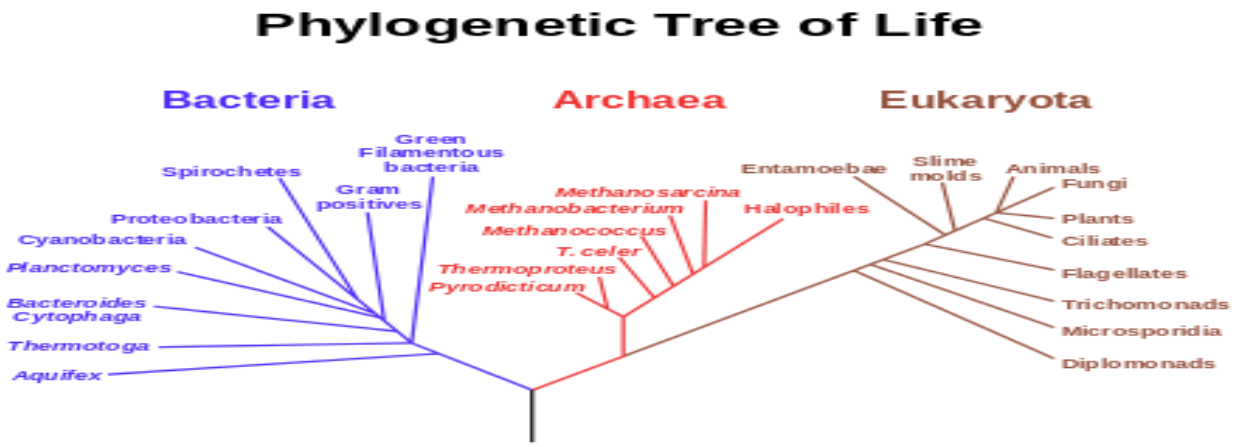
The differences between fungi and other organisms regarded as plants had long been recognized. For example, at one point Haeckel moved the fungi out of Plantae into Protista, before changing his mind.[5] Robert Whittaker recognized an additional kingdom for the Fungi. The resulting five-kingdom system, proposed in 1969 by Whittaker, has become a popular standard and with some refinement is still used in many works and forms the basis for new multi-kingdom systems. It is based mainly on differences in nutrition; his Plantae were mostly multicellular autotrophs, his Animalia multicellular heterotrophs, and his Fungi multicellular saprotrophs. The remaining two kingdoms, Protista and Monera, included unicellular and simple cellular colonies.[7] The five kingdom system may be combined with the two empire system.



In the Whittaker system, Plantae included some algae. In other systems (e.g., Margulis system), Plantae included just the land plants (Embryophyta).

Recent developments: six kingdoms or more?

The three domains of life



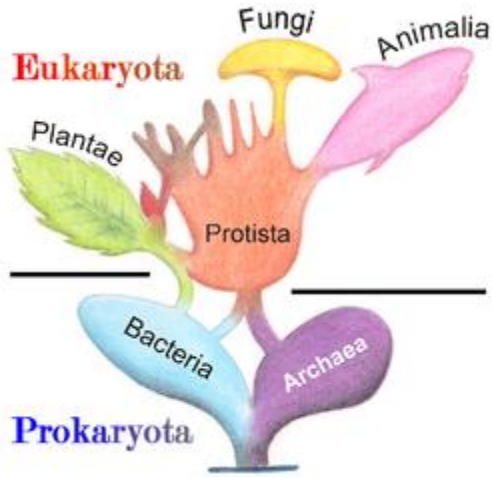
A phylogenetic tree based on rRNA data showing Woese's three-domain system

From around the mid-1970s onwards, there was an increasing emphasis on comparisons of genes on the molecular level (initially ribosomal RNA genes) as the primary factor in classification; genetic similarity was stressed over outward appearances and behavior. Taxonomic ranks, including kingdoms, were to be groups of organisms with a common ancestor, whether monophyletic (*all* descendants of a common ancestor) or paraphyletic (*only some* descendants of a common ancestor).

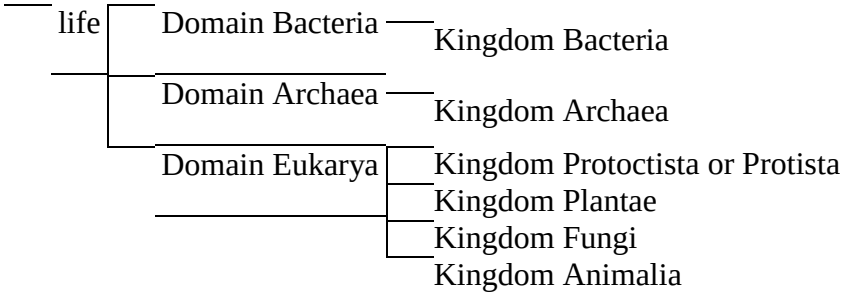
Based on such RNA studies, Carl Woese divided the prokaryotes (hitherto classified as the Kingdom Monera) into two groups, called Eubacteria and Archaebacteria, stressing that there was as much genetic difference between these two groups as between either of them and all eukaryotes. Similarly, though eukaryote groups such as plants, fungi and animals may look different, they are more closely related to each other from a genetic standpoint than they are to either the Eubacteria or Archaebacteria. It was also found that the eukaryotes are more closely related, genetically, to the Archaebacteria than they are to the Eubacteria.

Although the primacy of the eubacteria-archaebacteria divide has been questioned, it has been upheld by subsequent research.[8]

Woese attempted to establish a "three primary kingdom" or "urkingdom" system.[9] In 1990, the name "domain" was proposed for the highest rank.[10] The six-kingdom system shown below represents a blending of the classic five-kingdom system and Woese's three-domain system. Such six-kingdom systems have become standard in many works.

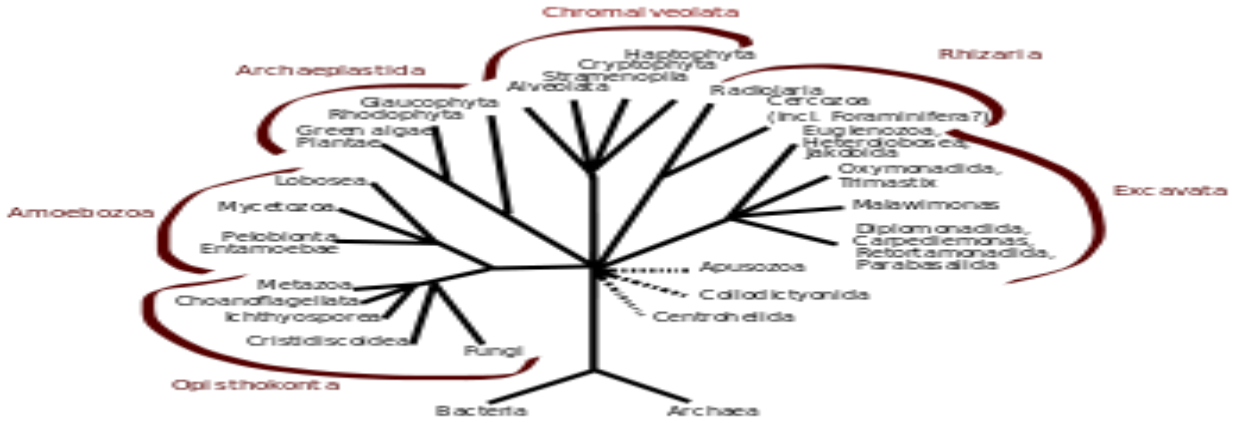


Phylogenetic and symbiogenetic tree of living organisms, showing the origins of eukaryotes[11] and evolutionary relationships between groups.



Woese also recognized that the kingdom Protista was not a monophyletic group and might be further divided at the level of kingdom.

International Society of Protistologists Classification (2005)



One hypothesis of eukaryotic relationships, modified from Simpson and Roger (2004).

The "classic" six-kingdom system is still recognizably a modification of the original two-kingdom system: Animalia remains; the original category of plants has been split into Plantae and Fungi; and single-celled organisms have been introduced and split into Bacteria, Archaea and Protista.

- life
  - Domain Bacteria
    - Bacteria
  - Domain Archaea
    - Archaea
  - Domain Eukarya
    - Excavata — Various flagellate protozoa
    - Amoebozoa — most lobose amoeboids and slime moulds
    - Opisthokonta — animals, fungi, choanoflagellates, etc.
    - Rhizaria — Foraminifera, Radiolaria, and various other amoeboid protozoa
    - Chromalveolata — Stramenopiles (or Heterokonta), Haptophyta, Cryptophyta (or cryptomonads), and Alveolata
    - Archaeplastida (or Primoplantae) — Land plants, green algae, red algae, and glaucophytes

In this system, the traditional kingdoms have vanished. For example, research shows that the multicellular animals (Metazoa) are descended from the same ancestor as the unicellular choanoflagellates and the fungi. A classification system which places these three groups into different kingdoms (with multicellular animals forming Animalia, choanoflagellates part of Protista and Fungi a separate kingdom) is not monophyletic. The monophyletic group is the Opisthokonta, made up of all those organisms believed to have descended from a common ancestor, some of which are unicellular (choanoflagellates), some of which are multicellular but

not closely related to animals (some fungi), and others of which are traditional multicellular animals.[16]

Summary

The sequence from the two-kingdom system up to Cavalier-Smith's six-kingdom system can be summarized in the table below.

Linnaeus 1735[1]	**Haeckel 1866**[26]	**Chatton 1925**[27][28]	**Copeland 1938**[29][30]	**Whittaker 1969**[7]	**Woese et al. 1977**[9][31]	**Woese et al. 1990**[32]	**Cavalier-Smith 1993**[33][34][35]	**Cavalier-Smith 1998**[36][14][37]
2 kingdoms	3 kingdoms	2 empires	4 kingdoms	5 kingdoms	6 kingdoms	3 domains	8 kingdoms	6 kingdoms
(not treated)	Protista	Prokaryota	Monera	Monera	Eubacteria	Bacteria	Eubacteria	Bacteria
					Archaebacteria	Archaea	Archaebacteria	
		Eukaryota	Protoctista	Protista	Protista	Eucarya	Archezoa	Protozoa
							Protozoa	
							Chromista	Chromista
Vegetabilia	Plantae		Plantae	Plantae	Plantae		Plantae	Plantae
				Fungi	Fungi		Fungi	Fungi
Animalia	Animalia		Animalia	Animalia	Animalia		Animalia	Animalia

- Kingdoms such as Bacteria represent grades rather than clades, and so are rejected by phylogenetic classification systems.
- The most recent research does not support the classification of the eukaryotes into any of the standard systems. As of April 2010, no set of kingdoms is sufficiently supported by research to attain widespread acceptance. In 2009, Andrew Roger and Alastair Simpson emphasized the need for diligence in analyzing new discoveries: "With the current pace of change in our understanding of the eukaryote tree of life, we should proceed with caution."[38]

Viruses

There is ongoing debate as to whether viruses, obligate intracellular parasites that are not capable of replication outside of a host, can be included in the tree of life.[39] [40] A principal reason for

inclusion comes from the discovery of unusually large and complex viruses, such as Mimivirus, that possess typical cellular genes.[41]

Current classification

Knowledge of the phenotypic, genotypic and biological characteristics of a microorganism is imperative in differentiating it from its pathogenic and/or toxigenic relatives or other microorganisms that are detrimental to the health of plants, animals, humans and the environment.

Polyphasic approach to microbial identification

The selection of method(s) used for microbial identification depends on the type and nature of the microorganism. The method(s) chosen should be well-described in scientific literature and consistent with those currently used in the field of microbial identification and taxonomic classification and they must enable identification of the organisms to the genus and species and, if possible, strain level. The robustness, precision and validity of the methodologies used to identify the microorganism are critical elements in the assessment of the safety of the product.

The choice of methods for microbial identification is at the discretion of the product proponent. However, the Fertilizer Safety Office recommends that applicants adopt an integrated polyphasic approach that includes classical microbiological and phenotypic analysis along with molecular tools, to accurately identify the active microorganism(s). The strengths and weaknesses of the various identification methods should be taken into consideration, such that the methods chosen complement each other to result in a conclusive and definitive identification of the microorganism, and allow for clear differentiation of the organism from any closely related pathogenic and/or toxigenic species and strains. The methods commonly used in identification and substantiation of taxonomic classification of microorganisms are summarized below.

1. Phenotypic analysis

Preliminary analysis in microbial identification often involves one or more phenotypic methods. Phenotypic methods are suitable for microorganisms that are culturable (i.e., can grow as pure culture on artificial media), have well-established growth parameters, and physiological and biochemical profiles.

a. Analysis of morphological traits

These methods utilize colony and cell morphology to obtain an initial identification of a microorganism. This is accomplished through simple isolation and culturing of the microorganism and subsequent visual observation using microscopy. The morphological properties include:

1. shape,
2. size,
3. surface characteristics and pigmentation,
4. cell wall characteristics (Gram-staining),
5. sporulation characteristics,
6. mechanisms of motility, and
7. other cellular inclusions and ultrastructural characteristics.

b. Analysis of biochemical, physiological and metabolic characteristics

Phenotypic identification methods include the study of the biochemical profile and metabolic properties of a microorganism by testing its growth requirements, enzymatic activities and cellular fatty acid composition.

The biochemical tests use specific growth media, nutrients, chemicals or growth conditions to elicit an observable or measurable biochemical response from the microorganism, thereby enabling its identification and characterization. These tests include: utilization of carbon and nitrogen sources, growth requirements (anaerobic or aerobic; temperature-optimum and range, pH optimum and range), preferred osmotic conditions, generation of fermentation products, production of enzymes, production of antimicrobial compounds, as well as sensitivity to metabolic inhibitors and antibiotics. Examples of recognized tests include: phenol red carbohydrate, catalase and oxidase tests, oxidation-fermentation tests, methyl red tests, Voges-Proskauer tests, nitrate reduction, starch hydrolysis, tryptophan hydrolysis, hydrogen sulfide production, citrate utilization, litmus milk reactions, etc. Several miniaturized and automated commercial systems are currently available with well-defined quality control procedures that allow for rapid identification of microorganisms.

c. Analysis of Fatty Acid Methyl Ester composition (FAME analysis)

Microorganisms can be identified by analysing the fatty acid profiles of whole cells or cell membranes using gas-liquid chromatography or mass spectrometry. The data on the type, content, proportion and variation in the fatty acid profile are used to identify and characterize the genus and species by comparing it against the fatty acid profiles of known organisms.

The expression of microbial phenotypes is highly dependent on environmental variables (e.g., culture pH, temperature, selective vs non-selective media, depletion of nutrients, presence of stressors etc.), and thus, may introduce inconsistencies in the identification process. The phenotypic methods are only acceptable if the response criteria are sufficient to identify the microorganism with a high level of confidence and distinguish it from phylogenetically close relatives that potentially pose safety concerns. Also, the applicability of the method is based on the robustness of information in reference databases. As such, results from phenotypic methods may require supporting data from other methods to accurately identify a microorganism.

2. Molecular Methods

Development of molecular methods has greatly improved the ability to rapidly detect, identify and classify microorganisms and also establish the taxonomic relationship among closely related genera and species. Identification, using molecular methods, relies on the comparison of the nucleic acid sequences (DNA, RNA) or protein profiles of a microorganism with documented data on known organisms. The molecular methods are considered sensitive enough to allow detection of low concentrations of viable or non-viable microorganisms in both pure cultures and complex samples (e.g., soil, peat, wateretc.).

a. Genotypic methods

These include methods such as nucleic acid hybridization (Southern blot analysis or Solution-phase hybridization) and amplification-based or polymerase chain reaction (PCR) technologies. The latter consist of sequence comparisons of conserved genomic regions such as 16S or 18S rRNA, or comparisons of restriction fragment length polymorphisms (RFLP), amplified

fragment length polymorphisms (AFLP) or G+C % content in the genomic DNA with corresponding data on known organisms.

Reliable genotypic identification requires databases with accurate and complete sequence information from a large number of taxa. The commonly used gene sequence databases include:

- GenBank®[Footnote 2];
- Ribosomal Database Project (RDP)[Footnote 3];
- Europe's collection of nucleotide sequence data (EMBL)[Footnote 4]; and
- Universal Protein Resource (UniProt)[Footnote 5].

Product proponents are not limited to using the reference materials listed above; these are intended as guidance only.

Some of the limitations associated with genotypic methods also include: difficulties in differentiating between species that share identical and/or similar conserved region sequences, limited information on the quality of sequence data available in public databases and the complexity of taxonomic nomenclature overall. Considering the above, it is important to validate the results of genotypic microbial identification methods with data from other sources (e.g., morphological and/or phenotypic analysis).

b. Protein based methods

Serological methods such as Western blotting, Immuno-precipitation and Enzyme Linked Immuno-sorbant Assay (ELISA) use antibodies to detect specific proteins that are unique and/or characteristic of a microorganism. The applicability of serological methods is dependent on the availability, sensitivity and specificity of the antibodies used. There are commercial kits available for immuno-detection of several microorganisms. Protein based methods also include gel electrophoresis (SDS-PAGE, 2D-gels, etc.) that can separate cellular proteins on a defined matrix and identify microbial proteins of interest by comparing with microorganisms with known protein profiles.

3. Genomics

More recently, complete profiling of the transcriptome, genome, proteome or metabolome have been used to identify and characterise organisms. Several modern technologies such as DNA and protein microarray analyses, mass spectral protein profiling, nuclear magnetic resonance (NMR) spectral analysis, in-silico microbial metabolome platforms are increasingly used in identification and characterization of microorganisms.

The knowledge of the sensitivity and specificity of genomic tools and their application in microbial identification is rapidly evolving. However, challenges related to standardization of genomics methodologies (including optimization of protocols and bioinformatics tools for reliable data annotation, interpretation etc.) continue to hinder their applicability in safety (risk) assessment and regulatory decision making. The Fertilizer Safety Office will consider data generated by genomics-methods on a case-by-case basis. However, validation of genomics data using alternate methods is currently required to substantiate the identification and taxonomic classification of an active microorganism(s) in a supplement product.

II. Taxonomic Classification and Nomenclature:

The taxonomic identification of the microorganism(s) should be based on the currently used and internationally accepted taxonomic classification system. The description of the microorganism(s) in the product and its characteristics must correspond to the characteristics described in standard resources and/or references that are commonly used by the scientific community to validate taxonomic classification. These can include but are not limited to:

- textbooks such as the Bergey's Manual of Systematic Bacteriology[Footnote 6];
- The Prokaryotes[Footnote 7];
- Applied Microbial Systematics[Footnote 8];
- Principles of fungal taxonomy[Footnote 9] etc.;
- online resources such as the Catalogue of Life[Footnote 10];
- PubMed Taxonomy[Footnote 11] and UniProt Taxonomy[Footnote 12] etc.; and
- peer reviewed journals.

The taxonomic name should follow the nomenclature code officially recognized by the International Committee on Systematics of Prokaryotes (ICSP). Applicants should verify the "Approved List of Bacterial Names" to ensure that the nomenclature is in accordance with the latest Validation List developed and updated by the International Journal of Systematic and Evolutionary Microbiology (IJSEM).

Please note that microbial taxonomic classification and nomenclature, particularly for bacteria, is in a constant state of flux as methodologies evolve to generate more reliable information to identify/classify and/or reclassify the current taxonomic scheme. Cross referencing more than one resource/reference will help in validating the current taxonomic designation and classification of a microorganism.

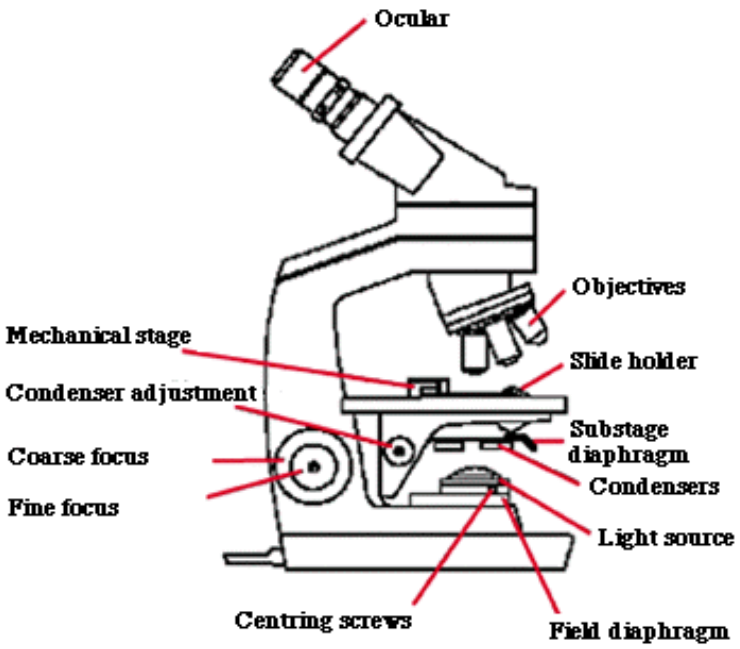
Methods of Identifying microbes

Based on

1. **Morphological characteristics – shape and size – staining techniques**
2. **Chemical characteristics - microscopic observation**
3. **Cultural characteristics - spread , pour and streak plate method**
4. **Metabolic characteristics – biochemical tests**
5. **Antigenic characteristics – Ag –Ab reactions**
6. **Genetic characteristics - DNA sequencing**
7. **Pathogenic characteristics - Infection to human and plants**
8. **Ecological characteristics - climatic conditions**

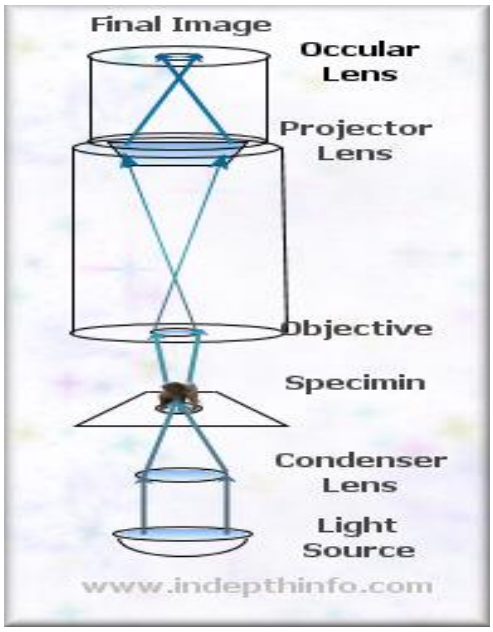
The Basics of Microscopy.

Brightfield Microscopy



The Basics of Brightfield Microscopy

Bright field microscopy is the simplest of all the optical microscopy illumination techniques. Sample illumination is transmitted (i.e., illuminated from below and observed from above) white light and contrast in the sample is caused by absorbance of some of the transmitted light in dense areas of the sample. Bright field microscopy is the simplest of a range of techniques used for illumination of samples in light microscopes and its simplicity makes it a popular technique. The typical appearance of a bright field microscopy image is a dark sample on a bright background, hence the name.



Light path

The light path of a bright field microscope is extremely simple, no additional components are required beyond the normal light microscope setup. The light path therefore consists of:

light source

1. a transillumination light source, commonly a halogen lamp in the microscope stand; A **halogen lamp**, also known as a **tungsten halogen lamp** or **quartz iodine lamp**, is an incandescent lamp that has a small amount of a halogen such as iodine or bromine added. The combination of the halogen gas and the tungsten filament produces a **halogen cycle** chemical reaction which redeposits evaporated tungsten back onto the filament, increasing its life and maintaining the clarity of the envelope
2. a **condenser lens** which focuses light from the light source onto the sample. A **condenser** is one of the main components of the optical system of many transmitted light compound microscopes. A condenser is a lens that serves to concentrate light from the illumination source that is in turn focused through the object and magnified by the objective lens.
3. **objective lens** : In an optical instrument, the **objective** is the optical element that gathers light from the object being observed and focuses the light rays to produce a real image. Objectives can be single lenses or mirrors, or combinations of several optical elements. Microscope objectives are characterized by two parameters: magnification and numerical aperture. The typically ranges are 4× , 10x , 40x and 100×.
4. **oculars** to view the sample image. An **eyepiece**, or **ocular lens**, is a type of lens that is attached to a variety of optical devices such as microscopes. It is so named because it is usually the lens that is closest to the eye when someone looks through the device. The objective lens or mirror collects light and brings it to focus creating an image. The eyepiece is placed near the focal point of the objective to magnify this image. The amount of magnification depends on the focal length of the eyepiece.

Magnification is the process of enlarging something only in appearance, not in physical size. Typically magnification is related to scaling up visuals or images to be able to see more detail, increasing resolution.

Resolving power is the ability of an imaging device to separate (i.e., to see as distinct) points of an object that are located at a small angular distance..

In optics, the **numerical aperture** (**NA**) of an optical system is a dimensionless number that characterizes the range of angles over which the system can accept or emit light. In most areas of optics, and especially in microscopy, the numerical aperture of an optical system such as an objective lens is defined by

$$\mathrm{NA} = n \sin\theta$$

where n is the index of refraction of the medium in which the lens is working (1.0 for air, 1.33 for pure water, and up to 1.56 for oils; see also list of refractive indices), and θ is the half-angle of the maximum cone of light that can enter or exit the lens. In general, this is the angle of the real marginal ray in the system

Working Performance

Bright field microscopy typically has low contrast with most biological samples as few absorb light to a great extent. Staining is often required to increase contrast, which prevents use on live cells in many situations. Bright field illumination is useful for samples which have an intrinsic colour, for example chloroplasts in plant cells.

Light is first emitted by the light **source** and is directed by the **condenser lens** on to the specimen, which might be a loose object, a prepared plate or almost anything. A microscope can even be applied to small parts of larger objects, though with a bit more difficulty. (The light does not absolutely need to originate below the specimen.)

The light from the specimen then passes through the objective lens. This lens is often selected from among three or four and is the main determinant for the level of magnification. It bends the light rays and in the case of this example sends them to a **projector lens**, which reverses their direction so that when the image reaches the eye it will not appear "upside-down". Not all microscopes have a projector lens, so the viewer may be seeing a reverse image. In these cases, when the slide is moved, it will appear to be moving in the opposite direction to the viewer.

The light rays then travel to the **oracular lens** or "eye piece". This is often a 10X magnification lens, meaning it magnifies the magnified image an additional ten times. The image is then projected into the eye. It is very seldom that a specimen is in focus the moment it is placed beneath a microscope. This means that some adjustment will have to be made. Unlike in telescopes, the focal length between lenses remains constant when adjusting the focus. The lens apparatus is brought closer to or further from the object. The focus adjustment is often along the

neck of the tube containing the lenses, but it might just as well move the slide up and down. The best way to make this adjustment is to make a course adjustment so that it is too close to the object and then back off with the fine adjustment[2]. This helps to ensure that the specimen is not inadvertently smashed by the lens.

Advantages

The name "brightfield" is derived from the fact that the specimen is dark and contrasted by the surrounding bright viewing field. Simple light microscopes are sometimes referred to as bright field microscopes.

Brightfield microscopy is very simple to use with fewer adjustments needed to be made to view specimens.

Some specimens can be viewed without staining and the optics used in the brightfield technique don't alter the color of the specimen.

It is adaptable with new technology and optional pieces of equipment can be implemented with brightfield illumination to give versatility in the tasks it can perform.

Disadvantages

Certain disadvantages are inherent in any optical imaging technique.

- By using an aperture diaphragm for contrast, past a certain point, greater contrast adds distortion. However, employing an iris diaphragm will help compensate for this problem.
- Brightfield microscopy can't be used to observe living specimens of bacteria, although when using fixed specimens, bacteria have an optimum viewing magnification of 1000x.

Brightfield microscopy has very low contrast and most cells absolutely have to be stained to be seen; staining may introduce extraneous details into the specimen that should not be present.

Also, the user will need to be knowledgeable in proper staining techniques.

Lastly, this method requires a strong light source for high magnification applications and intense lighting can produce heat that will damage specimens or kill living microorganisms.

The most important feature of a microscope is its **resolution**, the ability to see fine details. Once you can resolve fine details then you can magnify them.
Every optical system has a finite resolution, if you magnify objects beyond the resolution the result will be **empty magnification**. So, the actual purpose of a microscope is to see small things clearly.

A second, desirable attribute of a microscope is **depth of field**, which is the range of depth that a specimen is in acceptable focus. A microscope that has a thin depth of field will have to be continuously focused up and down to view a thick specimen.

A third feature that a microscope has its mechanism for **contrast** formation. In order to distinguish a feature from its surrounding background the human eye needs a difference of 2 percent in intensity. Contrast is the ratio between the dark and the light. Typically, most microscopes use absorption contrast, that is the specimen is subjected to stains in order to be seen. This is called **bright field microscopy**.
There are other types of microscope that use more exotic means to generate contrast, such as phase contrast, dark field, differential interference contrast.

The fourth desirable feature is a strong **illumination** source. The higher a microscope magnifies the more light will be required. Also, there will be more optical trade off leeway when there is more light present. The illumination source should also be at a wavelength (color) that will facilitate the interaction with the specimen. All microscopes fall into either of two categories based on how the specimen is illuminated. In the typical compound microscope the light passes through the specimen and is collected by the image forming optics. This is called diascopic illumination. Dissecting (stereo) microscopes generally use episcopic illumination for use with opaque specimen. The light is reflected onto the specimen and then into the objective lens.

The four attributes of an optical system trade off with each other. Resolution and brightness is antagonistic towards contrast and depth of field. For example, you can not have maximum resolution and maximum contrast simultaneously. Theoretically speaking, if you had an infinite resolving system there would be no contrast to discern the image. It is up to the microscopist to decide which attribute is needed to view a particular specimen. All of which are controlled be the iris diaphragm, see Koehler illumination.

Electron Microscope

You've probably used a microscope in school -- maybe to observe the wings of an insect or to get a closer look at a leaf. If so, then you know microscopes are used in the classroom to illuminate the surface of your subject of study. These microscopes use transparent glass lenses to magnify the image of whatever you are observing.

However, the size of the smallest features that we can distinguish under the microscope is on the order of the wavelength of the light used. Visible light, which is the one our eyes are sensitive to, ranges between 390 and 700 nanometers (one nanometer is one billionth of a meter). This means that we cannot observe things that are smaller than a few hundred nanometers using our eyes and visible light.

With the advancement of science and technology, we realized there is a whole world of things that we can observe and study at small scales. For example, the size of atoms and molecules is just fractions of a nanometer. An **electron microscope** allows us to see at these small scales.

Electron microscopes work by using an electron beam instead of visible light and an electron detector instead of our eyes. An **electron beam** allows us to see at very small scales because electrons can also behave as light. It has the properties of a wave with a wavelength that is much smaller than visible light (a few trillionths of a meter!). With this wavelength we can distinguish

features down to a fraction of a nanometer. Let's explore the different types of electron microscopes, how they work and some of their applications.

Types of Electron Microscopes

Scanning Electron Microscope (SEM)

In a **scanning electron microscope** or **SEM**, a beam of electrons scans the surface of a sample (Figure 1). The electrons interact with the material in a way that triggers the emission of **secondary electrons**. These secondary electrons are captured by a detector, which forms an image of the surface of the sample. The direction of the emission of the secondary electrons depends on the orientation of the features of the surface. There, the image formed will reflect the characteristic feature of the region of the surface that was exposed to the electron beam.

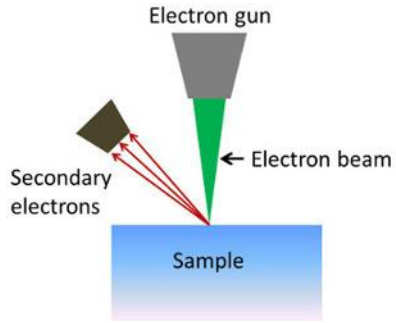


Figure 1. A Scanning Electron Microscope focuses a beam of electrons on the surface of a sample. Secondary electrons are emitted from the sample surface. A detector is used to form an image from the secondary electrons.

Transmission Electron Microscope (TEM)

In a **transmission electron microscope** or **TEM**, a beam of electrons hits a very thin sample (usually no more than 100 nm thick). The electrons are transmitted through the sample (Figure 2). After the sample, the electrons hit a fluorescence screen that forms an image with the electrons that were transmitted. You can better understand this process by imagining how a movie projector works. In a projector, you have a film that has the negative image that will be projected. The projector shines white light on the negative and the light transmitted forms the image contained in the negative.

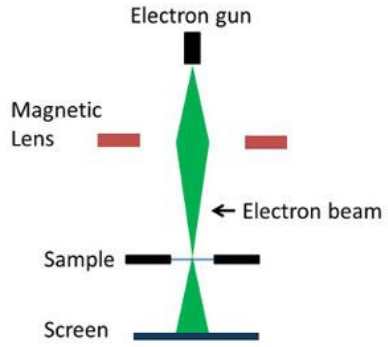


Figure 2. Transmission Electron Microscope. A beam of electrons is focused on a sample. The electrons pass through the sample to form an image on a fluorescent screen.

Scanning Transmission Electron Microscope (STEM)

A **scanning transmission electron microscope** or **STEM** combines the capabilities of both an SEM and a TEM. The electron beam is transmitted across the sample to create an image (TEM) while it also scans a small region on the sample (SEM). The ability to scan the electron beams allows the user to analyze the sample with various techniques such as **Electron Energy Loss Spectroscopy (EELS)** and **Energy Dispersive X-ray (EDX)** Spectroscopy which are useful tools to understand the nature of the materials in the sample.

Uses of the Electron Microscope

With electron microscopes we can observe the small scale world that makes up most of the things around us. Before the development of the electron microscope we did not know how all these things looked (shape, size, etc.). We were relying on our imagination to picture these objects in our minds.

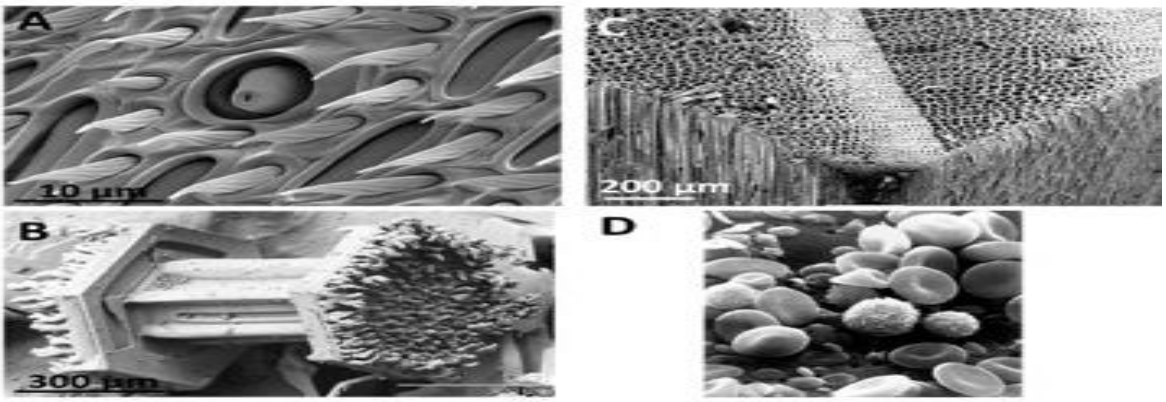


Figure 3. SEM Images. A) The surface of the antenna of a wasp. B) A snow flake. C) Wood. D) Blood cells (size of 1-6 micrometers).

Simple Staining Technique

In a **simple staining technique**, a basic, cationic dye is flooded across a sample, adding color to the cells. Before we move on, let's define the word cationic. A **cation** is simply a positively charged ion. The molecules that make up basic dyes have a positive charge. This is important because the cell wall and cytoplasm of bacterial cells have a negative charge. The positively charged dye is attracted to the negatively charged cells, enhancing the ability of the stain to stick to and color the cells. Now, those nearly colorless cells should pop off the slide in any number of colors.

It is important to note that before a sample can be stained with a simple stain, it must be heat fixed to the slide. During heat fixation, a glass slide is waved over an open flame. This kills the bacteria, attaches the cells to the slide, and enhances the stain uptake. This process makes staining more effective but can damage or distort the cells, changing their appearance from a truly natural, free-living state.

Methylene blue is a classic example of a simple stain. This blue stain will color all cells blue, making them stand out against the bright background of the light microscope. Notice how the background remains generally clear, while the bacterial cells are a deep blue.

Procedure:

1. Clean and dry microscope slides thoroughly.
2. Flame the surface in which the smear is to be spread.
3. Flame the inoculating loop.
4. Transfer a loop full of tap water to the flamed slide surface.
5. Reflame the loop making sure the entire length of the wire that will enter the tube has been heated to redness
6. Remove the tube cap with the fingers of the hand holding the loop.
7. Flame the tube mouth.
8. Touch the inoculating loop to the inside of the tube to make sure it is not so hot that it will distort the bacterial cells; then pick up a pinhead size sample of the bacterial growth without digging into the agar
9. Reflame the tube mouth, replace the can, and put the tube back in the holder.
10. Disperse the bacteria on the loop in the drop of water on the slide and spread the drop over an area the siz of a dime. It should be a thin, even smear.
11. Reflame the inoculating loop to redness including the entire length that entered the tube.
12. Allow the smear to dry thoroughly.
13. Heat-fix the smear cautiously by passing the underside of the slide through the burner flame two or three times. Test the temperature of the slide after each pass against the back of the hand. It has been heated sufficiently when it feels hot but can still be held against the skin for several seconds. Overheating will distort the cells
14. Stain the smear by flooding it with one of the staining solutions and allowing it to remain covered with the stain for the time designated below.

Methylene blue - 1 minute

Crystal violet - 30 seconds

Carbol fuchsin - 20 seconds

During the staining the slide may be placed on the rack or held in the fingers.

15. At the end of the designated time rinse off the excess stain with gently running tap water. Rinse thoroughly.

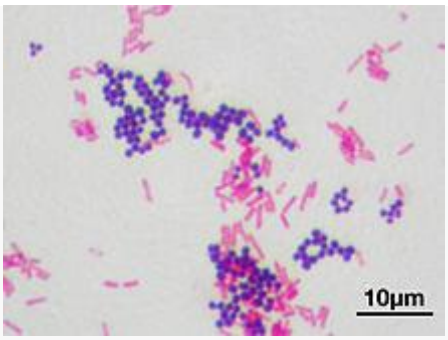
16. Wipe the back of the slide and blot the stained surface with bibulous paper or with a paper towel.

17. Place the stained smear on the microscope stage smear side up and focus the smear using the 10X objective.

18. Choose an area of the smear in which the cells are well spread in a monolayer. Center the area to be studied, apply oil directly to the smear, and focus the smear under oil with the 100X objective.

19. Draw the cells observed.

Gram staining



A Gram stain of mixed*Staphylococcus aureus*(Staphylococcus aureus ATCC 25923, Gram-positive cocci, in purple) and*Escherichia coli* (Escherichia coli ATCC 11775, Gram-negative bacilli, in red), the most common Gram stain reference bacteria

Gram staining, also called **Gram's method**, is a method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative). The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique.

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in a thick layer in Gram-positive bacteria.[1] In a Gram stain test, Gram-positive bacteria retain the crystal violet dye, while a counterstain (commonly safranin or fuchsine) added after the crystal violet gives all Gram-negative bacteria a red or pink coloring.

The Gram stain is almost always the first step in the identification of a bacterial organism. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique. This gives rise to *Gram-variable* and *Gram-indeterminate* groups as well.

History

The method is named after its inventor, the Danish scientist Hans Christian Gram (1853–1938), who developed the technique,

Process

- **Step 1.** Prepare a slide with the culture by transferring the specimen to be examined onto a drop of suspension medium (distilled water) using an inoculation loop. Spread the specimen on the slide to ensure that it is not clumped.

- **Step 2.** Fix the culture by heating the slide over a Bunsen burner to evaporate the water -- make sure not to hold the slide over the flame too long or it will denature the specimen.

- **Step 3.** Drop a few drops of crystal violet stain onto the fixed culture (enough to cover the specimen) and let it stand for 20 seconds. Then pour off the crystal violet stain and gently rinse the excess stain with distilled water.
  - The objective of this step is:
    - To allow the crystal violet stain to bind to the peptidoglycan molecules of the Gram + bacteria (if present). Remember, Gram + bacteria have a large peptidoglycan layer located outside the bacterial "inner membrane".
    - To allow the crystal violet stain to bind to the lipopolysaccharide molecules attached to the "outer membrane" of the Gram - bacteria (if present). Remember, while Gram + bacteria have no "outer membrane", Gram - bacteria have lipopolysaccharide molecules attached to their bacterial "outer membrane".

- **Step 4.** Drop a few drops of iodine solution on the smear and let it stand for 20 seconds. Then pour off the iodine solution and rinse the slide with distilled water.
  - The objective of this step is to fix the crystal violet to the peptidoglycan molecules on the Gram + bacteria (if present).

- **Step 5.** Drop a few drops of decolorizer (acetone or ethanol) and let the solution trickle down off the slide until the decolorizer has removed enough of the color to drip off clear. Then IMMEDIATELY rinse the slide off with distilled water after 5 seconds. Note that pouring too much decolorier will cause the decolorization of the Gram + bacterial cells (in addition to the Gram - bacteria), and the purpose of staining will be defeated.
  - The objective of this step is to dissolve the lipopolysaccharide membraine in the Gram - bacteria and expose the thin peptidoglycan layer below.

- **Step 6.** Drop a few drops of basic counterstain (fuchsin or safranin) on the slide and let it sit for 20 seconds, then wash off the solution with distilled water.
  - The objective of this step is to stain the peptidoglycan layer of the Gram - bacteria a pink / red color. Remember that the addition of iodine to the crystal violet in Step 4 binds the crystal violet stain in the Gram + bacteria, so the counterstain is unable to bind to the peptidoglycan wall in the Gram + bacteria in the specimen.

- **Step 7.** Observe the slide under light microscope.

Uses

Gram staining is a bacteriological laboratory technique[4] used to differentiate bacterial species into two large groups (gram-positive and gram-negative) based on the physical properties of

their cell walls.[5] Gram staining is not used to classify archaea, formerly archaeabacteria, since these microorganisms yield widely varying responses that do not follow their phylogenetic groups.[6]

Staining mechanism

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas Gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by the Safranin. There are four basic steps of the Gram stain:

- Applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture. Heat fixation kills some bacteria but is mostly used to affix the bacteria to the slide so that they don't rinse out during the staining procedure.
- The addition of iodide, which binds to crystal violet and traps it in the cell,
- Rapid decolorization with ethanol or acetone, and
- *Counterstaining* with safranin.[9] Carbol fuchsin is sometimes substituted for safranin since it more intensely stains anaerobic bacteria, but it is less commonly used as a counterstain.[10]

Crystal violet (CV) dissociates in aqueous solutions into CV+ and chloride (Cl−) ions. These ions penetrate through the cell wall and cell membrane of both Gram-positive and Gram-negative cells. The CV+ ion interacts with negatively charged components of bacterial cells and stains the cells purple. Iodide (I^- or I^-_3) interacts with CV+ and forms large complexes of crystal violet and iodine (CV–I) within the inner and outer layers of the cell. Iodine is often referred to as a mordant, but is a trapping agent that prevents the removal of the CV–I complex and, therefore, color the cell.[11]

When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane. A gram-negative cell loses its outer lipopolysaccharide membrane, and the inner peptidoglycan layer is left exposed. The CV–I complexes are washed from the gram-negative cell along with the outer membrane. In contrast, a gram-positive cell becomes dehydrated from an ethanol treatment. The large CV–I complexes become trapped within the gram-positive cell due to the multilayered nature of its peptidoglycan. The decolorization step is critical and must be timed correctly; the crystal violet stain is removed from both gram-positive and negative cells if the decolorizing agent is left on too long (a matter of seconds).

After decolorization, the gram-positive cell remains purple and the gram-negative cell loses its purple color. Counterstain, which is usually positively charged safranin or basic fuchsine, is applied last to give decolorized gram-negative bacteria a pink or red color.[12][13]

Some bacteria, after staining with the Gram stain, yield a *gram-variable* pattern: a mix of pink and purple cells are seen. The genera *Actinomyces*, *Arthobacter*,*Corynebacterium*, *Mycobacterium*, and *Propionibacterium* have cell walls particularly sensitive to breakage during cell division, resulting in gram-negative staining of these gram-positive cells. In cultures of *Bacillus*, *Butyrivibrio*, and *Clostridium*, a decrease in peptidoglycan thickness during growth coincides with an increase in the number of cells that stain gram-negative.[14] In addition, in all bacteria stained using the Gram stain, the age of the culture may influence the results of the stain.

Examples

Gram-positive bacteria

Historically, the gram-positive forms made up the phylum Firmicutes, a name now used for the largest group. It includes many well-known genera such as *Bacillus*,*Listeria*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Clostridium*. It has also been expanded to include the Mollicutes, bacteria like *Mycoplasma* that lack cell walls and so cannot be stained by Gram, but are derived from such forms.

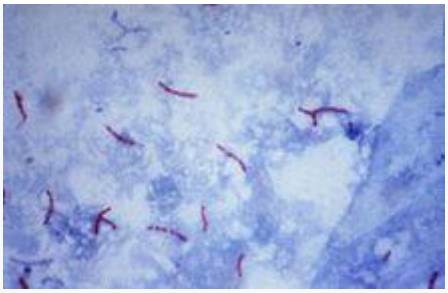
Gram-negative bacteria

Gram-negative bacteria generally possess a thin layer of peptidoglycan between two membranes (*diderms*). Most bacterial phyla are gram-negative, including thecyanobacteria, spirochaetes, and green sulfur bacteria, and most Proteobacteria and *Escherichia coli*. (exceptions being some members of the *Rickettsiales* and the insect-endosymbionts of the *Enterobacteriales*).[5][15]

Gram-indeterminate bacteria

Gram-indeterminate bacteria do not respond predictably to Gram staining and, therefore, cannot be determined as either gram-positive or gram-negative. They tend to stain unevenly, appearing partially gram positive and partially gram negative, or unstained by either crystal violet or safranin. Staining older cultures (over 48 hours) can lead to false Gram-variable results, probably due to changes in the cell wall with aging. Gram-indeterminate bacteria are best stained using acid-fast staining techniques. Examples include many species of *Mycobacterium*, including *M. tuberculosis* and *M. leprae*.[16][17]

Ziehl–Neelsen stain - Acid-fast stain



The **Ziehl–Neelsen stain**, also known as the **acid-fast stain**, was first described by two German doctors: the bacteriologistFranz Ziehl (1859–1926) and the pathologist Friedrich Neelsen (1854–1898). It is a special bacteriological stain used to identify acid-fast organisms, mainly Mycobacteria. *Mycobacterium tuberculosis* is the most important of this group because it is responsible for tuberculosis (TB). Other important Mycobacterium species involved in human disease are *Mycobacterium leprae*, *Mycobacterium kansasii*, *Mycobacterium marinum*, *Mycobacterium bovis*, *Mycobacterium africanum* and members of the *Mycobacterium avium* complex. Acid fast organisms like Mycobacterium contain large amounts of lipid substances within their cell walls called mycolic acids. These acids resist staining by ordinary methods such as a Gram stain.[1] It can also be used to stain a few other bacteria, such

as *Nocardia*. The reagents used are Ziehl–Neelsen carbol fuchsin, acid alcohol, andmethylene blue. Acid-fast bacilli will be bright red after staining.

A variation on this staining method is used in mycology to differentially stain acid-fast incrustations in the cuticular hyphae of certain species of fungi in the genus *Russula*.[2][3] It is also useful in the identification of some protozoa, namelyCryptosporidium and Isospora. The Ziehl–Neelsen stain can also hinder diagnosis in the case of paragonimiasis because the eggs in an ovum and parasite sputum sample (OnP) can be dissolved by the stain, and is often used in this clinical setting because signs and symptoms of paragonimiasis closely resemble those of TB.

Procedure

A typical AFB stain procedure involves dropping the cells in suspension onto a slide, then air drying the liquid and heat fixing the cells. The slide is flooded with Carbol Fuchsin, which is then heated to dry and rinsed off in tap water. The slide is then flooded with a 1% solution of hydrochloric acid in isopropyl alcohol (or methanol) to remove the carbol fuchsin, thus removing the stain from cells that are unprotected by a waxy lipid layer. Thereafter, the cells are stained in methylene blue and viewed on a microscope under oil immersion.

Studies have shown that an AFB stain without a culture has a poor negative predictive value. An AFB Culture should be performed along with an AFB stain; this has a much higher negative predictive value.

Mechanism explanation

Initially, Carbol Fuchsin stains every cell. When they are destained with acid-alcohol, only non-acid-fast bacteria get destained since they do not have a thick, waxy lipid layer like acid-fast bacteria. When counter stain is applied, non-acid-fast bacteria pick it up and become blue when viewed under the microscope. Acid-fast bacteria retain Carbol Fuchsin so they appear red.

Modifications

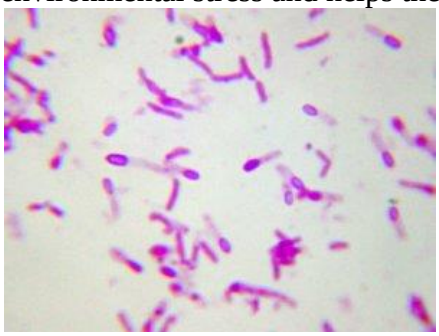
- 1% sulfuric acid alcohol for actinomycetes, nocardia.
- 0.5-1% sulfuric acid alcohol for oocysts of isospora, cyclospora.
- 0.25–0.5% sulfuric acid alcohol for bacterial endospores.
- Brucella differential stain – glacial acetic acid used, no heat applied, secondary stain is loeffler's methylene blue.
- Kinyoun modification (or cold Ziehl–Neelsen technique) is also available.
- A protocol in which a detergent is substituted for the highly toxic phenol in the fuchsin staining solution

Endospore staining

Endospore staining is a technique used in bacteriology to identify the presence of endospores in a bacterial sample, which can be useful for classifying bacteria.[1]Within bacteria, endospores are quite protective structures used to survive extreme conditions, but this protective nature makes them difficult to stain using normal techniques. Special techniques for endospore staining include the Schaeffer–Fulton stain and the Moeller stain. A good stain to use for spore staining is malachite green. It takes a long time for the spores to stain due to their density, so time acts as the mordant when doing this differential stain; the slide with the bacterium should be soaked in malachite green for at least 30 minutes. Water acts as the decolorizer. A counterstain to differentiate the vegetative cells is 0.5% safranin. Types of endospores that could be identified are free endospores, central endospores, central and swollen endospores, and subterminal endospores. One obstacle of this stain is if staining *Mycobacterium* because due to the its thick, wax coats, some cells will stain green, looking positive for spores although this particular bacterium does not produce .

Endospore Staining: Principle, Procedure and Results

When vegetative cells of certain bacteria such as *Bacillus* spp and *Clostridium* spp. are subjected to environmental stresses such as nutrient deprivation they produces metabolically inactive or dormant form-Endospore. Formation of Endospore circumvent the problems associated with environmental stress and helps them to survive.



Most endospore forming bacteria are found in soil or aquatic environments. However, some species of *Bacillus* and *Clostridium*have **medical significance**. *Clostridium perfringens, C. botulinum* and*C. tetani* are the causative agents of gas gangrene, botulism and tetanus, respectively. *Bacillus anthracis* and *Bacillus cereus* are the causative agents of anthrax and a self limiting food poisoning, respectively.

Principle of Spore Staining:

A **differential staining** technique (the Schaeffer-Fulton method) is used to distinguish between the vegetative cells and the endospores. A **primary stain (malachite green) is used to stain the endospores**. Because endospores resist staining, the malachite green will be **forced** into the endospores by **heating**. In this technique heating acts as a**mordant**.

Water is used to decolorize the cells; as the endospores are resistant to staining, the endospores are equally resistant to de-staining and will retain the primary dye while the vegetative cells will

lose the stain. The addition of a counterstain or **secondary stain (safranin) is used to stain the decolorized vegetative cells**.

When visualized under microscopy the cells should have three characteristics:

1. the vegetative cells should appear pink,
2. the vegetative cells that contain endospores should stain pink while the spores should be seen as green ellipses within the cells.
3. Mature, free endospores should not be associated with the vegetative bacteria and should be seen as green ellipses.

Procedure of endospore stain:

1. Prepare smears of organisms to be tested for endospores on a clean microscope slide and air dry it.
2. Heat fix the smear.
2. Place a small piece of blotting paper (absorbent paper) over the smear and place the slide (smear side up) on a wire gauze on a ring stand.
3. Heat the slide gently till it starts to evaporate (either by putting the slide on a staining rack that has been placed over a boiling water bath or via bunsen burner).
4. Remove the heat and reheat the slide as needed to keep the slide steaming for about 3-5 minutes. As the paper begins to dry add a drop or two of malachite green to keep it moist, but don't add so much at one time that the temperature is appreciably reduced. DO NOT OVERHEAT. The process is steaming and not baking.
7. After 5 minutes carefully remove the slide from the rack using a clothespin
8. Remove the blotting paper and allow the slide to cool to room temperature for 2 minutes.
9. Rinse the slide thoroughly with tap water (to wash the malachite green from both sides of the microscope slide).
8. Stain the smear with safranin for 2 minutes.
9. Rinse both side of the slide to remove the secondary stain and blot the slide/ air dry.

Observe the bacteria under 1000X (oil immersion) total magnification.

Results: The vegetative cells will appear pink and the spores will appear green.

Flagella Stain Procedure

FLAGELLAR STAINING

A. Preparation of Cultures

Background

Solid or liquid media cultures can be used for flagella staining (7). Cultures should incubate between 16 and 20 hours before staining, as older cultures tend to lose flagella. For example, newer cultures are particularly important for *Bacillus* spp. that undergo spore formation and lose flagella during this developmental process. If vortexing is necessary for suspension after centrifugation of liquid cultures, or because cultures clump, do so gently as flagella are easily sheared from the bacterium.

Method

From an agar plate or slant cultures, prepare a suspension by removing a small amount of growth, approximately one-fourth of the colony, with an inoculating loop using proper aseptic technique. Emulsify in 100 ml of distilled water in a microcentrifuge tube by gently vortexing. The emulsion should be only slightly cloudy. Using too much inoculum results in the inability to visualize the flagella.

For staining from liquid cultures, Leifson (7) recommends two rounds of centrifugation and final suspension in distilled water to remove any medium components. Place 100 ml of the liquid culture in a microcentrifuge tube, centrifuge, and remove spent medium. Resuspend in 100 ml of distilled water by gently vortexing, again centrifuge, and remove supernatant. Form a slightly cloudy emulsion by resuspending in ~200 ml of distilled water. Gently vortex. Again, emulsions should be only slightly cloudy prior to proceeding to staining. Optimization of the washing procedure will most likely be necessary to maximize quality of flagella stain.

B. Preperation of Slides

1. Wipe clean a new microscope slide with 95% ethanol and a Kimwipe. Flame to dry thoroughly. Use slides immediately.

2. When the slide is cool enough to handle, label it using tape with the name of the organism you will be staining.

3. Place 5 to 10 ml of the culture emulsion on one end of the slide using a micropipettor and spread the emulsion using the same pipette tip held parallel to the microscope slide.

4. Allow the sample to dry at room temperature. Do not heat fix as this will destroy the proteinaceous flagella structure.

C. Flagella Staining

Leifson flagella stain (14)

1. Take a prepared slide and using a wax pencil draw a rectangle around the dried sample. Place slide on staining rack.

2. Flood Leifson dye solution on the slide within the confines of the wax lines. Incubate at room temperature for 7 to 15 minutes. The best time for a particular preparation will require trial and error.

3. As soon as a golden film develops on the dye surface and a precipitate appears throughout the sample, as determined by illumination under the slide, remove the stain by floating off the film with gently flowing tap water. Air dry.

4. View using oil immersion, at 1,000x magnification, by bright-field microscopy. Bacterial bodies and flagella will stain red.

Presque Isle Cultures flagella stain

1. Prepare slide as described above. Place slide on staining rack.

2. Flood slide with Presque Isle Cultures Solution I, the mordant. Incubate at room temperature for 4 minutes.

3. Gently rinse with distilled water. Shake excess water from slide.

4. Flood with Presque Isle Cultures Solution II, the silver stain.

5. Heat over Bunsen burner by moving slide back and forth, just until steam is emitted. If a Bunsen burner is not available then an alternate heat source can be used, but optimization will be necessary. Be careful not to overheat sample, as excess heat will destroy the flagella. Incubate at

room temperature for 4 minutes.

6. Rinse with distilled water. Carefully blot dry with bibulous paper.

7. View using oil immersion, at 1,000x magnification, by bright-field microscopy. Bacteria and flagella will appear golden brown. Excess stain is often observed on the slides and illustrates the necessity of beginning the procedure with thoroughly cleaned microscope slides.

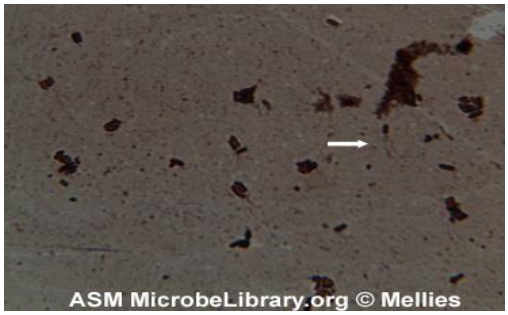


FIG. 1. Arrow points to lophotrichous flagella of *Pseudomonas fluorescens*cultured on nutrient agar, stained using the Presque Isle flagella stain, and visualized under bright-field microscopy using oil immersion at 1,000x magnification.

Capsule Staining

Principle, Reagents, Procedure and Result

The main purpose of capsule stain is to distinguish capsular material from the bacterial cell. A **capsule** is a gelatinous outer layer secreted by bacterial cell and that surrounds and adheres to the cell wall. Most capsules are composed of polysaccharides, but some are composed of polypeptides. The **capsule** differs from the **slime layer** that most bacterial cells produce in that it is a thick, detectable, discrete layer outside the cell wall. The capsule stain employs an acidic stain and a basic stain to detect capsule production.

Principle of Capsule Staining

Capsules stain very poorly with reagents used in simple staining and a capsule stain can be, depending on the method, a misnomer because the capsule may or may not be stained.

Negative staining methods contrast a translucent, darker colored, background with stained cells but an unstained capsule. The background is formed with **india ink or nigrosin or congo red**. India ink is difficult to obtain nowadays; however, nigrosin is easily acquired.

A positive capsule stain requires a mordant that precipitates the capsule. By counterstaining with dyes like**crystal violet or methylene blue**, bacterial cell wall takes up the dye. Capsules appear colourless with stained cells against dark background.

Capsules are fragile and can be diminished, desiccated, distorted, or destroyed by heating. A drop of serum can be used during smearing to enhance the size of the capsule and make it more easily observed with a typical compound light microscope.

Reagents used for Capsule Staining

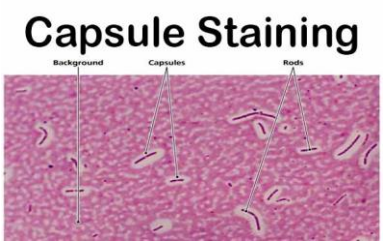
Crystal Violet (1%)
Crystal Violet (85% dye content) = 1 gm
Distilled Water = 100 ml

Nigrosin
Nigrosine, water soluble = 10 gm
Distilled Water = 100 ml

Procedure of Capsule Staining

1. Place a small drop of a **negative stain** (India Ink, Congo Red, Nigrosin, or Eosin) on the slide. ***Congo Red*** *is easier to see, but it does not work well with some strains.* ***India Ink*** *generally works, but it has tiny particles that display Brownian motion that must be differentiated from your bacteria.****Nigrosin*** *may need to be kept very thin or diluted.*
2. Using sterile technique, add a loopful of bacterial culture to slide, smearing it in the dye.
3. Use the other slide to drag the ink-cell mixture into a thin film along the first slide and let stand for **5-7 minutes.**
4. Allow to air dry (do not heat fix).
5. Flood the smear with **crystal violet stain** (this will stain the cells but not the capsules) for about **1 minutes**. Drain the crystal violet by tilting the slide at a 45 degree angle and let stain run off until it air dries .
6. Examine the smear microscopically (100X) for the presence of encapsulated cells as indicated by clear zones surrounding the cells.

Result of Capsule Staining



Capsule: Clear halos zone against dark background
No Capsule: No Clear halos zone

Examples of Capsule Positive and Negative

Positive
*Bacillus anthracis, Klebsiella pneumoniae, Streptococcus pneumonia Neisseria meningitidis Clostridium*spp, *Rhizaobium* spp, etc.

Negative
Neisseria gonorrhoreae, etc.

Mneomonics to remember capsulated bacteria- Some Killers Have Pretty Nice Capsule

S*treptococcus pneumoniae*
K*lebsiella pneumoniae*

H*aemophilus influenzae*
P*seudomonas aeruginosa*
N*eisseria meningitidis*
C*ryptococcus neoformans*