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Microbiology (from Greek , *mīkros*, "small", *bios*, "life"; and *-logia*) is the study of microscopic organisms, those being unicellular (single cell), multicellular (cell colony), or acellular (lacking cells). 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 prokaryotic organisms—all of which are microorganisms—are conventionally classified as lacking membrane-bound organelles and include eubacteria andarchaebacteria. 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. Microbiologists often rely on extraction or detection of nucleic acid, either DNA or RNA sequences.

Viruses have been variably classified as organisms,[1] 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.

Louis Pasteur contribution in microbiology:-

Louis Pasteur 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 remembered for his remarkable breakthroughs in the causes and preventions of diseases, and his discoveries have saved countless lives ever since. He reduced mortality from puerperal fever, and created the first vaccines for rabies and anthrax. His medical discoveries provided direct support for the germ theory of disease and its application in clinical medicine. 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".

Pasteur was responsible for disproving the doctrine of spontaneous generation. Although Pasteur was not the first to propose the germ theory of disease.

Research:-

Tartaric:-

In Pasteur's early work as a chemist, beginning at the and continuing at Strasbourg and Lille, he examined the chemical, optical and crystallographic properties of a group of compounds known as tartrates He resolved a problem concerning the nature of tartaric acid.

Discovery of microbial fermentation:-

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 ("all life from life"). He was motivated to investigate the matter while working at Lille. 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 makes wines sour. he also showed that bacteria are responsible for turning the wine sour.

Disproving the theory of Spontaneous generation:-

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.

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. 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.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. 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.

Anthrax vaccine

Pasteur had given a misleading account of the preparation of the anthrax vaccine used in the experiment at Pouilly-le-Fort. The fact is that Pasteur publicly claimed his success in developing anthrax vaccine in 1881.[42] 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. Pasteur purely on grounds of jealousy contested the discovery by publicly displaying his vaccination method in Pouilly-le-Fort on 5 May 1881.

Pasteurization:-the process of partial killing of microbes called pasteurization which is now widely employed in dairies was originally devised by Pasteur to prevent spoilage of wine & beer.

Robert Koch contribution in microbiology:-

Robert Heinrich Herman Koch (German:]; 11 December 1843 – 27 May 1910) was a celebrated German physicianand 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. 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. 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. As a result of his groundbreaking research on tuberculosis, Koch received the Nobel Prize in Physiology or Medicine in 1905.

Research contribution

Anthrax

Robert Koch is widely known for his work with anthrax, discovering the causative agent of the fatal disease to be *Bacillus anthracis*. Koch discovered the formation of spores in anthrax bacteria that could remain dormant under specific conditions. 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. 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.

Koch's four postulates

Koch accepted a position as government advisor with the Imperial Department of Health in 1880. 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 inKoch's four postulates. 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. 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:

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. In an attempt to grow bacteria, Koch began to use solid nutrients such as potato slices. Through these initial experiments, Koch observed individual colonies of identical, pure cells. Coming to the conclusion that potato slices were not suitable media for all organisms, Koch later began to use nutrient solutions with gelatin.[1] 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. 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.

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. 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*. The bacterium had originally been isolated in 1854 by Italian anatomist Filippo Pacini,[1] 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.At the time, it was widely believed that tuberculosis was an inherited disease.However, Koch was convinced that the disease was caused by a bacterium and was infectious, and tested his four postulates using guinea pigs. Through these experiments, he found that his experiments with tuberculosis satisfied all four of his postulates 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* His work with this disease won Koch the Nobel Prize in Physiology and Medicine in 1905. 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.

Antonie van Leeuwenhoek contribution in microbiology:-

Antonie Philips van Leeuwenhoek[2] (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.

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 lensmaking. Using his handcrafted microscopes, he was the first to observe and describe microorganisms, which he originally referred to as *animalcules* (from Latin *animalculum* = "tiny animal"). Most of the "animalcules" are now referred to as unicellular organisms, though he observed multicellular organisms in pond water. He was also the first to document microscopic observations of muscle fibers, bacteria, spermatozoa, and blood flow in capillaries.

Techniques & 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.

Van Leeuwenhoek's single-lens microscopes were relatively small devices, the biggest being about 5 cm long.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 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'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;
- the vacuole of the cell
- the spermatozoa in 1677
- the banded pattern of muscular fibers, in 1682

In 1687 Van Leeuwenhoek reported his research on the coffee bean.

Optical microscope

The **optical microscope**, often referred to as **light microscope**, is a type of microscope which uses visible light and a system oflenses to magnify images of small samples. Optical microscopes are the oldest design of microscope and were possibly invented in their present compound form in the 17th century. Basic optical microscopes can be very simple, although there are many complex designs which aim to improve resolution and sample contrast.

The image from an optical microscope can be captured by normal light-sensitive cameras to generate a micrograph. Originally images were captured by photographic film but modern developments in CMOS and charge-coupled device (CCD) cameras allow the capture of digital images. Purely digital microscopes are now available which use a CCD camera to examine a sample, showing the resulting image directly on a computer screen without the need for eyepieces.

Alternatives to optical microscopy which do not use visible light include scanning electron microscopy and transmission electron microscopy.

On 8 October 2014, the Nobel Prize in Chemistry was awarded to Eric Betzig, William Moerner and Stefan Hell for "the development of super-resolved fluorescence microscopy," which brings "optical microscopy into the nanodimension"

Optical Configuration:-

There are two basic configurations of the conventional optical microscope: the simple microscope and the compound microscope. The vast majority of modern research microscopes are compound microscopes while some cheaper commercial digital microscopes are simple single lens microscopes. A magnifying glass is, in essence, a single lens simple microscope. In general, microscope optics are static; to focus at different focal depths the lens to sample distance is adjusted, and to get a wider or narrower field of view a different magnification objective lens must be used.

Simple microscope

A **simple microscope** is a microscope that uses a lens or set of lenses to enlarge an object through angular magnification alone, giving the viewer an erect enlarged virtual image. Simple microscopes are not capable of high magnification. The use of a single convex lens or groups of lenses are still found in simple magnification devices such as the magnifying glass, loupes, and eyepieces for telescopes and microscopes.

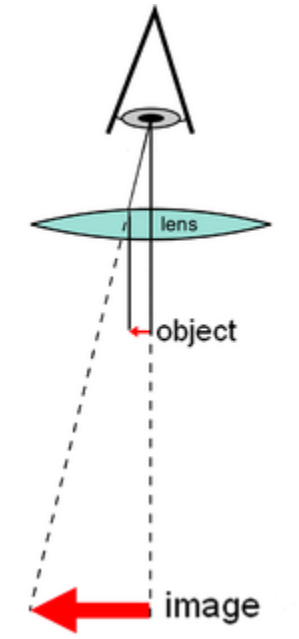


Diagram of a simple microscope

Compound microscope

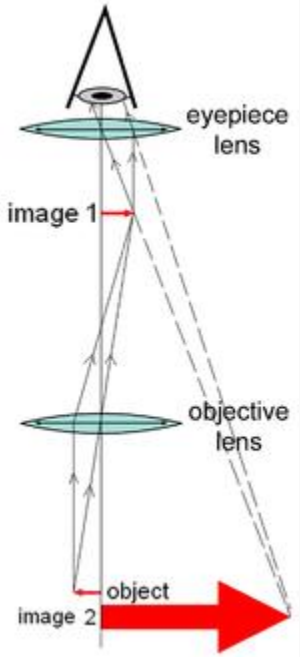


Diagram of a compound microscope

A **compound microscope** is a microscope which uses a lens close to the object being viewed to collect light (called the objective lens) which focuses a real image of the object inside the microscope That image is then magnified by a second lens or group of lenses (called the eyepiece) that gives the viewer an enlarged inverted virtual image of the object The use of a compound objective/eyepiece combination allows for much higher magnification, reduced chromatic aberration and exchangeable objective lenses to adjust the magnification. A compound microscope also enables more advanced illumination setups, such as phase contrast.

Component:-

All modern optical microscopes designed for viewing samples by transmitted light share the same basic components of the light path. In addition, the vast majority of microscopes have the same 'structural' components.

Basic optical transmission microscope elements (1990s)

All modern optical microscopes designed for viewing samples by transmitted light

- Eyepiece (ocular lens) (1)
- Objective turret, revolver, or revolving nose piece (to hold multiple objective lenses) (2)
- Objective lenses (3)
- Focus knobs (to move the stage)
  - Coarse adjustment (4)
  - Fine adjustment (5)
- Stage (to hold the specimen) (6)
- Light source (a light or a mirror) (7)
- Diaphragm and condenser (8)
- Mechanical stage (9)

Operation

The optical components of a modern microscope are very complex and for a microscope to work well, the whole optical path has to be very accurately set up and controlled. Despite this, the basic operating principles of a microscope are quite simple.

The objective lens is, at its simplest, a very high powered magnifying glass *i.e.* a lens with a very short focal length. This is brought very close to the specimen being examined so that the light from the specimen comes to a focus about 160 mm inside the microscope tube. This creates an enlarged image of the subject. This image is inverted and can be seen by removing the eyepiece and placing a piece of tracing paper over the end of the tube. By carefully focusing a brightly lit specimen, a highly enlarged image can be seen.

Application

Optical microscopy is used extensively in microelectronics, nanophysics, biotechnology, pharmaceutic research, mineralogy and microbiology.

Optical microscopy is used for medical diagnosis, the field being termed histopathology when dealing with tissues, or in smear tests on free cells or tissue fragments.

In industrial use, binocular microscopes are common. Aside from applications needing true depth perception, the use of dual eyepieces reduces eye strain associated with long workdays at a microscopy station. In certain applications, long-working-distance or long-focus microscopes are beneficial. An item may need to be examined behind a window, or industrial subjects may be a hazard to the objective. Such optics resemble telescopes with close-focus capabilities.

Electron microscope

An **electron microscope** is a microscope that uses a beam of accelerated electrons as a source of illumination. As the wavelength of an electron can be up to 100,000 times shorter than that of visible light photons, the electron microscope has a higher resolving power than a light microscope and can reveal the structure of smaller objects. A transmission electron microscope can achieve better than 50 pmr esolution and magnifications of up to about 10,000,000x whereas most light microscopes are limited by diffraction to about 200 nm resolution and useful magnifications below 2000x.

Types:-

Transmission electron microscope (TEM)

The original form of electron microscope, the transmission electron microscope (TEM) uses a high voltage electron beamto create an image. The electron beam is produced by an electron gun, commonly fitted with a tungsten filament cathodeas the electron source. The electron beam is accelerated by an anode typically at +100 keV (40 to 400 keV) with respect to the cathode, focused by electrostatic and electromagnetic lenses, and transmitted through the specimen that is in part transparent to electrons and in part scatters them out of the beam. When it emerges from the specimen, the electron beam carries information about the structure of the specimen that is magnified by the objective lens system of the microscope. The spatial variation in this information (the "image") may be viewed by projecting the magnified electron image onto a fluorescent viewing screen coated with a phosphor or scintillator material such as zinc sulfide. Alternatively, the image can be photographically recorded by exposing a photographic film or plate directly to the electron beam, or a high-resolution phosphor may be coupled by means of a lens optical system or a fibre optic light-guide to the sensor of a CCD (charge-coupled device) camera. The image detected by the CCD may be displayed on a monitor or computer.

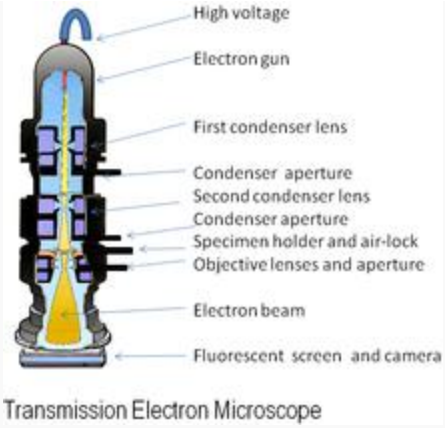


Diagram of a transmission electron microscope

Scanning electron microscope (SEM)

The SEM produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen (raster scanning). When the electron beam interacts with the specimen, it loses energy by a variety of mechanisms. The lost energy is converted into alternative forms such as heat, emission of low-energy secondary electrons and high-energy backscattered electrons, light emission (cathodoluminescence) or X-ray emission, all of which provide signals carrying information about the properties of the specimen surface, such as its topography and composition. The image displayed by an SEM maps the varying intensity of any of these signals into the image in a position corresponding to the position of the beam on the specimen when the signal was generated. In the SEM image of an ant shown below and to the right, the image was constructed from signals produced by a secondary electron detector, the normal or conventional imaging mode in most SEMs. Generally, the image resolution of an SEM is at least an order of magnitude poorer than that of a TEM.

Sample preparation

Materials to be viewed under an electron microscope may require processing to produce a suitable sample. The technique required varies depending on the specimen and the analysis required:

- *Chemical fixation* – for biological specimens aims to stabilize the specimen's mobile macromolecular structure by chemical crosslinking of proteins with aldehydes such as formaldehyde and glutaraldehyde, and lipids with osmium tetroxide.
- *Negative stain* – suspensions containing nanoparticles or fine biological material (such as viruses and bacteria) are briefly mixed with a dilute solution of an electron-opaque solution such as ammonium molybdate, uranyl acetate (or formate), or phosphotungstic acid. This mixture is applied to a suitably coated EM grid, blotted, then allowed to dry. Viewing of this preparation in the TEM should be carried out without delay for best results. The method is important in microbiology for fast but crude morphological identification, but can also be used as the basis for high resolution 3D reconstruction using EM tomography methodology when carbon films are used for support. Negative staining is also used for observation of nanoparticles.
- *Cryofixation* – freezing a specimen so rapidly, in liquid ethane, and maintained at liquid nitrogen or even liquid helium temperatures, so that the water forms vitreous (non-crystalline) ice. This preserves the specimen in a snapshot of its solution state. An entire field calledcryo-electron microscopy has branched

from this technique. With the development of cryo-electron microscopy of vitreous sections (CEMOVIS), it is now possible to observe samples from virtually any biological specimen close to its native state.

- *Dehydration* – or replacement of water with organic solvents such as ethanol or acetone, followed by critical point drying or infiltration with embedding resins. Also freeze drying.
- *Embedding, biological specimens* – after dehydration, tissue for observation in the transmission electron microscope is embedded so it can be sectioned ready for viewing. To do this the tissue is passed through a 'transition solvent' such as propylene oxide(epoxypropane) or acetone and then infiltrated with an epoxy resin such as Araldite, Epon, or Durcupan; tissues may also be embedded directly in water-miscible acrylic resin. After the resin has been polymerized (hardened) the sample is thin sectioned (ultrathin sections) and stained – it is then ready for viewing.
- *Embedding, materials* – after embedding in resin, the specimen is usually ground and polished to a mirror-like finish using ultra-fine abrasives. The polishing process must be performed carefully to minimize scratches and other polishing artifacts that reduce image quality.

Application:-

Semiconductor and data storage

- Circuit edit
- Defect analysis
- Failure analysis

Biology and life sciences

- Diagnostic electron microscopy
- Cryobiology
- Protein localization
- Electron tomography
- Cryo-electron microscopy
- Toxicology
- Biological production and viral load monitoring
- Particle analysis
- Pharmaceutical QC
- Structural biology
- 3D tissue imaging
- Virology
- Vitrification

Materials research

- Electron beam-induced deposition
- Materials qualification
- Medical research
- Nanoprototyping
- Nanometrology
- Device testing and characterization

Industry

- High-resolution imaging
- 2D & 3D micro-characterization
- Macro sample to nanometer metrology
- Particle detection and characterization
- Direct beam-writing fabrication
- Dynamic materials experiments
- Sample preparation
- Forensics
- Mining (mineral liberation analysis)
- Chemical/Petrochemical

Structure of bacterial cell

They are as unrelated to human beings as living things can be, but bacteria are essential to human life and life on planet Earth. Although they are notorious for their role in causing human diseases, from tooth decay to the Black Plague, there are beneficial species that are essential to good health.

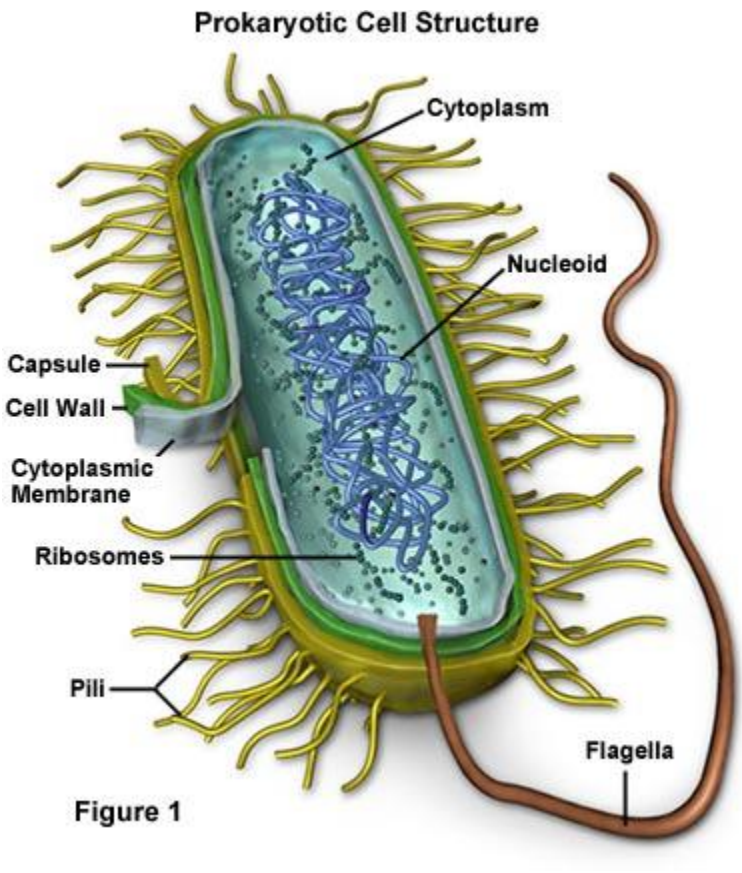


Figure 1

For example, one species that lives symbiotically in the large intestine manufactures vitamin K, an essential blood clotting factor. Other species are beneficial indirectly. Bacteria give yogurt its tangy flavor and sourdough bread its sour taste. They make it possible for ruminant animals (cows, sheep, goats) to digest plant cellulose and for some plants, (soybean, peas, alfalfa) to convert nitrogen to a more usable form.

Bacteria are prokaryotes, lacking well-defined nuclei and membrane-bound organelles, and with chromosomes composed of a single closed DNA circle. They come in many shapes and sizes, from minute spheres, cylinders and spiral threads, to flagellated rods, and filamentous chains. They are found practically everywhere on Earth and live in some of the most unusual and seemingly inhospitable places.

Evidence shows that bacteria were in existence as long as 3.5 billion years ago, making them one of the oldest living organisms on the Earth. Even older than the bacteria are the archeans (also called archaebacteria) tiny prokaryotic organisms that live only in extreme environments: boiling water, super-salty pools, sulfur-spewing volcanic vents, acidic water, and deep in the Antarctic ice. Many scientists now believe that the archaea and bacteria developed separately from a common ancestor nearly four billion years ago. Millions of years later, the ancestors of today's eukaryotes split off from the archaea. Despite the superficial resemblance to bacteria, biochemically and genetically, the archea are as different from bacteria as bacteria are from humans.

In the late 1600s, Antoni van Leeuwenhoek became the first to study bacteria under the microscope. During the nineteenth century, the French scientist Louis Pasteur and the German physician Robert Koch demonstrated the role of bacteria as pathogens (causing disease). The twentieth century saw numerous advances in bacteriology, indicating their diversity, ancient lineage, and general importance. Most notably, a number of scientists around the world made contributions to the field of microbial ecology, showing that bacteria were essential to food webs and for the overall health of the Earth's ecosystems. The discovery that

some bacteria produced compounds lethal to other bacteria led to the development of antibiotics, which revolutionized the field of medicine.

There are two different ways of grouping bacteria. They can be divided into three types based on their response to gaseous oxygen. Aerobic bacteria require oxygen for their health and existence and will die without it. Anerobic bacteria can't tolerate gaseous oxygen at all and die when exposed to it. Facultative aneraobes prefer oxygen, but can live without it.

The second way of grouping them is by how they obtain their energy. Bacteria that have to consume and break down complex organic compounds are heterotrophs. This includes species that are found in decaying material as well as those that utilize fermentation or respiration. Bacteria that create their own energy, fueled by light or through chemical reactions, are autotrophs.

- **Capsule** - Some species of bacteria have a third protective covering, a capsule made up of polysaccharides (complex carbohydrates). Capsules play a number of roles, but the most important are to keep the bacterium from drying out and to protect it from phagocytosis (engulfing) by larger microorganisms. The capsule is a major virulence factor in the major disease-causing bacteria, such as ***Escherichia coli*** and ***Streptococcus pneumoniae***. Nonencapsulated mutants of these organisms are avirulent, i.e. they don't cause disease.

- **Cell Envelope** - The cell envelope is made up of two to three layers: the interior cytoplasmic membrane, the cell wall, and -- in some species of bacteria -- an outer capsule.

- **Cell Wall** - Each bacterium is enclosed by a rigid cell wall composed of peptidoglycan, a protein-sugar (polysaccharide) molecule. The wall gives the cell its shape and surrounds the cytoplasmic membrane, protecting it from the environment. It also helps to anchor appendages like the pili and flagella, which originate in the cytoplasm membrane and protrude through the wall to the outside. The strength of the wall is responsible for keeping the cell from bursting when there are large differences in osmotic pressure between the cytoplasm and the environment.

  Cell wall composition varies widely amongst bacteria and is one of the most important factors in bacterial species analysis and differentiation. For example, a relatively thick, meshlike structure that makes it possible to distinguish two basic types of bacteria. A technique devised by Danish physician Hans Christian Gram in 1884, uses a staining and washing technique to differentiate between the two forms. When exposed to a gram stain, gram-positive bacteria retain the purple color of the stain because the structure of their cell walls traps the dye. In gram-negative bacteria, the cell wall is thin and releases the dye readily when washed with an alcohol or acetone solution.

- **Cytoplasm** - The cytoplasm, or protoplasm, of bacterial cells is where the functions for cell growth, metabolism, and replication are carried out. It is a gel-like matrix composed of water, enzymes, nutrients, wastes, and gases and contains cell structures such as ribosomes, a chromosome, and plasmids. The cell envelope encases the cytoplasm and all its components. Unlike the eukaryotic (true) cells, bacteria do not have a membrane enclosed nucleus. The chromosome, a single, continuous strand of DNA, is localized, but not contained, in a region of the cell called the nucleoid. All the other cellular components are scattered throughout the cytoplasm.

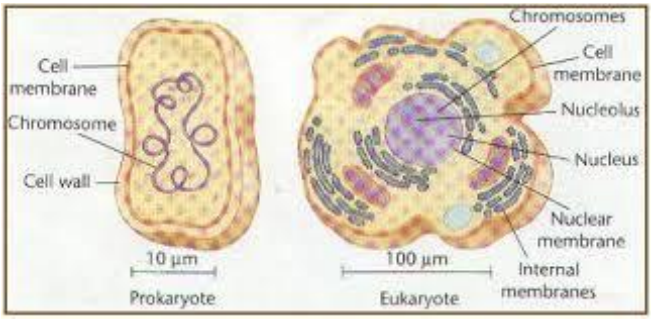
  One of those components, plasmids, are small, extrachromosomal genetic structures carried by many strains of bacteria. Like the chromosome, plasmids are made of a circular piece of DNA. Unlike the chromosome, they are not involved in reproduction. Only the chromosome has the genetic instructions for initiating and carrying out cell division, or binary fission, the primary means of reproduction in bacteria. Plasmids replicate independently of the chromosome and, while not essential for survival, appear to give bacteria a selective advantage.

Plasmids are passed on to other bacteria through two means. For most plasmid types, copies in the cytoplasm are passed on to daughter cells during binary fission. Other types of plasmids, however, form a tubelike structure at the surface called a pilus that passes copies of the plasmid to other bacteria during conjugation, a process by which bacteria exchange genetic information. Plasmids have been shown to be instrumental in the transmission of special properties, such as antibiotic drug resistance, resistance to heavy metals, and virulence factors necessary for infection of animal or plant hosts. The ability to insert specific genes into plasmids have made them extremely useful tools in the fields of molecular biology and genetics, specifically in the area of genetic engineering.

- **Cytoplasmic Membrane** - A layer of phospholipids and proteins, called the cytoplasmic membrane, encloses the interior of the bacterium, regulating the flow of materials in and out of the cell. This is a structural trait bacteria share with all other living cells; a barrier that allows them to selectively interact with their environment. Membranes are highly organized and asymmetric having two sides, each side with a different surface and different functions. Membranes are also dynamic, constantly adapting to different conditions.
- **Flagella** - Flagella (singular, flagellum) are hairlike structures that provide a means of locomotion for those bacteria that have them. They can be found at either or both ends of a bacterium or all over its surface. The flagella beat in a propeller-like motion to help the bacterium move toward nutrients; away from toxic chemicals; or, in the case of the photosynthetic cyanobacteria; toward the light.
- **Nucleoid** - The nucleoid is a region of cytoplasm where the chromosomal DNA is located. It is not a membrane bound nucleus, but simply an area of the cytoplasm where the strands of DNA are found. Most bacteria have a single, circular chromosome that is responsible for replication, although a few species do have two or more. Smaller circular auxiliary DNA strands, called plasmids, are also found in the cytoplasm.
- **Pili** - Many species of bacteria have pili (singular, pilus), small hairlike projections emerging from the outside cell surface. These outgrowths assist the bacteria in attaching to other cells and surfaces, such as teeth, intestines, and rocks. Without pili, many disease-causing bacteria lose their ability to infect because they're unable to attach to host tissue. Specialized pili are used for conjugation, during which two bacteria exchange fragments of plasmid DNA.
- **Ribosomes** - Ribosomes are microscopic "factories" found in all cells, including bacteria. They translate the genetic code from the molecular language of nucleic acid to that of amino acids—the building blocks of proteins. Proteins are the molecules that perform all the functions of cells and living organisms. Bacterial ribosomes are similar to those of eukaryotes, but are smaller and have a slightly different composition and molecular structure. Bacterial ribosomes are never bound to other organelles as they sometimes are (bound to the endoplasmic reticulum) in eukaryotes, but are free-standing structures distributed throughout the cytoplasm. There are sufficient differences between bacterial ribosomes and eukaryotic ribosomes that some antibiotics will inhibit the functioning of bacterial ribosomes, but not a eukaryote's, thus killing bacteria but not the eukaryotic organisms they are infecting.

Difference between prokaryotes & eukaryotes cell

Definitions / Description	Eukaryotic Cell	Prokaryotic Cell
Organisms:	Plants, animals and fungi have eukaryotic cells.	Only bacteria and cyanobacteria have prokaryotic cells.
Cell wall:	No (animals); Yes (plants)	Yes
Centrioles:	Yes (all animals and some lower plant forms)	No
Cilia and Flagella:	Yes, simple	Yes, complex
Golgi Complex:	Yes	No
Lysosomes:	Common in animals; Not present in plants	No
Peroxisomes:	Yes	No
Nucleus:	Yes	No
Plasma membrane:	Yes	Yes
Chromosomes:	Several chromosomes	One long DNA strand
Ribosomes:	Yes	Yes
Endoplasmic Reticulum:	Present	Absent



Bacteriophage

A **bacteriophage** is a virus that infects and replicates within a bacterium. The term is derived from "bacteria" and the Greek:, "to devour". Bacteriophages are composed of proteins that encapsulate a DNAor RNA genome, and may have relatively simple or elaborate structures. Their genomes may encode as few as four genes, and as many as hundreds of genes. Phages replicate within the bacterium following the injection of their genome into its cytoplasm. Bacteriophages are among the most common and diverse entities in the biosphere**.** Phages are widely distributed in locations populated by bacterial hosts, such as soil or the intestines of animals. One of the densest natural sources for phages and other viruses is sea water, where up to 9×10^{8} virions per milliliter have been found in microbial mats at the surface.

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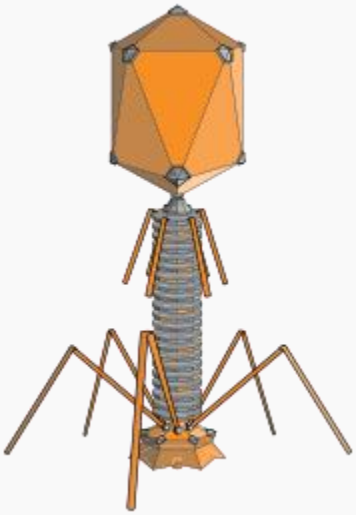
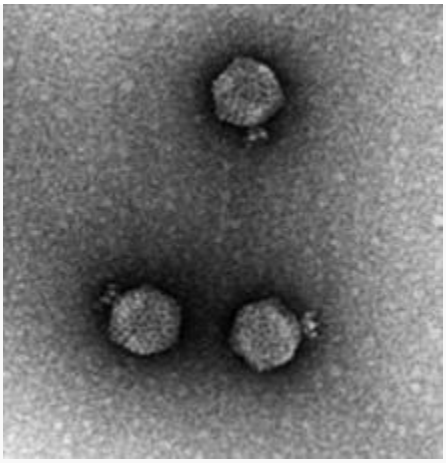


The structure of a typical myovirus bacteriophage

Classification

Bacteriophages occur abundantly in the biosphere, with different virions, genomes, and lifestyles. Phages are classified by the International Committee on Taxonomy of Viruses(ICTV) according to morphology and nucleic acid.

Nineteen families are currently recognized by the ICTV that infect bacteria and archaea. Of these, only two families have RNA genomes, and only five families are enveloped. Of the viral families with DNA genomes, only two have single-stranded genomes. Eight of the viral families with DNA genomes have circular genomes while nine have linear genomes. Nine families infect bacteria only, nine infect archaea only, and one (*Tectiviridae*) infects both bacteria and archaea.



Bacteriophage P22, a member of the *Podoviridae* by morphology due to its short, non-contractile tail.

ICTV classification of prokaryotic (bacterial and archaeal) viruses[1]

Order	Family	Morphology	Nucleic acid	Examples
Caudovirales	*Myoviridae*	Nonenveloped, contractile tail	Linear dsDNA	T4 phage, Mu, PBSX, P1Puna-like, P2, I3, Bcep 1, Bcep 43, Bcep 78
	Siphoviridae	Nonenveloped, noncontractile tail (long)	Linear dsDNA	λ phage, T5 phage, phi, C2, L5, HK97, N15
	Podoviridae	Nonenveloped, noncontractile tail (short)	Linear dsDNA	T7 phage, T3 phage, Φ29, P22, P37
Ligamenvirales	*Lipothrixviridae*	Enveloped, rod-shaped	Linear dsDNA	Acidianus filamentous virus 1
	Rudiviridae	Nonenveloped, rod-shaped	Linear dsDNA	Sulfolobus islandicus rod-shaped virus 1
Unassigned	*Ampullaviridae*	Enveloped, bottle-shaped	Linear dsDNA	
	Bicaudaviridae	Nonenveloped, lemon-shaped	Circular dsDNA	
	Clavaviridae	Nonenveloped, rod-shaped	Circular dsDNA	
	Corticoviridae	Nonenveloped, isometric	Circular dsDNA	
	Cystoviridae	Enveloped, spherical	Segmented dsRNA	
	Fuselloviridae	Nonenveloped, lemon-shaped	Circular dsDNA	

ICTV classification of prokaryotic (bacterial and archaeal) viruses[1]

Order	Family	Morphology	Nucleic acid	Examples
	Globuloviridae	Enveloped, isometric	Linear dsDNA	
	Guttaviridae	Nonenveloped, ovoid	Circular dsDNA	
	Inoviridae	Nonenveloped, filamentous	Circular ssDNA	M13
	Leviviridae	Nonenveloped, isometric	Linear ssRNA	MS2, Qβ
	Microviridae	Nonenveloped, isometric	Circular ssDNA	ΦX174
	Plasmaviridae	Enveloped, pleomorphic	Circular dsDNA	
	Tectiviridae	Nonenveloped, isometric	Linear dsDNA	

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image. Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes. Stains may be used to define and examine bulk tissues (highlighting, for example, muscle fibers or connective tissue), cell populations (classifying different blood cells, for instance), or organelles within individual cells.

Simple staining is staining with only one stain/dye. There are various kinds of multiple staining, many of which are examples of counterstaining, differential staining, or both, including double staining and triple staining.

Gram staining

Gram staining, also called **Gram's method**, is a method of staining used to differentiate 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.

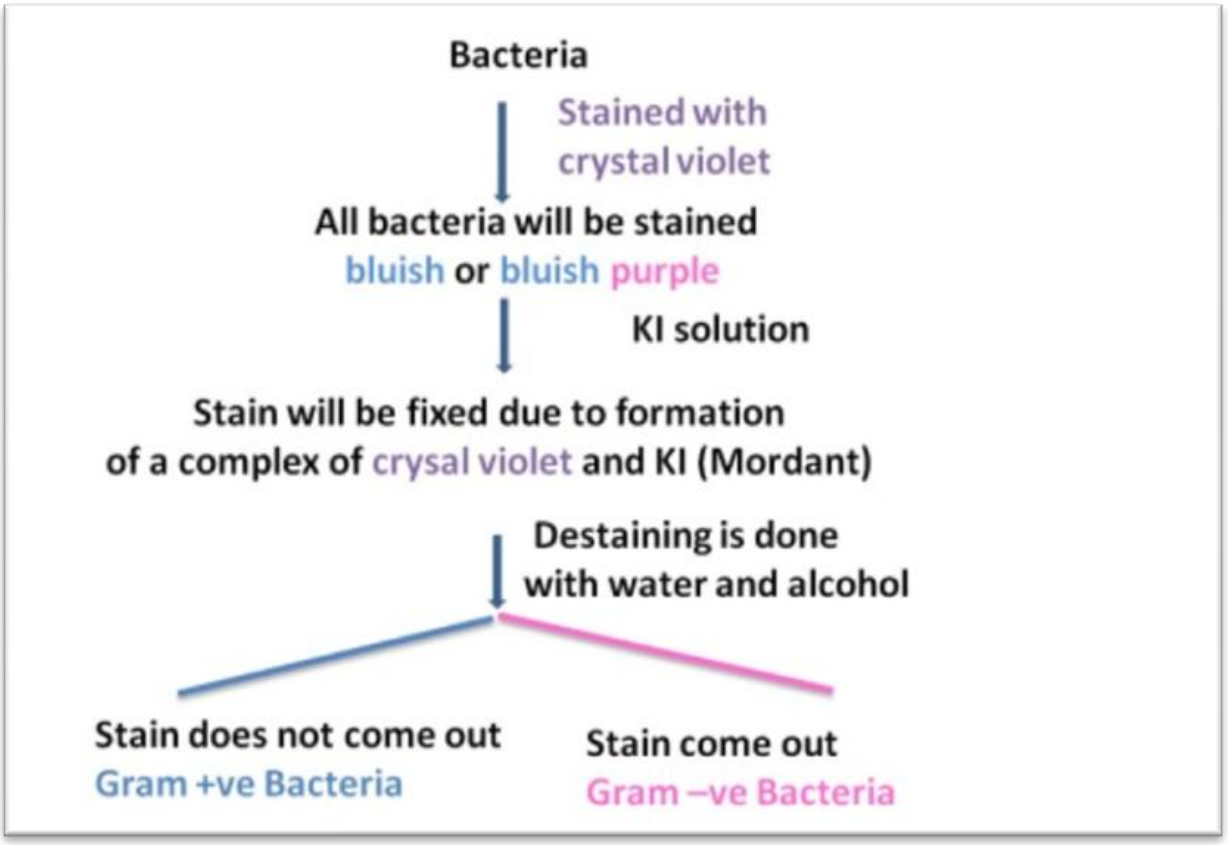
Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in a thick layer in gram-positive bacteria. Gram-positive bacteria retain the crystal violet dye, while a counterstain (commonly safranin or fuchsine) added after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

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 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
- *Counterstaining* with safranin. Carbol fuchsin is sometimes substituted for safranin since it more intensely stains anaerobic bacteria, but it is less commonly used as a counterstain.
    - 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.
    - 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.



Acid fast staining

The **Ziehl–Neelsen stain**, also known as the **acid-fast stain**, was first described by two German doctors: the bacteriologist Franz 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. It can also be used to stain a few other bacteria, such as *Nocardia*. The reagents used are Ziehl–Neelsen carbol fuchsin, acid alcohol, and methylene 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*. It is also useful in the identification of some protozoa, namely Cryptosporidium 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.

Negative staining

Negative staining is an established method, often used in diagnostic microscopy, for contrasting a thin specimen with an optically opaque fluid. In this technique, the background is stained, leaving the actual specimen untouched, and thus visible. This contrasts with 'positive staining', in which the actual specimen is stained.

For bright field microscopy, negative staining is typically performed using a black ink fluid such as nigrosin. The specimen, such as a wet bacterial culture spread on a glass slide, is mixed with the negative stain and allowed to dry. When viewed with the microscope the bacterial cells, and perhaps their spores, appear light against the dark surrounding background. An alternative method has been developed using an ordinary waterproof marking pen to deliver the negative stain.

In the case of transmission electron microscopy, opaqueness to electrons is related to the atomic number, i.e., the number of protons. Some suitable negative stains include ammonium molybdate, uranyl acetate, uranyl formate, phosphotungstic acid, osmium tetroxide, osmium ferricyanide and auroglucothionate. These have been chosen because they scatter electrons strongly and also adsorb to biological matter well. The structures which can be negatively stained are much smaller than those studied with the light microscope. Here, the method is used to view viruses, bacteria, bacterial flagella, biological membrane structures and proteins or protein aggregates, which all have a low electron-scattering power. Some stains, such as osmium tetroxide and osmium ferricyanide, are very chemically active. As strong oxidants, they cross-link lipids mainly by reacting with unsaturated carbon-carbon bonds, and thereby both fix biological membranes in place in tissue samples and simultaneously stain them.

Classification of bacteria on the basis of , PH

Bacteria can be The following classification

- A mesophile is an organism that grows best in moderate temperature, neither too hot nor too cold, typically between 20 and 45 °C (68 and 113 °F).The term is mainly applied to microorganisms.
- All bacteria have their own optimum environmental surroundings and temperatures in which they thrive the most.
- Thermophiles contain enzymes that can function at high temperatures. Some of these enzymes are used in molecular biology (for example, heat-stable DNA polymerases for PCR), and in washing agents.

neutrophile(ph)
an organism that grows best in a narrow range of neutral pH (between pH 6.5 and pH 7.5), same pH range as most tissues/organs in human body.

Acidophile(ph)
an organism that grows best in acidic environments, as low as pH 0.0
- can use acidophiles in the preservation of food b/c they generate acidic waste products that inhibit further microbial growth EX: Helicobacter pylori.

Alkalineophile(ph)
an organism that grows best in alkaline environments, as high as pH 11.5
- live in alkaline soil and water EX: Vibrio cholera

on the basis of temperature

psychrophile(temperature)
an organism that lives best at low temperatures (0º-15ºC)
- will die at temperatures above 20ºC
- lives in snowfields, ice, cold water
- can cause food spoilage in refrigerators
- does not cause disease in humans b/c cannot survive at body temperature

mesophile(temperature)
an organism that grow best in temperature between 20ºC to 40ºC
- human pathogens are mesophiles (body temp = 37ºC)

thermophile(temperature)
an organism that grows at temperatures above 45ºC
- live in compost piles, hot springs
- do not cause disease in humans b/c they "freeze" at normal body temperature

on the basis of oxygen

osmotolerant (facultative halophile)
an organism that can tolerate osmotic pressure (Great Salt Lake, salt ponds)
EX. Staphylococcus aureus, can tolerate up to 20% salt and survive/colonize surface of skin

halophile
an organism that is adapted to growth under high osmotic pressure (Dead Sea, Great Salt Lake, salt ponds)
- can grow in up to 30% salt, will burst if placed in freshwater

autotroph
an organism that uses an inorganic source of carbon (i.e., CO2) as sole source of carbon
- can "feed themselves" by making organic compounds from CO2

heterotroph
an organism that relies on other organisms to provide source of carbon from organic compounds
- can catabolize organic molecules (carbohydrates, proteins, amino acids, fatty acids) to acquire carbon from other organisms

obligate aerobe
an organism which must have oxygen in environment because it serves as the final electron acceptor of the electron transport chain which produces most of the ATP (source of energy)

obligate anaerobe
an organism which cannot tolerate oxygen, cannot undergo fermentation

facultative anaerobe
an aerobic organism that can use aerobic respiration, fermentation or anaerobic respiration to maintain life, although metabolic efficiency is reduced in absence of oxygen
- most adaptive type of microorganism
(EX. E. coli)

aerotolerant anaerobe / obligate fermenter

an organism that uses fermentation
does not use aerobic metabolism (no Krebs cycle or ETC) but can tolerate oxygen because it has some of the enzymes present that detoxify poisonous oxygen
(EX. lactobacilli that turn milk into cheese, and cucumbers into pickles)

microaerophile

an aerobic organism that requires a small amount of oxygen (2-10%)
- cannot tolerate normal atmospheric amounts of oxygen due to limited amount of necessary enzymes
(EX. H. pylori, found in stomach which contains low concentration of oxygen