GOVERNMENT OF TAMIL NADU

MICROBIOLOGY

HIGHER SECONDARY FIRST YEAR

Untouchability is Inhuman and a Crime

A publication under Free Textbook Programme of Government of Tamil Nadu

Department of School Education

Government of Tamil Nadu

First Edition - 2018

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Content Creation

State Council of Educational
Research and Training

Printing & Publishing

Tamil NaduTextbook and Educational
Services Corporation

www.textbooksonline.tn.nic.in

Contents

EC51BL
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HOW TO USE THE BOOK ?

Chapter Outline	Presents a complete overview of the chapter
Learning Objectives:	Goals to transform the classroom processes into learner centric with a list of bench marks
	Amazing facts, Rhetorical questions to lead students to biological inquiry
Activity	Directions are provided to students to conduct activities in order to explore, enrich the concept.
Infographics	Visual representation of the lesson to enrich learning .
	To motivate the students to further explore the content digitally and take them to virtual world
ICT	To enhance digital Science skills among students
Glossary	Explanation of scientific terms
Evaluation	Assess students to pause, think and check their understanding
Career corner	List of professions particular to that chapter
References	List of related books for further details of the topic
Web links	List of digital resources

Career Opportunities for Microbiologists

Microbiologists are biological scientists who study about organisms that are generally so small and can only be seen with a microscope. These microorganisms include bacteria, algae, yeasts, fungi, protozoa, viruses, and other microscopic forms of life. Some microbiologists specialize in one type of microorganism. For example, bacteriologists concentrate on bacteria and virologists study viruses. Microbiologists isolate and make cultures of microorganisms, identify their characteristics, and observe their reactions to chemicals and other kinds of stimuli. They also study how microorganisms develop and reproduce as well as their distribution in nature.

The Scope of Microbiology (Course Benefit / Advantages)

The whole ecosystem depends on bacterial activities. The modern microbiology is a large discipline with different specialities. Microbiology has a great impact on fields such as medicine, agriculture, food sciences, ecology, genetics, biochemistry and molecular biology. There are many possible avenues of advancement for microbiologists.

Medical Microbiology – Medical microbiologists are involved in identifying the microorganisms causing the infectious diseases. They work on identifying the pathogens and assist the medical practitioners for prescribing the apt antibiotics in right dosages. They also study the ways in which the micro organisms cause the infection. Medical microbiologists study the relationship between microorganisms and disease establishment.

Immunology – Those who work on immune system related work are called immunologists. Immunologist study the body's defensive responses to microorganisms. They learn how our immune system protects our body during the infection. They suggest possible ways to increase our immunity. It is one of the fastest growing areas in science.

Microbial Ecology - The microbial interactions with living and non-living matters of the environmental habitats is referred to as microbial ecology. Microbial ecologists study the contributions of microorganisms to the cycling of various nutrients or elements. The ecologists are employed in reducing the pollution of the environment which is the burning issue in all metro cities. They work on employing microorganisms in bioremediation to reduce pollution effects.

Food and Dairy Microbiology – Some of our foods are actually the by-products of microbial growth. Example: Cheese is produced by the growth of microorganisms such as *Leuconostoc citrovorum* and *Streptococcus lactis*. Yoghurt results from the growth of bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in milk. The leavening of bread is accomplished by *Saccharomyces cerevisiae* (Baker's yeast). Main work of the food and dairy microbiologists in the food industry is to prevent contamination during processing and the transmission

of food borne diseases. Microbiologists are currently employed in all food and dairy processing industries. They are also employed in Mineral water companies to check the quality of water.

Agricultural Microbiology – It is concerned with the impact of microorganisms on agriculture. Most bacteria and fungi live saprophytically on dead and organic matter of the soil. They decompose the complex organic matter into simpler form making it available for the soil microorganisms. Thus they form an important constituent of soil called humus. Certain microbes increase the fertility of the soil by converting the atmospheric nitrogen into ammonia, nitrites and nitrates. This is brought about by the microbes such as *Nitrosomonas*, *Nitrobacter* and *Rhizobium* sp. Agricultural microbiologists try to combat plant diseases that attack commercial food crops and they also work on methods to increase soil fertility and crop yields.

Industrial Microbiology – Microorganisms are used to make products such as antibiotics, vaccines, steroids, alcohols, vitamins, amino acids and enzymes. Some important drugs are synthesized by microorganisms such as streptomycin, penicillin, chloramphenicol, tetracycline. Industrial microbiologists work on improving the strains that produce the industrially important products and thereby increase the yield. The Research and Development (R&D) units in the industries provide various job opportunities to microbiologists.

Directors of Research Units and Universities – Many microbiologists work for universities, where they teach and do research. Microbiologists can become directors of research in medical centres, private firms, or government agencies. Those who hold a teaching and research position in a university can advance to the rank of full professor. They can also make significant discoveries in their research and gain the recognition of other microbiologists. Many scientists consider this to be the highest form of advancement.

Microbial Genetics and Molecular Biology – The use of micro organisms has been very helpful in understanding the functions of the genes. Microbial geneticists play an important role in applied microbiology by producing new microbial strains that are more efficient in synthesizing useful products. Genetic techniques are used to test substances for their ability to cause cancer. Microbiologists are in greater demand in genetic engineering companies and research units.

Biomining – Microbes are used in extracting valuable metals like uranium from rocks. *Thiobacillus ferrooxidans* unlocks energy from inorganic compounds like iron sulphide. During this process, it produces sulphuric acid and iron sulphate. The use of micro organisms in mining has considerably reduced the cost of mining to 75%. Microbiologists involved in Biomining research are highly paid in the Government sector.

Medical coding – Medical coding is the transformation of healthcare diagnosis, procedures, medical services and equipment into universal medical alphanumeric codes. The diagnoses and procedure codes are taken from

medical record documentation, such as transcription of physician's notes, laboratory and radiologic results. Medical coding jobs are assigned to Life Science, Paramedical and Medical Graduates and Post Graduates.

Editor in Scientific Journals – Editing, proof reading in scientific journals, handle manuscripts on topics ranging from Zoology, Biology, Plants and Animal sciences are few assignments that could be accomplished by microbiologists. Microbiologists review the research articles that are to be published in reputed National and International Journals. They are employed as Editors and Associate Editors of Scientific Publishing Companies.

Pharma companies – A microbiologist in a pharmaceutical company is a member of quality department. The role of the microbiologist is to ensure the quality of raw materials before they are processed in the production area, monitor the microbiological quality of environment and water and validate the test methods used in testing the finished products from microbiological perspective.

Eligibility Criteria for Undergraduate level courses in Microbiology

In order to apply for under graduate level courses in Microbiology, candidates should complete 12th class. It is important to opt Physics, Chemistry and Biology subjects in 12th class to join for Microbiology courses. Candidates need to score good percentage of marks in 12th class as the selection process for undergraduate level courses in this stream will be based on the marks scored. There are certain top universities which conduct selection through entrance examination. Candidates can choose any of the specialization streams in order to choose courses related to Microbiology

- Agricultural Microbiology
- Food microbiology
- Medical Microbiology
- Pharmaceutical Microbiology
- Microbial Genetics
- Environmental Microbiology
- Aero Microbiology
- Microbial Physiology

Different Courses in Microbiology

- Bachelor of Science in Microbiology
- Bachelor of Science in Microbiology and Microbial Technology
- Bachelor of Science in Clinical Microbiology
- Bachelor of Science in Medical Microbiology
- Bachelor of Science in Industrial Microbiology
- Bachelor of Arts in Microbiology
- Diploma Courses in Microbiology
- Post Graduate Diploma in Marine Microbiology
- M.Sc in Microbiology
- M.Sc in Applied Microbiology
- M.Sc in Microbial Genetics and Bioinformatics

Universities offering Courses in Microbiology

- Indian Institute of Technology
- Banaras Hindu University

- Aligarh Muslim University
- University of Mumbai
- Vinayaka Mission University
- Mahatma Gandhi University
- Indian Institute of Science
- Amity University
- Kurukshetra University

Para Medical Courses and certificate courses in Tamilnadu Government Medical Colleges

1 Year Certificate Courses

Courses	Educational Qualification	Age limit
Cardio Sonography Technician	Pass in H.Sc. with physics, Chemistry, Botany & Zoology (or) Biology and Microbiology	Should complete 17 yrs Should not exceed 32 yrs
ECG/ Tread Mill Technician		
Pump Technician		
Cardiac Catheterisation Lab Technician		
Emergency Care Technician		
Dialysis Technician		
Anaesthesia Technician		
Theatre Technician		
Orthopaedic Technician		
Audiometry Technician		
Hearing Language and Speech Technician		
Clinical, Therapeutic, Nutrition & Food Service Management Technician		
E.C.G/E.M.G Course Technician		
Multipurpose Hospital Worker Course	Pass in SSLC	

2 Years Diploma Courses

Courses	Educational Qualification	Age limit
Dental Mechanic(Male)	Pass in H.Sc. with physics, Chemistry, Botany & Zoology (or) Biology and Microbiology	Should complete 17 yrs Should not exceed 32 yrs
Dental Hygienist (Female)		
Diploma in Medical Lan Rechnology (Dmlt)		
Diploma in Radio Diagnosis Technology (Drdt)		
Diploma in Radio Therapy Technology (Drtt)		
Diploma in Optometry		

Medical Record Science

Courses	Educational Qualification	Age limit
Diploma in Medical Record Technician (Six Months)	Pass in H.Sc. with physics, Chemistry, Botany & Zoology (or) Biology and Microbiology	Should complete 17 yrs Should not exceed 32 yrs

Job Prospects

Candidates who have studied courses related to Microbiology have good scope for jobs in different sectors. Candidates can take up jobs in private sectors mainly in pharmaceutical companies, research firms. Candidates can get in to roles like Medical Microbiologists, Agricultural Microbiologists, and Marine Microbiologists. Candidates can join for teaching jobs as well. Jobs are also available in public sector after doing under graduate or post graduate level courses in Microbiology. Job opportunities occur in government controlled development laboratories, chemical industries, hospitals, food industry, pharmaceutical companies. Apart from this, candidates can also try for jobs abroad. Candidates who attain good experience in this field will get higher salary packages in jobs.

Career Prospects after completion of B.Sc Microbiology course

Candidates, who have completed their B.Sc Microbiology, can become microbiologists and there is wide range of employment opportunities available for microbiologists. They can find job placement in research laboratories and research organizations in public sector and private sector. They can also find job placement in pharmaceutical firms, chemical firms. Since there is many similarities between microbiology and biotechnology, the career options available for professionals in the field of biotechnology are applicable to the professionals in the field of microbiology as well.

Central Government jobs after M.Sc Microbiology

Post Graduates of Microbiology can find plenty of job opportunities in the Central Government sector. Several vacancies are available for them in the research institutes run by Central Government. These graduates can apply for Scientist, Research Assistant, Technical Assistant, Field Assistant or Project Assistant posts in these institutes whenever vacancies are available. Institute of Liver and Biliary Sciences, New Delhi offers Microbiologist job for these graduates. They can apply for this post when the notification gets published in the newspaper or website. Staff Selection Commission conducts Combined Graduate Level Exam for recruiting graduates to various departments in the Government. Those who have completed M.Sc Microbiology can apply for this exam, if they are interested to work in the Government sector. There are many laboratories working under the supervision of Council of Scientific and Industrial Research (CSIR). M.Sc

Microbiology graduates can apply for various posts available for them in these laboratories. There are also vacancies available for these graduates in Government hospitals.

Teaching Profession in Government Sector after M.Sc Microbiology

Candidates who want to work in the teaching field after M.Sc Microbiology can apply to various colleges or universities. An M. Phil / Ph.D degree is required for these graduates to apply for these posts. They also need to clear NET exam so as to be eligible for teaching posts available in various universities.

Microbiology in India

There are number of Institutes engaged in microbiological research in our country. The Indian Institute of Petroleum, Dehradun; Tata Energy Research Institute, Delhi and National Chemical Laboratory, Pune have worked on microbial dewaxing of heavier petroleum fractions. The Institutes has also played a vital role on the area of microbial enhanced oil recovery and production of biosurfactants. National Institute of Nutrition, Hyderabad and National Institute of Occupational Health, Ahmedabad have already completed a long time plan on monitoring and surveillance of food contaminants hazards in India while genome analysis and synthetic gene design for modulation of genome expression *invivo* was carried out by the scientists of Indian Institute of Science, Bangalore.

The Future of Microbiology

Microbiology has a clearer mission than other scientific disciplines. It is confident of its value because of its practical significance. The following brief list will give us some idea of what the future may hold:

- Everyday microbes are changing its nature (mutation) and new diseases are emerging. Microbiologists will have to respond to these threats.
- Microbiologists must find ways to stop the spread of established infectious diseases.
- Microbial diversity is another area requiring considerable research.
- Much work needs to be done on microorganisms living in extreme environmental conditions. The discovery of new microorganisms may lead to further advances in industrial processes and enhanced environmental control.
- The genomes of many micro organisms already have been sequenced, and many more will be determined in the coming years.
- Microorganisms are essential partners with higher organisms in symbiotic relationships. Greater knowledge of symbiotic relationships can help improve our appreciation of the living world. It also will lead to improvements in the health of plants, livestock and humans.

Chapter 1

Introduction to Microbiology

Chapter Outline

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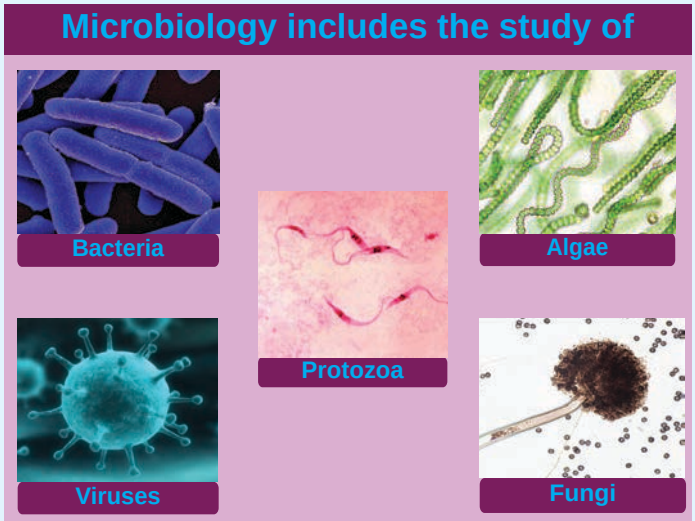

Microorganisms - Bacteria, Fungi, Algae, Protozoa and Viruses - have been around for at least 3,500 million years.Microbes affect every aspect of life on earth. They have an amazing variety of shapes and sizes. They can exist in a wide range of habitats.

Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world.

Louis Pasteur

Learning Objectives

After studying this chapter the student will be able,

- *To know the features of microorganisms.*
- *To know the contributions of different scientists.*
- *To know the branches of Microbiology.*

Microbiology is one of the fascinating fields of science. Microorganisms and their activities are the major concerns of society both nationally and internationally. The developments in biotechnology, genetic engineering and nanotechnology have placed Microbiology in the limelight. Microorganisms provide the model for interdisciplinary research and for studying fundamental life processes. There is growing recognition of microorganisms and their potential in many applied areas like Environmental science, Agriculture, Food and Pharmaceutical industries. The uses of microorganisms are becoming increasingly attractive. Some microorganisms are beneficial to us and we cannot live without them.

However microorganisms can be harmful in many ways and bring about undesirable changes. These microorganisms can cause diseases that can make us sick or even kill us. Although much more is known today about microbial life than ever before, the vast majority of this invisible world remains unexplored. Microbiologists continue to identify new ways that microbes benefit and threaten humans.

Microbiology is the study of living organisms of microscopic size, which include bacteria, fungi, algae, protozoa, and viruses. Microbiology is concerned with form, structure, reproduction, physiology, metabolism, and classification of microorganisms. It includes the study of

- their distribution in nature,
- their relationship to each other and to other living organisms,
- their effects on human beings, animals and plants,
- their abilities to make physical and chemical changes in our environment,
- their reaction to physical and chemical agents.

1.1 Groups of Microorganisms

There are many kinds of microorganisms present in the universe. They are broadly classified into the following groups.

Bacteria: They are unicellular prokaryotic organisms or simple association of similar cells. Cell multiplication usually happen by binary fission.

Example: *Escherichia coli, Bacillus subtilis*

Fungi: They are eukaryotic organisms which is devoid of chlorophyll. They are usually multicellular. They range in size and shape from single celled microscopic yeasts to giant multicellular mushrooms and puffballs.

Example: *Aspergillus niger, Agaricus bisporus*

Protozoa: They are unicellular eukaryotic organisms. Their role in nature are varied. The best known protozoa cause disease in human beings and animals.

Example: *Giardia lamblia, Plasmodium vivax*

Algae: They range from unicellular, colonial to multicellular forms. All algal cell contain chlorophyll and are capable of photosynthesis. They are found most commonly in aquatic environments and damp soil.

Example: *Spirogyra, Chlamydomonas*

Viruses: In the study of Microbiology, we encounter "organisms" which may represent the borderline of life. Viruses are simpler in structure and composition than other living cells. A virus is made up of nucleic acids and proteins. Viruses are obligate parasites. They grow only within an appropriate host cell (plant, animal, humans or microbe). They cannot multiply outside a host cell.

Example: HIV, Rabies virus

Prions are infectious agents composed entirely of protein material. Creutzfeldt–Jacob Disease (CJD) is one of the human prion diseases.

1.2 Contributors to Microbiology

Many scientists contributed to the science of Microbiology from the 17th century to the present day. Some prominent microbiologists who have made significant contribution to the study of microorganisms are given below:

1.2.1 Antony Van Leeuwenhoek

Antony Van Leeuwenhoek (1632-1723) of Holland (Figure 1.1) developed microscopes. He was a Dutch merchant and a skilled lens maker. He made a variety of lenses with magnifying power 50-300X.

He was the first person to invent simple microscope. It has a single biconvex lens with a magnification of about 200X (Figure 1.2). His microscopes resolved bodies with diameters measuring below 1micron. He examined water, mud, saliva and found living organisms. He called these microorganisms as **Animalcules** (little animals). Bacteria like cocci, bacilli and spirochetes were recognized. He proposed that the size of bacteria is one sixth the diameter of Red Blood Cells.

He observed the growth of bacteria in infusions. The existence of spermatozoa and RBC was revealed by him. Animal histology was established by him. He described capillary circulation and added a new dimension to Biology. All kinds of unicellular microorganisms were accurately described by him including human oral microbial flora. He is commonly known as the '**Father of Microbiology**'.

1.2.2 Louis Pasteur (1822-1895)

Louis Pasteur was a French chemist and a crystallographer (Figure 1.3). His greatest contribution to microbiology made him to be the '**Father of Modern Microbiology**'.

Figure 1.1:
Antony Van Leeuwenhoek

Antony Van Leeuwenhoek wrote many letters. He wrote them in Dutch, the only language that he knew. These letters, described his complete scientific output. Antony Van Leeuwenhoek in a letter dated 12th June 1716, wrote "... *my work, which I've done for a long time, was not pursued in order to gain the praise I now enjoy, but chiefly from a craving after knowledge, which I notice resides in me more than in most other men. And therewithal, whenever I found out anything remarkable, I have thought it my duty to put down my discovery on paper, so that all ingenious people might be informed thereof*".

Contribution to science as a chemist

Louis Pasteur was working with tartaric acid crystals. He could pick up the dextro and levo rotatory crystals by seeing the morphology of the crystals. Later he was called to solve some of the problems in fermentation industry and turned his attention to biological process of fermentation.

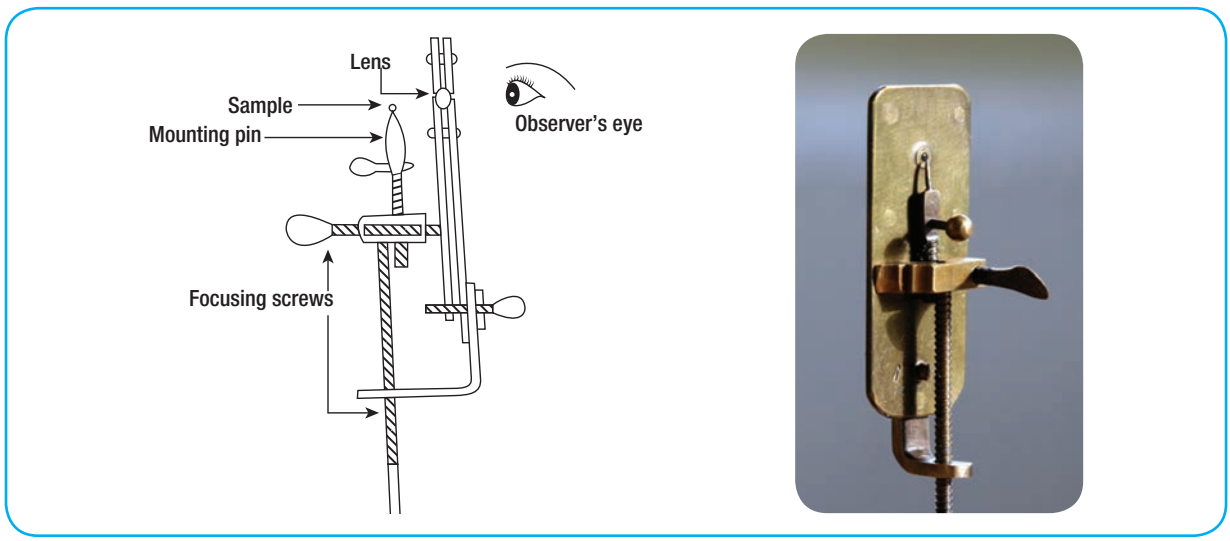

Figure 1.2: Leeuwenhoek's Microscope

Figure 1.3: Louis Pasteur (1822-1895)

Contribution to Microbiology

To wine industry

Louis Pasteur discovered alcohol production from grape juice was due to yeast. The presence or contamination of rod shaped bacteria resulted in large amounts of lactic acid production in wine. He also found that microorganisms in fermented fruits and grains, resulting in alcohol production. He coined the term "**fermentation**".

Pasteur disproved spontaneous generation

Spontaneous generation states that life could arise spontaneously from inanimate (non-living) materials (Abiogenesis). Pasteur disproved the theory of spontaneous generation. He strongly supported theory of Biogenesis (life orginates from pre-existing life forms). To prove this he carried out several experiments. Pasteur poured meat infusions into flasks and then drew the top of each flask into a long curved neck that would admit air but not dust (Figure 1.4). He found that if the infusions were heated, they remained sterile (free from any growth) until they were exposed to dust. After opening them on a dusty road and resealing them, he demonstrated the growth of microorganisms in all the flasks. The unopened flasks were sterile. Thus he disproved the theory of spontaneous generation.

Pasteurization

Louis Pasteur used heat to destroy undesirable microbes in fruit juices. He employed 62.8°C (145°F) for 30 mins to kill microbes. This process is called Pasteurization which is commonly used in distillaries and dairy industry.

Discovery of diseases

Louis Pasteur found that Pebrine disease in silk worm was caused by a protozoan parasite. He suggested that Pebrine disease could be eliminated by using only healthy,

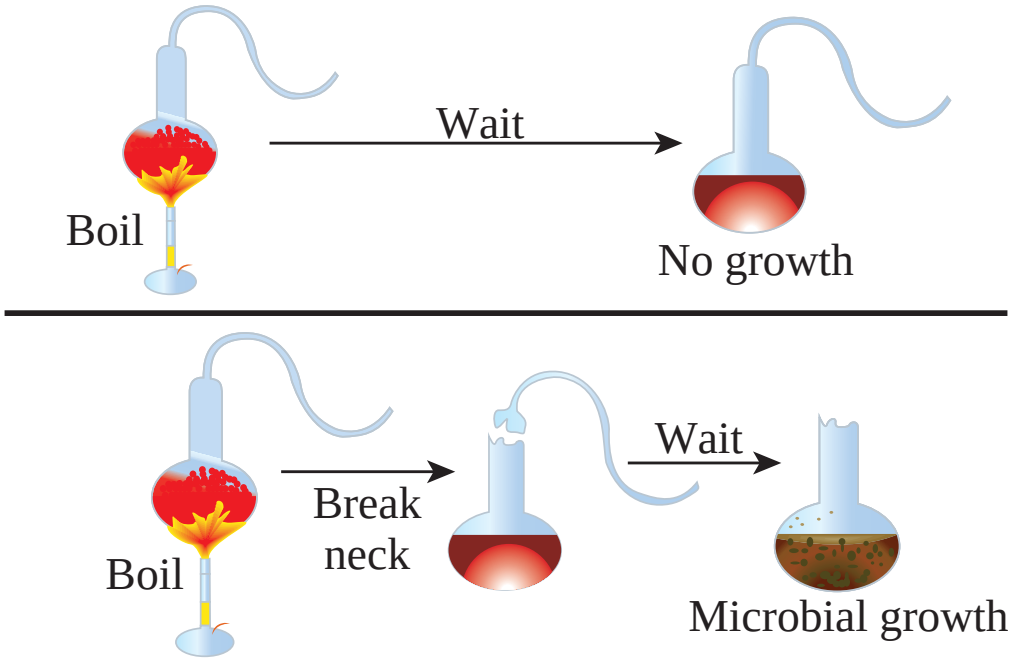

Figure 1.4: Pasteur's swan neck flask experiment

disease free silk worms. Wool Sorter's disease was named as "Anthrax" by him. He isolated *Bacillus anthracis* from the blood of infected animals. Chicken cholera bacterium was also isolated by Louis Pasteur using pure culture.

He proved that many diseases were caused by the presence of foreign microorganisms (**Germ theory of disease**). He discovered various infection causing microorganisms such as *Staphylococcus, Streptococcus* and *Pneumococcus.*

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Vaccination

Pasteur found out that bacteria could be attenuated by growing them in unnatural conditions. He coined the term "**attenuation**". It is a process wherein bacteria lose their virulence due to repeated subculturing under laboratory conditions. He used attenuated cultures as vaccines for immunizing and protecting an individual against the disease. He developed vaccines for anthrax and rabies.

1.2.3 Edward Jenner (1749-1823)

In ancient observation, persons who had suffered from a specific disease such as small pox (causative agent of small pox is varicella virus) or mumps, resisted the infection on subsequent exposures. They rarely contracted these infections for second time. Edward Jenner, a country doctor in England noted a pustular disease on the hooves of horses called the grease. This was carried by farm workers to the nipples of cows (cow pox). This was again carried by milk maids. They got inflamed spots on the hands and wrists. The people who got this cow pox were protected from small pox. He reported that 16 farm workers who had recovered from cow pox (causative agent of cow pox is vaccinia virus) were resistant to small pox infection.

He took the material (pus) from the cow pox and inoculated into the cut of 8 year old boy on 14th May 1796 (Figure 1.5). Two

months later Jenner inoculated the same boy with material taken from small pox patients. This was a dangerous but accepted procedure at that time. This procedure was called variolation. The boy was protected against small pox. His exposure to the mild cow pox disease had made him immune to the small pox disease. In this manner Jenner began the Science of Immunology, the study of the body's response to foreign substances. Edward Jenner was regarded as the **'Father of Immunology'**.

Figure 1.5: Dr. Edward Jenner performing his first vaccination (1796)

1.2.4 Robert Koch (1843-1910)

Robert Koch was a German physician and microbiologist (Figure 1.6). He was the founder of Modern Bacteriology. Robert Koch discovered *Bacillus anthracis* (Anthrax bacillus), *Mycobacterium tuberculosis*, and *Vibrio cholerae*. For the first time he showed the evidence that a specific germ (Anthrax bacillus) was the cause of a specific disease (splenic fever in sheep) and introduced scientific approach in Microbiology.

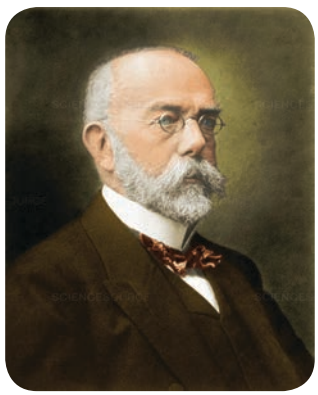


Figure 1.6: Robert Koch (1843-1910)

He modified Ziehl-Neelsen Acid Fast staining procedure which was introduced by Ehrlich. He devised solid medium to grow microorganism. He developed powerful method to isolate the microorganisms in pure culture from diseased tissue. He also perfected the techniques of identification of the isolated bacteria.

He introduced Koch's thread method to find out the efficacy of disinfectants. He established certain rules that must be followed to establish a cause and effect relationship between a microorganism and a disease. They are known as Koch's Postulates. He also described the Koch's Phenomenon. He was regarded as the **'Father of Medical Microbiology'**.

Infobits

Koch's Thread Method

Robert Koch carried out systematic experiments on disinfection, using pure cultures of bacteria. By means of his Thread Method, he investigated the effect on anthrax spores of the popular disinfectants at that time. Koch's Thread Method also called as carrier test. A carrier such as silk is contaminated by submerging in a liquid culture of the *Bacillus anthracis*, a test organism. The carrier is further dried and immersed in the disinfectant solution for a given exposure time. Thereafter the thread is cultured in a nutrient broth. No growth after incubation indicated that the product (disinfectant) is active.

Koch's Postulates

Four criteria were established by Robert Koch to identify the causative agents of an infectious disease. These include

1. A specific organisms can always be found in association with a given disease. If we take typhoid as an example it is caused by a bacterium *Salmonella typhi.*
2. The organism can be isolated and grown in pure culture in the laboratory. *Salmonella typhi* are grown in soild media under laboratory conditions.
3. The pure culture will produce the diseases when inoculated into a susceptible animal.

Almost all the pathogenic organisms produce the same disease in experimental animals. Usually rats, mice, rabbits or guinea pigs are used as experimental animals. *Pneumococci* produce pneumonia in animals. *Salmonella* species do not produce typhoid fever in rat, mice or rabbit. So chimpanzee is taken as experimental animal and it produces fever in chimpanzee.

4. It is possible to recover the organism in pure culture from the experimentally infected animals and it is observed to be the same as originally inoculated pathogen. Figure 1.7 explains the Koch's postulates.

Limitations

Some organisms have not yet been grown in artificial culture media

Example: *Mycobacterium leprae* and *Treponema pallidum*.

Modern addition to Koch's Postulates

Today we recognize additional criteria of causal relation between a microorganism and a disease. The important one is the demonstration of abnormally high concentration of specific circulating antibodies to the organism in the infected host or the presence of abnormally high degree of specific immunity or hypersensitivity to the infecting agent in

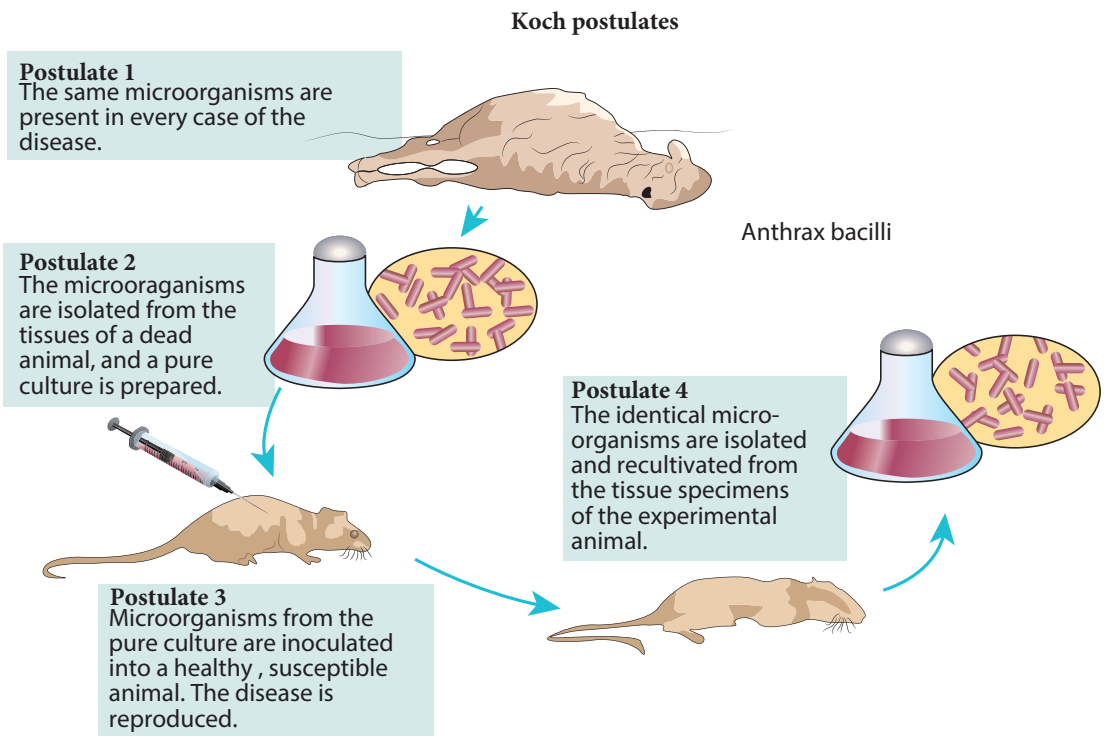


Figure 1.7: Koch's postulates for infectious diseases

a recently recovered host. In addition to culture techniques, serological techniques are also used for diagnosis of diseases.

Usefulness of Koch's Postulates

- It is useful in determining pathogenic organisms.
- To differentiate the pathogenic and nonpathogenic microorganism.
- For the classification of organisms.
- To detect the susceptibility or resistance of the laboratory animals.

1.2.5 Joseph Lister(1827-1912)

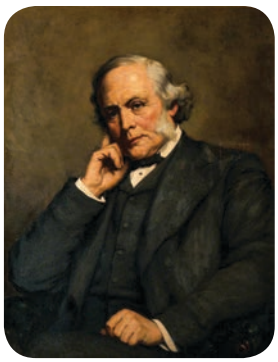


Figure 1.8: Joseph Lister (1827-1912)

Joseph Lister was a British surgeon (Figure 1.8). He found out that microorganisms were responsible for wound infections. He developed a system of antiseptic surgery. He used bandages soaked in phenol solution to prevent wound infection. He sterilized instruments by heat and sprayed diluted phenol over surgical area and prevented contamination of wounds. He was the first person to isolate bacteria in pure culture using liquid culture. Thus, he was considered as co-founder of Medical Microbiology with Koch, who later isolated bacteria on solid media.

1.2.6 Alexander Fleming (1881-1955)

He was a British Bacteriologist. He observed a mold (*Penicillium notatum*) growing on a plate of *Staphylococcus aureus*. The growth of *Staphylococcus aureus* around the mold colony was

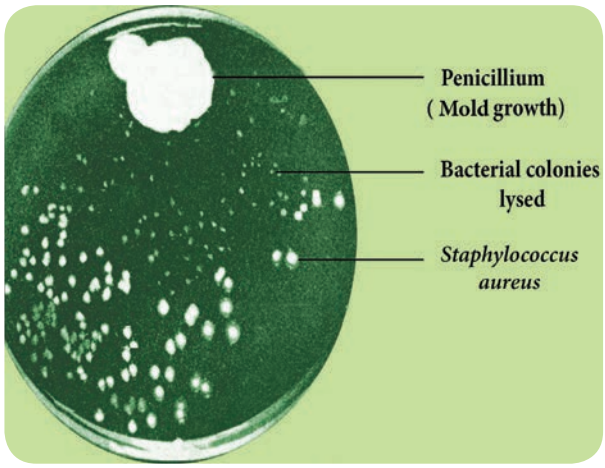


Figure 1.9: Original culture plate on which the observation of action of penicillin was made by Alexander Fleming

inhibited (Figure 1.9). He also showed that the culture filtrate of mold inhibited the growth of *Staphylococcus aureus*. He called this substance Penicillin, which acted on Gram positive bacteria. For the discovery of this antibiotic Fleming (Figure 1.10), Florey and Chain got Nobel Prize in 1945. Penicillin eventually came into use during world war II as a result of the work of a team of scientists led by Howard Florey of the University of Oxford.

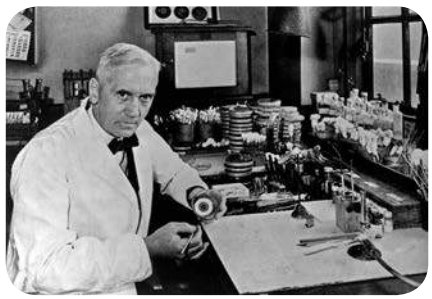


Figure 1.10: Alexander Fleming (1881-1955)

Alexander Fleming, the discoverer of penicillin warned about the possibility of antibiotic resistant bacteria due to antibiotics misuse, as early as in 1920s.

1.2.7 Selman Abraham Waksman (1888-1973)

Waksman was from Rutger University, USA (Figure 1.11). His research was largely on soil microorganisms. He showed antimicrobial activity of streptomyces that led to the discovery of Streptomycin and several other antibiotics.

Figure 1.11: Selman Abraham Waksman (1888-1973)

Waksman and his co-workers isolated Actinomycin in 1940, Streptothrecin in 1942, Streptomycin in 1943, and Neomycin in 1949.

Streptomycin is produced by *Streptomyces griseus*. It is a secondary metabolite produced by *Streptomyces griseus* which is not required for its growth but may help it to compete with other bacteria for food and space in the environment. Streptomycin is used in the treatment of tuberculosis. Waksman got Nobel Prize in 1952. for his work on Streptomycin

Antibiotics are usually not effective for sore throats and common colds. They are commonly caused by viruses rather than bacteria. Taking antibiotics for such illnesses is considered more harmful than beneficial.

1.3 Branches of Microbiology

Microbiology can be classified into Pure and Applied Microbiology. Pure Microbiology is classified based on taxonomical and integrative characteristics. Table 1.1 shows various branches of microbiology.

Table 1.1: Branches of Microbiology

Based on Taxonomical characteristics	
Bacteriology	The study of bacteria
Mycology	The study of fungi
Protozoology	The study of protozoa
Based on Taxonomical characteristics	
Phycology (or 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 the nematodes

Based on integrative characteristics	
Microbial Cytology	The study of microscopic and sub microscopic details of microorganisms
Microbial Physiology	The study of biochemical functions of microbial cell. It also includes the study of microbial growth, microbial metabolism and microbial cell structure
Microbial Ecology	The study of relationship between microorganisms and their environment
Microbial Genetics	The study of gene are organisation and regulation in microbes in relation to their cellular functions.
Cellular Microbiology	A discipline bridging microbiology and cell biology
Evolutionary Microbiology	The study of the evolution of microbes
Microbial Taxonomy	The study of naming and classification of microorganisms
Microbial Systematics	The study of the diversity and genetic relationship of microorganisms
Systems Microbiology	A discipline bridging systems biology and microbiology
Generation Microbiology	The study of microorganisms which have the same characters as their parents
Molecular Microbiology	The study of the molecular principles of physiological processes in microorganisms
Nano Microbiology	The study of microorganisms at nano level
Exo Microbiology (or Astro Microbiology)	The study of microorganisms in outer space
Biological Warfare	The study of microorganisms 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 and Epidemiology and is related to the study of disease, Pathology and Immunology
Pharmaceutical Microbiology	The study of microorganisms that are related to the production of antibiotics, enzymes, vitamins, vaccines, and other pharmaceutical products

Industrial Microbiology	The study of exploitation of microbes for use in industrial processes. Examples include industrial fermentation and waste water treatment. This field also includes brewing, an important application of microbiology
Microbial Biotechnology	The study of manipulation of microorganisms at the genetic and molecular level to generate useful products
Food Microbiology and Dairy Microbiology	The study of microorganisms in food spoilage, foodborne illness and food production.

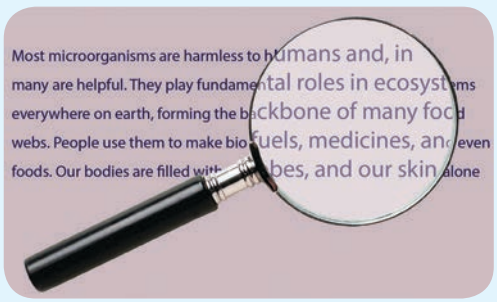
Summary

Microbiology is the study of microorganisms that includes bacteria, fungi,algae,protozoa and viruses. Many scientists contributed to the science of microbiology.

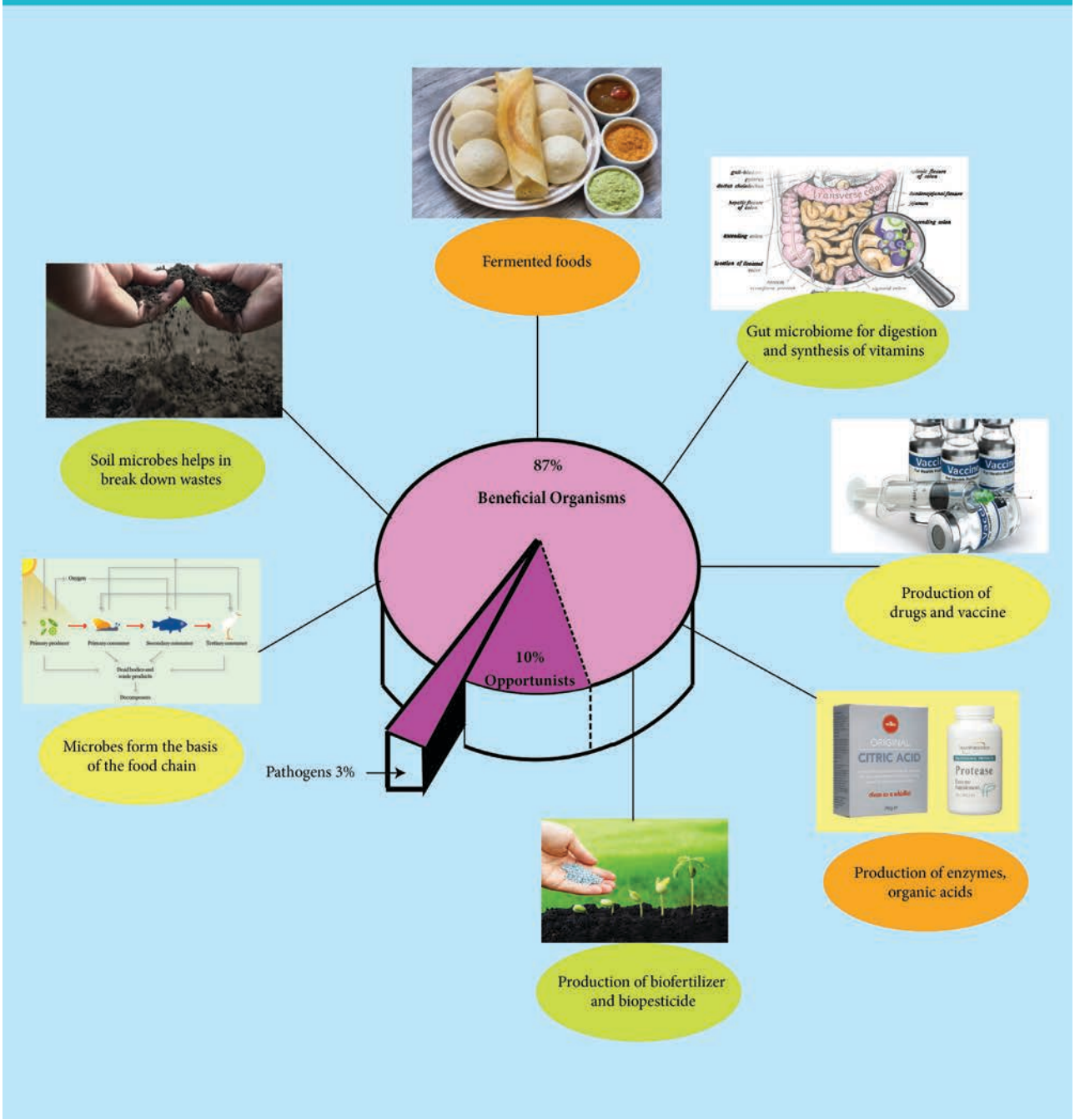
Antony Van Leuwenhoek made simple microscope. For the first time, Antony Van Leuwenhoek described the microorganisms. Louis Pasteur disproved the theory of spontaneous generation. Germ theory of disease came from the work of Pasteur and Robert Koch. Vaccines for Anthrax and rabies was developed by Pasteur. Direct relationship between the suspected pathogen and disease was established by Koch's postulates. Koch developed the technique of pure culture on solid medium. Joseph lister developed antiseptic surgery. Alexander Fleming discovered Penicillin. Waksman showed antimicrobian activity that led to the discovery of Streptomycin and other antibiotics. The branches of microbiology can be classified into pure and applied microbiology. Pure microbiology is classified based on taxonomical and integrated characteristics. Microbiology has got vast areas open for job opportunities.

Student Activity

1. Want to see spontaneous generation of life?
   Take chicken soup or meat soup boil it in a bottle. Keep it over the shadow of your window/or in a open place with mouth open. Observe for a week. You will see maggots (worms) growing. Observe and record your findings.
2. For you to enjoy-like Antony Van Leeuwenhoek !!
   Get a palmist lens, see through it a paper print. You will see letter becomes big, bigger, and at one point it is no longer magnifying the letter. A simple convex lens is magnifying things. Leeuweenhoek used such lens only. (as seen above) You know useful and useless magnification.



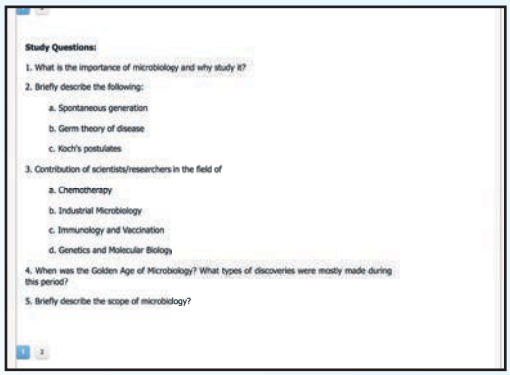
Not all Microorganisms are harmful.
Most Microorganisms are considered beneficial or harmless.



ICT CORNER

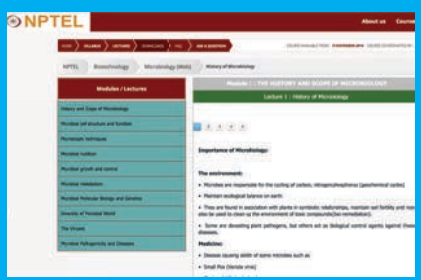
Microbiology

Lets meet our micro friends

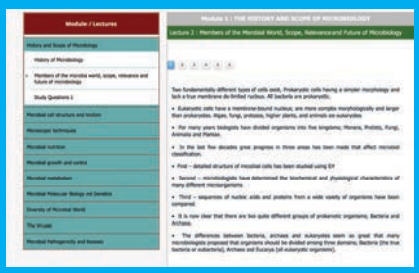


STEPS:

- Use the URL or scan the QR code to open 'NPTEL' page.
- Click 'History' and 'Scope of Microbiology' to know the history of microbiology.
- Select history of microbiology and click 'Start Course' at the bottom.
- Select 'Members of the Microbial world' to know about it.



Step1



Step2

Step3

URL:

http://nptel.ac.in/courses/102103015/41#

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Evaluation

Multiple choice questions

1. Theory of spontaneous generation was disproved by whom?
   a. Robert Koch
   b. Edward Jenner
   c. Louis Pasteur
   d. All of them
2. Which of the following did Edward Jenner used to protect the boy against small pox?
   a. Cow pox material
   b. Small pox material
   c. Both the above
   d. Rabbit pox
3. Among the following scientists, who discovered solid medium?
   a. Louis Pasteur
   b. Edward Jenner
   c. Robert Koch
   d. None of them
4. Which of the following organisms does not obey Koch's postulates?
   a. Cow pox virus
   b. Small pox virus
   c. Treponema pallidum
   d. M.Tuberculosis
5. Who modified Ziehl-Neelsen staining technique?
   a. Louis Pasteur
   b. Robert Koch
   c. Ziehl-Neelsen
   d. All the above
6. Which of the following fungi grow on Alexander Fleming's plate?
   a. *Penicillium chrysogenum*
   b. *Penicillium notatum*
   c. *Streptomyces griseus*
   d. *Penicillium mornefii*
7. Which of the following antibiotics was discovered by Waksman?
   a. Streptomycin
   b. Neomycin
   c. Actinomycin
   d. All the above

ED6JI7

Answer the following

1. Name the causative agent of cow pox and small pox.
2. Explain the method of Edward Jenner used to protect people against small pox.
3. List two organisms that do not obey Koch's postulates.
4. Give the usefulness of Koch's postulates.
5. What are the modern additions to Koch's postulates?
6. List the contribution of Alexander Fleming.
7. What is the theory of spontaneous generation?
8. How was spontaneous generation theory disproved?
9. Highlight the contribution of Waksman.
10. State the characteristics of streptomycin.
11. Give a list of contribution of Louis Pasteur to wine industry.
12. Explain Koch's postulates?
13. Describe the microscope made by Antony Van Leeuwenhoek.
14. What are the contributions of Antony Van Leeuwenhoek to microbiology?

Chapter 2

Microscopy

Chapter Outline

EDFF JU

Microorganisms are very small and cannot be viewed by human eye. The microscope helps in observing the microbial world which exists in a wide range of sizes. The prokaryotes (bacteria and archae) are smaller (~ 0.4-10µm) and the eukaryotes are larger (~ or >10µm). The word microscope is derived from the Latin word *micro*, which means small, and the Greek word skopos means *to look at.*

Learning Objectives

After studying this chapter the student will be able,

- *To know the properties of light and lens.*
- *To know the science of image formation in brightfield microscopy.*
- *To understand the design of light microscope.*
- *To learn and compare the principle, instrumentation and working of brightfield and darkfield microscopy.*

2.1 Historical Background

Antony Van Leeuwenhoek (1632-1723) was the first person to use a simple microscope with one lens similar to a magnifying glass. The lens is capable of 50X to 300X magnification.

Robert Hooke, built compound microscopes with multiple lenses. In 17th century, Dutch spectacle maker Zaccharias Janssen is given the credit for making first compound microscope. However, the early compound microscopes were poor in quality. In 1830, Joseph Jackson Lister (the father of Joseph Lister who practised antiseptic surgery) made significant development which resulted in the invention of modern compound microscope used in microbiology today.

2.2 Principles of Microscopy

All kind of microscopes use visible light to observe specimens. Light has a number of properties that affect our ability to visualise objects.

2.2.1 Properties of Light

Light is a part of the wide spectrum of electromagnetic radiation from the sun. It is a form of energy. The most important property of light is wavelength (the length

of light ray) (Figure 2.1).

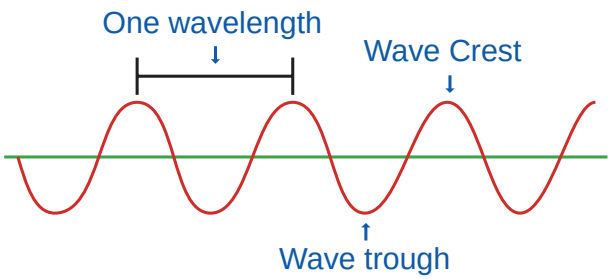


Figure 2.1: Wavelength-the distance between two adjacent crests or two adjacent troughs of the wave and denoted by greek letter (λ)

The sun produces a continuous spectrum of electromagnetic radiation with waves of various lengths (Figure 2.2). Radiation of longer wavelength includes Infrared (IR) and radiowaves, the shorter wavelengths include Ultra Violet (UV) rays and X-rays.

The physical behaviour of light can be caterigorised as either light rays, light waves or light particles. The combined properties of particle and wave enable light to interact with an object in several different ways like transmission, absorption, reflection, refraction, diffraction and scattering (Figure 2.3).

2.2.2 Lenses and its Properties

Lenses are optical devices which focus or disperse a light beam by means of refraction. A simple lens consists of a single piece of transparent material. Light rays from a distant source are focused at the focal point F. The focal point lies at a distance **f** (focal length) from the lens' centre (Figure 2.4).

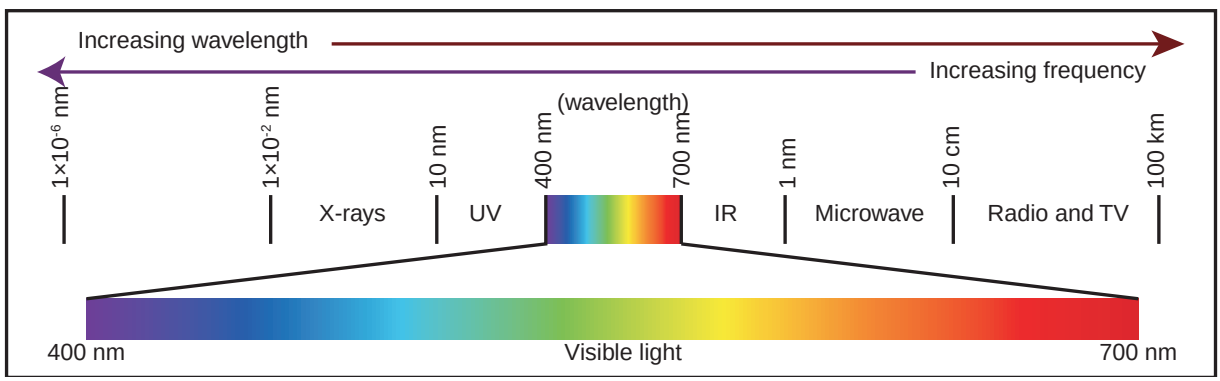


Figure 2.2: The electromagnetic spectrum-White light is a combination of all colours of visible spectrum

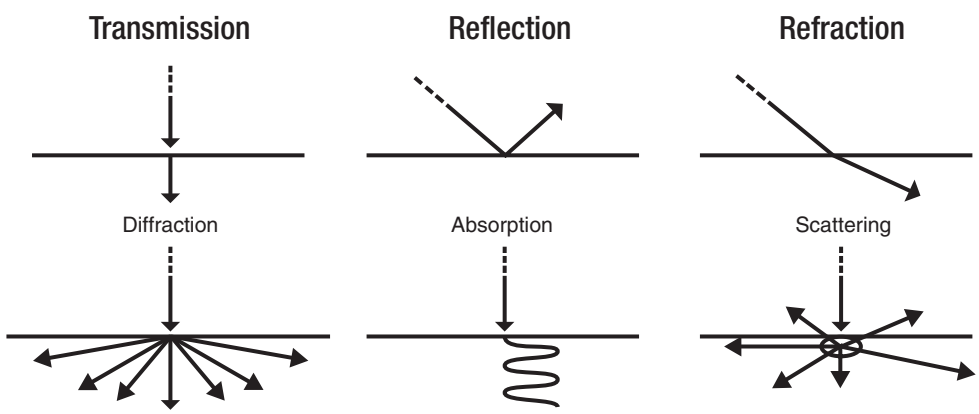


Figure 2.3: Interaction of light with matter

Microorganisms are measured in micrometers and nanometers. The average bacterial cell is 0.001mm in diameter.

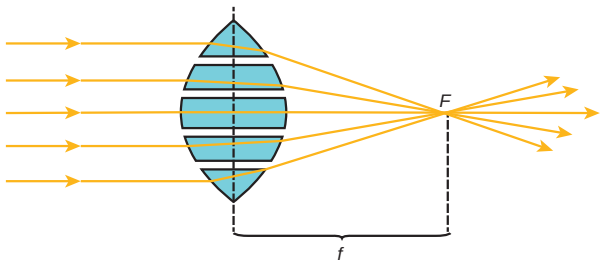


Figure 2.4: Lens function

Generating an image with a lens

When an object is placed outside the focal plane (the plane containing the focal point of the lens), all the light rays from the object are bent by the lens. The bent rays converge at the opposite focal point. At the focal point, the light rays continue and converge with nonparallel refracted light rays. The resultant reversed and magnified image is formed in the plane of convergence (Figure 2.5).

Microscope resolution

Objective is the important part in the microscope which is responsible to produce a clear image. The resolution of the objective is most important. Resolution is the capacity of a lens to separate or distinguish between small objects that are close together. The major factor in the resolution is the wave length of light used. The greatest resolution obtained with light of the shortest wave length, that is the light at the blue end of the visible spectrum are in the range of 450 to 500nm. The highest resolution possible in compound light microscope is about 0.2µm. That means, the two objects closer together than 0.2µm are not resolvable as distinct and separate. The light microscope is equipped with three or four objectives. The working distance of an objective is the distance between the front surface of the lens and the surface of the cover glass or the specimen. Objectives with large numerical apertures and great resolving power have short working distances.

Numerical aperture

Numerical Aperture (NA) is the value representing the light gathering capacity of an objective lens. NA was first described

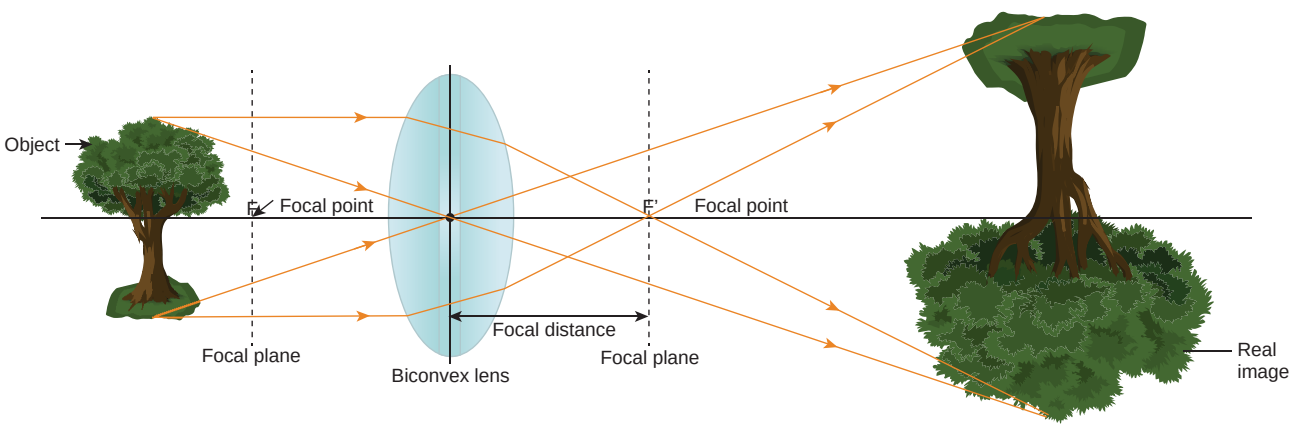
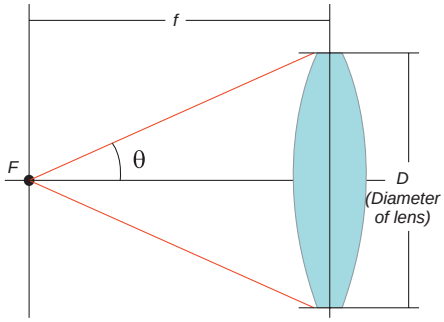


Figure 2.5: Generating an image with a lens

by Ernst Abbe, and is defined by the following expression



Numerical Aperture (NA) =n × sin(θ)

n = the refractive index of the medium between the specimen and objective; θ = half aperture angle or collection angle of the objective. (the maximum half angle of the cone of light that can enter or exit the lens).

Infobits

The smallest cells on the planet are some forms of *Mycoplasma* with dimensions of 0.2 to 0.3 µm, which is within the limit of resolution of light microscopes. Tiny cells that look like dwarf bacteria but are 10 times smaller than *Mycoplasma* and 100 times smaller than the average bacterial cell are called nanobacteria or nanobes (*Greek* nanos means one billionth).

The resolving power of a light microscope depends on the wavelength of light used and the NA of the objective lens.

The numerical aperture of a lens can be increased by

- Increasing the size of the lens opening and/or
- Increasing the refractive index of the material between the lens and the specimen.

The larger the numerical aperture the better the resolving power. It is important to illuminate the specimens properly to have higher resolution. The concave mirror in the microscope creates a narrow cone of light and has a small numerical aperture. However, the resolution can be improved with a sub stage condenser. A wide cone of light through the slide and into the objective lens increases the numerical aperture there by improves the resolution of the microscope.

Types of microscopes

In order to view microorganism and microbial structures of different sizes we require different kinds of microscopes.

- Light microscopes resolve images with the help of light. The specimen is viewed as dark object against a light background in bright field microscope. Dark field microscope uses a special condenser and the specimen appears light against a black background. The other types of mircoscopes are Phase contrast and Fluorescence microscope.
- Electron microscope uses a beam of electrons instead of light. Electrons pass through the specimen and form a two dimensional image in Transmission Electron Microscope (TEM). Electrons are reflected from the specimen and produce a three dimensional image in Scanning Electron Microscope (SEM).

2.3 Bright Field Microscope

The most commonly used microscope for general laboratory observations is the standard bright field microscope (Figure 2.6). It contains the following components

- A mirror or an electric illuminator is the light source which is located at the base of the microscope.

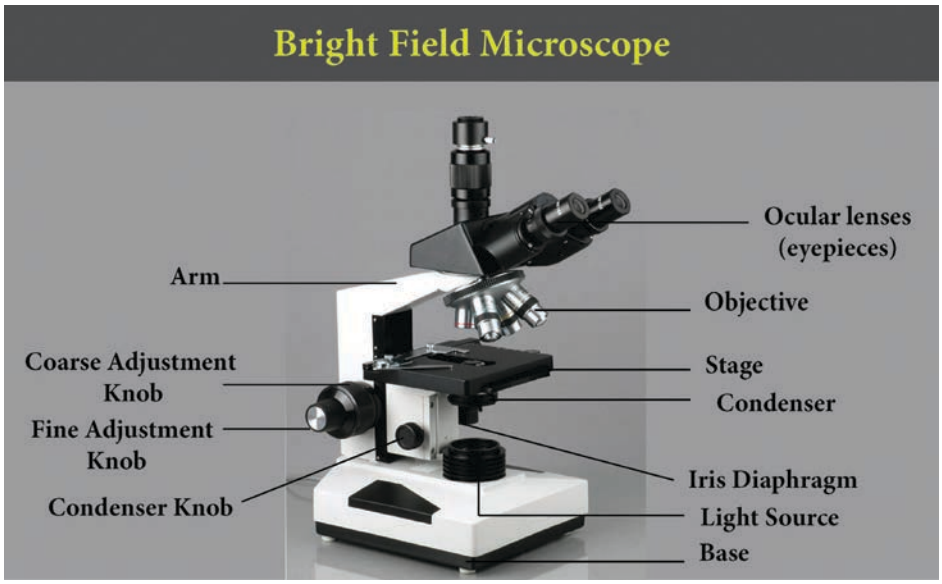


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Figure 2.6: Bright field Microscope

- There are two focusing knobs, the fine and the coarse adjustment knobs which are located on the arm. These are used to move either the stage or the nosepiece to focus the image.
- Mechanical stage is positioned about half way up the arm, which allows precise contact on moving the slide.
- The substage condenser is mounted within or beneath the stage and focuses a cone of light on the slide. In the simpler microscope, its position is fixed where as in advanced microscope it can be adjusted vertically.

The upper part of microscope arm holds the body assembly. The nose piece and one or more eyepieces or oculars are attached to it. The body assembly contains series of mirrors and prisms so that the barrel holding the eyepiece may be tilted for viewing. Three or five objectives with different magnification power are fixed to the nosepiece and can be rotated to the position beneath the body assembly. In bright field microscopy; the specimen is viewed against a bright background. The details of the image are defined by the surrounding light. A series of finely ground lenses forms an image which is many times larger than the real image. This magnification occurs when light rays from an illuminator (light source), pass through a condenser which has lenses that direct the light rays through the specimen. The light rays then pass into objective lens (the lens closest to the specimen). The image is again magnified by the ocular lens or the eyepiece. (Figure 2.7).

- Magnification is the process of enlarging the image of the specimen and can be calculated by multiplying the objective lens magnification power by ocular lens magnification power.

  Representative magnification values for a 10X ocular are:

  Scanning objective (4X) × (10X) = 40X magnification

  Low power objective (10X) × (10X) = 100X magnification

  High dry objective (40X) × (10X) = 400X magnification

  Oil immersion objective (100X) × (10X) = 1000X magnification

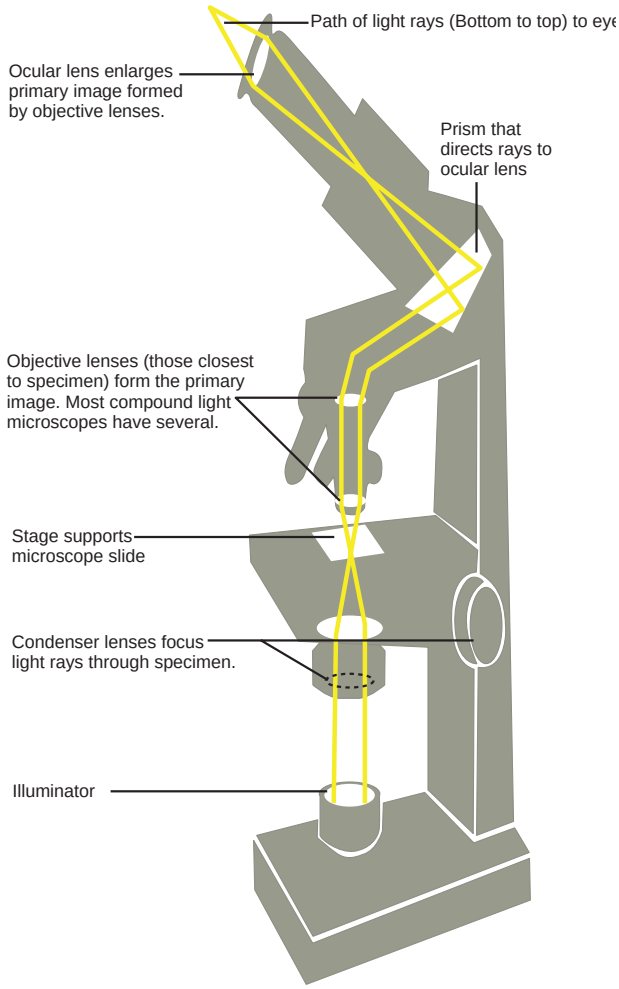


Figure 2.7: The path of light in light Microscopes

Oil Immersion

Oil immersion lens is designed to be in direct contact with oil placed on the cover slip. An oil immersion lens has a short focal length and hence there is a short working distance between the objective lens and the specimen. Immersion oil has a refractive index closer to that of glass than the refractive index of air, so the use of oil increases the cone of light that enters the objective lens. Figure 2.8 explains the working principle of oil immersion objective lens.

HOTS

- What are the two ways by which the resolving power of microscope can be enhanced?
- What are the advantages of the low-power objective over the oil immersion objective for viewing fungi or algae?
- What will happen if water is used instead of immersion oil under a 100X objective lens?

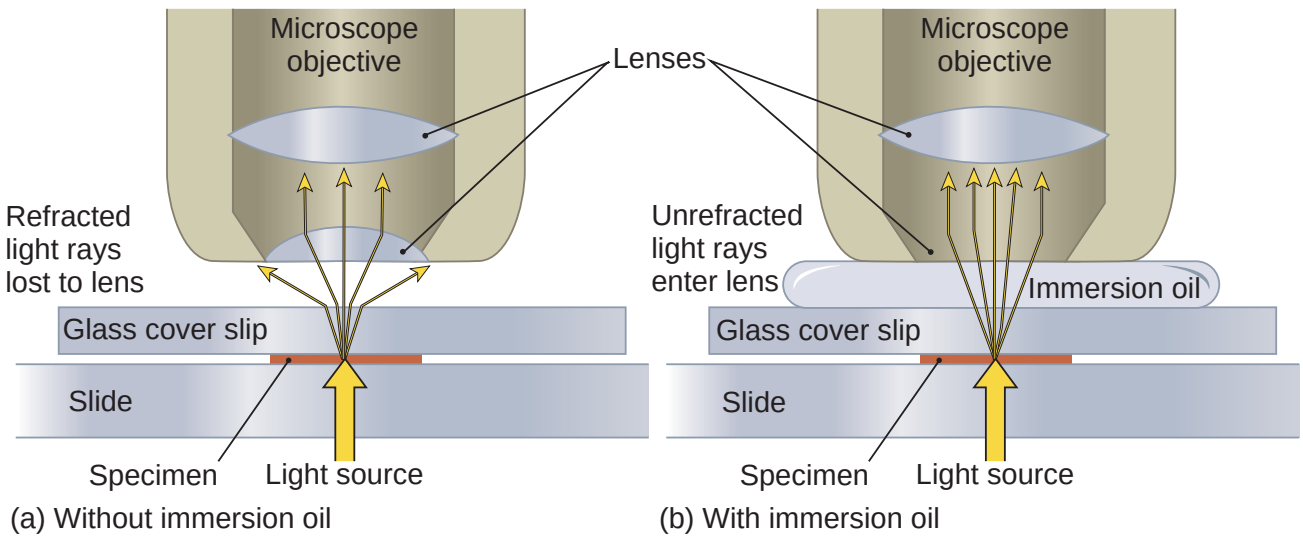
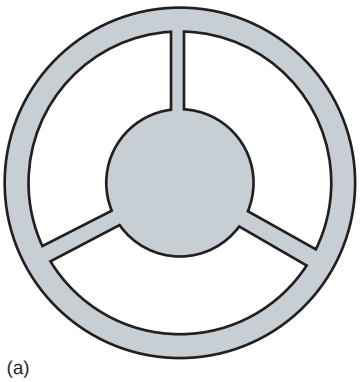


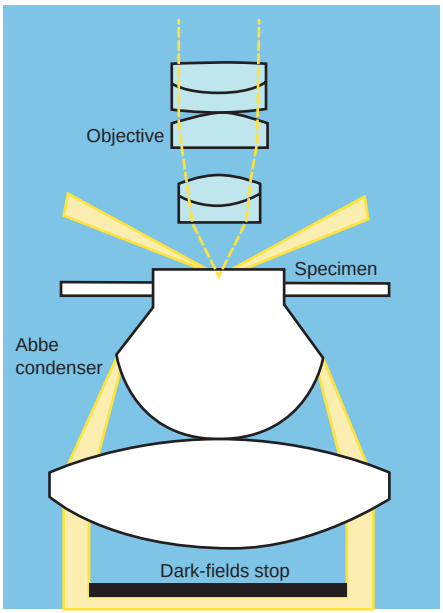
Figure 2.8: Oil Immersion Objective Working Principle

2.4 Dark Field Microscope

This is used for examining live microorganisms which are invisible in light microscope and cannot be stained by standard methods. It can be used to study samples which can get distorted by staining and cannot be identified further. The distinct feature is the dark field condenser that contains an opaque disc. The disc blocks direct entry of light to the objective lens. The light rays reflected off the specimen enter the objective lens and in the absence of direct background light, the specimen appears light against a dark background (Figure 2.9). The microbes are visualized as halos of bright light against the darkness, as stars are observed against the night sky (Figure 2.10).



(a)



(b)

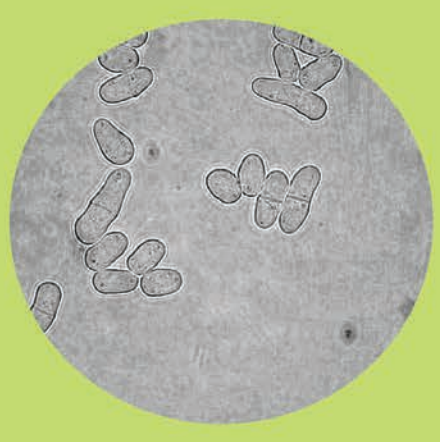
Figure 2.9: Dark Field Microscopy. The simplest way to convert a microscope to dark field microscope is to place. (a) a dark field stop underneath (b) the condenser lens system

Infobits

Compound microscope (also known as light microscope) produces a mono (2D) image and stereo microscope produces stereo (3D) image. 'Upright' life science microscopes are the most numerous of all microscopes. An inverted microscope is the kind of microscope that views objects from an inverted position. Digital microscopes are becoming widespread. These provide simple image and are convenient for electronic image capturing.

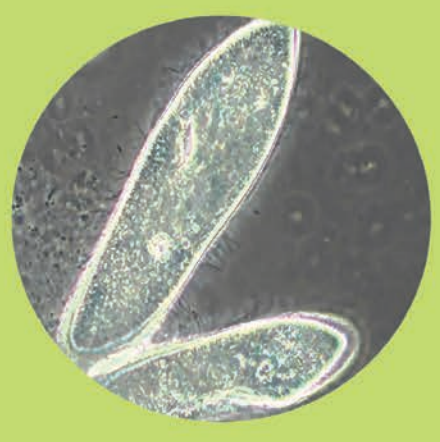
Figure 2.10: Dark field observation of bacteria *Treponema pallidum* specimen from a patient with Syphilis

Different types of Microscopic images : A comparison

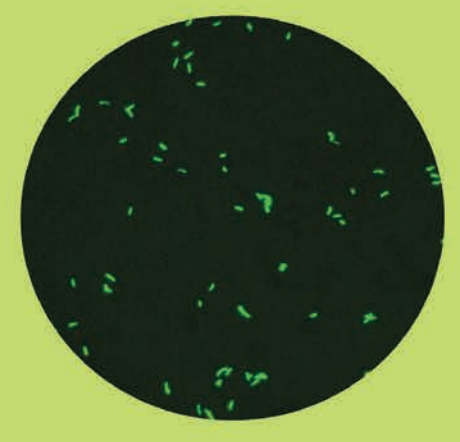


Yeast cells under
Bright field microscope

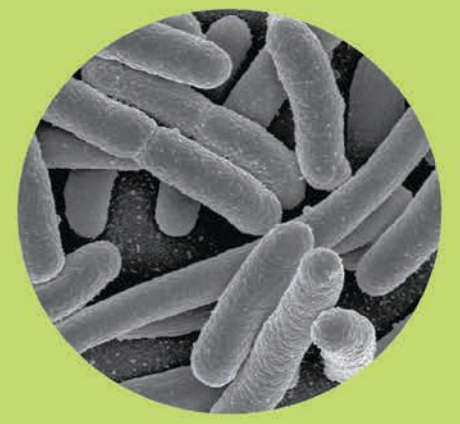
Spirillum under
Dark field microscope



Paramecium under
Phase contrast microscope



Mycobacterium under
Fluroscence microscope



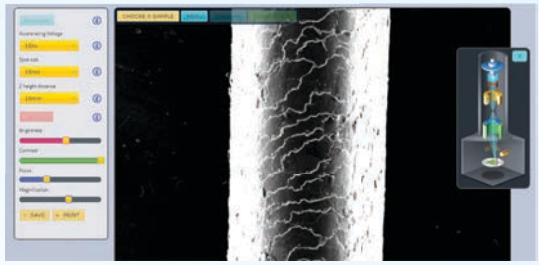
Escherichia coli under
Transmission Electron
microscope

Vibrio cholerae under
Scanning Electron
microscope

ICT CORNER

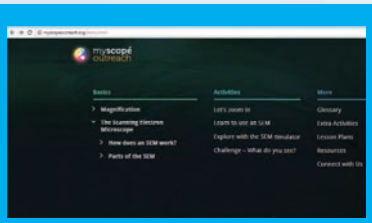
SEM

Lets focus with SEM

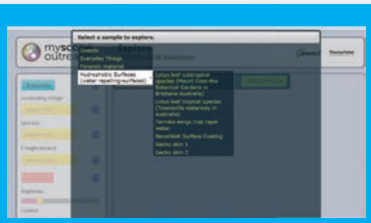


STEPS:

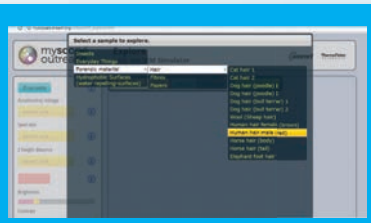
- Use the URL or scan the QR code to reach 'myscope outreach' interactive page.
- Click 'The Scanning Electron microscope' under 'Basic' menu to know about its parts and function.
- Follow the successive steps that lead to describe the n nuances of SEM.
- Select 'Let's Zoom in' under the activity to menu and explore the SEM stimulations.



Step1



Step2



Step3

URL:

http://myscopeoutreach.org

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Summary

The microscope is a tool to study small microscopic life forms. Zaccharias Janssen is given the credit for making first compound microscope. Light microscopy has undergone a renaissance during the later years of the 20th century and early stages of 21st century.

There are two main types of microscopes (i) Light microscope and (ii) Electron microscope. Light microscope makes use of light and Electron microscope uses the electrons.

Evaluation

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Multiple choice questions

1. The first compound microscope was invented by
   a. Robert Hook
   b. Anton von Leewenhoek
   c. Kepler and Galileo
   d. Zaccharias Janssen

2. All the following are components of compound microscope except
   a. Stage clips
   b. Fine adjustment knob

c. Electron gun
d. Binocular eye piece

3. Resolving power of an instrument can be increased by
   a. Using an illumination of longer wavelength and by decreasing the NA
   b. Using an illumination of longer wavelength and by increasing the NA
   c. Using an illumination of shorter wavelength and by increasing the NA
   d. Using an illumination of shorter wavelength and by decreasing the NA
4. The resolving power of unaided human eye is
   a. 1 cm
   b. 100 µm
   c. 200 µm
   d. 400 µm
5. Which of the following is false about dark field microscopy?
   a. Adding disc called "stop" to the condenser will make bright field to darkfield
   b. The stop disc prevents the entry of light from the central field and object is illuminated with beam of light
   c. The light gets reflected from the sides of the specimen and appears bright in dark background
   d. Image formation is without use of light

Answer the following

1. What is the importance of microscopy in microbiology?
2. Write down the names of different types of microscopes.
3. What principle defines an object as "microscope"?
4. What happens to light rays when they interact with an object?
5. Elucidate the lens function in image formation.
6. Define the characteristics of resolution, magnification and numerical aperture.
7. How do eukaryotic and prokaryotic cells differ in appearance under the light microscope?
8. Trace the pathway of light in brightfield microscopy.
9. Elaborate the role of condenser and image formation in dark field microscope.
10. Differentiate between Bright field and Dark field microscopy.

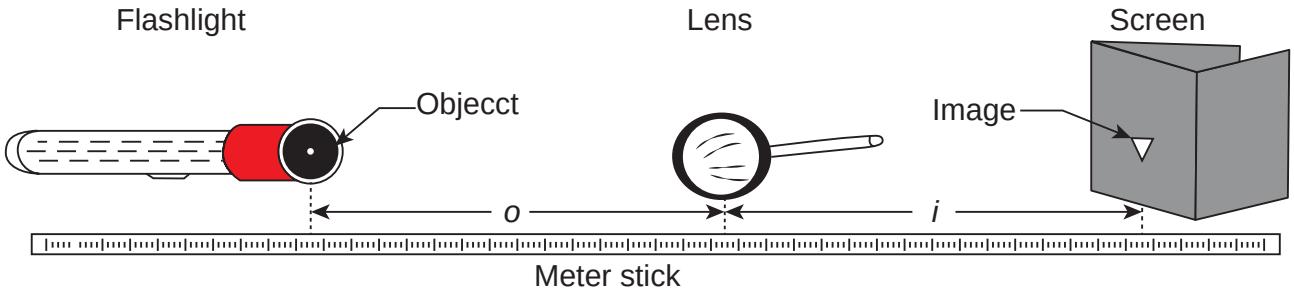
Student Activity

Experiment and enjoy……

Imaging Properties of a Simple Lens

Objective: In this experiment you will observe and measure the imaging properties of a simple lens.

Apparatus: You will need a good lens (magnifying glass), a flashlight, a viewing screen (tri-folded white copy paper), a meter stick and perhaps some modeling clay to hold things in place. Set all these things on a flat table about 1 meter wide in an area where the lighting can be dimmed.



Chapter 3

Stains and Staining Methods

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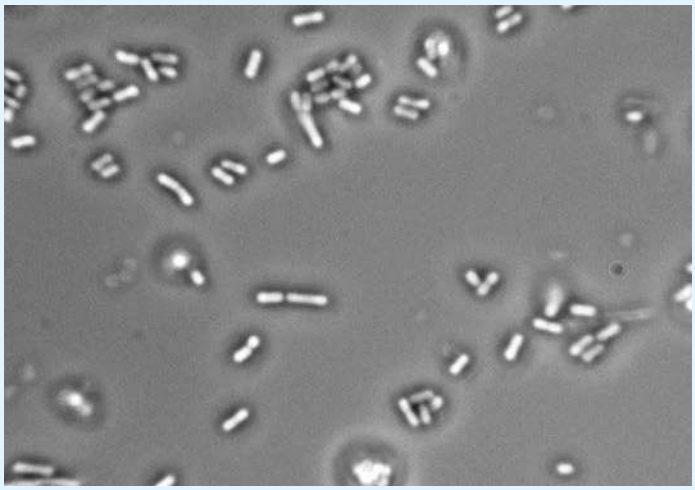
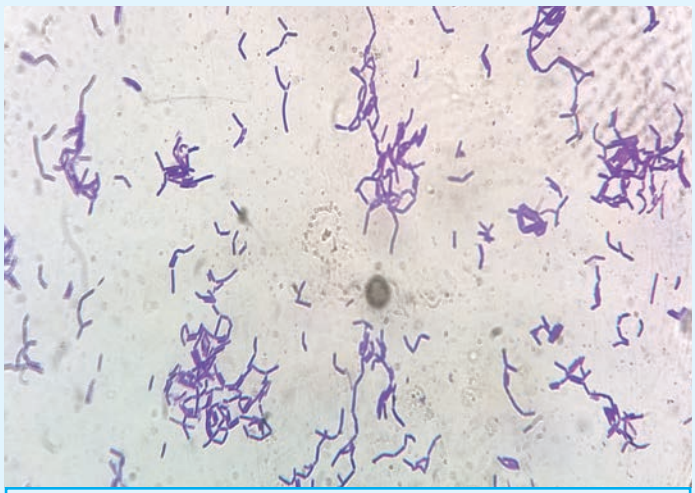
Chapter Outline





Unstained and stained *Lactobacillus sp.* in curd. *Lactobacillus* is a genus of bacteria which can convert lactose in milk into lactic acid by means of fermentation. Staining is used to visualize microbial cells under a microscope.

Learning Objectives

After studying this chapter the student will be able,

- *To appreciate the need for staining.*
- *To differentiate between an acidic dye and a basic dye and understand the principle of staining.*
- *To classify organisms based on staining reaction and differentiate between simple and differential stains.*
- *To know smear preparation and heat fixation.*
- *To describe the procedure of simple, Gram's and endospore staining methods.*
- *To describe the appearance of Gram positive and Gram negative cells after each step of Gram staining procedure.*
- *To know the importance of Gram staining and endospore staining in diagnosing and identifying bacteria.*
- *To learn a few staining solutions and names of bacteria.*

Have you ever thought of observing the microorganisms present in rain water when you play? Have you ever wondered how milk turns into curd and which microorganisms are involved? It is clearly understood from previous unit that microorganisms can be seen only under microscopes. But microorganisms do not show much of its structural details under the light microscope due to lack of contrast and poor resolution. To improve the visibility of these tiny living organisms, stains and staining methods are of great use.

3.1 Techniques for Observing Microorganism

A considerable amount of information can be gained by careful microscopic examination of microorganisms. There are two general techniques used in the preparation of microbial specimens to observe them under microscope. First technique employs the unstained preparation of living cells and second one employs stained preparations of killed microorganisms.

3.1.1 Examination of Unstained Preparation

Living microorganisms can be examined directly by wet mount or by hanging drop preparations. Both the techniques are very useful in determining size, shape and motility of the microorganisms. The spirochetes (spiral bacteria) are normally examined in wet preparation through Dark-field microscope. Some cell inclusion bodies such as vacuoles and spores can be readily observed even without staining.

- A wet mount is made by keeping a drop of liquid containing microorganisms (culture) on a microscope slide and placing a cover slip over the drop. (Figure 3.1a)
- A hanging drop mount is made by using a cover slip and a cavity slide. Vaseline is applied on each of the four corner of the cover slip or around the cavity using a match stick. A drop of culture (liquid containing microorganisms) is placed on a cover slip. The cavity slide is placed upside down on the cover slip and inverted such that the drop is hanging (Figure 3.1b).

Since microbial cells are colourless and transparent, observation of microorganisms in wet preparation by bright field microscope is difficult. But, dark-field and phase contrast microscopes give contrast and make structures within the cells to appear clear. Therefore, these microscopes are useful for examination of unstained preparation.

3.1.2 Examination of Stained Preparation

Staining enables better visualization of microorganisms under a microscope. Microscopic examination of stained cells helps to reveal the size, shape and arrangement of microbial cells. Microbial cell staining is important in the identification of infectious pathogens.

3.2 Purpose of Staining

Staining is very useful for the following reasons:

- To make the microscopic semi transparent microbial cell visible.
- To reveal the size and shape of microorganisms.
- To demonstrate the presence of internal and external structures of microbial cells.
- To distinguish between different types of microorganisms.
- To produce specific chemical and physical reactions.
- To preserve the stained microorganisms as specimen slide.

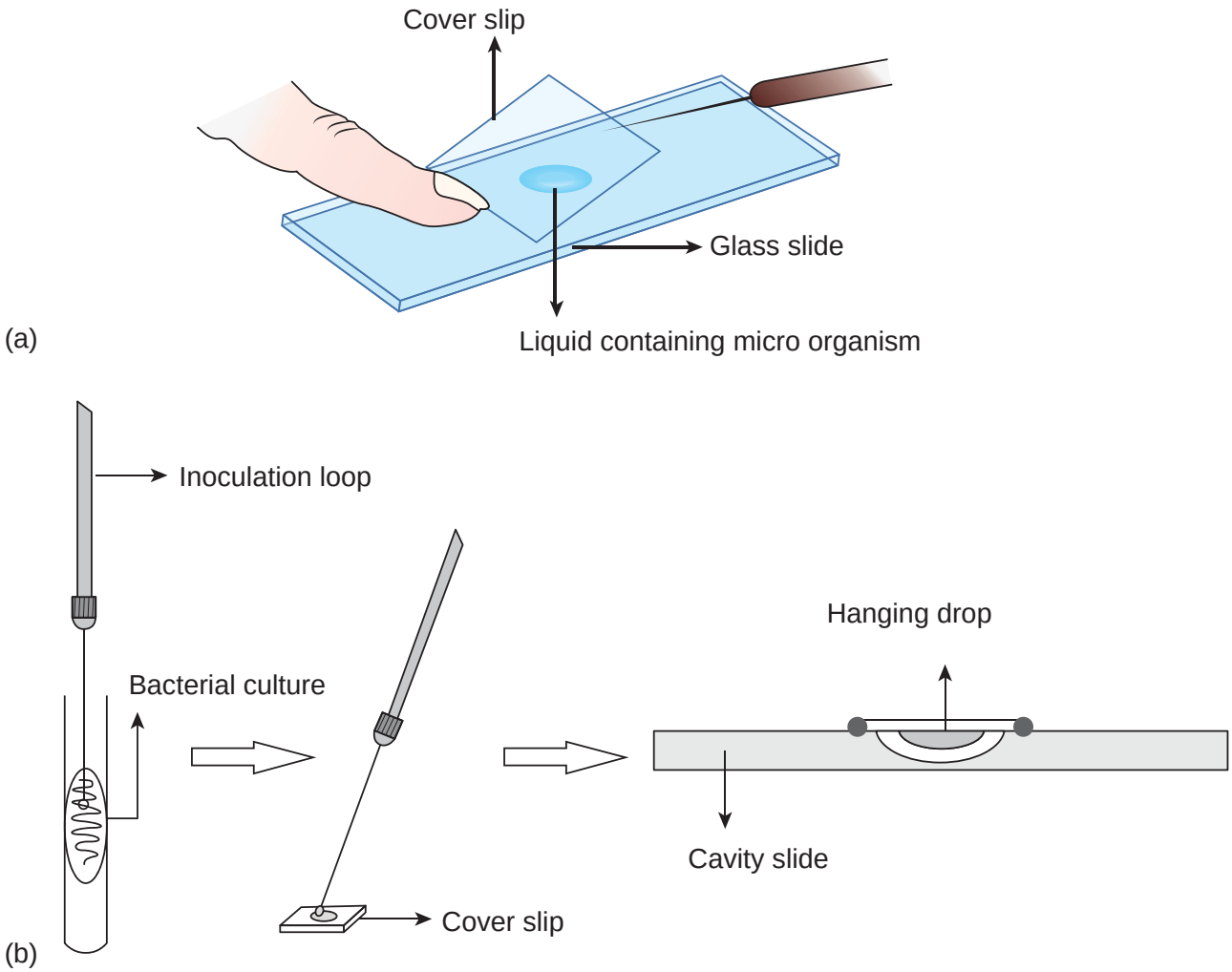


Figure 3.1: a) Wet mount and b) Hanging drop preparation

3.3 Stains

Stains are dyes used to increase colour contrast. Dye is a coloured organic compound that adheres to microbial cells, giving colour to the cell. Today several stains and staining procedures are available to study the morphological details of various microorganisms. The process of imparting colour to the microbial cell is known as staining.

Stains are organic compounds containing chromophore and auxochrome groups linked to benzene ring.

A chromophore group imparts colour to the compound. Compounds of benzene containing chromophore radicals are called chromogens. Such a compound, even though it is coloured, is not a dye. In order for a compound to be a dye, it must contain not only a chromophore group but also another group known as auxochrome that imparts the property of electrolytic dissociation. Auxochrome gives salt forming properties to the compound.

Hence, each stain or dye is composed of three components:

(i) Benzene ring: It is the basic colourless structural component of a stain or dye.

(ii) Chromophore: It is the functional group that gives colour.

(iii) Auxochrome: It is the group that gives ionic properties to the stain.

The term stain and dye are not the same. The basic differences between dye and stain are given in Table 3.1.

Table 3.1: Difference between dyes and stains.

Dyes	Stains
Dyes are a colouring agents used for general purposes.	Stains are colouring agents used for biological purposes.
Dyes are the textile colouring agents that are prepared with lesser specification and they may contain impurities.	Stains are pure. They are prepared with greater care and specification.

3.3.1 Classification of Stains

1. On the basis of origin, stains can be classified as natural and synthetic.

(i) Natural stains:

- These stains are obtained directly from natural products. For example, Haematoxylin is obtained from the heartwood of a tree (*Haematoxylon campechianum*).
- The natural stains are used mainly for histological purposes.

(ii) Synthetic stains:

- These are artificially produced mainly from coal tar products and hence popularly called coal-tar dyes.
- A majority of stains used in microbiology are the synthetic type and manufactured from Aniline. For example, Crystal violet, Safranin, Methylene blue and Acid fuchsin.

2. On the basis of chemical behavior, dyes are classified as acidic, basic and neutral.
   - An acidic dye is one in which the colour bearing ion, the chromophore, is an anion.
   - A basic dye is one in which the colour bearing ion, the chromophore, is a cation.
   - A neutral dye is a complex salt of a dye acid with a dye base.

Acid dyes generally combine more strongly with cytoplasmic (basic) elements of the cell, and basic dyes combine best with nucleic acid (acidic) elements of the cell. Table 3.2 shows the chemical characteristics of a stain or dye.

3.4 Principle of Staining

Positive Staining

In positive staining, the surface of the bacterial cell takes on the colour of the stain. When basic stain is applied, there is an attraction between the negatively charged cell surface and positively charged chromophore, which leads to staining of the cell (Figure 3.2).

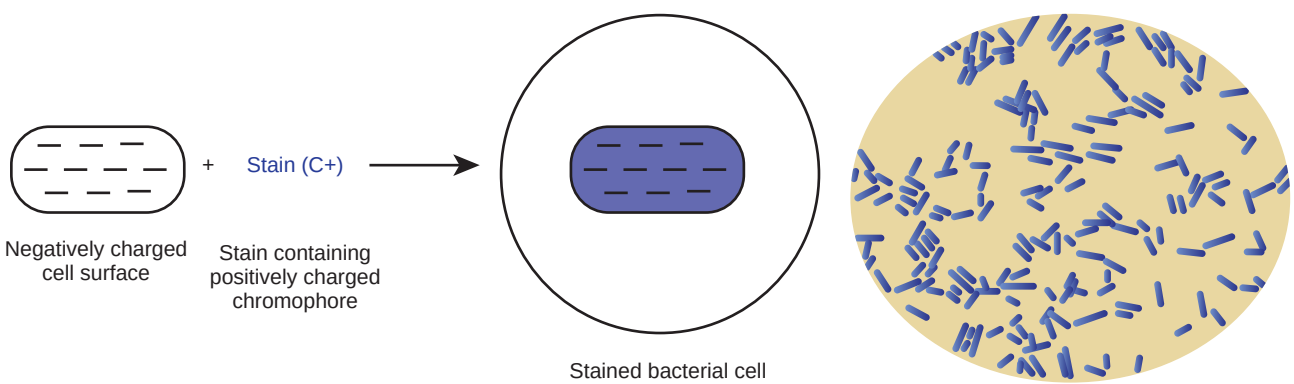


Figure 3.2: Positive staining

Table 3.2: Chemical characteristic of stain or dye

Acid stain	Basic stain	Neutral stain
Chromogen of acidic stain is negatively charged, so it is also known as anionic stain.	Chromogen or coloured part of basic stain is positively charged, so it is also known as cationic stain.	It is a complex salt of dye acid with dye base.
Used to stain the positively charged component of microbial cell.	Used to stain negatively charged component of microbial cell.	It stains both positive and negative charged components of microbial cell.
Example: Eosin, Nigrosin, India ink, Acid fuchsin, Congo red.	Example: Methylene blue, Safranin, Malachite green, Basic fuchsin, Crystal violet	Example: Giemsa stain, Leishmanstain.

Infobits

On the basis of demonstrating the living or non-living status of microorganisms, some stains are classified as vital stains. These stains differentiate between living and non-living microbial cells. For example, Tryphan blue selectively colour dead tissues or cells.

Certain stains will give a different colour to the cell inclusion bodies from its original colour. Such stains are called metachromatic stains. Metachromatic granules of *Corynebacterium diphtheriae* contain polymerized inorganic polyphosphate responsible for metachromasia with Toluidine blue or Methylene blue.

Negative Staining

In negative staining, the background is coloured and bacteria remains colourless. It is because the acidic dyes are repelled by the negatively charged bacterial surface. The background gets stained and the cell remains colourless. This technique is useful for revealing the cell shape, size and demonstrating capsule (Figure 3.3).

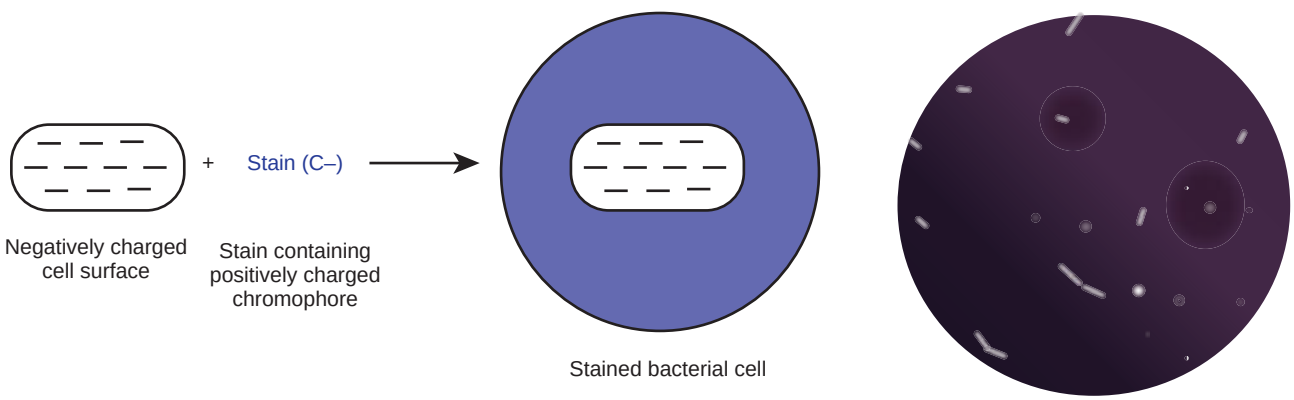


Figure 3.3: Negative staining

3.5 Preparation of Materials for Staining

The essential steps in the preparation of materials to be observed are

1) Preparation of smear
2) Fixation
3) Application of one or more staining solutions

3.5.1 Preparation of Smear

Smears can be made from liquid or solid cultures or from clinical specimens. Smear is prepared by placing a loopful of culture on a clear glass slide with an inoculation loop. The culture is spread on the glass slide so as to form a thin film. This film is allowed to air dry (Figure 3.4).

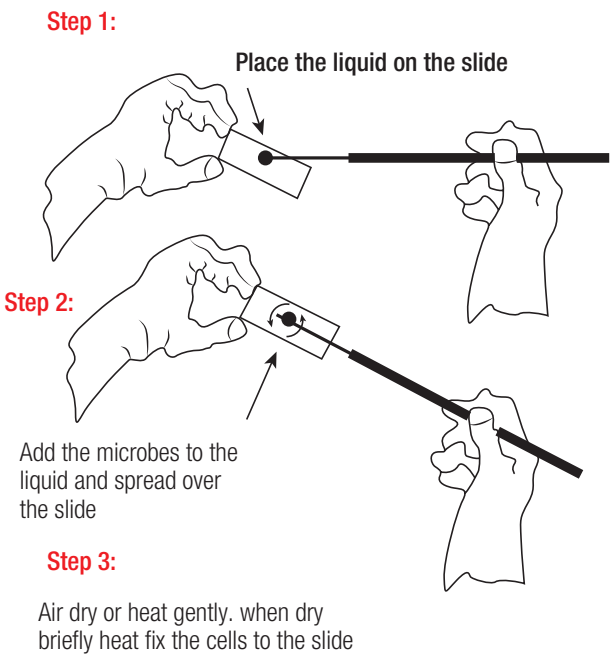


Figure 3.4: Preparation of smear

3.5.2 Fixation

Fixation kills the microorganisms and attaches them to the slide. This prevents washing away of microorganism in further steps of staining procedure. It also preserves various parts of microorganisms in their natural state with only minimal distortion. The two fixation methods that are used to fix microbial cells are heat fixation and chemical fixation.

Heat fixation

In this method the slide is gently heated by passed through a flame (Figure 3.5). Heat fixation will preserve the overall morphology of the cell without destroying the internal structures.

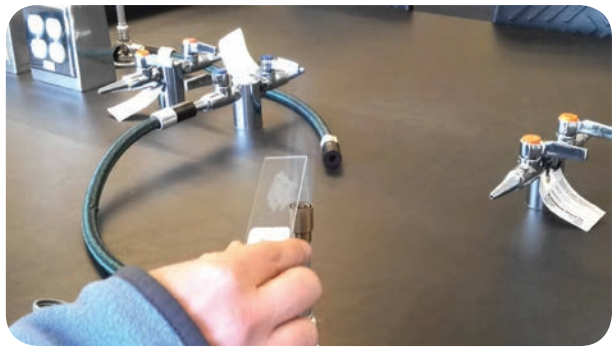


Figure 3.5: Fixation of smear by passing slide gently through the flame

Chemical fixation

It involves the use of chemical fixative to protect the fine cellular structures of delicate microorganisms. For this purpose, Ethanol, Acetic acid, Formaldehyde, Glutaraldehyde and Mercuric chloride are usually used.

3.5.3 Bacterial Staining Methods

Different staining methods are employed to study the bacterial morphology and to identify bacteria. Some methods are used for general purposes and others are used for special purposes. There are three categories of staining methods, they are:

Robert Hooke was the first to describe the appearance of stained objects under light microscope.

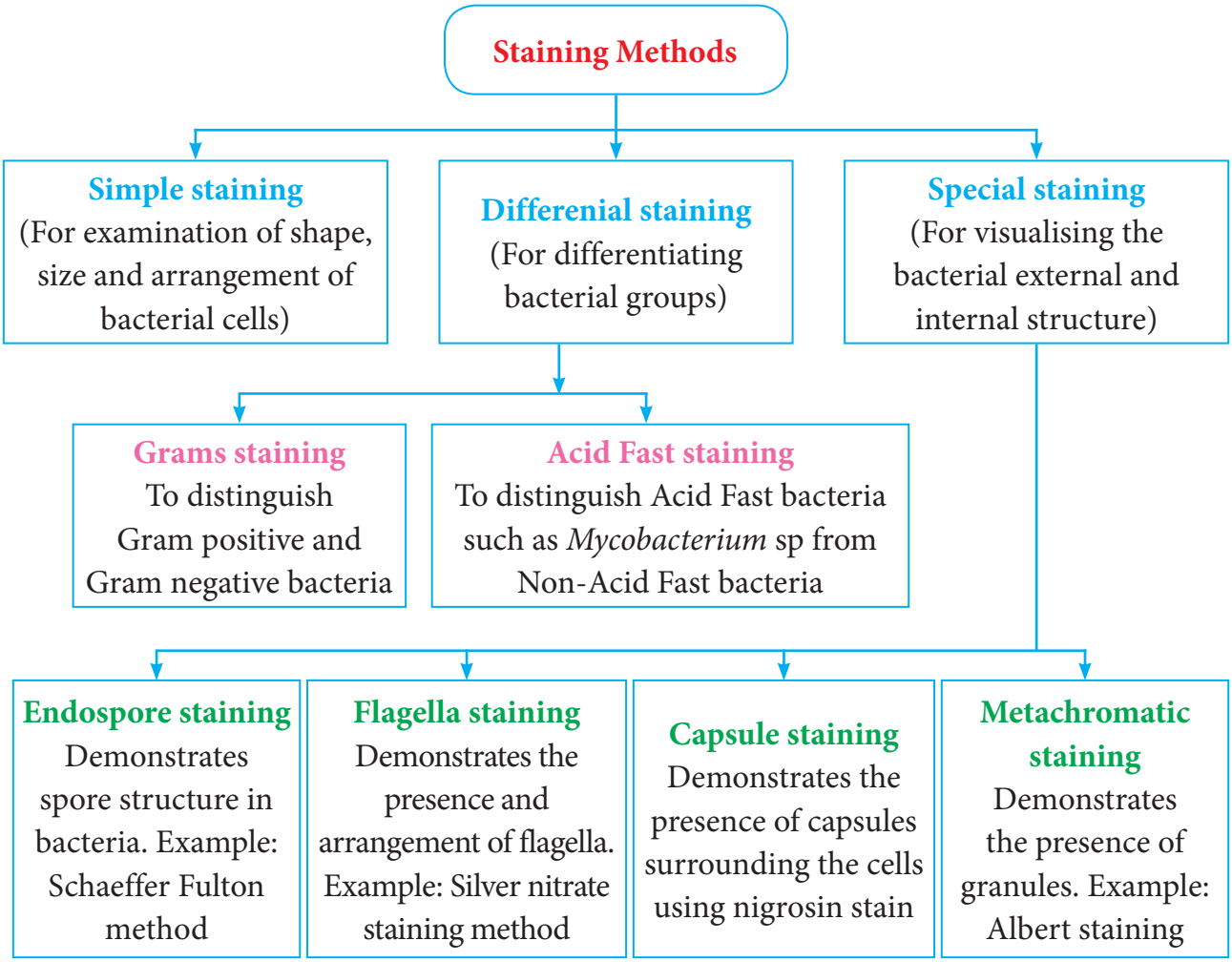
Professor Joseph Von Gerlach of Germany was the first to use stain in histology.

i) Simple staining method

ii) Differential staining method

iii) Special staining method.

Different types of bacterial staining methods are summarized in Flowchart 3.1



Flowchart 3.1: Types of Bacterial Staining methods

3.6 Simple Staining Method

In Simple Staining method only one stain is used. Stain is applied to the smear in one application. The fixed smear on the glass slide is flooded with a staining solution for about one minute. The solution is then washed off with water and the slide is blot dried. The stained slide is examined under a microscope (Figure 3.6). The cells stain uniformly. The simple stains used by the microbiologists for routine purposes are dilute solutions of Methylene blue, Crystal violet, Safranin and Carbol fuchsin.

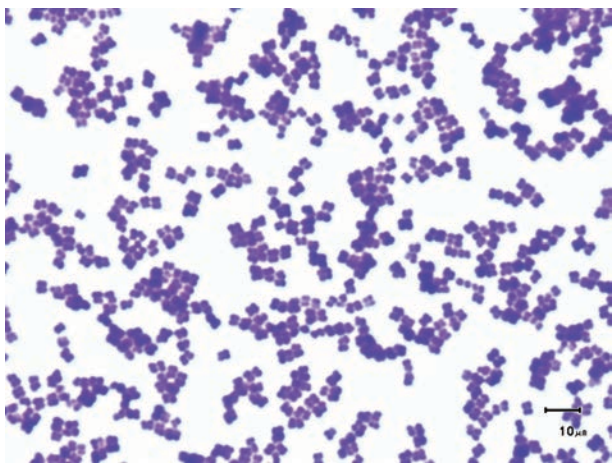
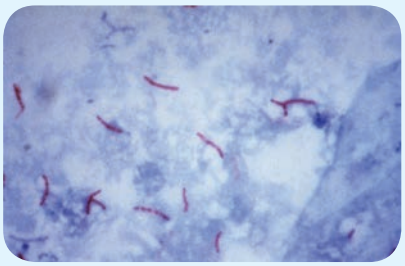


Figure 3.6: Simple stain – *Micrococcus sp.* stained with Methylene blue

Mycobacterium leprae which causes leprosy is an unculturable bacterium. It is primarily diagnosed by using a special bacteriological stain called Acid Fast stain.



Mycobacterium leprae (Acid Fast bacilli) stained with modified Ziehl Neelson stain.

Methylene blue is more frequently used than any other stain in Bacteriology. It is used for the rapid survey of bacterial population of milk. It is also used for the diagnosis of Diphtheria. This stain is incorporated along with Eosin in Lactose Agar to distinguish *Escherichia coli* from other fecal bacteria in contaminated water.

3.7 Differential Staining

In this method more than one stain is employed. In some method the stains are applied separately, while in other method they are mixed and applied in one application. These procedures show differences between the cells or parts of a cell and can be used for of identification. The two most important differential stains used by bacteriologists are Gram stain and Acid Fast stain. The differences between simple and differential staining are shown in Table 3.3.

3.7.1 Gram's Staining Method

The Gram's stain technique was developed by Danish Bacteriologist Hans Christian Gram in 1884. It is one of the most useful staining methods because it classifies bacteria into two large groups namely Gram positive and Gram negative. In this method, the fixed bacterial smear is subjected to staining reagents in the order of sequence listed below:

Crystal violet
(Primary stain)
↓
Iodine
(Mordant)
↓
Alcohol/Acetone
(Decolourising agent)
↓
Basic fuschsin/Safranin
(Counter stain)

Table 3.3: Differences between Simple and Differential Staining

Simple staining	Differential staining
1. This method uses only one stain.	This method uses more than one stain.
2. It imparts only one colour to all bacterial cells.	It imparts two or more different colours to bacterial cells.
3. It reveals the size, shape and arrangement of bacterial cells.	It reveals the size, shape and arrangement. In addition, it differentiates two groups of bacteria.
Example: Methylene blue staining method.	Example: 1. Gram's staining method 2. Acid Fast staining method

The organisms that retain the colour of the primary stain are called Gram positive and those that do not retain the primary stain when decolorised and take on the colour of the counter stain are called Gram negative.

Mordants: Mordants are not dyes. They are important to increase the biological specimen's affinity for a dye. Some stains never stain the cells or its components unless treated with a mordant. The mordant becomes attached to a cell or its components and then combines with the stain to form an insoluble colour complex.

3.7.2 Procedure of Gram's Staining

Gram's Staining comprises of four steps:

Step 1: A heat fixed smear is covered with a basic violet dye, Example: Crystal violet. This stain imparts its colour to all cells. It is referred to as a primary stain, since it is applied first.

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Step 2: After a short time, the slide is washed off and the smear is covered with iodine, a mordant. At this stage both Gram positive and Gram negative bacteria

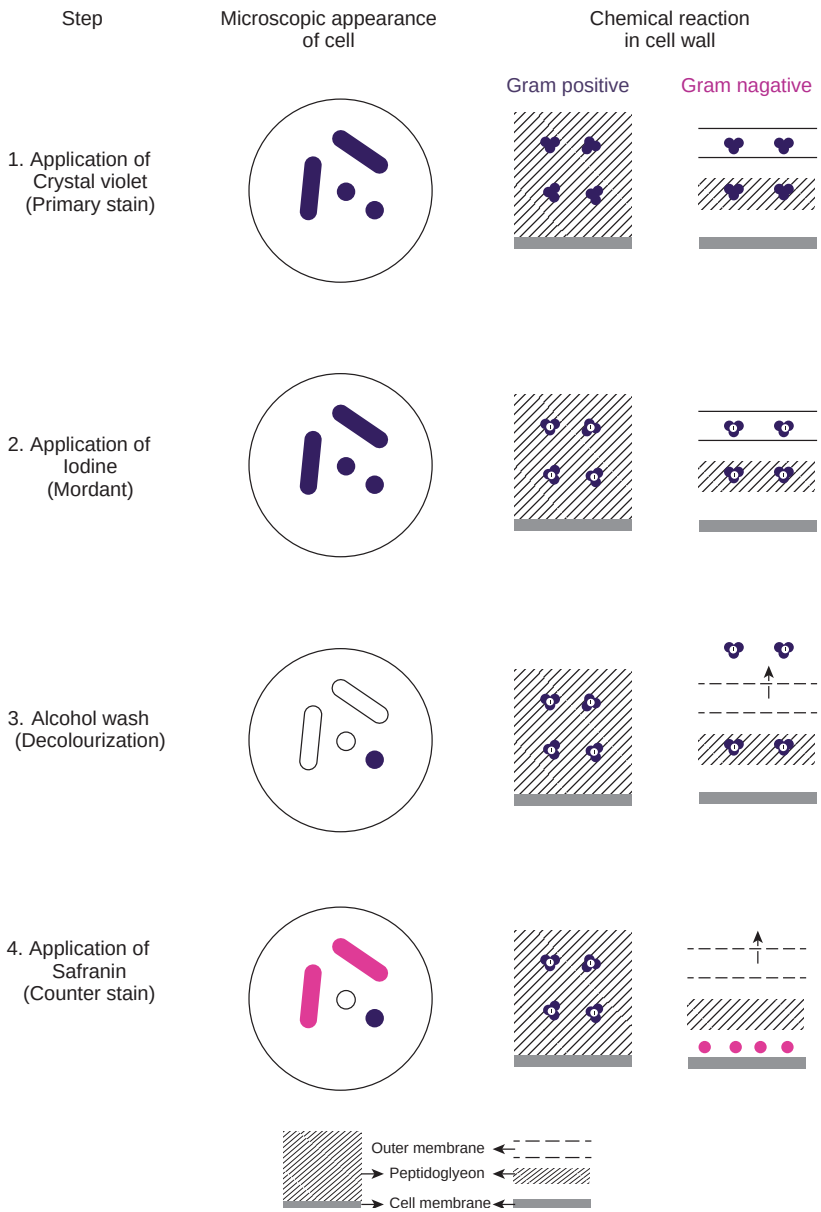


Figure 3.7: Steps, micrograph and chemical reaction of Gram Stained Bacteria

appear dark violet.

Step 3: Next, the slide is decolurized with alcohol or an acetone alcohol solution. This solution is a decolurizing agent, which removes the primary stain from the cells of some species but not from others.

Step 4: The slide is immediately washed after decolurization and the slide is then counter stained with basic fuchsin or safranin, a basic red dye. The smear is washed again, blot dried and examined under microscope (Figure 3.7).

3.7.3 Principle of Gram's Staining

The exact mechanism of action of this staining technique is not clearly understood. However, the most acceptable explanations are associated with the structure and composition of the cell wall.

The cell wall of Gram positive bacteria have a thicker peptidoglycan (consists of disaccharides and amino acids) than Gram negative bacteria. Figure 3.8 depicts the cell wall of Gram positive and Gram negative bacteria. In addition, Gram negative bacteria contain a layer of lipo polysaccharide (consists of lipids and polysaccharide) as part of their cell wall. When Crystal violet and subsequently Iodine is applied to both Gram positive and Gram negative cells, the two combine to form CV-I complex.

The cell wall of Gram positive bacteria with lower lipid content get dehydrated during alcohol treatment. The pore size decreases and the permeability is reduced. Thus, the CV-I complex cannot be extracted and the cells remain violet.

The alcohol treatment of Gram negative bacteria extracts the lipid which results in increased porosity or permeability of the cell wall. Thus, the crystal violet iodine [CV-I] complex is extracted and the bacteria are decolorized. These cells subsequently take on the colour of the counter stain basic fuchsin or safranin and appears red to pink.

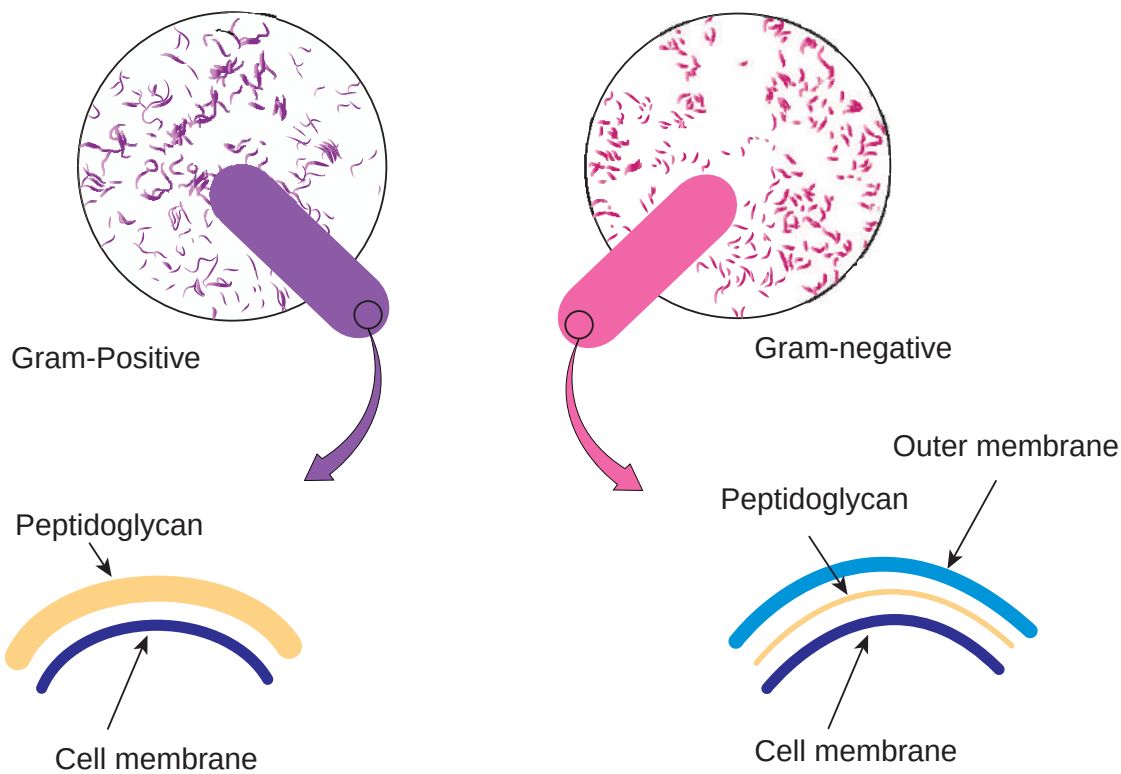


Figure 3.8: Cell wall of Gram positive and Gram negative Bacteria

HOTS

1. If the iodine step were omitted in the Gram's staining procedure, what colour would you expect Gram positive and Gram negative bacteria to stain?
   a. Gram positive : pink and
      Gram negative : purple
   b. Gram positive : purple and
      Gram negative : pink
   c. Gram positive : purple and
      Gram negative : purple
   d. Gram positive : pink and
      Gram negative : pink
2. In a Gram's staining method, a step could be omitted and still allow differentiation between Gram positive and Gram negative cells. Name the step.

3.7.4 Importance of Gram Staining

This century old staining method still remains as the universal basis for bacterial classification and identification. Even with today's elaborate and expensive medical technology, the Gram's staining remains an important, inexpensive and unbeatable tool in the identification of pathogens.

Examination of Gram stained organisms usually provides the basis for classifying, identifying and characterizing bacteria. Gram staining of clinical specimens, however provides only a preliminary indication of the identity of the etiological agent (the organism causing the disease). Gram nature of common pathogenic bacteria is given in Table 3.4.

Gram stains of clinical specimens or of growth on culture plates are especially important in determining the most effective antibiotic for the ill patients who required immediate therapy.

Infobits

There are several modifications of Gram's Stain

- Kopeloff and Beerman's modification.
- Jensen's modification.
- Weigert's modification.
- Preston and Morell's modification.

Prof. Hans Christian Gram
(September 13, 1853-November 14, 1938)

In 1884, Prof. Hans Christian Gram while examining lung tissue from patients who had died of pneumonia, discovered that certain stains were preferentially taken up and retained by bacterial cells. Gram was a modest man, and in his initial publication he remarked, "I have therefore published the method, although I am aware that as yet it is very defective and imperfect; but it is hoped that also in the hands of other investigators it will turn out to be useful". Dr. Gram used Bismarck brown instead of Safranin. It was a few years later, German pathologist Carl Weigert (1845-1904), added the final step of staining with Safranin.

Table 3.4: Gram nature of common pathogenic bacteria

	Gram positive bacteria	Gram negative bacteria
Cocci	*Staphylococcus aureus, Streptococcus pyogenes*	*Neisseria gonorrhoeae*
Rods(bacilli)	*Mycobacterium tuberculosis, Bacillus anthracis, Corynebacterium diphtheriae, Clostridium tetani*	*Escherichia coli, Shigella Salmonella, Pseudomonas aeruginosa*
Spirochaetes	—	*Leptospira, Treponema*

3.8 Special Staining – Endospore Staining

Endospores are highly resistant structures produced by some bacteria during unfavourable environment conditions. Endospore formation is a distinguishing feature of aerobic genera *Bacillus* and anaerobic genera *Clostridium*. The size, shape and position of the spore (Figure 3.9) are relatively constant characteristics of a given species and are important in identifying the species within genera. The position of spore in the cell may be terminal, central or sub-terminal. Figure 3.9 shows the position of spores in a vegetative cell.

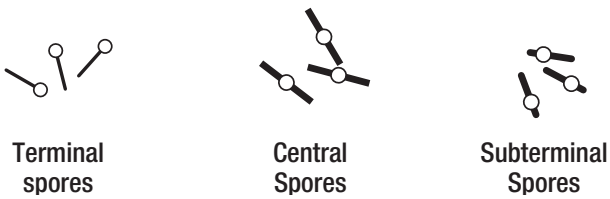


Figure 3.9: Position of spore in a vegetative cell.

Endospores cannot be stained by ordinary methods, such as simple staining and Gram staining, because the dyes do not penetrate the wall of the endospore. If simple stains are used, the vegetative body of the bacillus is deeply coloured, whereas the spore is unstained and appears as a clear area in the organism.

By vigorous staining procedure, the dye can be introduced into the spore. Once stained, the spore tends to retain the dye even after treatment with decolorizing agents. The most commonly used endospore staining procedure is the Schaeffer Fulton endospore staining method. Malachite green, the primary stain, is applied to a heat fixed smear and heated to steaming for about 5 minutes. Heat helps the stain to penetrate the endospore wall. Then the preparation is washed for about 30 seconds with water. Next safranin, a counterstain is applied to the smear to stain the portions of the cell other than endospores.

In a properly prepared smear, the endospores appear green within red cells (Figure 3.10). Endospores are highly refractive. They can be detected under the light microscope when unstained, but cannot be differentiated from inclusions of stored material without a special stain.

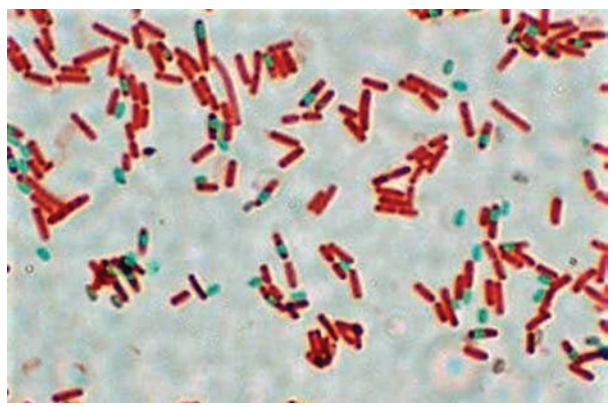
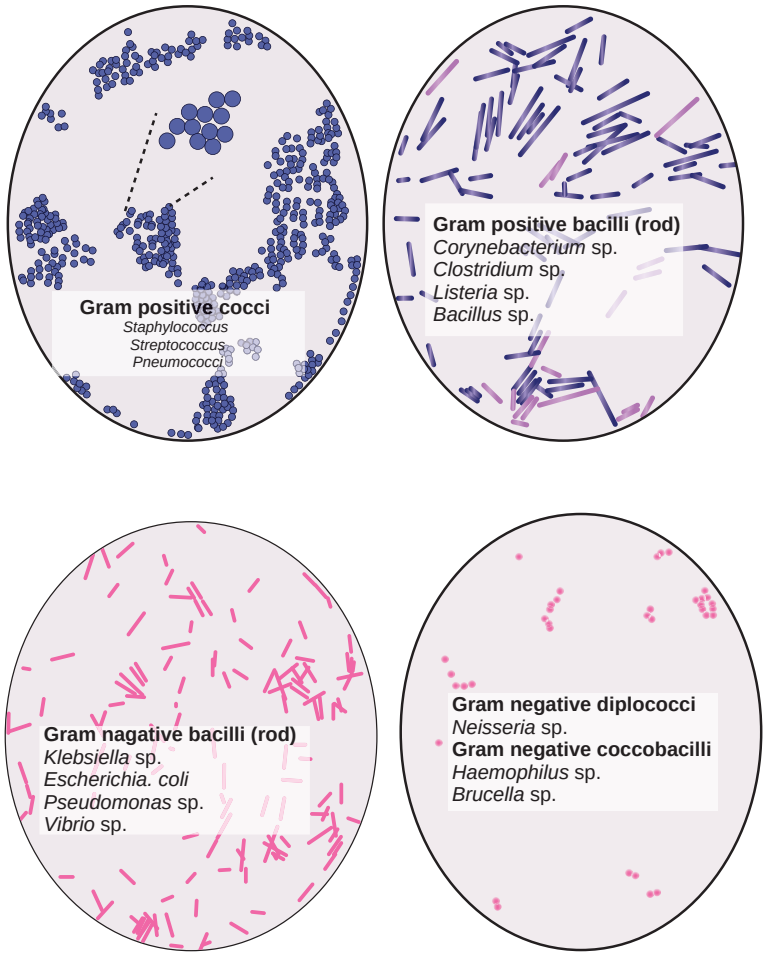


Figure 3.10: Schaeffer Fulton Endospore staining method- spores stained green and vegetative cell stained pink

Common Bacteria with their Gram reactions



3.9 Commonly used Stains and its Applications

Lactophenol cotton blue stain is the most widely used for staining and observing fungi. Giemsa stain is a Romanowsky stain, widely used in microbiology laboratory for staining of blood and blood parasites like malarial protozoans. Calcofluor white stain is commonly used stain to directly detect the fungal elements in tissues and in culture.

Acridine orange stain is used to confirm the presence of bacteria in blood cultures when Gram stain results are difficult to interpret using light microscopy. The stain binds to nucleic acid and stains them. It is also used for the detection of cell wall deficient bacteria example Mycoplasma. Fluorochrome stains such as auramine-rhodamine stains are readily available to detect the bacteria in the specimens through Fluorescent microscopy.

Summary

Staining makes microscopic semi transparent bacterial cell visible. It is a substance that adheres to a cell and impart colour. On the basis of the chemical composition, stains or dyes are classified as acidic, basic and neutral. Staining techniques are classified as simple, differential and special. Simple staining uses a single dye and can help to identify the shape and size of an organism. Differential staining use more than one dye to distinguish between structures in a cell or different types of cells. The Gram stain procedure divides bacteria into Gram positive and Gram negative bacteria. Specialized staining such as endospore staining is used to detect the presence of endospores in bacteria.

Evaluation

EEZQUQ

Multiple choice questions

1. An dye has negative charge.
   a. Basic
   b. Acidic
   c. Neutral d. None
2. _____ stain is incorporated with Eosin in Lactose agar to distinguish typical *Escherichia coli* in contaminated water.
   a. Crystal violet b. Acid fuchsin
   c. Methylene blue d. Safranin
3. Which of the following is not an anionic dye?
   a. Safranin b. Eosin
   c. Rose Bengal d. Acid fuchsin
4. Christian Gram discovered a staining technique to differentiate the bacteria of similar morphology in the year.
   a. 1857 b. 1880 c. 1884 d. 1881
5. Which of the following is used for negative staining of microbial cells?
   a. Nigrosin and Acid fuchsin
   b. Rose Bengal and malachite green
   c. Safranin and Eosin
   d. Nigrosin and Indian Ink
6. _____ is used as a mordant in Gram staining techniques.
   a. Iodine
   b. Crystal violet
   c. Methylene blue
   d. Safranin
7. Which of the following pairs is mismatched?
   a. Capsule-negative stain
   b. Cell arrangement-simple stain
   c. Cell size-albert stain
   d. Gram stain-bacterial identification
8. The order of reagents in the gram staining reactions are:
   a. Safranin, alcohol, methylene blue, iodine
   b. Crystal violet, iodine, alcohol, safranin
   c. Methylene blue, alcohol, iodine, safranin
   d. Crystal violet, alcohol, iodine, safranin
9. The Schaeffer-Fulton endospore staining usually shows
   a. Spore green within pink cells
   b. Spores pink within green cells
   c. Colourless spores within pink cells
   d. Colourless spores within green cells

Answer the following

1. Define stain.
2. Give examples for basic stain.
3. Why heat fixation is important?
4. What are endospores?
5. Distinguish between a dye and a stain.
6. List out few gram positive bacteria.
7. What is the purpose of a counterstain/ decolorizer in the gram stain?
8. Fill in the following table regarding the gram stain.

	Appearance after this step of gram staining	
Steps	Gram positive cells	Gram negative cells
Crystal violet		
Iodine		
Alcohol		
Safranin		

9. What is meant by negative staining?
10. What are the uses of staining?
11. Differentiate simple and differential stain.
12. What are acidic stains? Give examples.
13. Why do basic dyes stain bacterial cells? Why won't acidic dyes stain bacterial cells?

14. For what purpose would you use each of the following?
    a. Simple stain
    b. Negative stain
    c. Acid- fast stain
    d. Gram stain
15. The gram stain has been described as the most important stain for microbiologist. Explain why?
16. How will you appreciate the need of staining?
17. Classify staining technique based on their purpose.
18. Explain the principle of grams staining.
19. Diagrammatically explain Gram's staining procedure.
20. How to visualise an endospore.

ICT CORNER

Gram Staining of Bacteria

Know the Gram Staining process

STEPS:

- Use the URL OR Scan the QR code to reach 'Virtual Interactive bacteriology laboratory'.
- Click 'module' and select 'steps' and read the procedure to follow.
- Select 'start' to enter the 'Gram Stain' process and follow the procedure.
- Leave the slide to dry and heat fix with Bunsen burner and view under microscope

OBSERVATIONS :

- Select other examples and record your observation on Gram +ve and Gram –ve bacterial stains.

Step1

Step2

Step3

Step4

URL:

https://www.cellsalive.com/toc_micro.htm

B177_11_MBIO_EM

Chapter 4

Sterilization

Chapter Outline

EMZHNV

The inoculation loop is sterilized with flame or any other heat source, until it becomes red hot before and after each use.

Learning Objectives

After studying this chapter the student will be able,

- *To understand the concepts of sterilization to maintain aseptic conditions.*
- *To compare the effectiveness of dry heat (red heat, flamimg, incineration, hot air oven), and moist heat (boiling, autoclaving, pasteurization).*
- *To learn the uses of pasteurization in the field of food industry.*
- *To describe the role of radiation in killing pathogens.*
- *To describe how separation of microorganism is achieved through filtration.*

Microorganisms are ubiquitous. They can contaminate, infect or decay inorganic and organic matter. Hence, it becomes necessary to kill or remove them from materials or from areas around us. This is the objective of sterilization.

The process of sterilization is used in Microbiology

- for preventing contamination by extraneous organisms
- in surgery for maintaining asepsis
- in food and drug manufacture for ensuring safety from contaminating organisms

The choice of methods of sterilization depend on the purpose for which it is carried out: the material to be sterilized and the nature of the microorganisms that are to be removed or destroyed.

DO YOU KNOW?

As early as the stone age, humans used physical methods of microbial control to preserve foods, like drying (desiccation) and salting (osmotic pressure).

Sterilization is defined as the process of complete removal or destruction of all forms of microbial life, including vegetative cells and their spores.

4.1 Need for Sterilization

The aim of all sterilization strategies is to kill or remove the unwanted microorganisms. In certain cases, microbes are regarded as potential pathogens and therefore it is essential to eliminate these forms (vegetative and spores) of microbial life. All microbiological techniques require appropriate and adequate sterilization.

Sterilization of culture media, containers and instruments is essential in microbiological work for isolation and maintenance of microorganisms. In surgery and medicine, the sterilization of instruments, drugs and other supplies is important for the prevention of infection.

4.2 Methods of Sterilization

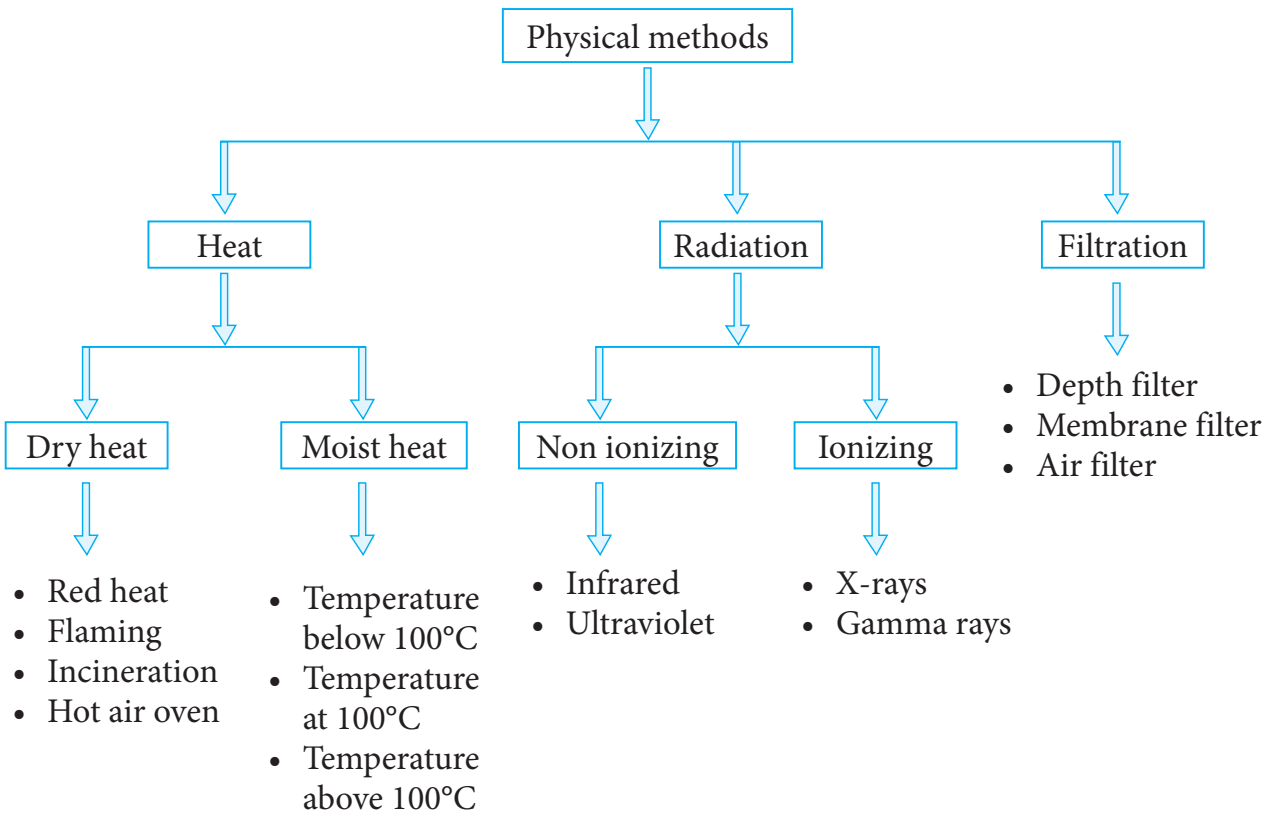
Growth and multiplication of microorganisms can be controlled by removing, killing or inhibiting them using various physical or chemical agents.

4.3 Physical Methods of Sterilization

The various physical methods of sterilization are given in flowchart 4.1

4.4 Sterilization by Heat

Heat is the most rapid and best method of sterilization. It is the method of choice that the material to be sterilized is stable enough to withstand the required temperature necessary to kill the microbes. The time needed for sterilization depends on the initial number of organisms present, type of materials to be sterilized (hence washed and cleaned items are easier to sterilize than dirty ones) and also on the temperature used. Spores need higher temperatures while vegetative bacteria can be destroyed at lower temperatures.



Flowchart 4.1: Physical Methods of Sterilization

Infobits

Heating process in canning was first used by **Nicholas Appert** in 1890. He described a safe means of preserving all kinds of food substances in containers or in cans. Appert is known as father of cannning.

Heat resistance varies among different microorganisms. These differences can be expressed in terms of thermal death point. **Thermal Death Point (TDP)** is the lowest temperature at which all the microorganisms in a particular liquid suspension will be killed in 10 minutes.

Another factor to be considered in sterilization is the duration of time required. This is expressed as **Thermal Death Time (TDT)**. TDT is the minimal time required for all microorganism in a particular liquid culture to be killed at a given temperature. Both TDP and TDT are useful guidelines that indicate the degree of treatment required to kill a given population of bacteria.

Decimal Reduction Time (DRT) is related to bacterial heat resistance. DRT is the time, in minutes, in which 90% of a population of microorganism at a given temperature will be killed.

Heat is employed either as dry heat or moist heat.

4.4.1 Sterilization by Dry Heat

Dry heat is frequently used for the sterilization of glassware and laboratory equipments. In dry heat sterilization, microbial cells are apparently killed by oxidation of their constituents and protein denaturation. Dry heat is applied in the following ways:

a) Red heat

Inoculating wires, points of forceps and searing spatulas are sterilized by holding them in the flame of a bunsen burner until they are seen to be red hot.

b) Flaming

This method is used for sterilizing scalpels, needles, mouths of culture tubes, slides and cover slips. It involves passing the article through the bunsen flame without allowing it to become red hot.

c) Incineration

This is an excellent method for destroying materials such as contaminated clothes, cotton wool stoppers, animal carcasses and pathological materials. It involves burning of materials in incinerators.

d) Hot air oven

This is the most widely used method of sterilization using dry heat. The oven is usually heated by electricity and it has a thermostat that maintains the chamber air constantly at the chosen temperature.

It has a fan or turbo-blower to assist the circulation of air and to ensure rapid, uniform heating of the load. In Hot Air Oven, the air is heated at a temperature of 160°C for one hour. Figure 4.1 shows laboratory hot air oven.

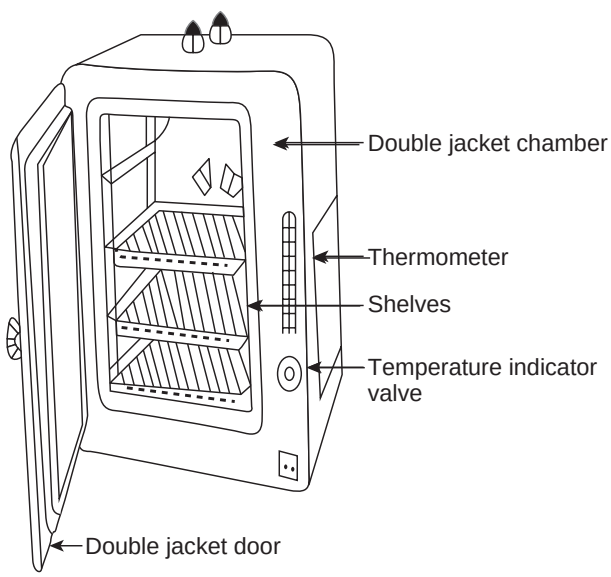


Figure 4.1: Hot Air Oven

This is the best method of sterilizing dry glass ware such as test tubes, petri dishes, flasks, pipettes and instruments such as forceps, scalpels and scissors. It is also used to sterilize some pharmaceutical products such as liquid paraffin, dusting powder, fats and grease.

Quality control of dry heat sterilization:

The spores of a nontoxigenic strain of *Clostridium tetani* are used to test the efficiency of dry heat sterlization.

4.4.2 Sterilization by Moist Heat

Moist heat kills microorganisms primarily by the coagulation of proteins (denaturation), which is caused by breakage of the hydrogen bonds that hold the proteins in three dimensional structure.

There are three methods employed in moist heat sterilization.

- Temperature below 100°C.
- Temperature at 100°C.
- Temperature above 100°C.

a) Temperature below 100°C:

Pasteurization

The process of heating a liquid food or beverage either at 62.8°C for 30 minutes or 72°C for 15 seconds to enhance their shelf life and destroy harmful microorganisms. It should be noted that pasteurization process kills only vegetative cells but not the spores. Pasteurization named in honour of its developer Louis Pasteur. Table 4.1 gives comparison between Sterilization and Pasteurization.

Raw milk can harbour dangerous microorganisms, such as *Salmonella, Escherichia coli* and *Listeria*, that can pose serious health risk, and children are particularly susceptible to the potential infection of unpasteurized or raw milk

Pasteurization can be done in the following methods,

- **Low Temperature Holding Method (LTH)**
  In this method milk, beer and fruit juices are maintained at 62.8°C for 30 minutes.
- **High Temperature Short Time Method (HTST)**
  Products are held at 72°C for 15 seconds.
- **Ultra High Temperature (UHT)**

Table 4.1: Comparison between Sterilization and Pasteurization

Sterilization	Pasteurization
Sterilized products have a longer shelf life	Pasteurized products have shorter shelf life
Discovered by Nicolas Appert	Discovered by Louis Pasteur
Eliminates all forms of microorganisms	Eliminates pathogenic microorganisms only
Can be accomplished in many ways	Can be accomplished with heat
Applied in food industry, medical, surgery and packaging	Mainly applied in food industry

Milk can be treated at 141°C for 2 seconds (This method employ temperature above 100°C).

b) Temperature at 100°C:

i) Water at 100°C (Boiling):

Boiling is one of the moist heat sterilization methods. It kills vegetative forms of bacterial pathogens, almost all viruses and fungi (including their spores) within 10 minutes, usually much faster.

- Most vegetative bacteria will die in 5-10 minutes when immersed in boiling water, but some spores will survive at this temperature for several hours.
- Articles sterilized by this method cannot be stored for a long time.

ii) Steaming at 100°C (Tyndallization):

It is a process discovered by John Tyndall in 19th century for sterilizing substances to kill the spores of bacteria. The process of exposure of materials to steam at 100°C for 20 min for three consecutive days is known as tyndallization. First exposure kills all the vegetative forms and in the intervals between heating, the remaining spores germinate into vegetative forms which are killed on subsequent heating. Tyndallization is also called fractional sterilization or intermittent boiling.

c) Temperature above 100°C:

Moist heat sterilization can be carried out at temperature above 100°C in order to destroy bacterial endospores. This requires the use of saturated steam under pressure. This is achieved using autoclave.

Autoclave

Sterilization using an autoclave is most effective when the organisms are either contacted by the steam directly or contained in a small volume of aqueous liquid (primarily water). The temperature used in autoclave is 121°C at 15 lbs (pounds) pressure for 15 minutes (Figure 4. 2a & b).

Autoclaving is used in sterilizing culture media, instruments, dressings, applicators, solutions, syringes, transfusion equipment, pharmaceutical products, aqueous solutions and numerous other items that can withstand high temperatures and pressures. The same principle of autoclaving applies for the common household pressure cooker used for cooking food.

Factors influencing sterilization by heat:

Sterilization by heat depends upon various factors such as time, temperature employed, number of microorganisms, spores and nature of material to be sterilized.

Quality control of moist heat sterilization:

To check the efficiency of moist heat sterlization, the indicator commonly used is the paper strips containing spores of *Bacillus sterothermophilus.*

4.5 Radiation

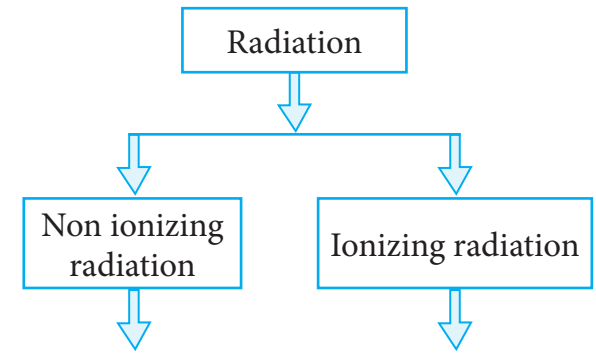
Radiation is commonly employed for sterilizing heat sensitive materials such as disposable plastic products and materials that cannot withstand moisture. The most effective type of radiation to sterilize or reduce the microbial burden in the substance is through the use of electromagnetic radiations. Figure 4.3 shows different types of electromagnetic radiations. Radiation has various effects on cells, depending on its wavelength, intensity and duration of explosure (Flowchart 4.2). Radiation that kills microorganism is of two types namely ionizing and nonionizing.

a) Non-ionizing radiation

Infra-red rays and ultra-violet rays are non ionizing radiation.

i) Infra-red radiation

These are electromagnetic rays with wavelengths longer than those of visible light. These are low energy type. It kills microorganisms by oxidation of molecules as a result of heat generated. Infrared radiation is used for rapid mass sterilization of pre-packed items such as syringes and catheters.



Flowchart 4.2: Radiation

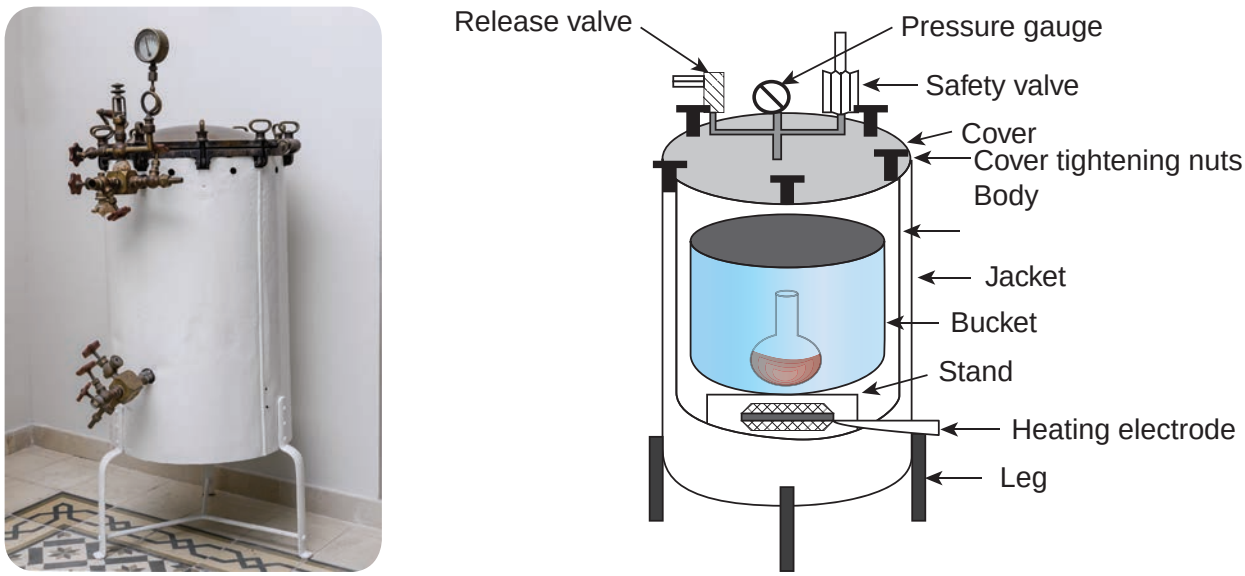


Figure 4.2: (a) Laboratory autoclave (b) Components of autoclave

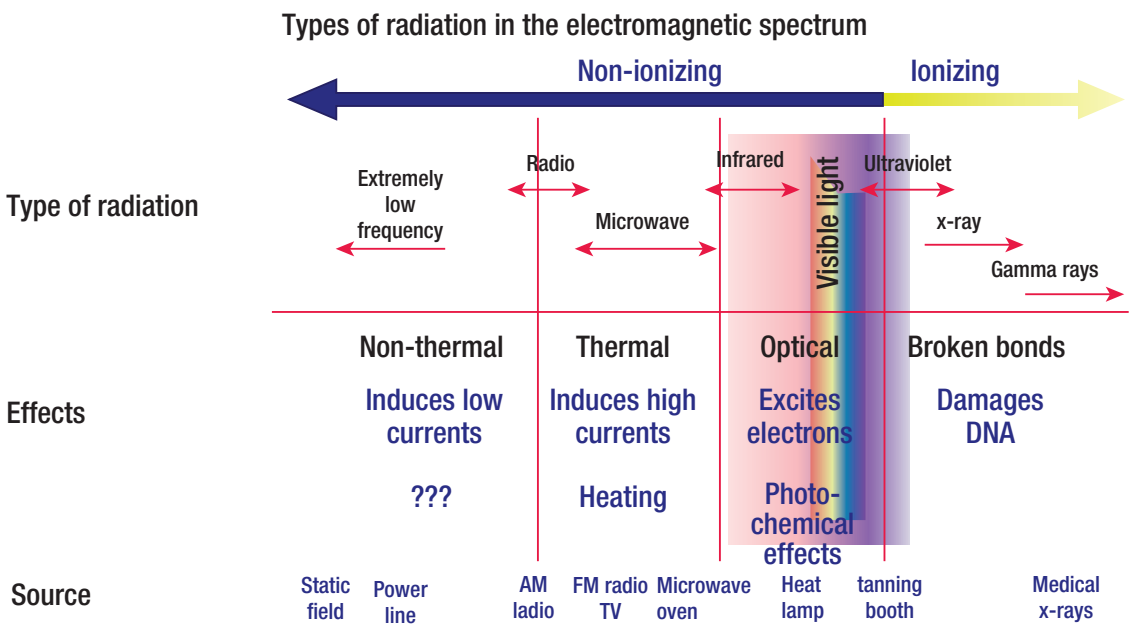


Figure 4.3: Types of radiation in electromagnetic spectrum

ii) Ultra-violet radiation

The ultraviolet (UV) portion of the electromagnetic spectrum includes all radiations from 150-3900A°, UV radiation around 2600A° is most lethal to microorganisms. UV has a very little ability to penetrate matter. Thus, only the microorganisms on the surface of an object, exposed directly to the ultraviolet light are susceptible to destruction. UV radiations are used to sterilize operation theaters, laboratories and entry ways.

b) Ionizing radiation

Ionizing radiations (X-rays, Gamma rays and Cosmic rays) are an excellent sterilizing agents and they penetrate deep into the objects. These radiations do not produce heat on the surface of materials. Hence, sterilization using ionizing radiations is referred as cold sterilization. It will destroy bacterial endospores and vegetative cells, both Prokaryotic and Eukaryotic; however ionizing radiation is not always effective against viruses. Gamma radiation from Cobalt 60 source is used in the cold sterilization of antibiotics, hormones, sutures and plastic disposables supplied such as syringes and in pasteurization of meat.

Deinococcus radiodurans is an extremophilic bacterium. It is one of the most radiation-resistant organisms known.

4.6 Filtration

Filtration is an effective and reasonably economical method of sterilization. It is used to sterilize heat-sensitive fluids, and air. It is particularly useful for solutions containing toxins, enzymes, drug, serum and sugars. Sugar solutions used for the cultivation of microorganisms tend to caramelise during autoclaving and so they are best sterilized by filtration. Filtration is also used extensively in beer and wine industries. Filters with known pore sizes which are sufficiently small to hold back bacteria are employed. Recently filters that can remove viruses are also available.

Filtration is an excellent way to remove the microbial population from solution containing heat sensitive material.

There are two types of filters namely (Figure 4.4):

i) Membrane filter (surface filtration) and

ii) Depth filter

Membrane filters

Membrane filtration is used for preparing heat-labile culture media components. It

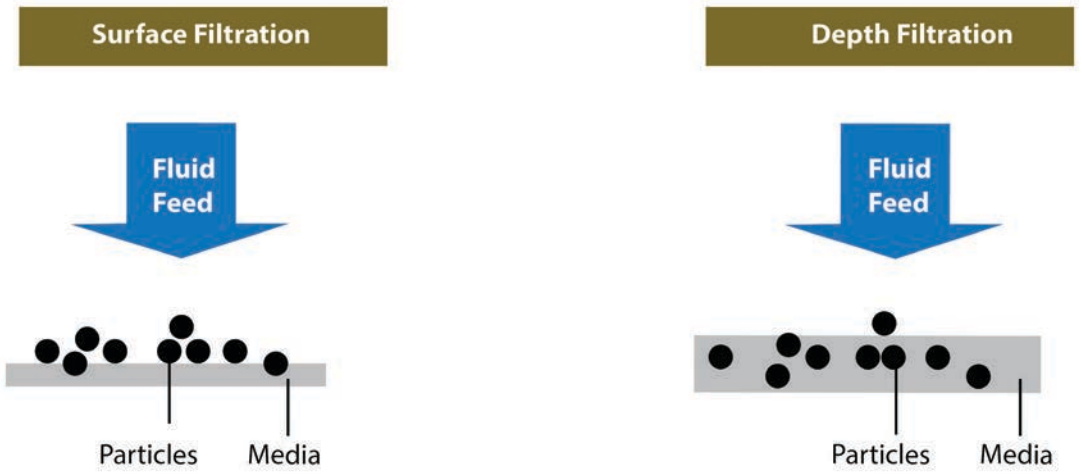


Figure 4.4: Principle of filtration

is also useful in removing bacteria from heat-sensitive pharmaceutical products and biological solutions.

Membrane filters are made up of either cellulose acetate, cellulose nitrate, polycarbonate, polyvinylidene fluoride or other synthetic porous materials. These filters remove microorganisms by screening them out, such as a sieve separates large sand particles from small ones. Membranes with pore size of 0.2µm in diameter are used to remove most vegetative cells but not viruses. These filters are used to sterilize pharmaceutical products, ophthalmic solutions, culture media, oils, antibiotics, and other heat sensitive solutions (Figure 4.5a, b & c).

Depth filters

Depth filters are the oldest type of filters and they consist of overlapping layers of fibrous sheets of paper, asbestos or glass fibers. The overlapping fibers create random paths through the filter that trap many particles. Depth filter are made up of diatomaceous earth (Berkefeld filters) which are used as water purifiers. Examples of types of depth filters (Figure 4.6) contains unglazed porcelain (Chamberl and filters) and asbestos (Seitz Filter).

Air filtration

Air also can be sterilized by filtration.

HOTS

Give a reasonable method of sterilization for the following.

1. Operation theatre 2. Serum 3. Pot of soil 4. Plastic Petri Dishes 5. Rubber gloves 6. Disposable syringes. 7. Metal instruments 8. Flask of nutrient agar 9. Milk 10. Papers with spores.

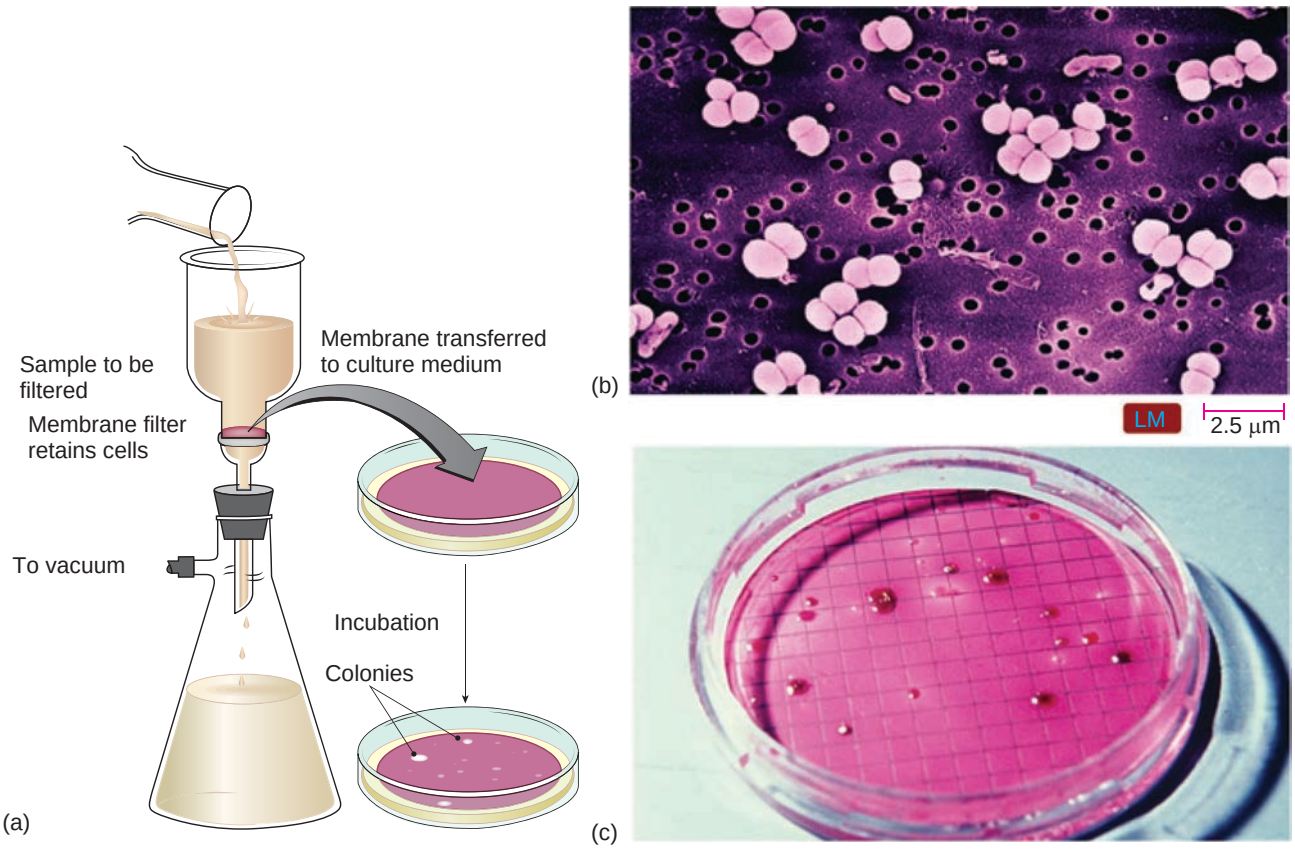


Figure 4.5: (a) Membrane filter apparatus (b) Light microscope image of microorganism filtered through membrane filter (c) Membrane filters showing microbial colonies on culture media

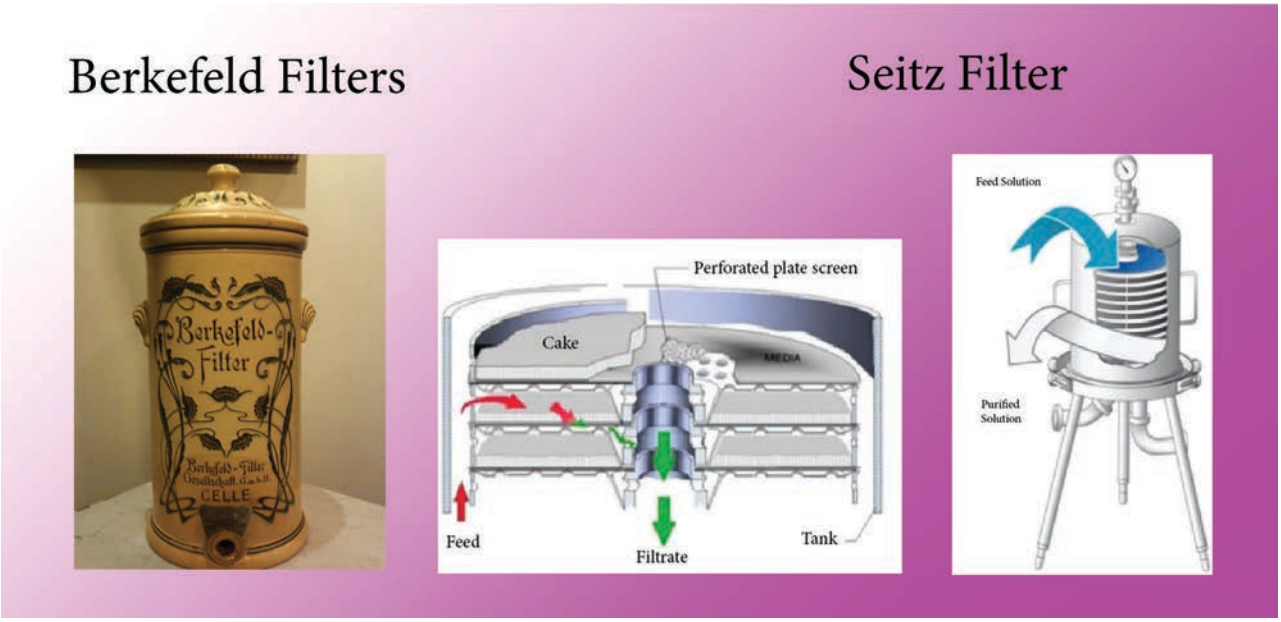


Figure 4.6: Types of depth filters

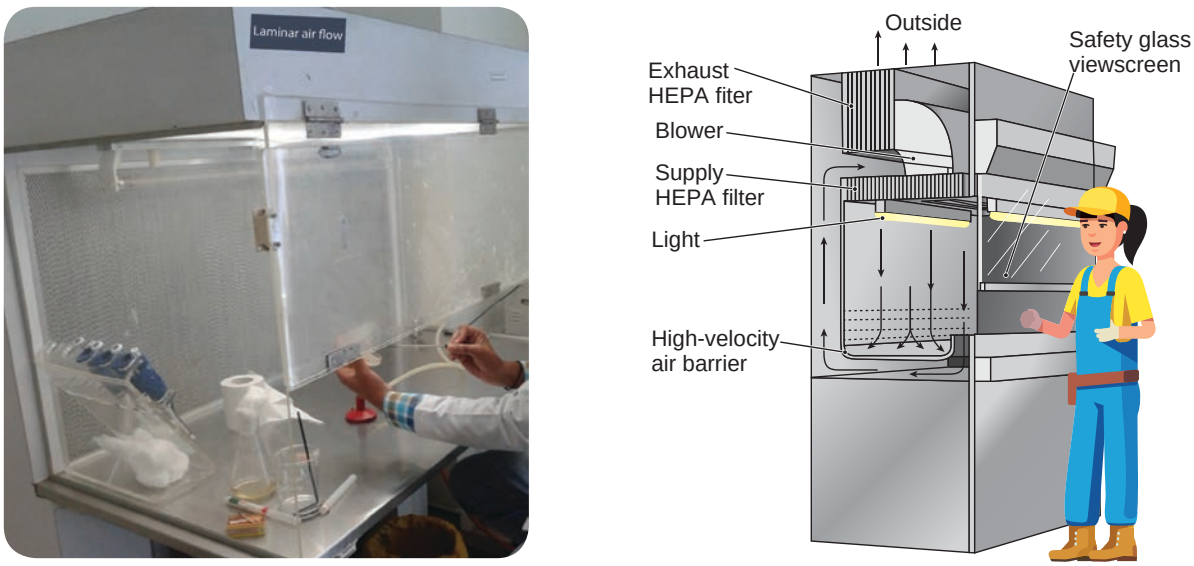


Figure 4.7: Laminar air flow

The air is freed from infection by passing it through High Efficiency Particle Arrester (HEPA) filter. Laminar air flow biological safety cabinets are one of the most important air filtration systems (Figure 4.7). It employes HEPA filters which remove 99.97% of 0.33µm particles size. Some operation theaters and rooms occupied by burn patients receive filtered air to lower the numbers of airborne microbes. HEPA filters remove almost all microorganisms above 0.3µm in diameter.

Various physical methods of sterlization is summarized in Table 4.2

Table 4.2: Physical methods used to control microbial growth

	Method	Mechanism of action	Comment	Preferred for sterilizing
Heat				
1	**Dry heat**			
	a. Direct flaming	Burning contaminants to ashes	Very effective method of sterilization	Inoculating loops
	b. Incineration	Burning to ashes	Very effective method of sterilization	Paper cups, contaminated dressings, animal carcasses, bags, and wipes
	c. Hot –air sterilization	Oxidation	Very effective method of sterilization, but requires temperature of 160°C for about 1 hour	Empty glassware, instruments, needles, and glass syringes
2	**Moist heat**			
	a. Boiling or flowing steam	Protein denaturation	Kills vegetative bacterial and fungal pathogens and almost all viruses within 10 min; less effective on endospores	Dishes, basins, pitchers, various equipment
	b. Autoclaving	Protein denaturation	Very effective method of sterilization; at about 15 lbs of pressure (121°C), all vegetative cells and their endospores are killed in about 15 min	Microbiological media, solutions, linens, utensils, dressings, equipment, and other items that can withstand temperature and presure
	c. Pasteurization	Protein denaturation	Heat treatment for milk (72°C for about 15 sec) that kills all pathogens and most nonpathogens	Milk, cream, and certain alcoholic beverages(beer and wine)
3	**Radiation**			
	a. Ionizing	Destruction of DNA	Not widespread in routine sterilization	Used for sterilizing pharmaceuticals and medical and dental supplies
	b. Nonionizing	Damage to DNA	Radiation not very penetrating (non penetrating)	Control of closed environment with UV

TYPES OF STERILIZATION AND THEIR USES

sterilization processes are used every day around the world to eliminate hazardous biological agent and bacteria.This process is especially critical to the Medical,Pharmaceutical and Food industries for public safety and regulation compliance

Steam (moist heat) Sterilization

Invented by Charles Chamberland in 1880

Steam Sterilization exposes each item to direct steam contact at the required temperature and pressure for the specified time.

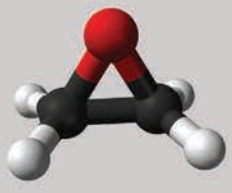
Steam Sterilization is primarily used for heat stable materials such as:

Glassware

Surgical Instruments

Medical waste

EtO/EO Sterilization



First used in the 1940s by the US military

EtO or EO sterilization is a method which utilizes Ethylene Oxide gas within a chamber to sterlize items or materials that cannot withstand the high temperatures or humidity that othersterilization methods employ.

Examples include devices or products including

Electrical components

Plastics

Gauze

Cardboard

Spices

Depyrogenation /Dry Heat sterilization

Dry Heat Sterilization is one of the earliest forms of Sterilization practiced

Used on products that may be degraded when exposed to steam or moisture,but which can withstand high temperatures.

Examples of items sterilization by dry heat sterilization

Metal Surgical Instruments

Needle

Petroleum product

Glassware

Powders

Oils

Summary

Physical methods of microbial control include heat, radiation, drying and filtration.

Heat is the most widely used method of microbial control. It is used in both forms: moist and dry. The thermal death time (TDT) is the time required to kill all microbes at a specific temperature. The thermal death point (TDP) is the lowest temperature at which all microbes are killed in a specified duration of time.

Autoclaving, or steam sterilization, is the process by which steam is heated under pressure to sterilize a wide range of materials in a comparatively short time.

Dry heat kills the microorganisms under specified time and temperature. Dry heat is applied in the following ways: Red heat, incineration and Hot air oven.

Ionizing radiation (cold sterilization) by X rays and gamma rays is used to sterilize medical products and meat. It damages DNA and cell organelles by producing disruptive ions. Ultraviolet light, or nonionizing radiation, has limited penetrating ability. It is therefore restricted to sterilize suface of the materials.

Decontamination by filtration removes microbes from heat sensitive liquids and circulating air. The pore size of the filter determines what kinds of microbes are removed.

Evaluation

Multiple choice questions

1. Which of the following does not kill endospores?
   a. Autoclaving
   b. Incineration
   c. Hot-air sterilization
   d. Pasteurization
2. Which of the following is most effective for sterilizing mattresses and plastic Petri dishes?
   a. Chlorine
   b. Ethylene oxide
   c. Autoclaving
   d. Nonionizing radiation
3. Which of the following cannot be used to sterilize a heat labile solution stored in a plastic container?
   a. Gamma radiation
   b. Ethylene oxide
   c. Nonionizing radiation
   d. Autoclaving
4. _____ kills organisms by coagulation and denaturing their proteins
   a. Dry heat
   b. Moist heat
   c. Both a & b
   d. None of the above
5. In which method, temperature of 160°C for 1 hour is employed?
   a. Red heat
   b. Infrared radiation
   c. Hot air oven
   d. Flaming
6. Which of the following temperature and time are employed in autoclave for sterilization of materials?
   a. 16 lbs 120°C for 18 mints
   b. 18 lbs 180°C for 20 mints
   c. 22 lbs 170°C for 35 mints
   d. 15 lbs 121°C for 15 mints

7. Wavelength used for the absorption of UV spectrum is
   a. 4000A°
   b. 2600A°
   c. 20A°
   d. None of the above

Answer the following

1. Define Pasteurization.
2. What is Incineration?
3. Define membrane filters?
4. What is Sterilization?
5. Explain the principle moist heat sterilization?
6. Differentiate the mechanism of operation employed in autoclave and hot air oven.
7. Discuss ionizing radiation.
8. How do you sterilize heat sensitive materials?
9. Define Tyndallization.
10. Describe the sterilization in an autoclave.
11. Explain the methods of sterilization by dry heat.
12. Explain the methods of radiation.

Student Activity

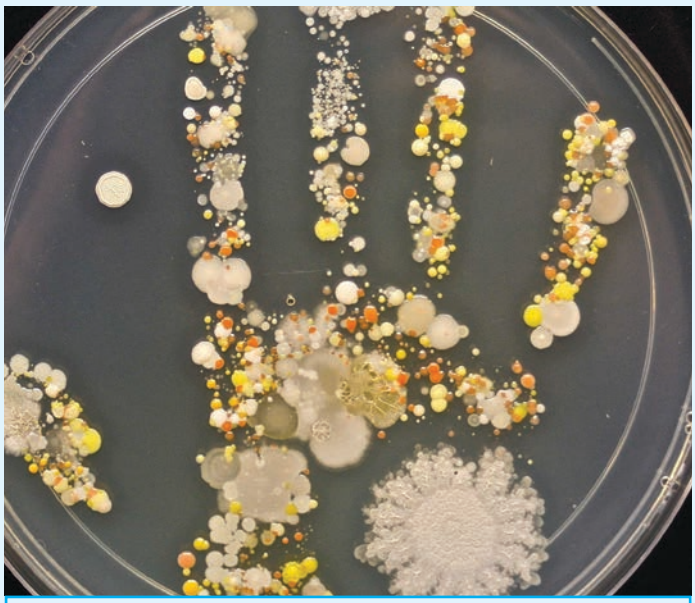
1. Collect samples of raw milk (unpasteurized) and boiled milk, place them in open containers separately. Observe the changes after a few hours in both and infer.
2. Making a working model of depth and membrane filters and demonstrating their uses.

Chapter 5

Cultivation of Microorganisms

ENI9S6

Chapter Outline



The microorganisms on the handprint of an eight-year-old boy. After incubation the plates showed coloured colonies of bacteria and fungi as you see above.

The cultivation of microorganisms under laboratory condition makes the microscopic cells to grow and form individual colonies macroscopically.

Learning Objectives

After studying this chapter the student will be able,

- *To know the importance of bacterial media for growth of microorganisms. To understand various types of media for differentiation and diagnosis of important pathogenic microorganisms.*
- *To know pure culture techniques*
- *To understand the methods involved in isolating pure culture of bacteria, which includes pour plate, spread plate and streak plate.*
- *To differentiate the growth characteristics of bacteria and fungi.*

Microorganisms are omnipresent and they exist in soil, air, water, spoiled food, decayed animal and plant residues. They are found in environment as pathogens and normal microflora. Excellent supporting factors are available in nature for microorganisms to survive in the environment. This leads to microbial proliferation as an extended community in nature. The term 'cultivation of microorganisms' means growing microorganisms in the laboratory with ample supply of specific nutrients (Figure 5.1). Obligate intracellular parasites like viruses, *Rickettsias* and *Chlamydias* are cultivated within living cells.

Survival and growth of microorganisms depend upon the favourable growth environment. Laboratory cultivation plays a crucial role in the isolation, identification and classification of microorganisms. Cultivation of bacteria and fungi by artificial formulated medium is one of the important milestones in the history of Microbiology.

Robert Koch devised the solid medium (by using gelatin) to grow and isolate the microorganisms.

5.1 Significance of Culturing Microorganisms

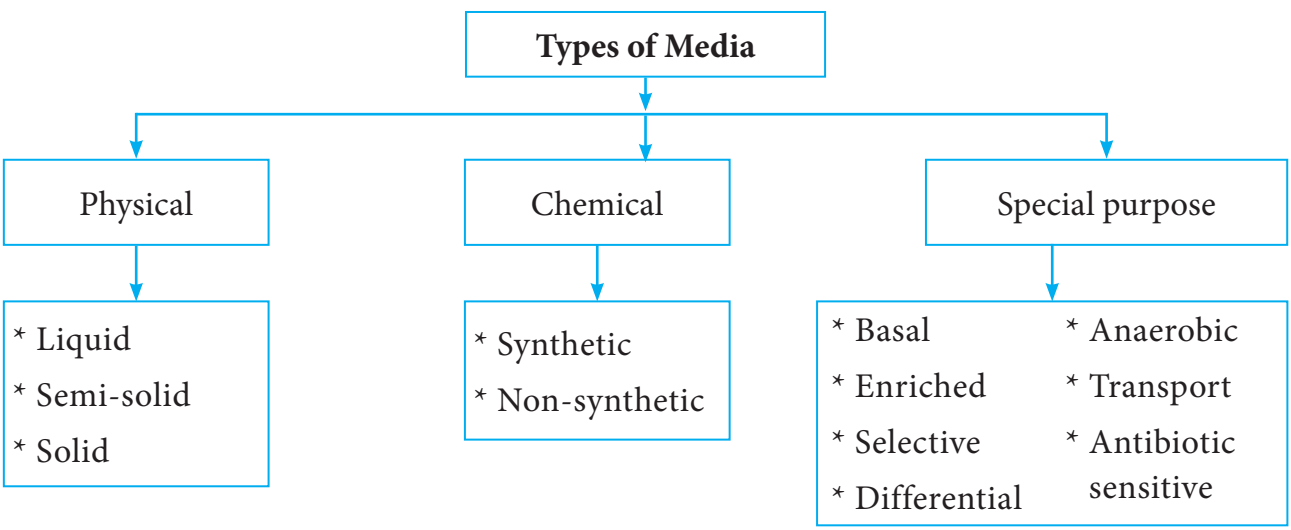
- To isolate microorganisms from any samples
- To study the morphology and biochemical characteristics of microorganisms
- To maintain the stock culture
- To identify disease causing microorganisms
- To study the role of microorganisms in the production of industrially important products

5.2 Bacteriological Media and its Types

Generally microorganisms occur as mixed culture in nature. Human beings, animal bodies and other natural resources harbour microbes in mixed population. By using appropriate media, microorganisms can be grown separately in pure form and can be studied. For successful cultivation of a given microorganism, it is necessary to understand the nutritional requirements of that microorganism and then supply the essential nutrients in proper form and proportions in culture medium. Flowchart 5.1 describes the types of media. A common bacteriological medium has Carbon and Nitrogen sources along with buffering agents. Most of the media are prepared using dehydrated components. The basic components are peptone, beef extract, meat extract, yeast extract and agar (Table 5.1).

Table 5.1: Common ingredients of a culture media

S.No	Ingredients	Source of
a.	Peptone (protein hydrolysates)	Carbon, nitrogen, energy
b.	Beef extract (Extract of lean beef)	Aminoacids, vitamins, minerals
c.	Yeast extract (Brewer's yeast)	Vitamin B, Carbon, Nitrogen
d.	Agar	Solidifying agent



Flowchart 5.1: Types of media

Uses of agar:

- It is one of the principle ingredients in the preparation of solid or semisolid media.
- It is used as a solidifying agent in culture medium.
- It is extracted from certain seaweeds belonging to genera of red algae like *Gelidium* and *Gracilaria* (Figure 5.1).

Figure 5.1: *Gelidium* – Red algae

- It is a sulphated polymer mainly consisting of D-galactose.
- Agar is a highly preferred solidifying agent because it does not affect the growth of microorganisms. Agar is also used in the food and pharmaceutical industries.
- The purified form of the agar is called Agarose. It is prepared by removing the pectin from the Agar. It is used in Molecular Biology laboratory for the separation of DNA molecules by gel electrophoresis.

Agar was first described for use in Microbiology in 1882 by the German microbiologist Walther Hesse, an assistant working in Rober Koch's laboratory, as suggested by his wife Fannie Hesse.

A cheap substitute for agar in microbial culture media is Guar gum, which can be used for the isolation and maintenance of thermophiles.

HOTS

Why is agar preferred to gelatin as a solidifying agent in culture media?

5.2.1 Physical Nature of Agar Medium

The concentration of agar plays a major role in determining the consistency of the medium. A medium with agar concentration of 2% or greater is said to be a solid medium and that of 0.5% is said to be a semisolid medium (jelly like appearance). Tabel 5.2 lists the concentration of agar in

Table 5.2: Concentration of agar in media

Nature of Medium	Concentration	Example	Uses
Solid	2%	Nutrient agar	To isolate microorganisms on petridish and forming agar slant
Semisolid	0.5%	SIM (Sulphur Indole Motility medium)	Agar stab to observe motility
Liquid	0%	Nutrient broth	To observe biochemical reaction.

media. However liquid media (broth) does not contain agar. Figure 5.2 shows types of media depending on physical nature.

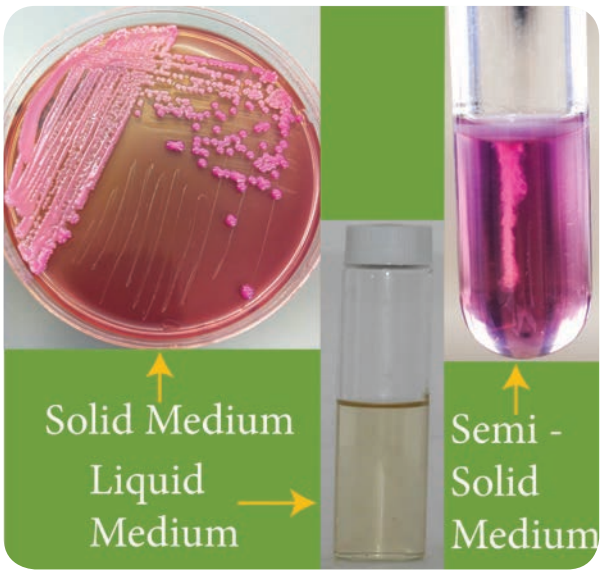


Figure 5.2: Solid, liquid and semi-solid media

5.2.2 Chemical Nature of Medium

- **Synthetic medium**

Chemically defined synthetic Medium is used for various experiments. This medium is prepared exclusively from pure substances with known chemical composition and concentrations. This is widely used in research to find the type of compound metabolized by the experimental organism.

- **Non-synthetic medium**

The medium in which the exact chemical composition and the concentration of each ingredient is not certainly known is called non-synthetic medium. In this medium, crude materials such as meat extract, yeast extract, various sugars, molasses and corn steep broth are used. This supports the growth of a variety of microorganisms. It is otherwise called as complex medium.

Infobits

Veggitone is a vegetable based product containing peptones. It is made from raw materials such as peas and fungal proteins that are digested using fungal and bacterial enzymes.

5.2.3 Special Purpose Medium

i) Basal medium

This medium promotes the growth of many types of microorganisms which do not require any special nutrient supplement. It is a routine laboratory medium with Carbon and Nitrogen sources along with some minerals. Example: Nutrient Agar or Nutrient Broth. It is also called general purpose medium. It is used for subculturing the pathogens. It is a non-selective medium, which is designed to support the growth of a wide spectrum of heterotrophic organisms. (Figure 5.3)

Figure 5.3: Growth of bacteria on Nutrient agar

ii) Enriched medium

In enriched medium, substances like blood, egg or serum are added along with the basal medium. It is used to grow fastidious organisms that are very particular in their nutritional needs. Fastidious organisms

have elaborate requirements of specific nutrients like vitamins and growth promoting subtances and or not easily pleased or satisfied by ordinary nutrients available in nature. Example: Blood agar is used to identify haemolytic bacteria (Figure 5.4) and Chocolate agar used to identify *Neisseria gonorrhoeae.*

Figure 5.4: Blood Agar showing alpha, beta & gamma – haemolytic colonies

In 1919, James Brown used blood agar as diagnostic medium to study the haemolytic patterns of bacteria.

iii) Selective medium

Selective medium contains one or more agents (selective components) that inhibit unwanted organisms but allow the desired organisms to grow. Growth of unwanted microbes is suppressed by adding bile salts, antibiotics and dyes. Example: Mannitol salt agar is selective for *Staphylococci.* This medium contains 7% Sodium chloride that inhibits the growth of other bacterial population but allows the growth of *Staphylococci* (Figure 5.5). Moreover it has Phenol red dye to indicate acid production. *Staphylococcus* utilizes Mannitol and produces acid which changes the colour of the Phenol red indicator to yellow. Salmonella-Shigella (SS) agar is selective for *Salmonella* (Figure 5.6).

Figure 5.5: Growth of *Staphylococcus aureus* on Mannitol salt agar

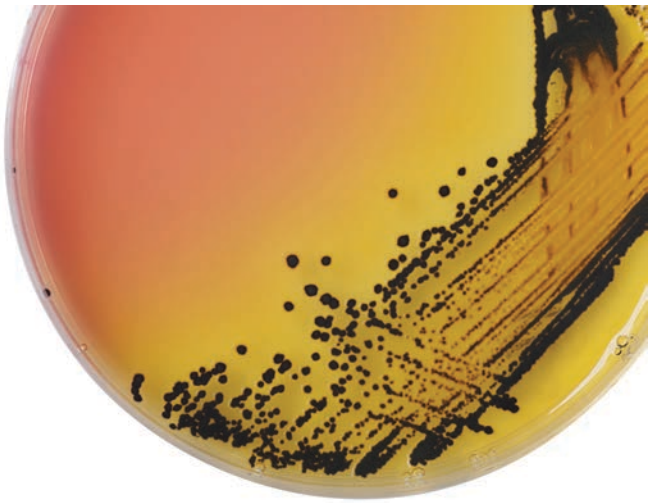


Figure 5.6: Growth of *Salmonella* on SS agar

It is nothing short of amazing and humbling fact that even after 120 years of trying to grow microbes in the laboratory, we have succeeded in culturing only 0.1% of the microorganisms around us.

iv) Differential medium

Differential medium distinguishes between different groups of bacteria and permit tentative identification of microorganisms based on their biological charaterstictics as they cause a visible change in the medium. We can differentiate haemolytic and non-haemolytic patterns of bacteria using blood agar. Differential medium is otherwise called indicator medium as it distinguishes one organism from another growing on the same plate by the formation of pigments due to its biochemical and physiological nature. Example: MacConkey agar medium has neutral red dye. Lactose fermentors form pink coloured colonies and non fermentors form colourless translucent colonies on it (Figure 5.7).

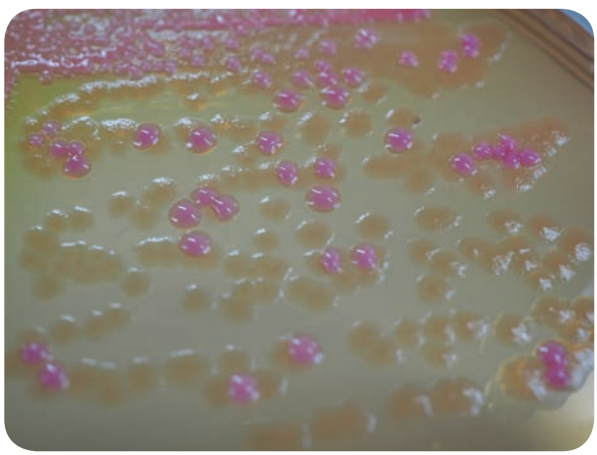


Figure 5.7: Growth of microorganisms on MacConkey agar (Lactose fermenting bacterial colonies appears pink)

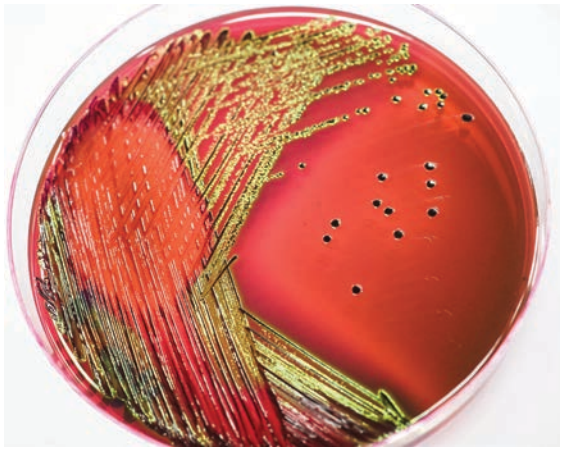
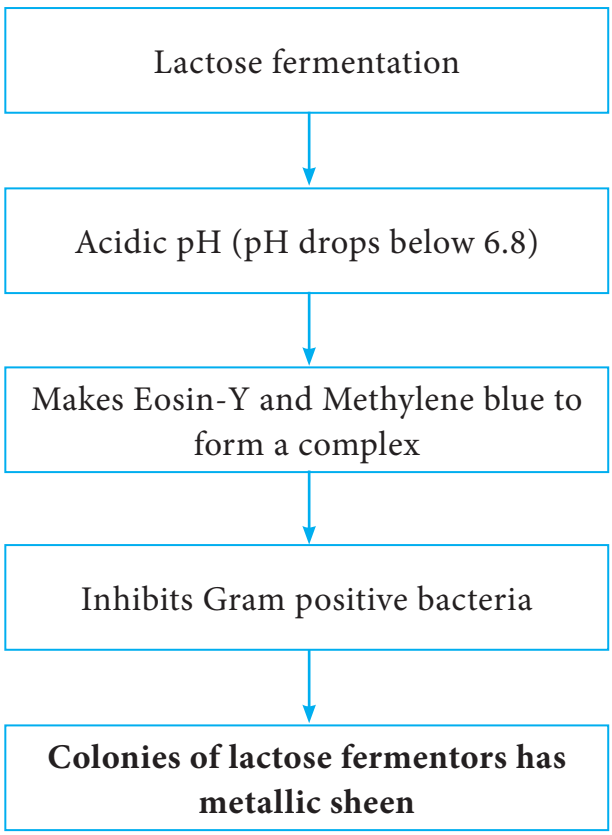


Figure 5.8: Growth of lactose fermenting bacteria on EMB Medium

Eosin Methylene Blue (EMB) agar medium is also a differential medium. It is used to differentiate lactose fermentors from non-lactose fermentors. It has lactose sugar and two dyes namely Eosin –Y and Methylene blue. These dyes act as inhibitory agent towards Gram positive bacteria. Example: Lactose fermentors such as faecal *Escherichia coli* show metallic sheen and non lactose fermentors such as *Enterococcus* do not show metallic sheen. (Figure 5.8).



Chromogenic medium is used for the simple and fast detection of transformed bacteria by using chromogenic substrates. The chromogenic mixture contains substrates such as Salmon-GAL, X-GAL. Certain bacterial enzymes cleave the chromogenic substrate resulting in the coloured colonies.

HOTS

Why is EMB medium called a selective, differential as well as complex medium?

v) Enrichment medium

Enrichment medium is a liquid medium. It is used to grow a particular microorganism that is present in much smaller number along with others present in sufficiently large numbers. An enrichment medium provides nutrients and environmental conditions that favour the growth of a desired microorganisms. It is used to culture microorganisms present in soil or faecal samples that are very small in number. Example: Selenite F Broth is used to isolate *Salmonella typhi* present in low density in faecal sample. It is cultured in an enrichment medium containing Selenium. Selenium supports the growth of the desired organism and increase it to detectable levels compared to intestinal flora. Sodium selenite inhibits many species of Gram positive and Gram negative bacteria including *Enterococci* and coliforms.

vi) Antibiotic sensitivity medium

Antibiotic sensitivity medium is a microbiological growth medium that is commonly used for antibiotic sensitivity testing. Example: Muller-Hinton agar medium. It is a non-selective and non-differential medium. It allows the growth of most type of microorganisms. It contains starch which absorbs toxins released from bacteria. Hence toxins do not interfere with antibiotics. Agar concentration of 1.7% is used in this media which allows better diffusion of antibiotics (Figure 5.9).

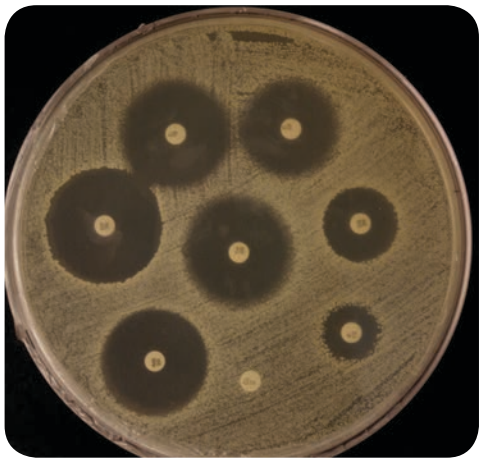


Figure 5.9: Antibiotic sensitivity on Muller Hinton agar

vii) Anaerobic medium

Anaerobic medium is a medium used for the cultivation of anaerobes, Example: i) Robertson cooked meat medium: This is used for the isolation of *Clostridium* ii) Thioglycolate broth: In this medium Sodium thioglycollate is used as a reducing agent which maintain a low Oxygen tension by removing the molecular Oxygen from the environment.

viii) Transport medium

Transport medium is used for the temporary storage of specimens that are being transported to the laboratory for cultivation. It maintains the viability of all

organisms in the specimen without altering their concentration. It mainly contains buffers and salts. Example: Stuart's transport medium that lacks Carbon, Nitrogen and organic growth factors. Other examples of transport media are Cary Blair and Amies.

Infobits

Viral Transport Medium is used to carry a specimen containing viruses. Universal Transport Viral Medium (UTVM). This liquid medium is stable at room temperature. It is used for collection, transport, and maintenance and long term freeze storage of viruses.

Exceptions in cultivation of microbes in artifical medium

Some bacteria like *Mycobacterium leprae* and *Treponema pallidum* cannot be cultivated in artificial medium.

ix) Media used for isolation of fungi

Apart from the bacteriological media, fungal media are used to study fungal morphology pigmentation and sporulation. Sabouraud's Dextrose Agar (SDA) is used as a common medium to isolate fungus. There are several other important fungal media used for fungal cultivation. Examples Niger Seed Agar and Potato Dextrose Agar

HOTS

1. Which medium is used to carry the sample when the sick person is unable to come to the laboratory?
2. Give a special medium to check the growth of anerobes in a burn wound infection with dead tissues.

5.3 Pure Culture

In nature, microorganisms usually exist as complex multispecies community. A single species has to be characterized in order to know the morphology, pathogenicity and molecular genomic pattern of the organism. For characterizing a species we have to isolate the organisms in pure form. Pure culture or axenic culture is a culture containing only one type of organism. The descendents of a single organism in pure culture is called a strain. A strain forms a single colony. Colony is a cluster of microorganisms in which all the characters of the family remain same. With the advent of the pure culture techniques many microorganisms are being identified.

5.3.1 Methods Employed in the Isolation of Microorganisms

Though there are many methods designed for isolation of microorganisms, pour plate method, spread plate method and streak plate method are widely used in the field of Microbiology.

i) Pour plate method

- It is the used for the isolation and counting of colony forming bacteria in the specified sample.
- In this technique a sample is diluted several times to reduce the density of the microbial population.
- A very small amount of diluted sample (1ml or 0.1ml) is mixed with the molten agar at a temperature of 45°C.
- The mixture is poured into the sterile petridish (In 1887, Juluis Richard Petri, a worker in Koch's laboratory, designed the Petriplate.) in an aseptic condition

Infobits

Nowadays media are available as contact plates, agar strips, media cassettes, contact slide and settle plates which are used for microbial air monitoring and compressed gas lines in food and beverage production plants. These media are also used for the enumeration of typical food contaminants such as coliforms, yeast and molds.

Colour coded MC-MEDIA pads are available for rapid and convenient microbial testing for *Escherichia coli*, yeast, mold, coliform and aerobes.

and plates are incubated at a specific temperature for a given period of time.

- Plates are incubated in an inverted manner.
- After incubation, the colonies are formed in a discrete pattern both on the surface of agar and also embedded within the medium.
- Pour plate can be also used to determine the number of cells in a population.(Figure 5.10)

Disadvantages of pour plate method

i) Loss of viability of heat sensitive organisms coming into contact with hot agar.

ii) Reduced growth of obligate aerobes in the depth of agar.

iii) Colonies embedded within the agar are much smaller than that of surface and may be confluent or invisible.

Basic five '**I**' steps one should follow in culturing micro organisms

a. **I**noculation
b. **I**ncubation
c. **I**solation
d. **I**nspection
e. **I**dentification

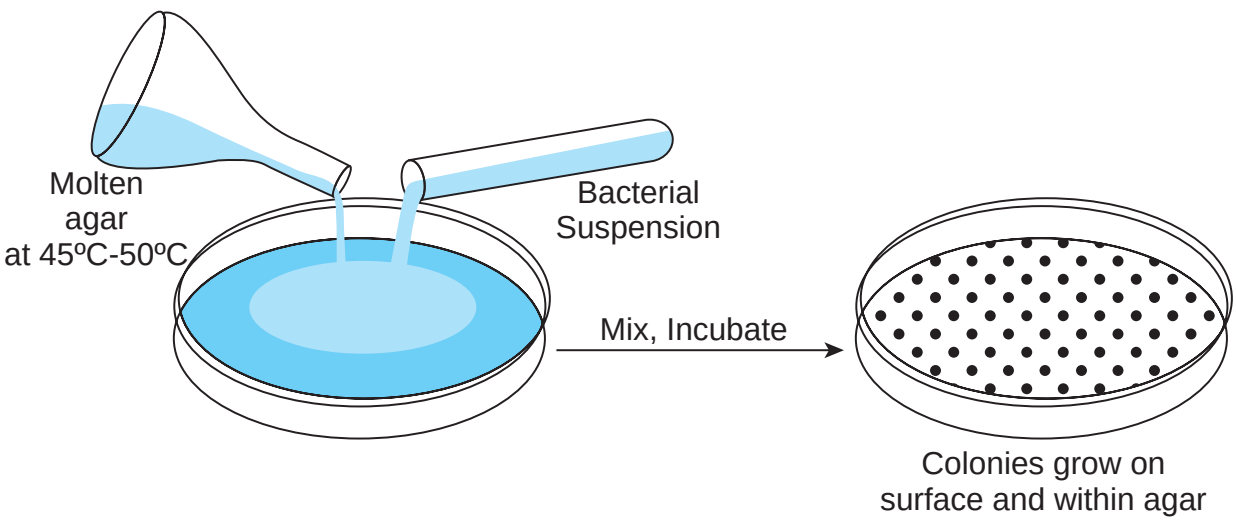


Figure 5.10: Pour plate method

ii) Spread plate method

- Spread plate method is an easy and direct method of isolating a pure culture.
- In this technique a specified amount of diluted inoculum (0.1ml or less) of microbial culture is seeded on agar plate.
- After inoculation of the sample on the agar medium, the inoculum is evenly spread on the surface with the help of a sterile glass L rod (a bent glass rod)
- Microorganisms are evenly distributed in the entire surface of agar.
- The dispersed microorganisms develop into isolated colonies.
- In this method, the plates are incubated at a specified temperature for a given period of time.
- After incubation the plates are observed for the growth of discrete colonies.
- The number of colonies are equal to the number of viable organism. This method can be used to count the microbial population (Figure 5.11).

iii) Streak plate technique

- The streak plate technique is one of the most commonly used methods for isolating pure culture of bacteria.

ENS5TT

- In this method, a loopful of inoculum from a sample is taken and it is streaked across the surface of the sterile solid medium.
- Different streaking patterns can be used to separate individual bacterial cell on the agar surface.
- After the first sector is streaked the inoculated loop is sterilized and inoculum for the second sector is obtained from the first sector.
- Similar process is followed for streaking the further areas in the sectors.
- Since the inoculum is serially diluted during streaking patterns the dilution gradient is established across the surface of the medium.
- After streaking, plates are incubated at a specific temperature for a given period of time.

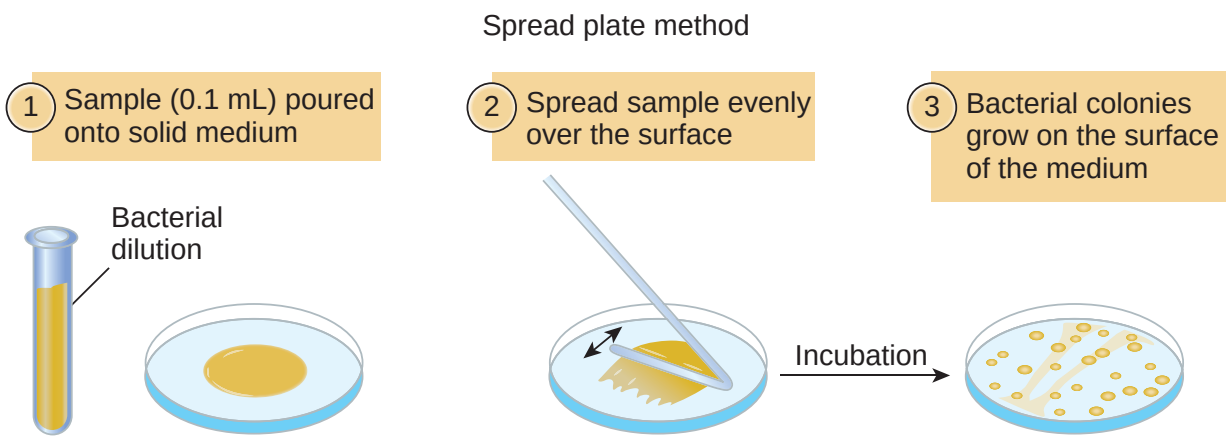


Figure 5.11: Spread plate method

- After incubation, plates are observed for growth of colonies (based on the streaking pattern and density of culture growth of microbes are abundant in the first sector in comparison with the formation of separated discrete colonies in the fourth sector of the agar medium).
- Each isolated colony is assumed to be grown from a single bacteria and thus represent a clone of pure culture.
- Successful isolation depends on spatial separation of single cells (Figure 5.12).

Infobits

Micro manipulator: It is a device used along with a microscope to pick a single bacterial cell from a mixed culture.

It has micropipette or microprobe so that a single cell can be picked up.

5.4 Growth and Colony Characteristics of Bacteria and Fungi

In the previous section we have learned the various types of media and specific purpose of each medium. Morphology is the basic criteria for the isolation, identification and classification of microorganisms. Colony characteristics are the basic tool in the field of taxonomy.

Bacteria grow in both solid and liquid medium, but identification will be easy on the solid medium. In solid medium bacteria form colonies. In liquid medium growth of bacteria are generally not distinctive because there is uniform turbidity or sediment at the bottom or pellicle is formed on the surface.

Some basic attributes such as shape, size, colour, pigmentation, texture, elevation and margin of the bacterial colony in the growth medium are explained below.

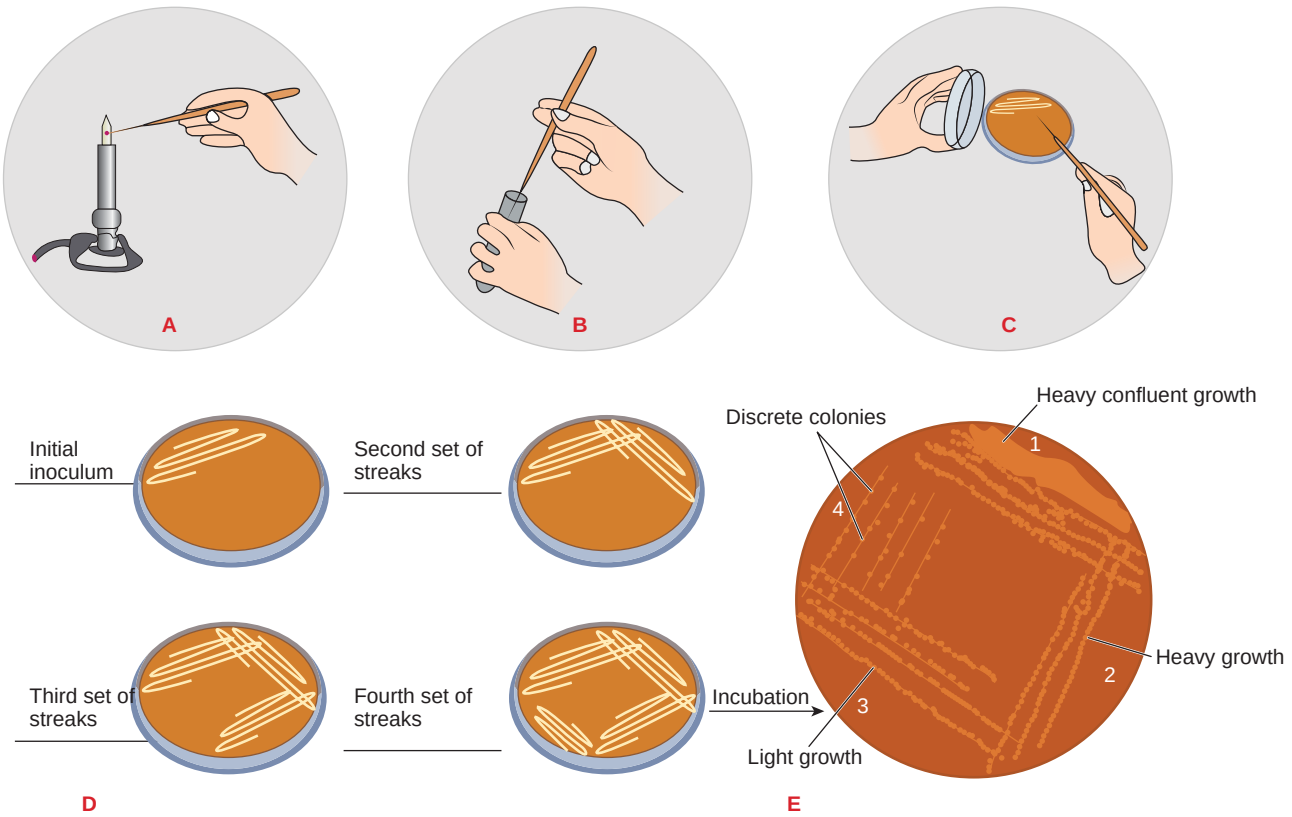


Figure 5.12: Steps in streak plate isolation method

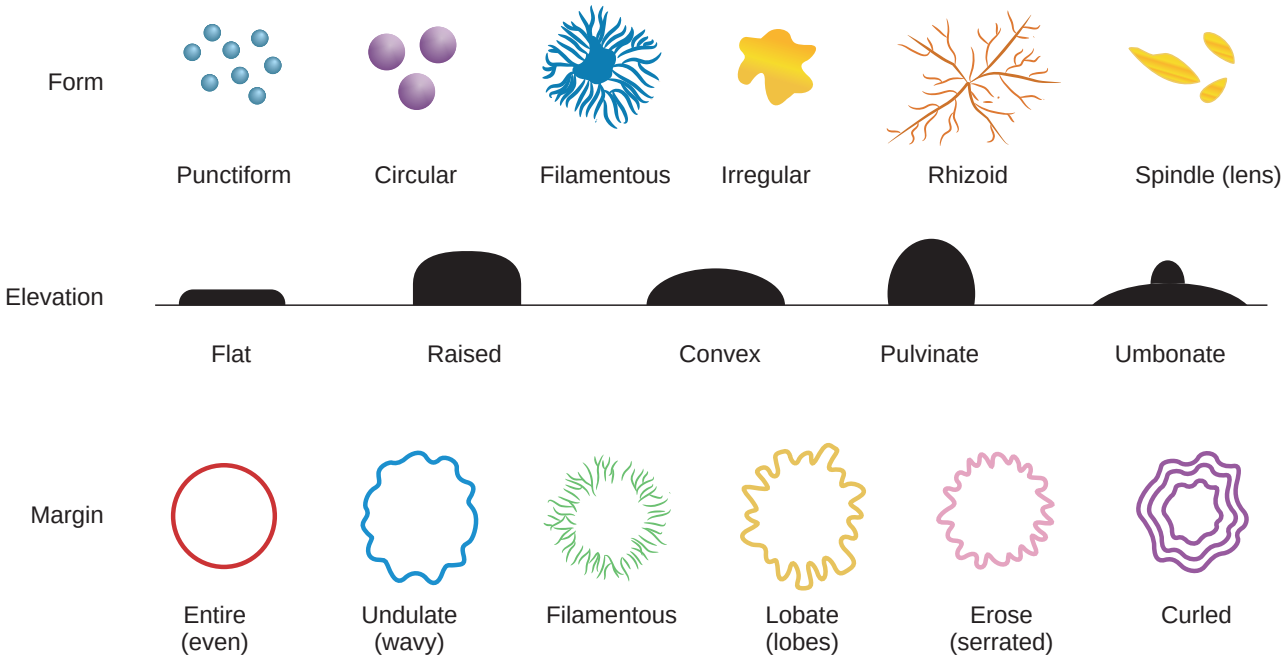


Figure 5.13: Colony morphology of bacteria

5.4.1 Colony Morphology of Bacteria on Solid Media

Shape: The shape of colony may be circular, irregular, filamentous, rhizoid.

Elevation: It is the side view of the colony. It may be flat, raised, umbonate (having a knobby protuberance) crateriform, convex pulvinate (cushion shaped)

Margin: The margin of the bacterial colony may be entire (smooth) irregular, undulate (ovary), lobate, curled, filiform. The irregular shape of the colony give irregular margin (Figure 5.13).

Colony Size: The diameter of the colony is measured in millimeter. It is described in relative terms such as pinpoint, small, medium and large.

Appearance of colony on the surface: The bacterial colonies are frequently shiny/ smooth in appearance. Colonies may be veined, rough, dull, wrinkled, or glistening.

Texture of the colony: Texture means consistency of the bacterial growth. It may be dry, moist, mucoid, brittle (dry breaks apart), viscid (sticks to loop, hard to get off), viscous, or butyrous (buttery).

Opacity of the bacterial Colony: Colonies may exhibit different optical density. It may be transparent (clear), opaque (not clear), translucent (almost clear), or iridescent (changing colour in reflected light).

Colony Odour: Some bacteria produce a characteristic smell, which sometimes helps in identifying the bacteria. Actinomycetes produce an earthy odour which is quite often experienced after rain. Many fungi produce fruity smell while *Escherichia coli* produce a faecal odour.

Smooth colonies of *Streptococcus pneumoniae* are usually virulent, where as rough colonies are non-virulent. But in *Mycobacterium tuberculosis* colonies with rough surface indicates a good factor of virulence.

Colony Colour: Many bacteria develop colonies which are pigmented.(Table 5.3) Some bacteria produce and retain water insoluble pigments and the colonies appear coloured by taking the pigment intracellularly (Figure 5.14). But some bacteria produce water soluble pigment which diffuse into the surrounding agar. Example: Pyocyanin pigment of *Pseudomonas aeruginosa* is a water soluble pigment and give blue colour to the medium.

Figure 5.14: Pigmentation of bacterial colonies on culture medium

Certain water soluble pigments are fluorescent in nature Example: Pyoverdin. Agar medium around the colonies glows white or blue green when exposed to ultraviolet light.

5.4.2 Nature of bacterial growth in liquid medium

1. If the entire broth appears milky and cloudy it is called turbid.
2. If deposit of cells are present at the bottom of the tube, the term sediment is used.
3. If the bacterial growth forms a continous or interrupted sheet over the broth it is called pellicle (Figure 5.15).

5.4.3 Growth and Colony Characteristics of Fungi

Fungi are eukaryotic organisms. They exist in both unicellular-yeast like form and in filamentous multicellular hyphae or mold form and some are dimorphic. Generally

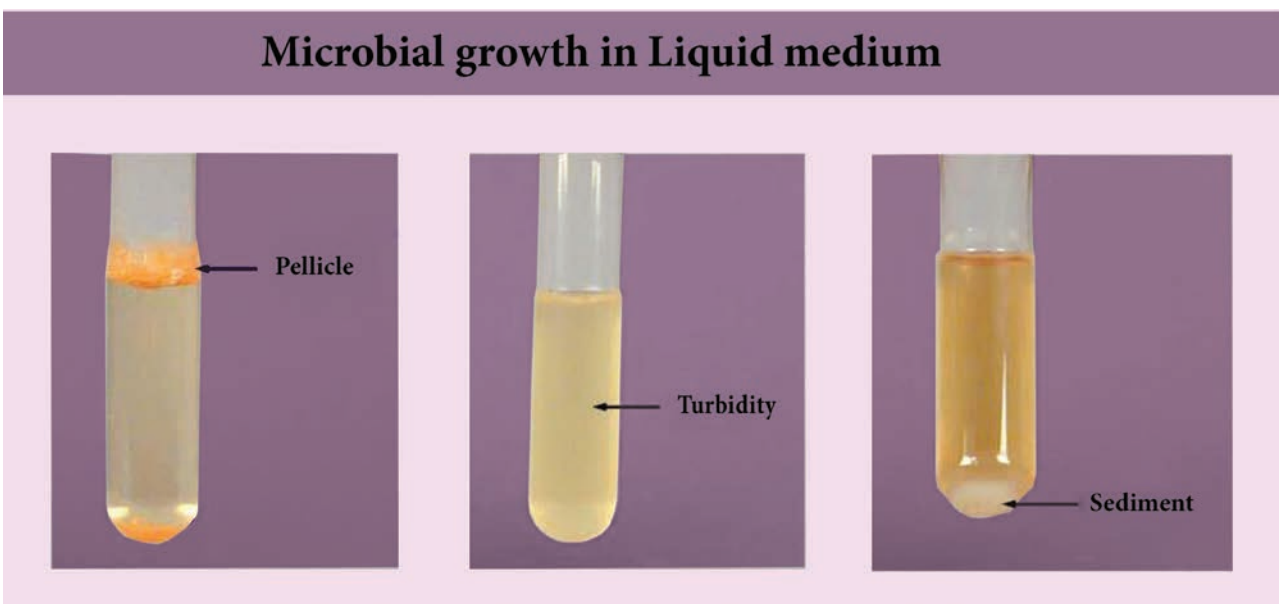


Figure 5.15: Microbial growth in Liquid medium

Table 5.3: Pigmentation of chromogenic bacteria

Bacteria	Pigment colour
Serratia marcescens	Red
Staphylococcus aureus	Golden yellow
Micrococcus luteus	Yellow
Pseudomonas aeruginosa	*Green*

fungi prefer to grow in the acidic medium. Sabourad Dextrose Agar (SDA) plates and Potato Agar plates are used for general cultivation of fungi. The acidic nature of SDA agar reduce the growth of bacteria.

The characters to be noticed in colony of fungi are colour of the surface and reverse of the colony, texture of the surface (powdery, granular, ecolly, cottony, velvety or glabrous), the topography (elevation, folding, margin) and the rate of growth.

Infobits

Dimorphic fungi are fungi that can exist in both mold and yeast form depending on environmental and physiological conditions. Example: *Histoplasma capsulatum,* a human pathogen, grows as a mold form at room temperature and as a yeast form at human body temperature.

- **Growth and colony characteristics of yeast *Candida***

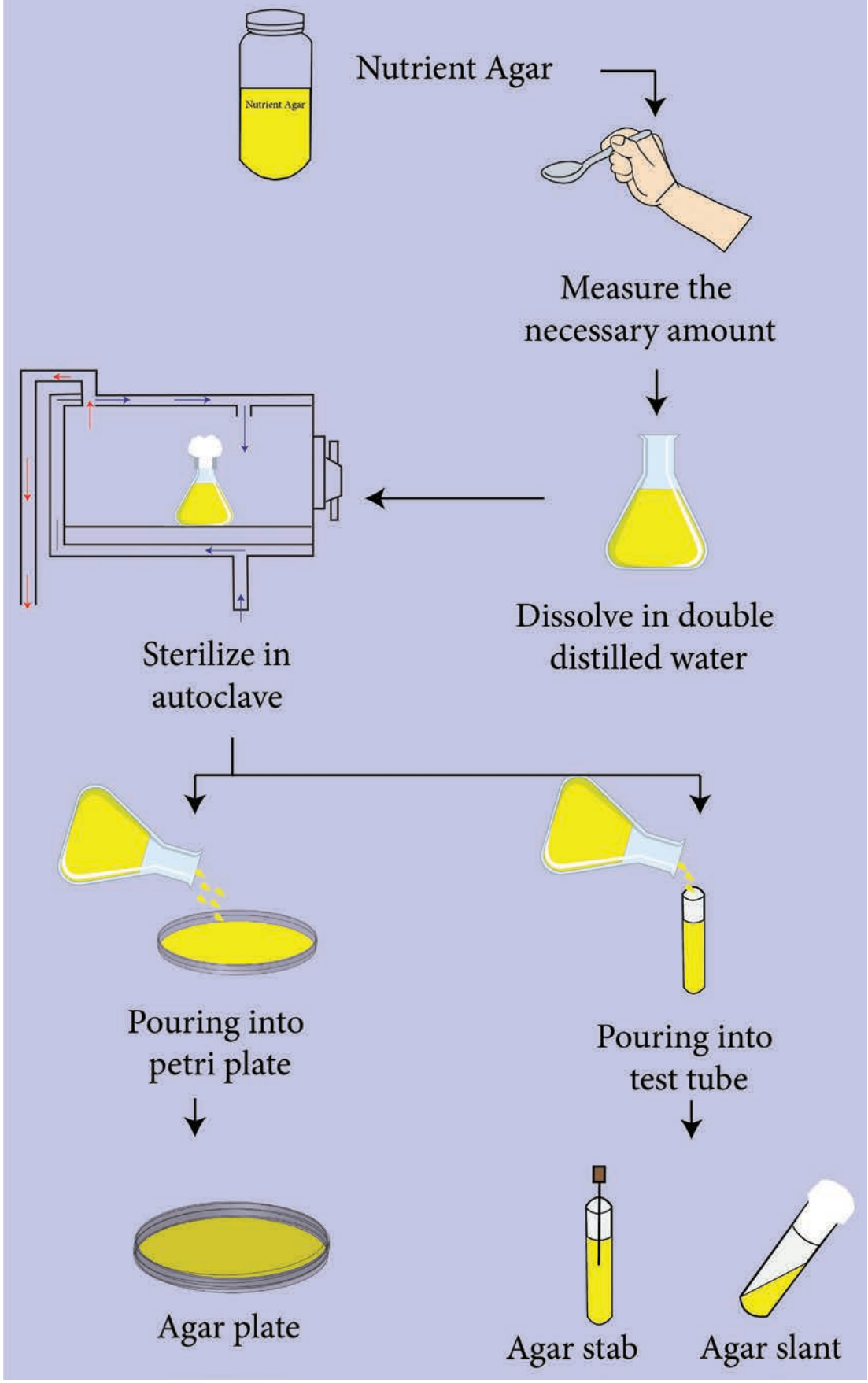
  Yeasts are grown on Sabourad Dextrose Agar aerobically. Yeasts grow as typical pasty colonies and give out yeasty odour. The colony morphology varies with different yeasts. Yeasts colonies generally have smooth texture and are larger than bacterial colonies on SDA medium (Figure 5.16a).

- **Growth and Colony characteristics of mold *Mucor***

  The genus *Mucor* is typically coloured white to brown or grey and is fast growing. Older colonies become grey to brown due to the development of spores. (Figure 5.16b).

Figure 5.16: Fungal growth on Sabouraud Dextrose Agar media a) yeast growth b) mold growth

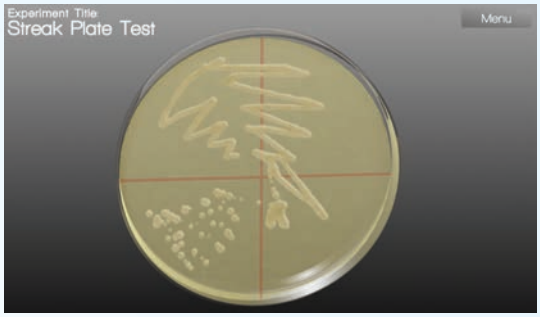
How to prepare medium in microbiology laboratory



ICT CORNER

Streak Plate Technique

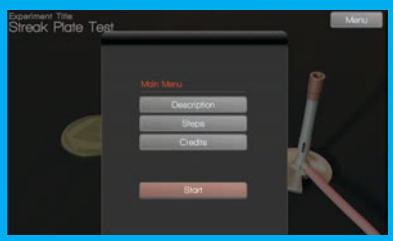
Isolation of pure culture of bacteria



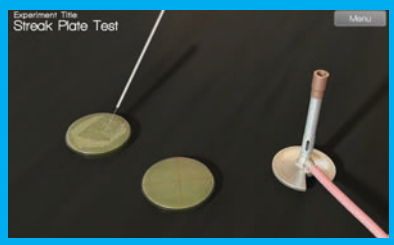
STEPS:

- Use the URL or scan the QR code to reach 'Virtual Interactive Bacteriology Laboratory.
- Click module and read the description and steps.
- Do the streak plating process from the top left part to the bottom left order.
- Heat and cool the loop between each steps.

Step1



Step2



Step3

Step4

URL:

http://learn.chm.msu.edu/vibl/content/streakplate.html

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Summary

In natural environments microogranisms exist as mixed cultures. Survival and growth of microorganisms depends upon the availability of favourable growth environment. Cultivation of microorganisms in the laboratory plays an important role in isolation, identification and classification of microorganisms. A medium is an environment which supplies the nutrients necessary for the growth of the microorganisms. Various kinds of media have been prepared to satisfy the need of the microorganism to be isolated as a pure culture. Based on the physical, chemical and special purposes, media are classified and are used to identify a particular organism from a clinical specimen or environment. In Microbiology there are many methods used for isolation of microorganisms. The methods commonly used for isolation are pour plate, spread plate method and streak plate method.

The growth of organisms on media is a basic criteria in the isolation, identification, and classification of microorganisms. Colony characterization of both bacteria and fungi highly depends upon the nutrients, temperature and pH.

Evaluation

Multiple choice questions

1. In a culture, the desired organism is low in number when compared with unwanted microorganism. Which media can be used to isolate the desired organism?
   a. Selective media
   b. Enriched media
   c. Basal media
   d. General purpose media
2. _____ is an example for differential media
   a. Blood agar
   b. EMB agar
   c. Both a and b
   d. None
3. A medium in which precise ingredients are clearly defined.
   a. Synthetic medium.
   b. Non synthetic medium.
   c. Complex medium
   d. Natural medium
4. A microbial inoculum of faecal specimen is subjected to isolation of typhoid bacilli species. Which medium can be used to select the bacilli?
   a. Selective medium
   b. Basal medium
   c. Enriched medium
   d. Differential medium
5. _____ is the method in which inoculum is not placed over the surface of agar plate.
   a. Pour plate method
   b. Spread plate method
   c. Streak plate method
   d. All the above
6. In perfect isolation of pure colonies, which method will be the most successful one?
   a. Pour plate method
   b. Pour and spread plate method
   c. Streak plate method
   d. All the above

7. Name the method in which the inoculums is mixed with the molten agar medium in the test tube and poured into the sterile petridish
   a. Pour plate method
   b. Spread plate method
   c. Streak plate
   d. All the above
8. The culture with only one type of organism in the colony is called _____
   a. Pure culture
   b. Mixed culture
   c. Semi mixed culture
   d. Contaminated culture
9. Identify the reason for the meager growth of aerobic colonies in pour plate isolation method
   a. Less oxygen availability
   b. More oxygen availability
   c. Carbon-di-oxide availability
   d. None of the above
10. If a microbial inoculum is with more contaminations, which method will be used for isolation?
    a. Spread plate method
    b. Pour plate method
    c. Streak plate method
    d. All the above
11. The plate has a culture of A and B with definite circular morphology If A is producing an inhibitory substance towards B, what will happen to the colony morphology of B?
    a. Change in the colony pattern of A
    b. Change in the colony pattern of a B
    c. Change in the colony pattern of both A and B
    d. No change
12. If a plate observing for colony morphology is subjected to contamination, what will happen to the colony?
    a. Growth will be clear
    b. Growth will not be clear
    c. Growth will be either clear or disturbed.
    d. None of the above
13. If agar seeded plates exposed to atmosphere are incubated at room temperature, which colony morphology will be predominantly present in the plate?
    a. Bacteria
    b. Fungi
    c. Virus
    d. None of the above
14. If chromogenic bacteria produce intracellular water insoluble pigment, it will stain _____
    a. Growth of a colony
    b. Agar medium
    c. Both a and b
    d. None of the above
15. If the water soluble pigment of the pigmented bacteria diffuses into the medium, _____
    a. Medium gets pigmented
    b. Colony get stained
    c. Both a and b
    d. None of the above

Answer the following

1. Define semisolid media with an example.
2. State basal media with an example.
3. What is synthetic medium? Give suitable example.
4. State a few aspects of enrichment medium.
5. State 3 fungal media used for isolation of fungi.
6. Define pure culture.
7. How do you differentiate pure culture from mixed culture?
8. Why are the colonies growth on surface in pour plate method are quite larger than those within the medium?
9. Why is it important to invert the petridish during incubation?
10. State the various forms in the appearance of the colony. Name the pigments produced by *Pseudomonas aeroginosa.*
11. Colony characteristics will be studied and identified clearly by using the nutrient agar medium in agar rather than agar slant. Why?
12. Write about the elevation of the bacterial colony?
13. Explain streak plate/pour plate/spread plate method.
14. Why is agar mainly used as a solidifying agent even though other solidifying agents are available?
15. How do you differentiate enrichment medium from selective medium?
16. Give a list of pigment producing bacteria.
17. Explain the opacity of a bacterial colony.
18. Explain the special purpose media in detail (any 5).
19. Why should we use a streak plate to grow a bacterium rather than on agar medium slant or in broth medium?
20. Expain the colony morphology of bacteria with diagrams.

Student Activity

1. The student will list out the substances which contain agar in their routine life and the role of agar in it.
2. Students will prepare chart/scrap book containing pictures of different types of media and colony types of bacteria and fungi.
3. Collect decayed/spoilt food for macroscopic observation.

Chapter 6

Microbial Nutrition and Growth

EPAHX4

Chapter Outline

Mold is a type of fungi that grows on food and other organic matters. It breaks down the complex substances into simpler ones and extracts nutrient for its growth from them.

Learning Objectives

After studying this chapter the student will be able,

- *To know the essential nutrients required by bacterial cell.*
- *To differentiate between macronutrients and micronutrients.*
- *To describe an organism based on the sources of carbon and energy.*
- *To compare the photosynthesis process in plant, algae and bacteria.*
- *To understand the phases of growth in bacterial growth curve.*
- *To know the methods of counting bacteria.*

6.1 Microbial Nutrition

All living organisms on this planet require energy for the normal functioning, growth and reproduction. Likewise, microorganisms acquire energy from various organic and inorganic compounds, light and CO_2. The requirement of energy depends on their need and metabolic ability.

6.2 Nutrient Requirement of Microorganisms

Microorganisms requires macronutrients, micronutrients and growth factors, for their growth. These nutrients help in constructing the cellular components like proteins, nucleic acids and lipids.

Macronutrients

Elements that are required in large amounts are called macronutrients. Nitrogen (N), Carbon (C), Oxygen (O), Hydrogen (H), Sulphur (S) and Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) and Iron (Fe) are macroelements.

Nitrogen is needed for the synthesis of amino acids, nucleotides like purines and pyrimidines which are part of nucleic acids (DNA and RNA).

Phosphorus is a part of phospholipids, nucleotides like ATP and phosphodiester bonds of nucleic acids.

Carbon, Hydrogen and Oxygen are the backbone of all organic macromolecules like peptidoglycan, proteins and lipids and nucleic acids.

Sulphur is needed for the synthesis of thiamin, biotin, and aminoacids like cysteine and methionine.

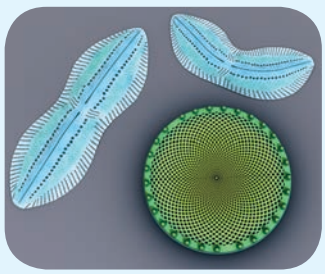
Potassium, Calcium, Magnesium and Iron exist as cations in the cell. These element plays vital role in the metabolic activity of microorganisms. Potassium (K^+) is needed for the activity of many enzymes Example: Pyruvate Kinase.

Calcium (Ca^{2+}) is involved in the heat resistance of bacterial endospores.

Magnesium (Mg^{2+}) binds with ATP and serves as a cofactor of enzymes like hexokinase.

Iron (Fe^{2+} or Fe^{3+}) is present in cytochromes and act as cofactors for cytochrome oxidase, catalase and peroxidase.

Diatoms (A group of algae) need silicon to construct their beautiful cell walls.



Micronutrients

Nutrients that are needed in trace quantities are called micronutrients. Example: Zinc (Zn), Molybdenum (Mo), Cobalt (Co), Manganese (Mn).

Besides macro and micronutrients, some microorganisms need growth factors like amino acids, purines and pyrimidines and vitamins. Example: Biotin is required by *Leuconostoc* sp and folic acid is required by *Enterococcus faecalis*.

HOTS

Is there a microbe that can grow in a medium that contains only the following compounds in water: calcium carbonate, magnesium nitrate, ferrous chloride, zinc sulphate and glucose. Defend your answer.

6.3 Nutritional Types of Microorganisms

Microorganisms can be classified into nutritional classes based on how they satisfy the requirements of carbon, energy and electrons for their growth and nutrition.

Based on the carbon source, microorganisms are able to utilize, they are classified into Autotrophs and Heterotrophs.

Autotrophs: These are organisms that utilize CO_2 as their sole source of carbon.

Heterotrophs: These are organisms that use preformed organic substances from other organisms as their carbon source.

Based on energy source, microorganisms are classified into Phototrophs and Chemotrophs.

Phototrophs: These are organisms that utilize light (radiant energy) as their energy source.

Chemotrophs: These are organisms that obtain energy by oxidation of organic or inorganic compounds.

Microorganisms are classified into **Lithotrophs** and **Organotrophs** based on the source from which they extract electrons. Lithotrophs are organisms that use reduced inorganic substances as their electron source whereas Organotrophs obtain electrons from organic compounds (Table 6.1).

All microorganisms fall into any one of the four nutritional classes based on their primary source of carbon, energy and electrons.

1. **Photoautotrophs:** Eukaryotic algae, Cyanobacteria (Blue Green Algae) (Figure 6.1) and Purple and Green Sulphur bacteria belong to this class. They are capable of using light energy and have carbondioxide as the sole source of carbon.

Figure 6.1: Microscopic view of *Cyanobacteria*

2. **Photoheterotrophs:** These organisms make use of light as energy source and organic compounds as electron and carbon source. Example: Purple and Green Non sulphur bacteria
3. **Chemoautotrophs:** These are ecologically important microorganisms. They oxidize inorganic compounds like nitrate, iron and sulphur to obtain energy and electrons.
4. **Chemoheterotrophs:** These organisms use organic compounds to satisfy their needs of energy, electron and carbon. (Table 6.2)

Table 6.1: Classification of microorganism based on carbon, energy and electron sources

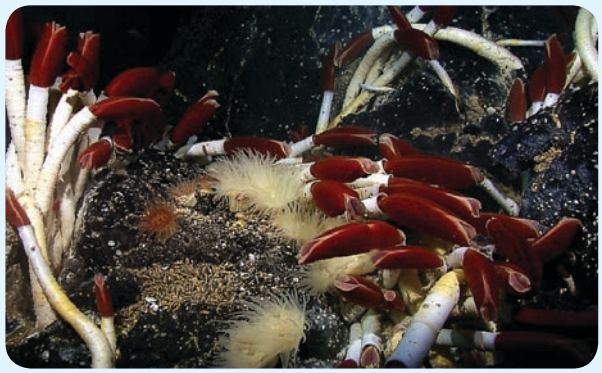
Carbon, Energy and Electron sources	
Carbon sources	
Autotrophs	CO_2 as sole carbon source
Heterotrophs	Organic substances from other organisms
Energy sources	
Phototroph	Light energy
Chemotrophs	Chemical energy source (Organic or Inorganic)
Electron sources	
Lithotrophs	Reduced inorganic substances
Organotrophs	Organic compounds

a. Blood lake in Texas-the blood red colour is due to the excess presence of purple sulphur bacteria.

b. Giant tube worms seen in deep sea hydrothermal vents survive on nutrients given by chemolithotrophic bacteria.

a)

b)



6.4 Photosynthesis

Photosynthesis is a process of capturing light energy and converting into chemical energy. The chemical energy produced in the form of ATP and NADPH is used to synthesise organic compounds (carbohydrates); to be used as food. This ability makes photosynthesis, a significant process taking place on earth.

EPJSYR

Eukaryotes (plants and algae) and prokaryotes (cyanobacteria and purple, green bacteria) are capable of carrying out photosynthesis. Cyanobacteria perform photosynthesis in a similar manner to plants.

Process of photosynthesis in cyanobacteria

The process of Photosynthesis is divided into

Table 6.2: Nutritional classes of Microorganisms

Nutritional class	Energy/Electron/Carbon source	Organisms
Photoautotrophs	Light energy Inorganic e^- donor CO_2	Cyanobacteria, Purple and Green sulphur Bacteria
Photoheterotrophs	Light energy Organic e^- donor Organic carbon source	Purple and Green Nonsulfur bacteria
Chemoautotrophs	Inorganic chemical compounds as energy source Inorganic e^- donor CO_2	Nitrifying bacteria, Iron bacteria
Chemoheterotrophs	Organic compounds as energy, electron and carbon source.	Most pathogenic bacteria, fungi and protozoa.

1. Light reaction
2. Dark reaction

In light reaction, light energy is captured by photosynthetic pigments and converted into chemical energy (ATP and NADPH).

In dark reaction, the chemical energy is used to fix CO_2 to construct organic compounds (carbohydrates); to be used as food. This reaction is also called CO_2 fixation or Calvin'scycle.

Light reactions in cyanobacteria

- **Photosynthetic pigments**

All photosynthetic organisms contain pigments to observe light. Chlorophyll is the main pigment involved in the absorption of light. In cyanobacteria, chlorophyll *a* is the predominant pigment present and it absorbs red light at a wavelength of 665nm. Cyanobacteria also contain accessory pigments like phycobiliprotein (Phycoerythrin and phycocyanin) which help to absorb light at a broader wavelength (470-630nm) and makes photosynthesis more efficient. These pigments are located in the cytoplasmic membrane of cyanobacteria.

- **Photosystems**

The pigment molecules are arranged in highly organized arrays called reaction center or Photosystems. Cyanobacteria and green plants have two photosystems namely Photosystem I (P700) and Photosystem II (P680) through which the electrons, excited by the capture of photons, flow.

When P700 (Photosystem I) absorbs energy and gets excited, the excited electron is transferred through a series of proteins like ferrodoxins and are eventually used to reduce $NADP^+$ to produce $NADPH^+$. (When a compound accepts electrons, it is said to be reduced). When P680 absorbs light and gets excited, the electron passes through pheophytin, plastaquinone, cytochromes and plastacyanin to be donated to the oxidised P700 in order to replenish the lost electron. It is during this process, ATP (Adenosine Tri phosphate) is generated (Figure 6.2). Oxidised P680 obtains electron when water is split (Photolysis) into oxygen atoms ($1/2\ O_2$) and hydrogen ions ($2H^+$) which results in evolution of oxygen. Hence it is called oxygenic photosynthesis.

$$CO_2 + H_2O \xrightarrow{\text{Light}} (CH_2O)_n + O_2$$

This process of electron flow where two photosystems are involved in the generation of ATP is called non cyclic photophosphorylation.

Photosynthesis in Bacteria

There are four groups of photosynthetic bacteria. They are green sulphur bacteria (Example: *Chlorobium*) and green non sulphur bacteria (Example: *Chloroflexus*) purple sulphur bacteria (Example: *Chromatium*) and purple non sulphur bacteria (Example: *Rhodospirillum*). These photosynthetic bacteria can fix atmospheric CO_2 in a similar fashion like cyanobacteria but using only one photosystem and using H_2S as the electron donor instead of H_2O.

Process of photosynthesis in bacteria

The electron transport system in purple and green bacteria consists of only one Photosystem PSI (P870). They do not possess photosystem II. When P870 gets excited upon capture of light energy, it donates the electron to bacteriopheophytin. Electrons flow through quinones and cytochromes and are reverted back to P870. This process is cyclic (since the electron

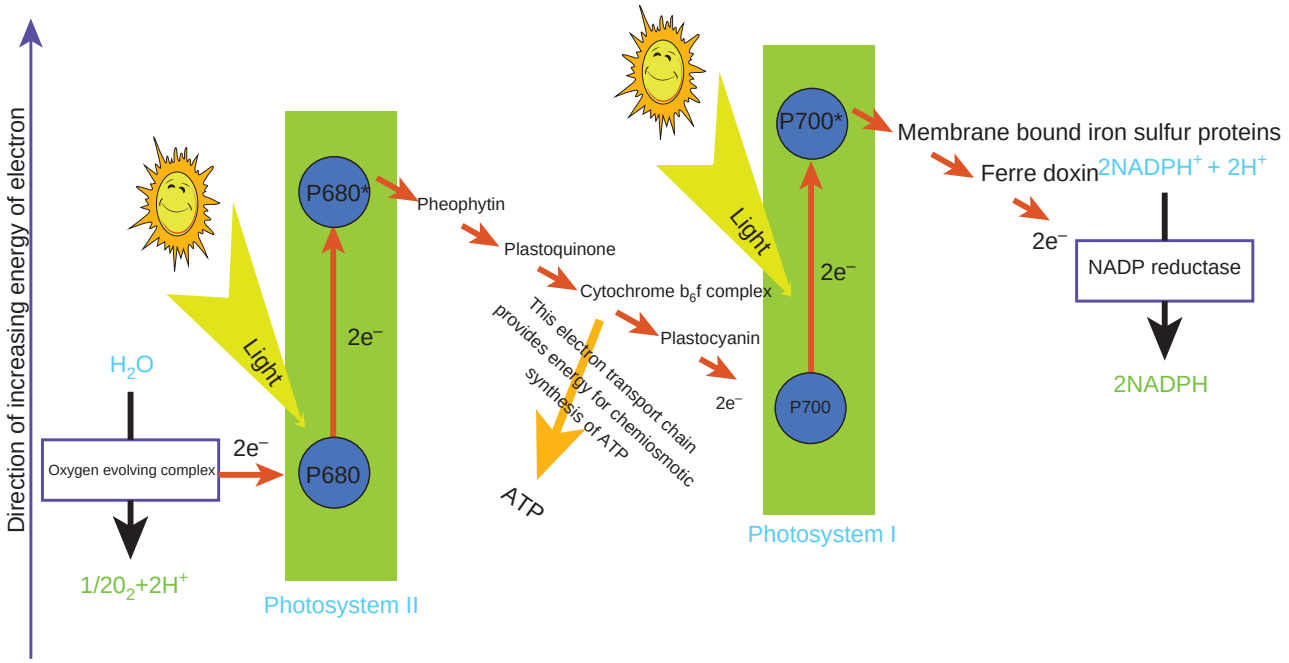


Figure 6.2: Simplified scheme of the light reactions of photosynthesis

excited from P870 comes back to P870) and generates ATP. A reversed electron flow operates in purple bacteria to reduce NAD^+ to NADH. Electrons are extracted from external electron donors like hydrogen sulphide, hydrogen, elemental sulphur and organic compounds to synthesise NADH. Since H_2O is not used as electron donor, oxygen is not evolved which explains the anoxygenic nature of the organisms involved (Figure 6.3). The sulphur evolved during this reaction is deposited as sulphur globules either outside or inside the cells. $CO_2 + H_2S \rightarrow (CH_2O)_n + S$. Table 6.3 compares the photosystheic process in plants, algae and bacteria.

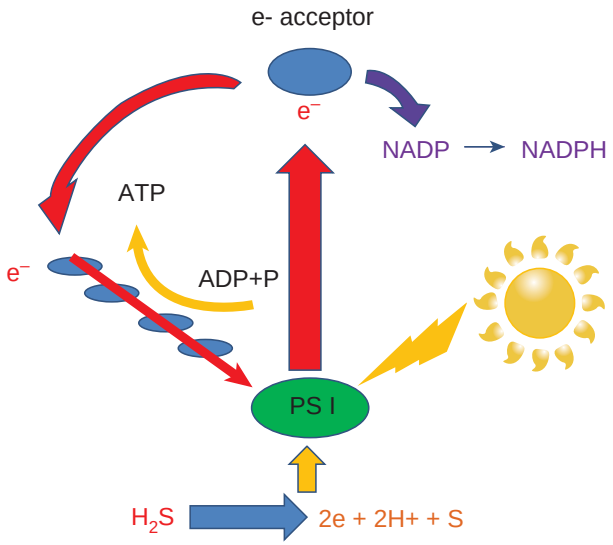


Figure 6.3: Green sulphur and purple sulphur bacteria that use sulphides as electron donors

HOTS

1. What will be the electron flow sequence of noncyclic and cyclic photo phosphorylation?
2. Chemical energy produced in photosynthesis is either ATP NADPH or ATP NADH. Why?

6.5 Microbial Growth

In bacteria, growth can be defined as an increase in cellular constituents. Growth results in increase of cell number.

When bacteria are cultivated in liquid medium and are grown as batch culture (Growth occurring in a single batch of medium with no fresh medium provided), cell multiplication happens till all the

Table 6.3: Photosynthetic process in plants, algae and bacteria

	Green plants and algae	Cyanobacteria	Purple and Green bacteria
Primary pigment	Chlorophyll a and b	Chlorophyll a	Bacteriochlorophyll
Accessory pigments	Carotenoids	Phycocyanin	-
Photosystem	Both Photosystem I and II present	Both Photosystem I and II present	Only Photosystem I present
Electron donors	H_2O	H_2O	H_2S
Oxygen evolution	O_2 is evolved Oxygenic	O_2 is evolved Oxygenic	S is evolved Non Oxygenic

nutrients are exhausted. After sometime, nutrient concentrations decline and bacterial cells begin to die. This growth pattern can be plotted in a graph as the logarithm of viable cells versus incubation time (Figure 6.4). The growth curve has four distinct phases.

1. Lag phase
2. Logrithmic phase/Exponential phase
3. Stationary phase
4. Death phase

1. Lag Phase

When bacteria are introduced into fresh medium, no immediate cell multiplication and increase in cell numbers occur. The cell prepares itself for cell division by synthesizing cell components and increase in cell mass. Since there is a lag in cell division, this phase is called lag phase.

2. Logrithmic Phase/Exponential Phase

During this phase, microorganisms rapidly divide and grow at a maximal rate possible utilizing all the nutrients present in the medium. The growth rate is constant during the exponential phase. The organism divides and doubles in number at regular intervals. The growth curve rises smoothly

3. Stationary Phase

As the nutrients get depleted, the cell growth stops and the growth curve become horizontal. The total number of viable cells remains constant which is due to a balance between cell division and cell death.

4. Death Phase

Nutrient deprivation and build up of wastes lead to the decline in cell numbers. The microbial population dies rapidly and logarithmically and the growth curve also stops down.

Batch culture

It is the growth of microorganisms in a fixed volume of culture medium in which nutrient supply is not renewed and wastes are not removed. It is a closed system. This can be used to study the various growth phases of microorganisms.

Continuous culture

A continuous culture is an open system with constant volume to which fresh

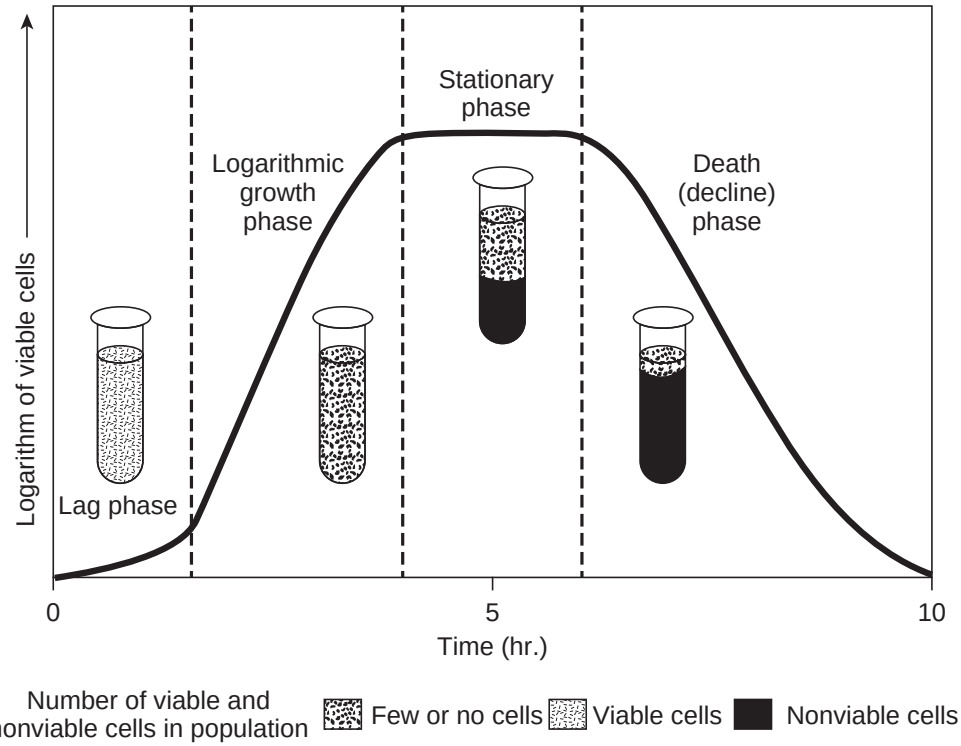


Figure 6.4: Bacterial growth curve showing phases of growth in laboratory conditions

medium is added and utilized (spent) medium are removed continuously at a constant rate. A microbial culture remains in exponential state for longer periods, for days and even weeks. This enables the researcher to learn about the physiological processes and enzymatic activities of organisms.

There are two ways by which continuous culture is operated.

a) Chemostat

b) Turbidostat

Chemostat

The chemostat operates so that the sterile nutrient medium enters the culture vessel at the same rate as the spent medium is removed. The chemostat can control growth rate and cell density simultaneously and independently of each other. Two factors play an important role in achieving this dilution rate and concentration of the limiting nutrient (a carbon or a nitrogen source like sugars or aminoacids). Growth rate can be controlled by adjusting the dilution rate and cell density is controlled by modifying the concentration of the limiting nutrient (Figure 6.5).

Turbidostat

This type of continuous culture system has a photocell that measures the turbidity of the culture vessel. This automatically regulates the flow rate of the culture medium. Turbidostat does not contain limiting nutrients (Figure 6.6).

6.5.1 Factors Influencing Growth

The growth and activities of microorganisms are greatly influenced by the physical and chemical conditions of their environment. Among all factors, four key factors play major roles in controlling the growth of microorganisms. They are

1. Temperature
2. pH

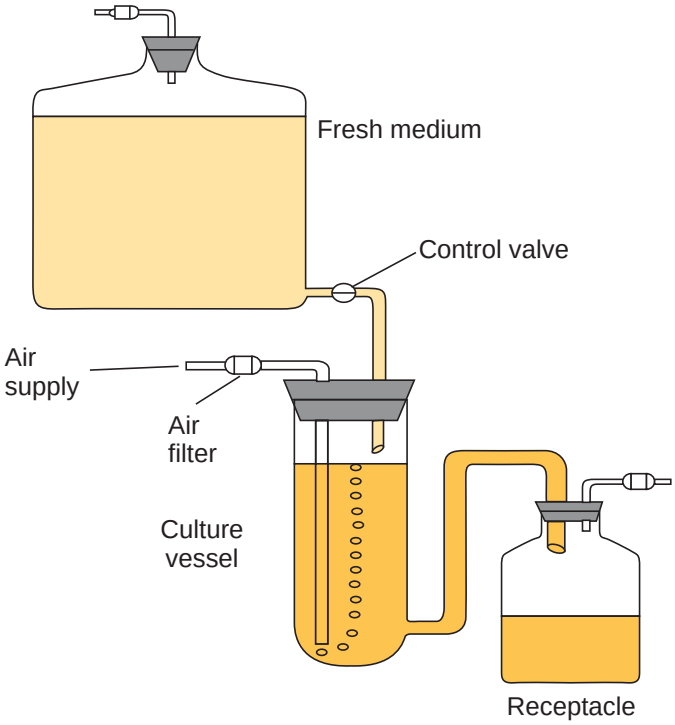


Figure 6.5: The Chemostat

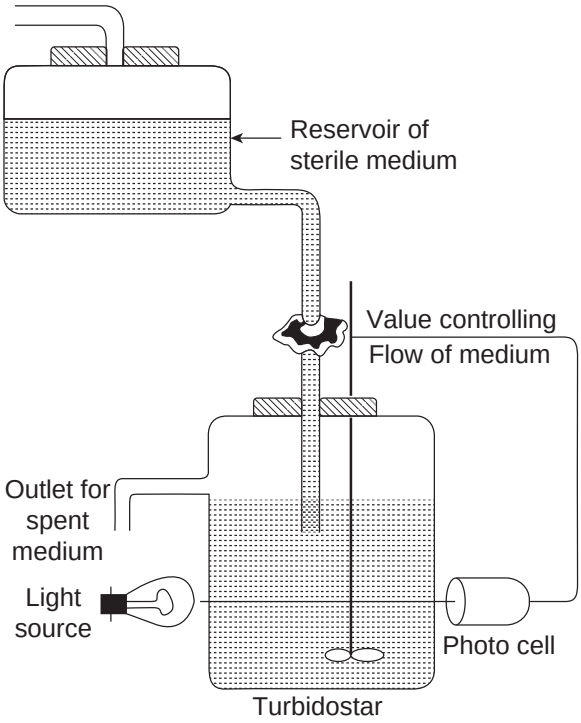


Figure 6.6: The Turbidostat

3. Water activity
4. Oxygen

1. Temperature

Temperature is one of the most important environmental factor affecting the growth and survival of microorganisms. Temperature can affect microorganisms because the enzyme catalysed reactions are sensitive to fluctuations in temperature.

For every microorganism, there is a minimum temperature below which no growth occurs, an optimum temperature at which growth is most rapid, and a maximum temperature above which no growth occur. These three temperatures are called cardinal temperatures.

Temperature classes of microorganisms

Microorganisms are broadly distinguished into four groups in relation to their temperature optima.

- Psychrophiles
- Mesophiles
- Thermophiles
- Hyperthermophiles

Psychrophiles

A psychrophile can be defined as an organism with an optimal growth temperature of 15°C, maximum growth temperature of 20°C and a minimum growth temperature at 0°C. These organisms are found in polar regions like Arctic and Antarctic oceans. They are rapidly killed as the temperature rises because the cellular constituents start to leak due to cell membrane disruption. Some examples of psychrophiles are *Moritella, Photobacterium* and *Pseudomonas.*

Psychrotolerant

Organisms that can grow at 0°C, but have temperature optimum growth temperature range of 20°C-40°C are called psychrotolerant.

Mesophiles

These are microorganisms that grow in optimum temperature between 20-45°C,

Snow alga – *Chlamydomonas nivalis* grows within the snow and its brilliant red coloured spores are responsible for the formation of pink snow.

HOTS

Why do unopened pasteurized milk spoil even under refrigeration?

they have a temperature minimum of 15-20°C and a maximum temperature of 45°C. All human pathogens are mesophiles.

Thermophiles

Organisms whose growth temperature optimum is between 55-65°C are called thermophiles. They have minimum growth temperature of 45°C. These organisms are found in compost stacks, hot water lines and hot springs. They contain enzymes that are heat stable and protein synthesis systems function well at high temperature.

Hyperthermophiles

Organisms whose growth optimum temperature is above 80°C are called hyperthermophiles. These are mostly bacteria and archaebacteria. They are found in boiling hot springs and hydrothermal vents on seafloor.

Infobits

Taq polymerase, a DNA polymerase enzyme which is of great applied importance used in DNA amplification. It is isolated from *Thermus aquaticus*, a thermophile.

2. pH

pH is defined as the negative logarithm of the hydrogen ion concentration. pH scale extends from pH 0.0 to pH 14.0 and each exchange of 1 pH unit represents a 10 fold change in hydrogen ion concentration. pH greatly influences microbial growth. Each organism has a definite pH range and well defined pH growth optimum. Most natural environments have pH values between 5 and 9.

Organisms are classified into Acidophiles, Neutrophiles and Alkalophiles based on their optimum growth pH.

Acidophiles are organisms that grow best at low pH (0.0–5.5) Example: Most fungi, bacteria like *Acidithiobacillus*, Archaebacteria like *Sulfolobus* and *Thermoplasma*.

Neutrophiles are organisms that grow well at an optimum pH between 5.5 and 8.0. Most bacteria and protozoa are neutrophiles.

Organisms that prefer to grow at pH between 8.5-11.5 are called alkalophiles. These microorganisms are typically found in soda lakes and high carbonate soils. Example: *Bacillus firmus.*

3. Water Activity and Osmosis

Water activity, (a_w) is the ratio of vapour pressure of the solution to the vapour pressure of pure water (a_w values vary between 0 and 1). Water activity is inversely related to osmotic pressure. Organisms that can grow in low a_w values are called osmotolerant. Example: *Staphylococcus*

Crenation:

Shriveling of cytoplasm in the cell is called crenation. This effect helps to preserve some foods.

aureus.

Only a few organisms are capable of tolerating high salt concentration and still growing optimally in low water activity. Such organisms are called halophiles. Halophiles can grow in 1-15% Sodium chloride (NaCl) concentrations. Organisms that can grow in very salty environments are called extreme halophiles. (They can grow in 15-30%) NaCl concentration. Example: *Halobacterium*.

4. Oxygen

Most of the microorganisms require oxygen for their optimal growth but some of them survive very well in total absence of oxygen and are killed when exposed to air.

Based on their need and tolerance for oxygen, microorganisms are classified into the following types.

(1) **Obligate aerobes** exhibit growth only at full oxygen level (21% O_2 on air) because O_2 is needed for their respiration and metabolic activities Example: *Micrococcus*, most Algae, Fungi and Protozoa.

(2) **Microaerophiles** are aerobes that require oxygen at levels lower than that of air. Example: *Azospirillum, Campylobacter, Treponema*

(3) Obligate anaerobes does not require oxygen for their respiration and growth. This group cannot tolerate O_2 and are killed in its presence. Example: Methanogens, *Clostridium*.

(4) **Aerotolerant anaerobes** can grow in the presence of oxygen though O_2 is not required for their growth. Example: *Streptococcus pyogenes*.

(5). **Facultative anaerobes** can grow either under oxic or anoxic conditions: Example: *Escherichia coli*. (Figure 6.7)

6.6 Measurement of Growth

Different methods are employed for measuring the cell growth of microorganisms. Cell growth is indicated by increase in the number of cells or increase in weight of cell mass. There are direct and indirect methods of measuring microbial growth.

1. Direct Measurements

Total count and viable count are the two methods widely employed to count cell numbers.

Total count:

The total number of cells in a population can be measured by counting a sample under the microscope. This is called direct microscopic count. This is done by using a specialized counting chamber called Petroff Hausser chamber which is a specially designed slide with a grid. The liquid sample is placed on the grid which has a total area of 1mm^2 and divided into 25 large squares. The number of cells in large square is counted and the total number of cells is calculated by multiplying it with a conversion factor based on the volume of the chamber (Figure 6.8).

Advantages

This is a quick method of estimating cell numbers.

Disadvantages

1. Dead cells are also counted
2. Special microscopes like phase contrast microscope are needed if unstained samples are used.
3. Small cells are difficult to count

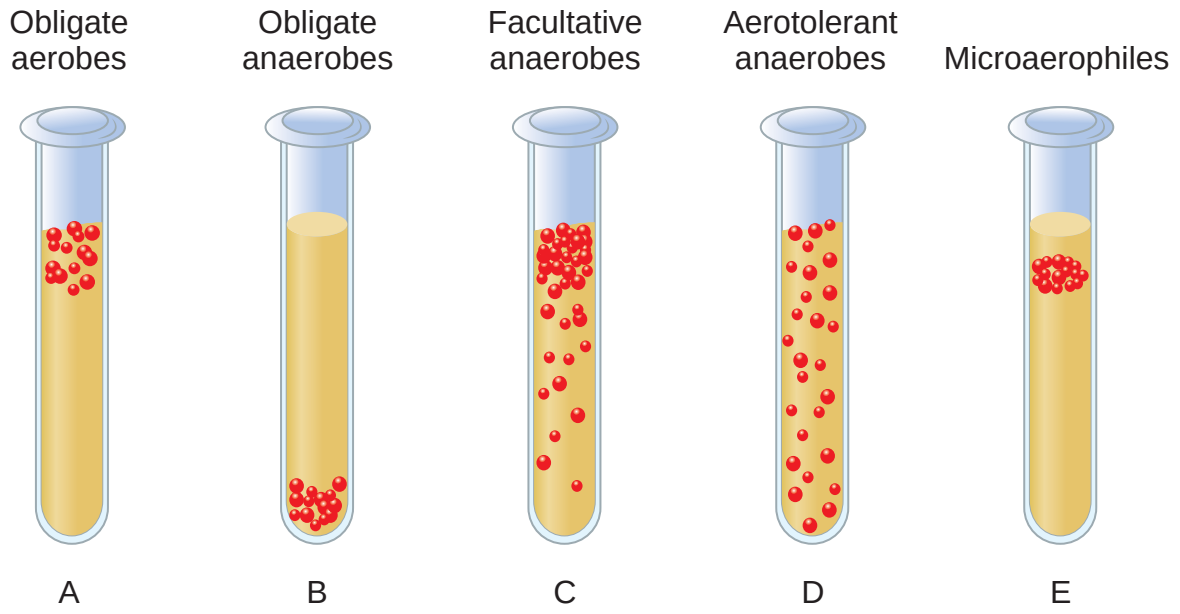


Figure 6.7: The effect of oxygen on the growth of various types of bacteria

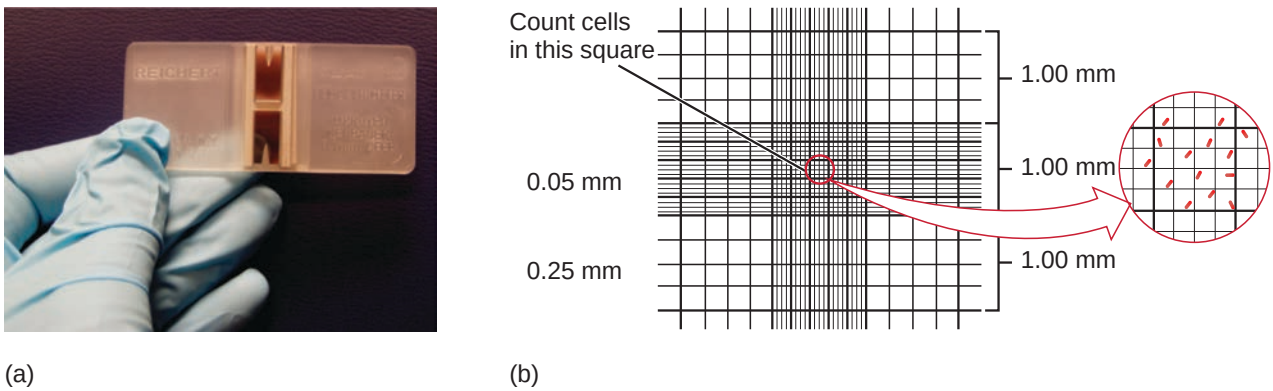


Figure 6.8: (a) Petroff-Hausser counting chamber
(b) Microscopic observation of bacterial cells

2. Viable Count

A viable cell is one that is able to divide and form a visible colony on the nutrient media. Viable cells are counted by methods pour plate and spread plate.

Pour plate method

In this method, a known volume (0.1 or 1.0ml) of the culture is pipetted into a sterile petri plate, then molten nutrient medium is poured over and incubated. Colonies will appear throughout the agar medium and are counted to obtain viable count.

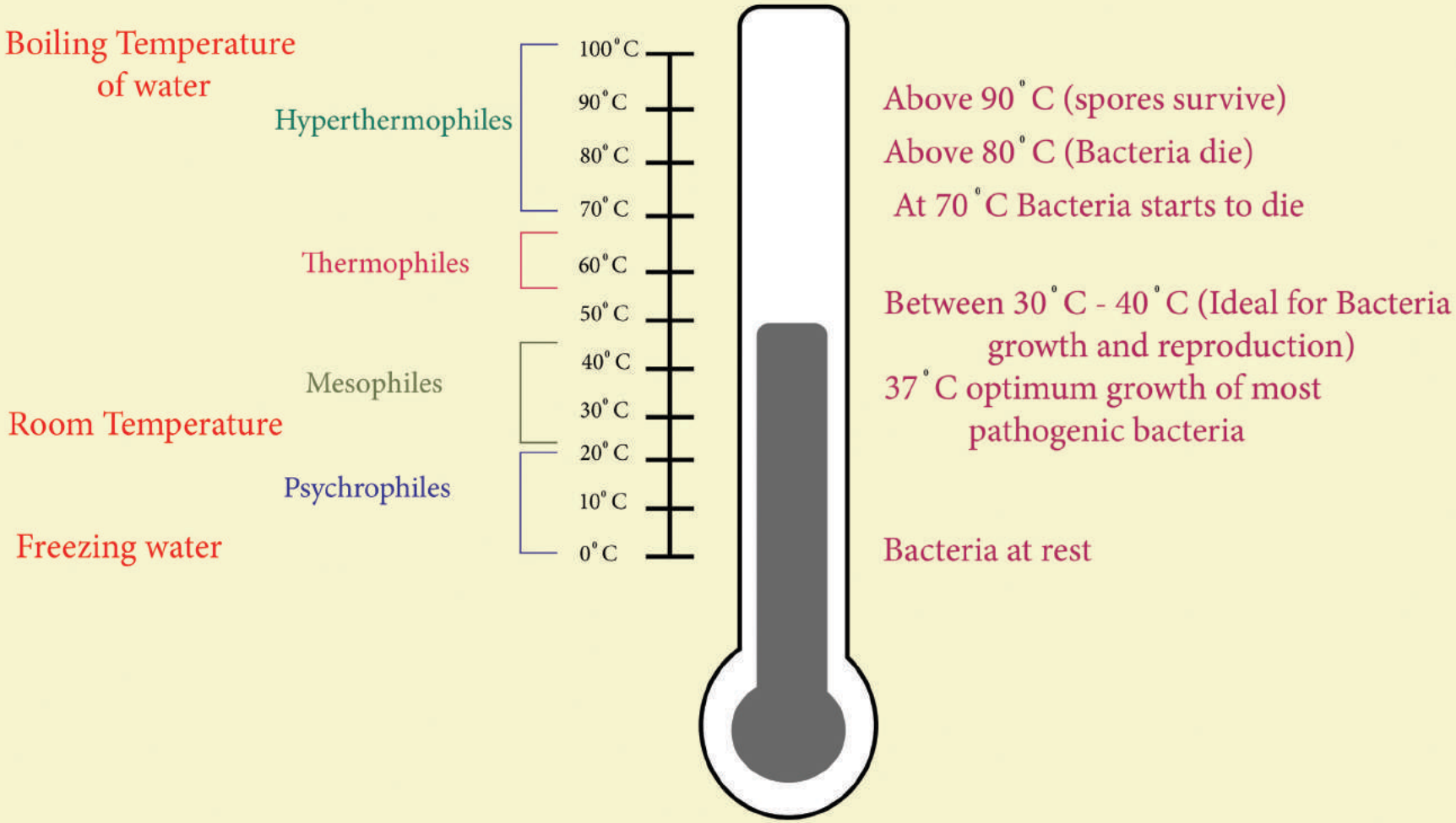
Spread plate method

In this method, a known volume of the culture (0.1ml) is plated and spread over solidified sterile agar medium, using a sterile spreader. The total number of colonies appearing on the plate after incubation represents the total number of viable cells in the culture.

3. Measurement of Cell Mass

A cell suspension appears turbid or cloudy due to active cell growth. When light is passed through this cell suspension, microbial cells scatter light striking them. As the concentration of cells and turbidity increases, more light is scattered and less light is transmitted through the suspension. The amount of unscattered light can be measured using a spectrophotometer, the values of which are indirectly related to cell numbers.

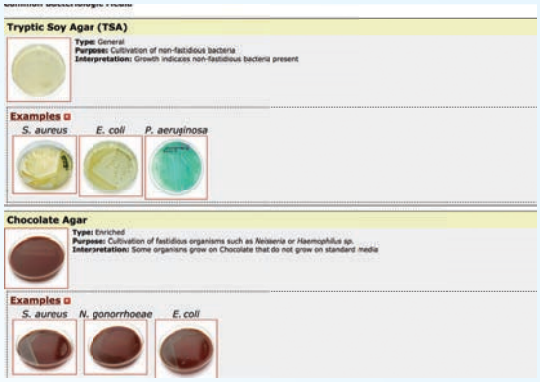
Growth of Bacteria Under Varing Temperature



ICT CORNER

Culture Media

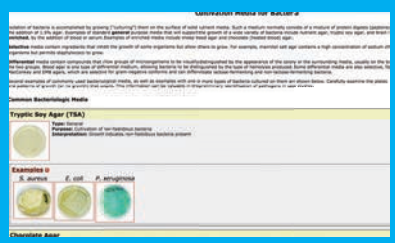
Preparation of Bacteriological Media



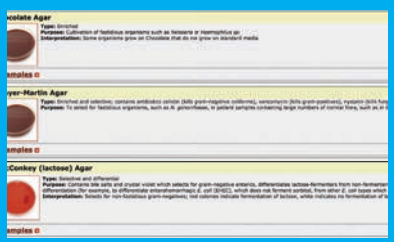
STEPS:

- Use the URL or scan the QR code to reach 'Virtual Interactive Bacterial Laboratory'.
- Click module at the bottom and read the description and steps.
- Follow the steps and open activities under' Common Bacteriologic Media' one by one and explore it.
- Record your observation of Differential Media. Click examples and record the specimen suitable for particular media

Step1



Step2



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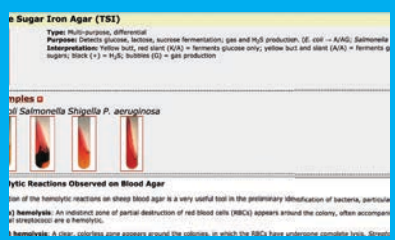

Step4

URL:

http://learn.chm.msu.edu/vibl/content/differential.html

B177_11_MBIO_EM

Summary

Microorganisms need macro and micronutrients for their growth. Based on the energy source, organisms are grouped into Phototrophs and Chemotrophs. Based on carbon source, they are classified into autotrophs and heterotrophs. Organisms are grouped into lithotrophs and organotrophs based on their electron source. The four nutritional classes of microbes are photoautotrophs, Photoheterotrophs, chemoautotrophs and chemoheterotrophs.

Cyanobacteria are prokaryotes that can perform photosynthesis. Chlorophyll is the pigment needed to capture light energy (photons). In cyanobacteria and green plants, non cyclic photophosphorylation takes place to generate ATP and NADPH during photosynthesis whereas cyclic photophosphorylation takes place in purple and green bacteria involving only one Photosystem (PS I).

In a batch culture, bacteria show a characteristic growth pattern which consists of lag phase, log phase and stationary phase and decline phase. In a chemostat, cultures can be maintained in an exponential phase for long periods. The most important factors affecting microbial growth are temperature, pH and oxygen level. Total count and viable count are the two widely used methods to measure cell numbers.

Evaluation

Multiple choice questions

1. An example of photoautotroph
   a. Cyanobacteria
   b. Algae
   c. Green plants
   d. All of the above

2. Magnesium is needed
   a. For cell wall synthesis
   b. As cofactor for enzymes
   c. For photosynthesis
   d. For protein synthesis
3. One of the following is an example for chemoautotroph
   a. For cell wall synthesis
   b. As cofactor for enzymes
   c. For photosynthesis
   d. For protein synthesis
4. The phase of growth in which the growth rate is equal to the death rate is
   a. Stationary phase
   b. Death phase
   c. Exponential phase
   d. Lag phase
5. Organisms that are capable of growing in 0°C are called
   a. Thermophiles
   b. Hyper thermophiles
   c. Barophiles
   d. Psychrophiles
6. Halophiles are organisms that can grow in
   a. Low water activity
   b. High salt concentration
   c. Low temperature
   d. High pH
7. An example of microaerophilic organism is
   a. Bacillus *b. Azospirillum*
   c. Pseudomonas *d. Escherichia.coli*
8. The specialized chamber used for the counting of microbial cells is
   a. Haemocytometer
   b. Counting chamber
   c. Petroff Hauss chamber
   d. Counting slide

Answer the following

1. Give notes on the nutritional classes of microorganisms.
2. Classify microorganisms based on energy and carbon source.
3. What are light and dark reactions in photosynthesis?
4. What is bacteriochlorophyll? Give its role.
5. Define chemoautotroph.
6. Define photosynthesis.
7. Give examples of photosynthetic bacteria.
8. What do mean by cardinal temperature?
9. Give notes on photosynthetic pigments.
10. What are halophiles?
11. Give reason for the ability of thermophiles to grow in high temperatures.
12. How bacterial cells are counted using counting chamber?
13. Classify microorganisms based on their temperature requirement.
14. Describe the role of macro and micronutrients in microorganisms. How do you think bacteria acquire their nutrients from their environment?
15. Explain the classification of microbes based on their nutrition. If H_2S is toxic to living organisms, how do purple and green bacteria survive and use H_2S in such environments.
16. Describe the photosystems of cyanobacteria.
17. Draw a schematic representation of Z scheme of non cyclic photophosporylation.
18. Compare photosynthesis between plants, cyanobacteria and purple green bacteria.
19. Explain the principle and uses of chemostat and turbidostat with diagrams.
20. Describe the classification of microorganism based on their oxygen requirement.
21. Explain the principle and uses of chemostat and turbidostat with diagrams.
22. Explain the relation of osmosis to water activity.
23. Define growth. Explain the phases of growth of bacteria with neat diagram.

Student Activity

- Expose a container with water to sunlight for a week. Observe the growth of cyanobacteria on water which explains the photoautotrophic mode of nutrition.
- Store a loaf of bread for a week after the expiry date. You can observe the growth of fungi/molds which demonstrates the mode of nutrition of chemoheterotrophs.
- Collect rusted iron pipes which contain chemolithotrophic *Thiobacillus sp* which can oxidize iron for their nutrients.
- Place two bowls of cooked rice/vegetables–one inside the refrigerator at 6°C, and another at room temperature at 30-35°C. Give reasons for the quick spoilage of the rice stored at 30-35°C. Check the pH of milk using a pH paper.

Chapter 7

Morphology of Bacteria

Chapter Outline

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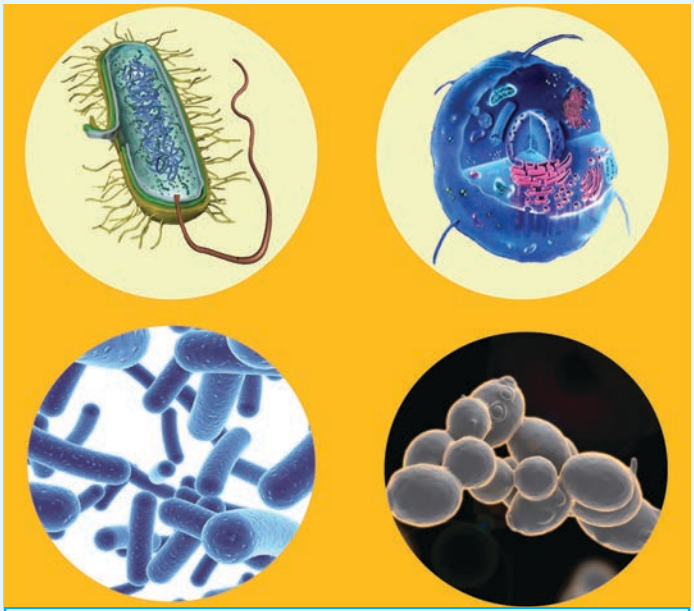


The distinction between **prokaryotes** and **eukaryotes** is considered to be the most important distinction among groups of organisms. Eukaryotic cells contain membrane bound organelles, such as mitochondria, while prokaryotic cells do not.

Learning Objectives

After studying this chapter the student will be able,

- *To know the size, shape and arrangement of bacteria.*
- *To list a few examples of bacteria with their shapes.*
- *To understand and describe the role of the structures external to the cell wall.*
- *To understand the structure, function and arrangement of bacterial flagella.*
- *To describe the role of capsule, slime layer, pili, flagella and fimbriae in a prokaryotic cell.*
- *To describe the structure and function of cell wall, outer membrane and cell membrane.*
- *To know the significance of Cell Envelope.*
- *To differentiate between Gram positive and Gram negative bacteria.*
- *To know the structures and functions internal to cell membrane.*
- *To differentiate between prokaryotic and eukaryotic cell structure.*

Living organisms are differentiated from non living matter by their (1) ability to reproduce (2) ability to ingest or assimilate food and metabolize them for energy and growth (3) ability to excrete waste products (4) ability to react to changes in their environment (irritability) and (5) susceptibility to mutation. The living organisms include a variety of micro and macro organisms of different size, shape, morphology and behaviour. They include tiny bacteria, protozoans, worms, plants and animals.

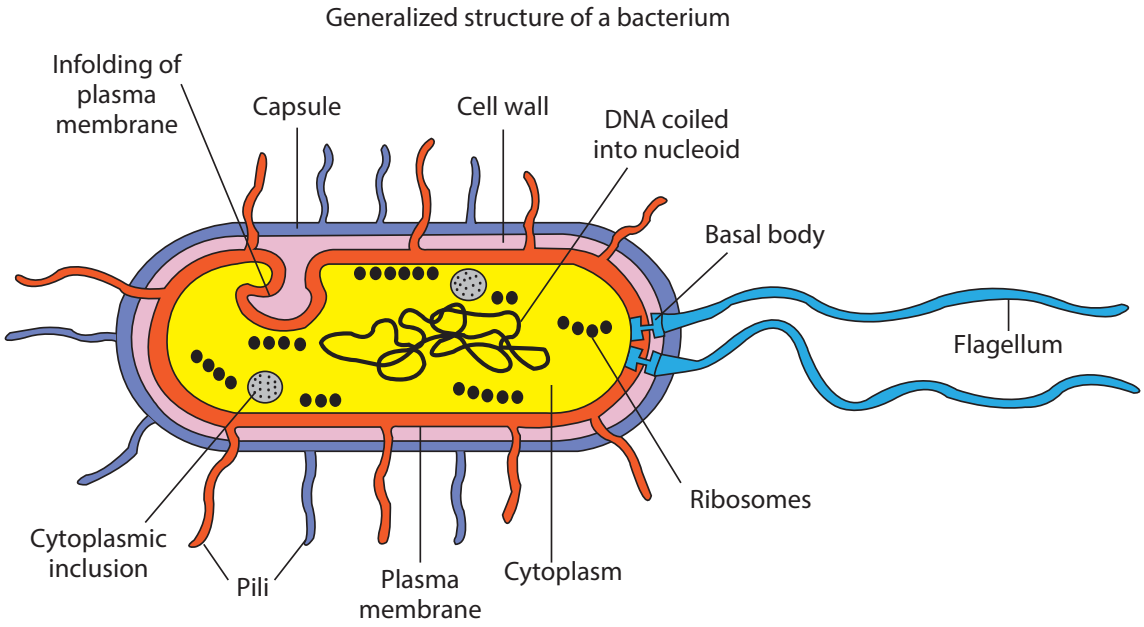


Figure 7.1: Generalized structure of a bacterium

Bacteria, cyanobacteria (blue green algae) microalgae, protozoa, yeasts and fungi represent the microorganisms. Prokaryotes are organisms with primitive type of nucleus lacking a well defined membrane (Figure 7.1). The nuclear material is a DNA molecule in prokaryotes compared to chromosomes of higher organisms. Eukaryotes are organisms with cells having true nuclei enclosed in a nuclear membrane and are structurally more complex than prokaryotes. There exists varying degree of localization of cellular functions in eukaryotes that occur in distinct membrane bound intracellular organelles like nuclei, mitochondria, chloroplasts. The cells of living organisms are either prokaryotic or eukaryotic in nature and there is not any intermediate condition. The size, shape, morphology and the internal cellular organizations are different in these two groups.

Satisfactory criteria to differentiate bacteria, fungi and algae could not be made until the development of electron microscope, which depicted the internal structure of these organisms. The absence of membrane bound internal structures in bacteria and their presence in fungi, algae, protozoa, plant and animal cells was taken as criterion to differentiate prokaryotes and eukaryotes.

7.1 Size, Shape and Arrangement of Bacteria

7.1.1 Size of Bacteria

Bacteria are minute living bodies and represent one of the lowest orders of living cells. The determination of size of the different forms is originally carried out by comparison with known RBC. A more accurate estimation is now obtained by the use of a special micrometer eye-piece, containing a graduated scale. The unit of measurement of bacteria is called micron (μ or μm). 1 micron is equal to 1 thousand of millimeter. Resolution of unaided eye is 200μm. The size of bacteria is constant but

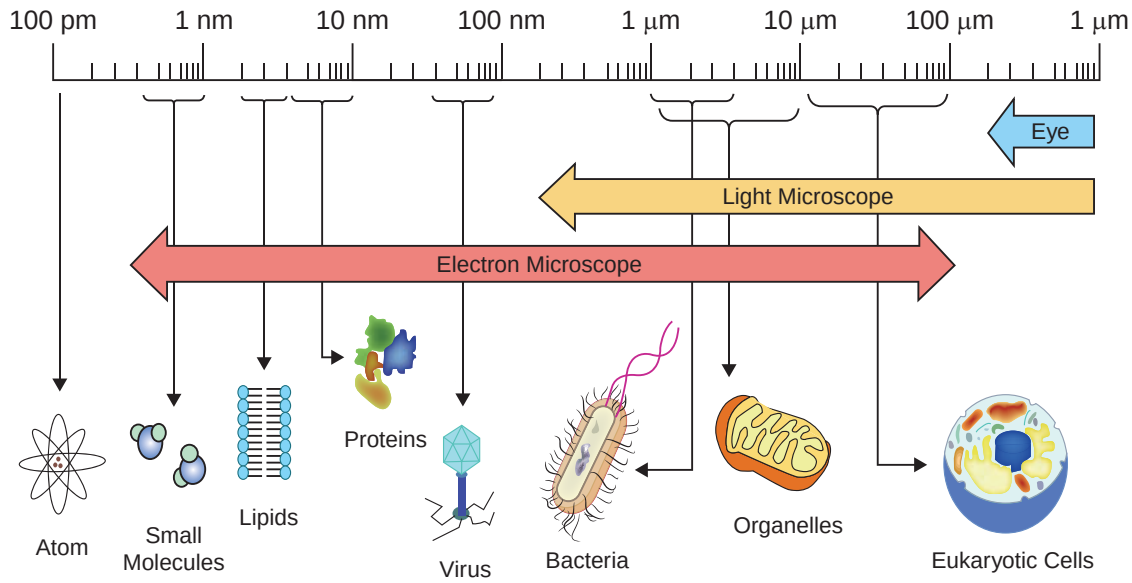


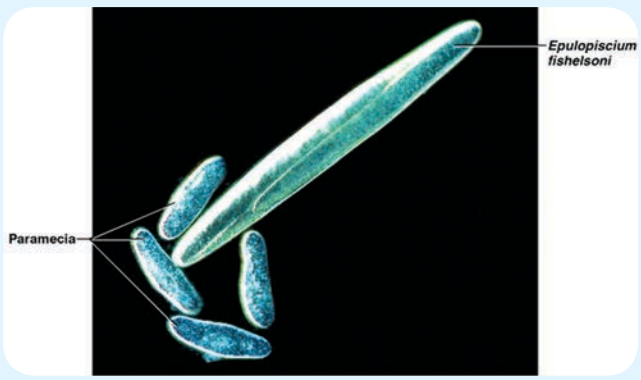
Figure 7.2: Metric unit of measurement

depends upon environmental and growth condition. Medically important bacteria ranges from 0.2 – 1.5 µm in diameter and 3-5µm in length (Figure 7.2).

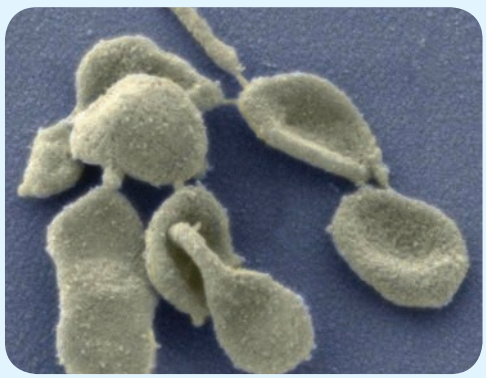
1 metre (m)	=	1000mm (millimeter)
1mm (10^{-3}m)	=	1000 µm (micrometer)
1 µm (10^{-6}m)	=	1000nm (nanometer)
1nm (10^{-9}m)	=	1000pm (picometer)
1A^0 (10^{-10}m) (angstrom)		

Infobits

The smallest bacteria is *Mycoplasma genitalium*, which has a diameter of 200-300nm. The largest and longest bacterium is *Thiomargarita namibiensis* (750µm) found in the ocean sediments in the continental shelf of Namibia. They are large enough to be visible to the naked eye. The previously known largest bacterial cell *Epulopiscium fishelsoni* is found only in the intestinal tract of certain topical fish over 500µm long. *Epulopiscium* means "guest at the table of fish".



Epulopiscium fishelsoni



Mycoplasma genitalium

7.1.2 Cell Shape and Arrangement of Bacteria

The shape of a bacterium is governed by its rigid cell wall. Typical bacterial cells are spherical (called cocci), straight rods (called bacilli) and helically curved rods (called spiral). These shapes are constant for the particular species or genus but there are bacterial cells that are pleomorphic in nature. They exhibit a variety of shapes.

- Cocci appear in several characteristic arrangements, depending on the plane of cellular division and whether daughter cells remain together with the parents even after cell division. The cells may occurs in pairs (diplococci), in groups of four (tetracocci), in clusters (*Staphylococcus*), in a bead like chain (*Streptococci*) or in cuboidal arrangement of cells (*Sarcinae*).
- Bacilli are rod shaped organism (Singular, bacillus = stick) usually ranging between 1 and 10 µm in length. Some bacilli are so short and stumpy that they appear ovoid and are referred to as coccobacilli. Bacilli are not arranged in patterns as complex as those of cocci and mostly occur as singles or in pairs (diplobacilli, Example: *Bacillus subtilis)* or in the form of chains (*Streptobacilli*). Some form trichomes, which are similar to chains. In other *Bacilli* such as *Corynebacterium diphtheria* the cells are lined side by side like matchsticks (pallisade arrangement). Some bacilli are curved into a form resembling a comma. These cells are called vibrios as in *Vibrio cholera*.
- Spiral bacteria: They are divided into two groups, spirilla (singular spirillum) and spirochetes (agent of syphilis). Although these two are similar in shape spirochetes are flexible in nature. Spiral bacteria are far too thin to be seen with the standard Brightfield microscope but are readily observed by Darkfield microscope (Figure 7.3).

Filamentous bacteria

Bacteria tend to form long strands composed of many cells. In these cases, an occasional single cell may be seen after it breaks away from a long filament. These organisms resemble the threadlike strands of fungi but their internal structure is typical of bacteria. Filamentous soil bacteria include *Streptomyces* species.

Pleomorphic bacteria

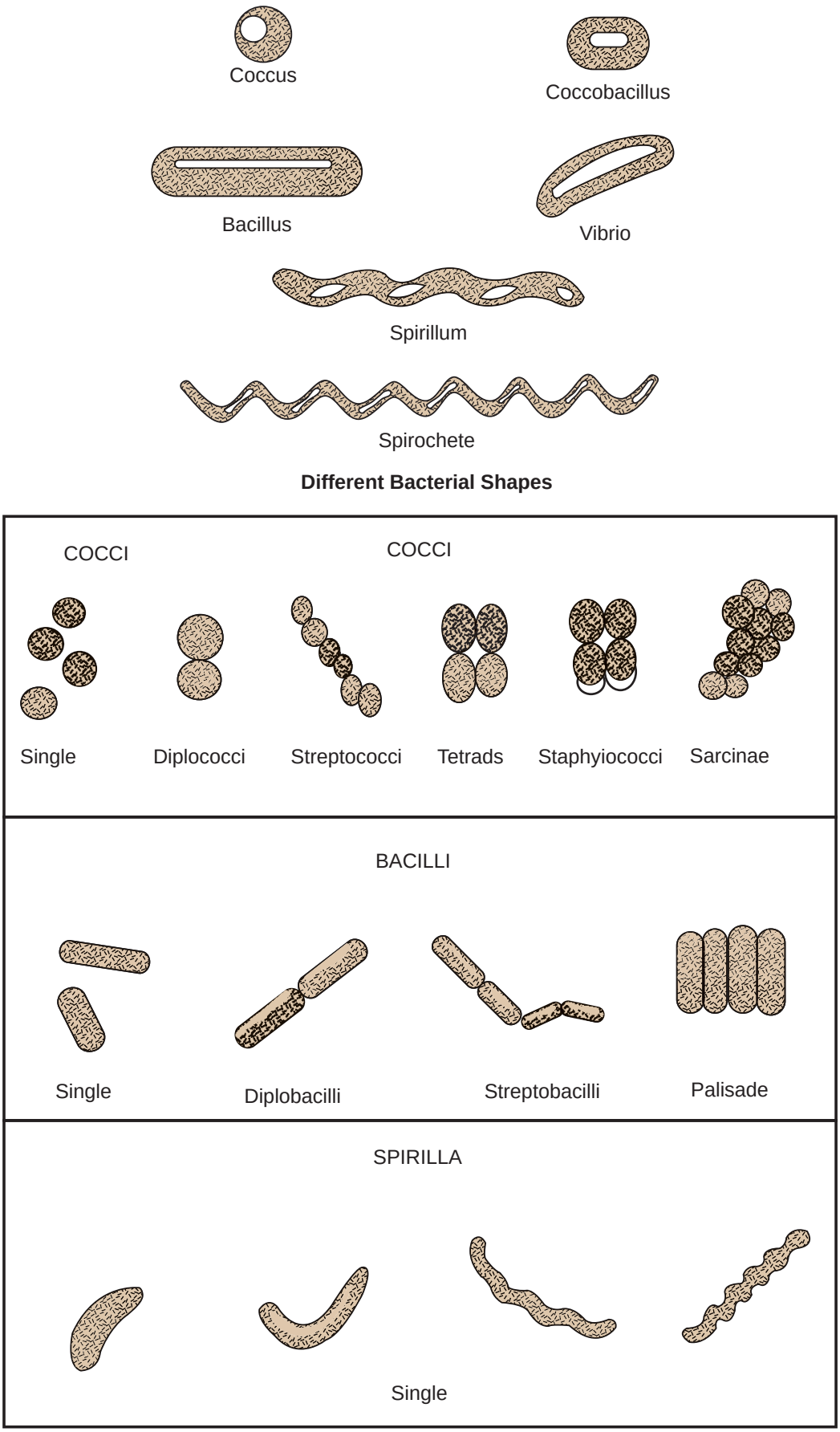
A few bacteria lack rigid cell walls, and their flexible plasma membrane allows them to change shape. These are called pleomorphic bacteria (pleo-more; morph-form). Example: *Mycoplasma*.

7.2 Structures External to Cell Wall of Bacetria

7.2.1 Appendages

Flagella

Flagella (singular flagellum) are threadlike, long, thin helical filaments measuring 0.01-0.02nm in diameter. These appendages extend outward from the plasma membrane and cell wall. Flagella are so thin that they cannot be observed directly with a bright field microscope, but must be stained with special techniques (example: Fontana's silver staining technique) that increase their thickness. The detailed structure of a flagellum can only be seen in the electron microscope.



Different Bacterial Shapes

Figure 7.3: Shapes and arrangement of bacteria

The bacterial flagellum is composed of three parts: a basal body (associated with the cytoplasmic membrane and cell wall), a short hook and a helical filament (which is usually several times as long as the cell). Filament is external to cell wall and is connected to the hook at cell surface; the hook and basal body are embedded in the cell envelope (Figure 7.4). Hook and filament are composed of protein subunits called as flagellin.

One can generalize that all spirilla, about half of the bacilli and a small number of cocci are flagellated. Some bacteria do not have flagella. Flagella vary both in number and arrangement on the cell surface. Flagella are arranged generally in two patterns.

1. In polar arrangement, the flagella are attached at one or both ends of the cell. Bacteria with polar flagellar arrangement are further classified into monotrichous, lophotrichous, and amphitrichous.
2. In lateral arrangement, flagella are arranged randomly all over the surface of the cell. Bacteria with lateral flagellar arrangement are called peritrichous. (Table 7.1)

Various types of mobility are observed based on the arrangement of the flagella. Serpentine motility is seen with *Salmonella*, darting motility with *Vibrio* and tumbling motility with *Listeria monocytogenes*. Some bacteria like *Cytophaga* exhibit a gliding motility, which is slow sinuous flexing motion. This occurs when the cells come in contact with solid surface.

Some bacteria have the ability to move toward or away from chemical substance. This movement is called chemotaxis. Positive chemotaxis is the movement of a cell in the direction of a favorable chemical stimulus (usually a nutrient). Negative chemotaxis is the movement away from a chemical substance (usually harmful compound). Some photosynthetic bacteria exhibit phototaxis, movement in response to light rather than chemicals.

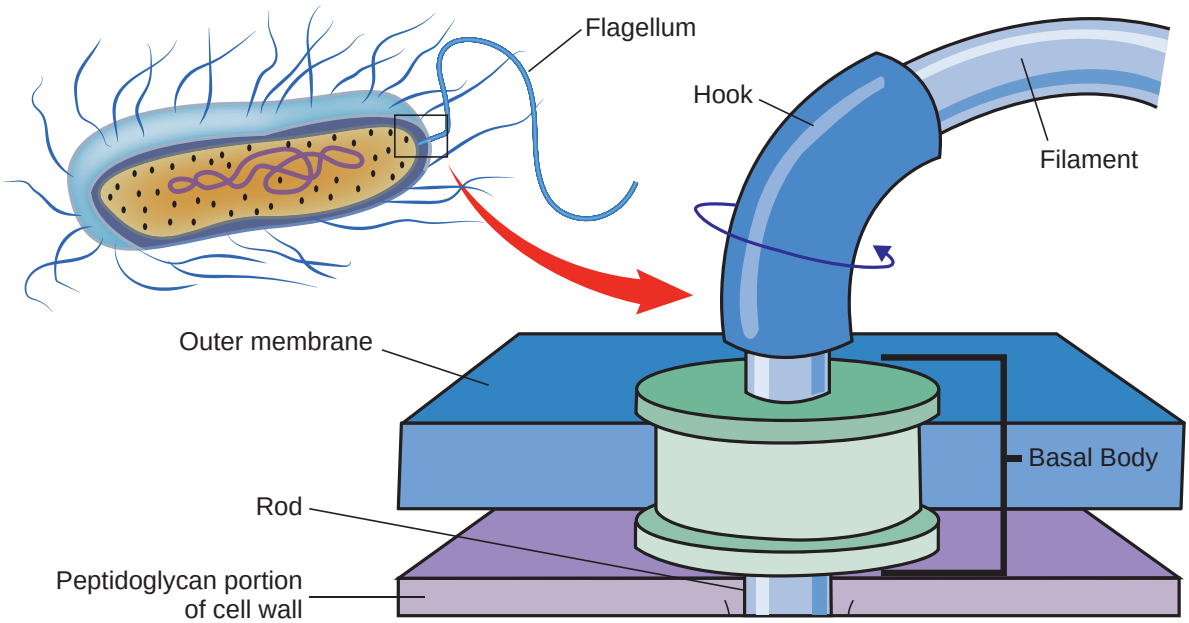
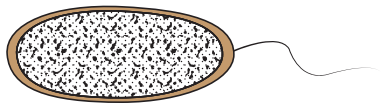
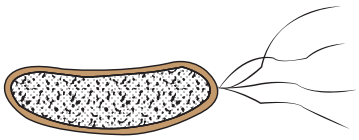
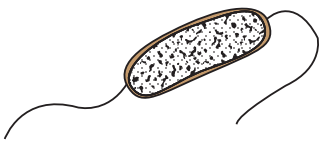
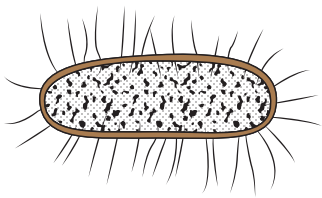


Figure 7.4: Structure of bacterial flagella

Table 7.1: Arrangement of bacterial flagella

Structure	Flagella type	Example
	Monotrichous(single flagella on one side)	*Vibrio cholera*
	Lophotrichous(tuft of flagella on one end)	*Pseudomonas fluorescens*
	Amphitrichous(single or tuft on both ends)	*Aquaspirillum serpens*
	Peritrichous(flagella throughout the cells)	*Salmonella typhi*

HOTS

A. If a bacterium loses its flagella, does it survive?

B. If you remove the cell wall from a flagellated bacterium, the organism loses the ability to move. Explain.

The presence of motility is one piece of information used to identify a pathogen in the laboratory. One way to detect motility is to stab a tiny mass of cells into soft (semi solid) medium in a test tube. Growth spreading rapidly through the entire medium is indicative of motility. Alternatively, cells can be observed microscopically by a hanging drop method.

Pili

Pili (singular pilus) are straight, short and thin and more numerous than flagella around the cell. They can be observed only by electron microscopy. They are found only in certain species of Gram negative bacteria. Pili play no role in motility. Pili originate from the plasma membrane and are made up of a special protein called pilin (Figure 7.5).

Pili play a major role in human infection by allowing pathogenic bacteria to attach to epithelial cells lining the respiratory, intestinal or genitourinary tracts. This attachment prevents the bacteria being washed away by body fluids, thus helps in establishment of infection. One specialized type of pilus (sex pilus) helps in the transfer of genetic material between the bacterial cells. This process is called conjugation.

Fimbriae

Fimbriae (singular: fimbria) is another term used for short pili that occur in great number around the cell. They enable bacteria to attach to surfaces and to each other, so that the bacteria form clumps or films called pellicles on the surface of liquid in which they are growing. Fimbriae are found in Gram positive as well as in Gram negative bacteria.

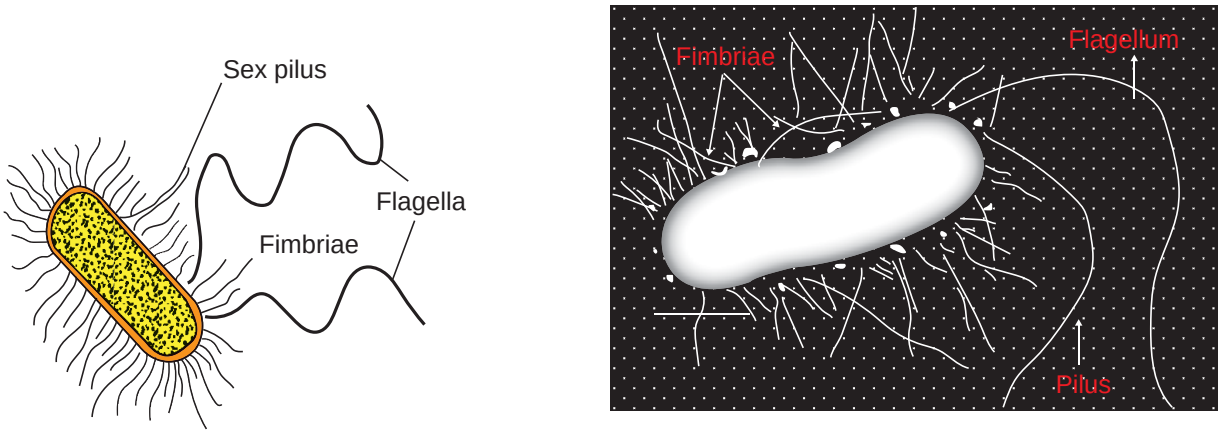


Figure 7.5: Structures of pili and fimbriae

Table 7.2 compares the pili and fimbriae.

7.2.2 Extracellular Polymeric Substance (EPS)

Many bacteria secrete high molecular weight polymers that adhere to the exterior of the cell wall to form a capsule or slime layer. Glycocalyx is often used to refer to any polysaccharide material outside the cell wall. Capsules and slime layer are considered to be glycocalyxes (Table 7.3).

Capsules

Some bacterial cells are surrounded by a viscous substance forming a covering layer or envelope around the cell wall called capsule (Figure 7.6). Capsule is usually made up of polysaccharide. It may be homopolysaccharide (made up of a single kind of sugar) or heteropolysaccharide (made up of several kinds of sugars). These are synthesized from sugars within the cell, transported and polymerized outside the cell. The capsule of some bacteria is made of polypeptides. The capsule of *Bacillus anthracis* has polymer of D-glutamic acid. Capsules are highly impermeable. Capsules can be demonstrated using special staining technique utilizing Indian ink or with Nigrosin stain. The presence of capsule in fresh isolates gives a moist and shiny appearance to the bacterial colonies on an agar medium. Capsular material is antigenic and may be demonstrated by serological methods.

Table 7.2: Comparison of pili and fimbriae

Characteristics	Pili	Fimbriae
Appearance	Hair like, straight appendages.	Tiny bristle like fibers arising from the surface of bacterial cell.
Length	Longer than fimbriae	Shorter than pili
Numbers per cell	1-10/cell	200-400/cell
Presence	Present only in Gram negative bacteria	Present in both Gram positive and Gram negative bacteria
Made -up of	Pilin protein	Fimbrillin protein