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GOVERNMENT OF TAMIL NADU

HIGHER SECONDARY FIRST YEAR

MICROBIOLOGY

A publication under Free Textbook Programme of Government of Tamil Nadu

Department of School Education

Untouchability is Inhuman and a Crime

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Government of Tamil Nadu

First Edition - 2018

Revised Edition - 2019, 2022

Reprint - 2020, 2021, 2023, 2024

(Published under New Education Syllabus)

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Content Creation

State Council of Educational
Research and Training

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Printing & Publishing

Tamil NaduTextbook and Educational
Services Corporation

www.textbooksonline.tn.nic.in

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Contents

Chapter 1 Introduction to Microbiology 01

Chapter 2 Microscopy 15

Chapter 3 Stains and Staining Methods 25

Chapter 4 Sterilization 40

Chapter 5 Cultivation of Microorganisms 53

Chapter 6 Microbial Nutrition and Growth 72

Chapter 7 Morphology of Bacteria 86

Chapter 8 Microbial Taxonomy 111

Chapter 9 Environmental Microbiology 122

Chapter 10 Soil Microbiology 149

Chapter 11 Agricultural Microbiology 162

Chapter 12 Medical Microbiology 181

Chapter 13 Immunology 213

Chapter 14 Microbial Genetics 253

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Assessment

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

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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

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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

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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

TN_GOVT_XI_Micro_Biology_FM.indd 7 16-02-2019 10.43.10 AM

- 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		

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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

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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.

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Chapter 1

Introduction to Microbiology

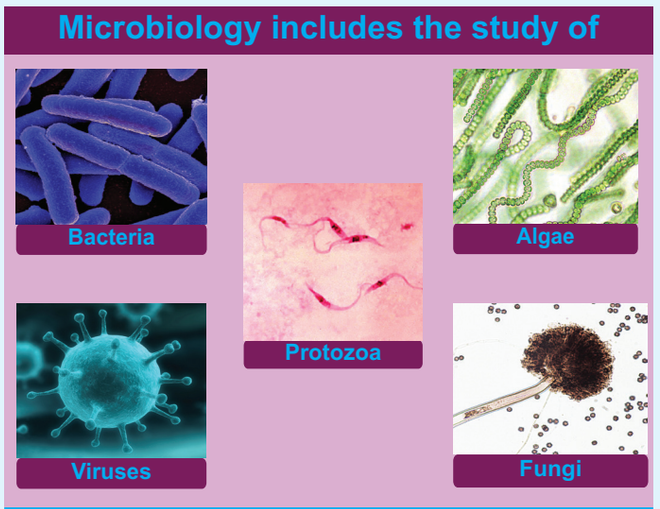
Chapter Outline

1.1 Groups of Microorganisms

1.2 Contributors to Microbiology

1.3 Branches of Microbiology

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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 human and 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.

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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.

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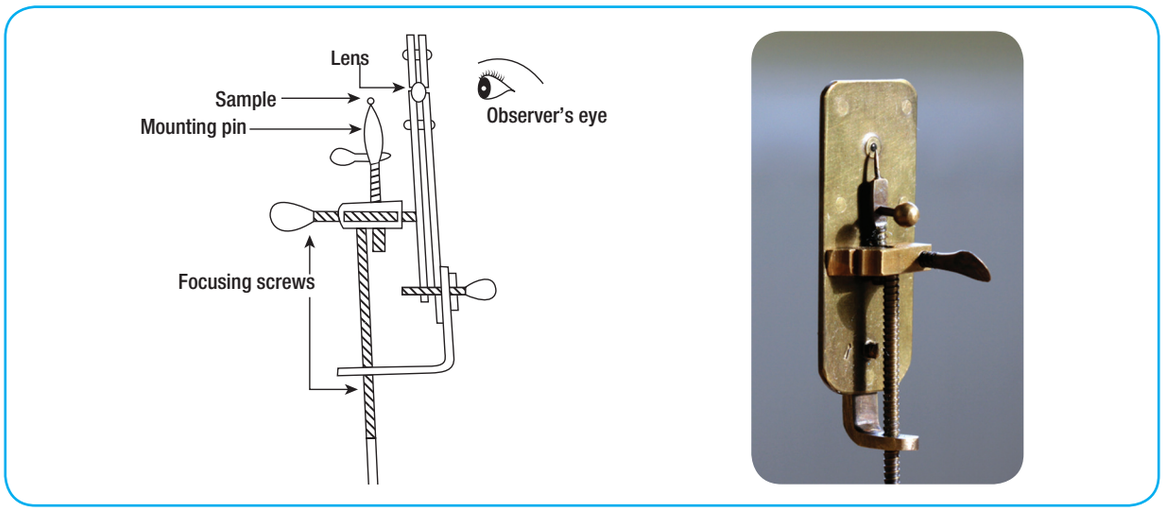


Figure 1.2: Leeuwenhoek's Microscope



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,

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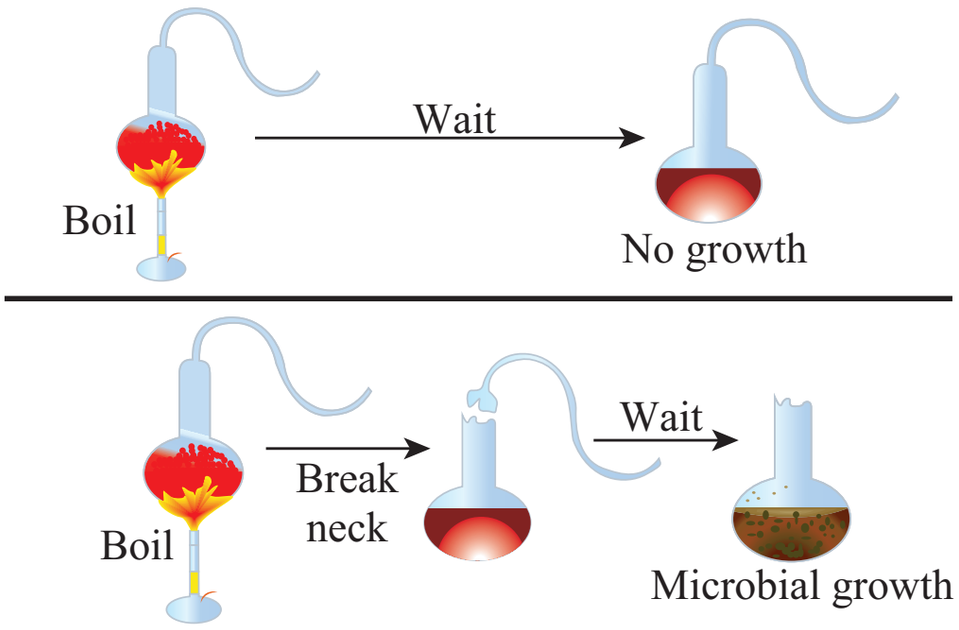


Figure 1.4: Pasteur's swan neck flask experiment

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.*

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

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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.

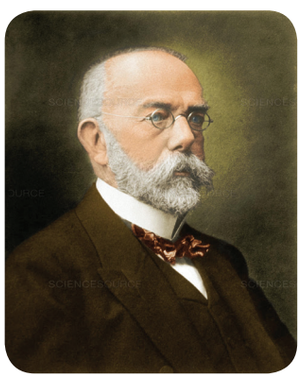


Figure 1.6: Robert Koch (1843-1910)

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

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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

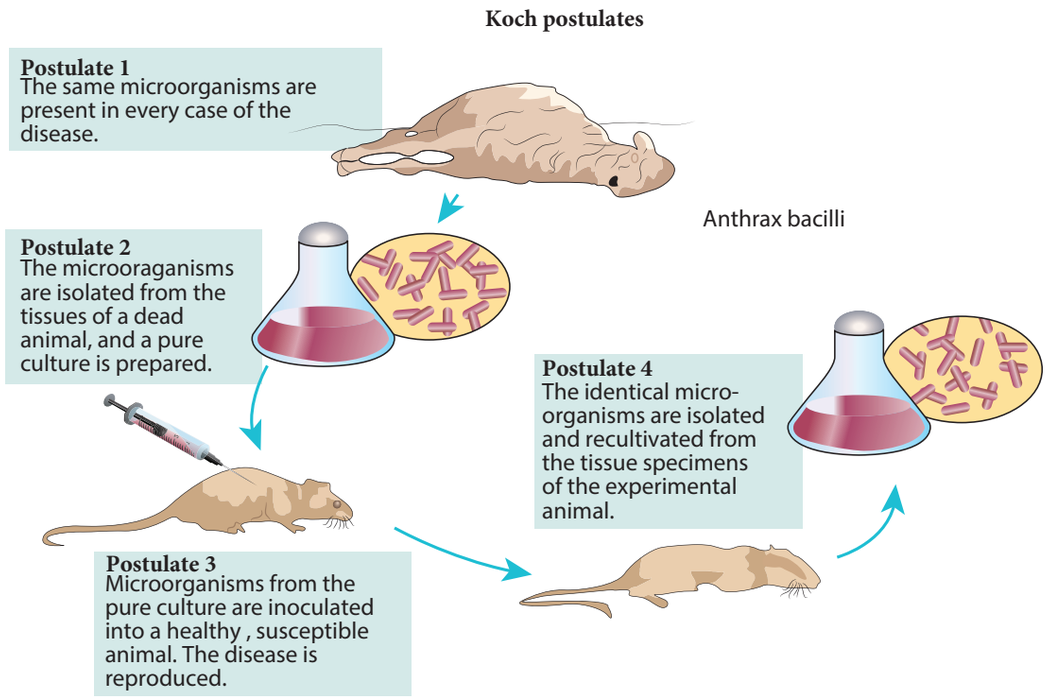


Figure 1.7: Koch's postulates for infectious diseases

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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)

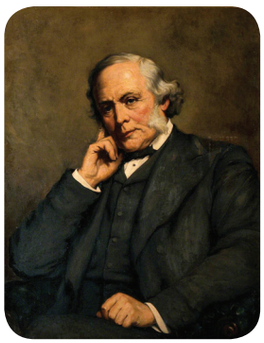


Figure 1.8: Joseph Lister (1827-1912)

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

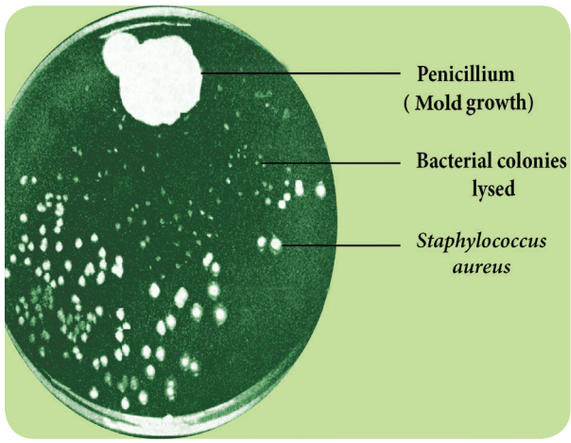


Figure 1.9: Original culture plate on which the observation of action of penicillin was made by Alexander Fleming

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.

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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.

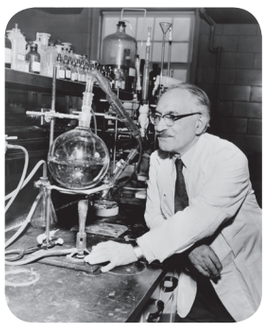


Figure 1.11: Selman Abraham Waksman (1888-1973)

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

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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

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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.

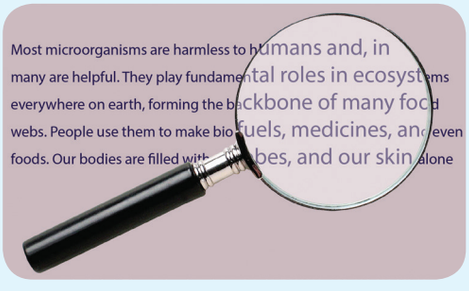
Summary

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.



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



Fermented foods



Gut microbiome for digestion and synthesis of vitamins



Soil microbes helps in break down wastes

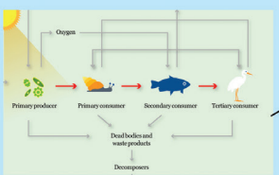
87%
Beneficial Organisms

10%
Opportunists

Pathogens 3%



Production of drugs and vaccine



Microbes form the basis of the food chain



Production of enzymes, organic acids



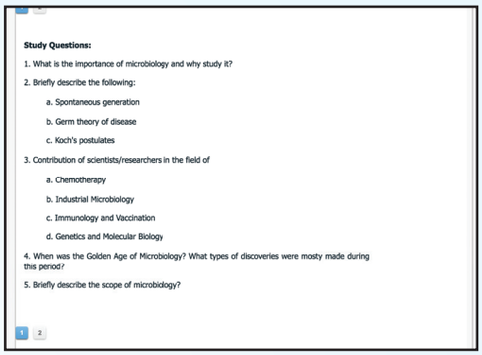
Production of biofertilizer and biopesticide

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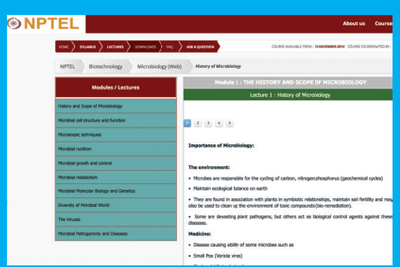
Microbiology

Lets meet our micro friends

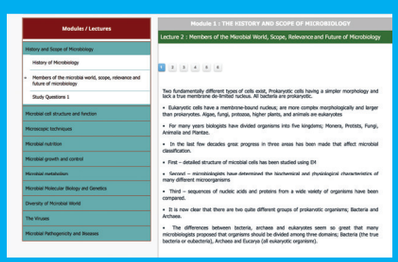


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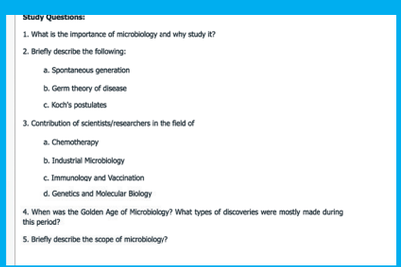
- Use the URL or scan the QR code to open 'NPTEL' page.
- Click 'History' and 'Scope of Microbiology' to know the history of microbiology.
- Select history of microbiology and click 'Start Course' at the bottom.
- Select 'Members of the Microbial world' to know about it.



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 were discovered by Selman Abraham Waksman?
   a. Streptomycin
   b. Neomycin
   c. Actinomycin
   d. All the above

E65DH

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?

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Chapter 2

Microscopy

Chapter Outline

2.1 Historical Background

2.2 Principles of Microscopy

2.3 Bright Field Microscope

2.4 Dark Field Microscope



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

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of light ray) (Figure 2.1).

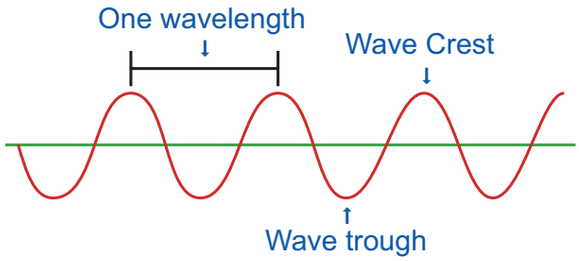


Figure 2.1: Wavelength-the distance between two adjacent crests or two adjacent troughs of the wave and denoted by greek letter (λ)

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).

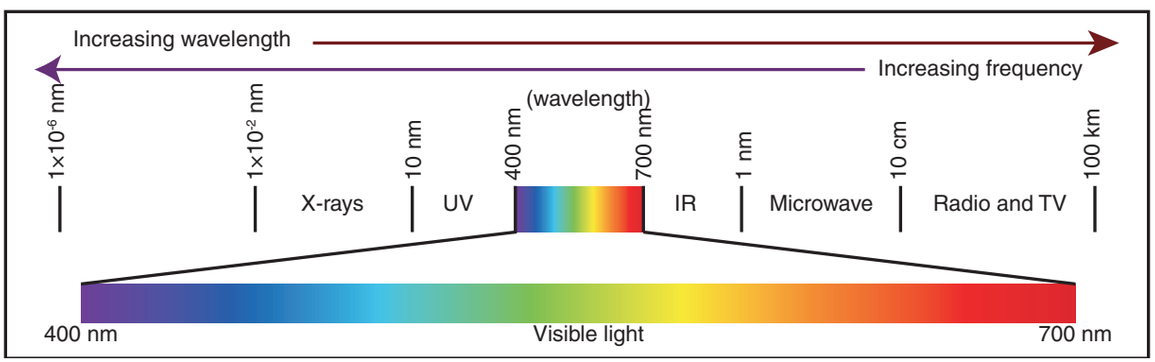


Figure 2.2: The electromagnetic spectrum-White light is a combination of all colours of visible spectrum

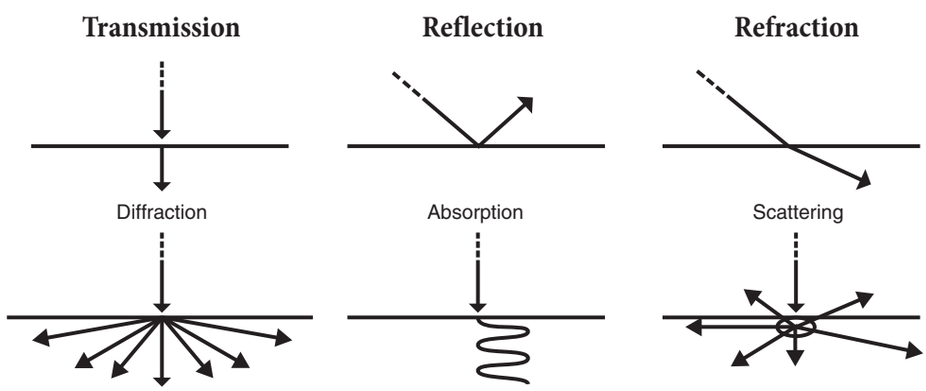


Figure 2.3: Interaction of light with matter

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

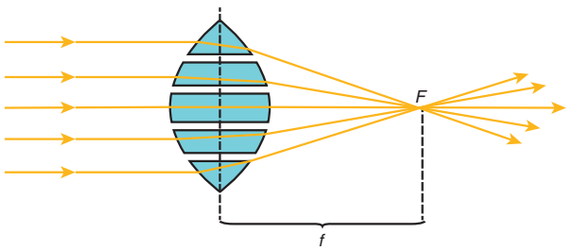


Figure 2.4: Lens function

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

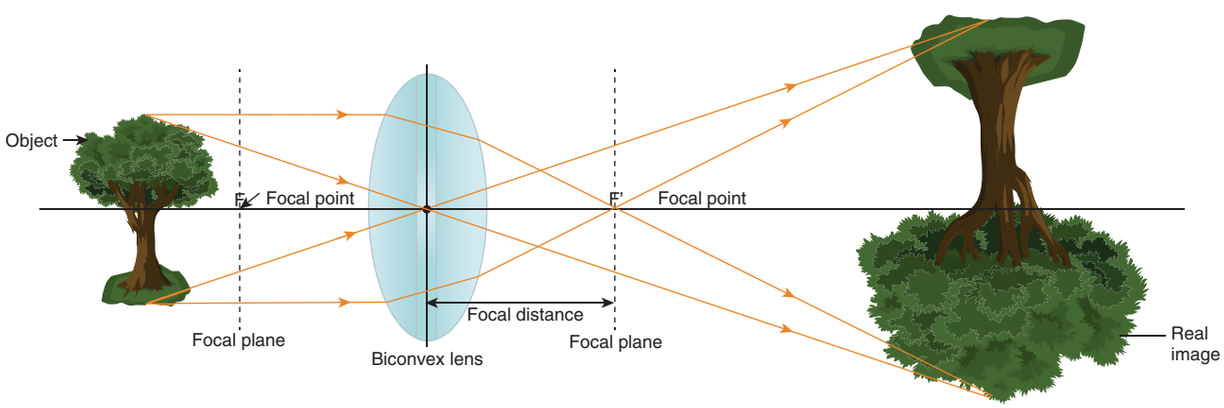
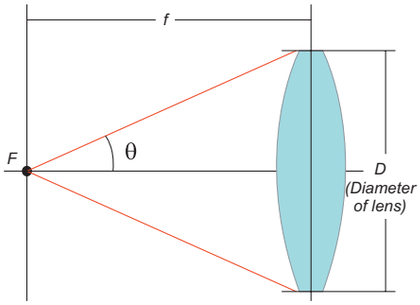


Figure 2.5: Generating an image with a lens

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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.

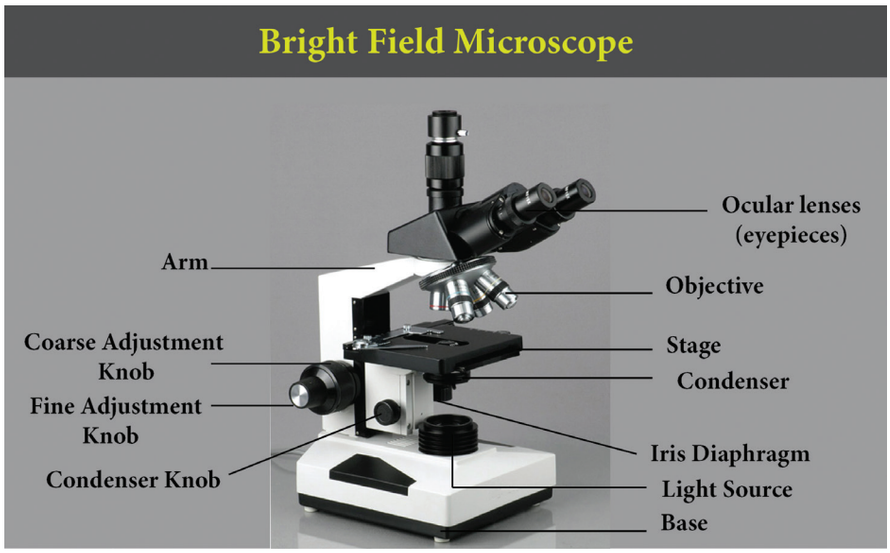
- Light microscopes resolve images with the help of light. The specimen is viewed as dark object against a light background in bright field microscope. Dark field microscope uses a special condenser and the specimen appears light against a black background. The other types of mircoscopes are Phase contrast 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

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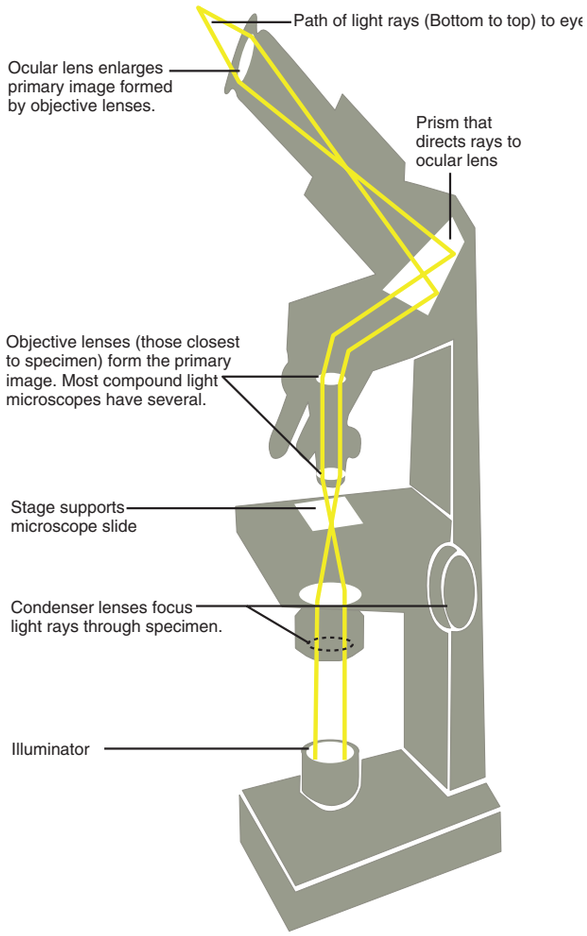


Figure 2.7: The path of light in light Microscopes

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?

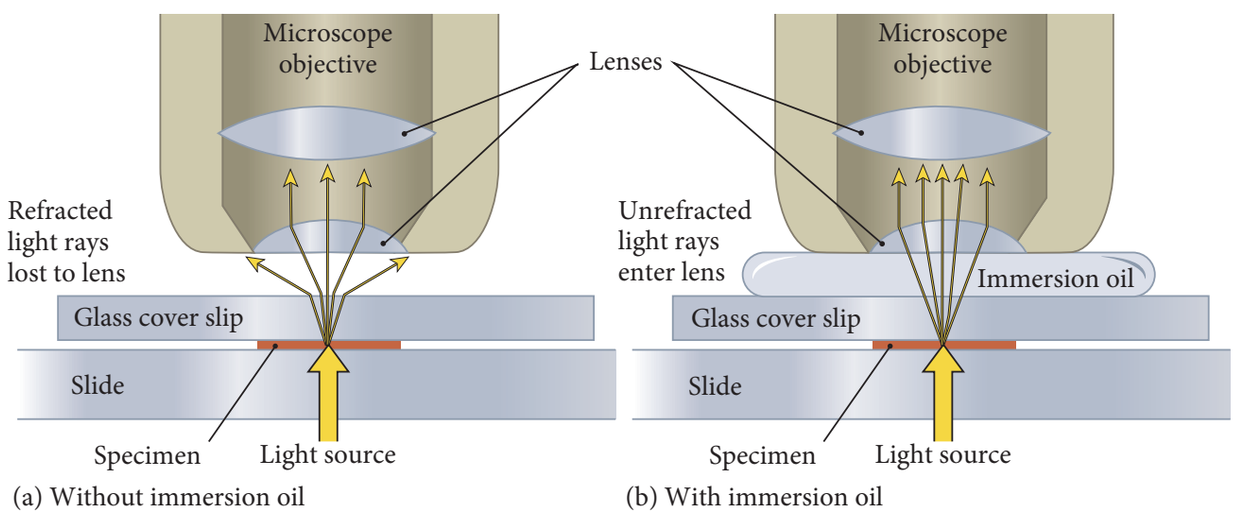
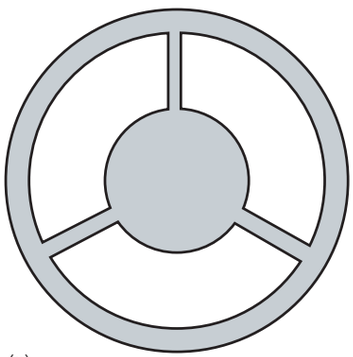


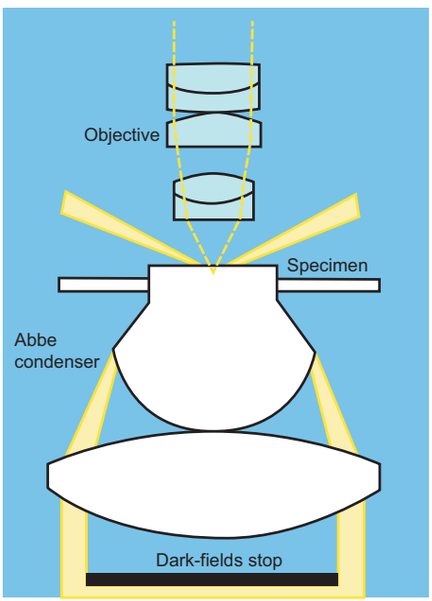
Figure 2.8: Oil Immersion Objective Working Principle

2.4 Dark Field Microscope

This is used for examining live unstained microorganisms. 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.

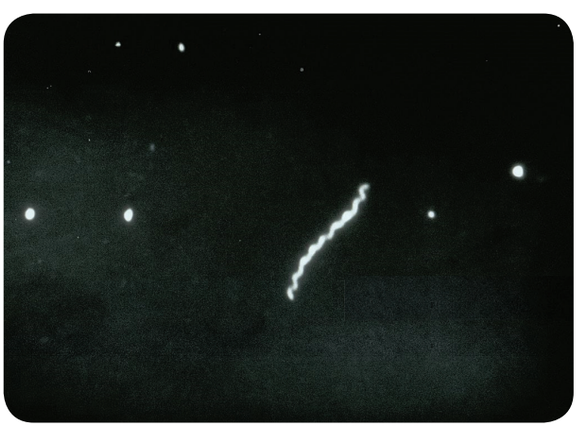
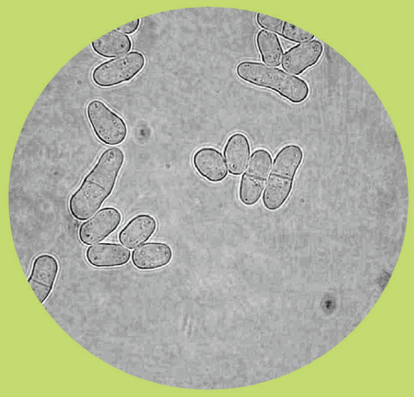


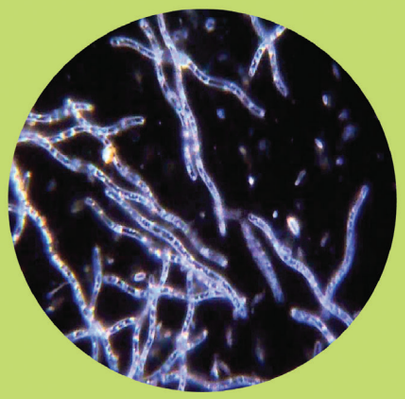
Figure 2.10: Dark field observation of bacteria *Treponema pallidum* specimen from a patient with Syphilis

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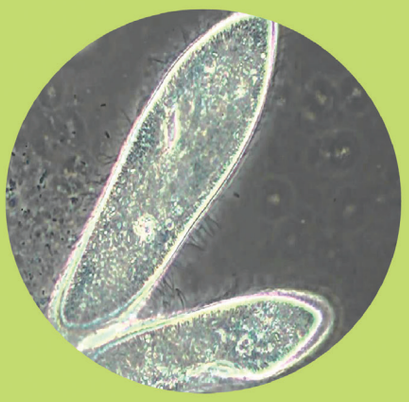
Different types of Microscopic images : A comparison



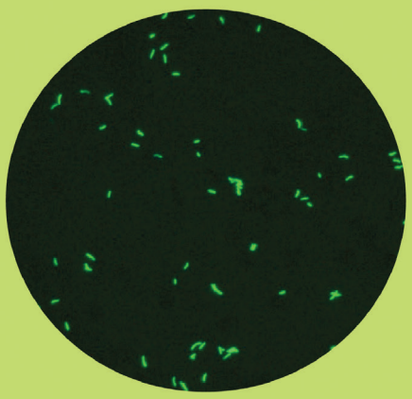
Yeast cells under
Bright field microscope



Spirillum under
Dark field microscope



Paramecium under
Phase contrast microscope



Mycobacterium under
Fluroscence microscope



Escherichia coli under
Transmission Electron
microscope



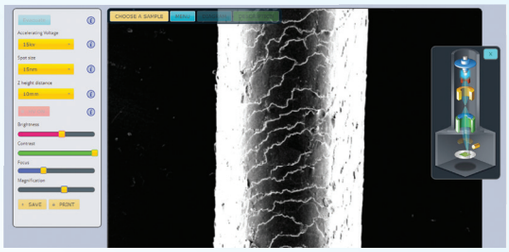
Vibrio cholerae under
Scanning Electron
microscope

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ICT CORNER

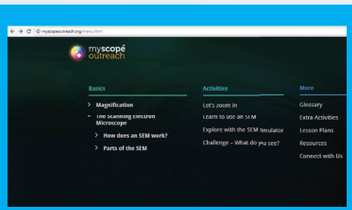
SEM

Lets focus with SEM

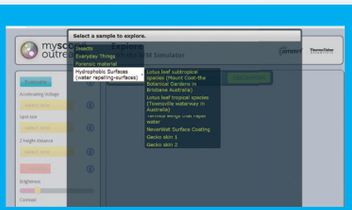


STEPS:

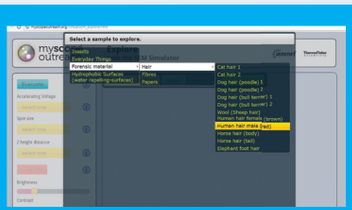
- Use the URL or scan the QR code to reach 'myscope outreach' interactive page.
- Click 'The Scanning Electron microscope' under 'Basic' menu to know about its parts and function.
- Follow the successive steps that lead to describe the n nuances of SEM.
- Select 'Let's Zoom in' under the activity to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



Step1



Step2



Step3

URL:

http://myscopeoutreach.org

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

Multiple choice questions

1. The credit for inventing the first compound microscope goes to

   a. Robert Hook
   b. Anton von Leewenhoek
   c. Kepler and Galileo
   d. Zaccharias Janssen

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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. Numerical aperture was first described by

   a. Robert Hook
   b. Anton von Leewenhoek
   c. Ernst Abbe
   d. Zaccharias Janssen

4. The resolving power of light microscope is

   a. 1 cm
   b. 1.0 µm
   c. 0.2 µm
   d. 2 nm

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.

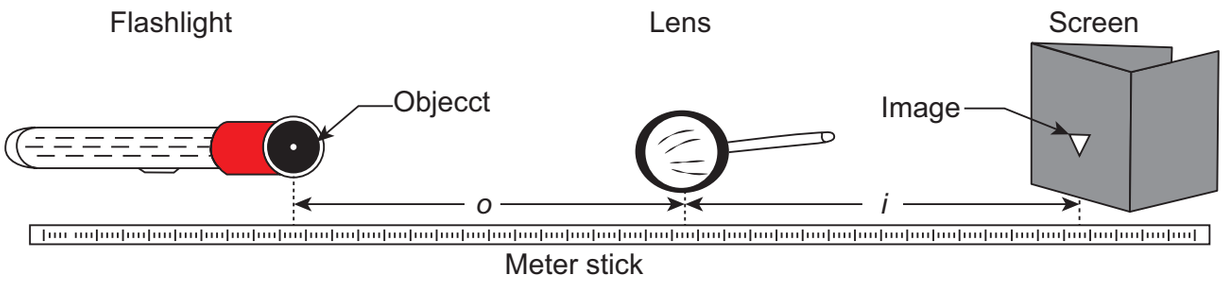
Student Activity

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.



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Chapter 3

Stains and Staining Methods

SMBCF

Chapter Outline

3.1 Techniques in Observing Microorganisms

3.2 Purpose of Staining

3.3 Stains

3.4 Principle of Staining

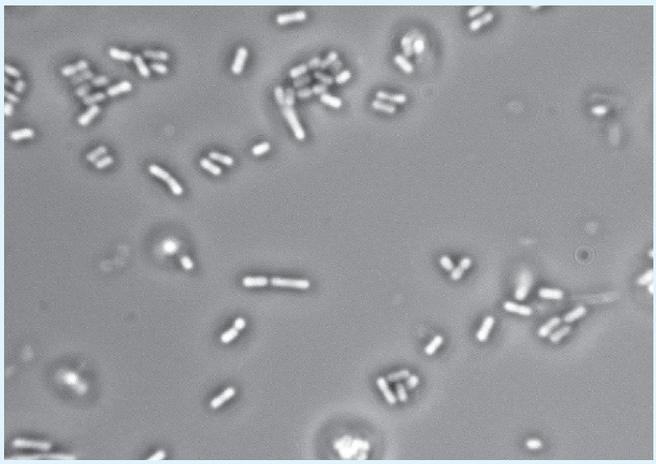
3.5 Preparation of Materials for Staining

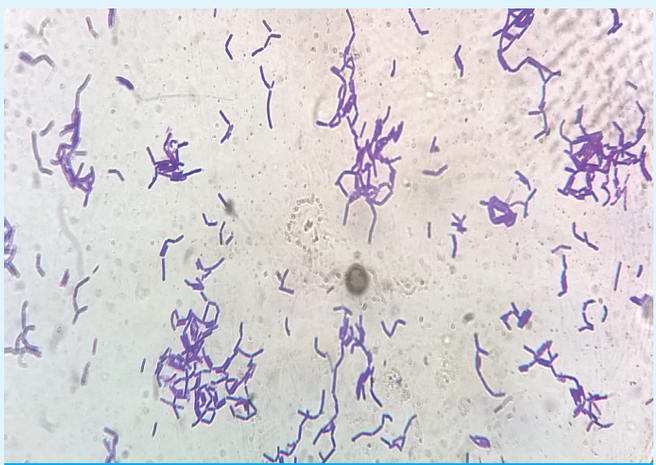
3.6 Simple Staining Method

3.7 Differential Staining

3.8 Special Staining – Endospore Staining

3.9 Commonly used Stains and its Applications





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.*

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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.

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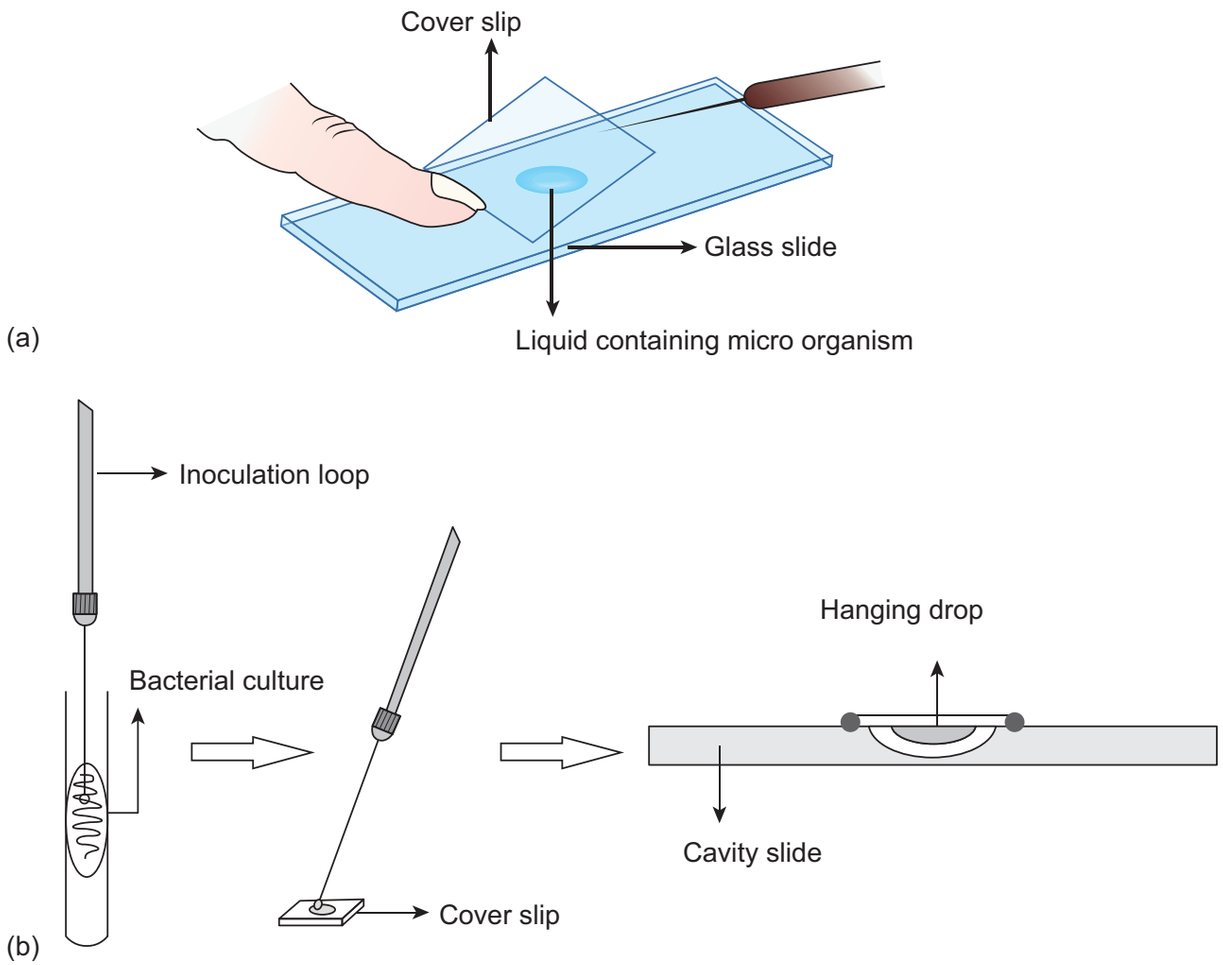


Figure 3.1: a) Wet mount and b) Hanging drop preparation

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.

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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).

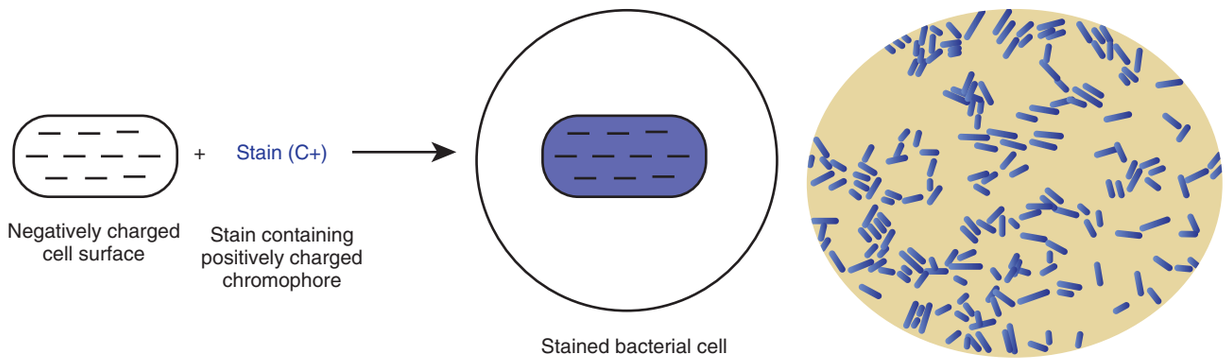


Figure 3.2: Positive staining

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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).

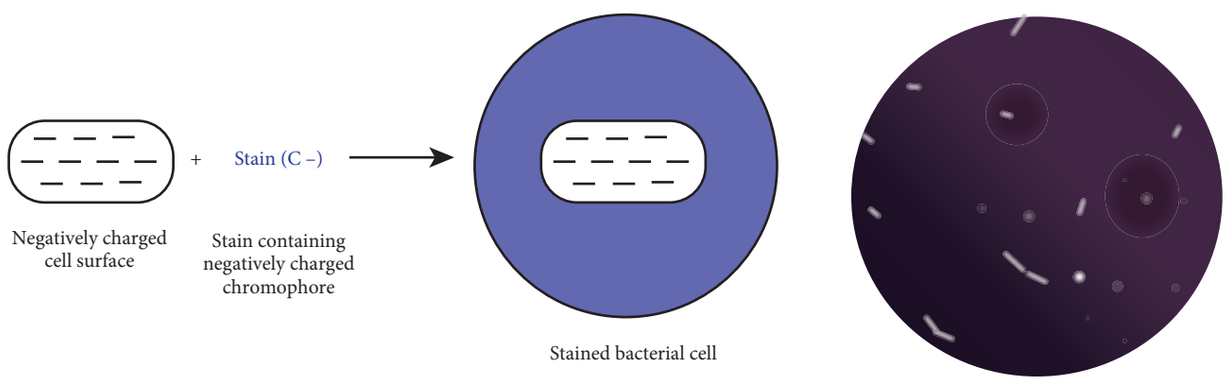


Figure 3.3: Negative staining

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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).

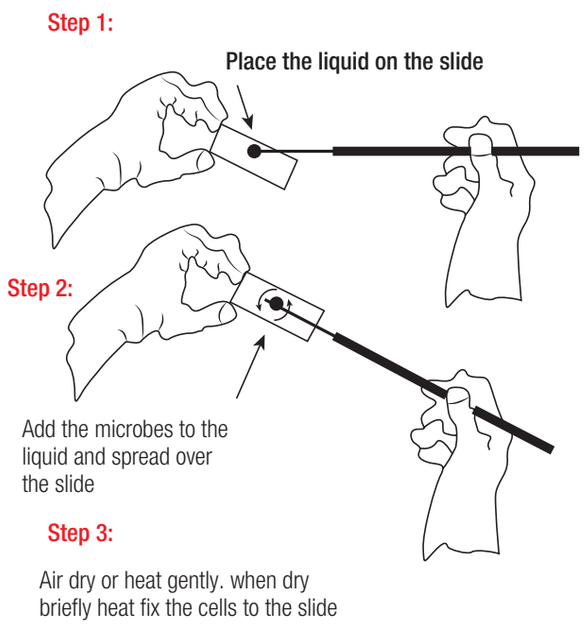


Figure 3.4: Preparation of smear

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.

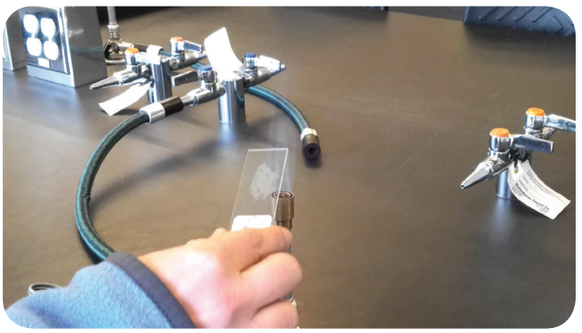


Figure 3.5: Fixation of smear by passing slide gently through the flame

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:

DO YOU KNOW?

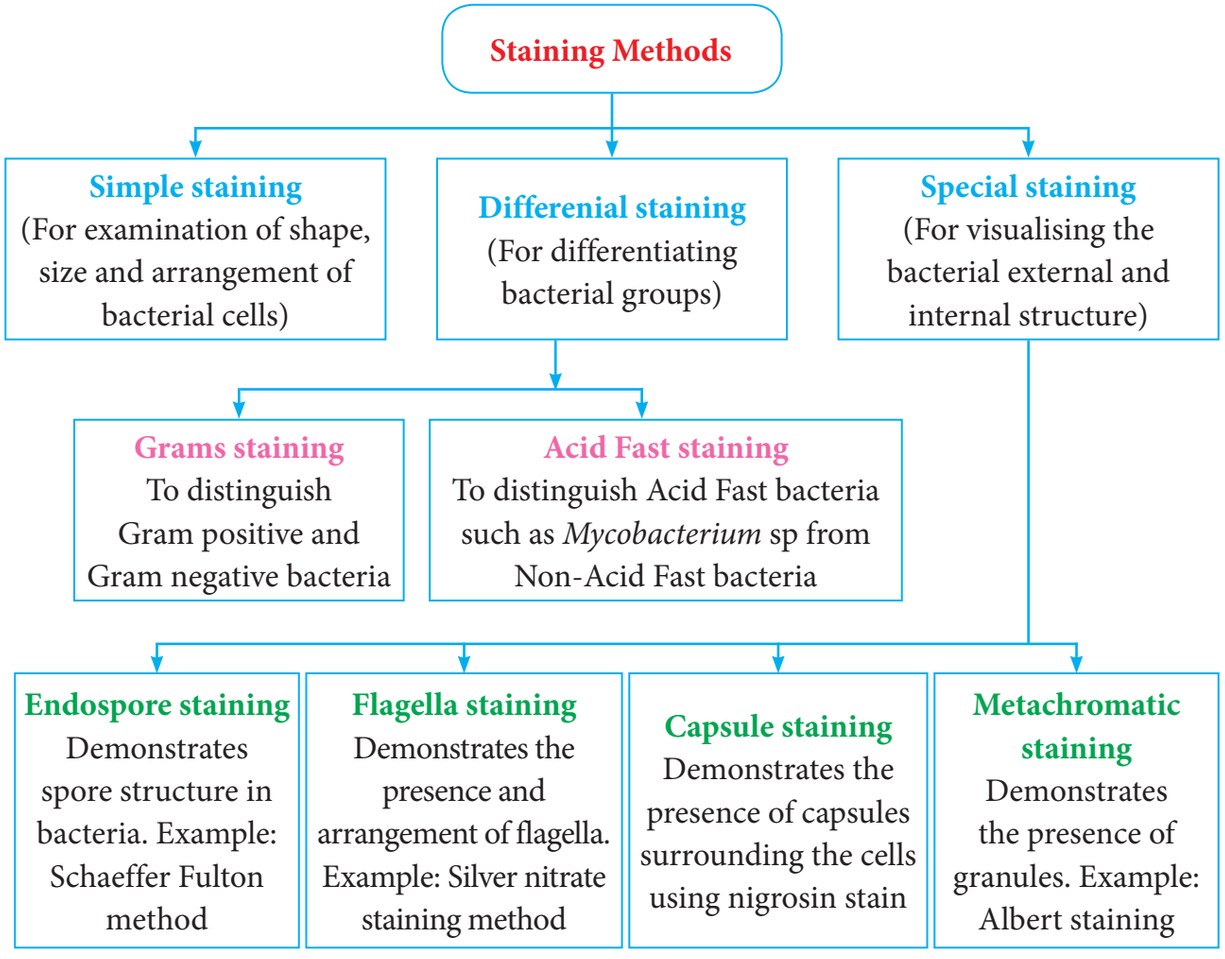
Robert Hooke was the first to describe the appearance of stained objects under light microscope.

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

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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.

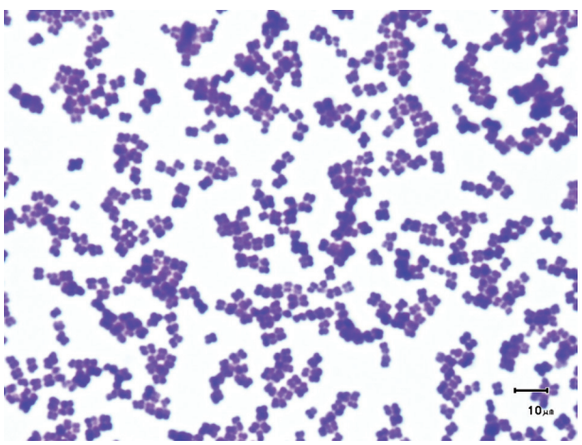
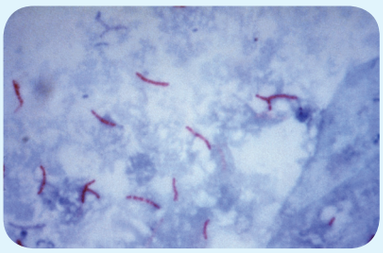


Figure 3.6: Simple stain – *Micrococcus sp.* stained with Methylene blue

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DO YOU KNOW?

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

TN_GOVT_XI_Micro_Biology_CH03.indd 32 16-02-2019 9.55.50 AM

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.

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

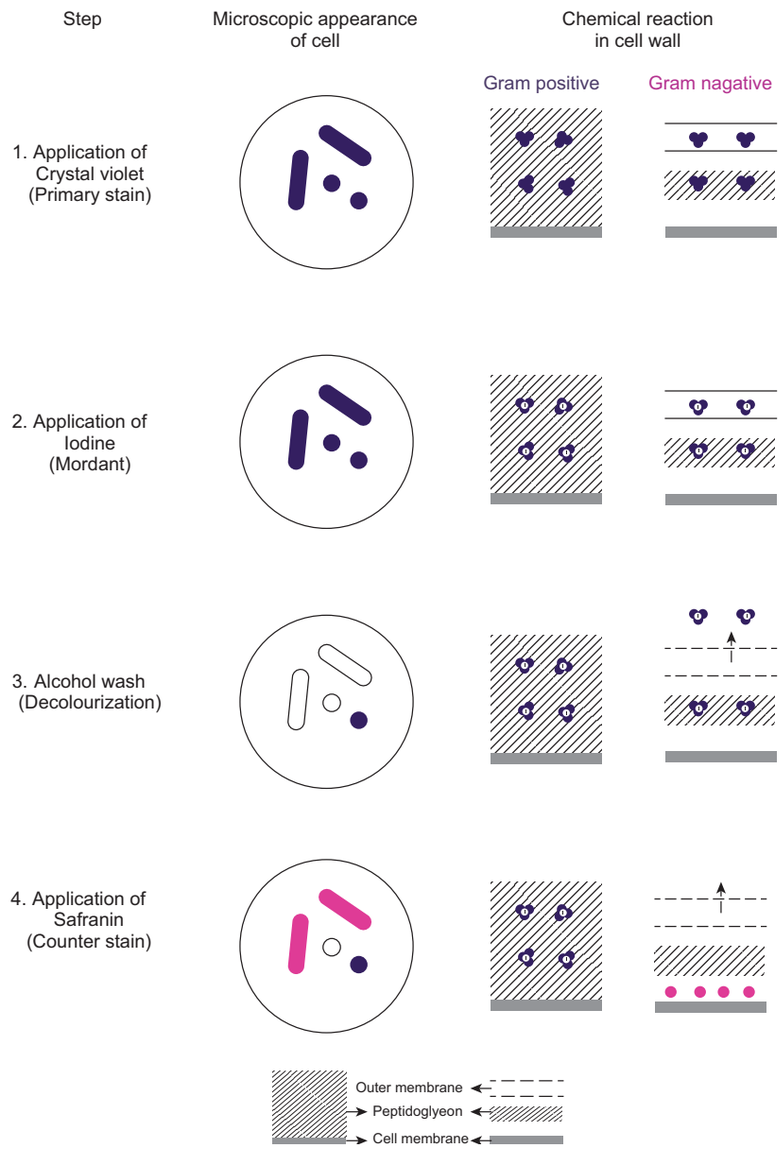


Figure 3.7: Steps, micrograph and chemical reaction of Gram Stained Bacteria

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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.

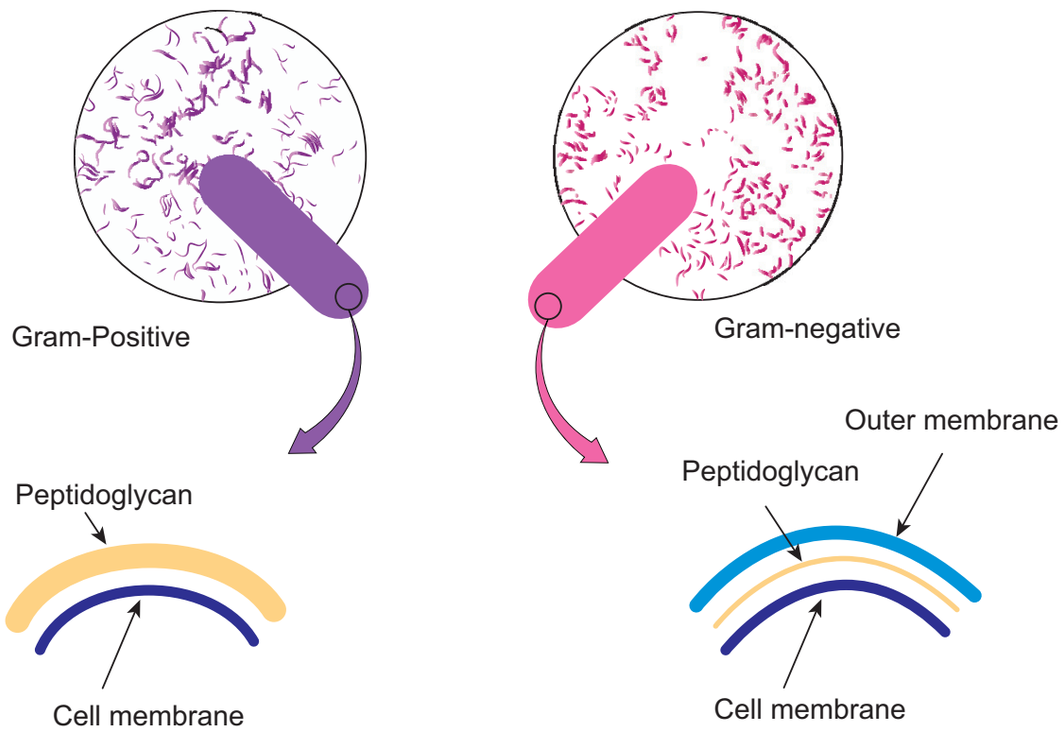


Figure 3.8: Cell wall of Gram positive and Gram negative Bacteria

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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.

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.

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.

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.

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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.

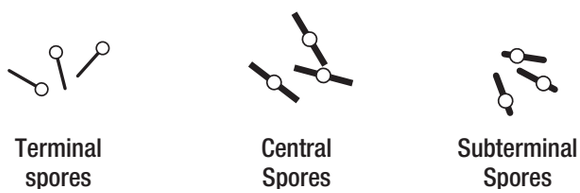


Figure 3.9: Position of spore in a vegetative cell.

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.

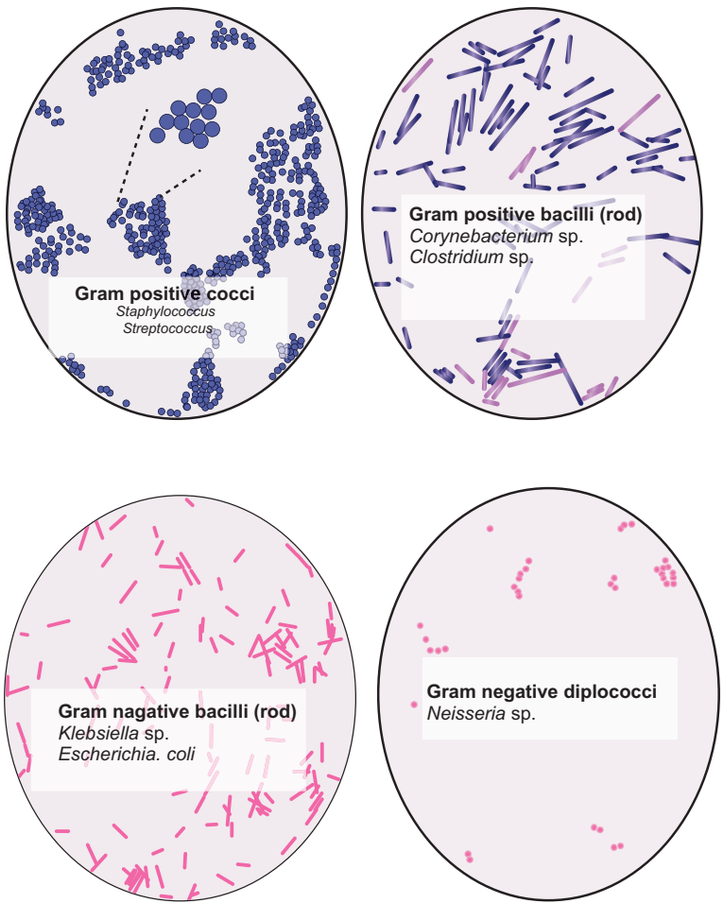
In a properly prepared smear, the endospores appear green within red cells (Figure 3.10). Endospores are highly refractive. They can be detected under the light microscope when unstained, but cannot be differentiated from inclusions of stored material without a special stain.



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

TN_GOVT_XI_Micro_Biology_CH03.indd 36 16-02-2019 9.55.51 AM

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.

TN_GOVT_XI_Micro_Biology_CH03.indd 37 16-02-2019 9.55.52 AM

Evaluation

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?

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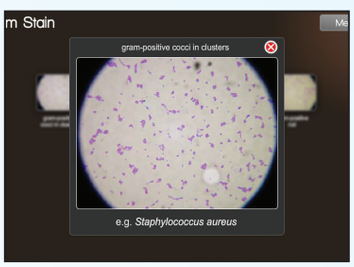
14. For what purpose would you use each of the following?
    a. Simple stain
    b. Negative stain
    c. Acid- fast stain
    d. Gram stain
15. The gram stain has been described as the most important stain for microbiologist. Explain why?
16. How will you appreciate the need of staining?
17. Classify 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

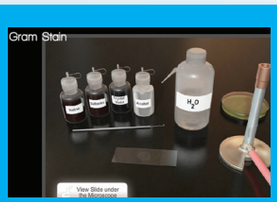


STEPS:

- 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

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www.tnschoolbooks.in

Chapter 4

Sterilization

Chapter Outline

4.1 Need for Sterilization

4.2 Methods of Sterilization

4.3 Physical Methods of Sterilization

4.4 Sterilization by Heat

4.5 Radiation

4.6 Filtration



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.



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

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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

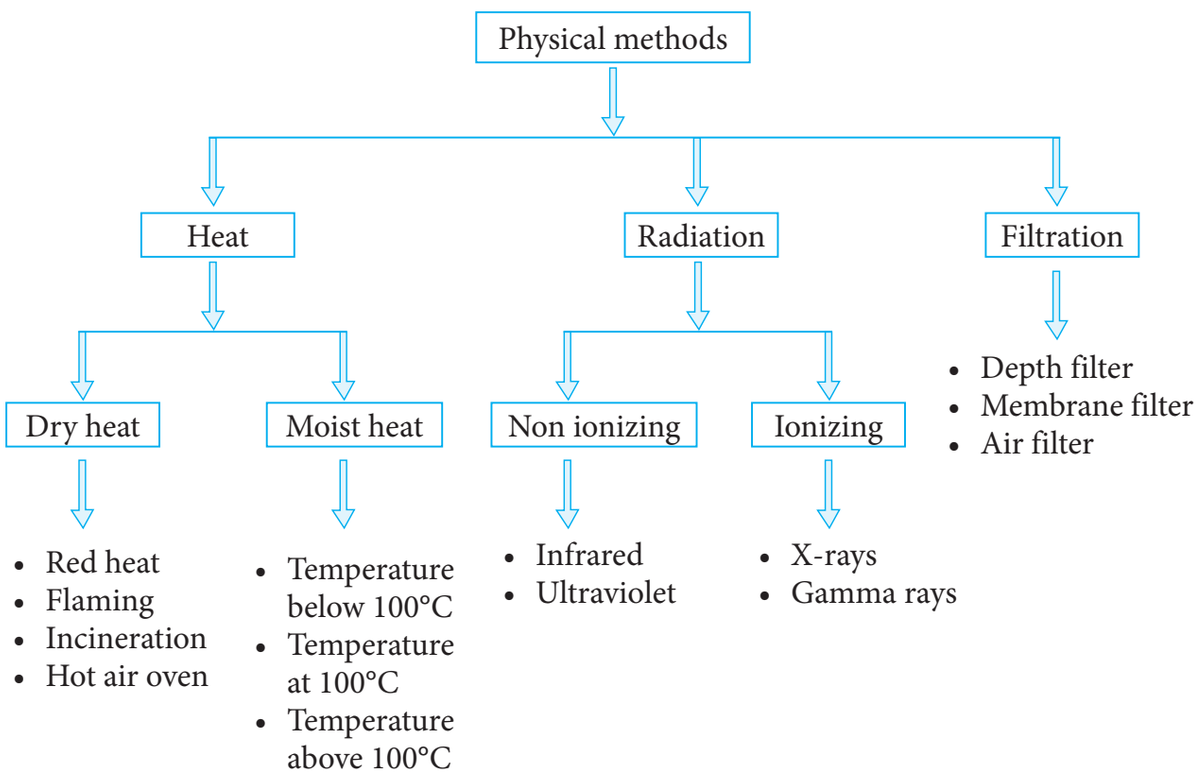
Growth and multiplication of microorganisms can be controlled by removing, killing or inhibiting them using various physical or chemical agents.

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

TN_GOVT_XI_Micro_Biology_CH04.indd 41 16-02-2019 9.56.34 AM

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.

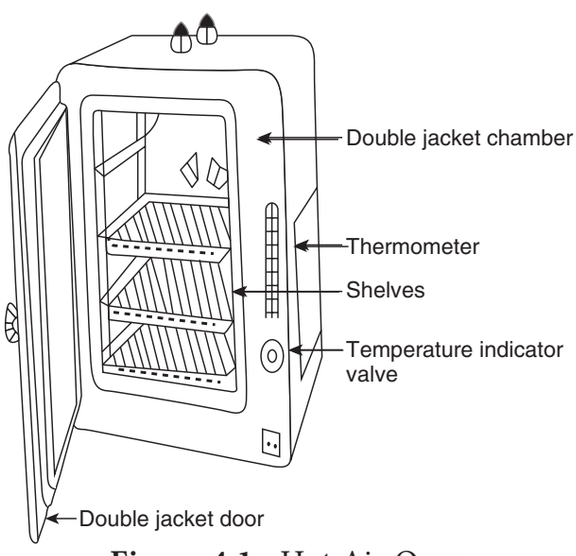


Figure 4.1: Hot Air Oven

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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

TN_GOVT_XI_Micro_Biology_CH04.indd 43 16-02-2019 9.56.34 AM

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 sterilization, the indicator commonly used is the paper strips containing spores of *Bacillus stearothermophilus.*

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.

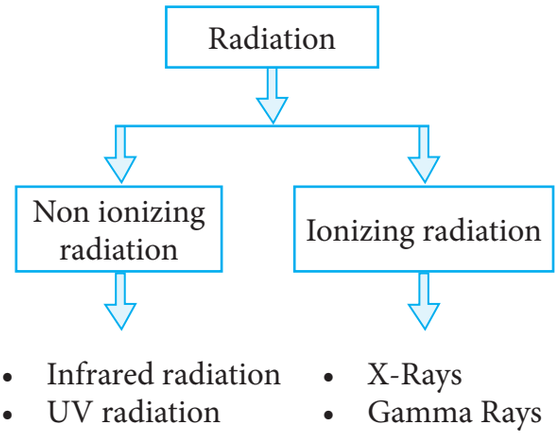
a) Non-ionizing radiation

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

TN_GOVT_XI_Micro_Biology_CH04.indd 44 16-02-2019 9.56.34 AM

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

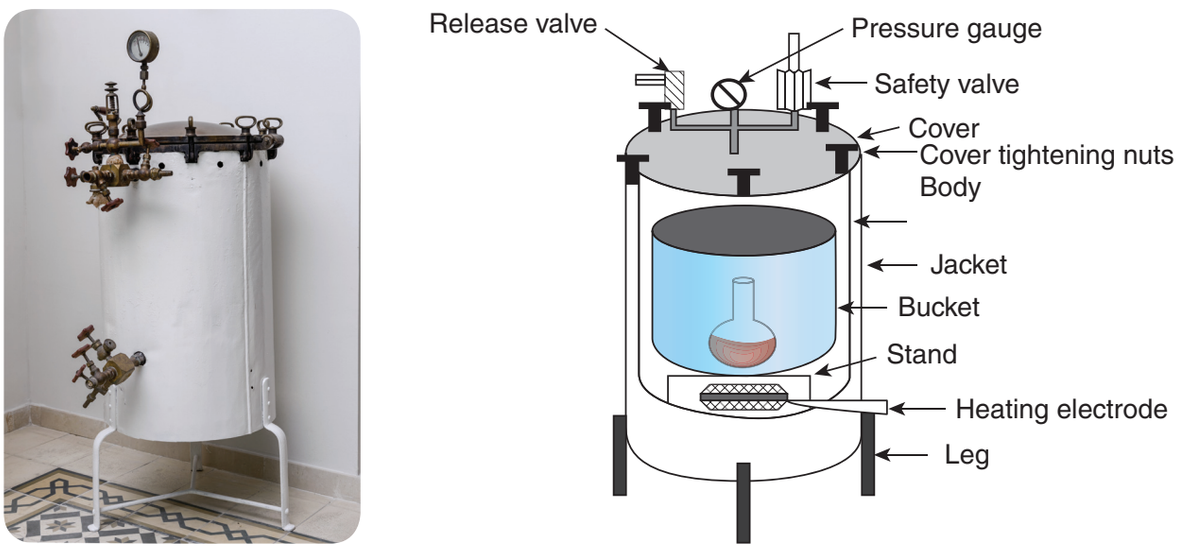


Figure 4.2: (a) Laboratory autoclave (b) Components of autoclave

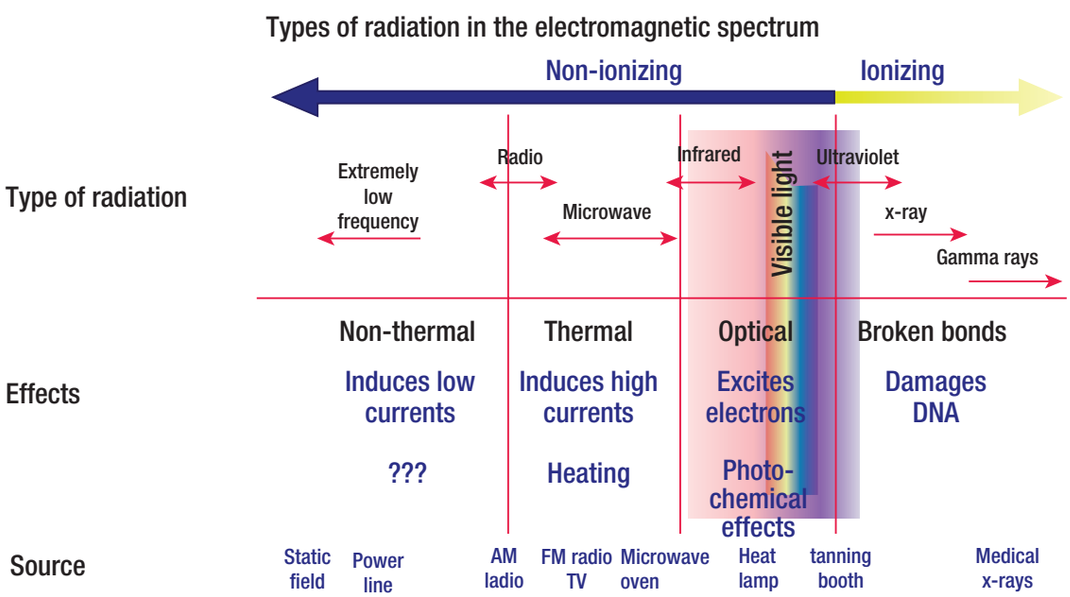


Figure 4.3: Types of radiation in electromagnetic spectrum

TN_GOVT_XI_Micro_Biology_CH04.indd 45 16-02-2019 9.56.36 AM

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

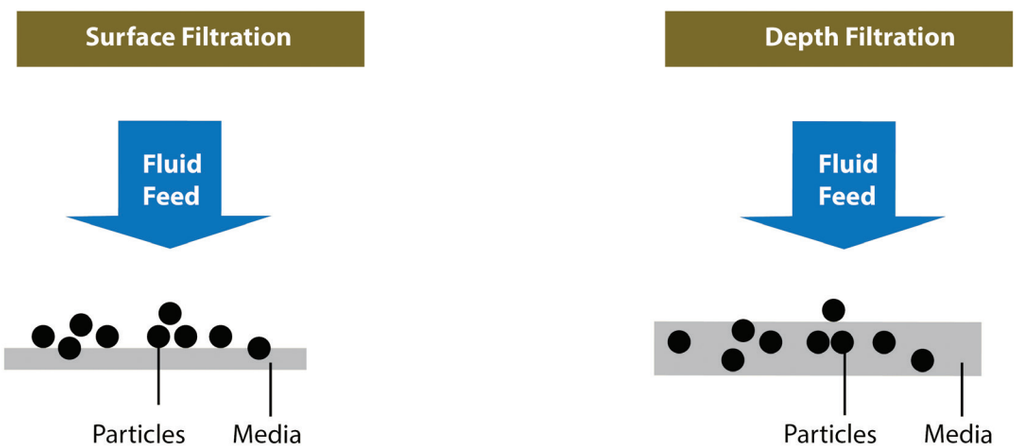


Figure 4.4: Principle of filtration

TN_GOVT_XI_Micro_Biology_CH04.indd 46 16-02-2019 9.56.36 AM

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.

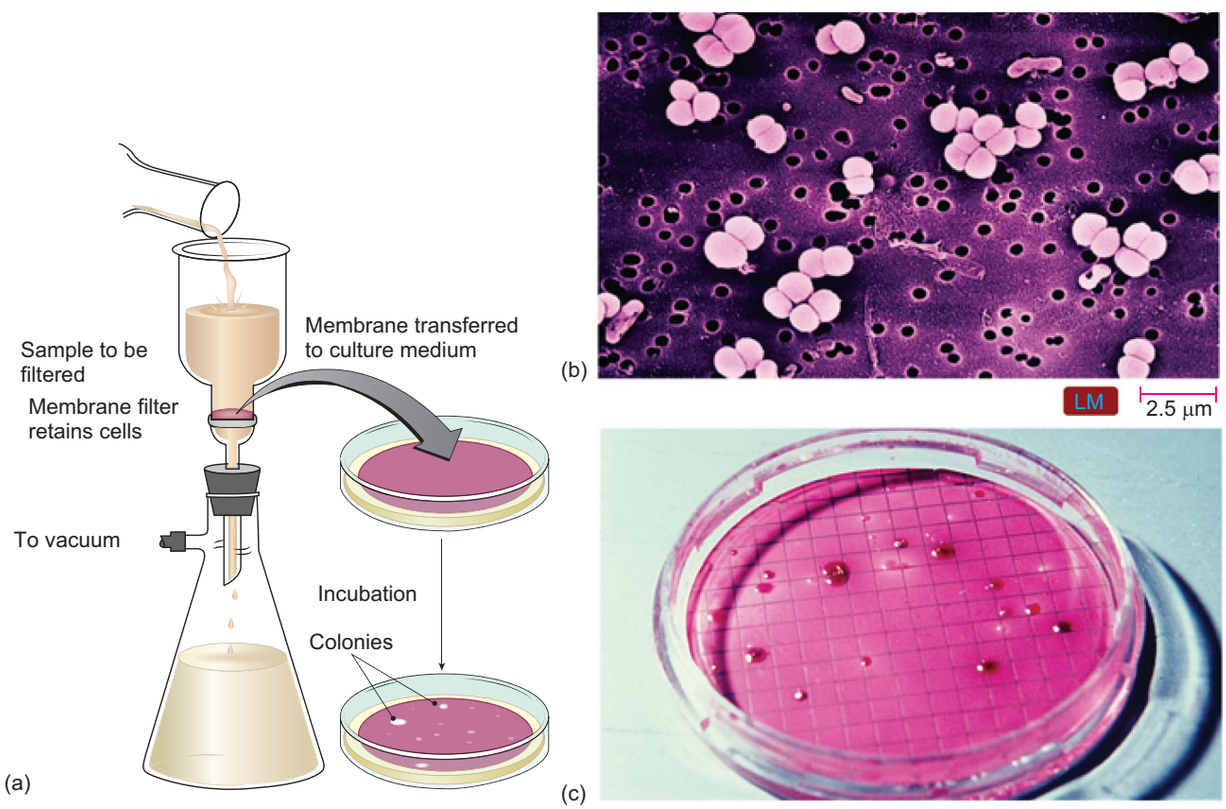


Figure 4.5: (a) Membrane filter apparatus (b) Light microscope image of microorganism filtered through membrane filter (c) Membrane filters showing microbial colonies on culture media

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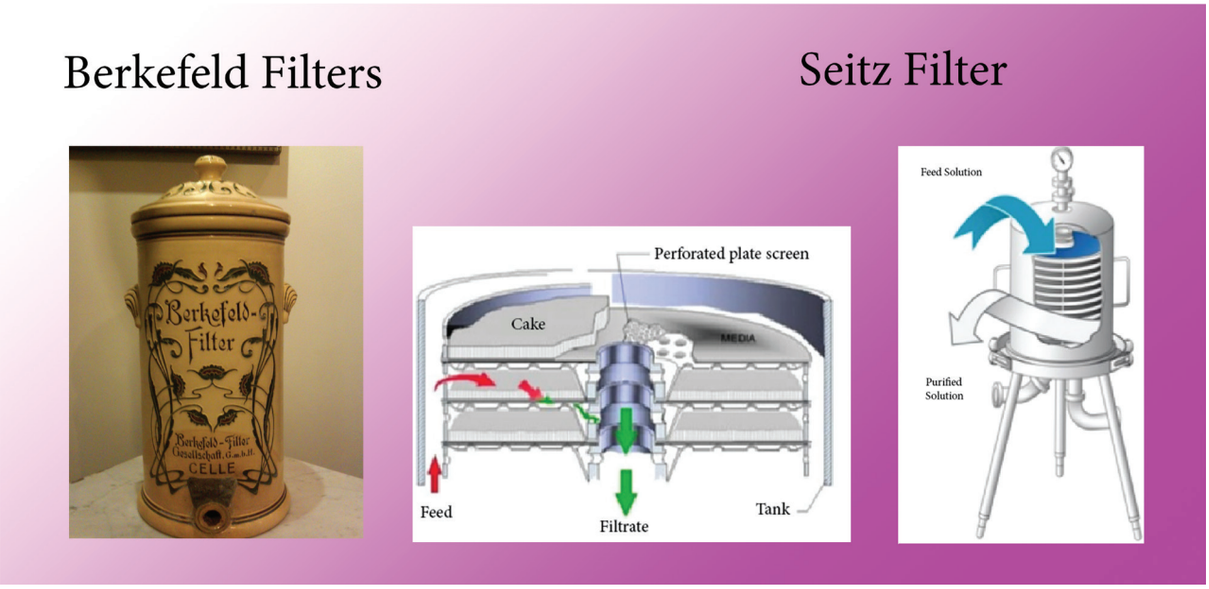


Figure 4.6: Types of depth filters

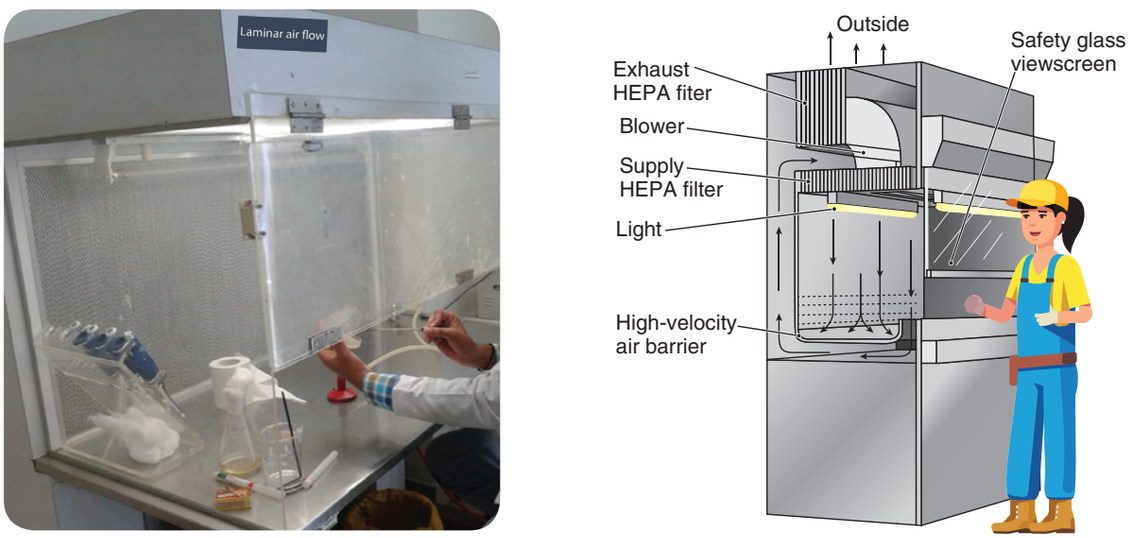


Figure 4.7: Laminar air flow

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

TN_GOVT_XI_Micro_Biology_CH04.indd 48 16-02-2019 9.56.38 AM

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

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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

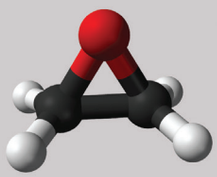


Surgical Instruments



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

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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 Petri dishes?
   a. Chlorine
   b. Ethylene oxide
   c. Autoclaving
   d. Nonionizing radiation
3. _____ kills organisms by coagulation and denaturing their proteins
   a. Dry heat
   b. Moist heat
   c. Both a & b
   d. None of the above
4. In which method, temperature of 160°C for 1 hour is employed?
   a. Red heat
   b. Infrared radiation
   c. Hot air oven
   d. Flaming
5. Which of the following temperature and time are employed in autoclave for sterilization of materials?
   a. 16 lbs 120°C for 18 min
   b. 18 lbs 180°C for 20 min
   c. 22 lbs 170°C for 35 min
   d. 15 lbs 121°C for 15 min
6. Wavelength used for the absorption of UV spectrum is
   a. 4000A°
   b. 2600A°
   c. 20A°
   d. None of the above

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Answer the following

1. Define Pasteurization.
2. What is Incineration?
3. Define membrane filters?
4. What is Sterilization?
5. Explain the principle of 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.

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Chapter 5

Cultivation of Microorganisms

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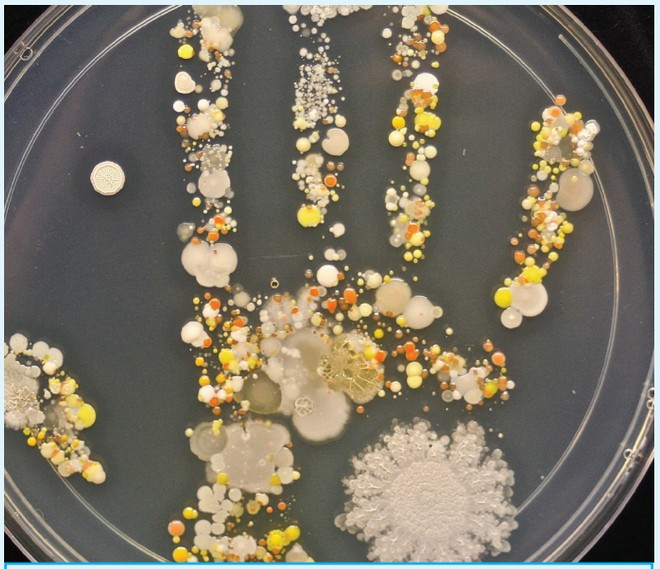
Chapter Outline

5.1 Significance of Culturing Microorganisms

5.2 Bacteriological Media and its Types

5.3 Pure Culture

5.4 Growth and Colony Characteristics of Bacteria and Fungi



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.

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

5.1 Significance of Culturing Microorganisms

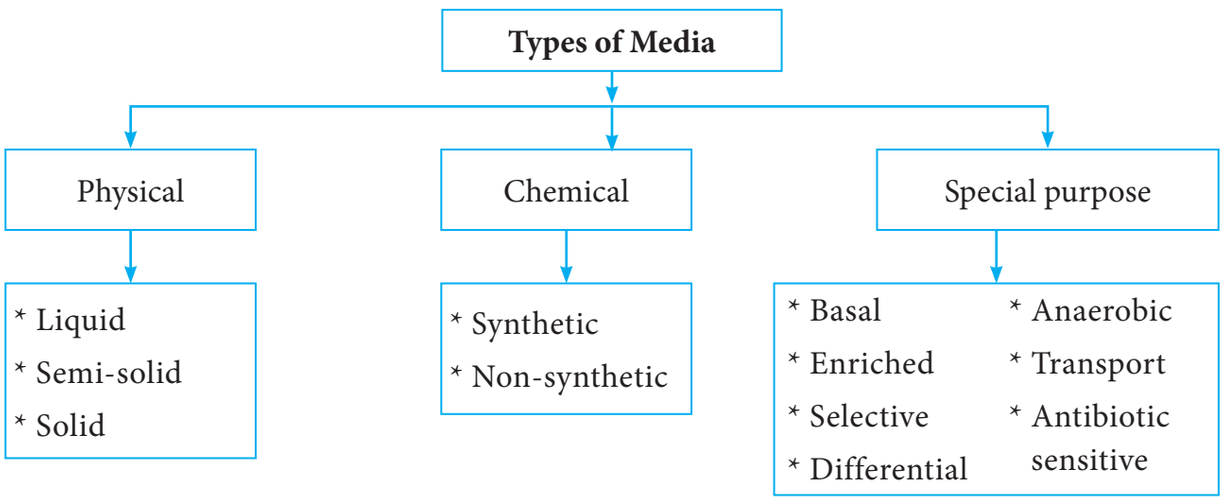
- To isolate microorganisms from any samples
- To study the morphology and biochemical characteristics of microorganisms
- To maintain the stock culture
- To identify disease causing microorganisms
- To study the role of microorganisms in the production of industrially important products

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

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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).

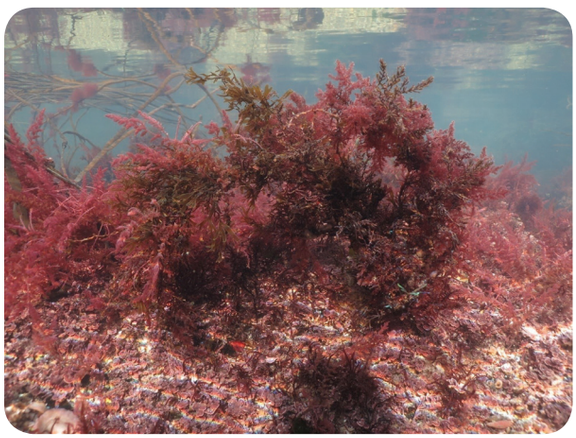


Figure 5.1: *Gelidium* – Red algae

- 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.

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

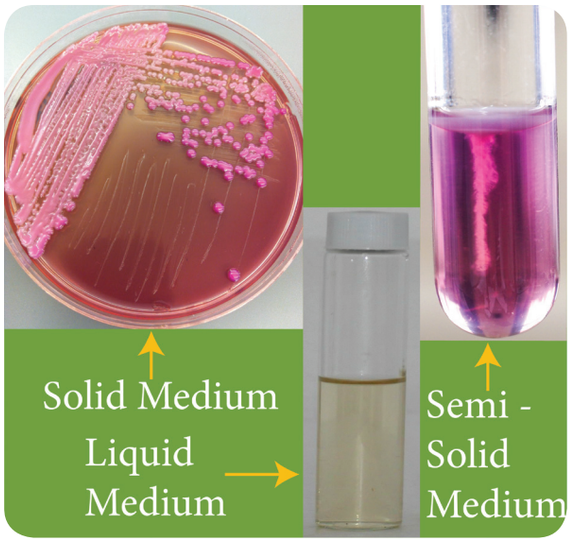


Figure 5.2: Solid, liquid and semi-solid media

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

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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

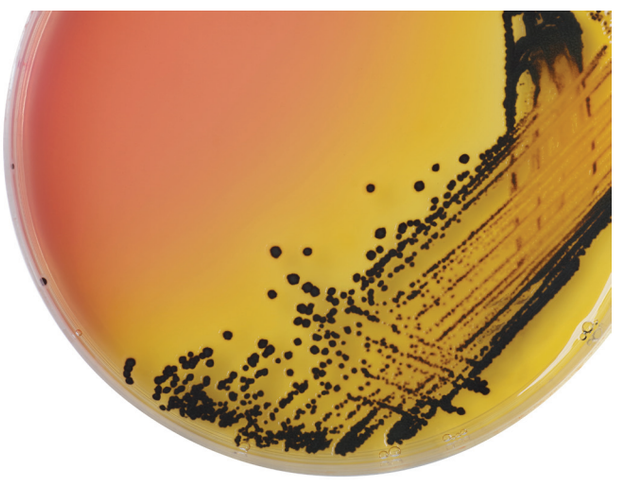


Figure 5.6: Growth of *Salmonella* on SS agar

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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).

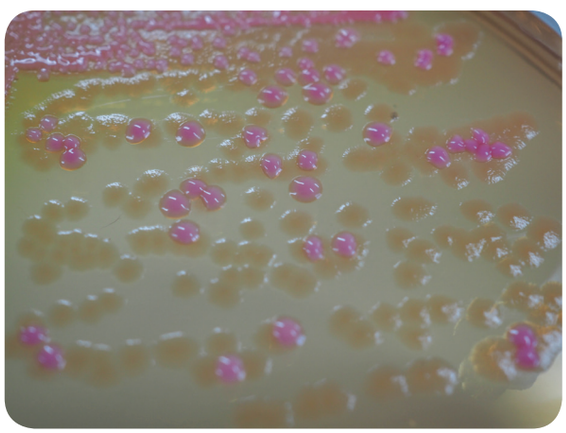


Figure 5.7: Growth of microorganisms on MacConkey agar (Lactose fermenting bacterial colonies appears pink)

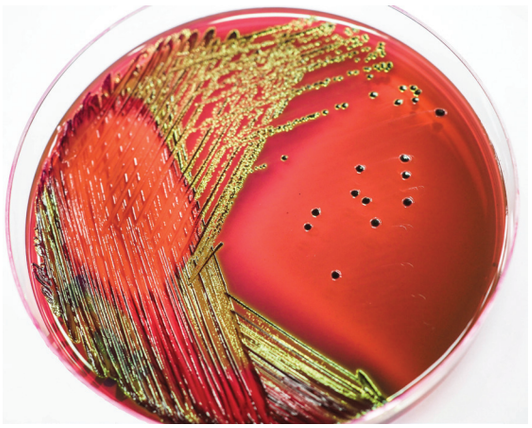
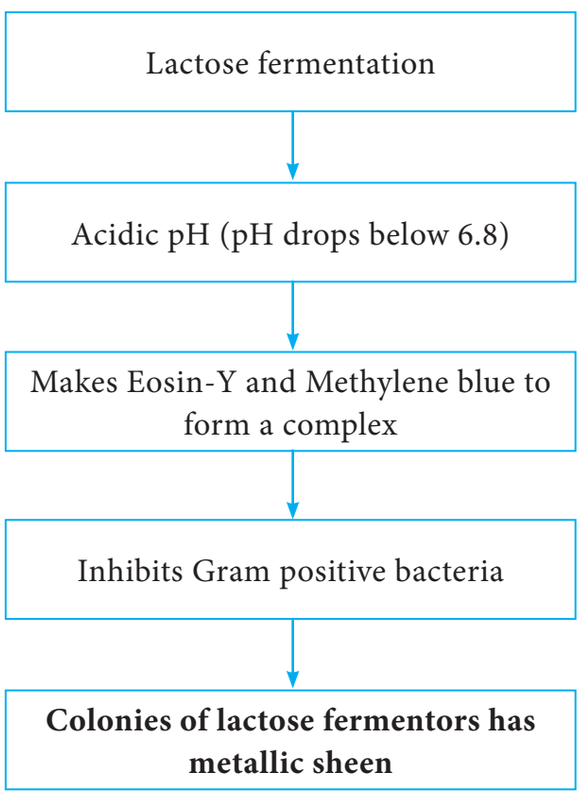


Figure 5.8: Growth of lactose fermenting bacteria on EMB Medium

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).



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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).

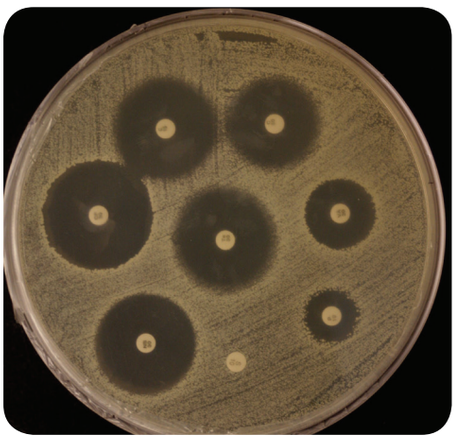


Figure 5.9: Antibiotic sensitivity on Muller Hinton agar

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

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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.

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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

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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

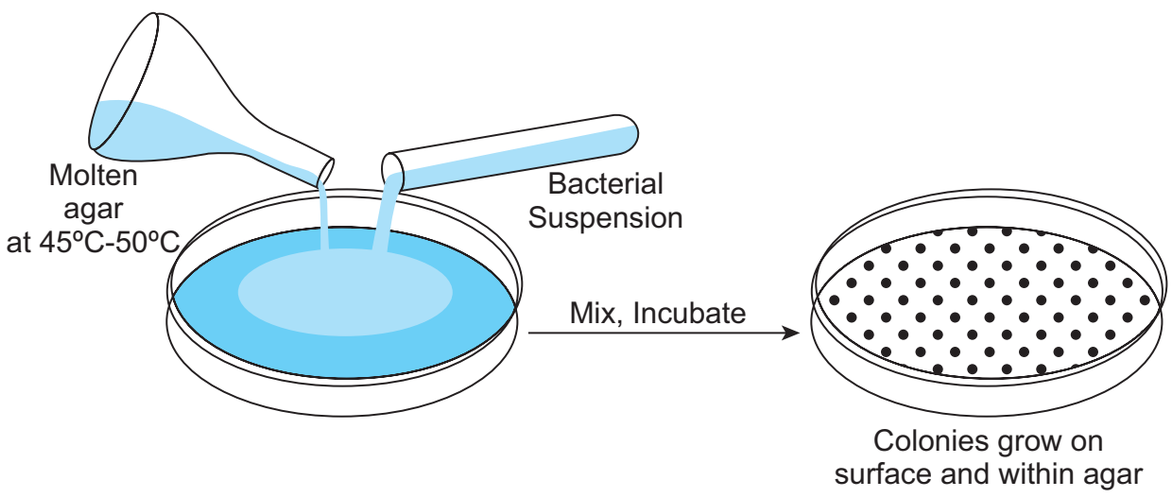


Figure 5.10: Pour plate method

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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.
- 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.

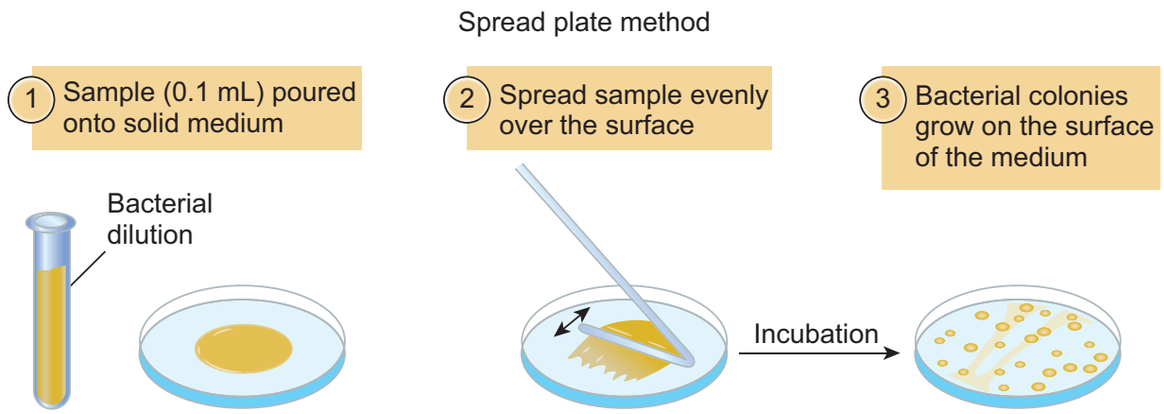


Figure 5.11: Spread plate method

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- 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.

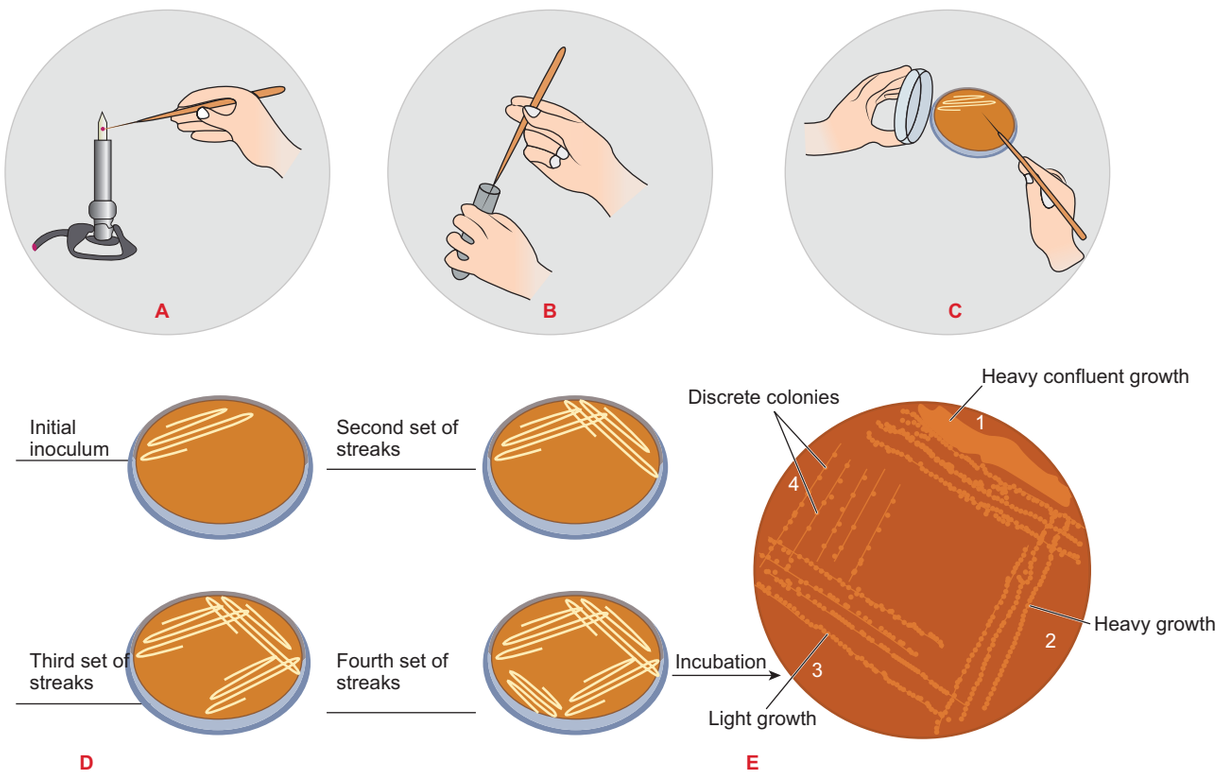


Figure 5.12: Steps in streak plate isolation method

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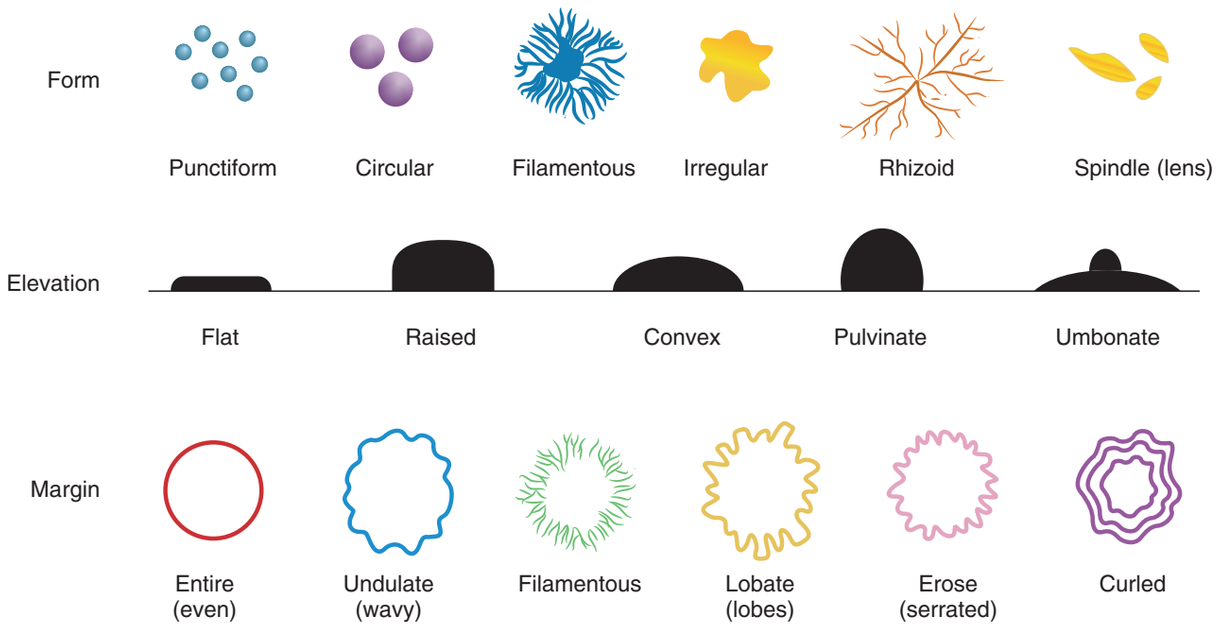


Figure 5.13: Colony morphology of bacteria

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.

TN_GOVT_XI_Micro_Biology_CH05.indd 64 16-02-2019 9.57.16 AM

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: Py-overdin. 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

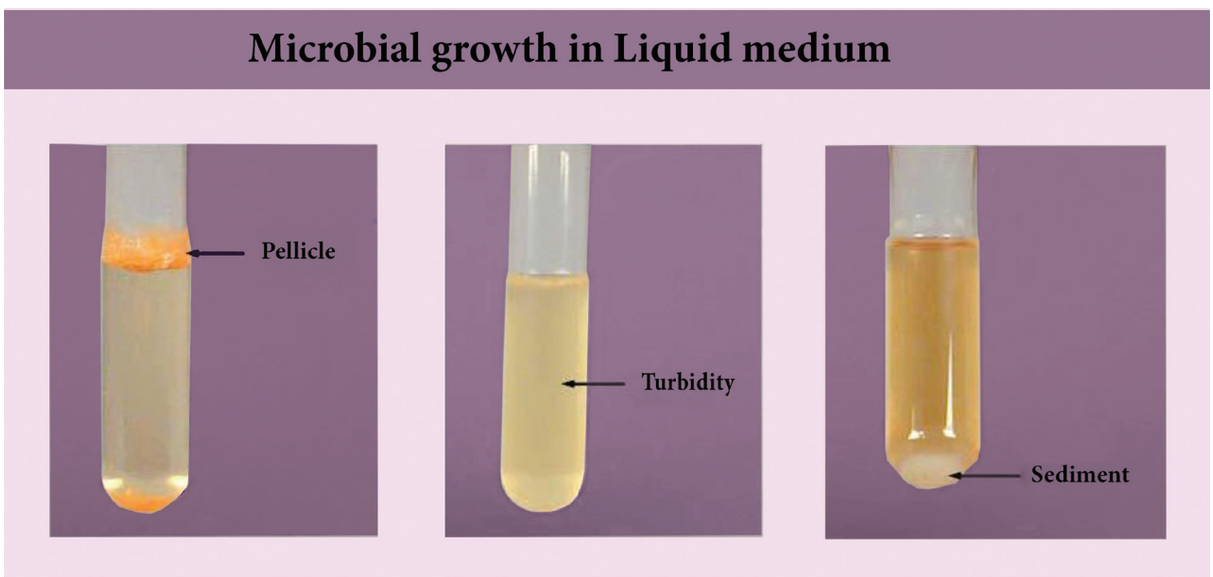


Figure 5.15: Microbial growth in Liquid medium

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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).

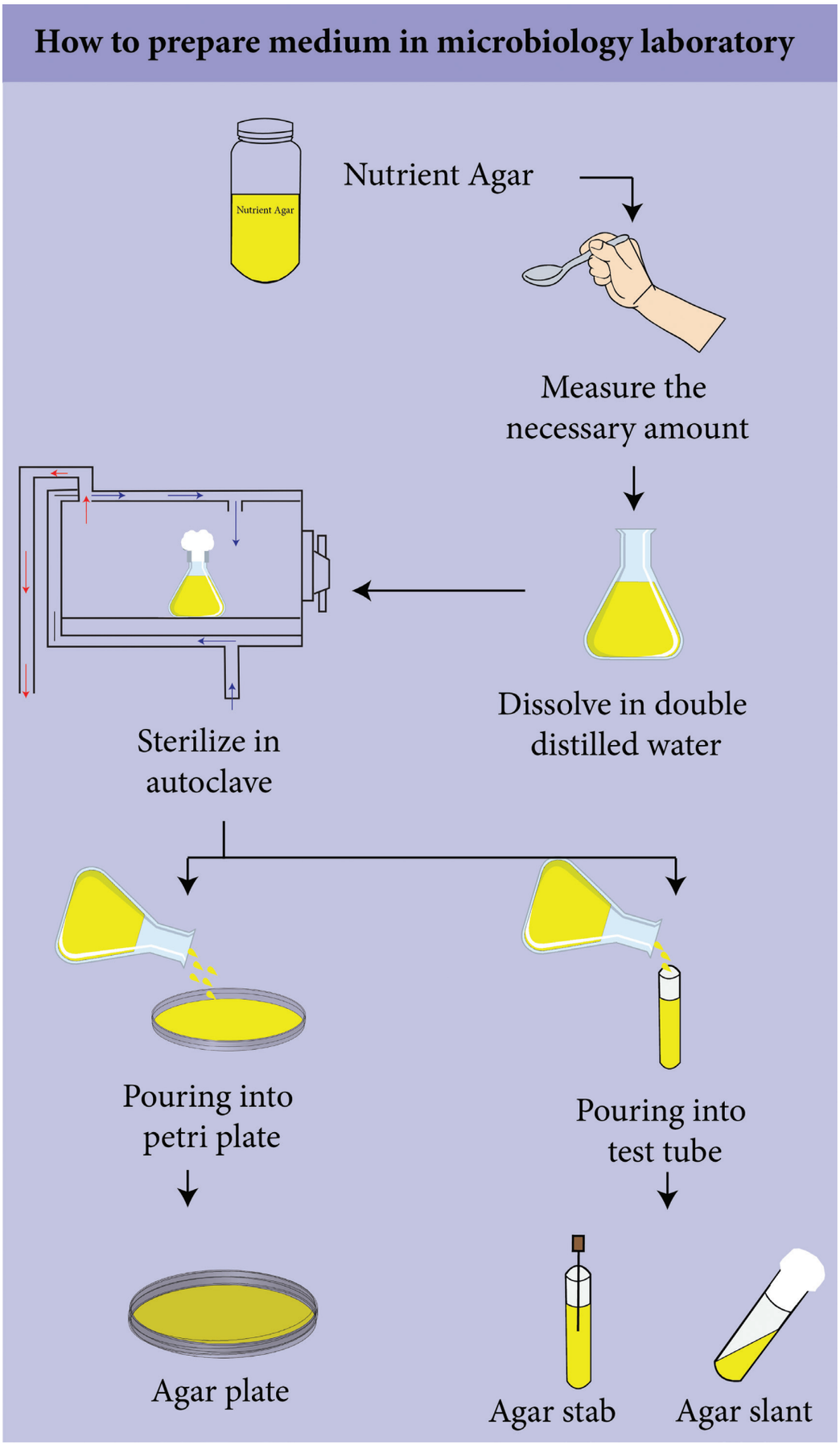




Figure 5.16: Fungal growth on Sabouraud Dextrose Agar media a) yeast growth b) mold growth

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How to prepare medium in microbiology laboratory

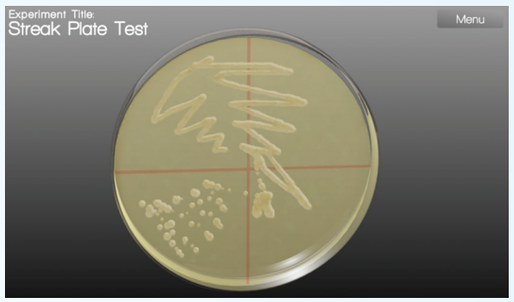


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ICT CORNER

Streak Plate Technique

Isolation of pure culture of bacteria

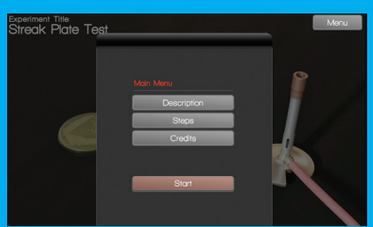


STEPS:

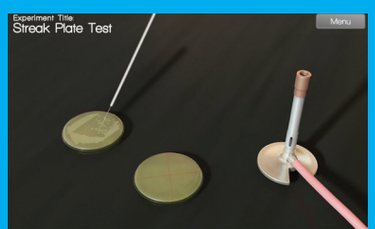
- Use the URL or scan the QR code to reach 'Virtual Interactive Bacteriology Laboratory.
- Click module and read the description and steps.
- Do the streak plating process from the top left part to the bottom left order.
- Heat and cool the loop between each steps.



Step1



Step2



Step3



Step4

URL:

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

TN_GOVT_XI_Micro_Biology_CH05.indd 68 16-02-2019 9.57.20 AM

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. 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

TN_GOVT_XI_Micro_Biology_CH05.indd 69 16-02-2019 9.57.20 AM

7. 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

8. 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

9. 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

10. 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

11. 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

12. 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

13. 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?

TN_GOVT_XI_Micro_Biology_CH05.indd 70 16-02-2019 9.57.20 AM

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.

TN_GOVT_XI_Micro_Biology_CH05.indd 71 16-02-2019 9.57.21 AM

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Chapter 6

Microbial Nutrition and Growth



Chapter Outline

6.1 Microbial Nutrients
6.2 Nutrient Requirement of Microorganisms
6.3 Nutritional Types of Microorganisms
6.4 Photosynthesis
6.5 Microbial Growth
6.6 Measurement of Microbial Growth



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.

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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.

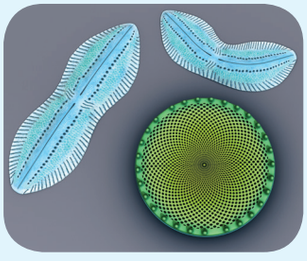
Calcium (Ca^{2+}) is involved in the heat resistance of bacterial endospores.

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.

TN_GOVT_XI_Micro_Biology_CH06.indd 73 16-02-2019 9.58.09 AM

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.

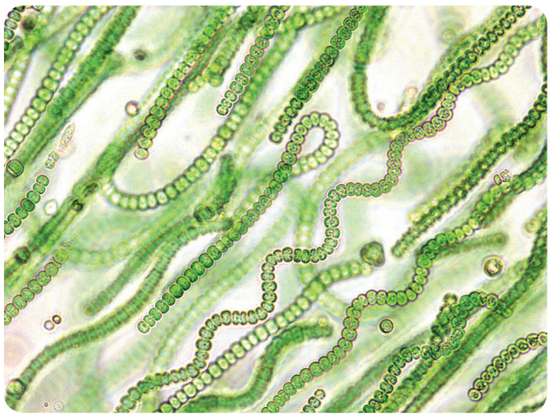


Figure 6.1: Microscopic view of *Cyanobacteria*

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

TN_GOVT_XI_Micro_Biology_CH06.indd 74 16-02-2019 9.58.09 AM

DO YOU KNOW?

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.

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.

Photosynthesis in Bacteria

There are four groups of photosynthetic bacteria. They are green sulphur bacteria

Table 6.2: Nutritional classes of Microorganisms

Nutritional class	Energy/Electron/Carbon source	Organisms
Litho photoautotrophs	Light energy Inorganic e^- donor CO_2	Cyanobacteria, Purple and Green sulphur Bacteria
Organo photoheterotrophs	Light energy Organic e^- donor Organic carbon source	Purple and Green Nonsulfur bacteria
Ltiho chemoautotrophs	Inorganic chemical compounds as energy source Inorganic e^- donor CO_2	Nitrifying bacteria, Iron bacteria
Organo chemoheterotrophs	Organic compounds as energy, electron and carbon source.	Most pathogenic bacteria, fungi and protozoa.

TN_GOVT_XI_Micro_Biology_CH06.indd 75 16-02-2019 9.58.09 AM

(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 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.

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 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.

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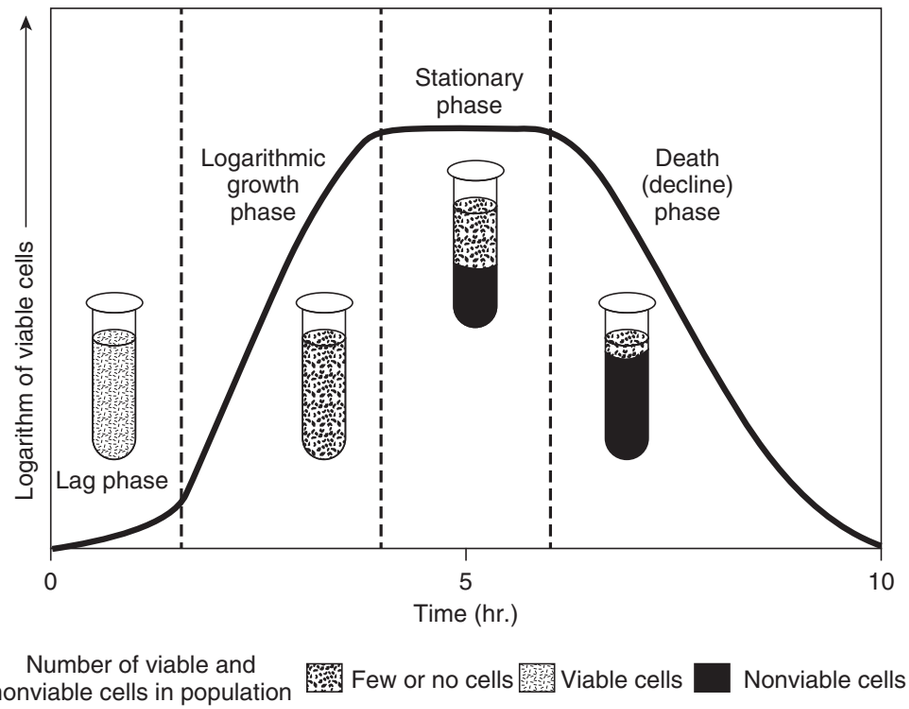


Figure 6.4: Bacterial growth curve showing phases of growth in laboratory conditions

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 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

TN_GOVT_XI_Micro_Biology_CH06.indd 77 16-02-2019 9.58.10 AM

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).

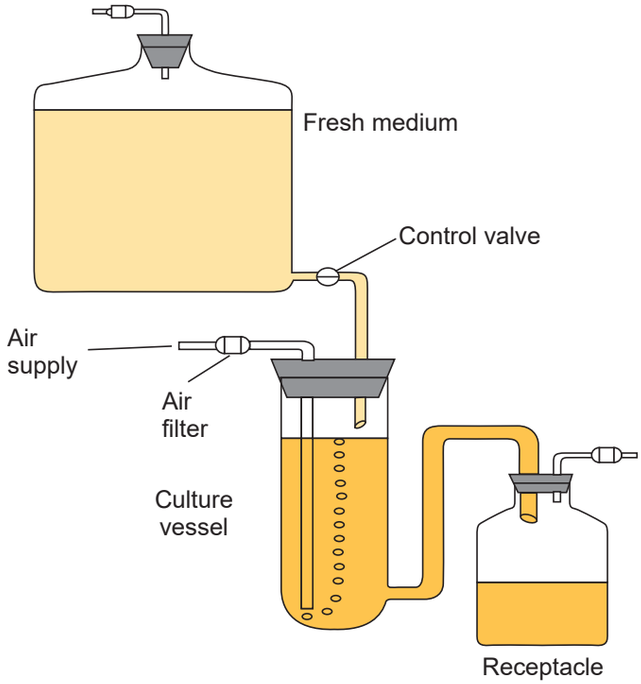


Figure 6.5: The Chemostat

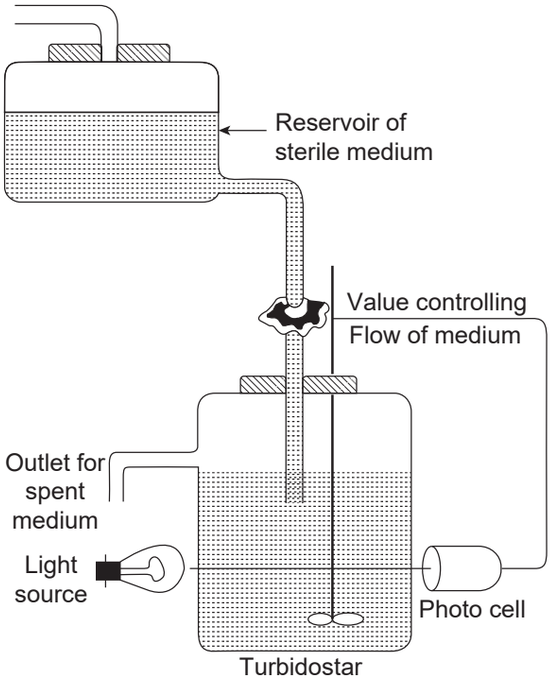


Figure 6.6: The Turbidostat

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
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

TN_GOVT_XI_Micro_Biology_CH06.indd 78 16-02-2019 9.58.10 AM

- 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.*



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?

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, 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.

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.

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.

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.

TN_GOVT_XI_Micro_Biology_CH06.indd 79 16-02-2019 9.58.11 AM

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 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.*



Crenation:

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

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.

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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

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.

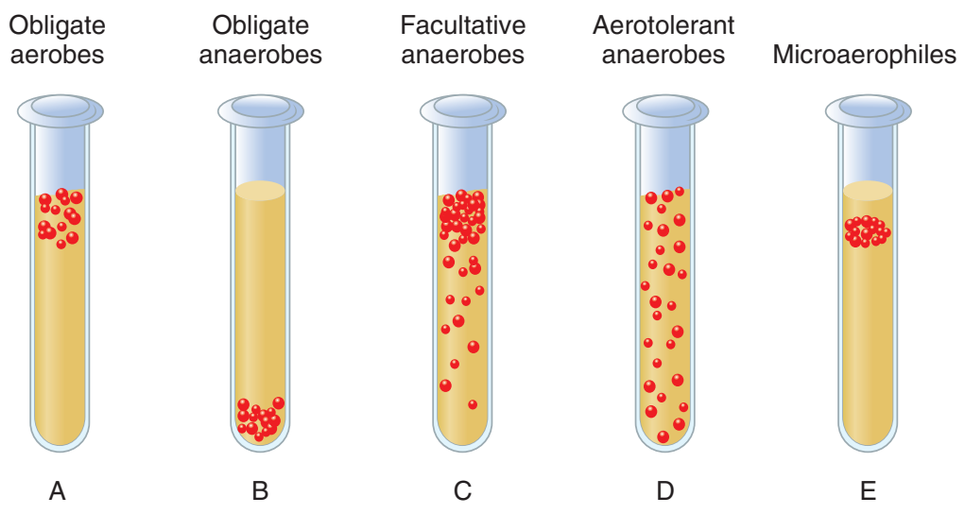


Figure 6.7: The effect of oxygen on the growth of various types of bacteria

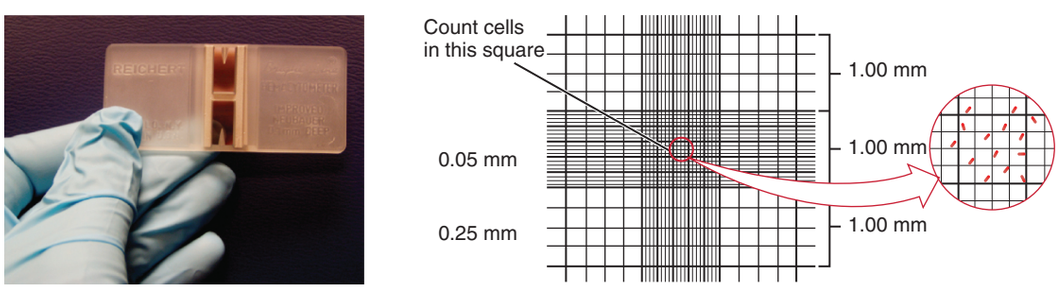


Figure 6.8: (a) Petroff-Hausser counting chamber
(b) Microscopic observation of bacterial cells

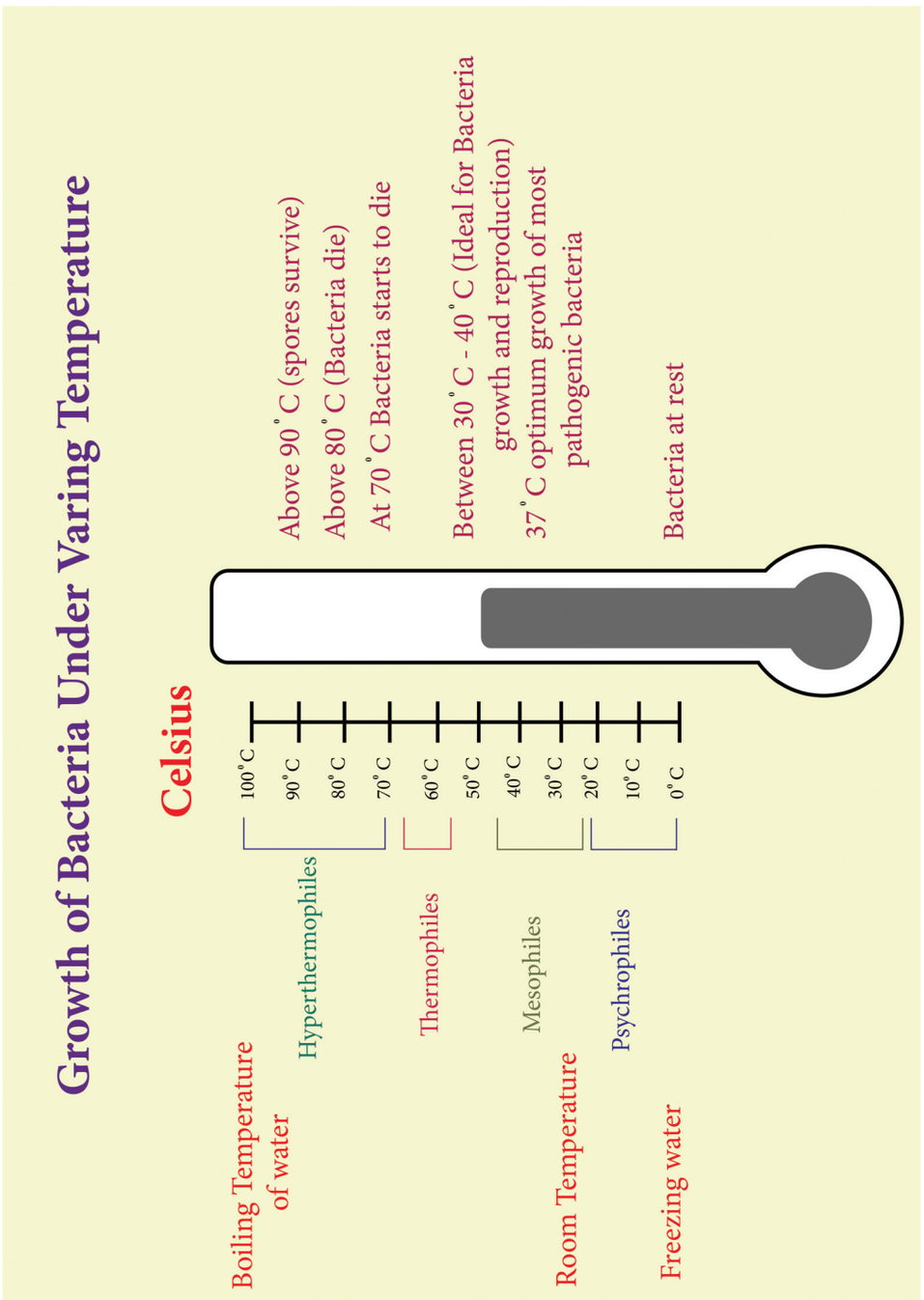
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Spread plate method

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.

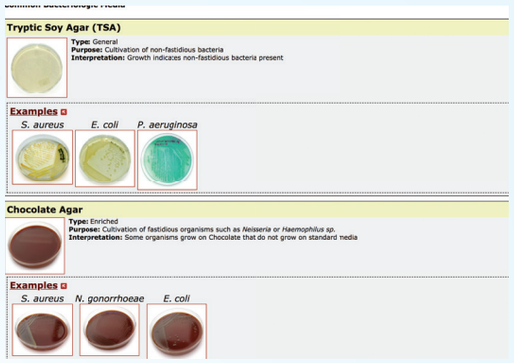


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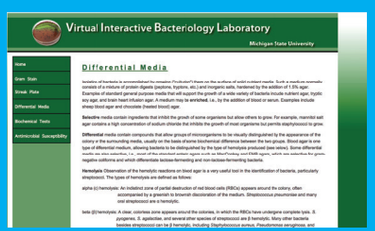
Culture Media

Preparation of Bacteriological Media

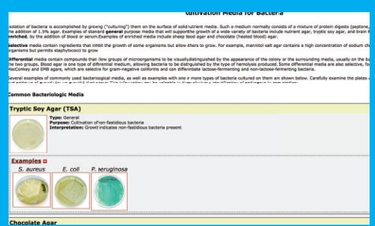


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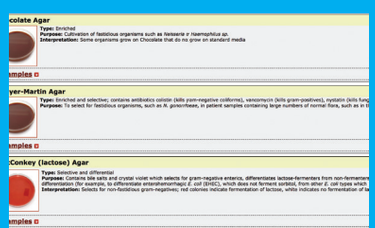
- Use the URL or scan the QR code to reach 'Virtual Interactive Bacterial Laboratory'.
- Click module at the bottom and read the description and steps.
- Follow the steps and open activities under' Common Bacteriologic Media' one by one and explore it.
- Record your observation of Differential Media.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



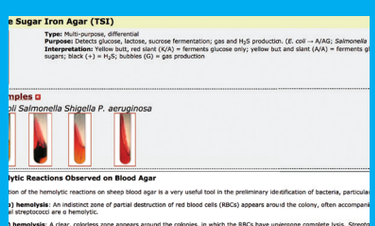
Step1



Step2



Step3



Step4

URL:

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

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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

QGHJV

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. Cyanobacteria
   b. Purple and green non sulphur bacteria
   c. Iron bacteria
   d. Protozoa
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*

TN_GOVT_XI_Micro_Biology_CH06.indd 84 16-02-2019 9.58.15 AM

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.

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Chapter 7

Morphology of Bacteria

Chapter Outline

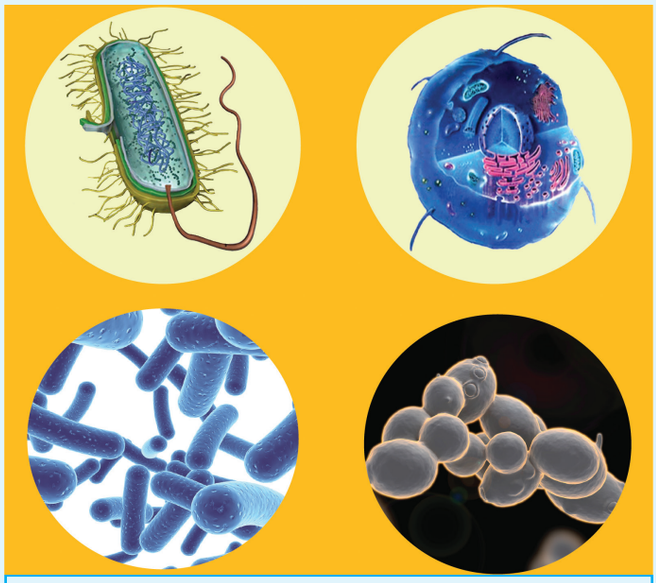
7.1 Bacterial Size, Shape and Arrangement

7.2 Structures External to Cell Wall of Bacteria

7.3 Cell Envelope of Bacteria

7.4 Structures Internal to Cell Membrane of Bacteria

7.5 Eukaryotic Cell Structure



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.

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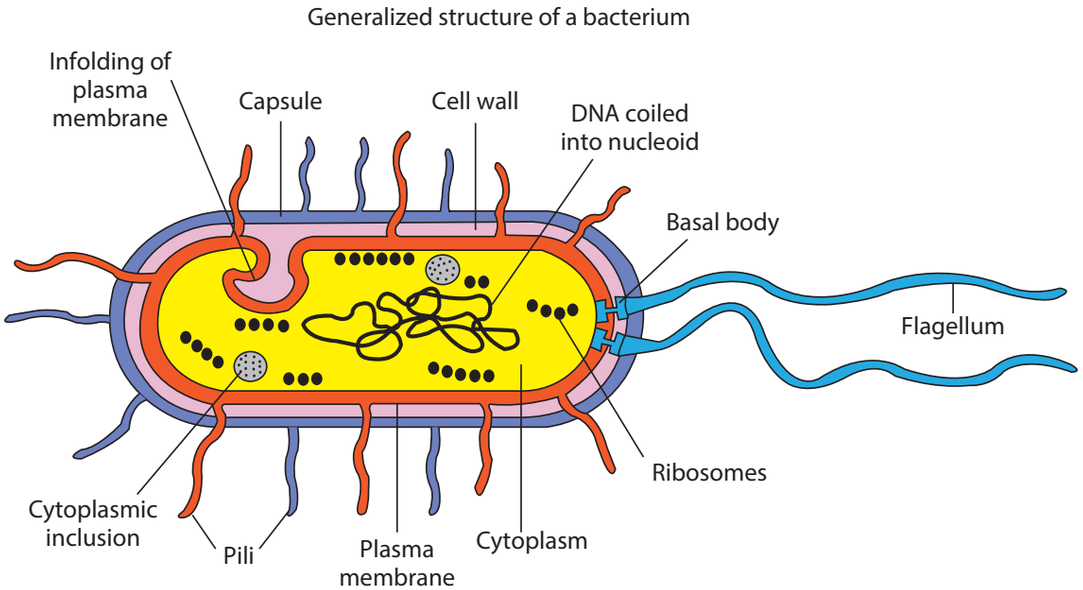


Figure 7.1: Generalized structure of a bacterium

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

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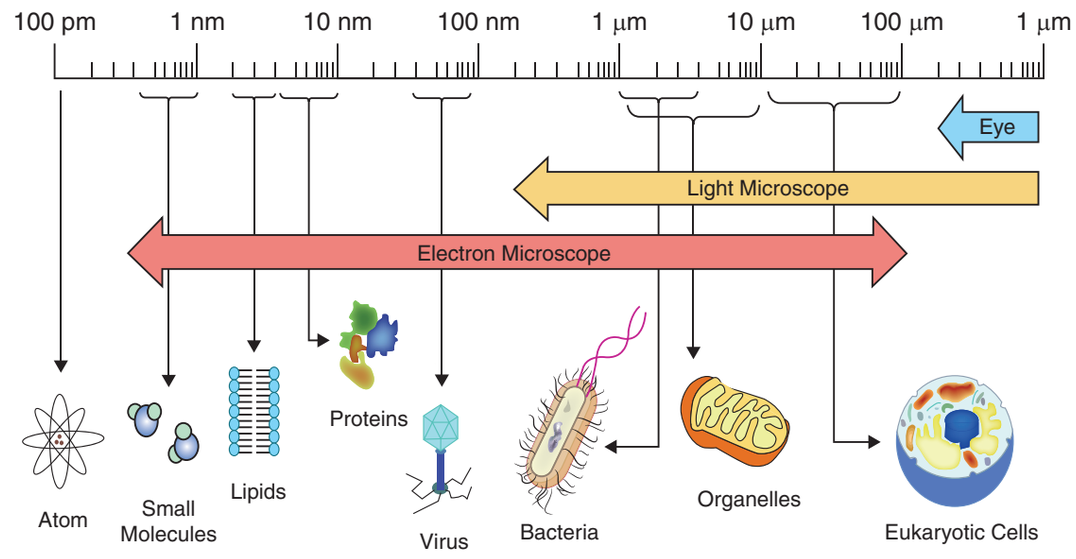


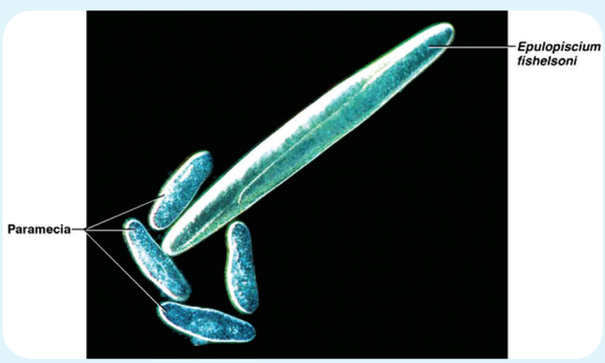
Figure 7.2: Metric unit of measurement

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).

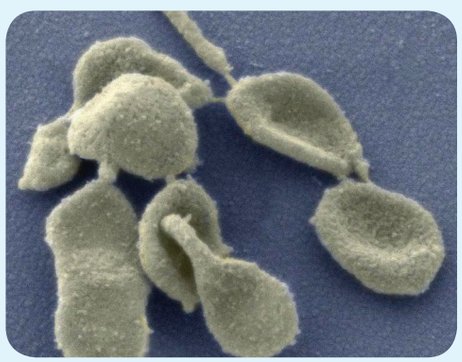
1 metre (m)	=	1000mm (millimeter)
1mm (10^{-3}m)	=	1000 µm (micrometer)
1 µm (10^{-6}m)	=	1000nm (nanometer)
1nm (10^{-9}m)	=	1000pm (picometer)
1A^0 (10^{-10}m) (angstrom)		

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

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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*.

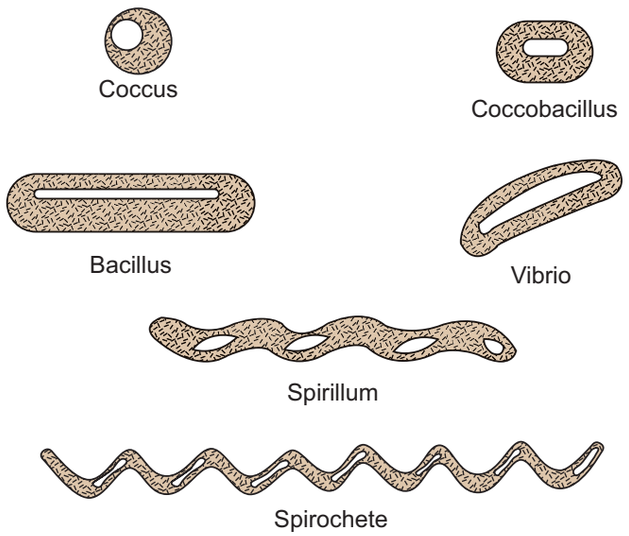
7.2 Structures External to Cell Wall of Bacetria

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.

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Different Bacterial Shapes

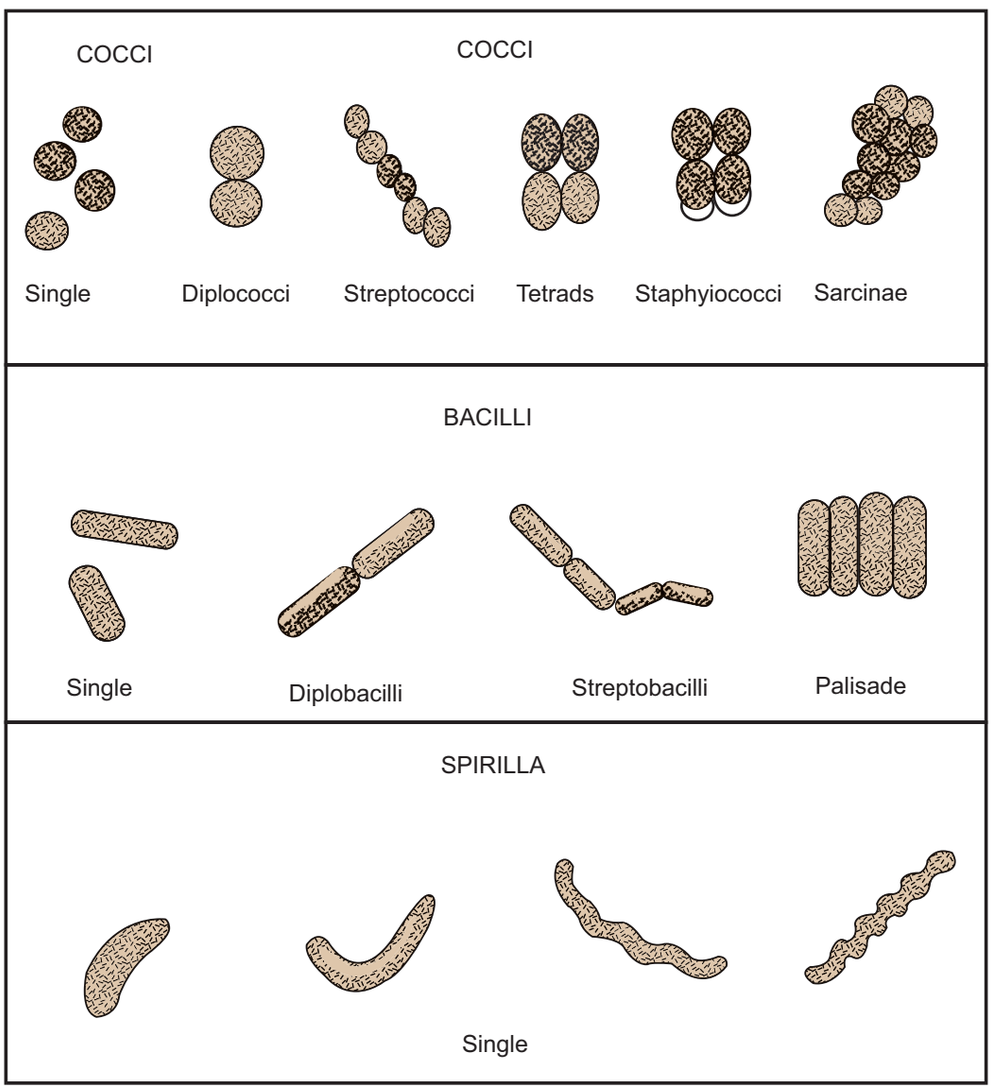


Figure 7.3: Shapes and arrangement of bacteria

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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.

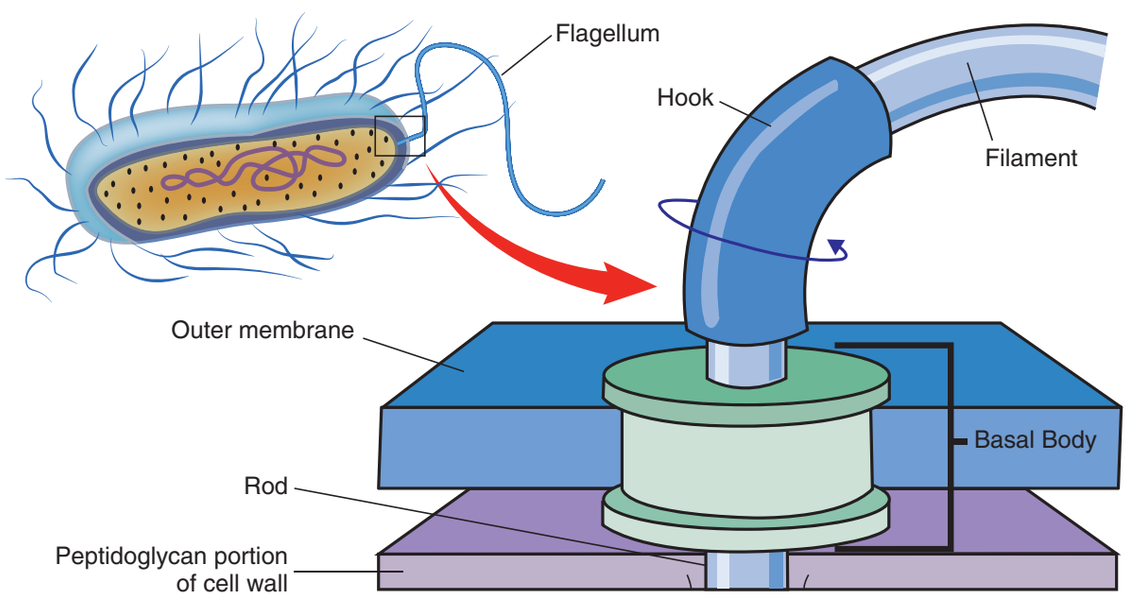
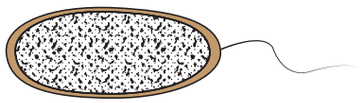
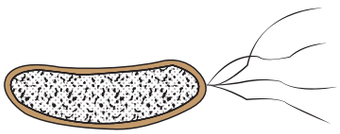
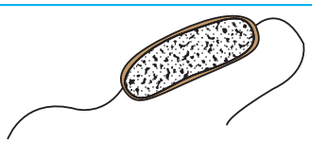
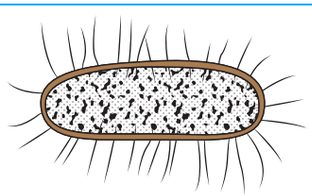


Figure 7.4: Structure of bacterial flagella

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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.

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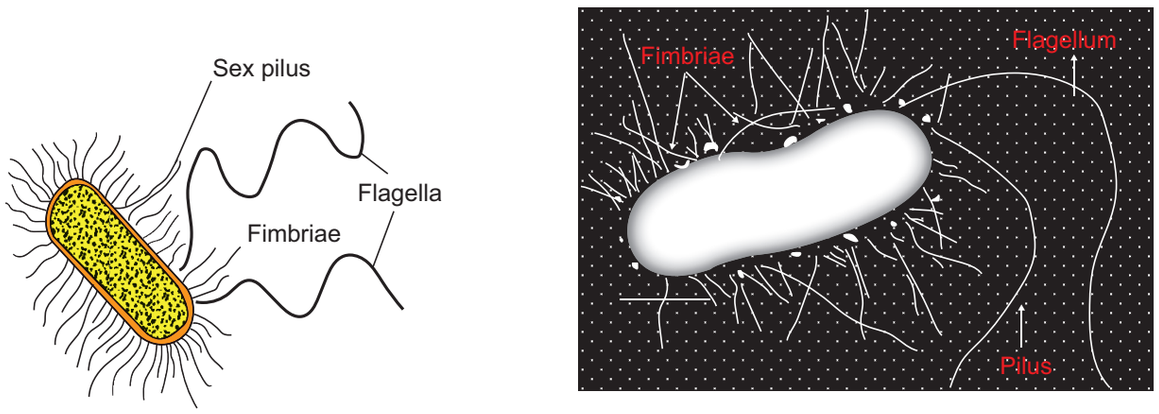


Figure 7.5: Structures of pili and fimbriae

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

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Table 7.3: Difference between Capsule and Slime layer

Capsule	Slime layer
Capsule is a glycocalyx layer, consisting of firmly associated polysaccharide molecules with the cell wall.	Slime layer is a glycocalyx layer that consists of loosely associated glycoprotein molecules.
It is a well-organized layer, difficult to be washed off.	It is an unorganized layer and can be easily washed off.
It is tightly bound to the cell wall.	It is loosely bound to the cell wall.
It is thicker than slime layer.	It is a thin glycocalyx layer.
It acts as a virulence factor that helps to escape phagocytosis.	It mainly helps in adherence. It protects the cell from dehydration and nutrient loss.

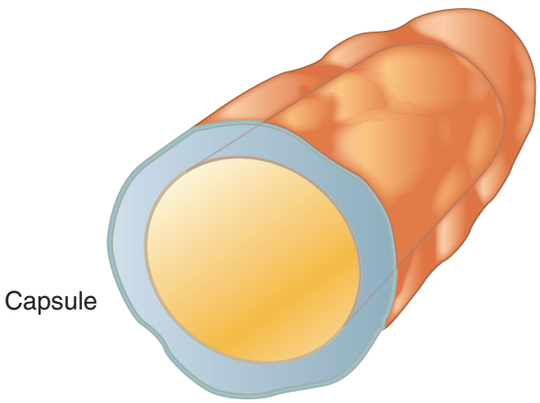


Figure 7.6: Structure of capsule

The role of the capsule varies depending on the bacterium.

- A thick capsule protects cells from dehydration.
- Capsules protect the pathogenic bacteria from being engulfed and destroyed by white blood cells (phagocytes).
- Capsules are virulence factors of many pathogenic bacteria, such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Bacillus anthracis*. Encapsulated bacterial cells generally have greater virulence.

Slime layer

Some bacteria are covered with a surface layer that is loosely distributed around the cell and diffuses into the medium, this surface layer is referred to as slime layer. (Figure 7.7) The slime layer is a structure that is easily washed off. Slime layer protects bacteria from loss of water and nutrients. Slime has little affinity for basic dyes and is ivisible in Gram stained smears.

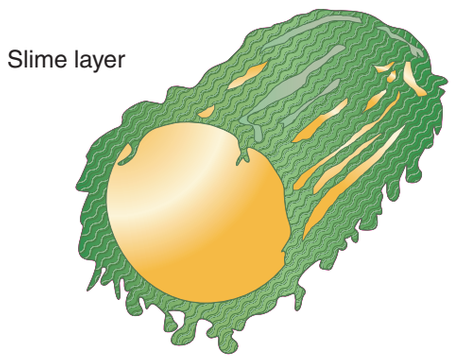


Figure 7.7: Structure of slime layer

7.2.3 Other Appendages

Sheath

Sheathed bacteria are bacteria that grow as long filaments in the form of chain or trichome. These bacteria are enclosed by a hollow tube like structure known as sheath

TN_GOVT_XI_Micro_Biology_CH07.indd 94 16-02-2019 9.59.25 AM

(Figure 7.8). Within the shealth, the bacteria are capable of growth and division. Aquatic bacteria mostly form sheath. Examples of sheathed bacteria include *Leptothrix discophora* (also known as iron bacteria), *Sphaerotilus* and *Clonothrix*.

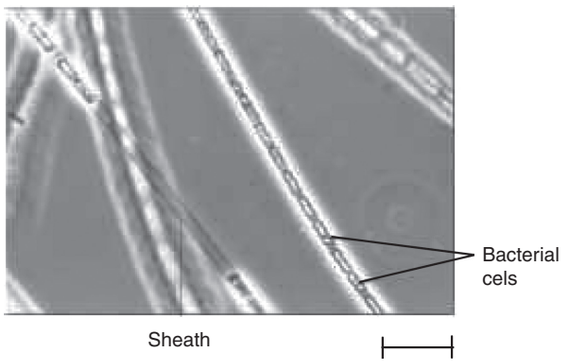


Figure 7.8: Sheathed bacterium

Function:

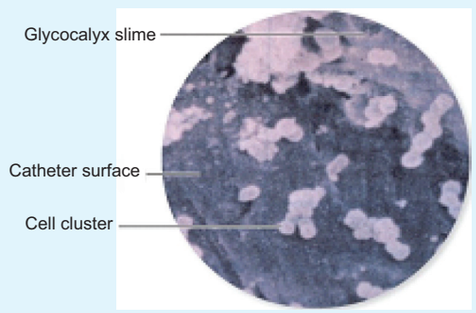
- It provides mechanical support.
- In a few bacteria, shealth is strengthened by the deposition of ferric and manganese hydroxides.

Prosthecae

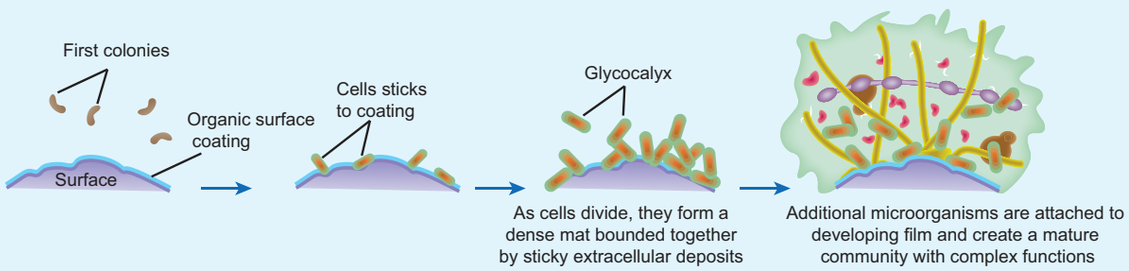
They are semi rigid extensions of cell wall and cell membrane. Some bacteria may contain more than one prosthecae (Figure 7.9). Aerobic bacteria in fresh water and marine environment possess prosthecae. Some of the prosthecate bacteria are *Caulobacter, Stellar, Prosthecobacter and Hyphomicrobium*.

Infobits

Biofilms:



Microbial adhesion to animate or inanimate surfaces can be mediated by polysaccharides capsules or slime. These adherence polymers are collectively called as adhesions. Microorganism tend to adhere to any surface and the layer they produce is called Biofilm. Biofilm can be harmful or beneficial to humans. Biofilm formation is a critical issue for almost all surfaces in health care and food preparation settings. Biofilms may form on a wide variety of surfaces, including living tissues, medical devices, industrial or portable water piping system, etc., Biofilm formation is a multi-step process starting with attachment to a surface, then formation of three dimensional structure and finally ending with maturation and detachment. During biofilm formation many species of bacteria are able to communicate with one another through specific mechanism called quorum sensing.



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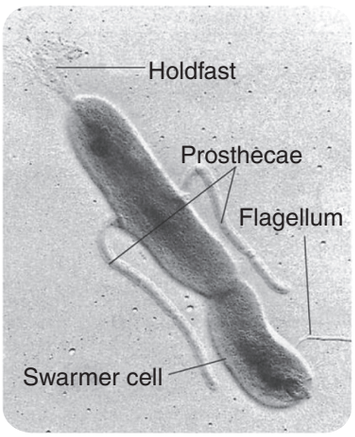


Figure 7.9: Prosthecate bacteria

Function:

- Prosthecae increase surface area for absorption of nutrients from the dilute aquatic environment.
- Helps in adhesion.
- Some prosthecae develop bud at the tip and helps in asexual reproduction.

Stalk

It is a nonliving ribbon like tubular structure. It is formed by excretory product of bacteria. Some of the stalked bacteria are *Gallionella, Planctomyces* (Figure 7.10).

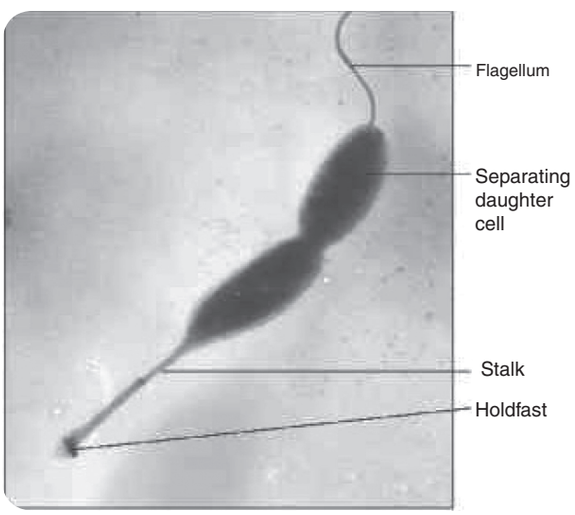


Figure 7.10: Stalked bacteria

Function:

- Stalk helps in attachment of cells to solid surface.

7.3 Cell Envelope of Bacteria

Cell envelope is an external covering that lies outside the cytoplasm. It is composed of two or three basic layers: the cell wall, the cell membrane and in some bacteria the outer membrane.

7.3.1 Structure of Prokaryotic Cell Wall

Prokaryotic cells almost always are bounded by a chemically complex cell wall. Cell wall lies beneath the external structures (capsules, sheaths and flagella). Cell wall lies external to the plasma membrane (cell membrane). Cell wall of eubacteria is made up of **peptidoglycan** or **murein**, whereas that of Archaeobacteria is composed of proteins, glycoproteins or polysaccharides. A few genera such as *Methanobacterium*, have cell walls composed of **pseudomurein**, a polymer whose structure superficially resemble eubacteria peptidoglycan of eubacteria but differs markedly in chemical composition.(Note: Ordinary or typical bacteria are sometimes called eubacteria to distinguish them from the phylogenetically distinct group known as archaeobacteria). Peptidoglycan is a cross linked polymer of enormous strength and rigidity. It is a polymer composed of many identical subunits (Figure 7.11). Peptidoglycan differs somewhat in composition and structure from one species to another, but it is basically a polymer of N-acetylglucosamine(NAG), N-acetylmuramic acid(NAM), L-alanine, D-alanine, D-glutamate, and a diamino acid (LL- or meso-diaminopimelic acid, L-lysine, L-ornithine, or L- diaminobutyric acid).

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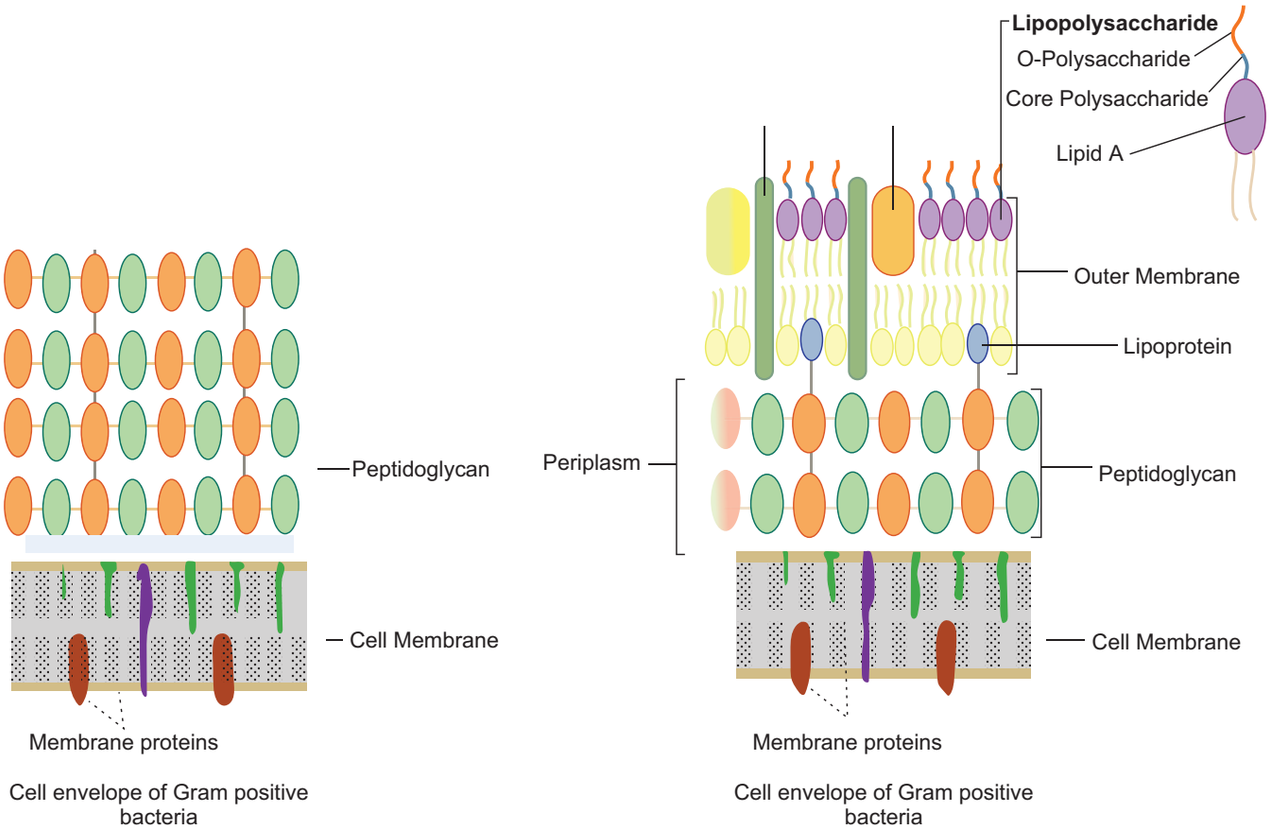


Figure 7.11: Cell envelope of Gram positive and Gram negative bacteria

Cell wall may contain other substances in addition to peptidoglycan. For instance, *Staphylococcus aureus* and *Streptococcus fecalis* contain **teichoic acids** (polymer of acidic polysaccharides) covalently linked to peptidoglycan. Cell wall of Gram positive bacteria contain very little lipid but *Mycobacterium* and *Corynebacterium* cell walls are rich in **mycolic acid** (or Cord factor) which make them acid fast. When stained, the cells cannot be decolorized easily despite treatment with dilute acids. *Mycoplasma* lack cell wall.

Protoplast is a bacterial cell consisting of cell material bound by a cytoplasmic membrane.

Spheroplast is a bacterial cell with two membranes namely the cytoplasmic membrane and the outer membrane but no cell wall.

Functions of cell wall

- It gives shape to bacteria like a bicycle tyre that maintains the necessary shape and prevents the more delicate inner tube (the cytoplasmic membrane) from bursting when it is expanded.
- It protects bacteria from osmotic lysis in dilute solutions (hypotonic environment).
- It protects cell from toxic substances.

HOTS

How do bacteria maintain their shape?

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7.3.2 Structure of Outer Membrane

Eubacteria and Archaeobacteria (Gram positive and Gram negative) differ with respect to their cell walls. Gram negative cell walls are more complex. An outer membrane surrounds a thin underlying layer of peptidoglycan (Table 7.4). Outer membrane is bilayered, consisting mainly of phospholipids, proteins and lipopolysaccharide (LPS).

LPS is composed of three parts which are covalently linked to each other. They are

1. **Lipid A** which is firmly embedded in the membrane,
2. **Core polysaccharide** that is located at the membrane surface and
3. **Polysaccharide O antigens** that extend like whiskers from the membrane surface into the surrounding medium

Special protein channels called porins span the membrane. The points of contact between outermembrane and cytoplasmic membrane are known as adhesions. Outer membrane is anchored to peptidoglycan layer by means of **Braun's** lipoprotein. Periplasmic space between the cell membrane and the outer membrane.

Functions of outer membrane

- It serves as an impermeable barrier to prevent the escape of important enzymes (such as those involved in cell wall growth) from the periplasmic space.
- It serves as a barrier to various external chemicals and enzymes that could damage the cell. For example, the walls of many Gram positive bacteria can be easily destroyed by treatment with an enzyme called lysozyme, which selectively

Table 7.4: Difference between Gram positive and Gram negative bacteria

	Gram positive bacteria	Gram negative bacteria
Gram reaction	The bacteria that retain the colour of the primary stain (crystal violet) are Gram positive	The bacteria that cannot retain the primary stain but takes on the colour of the counterstain safranin are called Gram negative
Cell wall	The cell wall is thick (20-30nm thick)	The cell wall is thin (8-12nm thick)
Peptidoglycan layer	Thick (multilayered)	Thin (single layered)
LPS content Lipopolysaccharide	None	High
Periplasmic space	Absent	Present
Outer membrane	Absent	Present
Lipid and lipoprotein content	Low (acid fast bacteria have lipids linked to peptidoglycan)	High due to the presence of outer membrane
Teichoic acids	Present in many	Absent
Example:	*Streptococcus, Staphylococcus, Corynebacterium, Bacillus, Clostridium*	*Escherichia coli, Pseudomonas, Haemophilus, Salmonella, Shigella.*

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dissolves peptidoglycan. However, Gram negative bacteria are refractory to this enzyme because large protein molecules of enzyme cannot penetrate the outer membrane. Only when outer membrane is damaged the enzyme can penetrate.

- Porins allow the smaller molecules, such as amino acids, monosaccharides to pass across.
- Adhesions are export sites for newly synthesised LPS and porins, and are sites at which pili and flagella are made.

7.3.3 Structure of Cytoplasmic Membrane

Immediately beneath the cell wall is the cytoplasmic membrane also known as plasma membrane or cell membrane. It is composed of phospholipids and proteins. The phospholipids form a bilayer. Integral proteins are embedded within this bilayer. Surface proteins or peripheral proteins are loosely attached to the bilayer. The lipid matrix of the membrane has fluidity, allowing the components to move around laterally. In eubacteria, the phospholipids are phosphoglycerides, in which straight chain fatty acids are ester linked to glycerol. In archaeobacteria, the lipids are polyisoprenoid branched-chain lipids, in which long-chain branched alcohols (phytanols) are ether linked to glycerol.

Functions of the cell membrane

- Prokaryotes do not have intracellular membrane bound organelles as present in eukaryotic organelles. Thus cell membrane provides a site for functions such as energy reactions, nutrient processing and synthesis.
- It regulates transport, the passage of nutrients into the cell and the discharge of wastes. It is a selectively permeable membrane.
- It is also involved in secretion or discharge of a metabolic product into extracellular environment.
- Cell membrane is an important site for a number of metabolic activities. Most enzymes of respiration and ATP synthesis reside in the cell membrane since prokaryotes lack mitochondria.

Significance of cell envelope

- It has toxic properties (Example: LPS)
- It stimulates antibody production by immune system
- The cell walls of many pathogens have components that contribute to their pathogenicity. Example mycolic acids of *Mycobacterium tuberculosis*
- Cell wall is a site of action of several antibiotics.
- Many of the serological properties of Gram negative bacteria are attributable to O antigens; they can also serve as receptors for bacteriophage attachment.

7.4 Structures Internal to Cell Membrane of Bacteria

Cytoplasm is called as the internal matrix of the cell inside the cell membrane. Its major component is water (70-80%). It also contains proteins carbohydrates, lipids, inorganic ions, and certain low molecular weight compounds. Inorganic ions are present in much higher concentrations in cytoplasm than in most media.

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Cytoplasm is thick, aqueous, semi-transparent and elastic. The major structures in the cytoplasm of prokaryotes are nucleoid (containing DNA), ribosomes and reserve deposits called inclusions. Prokaryotic cytoplasm lacks certain features of eukaryotic cytoplasm such as a cytoskeleton and cytoplasmic streaming.

Ribosomes

All living cells contain ribosomes. They are the sites of protein synthesis. High number of ribosomes represents the high rate of protein synthesis. Prokaryotic ribosomes are freely found in the cytoplasm, whereas eukaryotic ribosomes are attached to the cell membrane. Prokaryotic ribosomes consists of protein and a type of RNA called ribosomal RNA. They are smaller and less dense than the eukaryotic ribosomes. The ribosomes of prokaryotes are 70S where as that of eukaryote are 80S (Figure 7.12).

Nucleus

The nuclear area has the hereditary material of most bacteria. It contains a single, circular, long, continuous, thread like double stranded DNA called the bacterial chromosome. Some bacteria with linear chromosome also exist. It carries the information required for the cells structure and function. They are not surrounded by a nuclear envelope and are devoid of highly conserved histone proteins. The nuclear area can be spherical, elongated or dumbbell shaped. In actively growing bacteria, as much as 20% of the cell volume is occupied by DNA, because such cells presynthesize nuclear material for future cells. The chromosome is attached to the cell membrane. Proteins in the plasma membrane are believed to be responsible for the replication of the DNA and segregation of the new chromosomes to daughter cells in cell division.

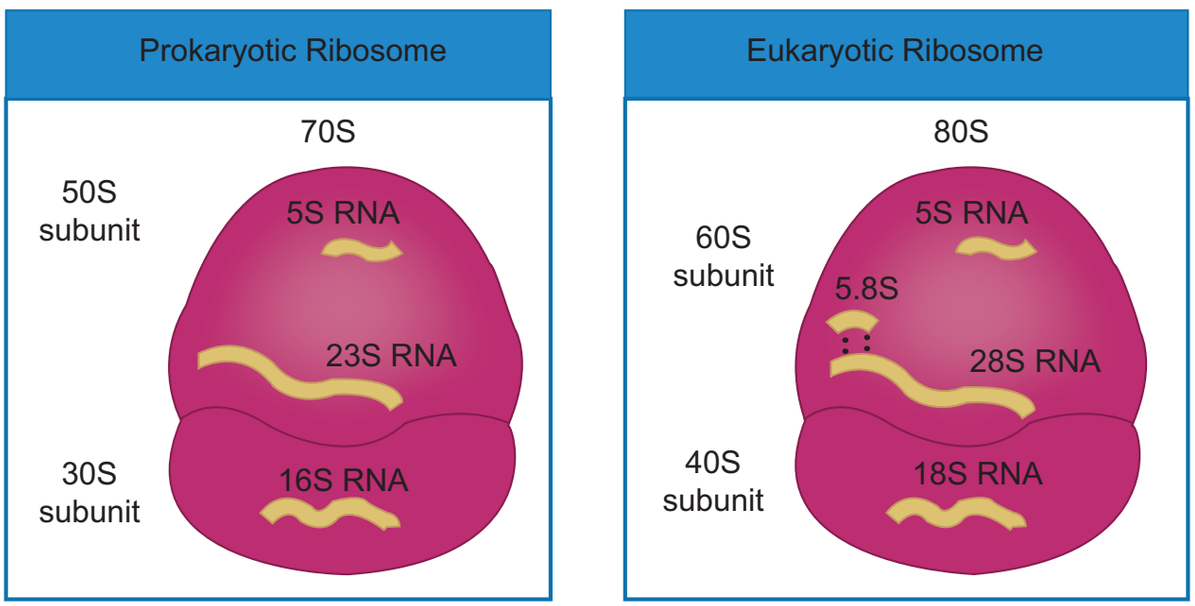


Figure 7.12: Prokaryotic and Eukaryotic Ribosomes

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Plasmids

Apart from the bacterial chromosome, bacteria also contain small circular, double stranded DNA molecules called plasmids (Figure 7.13). Plasmids are self replicating extra chromosomal genetic elements. Plasmids may carry genes for activities such as antibiotic resistance and tolerance to toxic metals. Examples: Fertility plasmid (F plasmid), Resistance plasmid (R plasmid) and colicin plasmid (Col plasmid).

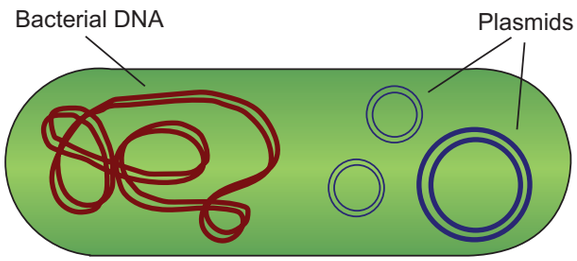


Figure 7.13: Plasmids in Prokaryotes

Molecular Chaperones

They are the helper proteins which recognize the newly formed polypeptides and fold them into their proper shape of secondary and tertiary structure. Many chaperones are involved in proper folding of bacteria. They were first identified in *Escherichia coli* mutant. Example: Heat shock proteins are produced in *Escherichia coli* cells subjected to live at high temperatures, or in any other stressful unfavorable conditions.

Inclusions

The cytoplasm of prokaryotic cells has several kinds of reserve deposits known as inclusions. Cells may accumulate certain nutrients when they are plentiful and use them when they are deficient. Some inclusions are common to a wide variety of bacteria whereas others are limited to certain species (Table 7.5).

Endospores:

Some species of bacteria produce metabolically dormant structures called spores. They are highly durable and dehydrated resting bodies produced inside the cells. They are formed by bacteria only when there is lack of water or depletion of essential nutrients in the environment. Endospores are coated with a specific chemical compound diaminopimelic acid. It binds with the Calcium and forms Calcium dipicolinate which removes the water from it and makes the spore resistant to extreme conditions Example: *Bacillus anthracis* and *Clostridium tetani* possess endospores.

Mesosomes

Generally prokaryotes do not have cytoplasmic organelles like mitochondria and chloroplast. It contains mesosome as their organelle. They are the invaginations of the cell membrane and they are in the form of tubules, vesicles or lamellae. They are seen in both Gram positive and Gram negative bacteria, generally more in Gram positive bacteria. They are located next to the septa or cross walls in dividing bacteria (Figure 7.14). They may be involved in cell wall formation during division or play a role in chromosome replication and distribution to daughter cells. If they are located near to the surface they are called peripheral mesosomes and if they are located deep into the cytoplasm they are called central mesosomes.

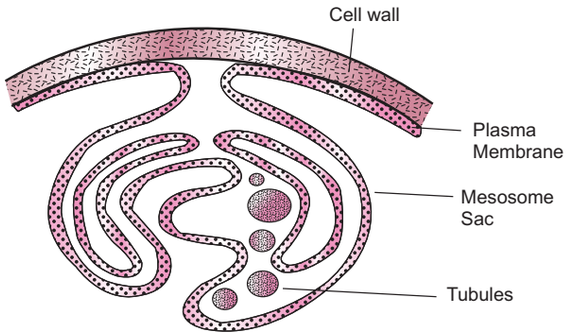


Figure 7.14: Bacterial Mesosome

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Table 7.5: Different types of inclusion bodies in bacteria

Type of inclusion bodies	Example of organisms possessing	Significance
Polyhydroxybutyrate (PHB)	*Bacillus megaterium*	Reserve of Carbon and energy sources. Sudan dye is used to observe lipid inclusions
Polyphosphate (volutin granules) or metachromatic granules	*Corynebacterium diphtheriae*	Reserve of phosphate
Sulphur globules	Phototrophic bacteria Like purple and green Sulphur bacteria Example: *Thiobacillus*	Elemental sulphur, reserve of electrons in phototrophs. Reserve of energy source in lithotrophs
Gas vesicles	Aquatic bacteria, *Cyanobacterium*	They are protein shells filled with gases. They provide buoyancy and keep the cells floating in vertical water column
Parasporal crystals	Genus *Bacillus*	It is a proteinaceous compound, It is toxic to certain insects
Magnetosomes	*Aquaspirillum magnetotacticum*	They are like intracellular chains of magnetite particles. They help the bacteria to swim to nutrient rich sediments. It protects the cell against H_2O_2 accumulation
Carboxysomes	Photosynthetic Bacteria, cyanobacteria Autotrophic bacteria	They contain the enzyme Ribulose 1-5 bisphosphate carboxylase which is involved in Carbon dioxide fixation during photosynthesis
Phycobilisomes or cyanophycin Granules	Cyanobacteria	They have a long polypeptide with equal proportion of Arginine and Aspartic acid. They store Nitrogen
Chlorosomes	Green bacteria	They contain bacteriocholorophyll pigments which are involved in bacterial photosynthesis

HOTS

Why are endospores so difficult to destory?

HE3R7

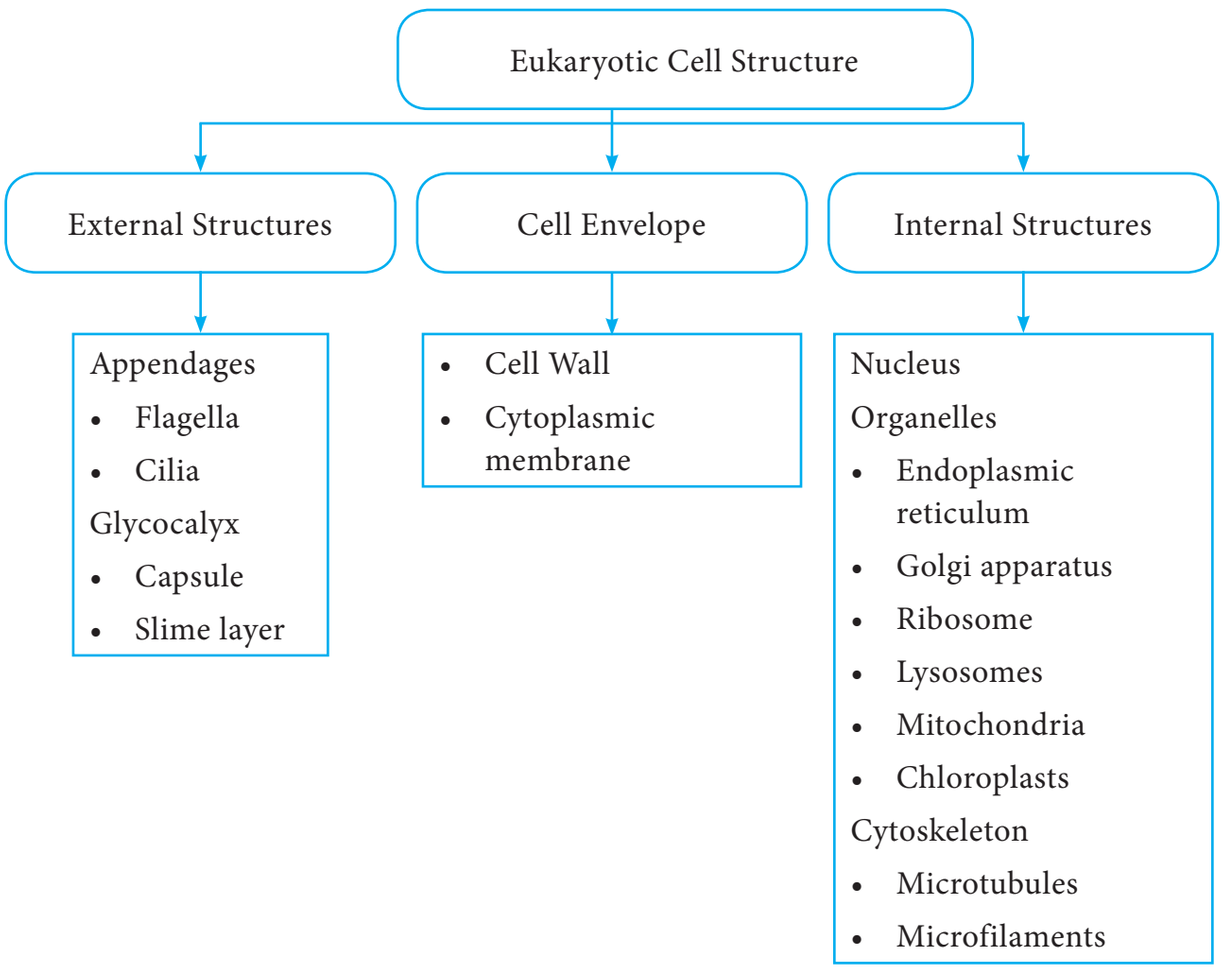
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7.5 Eukaryotic Cell Structure

As mentioned earlier, eukaryotic organisms include algae, protozoa, fungi, higher plants and animals. The eukaryotic cell is typically larger and structurally more complex than the prokaryotic cell (Flowchart 7.1).

Prokaryotes and Eukaryotes are chemically similar, in the sense that they both contain nucleic acids, proteins, lipids, and carbohydrates (Figure 7.15). They use the same kinds of chemical reactions to metabolize food, build proteins, and store energy. It is primarily the structure of cell walls and membranes, and the absence of organelles (specialized cellular structures that have specific functions), that distinguish prokaryotes from eukaryotes (Table 7.6).

The general, eukaryotic microbial cells have a cytoplasmic membrane, nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, cytoskeleton, and glycocalyx. A cell wall, locomotor appendages and chloroplasts are found only in some groups. The structure and functions of the eukaryotic cells are discussed in (Table 7.7).



Flowchart 7.1: Eukaryotic Cell Structure

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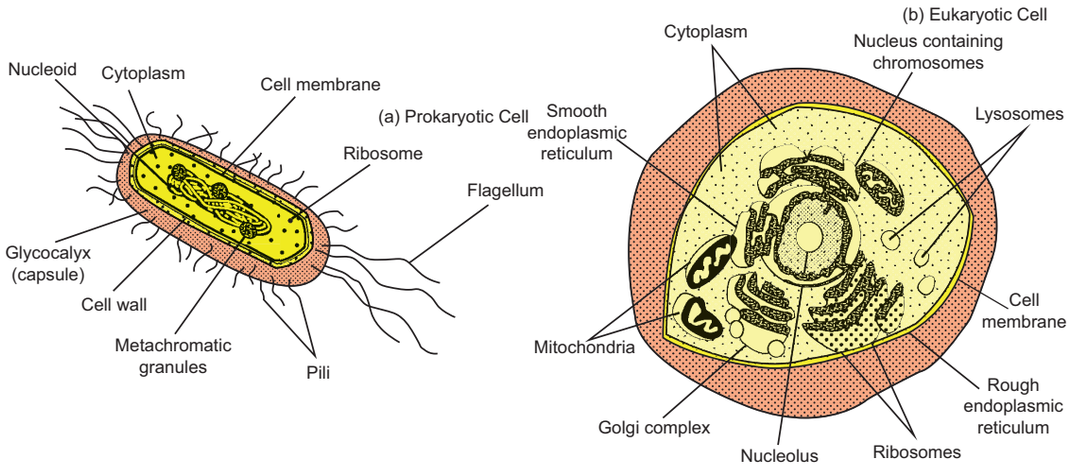


Figure 7.15: Structure of prokaryotic and eukaryotic cell

Table 7.6: Differences between prokaryotic and eukaryotic cell

S. No	Characteristic	Prokaryotic	Eukaryotic
1	Size of cell	Typically 0.2-2.0nm in diameter	Typically 10-100 nm diameters
2	Nucleus	No nuclear membrane or nucleoli	True nucleus, consisting of nuclear membrane and nucleoli
3	Membrane enclosed organelles	Absent	Present. Example: lysosomes, Golgi complex, endoplasmic reticulum, mitochondria and chloroplasts
4	Flagella	Consist of two protein building blocks	Complex, consist of multiple micro tubules
5	Glycocalyx	Present as a capsule or slime layer	Present in some cells that lack cell wall
6	Cell wall	Usually present and is chemically complex (typical bacterial cell wall includes peptidoglycan)	When present, chemically simple
7	Plasma membrane	No carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors are present
8	Cytoplasm	No cytoskeleton or cytoplasmic streaming	Has cytoskeleton and shows cytoplasmic streaming
9	Ribosomes	70S	80S (70S in organelles)

(Continued)

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Table 7.6: Differences between prokaryotic and eukaryotic cell *(Continued)*

10	Chromosome (DNA)	Single circular chromosome, lacks histone	Multiple linear chromosomes with histone arrangement
11	Cell division	Binary fission	Mitosis
12	Sexual recombination	No meiosis (transfer of DNA fragments only)	Involves meiosis

Table 7.7: Functions of Eukaryotic organelles

Eukaryotic organelles	**Functions**
Plasma membrane	Mechanical cell boundary, selectively permeable barrier with transport systems, mediates cell to cell interactions and adhesion to surfaces, secretion
Cytoplasmic matrix	Environment for other organelles, location of many metabolic processes
Microfilaments, intermediate filaments, and Microtubules.	Cell structure and movements from the cytoskeleton
Endoplasmic reticulum	Transport of materials, protein and lipid synthesis
Ribosome	Proteins synthesis
Golgi apparatus	Packaging and secretion of materials for various purposes, lysosome formation
Lysosomes	Intracelluar digestion
Mitochondria	Energy production through use of the tricarboxylic acid cycle, electron transport, oxidative hosphorylation, and other path ways
Chloroplasts	Photosynthesis, trapping light energy and formation of carbohydrate from CO_2 and water
Nucleus	Repository for genetic information, control center for cell
Cell wall and pellicle	Strengthen and give shape to the cell
Cilia and flagella	Cell attachment and Cell movement
Vacuole	Temporary storage and transport, digestion (food vacuoles), water balance(contractile vacuole)

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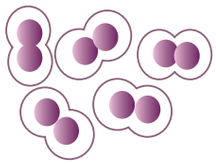
Different Shapes of Bacteria



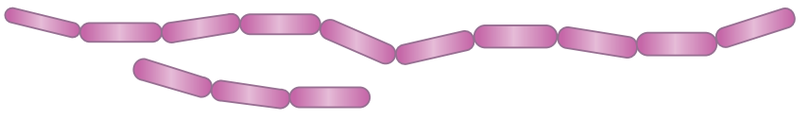
Staphylococcus aureus

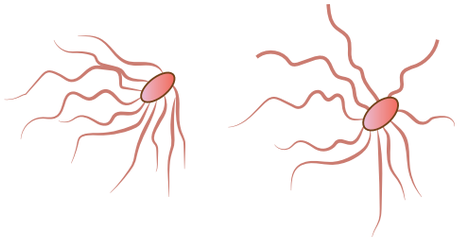


Streptococcus pyogenes

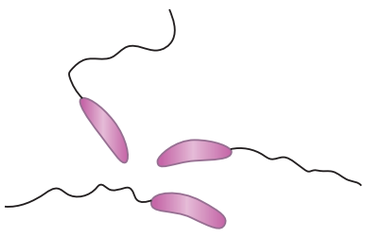


Streptococcus pneumoniae

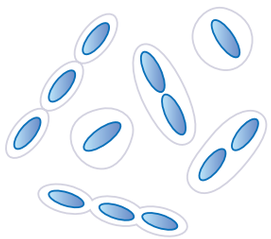




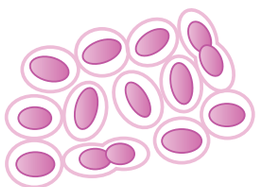
Escherichia coli; Salmonella



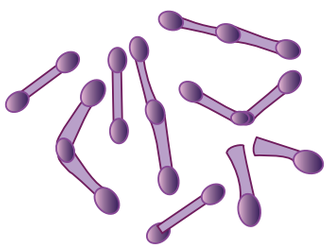
Vibrio cholerae



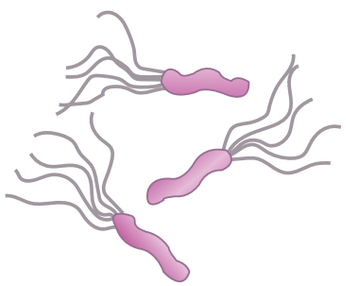
Klebsiella pneumoniae



Bordetella pertussis



Corynebacterium diphtheriae



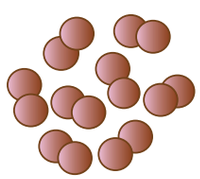
Helicobacter pylori



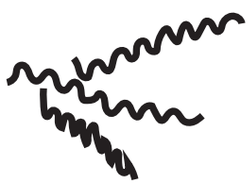
Clostridium botulinum



Clostridium tetani



Neisseria gonorrhoeae



Treponema pallidum

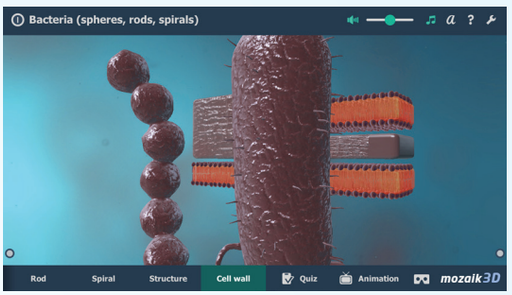
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ICT CORNER

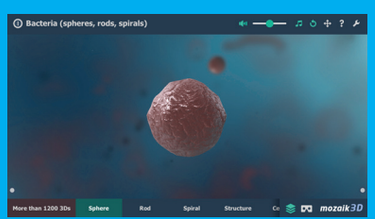
Bacteria

Know the various shapes of Bacteria

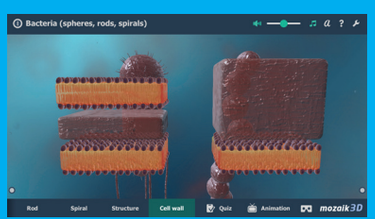


STEPS:

- Use the URL or scan the QR code to download the Bacteria interactive educational VR 3D app.
- Select sphere, rod and spiral to observe the structure of bacteria shapes.
- Select 'structure' tab and note the internal structure of bacteria.
- Click cell wall and note the difference between different shapes.



Step1



Step2



Step3

URL:

https://play.google.com/store/apps/details?id=com.rendernet.bacteria&hl=en

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Summary

Most prokaryotes have one of three general shapes coccus (round), bacillus (rod), or spiral, based on the configuration of the cell wall. Two types of spiral cells are spirochetes and spirlla. Shape and arrangement of cells are key factors for describing prokaryotes. Arrangements of cells are based on the number of planes in which a given species divides.

Cocci can divide in many planes to form pairs, chains, packets, or clusters. Bacilli divide only in the transverse plane. If they remain attached, they form chains or palisades.

Some bacterial cell walls are covered by capsules or slime which protect the cell from phagocytosis, drying and nutrient loss. Fimbriae and Pili are involved in attachment and in transfer of genetic information between bacterial cells. Flagella are involved in cell motility.

The cell envelope is the complex boundary structure surrounding a bacterial cell. In Gram negative bacteria, the envelope consists of outer membrane, the cell wall, and the cell membrane. Gram positive bacteria have only cell wall and cell membrane. Gram positive bacteria have thick murein and teichoic acid. The cell walls of Gram negative bacteria are thinner and have wide periplasmic space. The outer membrane of Gram negative cells contains LPS toxic to mammalian hosts. The cell membrane is typically composed of phospholipids and proteins, and it performs many metabolic functions as well as transport activities.

The cytoplasm of bacterial cell serve as a solvent for material used in all functions. The genetic material of bacteria is DNA and the genes are arranged on larger, circular chromosomes. Additional genes are carried on plasmid. Bacterial ribosomes are dispersed is the cytoplasm in chains and are also embedded in the cell membrane.

Bacteria may store nutrients in their cytoplasm, in form of inclusions. Inclusion vary in structure and the materials that are stored. A few bacteria produce dormant bodies called endospores, which are the hardiest of all life forms, survival for hundreds or thousands of years. The genus *Bacillus* and *Clostridium* are spore forming deadly pathogens.

Eukaryotes are cells with nucleus and membrane bound organelles. Cell structures common to most eukaryotes are the cell membrane, nucleus, vacuoles, mitochondria, endoplasmic reticulum, golgi apparatus and a cytoskeleton. Cell wall, chloroplast and locomotory organelles are present in some eukaryote groups.

Evaluation

Multiple choice questions

1. The arrangement of flagella on cell surface can sometimes help in the identification of an organism for example, *Escherichia coli* to has flagella throughout the cell surface that is referred to as.
   a. Lophotrichous
   b. Monotrichous
   c. Peritrichous
   d. None of the above

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2. The movement of an organism toward or away from a chemical substance in its environmentis called.
   a. Tracking
   b. Chemotaxis
   c. Tumbling
   d. Tumbling of none of the above.
3. Bacterial cell wall is composed of
   a. Lipid
   b. Murein
   c. Cellulose
   d. Chitin
4. Cell wall shows
   a. Semipermeability
   b. Complete permeability
   c. Differential permeability
   d. Impermeability
5. Gram positive differ from Gram negative in having
   a. Thick wall
   b. Absence of wall lipids
   c. Complete wall
   d. Simple wall
6. Lipopolysaccharide is found in cell wall of
   a. Gram positive bacteria
   b. Gram negative bacteria
   c. Both Gram positive and Gram negative
   d. Algae
7. Cell wall of archaeobateria contain
   a. Peptidoglycan
   b. Murein
   c. Pseudomurein
   d. All the above
8. Endotoxin present in
   a. Outer membrane
   b. Plasma membrane
   c. Murein
   d. All the above.
9. _____ has fluidity
   a. Cell wall
   b. Cell membrane
   c. Outer membrane
   d. All the above
10. The organelle of prokaryote involved in active cell division.
    a. Mesosomes
    b. Mitochondria
    c. Ribosomes
    d. Endoplasmic reticulum
11. The metachromaticgranules are seen in the bacteria
    a. *Escherichia coli*
    b. *Corynebacterium diptheriae*
    c. *Pseudomonas aeroginosa*
    d. *Bacillus antharcis*
12. The extra chromosomal DNA is called
    a. Plasmid
    b. Episome
    c. Nucleus
    d. Nucleoid
13. The siderophores has high affinity towards.
    a. Iron
    b. Magnesium
    c. Chloride
    d. Copper
14. The molecular chaperones are involved in
    a. Folding of proteins
    b. Folding of carbohydrates
    c. Folding of lipids
    d. Folding of fatty aids

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Answer the following

1. What is Glycocalyx?
2. What is a capsule? What are its functions?
3. What is a pilus, what is its function?
4. What is chemotaxis?
5. Explain the arrangement of flagella in bacteria with example.
6. Define carboxysomes
7. State volutin granules
8. What is called magnetosomes?
9. What is the role of ribosomes involved in protein synthesis?
10. Define periplasmic space.
11. What is LPS composed of?
12. List functions of cell wall
    a. Cell membrane
    b. Outer membrane
13. Discuss why a cell lyses without cell wall
14. Give the significance of cell envelope.
15. What is the role of siderophores?
16. Differentiate between capsule and slime/pili and fimbriae.
17. Diagrammatically explain structure of cell wall.
18. Differentiate Gram positive and Gram negative bacteria.
19. Explain any five cytoplasmic inclusions
20. Differentiate between Prokaryotes and Eukaryotes.

Student Activity

1. Students will prepare clay model of bacteria.
2. Students will collect the pictures of different Eukaryotic microorganisms.

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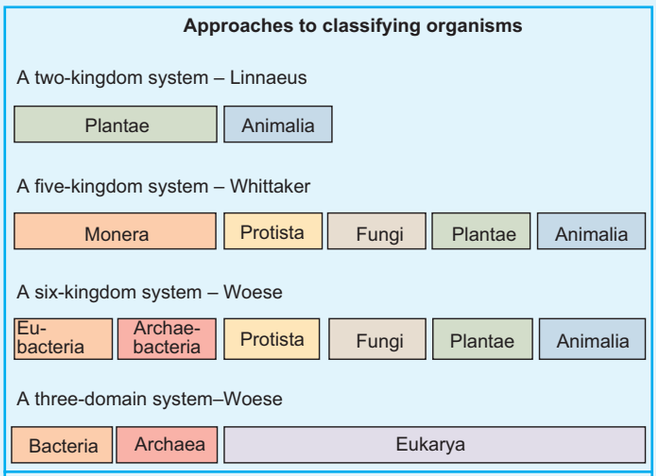
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Chapter 8

Microbial Taxonomy

Chapter Outline

8.1 Diversity of Living Organisms is Fascinating
8.2 Binomial Nomenclature
8.3 Whittaker's System of Classification
8.4 Taxonomic Systems
8.5 The Three Domain System
8.6 The Past and Present Status of Bacterial Taxonomy



The correct identification of micro organisms is of fundamental importance to microbial systematists as well as to scientists involved in many other areas of applied research and industry (Example: agriculture, clinical microbiology and food production).

Learning Objectives

After studying this chapter the student will be able,

- *To understand the concept of taxonomy, taxon and phylogeny.*
- *To appreciate the contribution of Linnaeus and Whittaker.*
- *To learn the characteristics of Kingdom Monera, Protista, Fungi, Plantae and Animalia.*
- *To know some special methods used in classification of microorganisms.*

8.1 Diversity of Living Organisms is Fascinating

The branch of science which deals with the classification, nomenclature and identification of all living organisms is called Taxonomy. (Greek taxis means arrangements and nomos means law or to distribute). Because of large number and great diversity of organisms, biologists use the characteristics of different organisms to identify and group them. To understand life, it is essential to understand taxonomy. The method of grouping related organisms is the basis of classification (Figure 8.1). The objectives of taxonomy are:

- To establish the criteria for identifying organisms
- To arrange related organisms into groups
- To provide evolutionary information of the organism

The system of naming living organism is called Nomenclature.

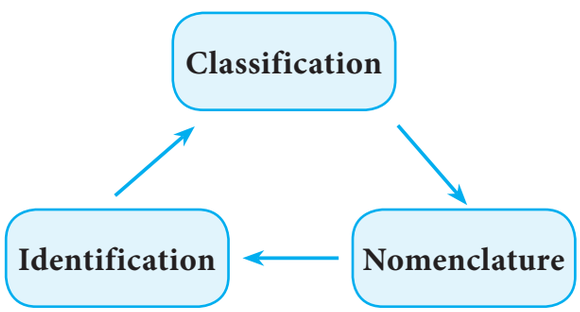


Figure 8.1: The three facets of taxonomy

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8.2 Binomial Nomenclature

Swedish botanist Carolus Linnaeus in 1735 introduced a formal system of classification which divided all living organism into two kingdoms-Animalia and Plantae. He introduced "two name" system, the first name, genus and second name species. The name often gives information on something special about it. Taxa (the basic taxonomic group) are constructed from strains which are successions of cultures derived from an initial colony. The basic taxonomic group is called the species (a collection of strains having similar characteristics). The special bacterial strain which is the permanent reference specimen for the species is called the "**type strain**" (Figure 8.2).

HOTS

Why is type strain refered as the most important strain in a bacterial species?

A variant strain that differ physiologically and biologically from other strains in a particular species is called as "Biovar". Variations in a species is biological in nature. One biovar in a species may grow on sucrose, while another cannot. If the biovars are very similar except for one property, they belong to the same genus and species, though vary in biological growth properties.

The strain that differ morphologically are called as Morphovar or Morphotypes. Serovars or Serotypes are those strains that differ in their antigenic properties. It refers to immunological variations in a species. An example of differing serovars is *Salmonella*. Cell surface of *Salmonella* varies slightly from one serovar to another. Because of this cell surface change, a person who has been infected by or become resistant or immune to one serovar will not be immune to a second type, because the immune system cannot recognize a similar bacterium with a new surface cover.

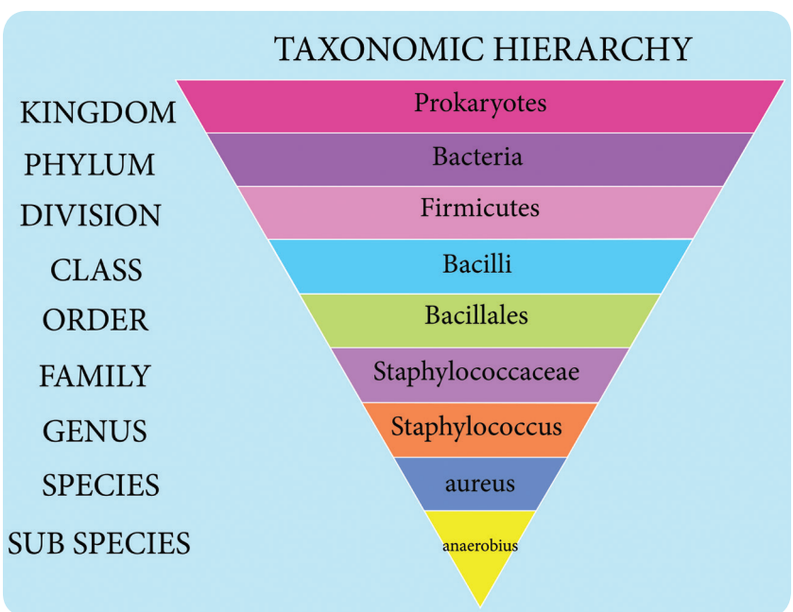


Figure 8.2: Taxonomic hierarchy-an example of hierarchy in microbial taxonomy

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Infobits

The Microbial Type Culture Collection and Gene Bank (MTCC), a national facility established in 1986 is funded jointly by the Department of Biotechnology (DBT) and the Council of Scientific and Industrial Research (CSIR), Government of India. The MTCC, housed at the Institute of Microbial Technology (IMTECH), Chandigarh, has established itself as a distinguished culture collection centre for microbial resources in India. It is an affiliate member of the World Federation for Culture Collections (WFCC) and is registered with the World Data Centre for Microorganisms (WDCM). The main objectives of this national facility are to act as a depository, to supply authentic microbial cultures and to provide related services to the scientists working in research institutions, universities and industries.

8.3 Whittaker's System of Classification

It is the five kingdom classification. In the 20th century, advances in cell biology and interest in evolutionary biology led scientists to question the two or three-kingdom classification schemes. In 1969, Robert H. Whittaker proposed a system which recognizes five kingdoms of living things: Monera (Bacteria), Protista, Fungi, Plantae and Animalia (Table 8.1).

Whittaker's system of classification is based on 1) complexity of cell structure 2) mode of nutrition 3) body organization 4) phylogenetic or evolutionary relationship.

Monera: This kingdom includes all prokaryotic organisms. Unicellular microorganism such as Mycoplasma, Bacteria, Actinomycetes and Cyanobacteria are grouped under kingdom Monera.



Phylogeny is the evolutionary history of organisms that refer to the relationship between life forms.

Table 8.1 Properties of Whittaker's five kingdoms

Kingdom	Monera	Protista	Fungi	Plantae	Animalia
Cell type	Prokaryotic	Eukaryotic	Eukaryotic	Eukaryotic	Eukaryotic
Cell organization	unicellular	unicellular	Multicellular and unicellular	Multicellular	Multicellular
Cell Wall	Present in most	Present in some absent in others	Present	Present	Absent
Nutritional Class	Phototrophic, heterotrophicor chemoautotrophic	Heterotrophic and phototrophic	Heterotrophic	Phototrophic	Heterotrophic
Mode of nutrition	Absorptive	Absorptive or ingestive	Absorptive	Mostly Absorptive	Mostly ingestive

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Infobits

Hints of life:

The Precambrian was the age of microorganisms. They were macroscopically expressed in a colonial structure called **stromatolite**. It is a layer produced by live or fossilized mats of photosynthetic prokaryotes (cyanobacteria) associated with warm lagoons or hot springs. The ancient **stromalite** belongs to anoxygenic phototrophic filamentous bacteria and modern **stromalite** belongs to oxygenic photo trophic cyanobacteria.

Protista: This kingdom includes eukaryotic unicellular Protozoans, slime molds and algae. The kingdom is made up of more than 250000 species. These organisms have typical eukaryotic cell organization.

Fungi: This kingdom includes non green, non photosynthetic eukaryotic fungi. molds, mushroom, toad stools, puffballs and bracket fungi are grouped under this kingdom. They are multicellular and consist of specialized eukaryotic cells arranged in a filamentous form.

Plantae: It includes all multicellular plants of land and water. They use photosynthesis to synthesize their organic molecules.

Animalia: This kingdom includes all multicellular eukaryotic animals. They are also referred to as Metazoans. Animals ingest their food through one of any ingestion portal and then use digestive enzymes to break food particles into absorbable fragments (Figure 8.3).

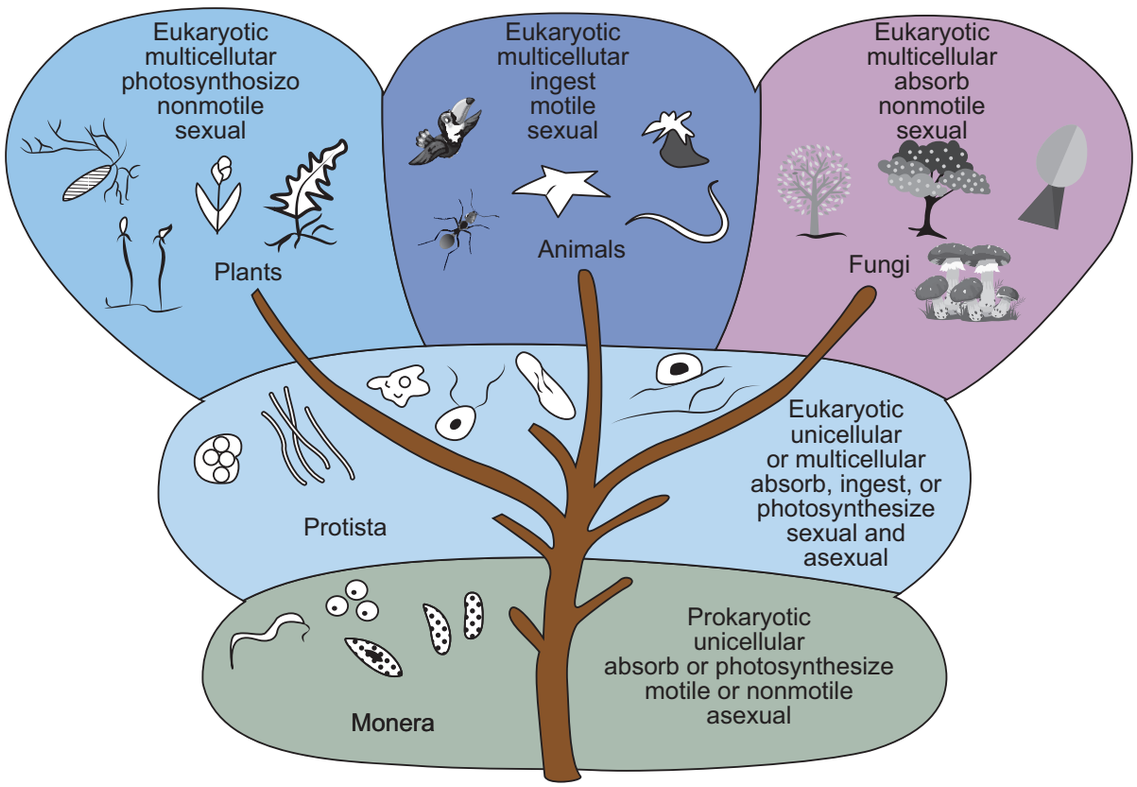


Figure 8.3: Whittaker's Five Kingdom classification

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8.4 Taxonomic Systems

Classical Taxonomy

Classical taxonomy is a method of classification based on morphology, physiology, biochemical and ecological characteristics of the microorganisms.

- **Morphological Characteristics:** The structural characteristics are the usual tools which help in classification. Cell morphology gives little information about phylogenetic relationship. The first step in identification of bacteria is differential staining.
- **Physiological and metabolic characteristics:** These characteristics are useful because they are directly related to nature and activity of microbial enzymes and transport protein. Since proteins are gene products, analysis of these characteristics provides an indirect comparison of microbial genomes.
- **Biochemical characteristics:** Enzymatic activities are widely used to differentiate bacteria. Bacteria can be separated into separate species by various biochemical tests. Example: Carbohydrate fermentation ability of bacteria.
- **Ecological characteristics:** Many properties are ecological in nature since they alter the relation of microorganism to their environment. Microorganisms living in various parts of the human body markedly differ from one another and from those growing in freshwater, terrestrial and marine environments.

Prokaryotes have only a few structural characteristics and these characteristics are subject to rapid change due to change in environment. In classifying prokaryotes, metabolic reactions, genetic relatedness and other specialized properties are used (Figure 8.4).

HOTS

If two microorganisms have an identical mol% G+C value for their DNA, are they necessarily related? Explain

If two micro organisms have very different mol% G+C values for their DNA, are they necessarily unrelated? Explain

Numerical Taxonomy

The objective classification system deals with the grouping by numerical methods of taxonomic units based on their character and does not use subjective evaluation of their properties. To be more objective about grouping bacteria, the scientists determine many characteristics (usually 100 to 200) for each strain studied, giving equal weightage for each character. Then by using computer **%similarity** is calculated **(%S** of each strain to every other strain). For any two strains, this is

$$\%S = \frac{NS}{NS + ND}$$

where, NS is the number of characteristics that are the same (positive or negative) for the two strains, and ND is the number of characteristics that are different. Those strains having a high %S to each other are placed into groups; and those groups having a high %S to each other are in turn placed into larger

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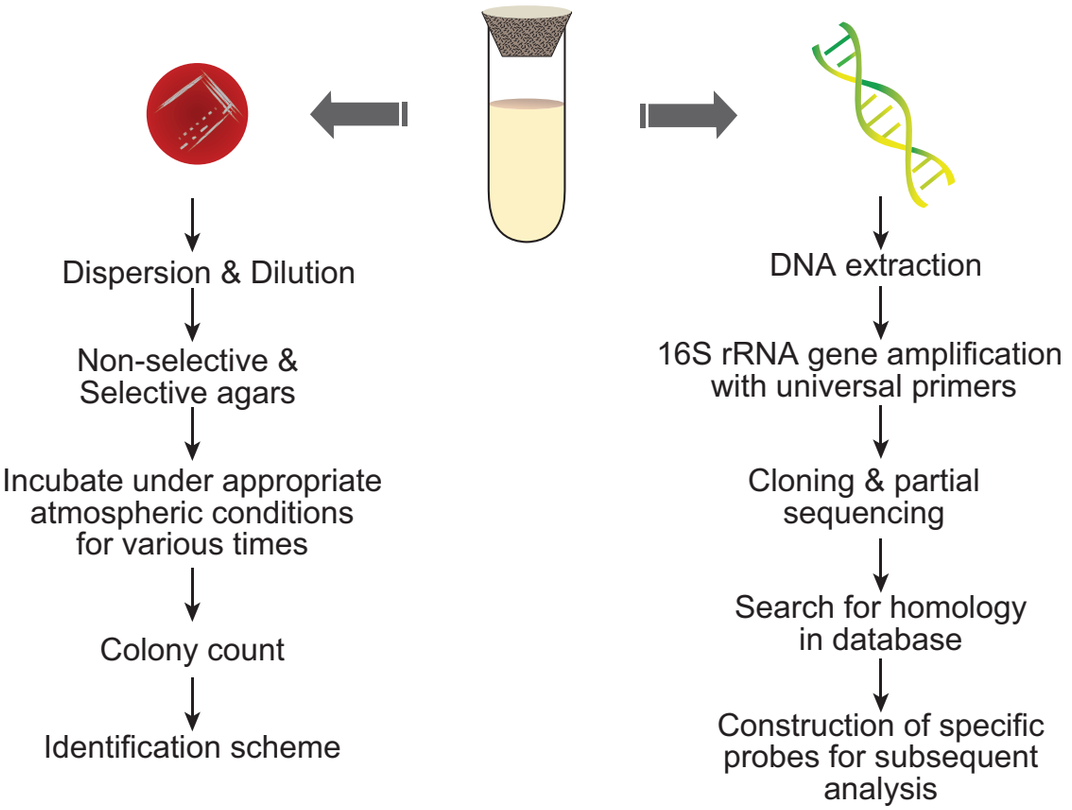


Figure 8.4: General scheme for classification and identification in microbial taxonomy

groups. Numerical taxonomy also yields classification that has a high degree of stability and predictability.



In Numerical Taxonomy, which was defined by Peter H.A.Sneath and Robert Sokal, each characteristic is given equal weightage and it is converted into numerical form and compared by means of a computer.

Atleast 50 and preferably several hundred characterictics are compared.

The presence and absence of selected characters in the group of organism is calculated by simple matching coefficient (SSM), called Jaccard coefficient.

Molecular Taxonomy



Molecular techniques in the field of biology has helped to understand genetic relationship between the numbers of different taxonomic categories.

DNA and protein sequencing, immunological methods, DNA-DNA or DNA-RNA hybridization methods are very helpful in studying different species.

The data or information from such studies are used to construct phylogenetic tree (a branching diagram showing the evolutionary relationship among various biological species based on similarities and difference in their physical or genetic characteristics).

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A classification technique that is widely used is DNA base composition which is expressed as the percentage of Guanine plus Cytosine (G+C). It is a fixed property that reveals the degree of species relatedness. Ribosomal RNA sequencing is used to determine the diversity of organisms and the phylogenetic relationship. Basically ribosomes consists of two subunits, each of which is composed of protein and a type of RNA. Specific base sequences called as signature sequences are found in all groups of organisms. These unique DNA sequences are 5-10 bases long and found in 16s rRNA location and unique to major groups of prokaryotic organisms.

Nucleic acid based detection methods help in the detection of genomic materials. The 16s rRNA gene sequencing has been established as the "gold standard" for identification & taxonomic classification of microbial species.

8.5 The Three Domain System

This system of classification was introduced by C.Woese, O. Kandler and M.L.Wheelis, is an evolutionary model of phylogeny based on cells rRNA sequences(differences in the sequences of nucleotides) studies. They grouped all living organisms into three domains: Bacteria, Archaea and Eukaryota (Figure 8.5).

Bacteria and Archaea are two different groups of prokaryotes. The domain Bacteria comprise the vast majority of prokaryotes. The domain Archaea contains prokaryotes that live mostly in extreme environments. The domain Eukaryota contains living organisms that includes Kingdom Protista, Kingdom Fungi, Kingdom Plantae and Kingdom

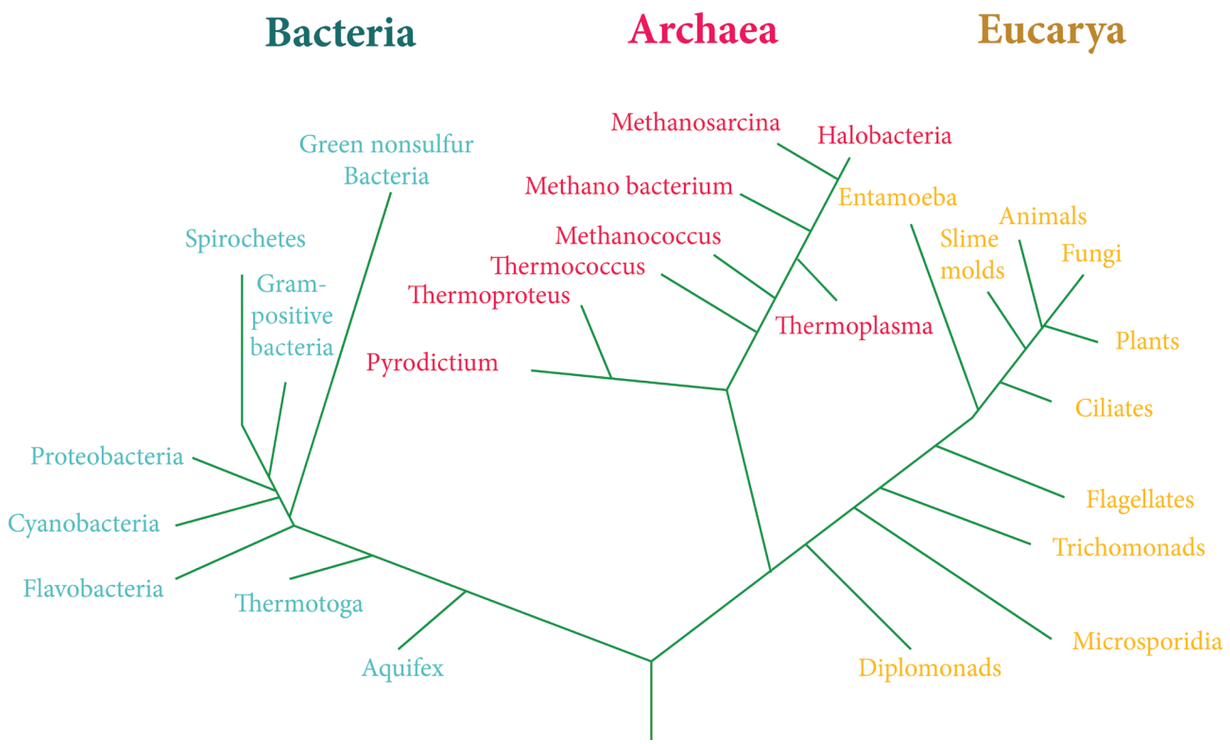


Figure 8.5: A phylogenetic tree based on rRNA analysis. Organisms are classified into three domains: Bacteria, Archaea and Eukaryotes as proposed by Carl Woese et al.

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Animalia. This system of classification is currently accepted by most biologist.

The three domain system is based on the current state of knowledge. As knowledge of organisms increase in the future, classification will undoubtedly continue to change.

Infobits

The ***Bergey's Manual of Systematic Bacteriology***'s first edition was published initially in four volumes. Volume 1 included Gram negative bacteria of general, medical or industrial importance, Volume 2 included Gram positive bacteria other than actinomycetes, Volume 3 included Cyanobacteria, Archaebacteria and remaining Gram negative bacteria and Volume 4 included Actinomycetes.

The current grouping edition 2 (2012) has five volumes based on 16S rRNA sequencing:

Volume 1 (2001) includes Archaea and the deeply branching and phototrophic Bacteria.

Volume 2 (2005) includes Proteobacteria.

Volume 3 (2009) includes Firmicutes.

Volume 4 (2011) includes Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes.

Volume 5 (in two parts) (2012) includes Actinobacteria.

8.6 The Past and Present State of Bacterial Taxonomy

The first classification scheme for bacteria was published in 1773 based on morphological characteristics. One of the unique, broadscope and widely accepted classification scheme was published in 1927 by David Bergey & colleagues is Bergey's Manual of Determinative Bacteriology.

It provides identification schemes for identifying Bacteria and Archae based on their morphology, differential staining and biochemical tests. Whereas in 1984, a more detailed work was entitled. Bergey's manual of Systematic Bacteriology provides information on Bacteria and Archaea based on rRNA sequencing. The classification in Bergey's Manual is accepted by the most microbiologists as the best consensus for prokaryotic taxonomy.

The present classification scheme based on genetic relatedness has more practical value. This is expected to provide greater stability and predictability. It would lead to improved identification schemes, and to aid our understanding of the origin of present day genera and species.

Summary

The branch of science which deals with the classification, nomenclature and identification of all living organisms is called taxonomy. The system of naming living organisms is called as nomenclature. Carolus Linnaeus divided all living organisms into two kingdoms- Animalia and Plantae. He introduced Binomial Nomenclature for naming living organisms. Whittaker

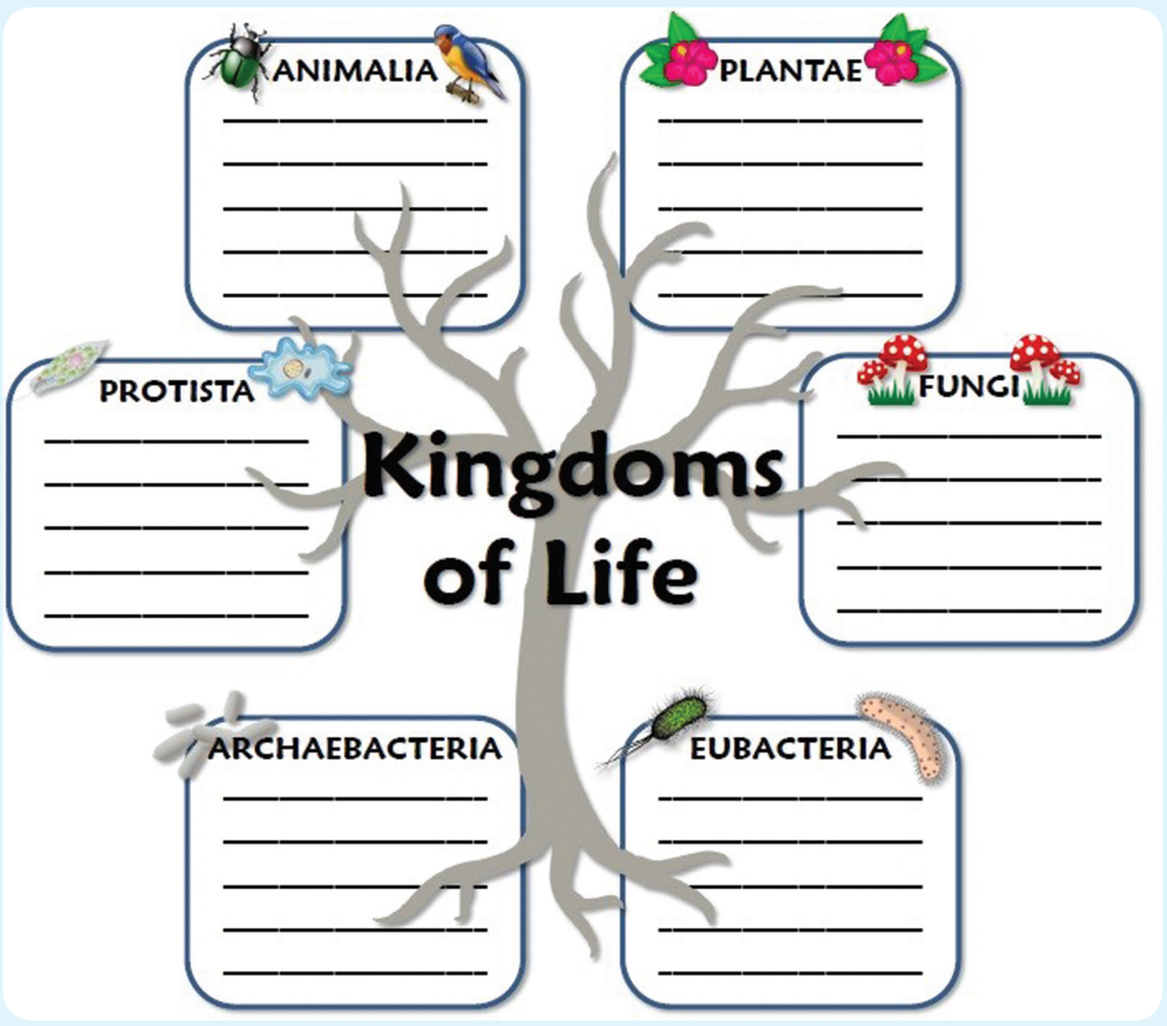
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proposed five kingdom classification based on various properties of living organisms. Currently accepted classification proposed by Woese, Kandler and Wheelis is the three domain classification. Modern developments of sequencing technologies and recognition of rDNA sequences are of now cornerstone for identification purposes.

Overall, it is important to recognize that microbial diversity is very much linked to its environment and the correlation has to be established by description of environmental parameters whenever sampling is carried out. It is also important to study the phenotypic characteristics and link them to the observations obtained from genotyping techniques. The link between habitat and diversity then becomes easier to understand for future studies.

Student Activity

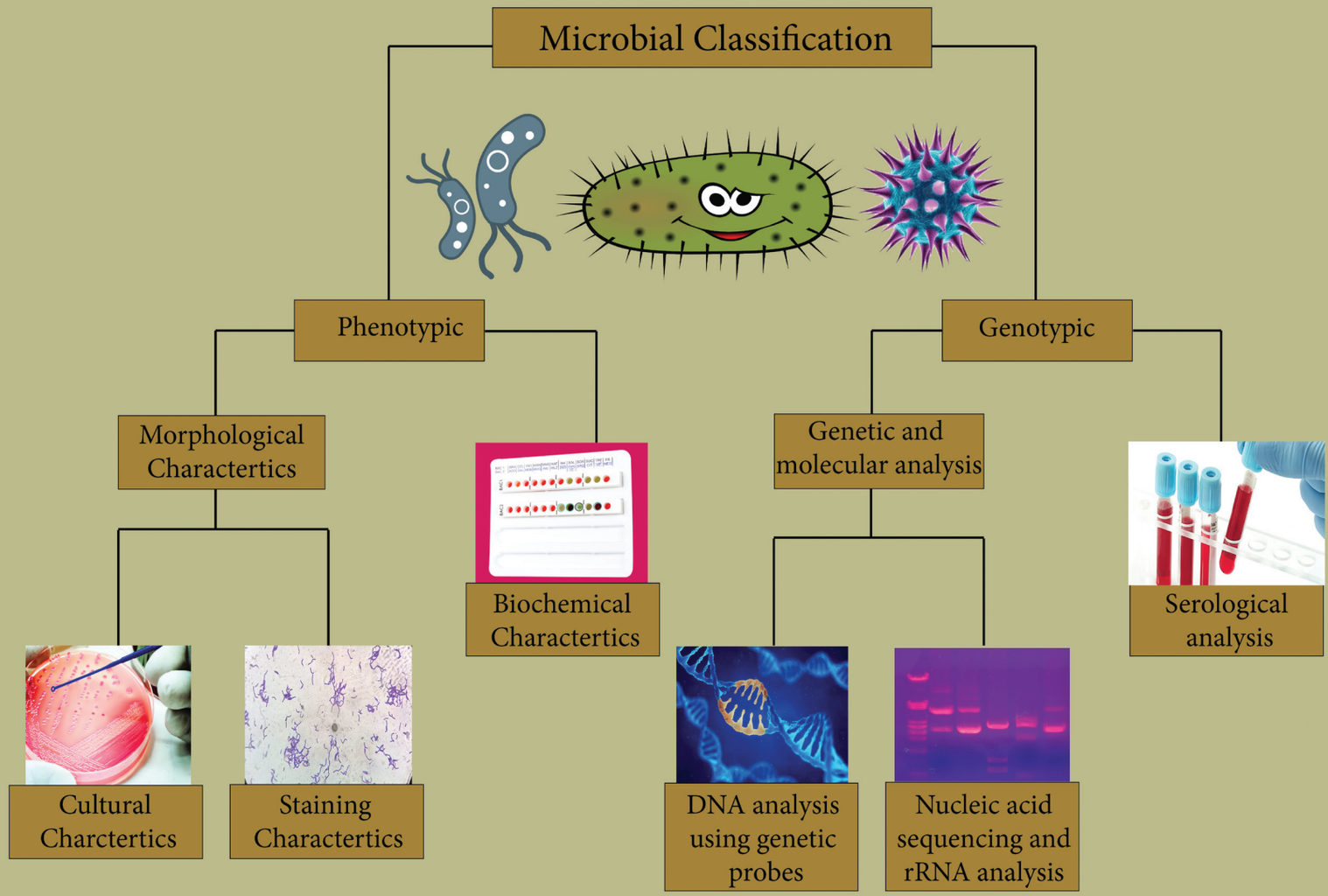
The student must understand the characteristics of each domain under the five kingdom classification and fill in the chart below.



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Classification system of Microorganism



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Evaluation

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Multiple choice questions

1. Which of the following is a reasonable representation of phylogenetic diversity?
   a. The Chain of Being
   b. The Ladder of Life
   c. The 5-Kingdom Tree
   d. The 3-Domain Tree
2. Microorganisms belonging to the same are expected to have the most characteristics in common with eachother.
   a. Order
   b. Species
   c. Family
   d. Kingdom
3. What was the first and most useful microscopic tests for classifying bacteria?
   a. Gramstain
   b. Flagellar stain
   c. Simple stain
   d. Capsular stain
4. Which of the following is the arrangement of organisum into groups or taxa?
   a. Nomenclature
   b. Identification
   c. Systematics
   d. Classification
5. Binomial nomenclature means writing the name of microorganism in two words is
   a. Order and family
   b. Family and genus
   c. Species and variety
   d. Genus and species

Answer the following

1. Define: Taxonomy and what is the here inter related parts of taxonomy?
2. Define: Classification, Nomenclature and Identification.
3. What is meant by binomial system?
4. Who developed the Bionomial system in the year?
5. What is taxonomic rank and why are we using this?
6. What is the difference between biovars, serovars and morphovars?
7. What is type strain and why it is called as type strain?
8. Write down the techniques which are used to identify the taxonomic characters of an organism?
9. Explain in detail about the molecular characteristics which are used to identify the taxonomic orders?
10. What are the five kingdom classifications?

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Chapter 9

Environmental Microbiology

DQ3IT

Chapter Outline

9.1 Interrelationship with other Fields

9.2 Air Microbiology

9.3 Microbiology of Water

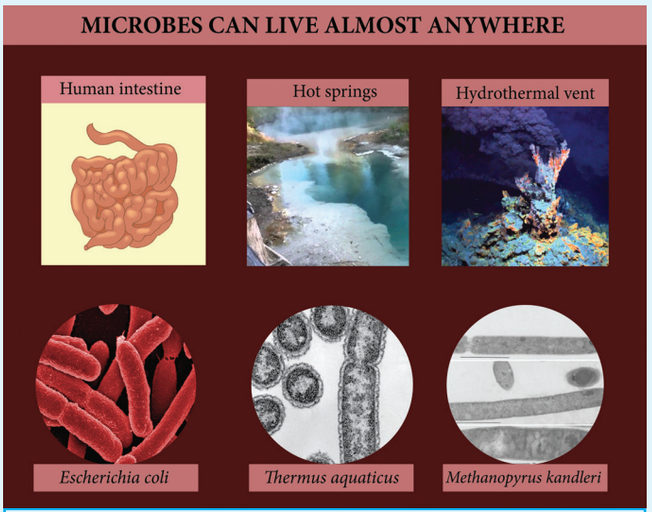
9.4 Water Pollution and Microbial Contamination

9.5 Sewage Treatment

9.6 Recycling of Treated Sewage

9.7 Composting

9.8 Biogas Production



Microorganisms are found in every corner of the earth from miles below the surface to boiling hot springs to Antartic ice. Every ecosystem on earth contains microorganisms thay occupy unique niches based on their specific metabolic properties.

Learning Objectives

After studying this chapter the student will be able,

- *To gain knowledge of various layers of atmosphere and micro fauna of air.*
- *To understand air pollution and air borne diseases.*
- *To learn water borne diseases and water treatment procedures.*
- *To know Eutrophication.*
- *To know composting techniques.*
- *To gain knowledge of biogas production.*

Environmental Microbiology is the field of science that examines the relationship between microorganisms and their biotic and abiotic environments. Microorganisms in the environment are diverse in origin and ubiquitous. Environmental Microbiology involves the study of the applied effects of microorganisms on the environment and on human activity, health and welfare.

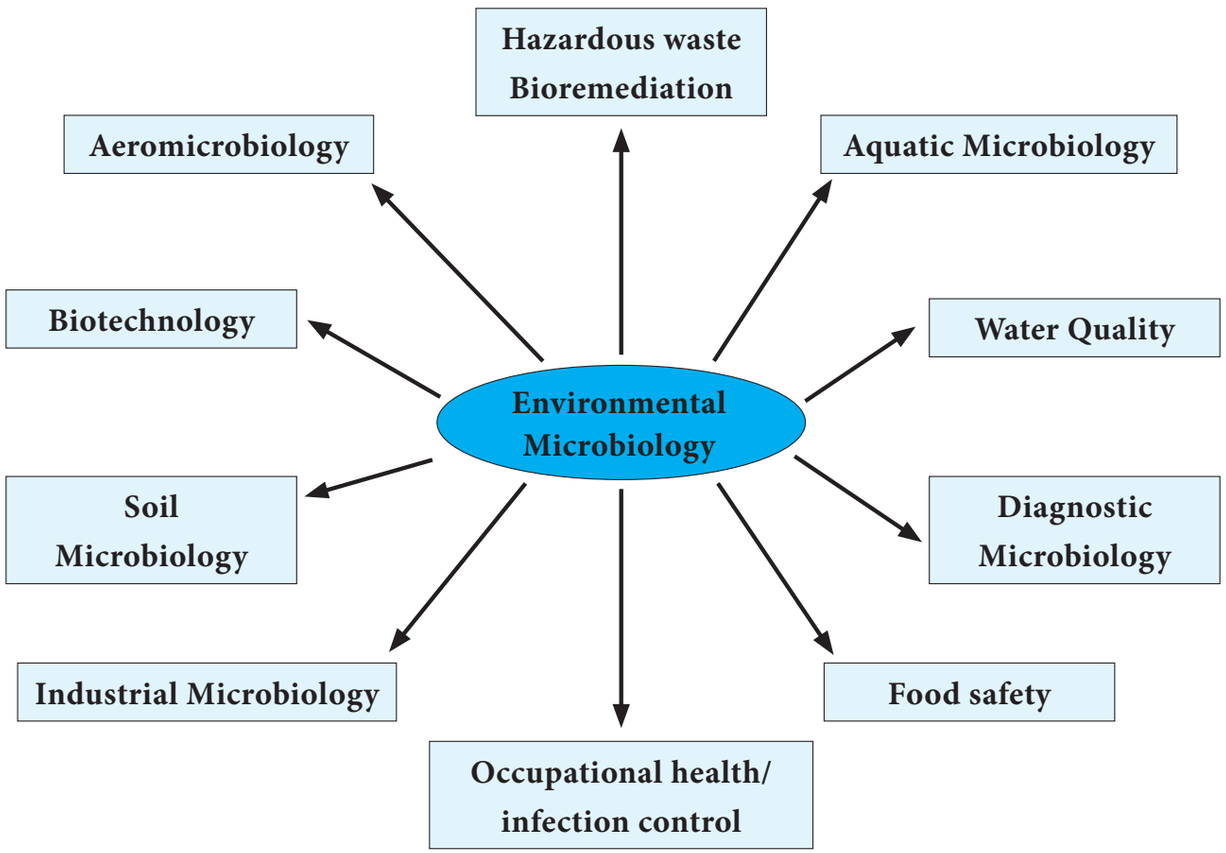
It was in the 1970s that a new area of Microbiology emerged and developed into the field of Environmental Microbiology. The initial focus was on water quality pathogens in the environment in the context of public health safety. The developing field of environmental microbiology expanded to several other areas of applied research. These include microbial interactions with chemical pollutants in the environment and the use of microorganisms for resource production and resource recovery.

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Chemical pollutants in soil and ground water have pronounced effects on human population. The cost of cleanup or remediation of the contaminated sites is too high. The overall objective of this chapter is to define the important microbes involved in environmental microbiology, the nature of the different possible environments in which the microbes are situated, the methodologies used to monitor the microbes and their activities and finally the possible effects of the microbes on human activities.

9.1 Interrelationship with other Fields

Since environmental microorganisms affect so many aspects of life and are easily transported between environments, the field of Environmental Microbiology interfaces with a number of subspecialities. It includes soil, aquatic, aeromicrobiology, as well as bioremediation, Water quality, Occupational health, Infection control, Food safety and Industrial Microbiology.



Modern environmental microbiology has a much wider scope. There are many different fields which recognises the problems in various fileds of Environmental Microbiology. It includes discovery and identification of new microbes and their products that may have practical application for protection of environment and human health and commercial applications.

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9.2 Air Microbiology

Earth's environment is endowed with atmosphere, hydrosphere, lithosphere and biosphere. In the 1930s, **F.C. Meier** coined the term "Aerobiology". Air is a natural resource and is fundamental to human life as it makes breathing possible. Its universal presence and requirement for the survival of human beings make air an important environmental factor. Air contains significant number of microorganisms. It also acts as a medium for the transmission of microorganisms including bacteria, viruses, fungi, yeast and protozoa. Air borne transmission is one of the important modes for the transmission of infectious diseases. Aeromicrobiology is the study of air borne microorganisms.

Infobits

Miquel (1883) and Carnally & colleagues (1887) carried out the most systematic studies in airborne microorganisms.

9.2.1 Layers of Atmosphere

Earth's atmosphere is divided into five major layers (Figure 9.1) they are:

- Exosphere : 700 to 10,000 km
- Thermosphere : 80 to 700 km
- Mesosphere : 50 to 80 km
- Stratosphere : 12 to 50 km
- Troposphere : 0 to 12 km

The air in the troposphere, the layer close to the earth is constantly in motion and the temperature decreases with increasing altitude, reaching a low of -57°C at the apex of this region. Microorganisms are frequently found in the lower portion of the troposphere, where they are dispersed by the air currents. Most of the microbes present in the troposphere are either spore formers or microbes that are easily dispersed in air. The stratosphere has a temperature range of -80°C to -10°C. The temperature in the stratosphere can reach a maximum of several thousand degrees. Microorganisms are not found in the upper regions of the atmosphere because of the temperature extremes, scarcity of available oxygen, absence of nutrients and moisture, and low atmospheric pressure. The relatively low humidity in the atmosphere (especially during daylight hours) and UV rays from the sun, limit the types and number of microorganisms that are able to survive in this part of the biosphere.

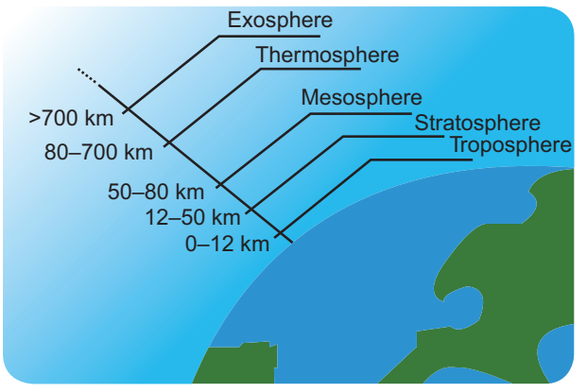


Figure 9.1: Diagram showing layers of atmosphere

9.2.2 Composition of Air

The air in our atmosphere is composed of different gaseous molecules. The most common gases are nitrogen (78%), CO_2 (0.034%) oxygen (21%) argon (1%) and other molecules in trace level are present in the atmosphere.

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Table 9.1: Composition of Air

Gas	Symbol	Content	
Nitrogen	N_2	78.084%	99.99%
Oxygen	O_2	20.947%	
Argon	Ar	0.934%	
Carbon dioxide	CO_2	0.033%	
Neon	Ne	18.20 ppm	
Helium	He	5.20 ppm	
Krypton	Kr	1.10 ppm	
Sulphur dioxide	SO_2	1.00 ppm	
Methane	CH_4	2.00 ppm	
Hydrogen	H_2	0.50 ppm	
Nitrogen oxide	N_2O	0.50 ppm	
Xenon	Xe	0.09 ppm	
Ozone	O_3	0.07 ppm	
Nitrogen dioxide	NO_2	0.02 ppm	
Iodine	I_2	0.01 ppm	

9.2.3 Microflora of Air

Human beings and animals are continuously inhaling the microbes present in the air that cause various infectious diseases. Most of the respiratory tract infections are acquired by inhaling the air pathogen. The microflora of air is studied under two categories such as indoor and outdoor microflora.

Indoor microflora

The air found inside the closed environment (building/ room) is referred as indoor air and the microbes present in this region is called indoor microflora.

Example: *Staphylococcus, Bacillus, Penicillium*.

Outdoor Microflora

The air in the exterior environment is called outdoor air and the microbes that reside there are called outdoor microflora.

Example: *Bacillus, Aspergillus*.

9.2.4 Sources of Microorganisms in Air

Air is not a natural environment for microorganisms as it doesn't contain enough moisture and nutrients to support their growth and reproduction. Soil microorganisms when disturbed by the blow of wind, gets liberated into air and remain suspended there for a long period.

Man-made actions like digging, ploughing the soil may also release soil borne microbes into the air. Microorganisms found in water may also enter air by wind action or tidal action in the form of droplets or aerosols. Air currents may bring the microorganism from plant or animal surfaces into air.

Commensal as well as pathogenic flora of the human beings may enter the air by activities like coughing, sneezing, talking and laughing. The microorganisms are discharged out in different forms of particles which are grouped on the basis of their relative size and moisture content. These are aerosols, droplets, droplet nuclei and infectious dust.

Aerosols

An aerosols are mixture of small liquid or solid particles dispresed in air. Aerosols can be natural or anthropogenic. Example: Dust and smoke.

Droplets

Droplets are formed by sneezing, coughing and talking which consists of saliva and

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mucus (Figure 9.2). Droplets are relatively large, about 10µm or more in size, and they tend to settle rapidly in still air. When inhaled these droplets are trapped on the moist surfaces of the respiratory tract. Thus, the droplets containing pathogenic microorganisms may be a source of infectious disease.



Figure 9.2: Droplets released during sneezing

Droplet nuclei

Small droplets in a warm, dry atmosphere tend to evaporate rapidly and become droplet nuclei. These are 1-4µm in size. They can remain in air for longer period and transmit various infectious airborne diseases.

Infectious Dust

Large aerosol droplets settle out rapidly from air on to various surfaces and get dried. Nasal and throat discharges from patient can also contaminate surfaces and become dry. Microorganisms can survive for longer period in dust. This creates a significant hazard, especially in hospital areas.

9.2.5 Air Borne Diseases

Many microbial diseases are transmitted through the air (Table 9.2). The incidence of diseases caused by airborne transmission can be reduced by covering one's nose and mouth during coughing or sneezing and by the use of face masks.

Table 9.2: Important airborne human diseases and causative agents (pathogens)

Human Diseases	Pathogens
Bacterial diseases	
Pulmonary tuberculosis	*Mycobacterium tuberculosis*
Pneumonia	*Klebsiella pneumoniae*
Streptococcal respiratory infections	*Streptococcus pyogenes*
Fungal diseases	
Aspergillosis	*Aspergillus fumigatus*
Cryptococcosis	*Cryptococcus neoformans*
Viral diseases	
Influenza	*Influenza Virus*
Common cold	*Picorna Virus*
Protozoal diseases	
Pneumocystosis	*Pneumocystis carinii*

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Nosocomial infection

Hospital acquired infection are also known as a nosocomial infection. It is acquired in a hospital or other health care facility. Infection is spread to the susceptible patient in the clinical setting by various means, one of them being air droplets. The infection can originate from another infected patient, staff, or in some cases, the source of the infection cannot be determined. The most common pathogens that cause nosocomial infections are *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli.* One of the common nosocomial infections is respiratory pneumonia.

9.2.6 Enumeration of Microorganisms in Air

There are several methods adopted to enumerate microorganisms in air. The most important methods are solid impingement and liquid impingement, filtration, sedimentation, centrifugation electrostatic precipitation. Many tools have been designed for the collection of air samples. Choosing an appropriate sampling device is based on many factors, such as availability, cost, volume of air to be sampled, sampling efficiency and the environmental conditions under which sampling will be conducted. One of the methods is Settle plate technique, where the microorganism carrying particles are allowed to settle onto the medium (solid impingement) for a given period of time and incubated at the optimum temperature (Figure 9.3). It works under the principle of gravitational force.



Figure 9.3: Settle plate technique showing bacterial and fungal growth

Choice of the medium depends upon the kind of microorganisms to be enumerated. For bacterial isolation Nutrient agar and for fungal isolation Sabourauds dextrose agar (SDA) can be used.

During sampling it is better to keep the plates about one meter above the ground level. Then the plates are uncovered in the selected position for the required period of time. Immediately after exposure for the given period of time the plates are closed with the lids. Then the plates are incubated for 24 hrs at 37°C for aerobic bacteria and for 2 days at room temperature (27°C) for fungi. Enumeration results are expressed as numbers of viable organisms per unit area of air colony forming unit (CFU/ mm^3).

HOTS

Can microorganisms grow in clouds?

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9.3 Microbiology of Water

Aquatic microbiology is the study of microorganisms and microbial communities in water environments. Aquatic environments occupy more than 70% of the earth's surface. Water is essential for life and one of the most important natural resource. Thus, protection and preservation of aquatic environments are vital for the continuation of life. There are two kinds of water found on earth:

1. Salt water (97%)
2. Fresh water (3%)

9.3.1 Salt Water Microflora

Salt water contains a significant level of dissolved salts. The major bodies of salt water are oceans, seas, estuaries, and certain salt water lakes. Average salinity of ocean is 3.5 grams per 100 grams of water. The pH of salt water remains relatively constant at a range of 8.3 to 8.5. The temperature of seawater fluctuates depending on location, season and depth.

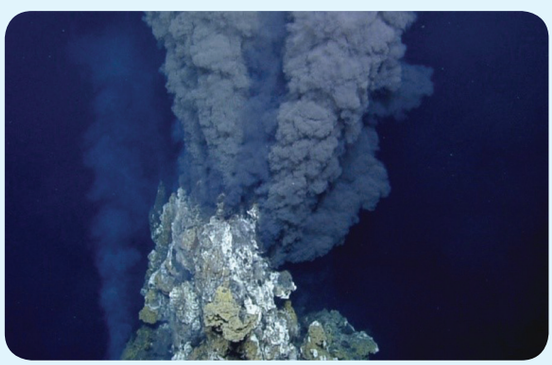
9.3.2 Estuaries

A partly enclosed coastal body of water in which fresh water is mixed with seawater is called an estuary. As a consequence of this geographical location, estuaries are of salinity levels that range from less than 0.5 to 2.5 grams of dissolved salts per 100 grams of water at their mouths, where the water flows into the marine environment. Estuaries are ecologically sensitive environments and serve as habitats for fish and wide variety of marine life. There is an increasing concern about the quality of life in estuaries seriously damaged by human impact, including over development and pollution from industrial and waste water discharges. The bacterial population in estuaries consists of *Pseudomonas, Flavobacterium,* and *Vibrio*, as well as enteric organisms. The quantities and types of organisms vary, and depend on the tide, rainfall, salinity, depth and temperature. Most of the bacteria found in water runoff come from animal and



The hot, sulphur-rich, acidic pool of yellow stone national park (U.S.) is home to many Archaebacteria. The colour differences in the pool result from the different communities of microbes that are able to thrive at extreme water temperatures. *Pyrolobus fumarii* is a unique Archaebacteria, which is hyperthermophilic that can grow at the temperature of 113°C. Some Archaebacteria live in thousands of miles deep in ocean near superheated volcanic vents.





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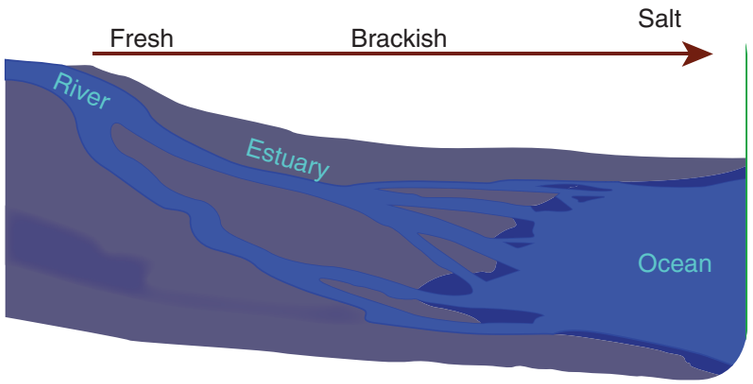


Figure 9.4: Estuary

fowl fecal matter deposited on the ground. Sometimes overflow from sewage systems contributes to these higher number of bacteria in the water. Microbiota are the primary producers in the aquatic ecosystem since they play a major role in food chain in water. Drifting microbial life of aquatic environment is called plankton. It is composed of phytoplankton and zooplankton. The bottom region of the water harbours largest number of microorganisms called as benthic microorganisms. Microorganisms have been found at all depths and at all latitudes within the ocean. Microorganisms are abundant near shore regions, where organic pollution as well as microbial pollution from the surrounding environment occurs.

Algae are common in these environments. Because of their dependence on light as a source of energy, algae occur primarily in the upper strata of the oceans and seas. Although they constitute a vital part of the food chain in the marine environments, they also can be a nuisance and threat to other forms of life. Nutrient levels in such environments can significantly increase as a result of sewage plant discharges. Under such conditions algal blooms are common.



Barophiles are bacteria that thrive deep in the ocean and live at pressures of 400-700 atmosphere but die at 1 atmosphere. A strain MT-41, a bacteria is isolated from the marine sediments in the Mariana Trench near the Phillippines where the depth exceeds 10 kilometres. This strain has optimum growth at a pressure of 709 bar.

9.3.3 Freshwater Microflora

The study of fresh water ecosystem is referred to as Limnology. The major bodies of fresh water are rivers, streams, swamps, marshes and lakes. The fresh water ecosystem is divided into lentic (still water) and lotic (flowing water) ecosystems. Lentic ecosystem is divided into zones based on light penetration and temperature. They are littoral, limnetic and profundal zones (Figure 9.5).

Most lakes are surrounded by rooted vegetation in a large littoral zone along the shore. Light penetrates the shallow littoral and open-water limnetic zone but is unable to reach the profundal zone in the deep portions of many lakes.

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As lake vegetation and animal life decompose, their organic matter provides a source of nutrients. Lakes that have very high concentrations of nutrients (particularly nitrogen and phosphorus) are termed eutrophic and have low oxygen concentrations because of extensive microbial decomposition of organic matter. In comparison, the lakes that receive small amounts of nutrients and are nutrient sparse are oligotrophic. Caulobacter grows well in oligotrophic lakes.

The common microorganisms found in fresh water are *Pseudomonas, Flavobacterium, Serratia, Chromobacterium, Micrococcus, Aeromonas* and *Alcaligenes*.

As these waters reach the surface or inland bodies of water, they become contaminated with different types of microorganisms. Rivers flowing water (lotic) in close contact with the soil may contain large numbers of soil bacteria (Bacillus, *Actinomyces* and *Streptomyces*), Fungi (*Polyphagus, Penicillium* and *Aspergillus*), and algae (*Microcystis* and *Nostoc*). These microorganisms frequently impart an earthy odour and taste to river water. Rivers also receive high concentration of bacteria and agricultural chemicals through surface runoff water from adjoining soil during heavy rains and irrigation. In many countries, rivers are heavily polluted with sewage bacteria, especially *Escherichia coli, Enterobacter fecalis, Proteus vulgaris* and other intestinal bacteria.

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Red Tides

Red tide is a common name for a phenomenon known as an algal bloom which is caused by a species of Dinoflagellates (*Gymnodinium*) and the bloom takes on a red colour due to the presence of photosynthetic pigments with the production of toxins. The most conspicuous effects of red tides are associated with mortalities of marine species



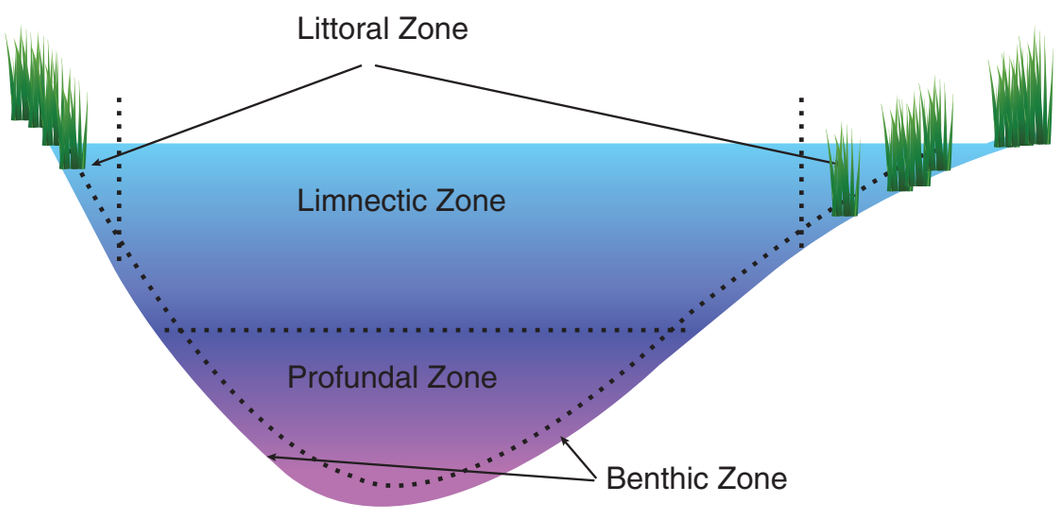


Figure 9.5: Light penetration zones of a freshwater lake

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9.3.4 Eutrophication

The addition of excess quantities of nutrients to aquatic ecosystems termed eutrophication often cause damage on the communities involved. Eutrophication is an enrichment of water by nutrients, especially nitrogen and phosphorus which are maintained at high levels that causes structural changes to the eco system. The sudden influx of abundant nutrients encourages a heavy surface growth of cyanobacteria and algae called a bloom (Figure 9.6). This heavy matt of biomass effectively shuts off the oxygen supply to the lakes below. The lack of oxygen greatly disturbs the ecological balance of the community. It causes massive death of strict aerobes. Fish invertebrates and anaerobic or facultative microbes will survive.

Effects of eutrophication

- Eutrophication can have serious, long term effect. The most notable effect of eutrophication is **algal blooms**.
- When a bloom occurs, the river, lake or ocean becomes covered with algae, which is usually bright green.
- Blue green algae like *Microcystis, Anabaena, Gonyaulax* and other Dinoflagellates produce toxins which are poisonous to aquatic lives such as fish.
- Algal toxin may also contaminate the drinking water supply.
- Plankton blooms of green alga create problems of O_2 supply in the water.
- Musty tastes and odors in drinking water are the other effects of Eutrophication.
- Excessive growth of aquatic weeds which impair fishing, swimming, boating, shell fish production.
- Irrigation canals may become clogged.

Control measures

Five different methods have been suggested to control eutrophication they are,

1. Ecological management (control the flow of nutrients into natural wastes)
2. Advanced treatments Example: Phosphate removal is effected by precipitation with lime.
3. Chemical algicides Example: copper sulphate



Figure 9.6: Algal blooms in a polluted eutrophic lake

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4. Biological algicides Example: Bacteria
5. Destratification Example: physical/ mixing / forced aeration.

9.4 Water Pollution and Microbial Contamination

Water is polluted by both natural as well as man-made activities. Polluted water is one which consists of undesirable substances rendering it unfit for drinking and domestic use.

Sources of water pollutants

1. Industrial waste
2. Sewage waste
3. Mining activities
4. Marine dumping
5. Accidental oil leakage
6. Burning of fossil fuels
7. Chemical fertilizers and pesticides
8. Radio-active waste

The most prevalent biological contaminants in water are microbes, particularly bacteria and viruses. Most of the bacteria carried by storm runoff originate from animal fecal matter. Studies have shown that during storms, the water that drains off the land and into sewage systems also carries large quantity of bacteria and chemicals. The chemicals include pesticides applied to lawns, chemical wastes near industrial plants, and organic matter deposited o the ground by different sources. In addition to the chemical and biological contaminants, physical properties also affect the quality of biological life in water. Among these are pH, temperature, dissolved oxygen concentration and salinity.

Potable Water

Clean water free from odour, disagreeable taste, harmful chemicals, turbidity and microorganisms is called potable water, which is safe to drink and can use for food preparation without risk of health problems.

Biological oxygen demand (BOD)

Biological oxygen demand is one of the common parameter to monitor water quality and purity. BOD is the amount of dissolved oxygen needed by aerobic organisms to break down organic material present in a given water sample at certain temperature over a specific period of time. The amount of decomposable organic material in sewage is measured by the biochemical oxygen demand, or BOD. The BOD of the water sample is determined by aerating it, measuring the amount of oxygen in sample before incubation, placing the sample in a sealed container (BOD bottle) and incubating the container for five days at 20°C. During this five day period, microorganisms in the water grow and oxidize any organic materials in it. After incubation period, the BOD of the water can be determined by measuring the quantity of residual oxygen in the container. BOD of drinking water should be below 3ppm or 3mg/litre.

Indicator Microorganisms

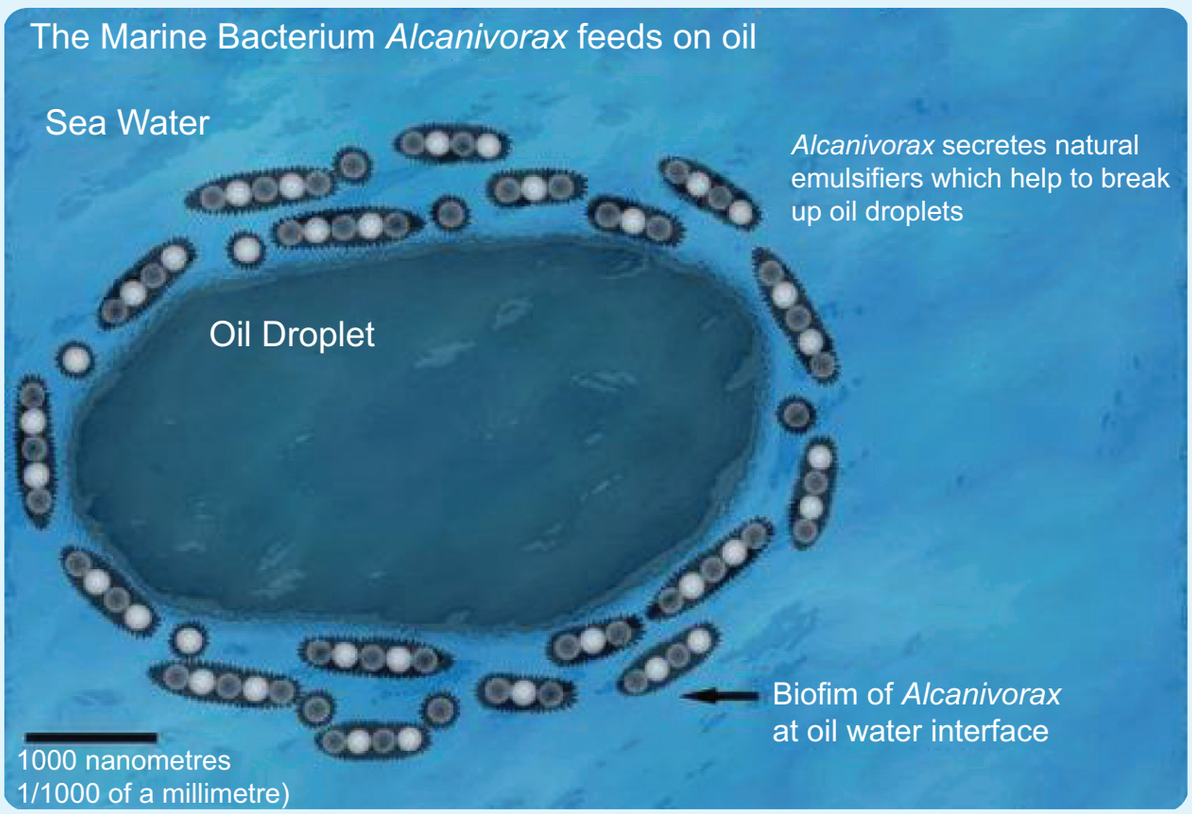
Indicator organism are frequently used to monitor bacterial contamination of water. These indicator organisms provide a representative index of the water contamination by pathogenic microbes. The indicator organisms generally used in water quality monitoring are those that are associated with the gastrointestinal tract

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Microbes at work:

One of the major environmental problems today is hydrocarbon contamination resulting from the activities related to the Petrochemical Iindustry. Accidental releases of petroleum products are of particular concern in the environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants. Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. There are the two main approaches to oil spill bioremediation: (a) Bioaugmentation, in which known oil-degrading bacteria are added to supplement the existing microbial population, and (b) Biostimulation, in which the growth of indigenous oil degraders is stimulated by the addition of nutrients or other growth-limiting cosubstrates. Bacteria like *Pseudomonas putida* and *Alcanivorax borkumens* are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment. Bioremediation is a potential source for clean and green environment.



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and fecal matter. The most common group of indicator organisms used in water quality monitoring are the coliforms, bacteria that are Gram negative, aerobic or facultative anaerobic, non-spore forming rods that ferment lactose with gas production within 48 hours at 35°C. Examples of coliforms are *Escherichia coli, Enterobacter aerogenes* and *Klebsiella pneumonia*. Two analytical procedures were followed to check the presence of coliforms in water. They are Most Probable Number (MPN) and Membrane Filteration (MF) technique. The number of coliforms per 100ml of water sample is estimated to find the quality of water and its suitability for drinking purposes. In addition to coliforms, coli phages, *Clostridia* and human enteric viruses are also monitored in drinking water.

Waterborne diseases

Waterborne diseases are posing a serious threat to health (Table 9.3).

Table 9.3: Waterborne diseases

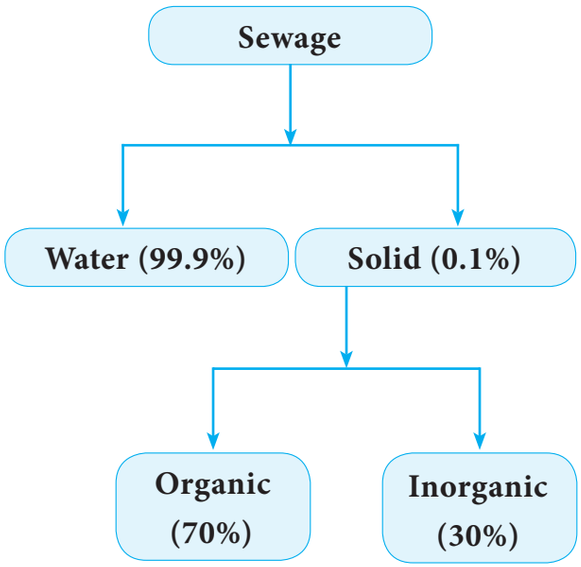
Waterborne diseases	Causative agent	Symptoms
Bacterial diseases		
Enteric fever	*Salmonella typhi*	Fever & enlargement of spleen
Cholera	*Vibrio cholerae*	Vomiting & watery diarrhea
Leptospirosis (Weils Disease)	*Leptospira interrogans*	High fever, red eyes, muscle pain and vomiting
Viral diseases		
Infectious Hepatitis	*Hepatitis A*	Jaundice, vomiting & abdominal pain
Gastroenteritis	*Rotavirus*	Diarrhoea, vomiting
Poliomyelitis	*Coxsackie Virus*	Head ache, neck stiffness, flaccid paralysis.
Protozoan diseases		
Giardiasis	*Giardia lamblia*	Chronic diarrhoea, abdominal cramp, fatigue & weight loss
Amoebiasis	*Entamoeba histolytica*	Stomach pain, bloody stools, fever
Meningoencephalitis	*Naegleria fowleri*	Ulceration, watery, bloody diarrhoea
Treamatode disease		
Schistosomiasis	*Schistosoma*	Drowsy, confusion, head ache, stiff neck, Diarrhoea

9.5 Sewage Treatment

Wastewater treatments also called **sewage treatment** which removes the impurities from wastewater, or sewage, before disposal into natural bodies of water. In broad terms, water is said to be polluted when it contains enough impurities to make it unfit for a particular use, such as

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drinking, swimming, or fishing. Water pollution is caused primarily by the drainage of contaminated wastewater into surface water or groundwater. The wastewater treatment is a major element of water pollution control.



Goal of Sewage Treatment

- To convert waste water into a reusable resources.
- To reduce the spread of pathogenic microorganisms
- To avoid health hazards while swimming and boating in the water.
- To prevent the development of objectionable colours and tastes

The predominant method of wastewater disposal in large cities and towns is discharge into a body of surface water. Suburban and rural areas rely more on subsurface disposal. In either case, wastewater must be purified or treated to some degree in order to protect both public health and water quality. Suspended particulates and biodegradable organics must be removed to varying extents. Pathogenic bacteria must be destroyed. It may also be necessary to remove nitrates, phosphates (plant nutrients) to neutralize or remove industrial wastes and toxic chemicals. The degree to which wastewater must be treated varies, depending on local environmental conditions and governmental standards.

HOTS

List the major oil spills that occurred recently in India and other countries. Is it practically possible to clean up these oil spills using bacteria?



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Sewage Treatment Methods

Sewage treatment defined as an artificial process in which sewage is subjected to remove / alter its constituents to render it less offensive. There are three levels of wastewater treatment (Figure 9.7): primary, secondary, and tertiary (or advanced).

1. Primary treatment
2. Secondary treatment
3. Tertiary / Final treatment

Primary treatment

Primary treatment removes about 60 percent of total suspended solids and about 35 percent of BOD; dissolved impurities are not removed. It is the physical method which remove large floating and suspended solids from sewage water. Example: papers, leaves, bottles, rocks, pieces of metal or wood. These objects are removed by passing the sewage through screens (Figure 9.8) and grit chambers. The screened water is then sent to settling tanks or basin, where the suspended solids are allowed to settle as primary sludge. Materials such as oil or grease, which float on the surface, are removed with a skimmer. The liquid wastewater remaining in the settling tank or basin is then ready for secondary treatment. The fluid from primary treatment is called primary effluent.



Figure 9.8: Bar Screening in Sewage Treatment

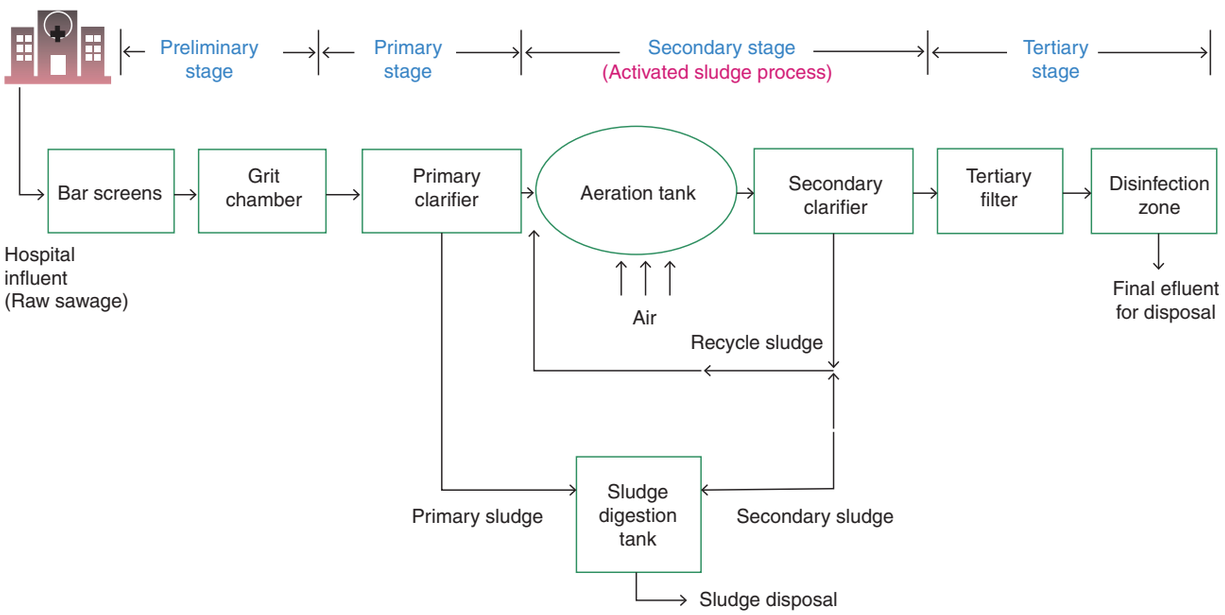


Figure 9.7: Schematic diagram of waste water treatment

Secondary treatment

Secondary treatment removes more than 85 percent of both suspended solids and BOD. Secondary treatment involves the oxidation of the primary effluent by microorganisms. The common types processes used are:

1. Trickling filter process
2. Activated Sludge process
3. Oxidation ponds

A trickling filter consists of a large tank or basin filled with a bed of crushed stone, gravel, slag or other porous material. Sewage is sprayed in a fine mist over the rocks. As the sewage trickles through the bed, organic matter clings to the rocks, where it is digested by heterotrophic microorganisms (Figure 9.9). The microorganisms are contained within a biofilm which are produced by slime forming bacteria such as Zooglea and the organic matter, is oxidized to gases and inorganic products.

In the activated sludge process sewage is mixed with a slime forming bacteria (Zooglea) in a large aeration tank. As the mixture is aerated, large flocs, or clumps, form. These clumps contain not only the original slime forming bacteria but also large population of heterotrophs, which oxidize organic matter within these clumps. In this system wastewater is continuously pumped into the tank and the treated water is removed into a holding tank and the flocs are allowed to settle. The settled floc material is recycled into the tank as an inoculums to continue the process. The remaining floc is further treated or removed for burial or incineration.

Oxidation ponds (Figure 9.10) are used in some communities to handle small loads of sewage. Small communities and isolated areas frequently use oxidation ponds for treatment of waste water. Sewage is channeled into an initial pond where the sludge settles out. The liquid portion of the sewage is then pumped into an adjacent series of ponds where aeration allows bacterial growth and degradation of organic matter. These secondary ponds often are seeded with algae which provide oxygen for the growth of aerobic, heterotrophic bacteria.

Tertiary treatment

Tertiary processes can remove 99 percent of all the impurities from sewage. Although effluents from secondary treatment have a low BOD, they may contain eutrophication inducing salts (Phosphorus and Nitrogen compounds), organic and inorganic suspended solids, and poorly biodegradable organic materials. Advanced or tertiary treatment process are designed to reduce or eliminate these materials depends more on physical and chemical processes than biological processes. For phosphorus elimination the phosphates are converted to poorly soluble aluminum, calcium or iron compounds and removed by precipitation. Nitrogen in sewage effluent is removed primarily through nitrification by microorganisms. The extent of nitrification during tertiary treatment depends on adequate treatment plant designed and the proper removal of sludge so that these bacteria are grown under optimal conditions. Otherwise, large amounts of nitrogenous compounds may escape tertiary treatment and released in the effluent. Suspended

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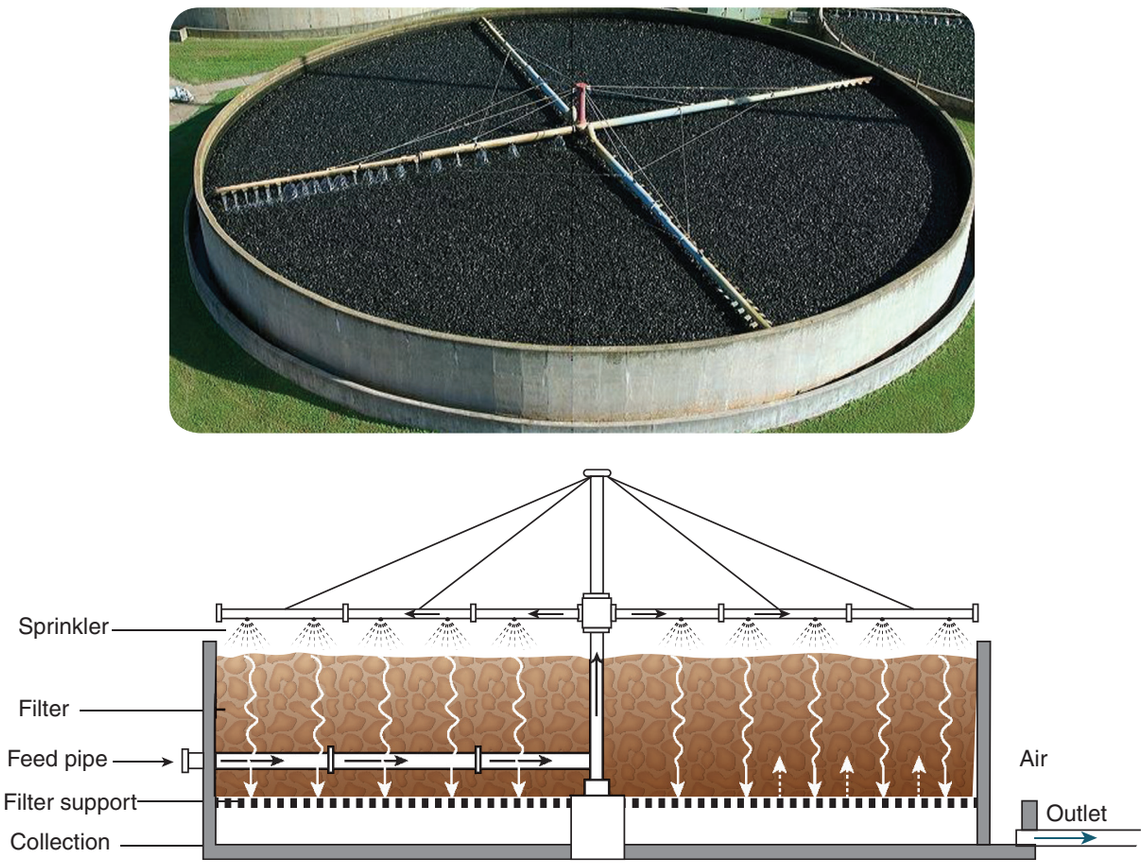


Figure 9.9: Trickling Filter in sewage treatment

solids are eliminated in sewage through filtration or sedimentation. Poorly biodegradable substances can be removed by the use of specialized microorganisms capable of using them as substrates. Chlorine is frequently added to tertiary treated effluent to kill any remaining microorganism.

9.6 Recycling of Treated Sewage

Water recycling is reusing treated wastewater for beneficial purposes such as agricultural and landscape irrigation, industrial processes, toilet flushing, and replenishing a ground water basin. Recycled water can satisfy most water demands, as long as it is adequately treated to ensure water quality appropriate for the use.

The residue that accumulates in sewage treatment plants is called sludge.

Sludge Digestion

Treatment of sewage sludge may include a combination of thickening, digestion, and dewatering processes. Among these digestion is mediated by microbes. Sludge digestion is a biological process in which organic solids are decomposed into stable substances. Digestion reduces the total mass of solids, destroys pathogens, and makes it easier to dewater or dry the sludge. Digested sludge is inoffensive, having the appearance and characteristics of a rich potting soil.

Most large sewage treatment plants use a two-stage digestion system in which organics are metabolized by bacteria anaerobically.

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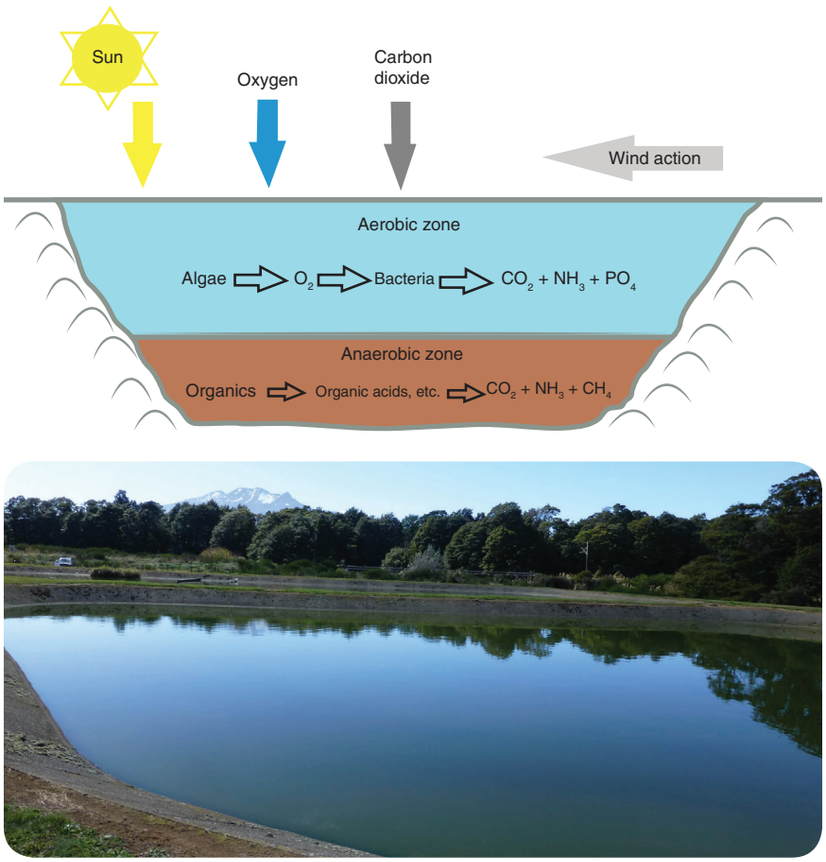


Figure 9.10: Oxidation Ponds

Sludge digestion may also take place aerobically The sludge is vigorously aerated in an open tank for about 20 days. Methane gas is not formed in this process.

Digested sewage sludge is usually dewatered before disposal. Sludge-drying beds provide the simplest method of dewatering. A digested sludge slurry is spread on an open bed of sand and allowed



Septic tank is a small scale anaerobic treatment process. It is commonly used in rural areas. It is simple, inexpensive and satisfactory if properly operated. The septic tank consists of an underground sedimentation container into which sewage from a home enter and is retained for a short time. The organic matter in the sewage settles to the bottom of the tank where it is covered by a thin organic film that excludes oxygen. Anaerobic bacteria in the sediment digests the organic matter into simpler chemical compounds and gases. The gases are then discharged through a vent in the tank. Liquids in the tank rise and overflow through an outlet pipe and are distribute in the surrounding soil. As the water trickles through the soil any remaining organic matter is decomposed by aerobic prokaryotes. Septic tank should not be located near water supplies because not all bacterial pathogens are removed by this treatment. Undigested solids in the bottom of the septic tanks must be periodically removed.

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to remain until dry. Drying takes place by a combination of evaporation and gravity drainage through the sand.

Sludge Disposal

The final destination of treated sewage sludge usually is the land. Dewatered sludge can be buried underground in a sanitary landfill. It also may be spread on agricultural land in order to make use of its value as a soil conditioner and fertilizer. Since sludge may contain toxic industrial chemicals, it is not spread on land where crops are grown for human consumption.

When a suitable site for land disposal is not available, as in urban areas, sludge may be incinerated. Incineration completely evaporates the moisture and converts the organic solids into inert ash. The ash must be disposed of, but the reduced volume makes disposal more economical. Air pollution control is a very important consideration when sewage sludge is incinerated. Appropriate air-cleaning devices such as scrubbers and filters must be used.

Benefits of Sewage Treatment

- Water recycling has proven to be effective and successful in creating a new and reliable water supply, while not compromising on public health.
- Water recycling can help us find ways to decrease the diversion of water from sensitive ecosystems
- Water users can supplement their demands by using recycled water.
- Decreases wastewater discharges
- Reduces and prevents water pollution
- Recycled water can be further used in Thermal power plant (for cooling), Municipal use, Irrigation and Agricultural use.

Infobits

There is a huge business opportunity in finding ways to use these waste dumps for productive purposes - energy, organic fertilizer. This requires methods of dealing with **old waste** that has been accumulating over the years as opposed to new/fresh waste.

9.7 Composting

Compost is organic matter that has been decomposed and recycled as a fertilizer and soil amendment. It is a mass of rotted organic matter made from waste. Example: garbage, paper, sugarcane trash, paddy straw, aquatic weeds, other agricultural waste.

Composting is a natural process in which aerobic and anaerobic microorganisms decomposes organic matter into valuable manure called as compost. The primary objective of composting is to convert an unstable material into stable end product (Figure 9.11).

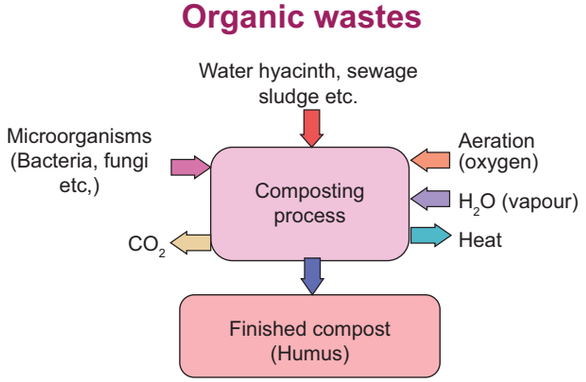


Figure 9.11: Composting process

The humification of organic material occurs in three stages

1. **Mesophilic stage** - Mesophilic is the initial stage of decomposition, lasting

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for about a week, during which sugars and other simple carbohydrates are rapidly metabolized. This is an exothermic process and may cause an increase in temperature by 40°C. Example: *Bacillus subtilis*

2. **Thermophilic stage** - Thermophilic is the second stage, lasting for about two weeks, during which the temperature may rise to about 50 to 75°C. Such a drastic increase in temperature is accompanied by the decomposition of cellulose and other resistant materials. It is important that the material be thoroughly mixed and kept aerated during this stage. Example: *Bacillus stearothermophilus*
3. **Curing stage** - The temperature decreases during this final stage and the material being composted is recolonized by mesophillic organisms, which often produce plant-growth stimulating compounds.

The humification of organic material is characterized by an increase in concentration of humic acids approximately from 4 to 12 percent, and decreases during the composting process.



What should you compost?

When selecting materials for your compost pile avoid the following:

- Wastes that attract pests
- Diseased / insect ridden plants
- Non-biodegradable things



Compost bed types

1. Pit method
2. Heap method

Pit method

The compost pits dug in soil with dimension of 3.5m × 2.5m × 1.5m (L×B×H). The pits are filled layer by layer using green plants and animal excreta. The layering is repeated until the pit is filled. Finally a layer of mud is plastered on the top of the pit (Figure 9.12).



Figure 9.12: Pit method

Heap method

In regions with heavy rainfall, the compost may be prepared in heaps above the ground level and protected by a shed. The pile is made with dimension of 2m × 2m × 1.5m (L×B×H) (Figure 9.13).



Figure 9.13: Heap method

Methods of compost preparation

1. Indore method
2. Bangalore method

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Indore method

This method was developed at Indore, India. In this method organic wastes are spread in the cattle shed to serve as bedding. Trenches are dug with dimension of 10ft × 6ft × 2ft.

Dry wastes with cattle dung and soil are added in ratio of 4: 2: 1 up to 2 inches layer in composting pit. A moisture level of about 40-50% is ideal for good composting. Odour and insect problems can be controlled by covering the piles with a layer of soil or wood chips.

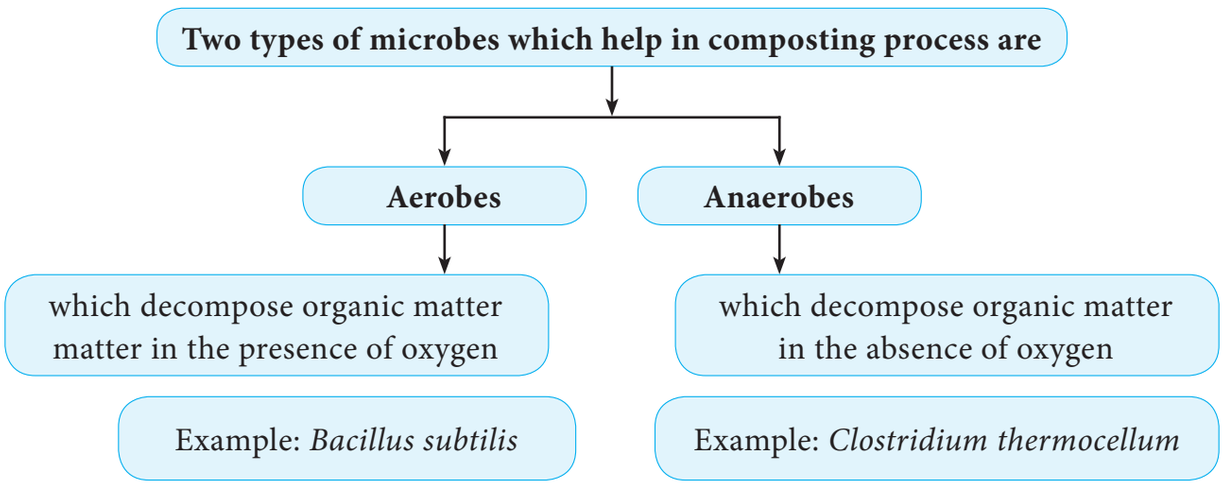
The heap is left undisturbed for about 8 to 9 months. Turning the pile for every 15 days is important for coplete composting because pile needs a periodic influx of O_2. Plant residues, weeds, sugarcane leaves, grass, wood ashes, animal dung, and water urine soaked mud can also be used as raw materials for this type of composting.

Bangalore method

- This method was developed at Bangalore, India. It is recommended as a satisfactory method for disposal of town wastes and night soil.
- The compost pits dug in soil with dimension of 4.5m × 2.5m × 90cm (L×B×H)
- In the Bangalore method of composting, dry waste material of 25cm thick is spread in a pit and a thick suspension of cow dung in water is sprinkled over for moistening.
- A thin layer of dry waste is laid over the moistened layer
- The pit is filled alternatively with dry layer of material and cow dung suspension till it rises 0.5m above the ground level and plastered with wet mud and left undisturbed for about 4-6 months or till required.
- This method saves labour cost because there is no need of turning & regular sprinkling of water.

Benefits of compost

- Compost improves the quality of soil hence called as a soil conditioner.
- Compost contains a variety of the basic nutrients required for healthy growth of the plant.
- Nitrogen, phosphorous, potassium and certain micronutrients *viz*, manganese, copper, iron and zinc are found in compost.



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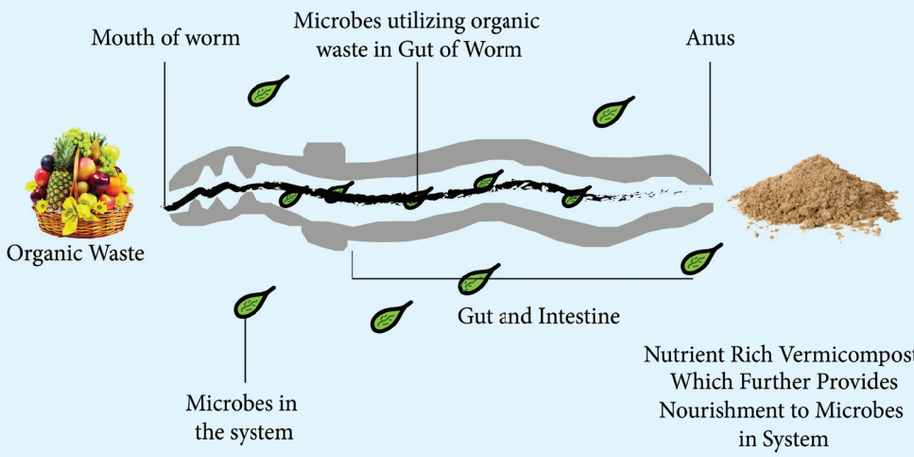
Infobits

Role of microbes in vermicomposting:

Recycling organic wastes through Vermiculture Biotechnology (VBT) is being considered an economically viable solution. Earthworms are regarded as natural bioreactors which proliferate along with other microorganisms and provide required conditions for the biodegradation of wastes. Vermicomposting involves bio oxidation and stabilization of organic material through the interactions between earthworms and microorganisms.

Worms like to feed on slowly decomposing organic materials like fruit and vegetable scraps. Worms produce castings that contain beneficial microbes and nutrients, which makes a great soil amendment. Worms are very efficient at breaking down food scraps and can eat over half their body weight in organic matter every day. Vermicasting, also called vermicomposting, is the processing of organic wastes through earthworms . It is a natural, odourless, aerobic process, much different from traditional composting. Earthworms ingest waste, then excrete casts – dark, odourless, nutrient- and organically rich, soil mud granules that make an excellent soil conditioner.





- The composted product is safe and easy to handle, and does not induce nitrogen deficiency in recipient plants by nitrogen stabilization in the compost.
- It suppresses disease infestation by partial sterilization and detoxifies pollutants.
- Compost material is principally used for the reclamation of drastically disturbed. Example: mined soil, landscaping and agriculture.
- Compost finds unrestricted application in parks and gardens for ornamental plants, in land reclamation and highway beautification projects.

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9.8 Biogas Production

Worldwide energy consumption and demand are growing up since past 50 years. With the growth of population, demand for energy is also increasing leading to an uneven supply and distribution of resources. Therefore, the requirement of sustainable and eco friendly energy in India to satisfy the energy demand is inevitable. Along with the source of sustainable green energy, biogas production is an alternative way to produce clean energy through solid waste management.

Biogas is a type of renewable energy that can be produced from decomposition of animal and plant waste. It is composed of 50–75% methane, 25–50% carbon dioxide, 0–10% nitrogen, 0–3% hydrogen sulphide, 0–1% hydrogen and traces of other gases. The term "anaerobic" suggests that the process occurs in the absence of free oxygen and produces CH_4 through decomposition of waste in nature and reduces environmental pollution.

Biogas generating technology is a favorable dual purpose technology at present since used as fuel and fertiliser.

Leftover foods fruit & vegetable wastes and cow dung can be subjected to anaerobic digestion for energy production in a variety of ways.

Production of Biogas

Biogas production is carried out in an airtight cylindrical tank called biogas digester Cow dung is mixed with equal volume of water and made into slurry and fed through the inlet of the biogas unit. The digestion proceeds at 37°C with sufficent amount of nitrogen and phosphorus. The production of biogas sets around 40-50 days, under anaerobic conditions. Production of biogas accomplished in 3 stages namely Hydrolysis, Acetogenesis and Methanogenesis

Steps

Hydrolytic fermentative stage

In this step, several microbes secrete different enzymes, which cleave the complex macromolecules into simpler forms. Organisms that are active in a biogas process during the hydrolysis of polysaccharides include various bacterial groups such as *Bacillus*, *Clostridium*, *Cellulomonas*.



Figure 9.14(a): Biogas production schematic diagram

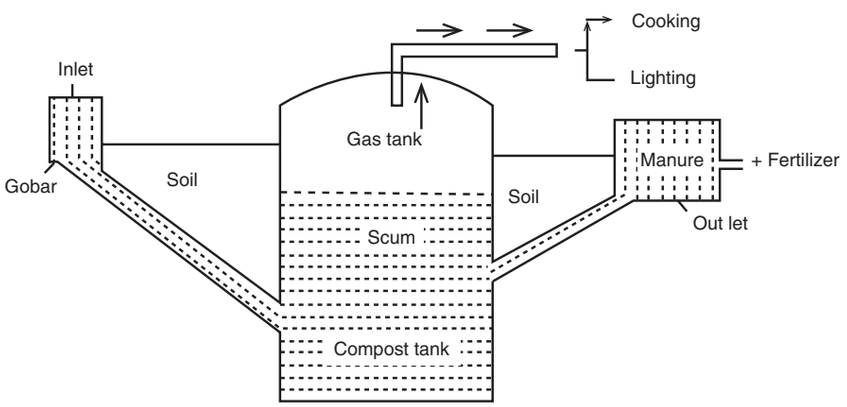
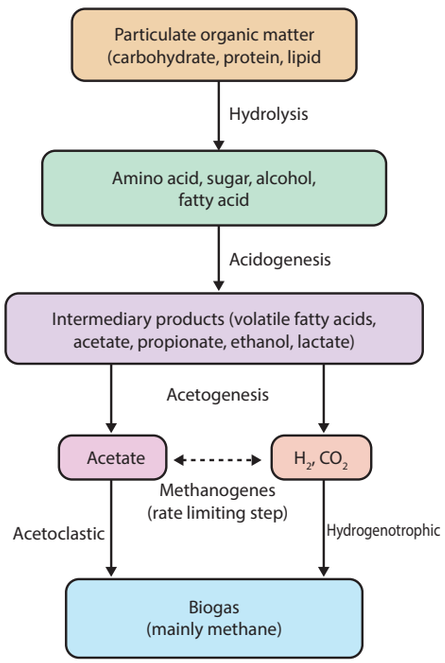


Figure 9.14(b): Biogas Digester

Acetogenic stage

Through various fermentation reactions, the products from hydrolysis are converted mainly into various organic acids (acetic acid, propionic acid, butyric acid, succinic acid, lactic acid), alcohols, ammonia (from amino acids), carbon dioxide and hydrogen. Facultative anaerobes and hydrogen producing bacteria Example: *Acetovibrio cellulosolvens, Bacteroid cellulosolvens* are involved.



Methanogenic stage

In this step, obligate anaerobic methane producing bacteria produce Methane gas as the major end product along with Carbon dioxide, Hydrogen and traces of other gases. Methanogenesis has six major pathways, each converting a different substrate into Methane gas. The six major substrates used are Carbon dioxide, Formic acid, Acetic acid, Methanol, Methylamine, and Dimethyl sulphate. The methanogenic bacteria include *Methanococcus voltae* and *Methanobacterium formicum* (Figure 9.14 a, b).



Deenabandhu model

It is a biogas production model popular in India which means "Friend of the helpless"

Small scale biogas unit

The biogas production is carried out in an air tight cylindrical tank called biogas digester (Figure 9.15).

Applications

1. Biogas used as fuel
2. Used to generate electricity
3. Biogas is used to run any type of heat engine in order to generate electrical and mechanical power.
4. Producing high quality fertilizer.
5. Reducing water and air pollution.

Summary

Environmental microbiology is the field of science that examines the relationship between microorganism and their biotic and abiotic environments.

Identification of new microbes and their products have practical application on protecting the environment as well as human health. Areomicrobiology is the study of airborne microorganisms and is one of the important modes for the transmission of infectious diseases. The air in our atmosphere is composed of different gaseous molecules. The air present both interior and exterior of the environment is called indoor air and outdoor air. The microorganisms are discharged out in different forms which are grouped on the basis of their relative size and moisture content. They are aerosols, droplet, droplet nuclei and infectious dust. Hospital – acquired infection are also known

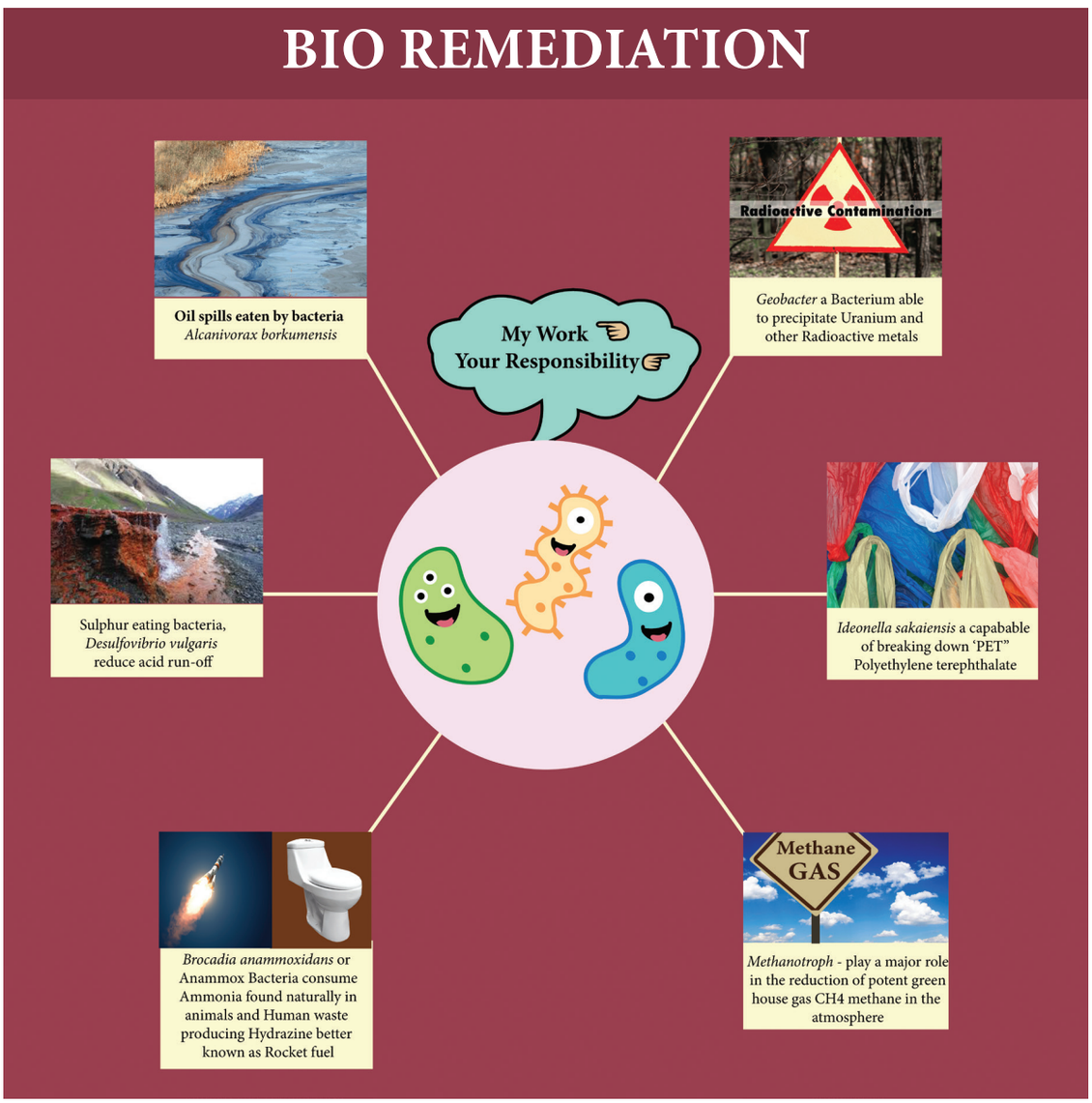
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as nosocomial infection. Solid and liquid impingement, filtration, sedimentation, centrifugation, electrostatic, precipitation are used to enumerate microorganisms in air.

The Aquatic Microbiology is the study of microorgansims and microbial communities in the water environment. Eutrophication is an enrichment of water by nutrients, especially nitrogen and phosphorus, which makes the overgrowth of the "algal bloom". Apart from microbes and chemicals, pH, temperature, dissolved oxygen concentration and salinity are the physical properties that affect the quality of biological life.

BOD is the amount of dissolved oxygen needed by aerobic organisms to breakdown organic material present in a given water sample at certain temperature over a specific period of time. Indicator organisms are frequently used to monitor bacterial contamination of water.

Waste water treatment are called sewage treatment which removes the impurities from waste water and sewage. Trickling filter, activated sludge, oxidation ponds are generally used. Compost is a natural process in which aerobic and anaerobic microorganisms decomposes organic matter in to valuable manure called as compost.



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Evalution

Multiple choice questions

1. In 1930, the term aerobiology was coined by _____.
   a. F.C.Meier b. Miquel
   c. Carnelly and colleagues
   d. None of the above
2. The gas molecules which are more in atmosphere.
   a. Nitrogen b. Oxygen
   c. Carbon dioxide d. Neon
3. _____ are water droplets containing several types of microorganisms released in to the air from the water sources.
   a. Droplet nuclei
   b. Infectious dust
   c. Droplet d. Aerosols
4. Hospital acquired infections are otherwise called as
   a. Nosocomial infection
   b. Gastro intestinal infections
   c. Ocular infection
   d. All the above
6. _____ Is the amount of dissolved oxygen needed by aerobic organisms to breakdown organic materials?
   a. BOD b. COD
   c. DOB d. DOC
7. _____ is called as Indicator organisms
   a. *Escherichia coli*
   b. *Staphylococcus aureus*
   c. *Pseudomonas aeruginosa*
   d. None of the above
8. In which process of treating sewage,99 percent of all the impurities from the sewage are removed.
   a. Primary treatment process
   b. Secondary treatment process
   c. Tertiary treatment process
   d. None of the above
9. Primary treatment is a _____ method
   a. Physical b. Chemical
   c. Biological d. All of the above
10. Activated sludge process is an example for_____treatment
    a. Physical b. Chemical
    c. Biological d. Composting
11. Chlorination is an example for _____ treatment
    a. Physical b. Chemical
    c. Biological
    d. None of the above
12. _____ is an open tank where algal forms are allowed to grow
    a. Trickling filter
    b. Oxidation pond
    c. Sludge digester
    d. None of the above
13. Trickling filter is an example for _____ treatment
    a. Physical b. Chemical
    c. Biological d. None of the above
14. Which one of the following is a good source for making compost?
    a. Plastic b. Aluminum foil
    c. Vegetable peel d. Polythene
15. Algal boom in pond water is called
    a. Eutrophication
    b. Acclimatization
    c. Algalization
    d. Green manuring

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16. The most common toxic algal bloom among the following
    a. Euglena b. Microcystis
    c. Paramaecium d. Hydra
17. Organism inhabiting the bottom sediment of aquatic environments constitute _____ community
    a. Pelagic b. Benthic
    c. Abyssopelagic d. Episammon
18. Study of flora and fauna of fresh water
    a. Ornithology b. Zoology
    c. Paleontology d. Limnology
19. A partly enclosed coastal body of water in which river water is mixed with sea water
    a. Lake b. Estuary
    c. Creek d. Bay
20. Chemical agent used for disinfecting water
    a. Glycols b. Chlorine
    c. Hydrogen peroxide
    d. None of the above
21. The main component of natural gas is_____
    a. CO_2 b. Carbon monoxide
    c. O_2 d. Methane
22. Biogas is a mixture of
    a. Methane, Nitrogen, Hydrogen
    b. Methane, Nitrogen, Oxygen
    c. O_2, CO_2, N_2
    d. None of these
23. Which compost method is employed in the regions with heavy rainfall?
    a. Heap method
    b. Pit method
    c. Indore method
    d. Banglore method

Answer the following

1. What are properties favour survival of microorganisms in the atmosphere?
2. Define Nosocomial infections
3. What is droplet nuclei?
4. What is the purpose of bacteriological analysis of water?
5. What is potable water?
6. What is sewage?
7. Define sludge.
8. Explain the sewage treatment processes.
9. Is compost a fertilizer?
10. Describe the process of composting?
11. What is Biogas?
12. What role can biogas play in supplying our energy needs?
13. Draw the light penetration zones of a fresh water lake.
14. What is Eutrophication?
15. Write a note on trickling filter.
16. Discuss the air borne diseases.
17. Write in detail about settle plate technique.
18. Write in detail about biogas production processes.
19. Discuss the Indore method of composting in detail.
20. Discuss the sludge digestion methods.
21. Explain the benefits of waste water treatment?

Student Activity

1. Set up a small scale anaerobic digester for anaerobic digestion using cow dung / fruits & vegetable waste.
2. Instruct the students to bring algal bloom sample from their residential area.
3. Visit nearby sewage treatment plant.

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Chapter 10

Soil Microbiology

Chapter Outline

10.1 Soil in General

10.2 Pioneers of Soil Microbiology

10.3 Components of Soil

10.4 Soil Microorganisms

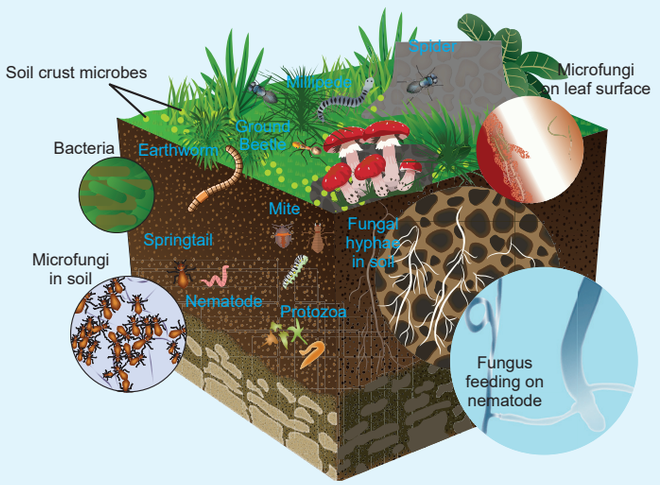
10.5 Microbial Interactions

10.6 Rhizosphere

10.7 Phyllospere

10.8 Spermosphere

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Soil is inhabited by a living microscopic population which is responsible for the numerous reactions taking place in the soil. The soil microorganisms affects the life economy of man in many ways.

Learning Objectives

After studying this chapter the student will be able,

- *To know the composition of soil.*
- *To understand the importance of soil microorganisms in soil fertility.*
- *To learn about the beneficial and harmful interaction between soil microorganisms.*

Knowledge of Soil Microbiology is essential to understand the agricultural and environmental science. Without soil microorganisms, life as we know could not exist on earth. Organic matter would accumulate in the form of undecomposed substances. Why should we study soil Microbiology? If we understand what is happening in soil, we get a better idea of how other biological systems work on earth.

10.1 Soil in General

Soil is the outer covering of the earth. It consists of loosely arranged layer of materials composed of inorganic and organic constituents. Soil provides the physical support needed for the anchorage of root system and serves as the reservoir of air, water and nutrients that are essential for plant growth.

10.1.1 Formation of Soil

The processes involved in the formation of soil are slow, gradual and continuous. The sum total of environmental effects on rocks collectively known as the weathering of rocks. Weathering of rocks is a continuous phenomenon and add more and more soil to the surface of the earth. There are different types of parent materials of rocks available for the formation of soil.

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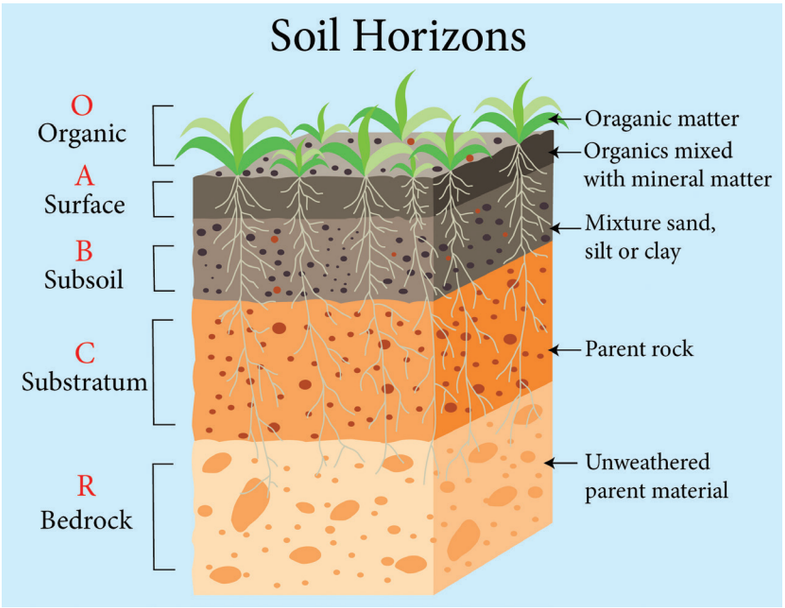


Figure 10.1: Soil Horizons

10.1.2 Soil Horizons

Each type of soil is characterized by the presence of different horizons which can be seen in a soil profile (Figure 10.1). The formation of soil horizons depends on climate, living organisms, parent rock material, topography and time; all of which control the weathering of rocks.

10.1.3 Physical and Chemical Properties of Soil

Physical properties of a soil type depends on the size of particles, soil texture, soil temperature and soil pH.

Chemical properties of soil includes three main components which provides nutrients for plant growth. The three components are the organic matter, the derivatives of parent rock materials and the clay fraction.

The fertility of soil depends not only on its chemical composition, but also on the qualitative and quantitative nature of microorganisms inhabiting it. The branch of science dealing with study of soil microorganisms and their activities in soil is known as '**Soil Microbiology**'.

10.2 Pioneers of Soil Microbiology

Scientists studied the microorganisms from water, air and soil. They recognized the role of microorganisms in natural processes. They realized the importance of soil microorganisms in growth and development of plants. Soil Microbiology emerged as a distinct branch of soil science during first half of the 19th century.

Sergei N. Winogradsky discovered the autotrophic mode of life among bacteria and established the microbiological transformation of Nitrogen and Sulphur. He isolated nitrifying bacteria for the first time and demonstrated the role of these bacteria in nitrification (1890). Further he demonstrated that free-living *Clostridium pasteurianum* could fix atmospheric Nitrogen (1893). He developed the Winogradsky column (Figure 10.2), a self contained ecosystem for studying the

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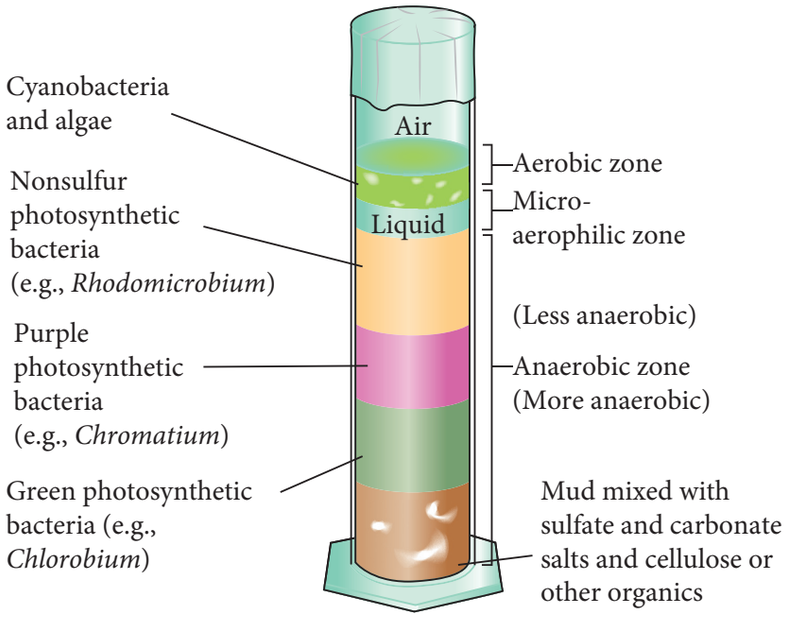


Figure 10.2: Winogradsky column

Sulphur cycle. Therefore, he is considered as the '**Father of Soil Microbiology**'.

M. W. Beijerinck (1888) isolated root nodule bacteria in pure culture from nodules in legumes and named them as *Bacillus radicola*. Thus, he is considered as the '**Father of Microbial ecology**'.

Beijerinck and Winogradsky (1890) developed the enrichment culture technique for isolation of soil organisms, proved independently that transformation of nitrogen in nature is largely due to the activities of various groups of soil microorganisms (1891). Therefore, they are considered as '**Pioneers in Soil Bacteriology**'.

10.3 Components of Soil

The soil is composed of five major components

- Mineral matter
- Water
- Air
- Organic matter
- Living organisms

One gram of soil contains approximately one million microorganisms. The soil has organic matter, soil solution and soil air. All these components are affected by the activities of microorganisms. Soil is a constantly changing medium. The soil solution in agricultural soil has ions like K^+, Na^{++}, Mg^{++}, Ca^{++}, Fe^+, S^-, NO_3, SO_4, PO_4 and others.

These ions are very essential in culture media. In a fertile soil, these elements in mineral form are supplemented by organic compound, derived from the decomposition of animal and plant residues. Thus the soil is an excellent natural medium for growth of microorganisms.

10.4 Soil Microorganisms

Soil contain five major groups of microorganisms. They are Bacteria, Actinomycetes, Fungi, Algae and Protozoa (Table 10.1).

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Table 10.1: Soil Microorganisms with example

Soil Microorganisms	Examples
Bacteria	*Agrobacterium* *Bacillus* *Clostridium* *Pseudomonas*
Actinomycetes	*Actinomyces* *Nocardia* *Streptomyces*
Fungi	*Aspergillus* *Fusarium* *Alternaria* *Cladosporum*
Soil algae	*Anabaena* *Oscillatoria* *Nostoc*
Protozoa	*Colpoda* *Nematodes* *Pleurotricha* *Heteromita*
Bacteriophages	T4 Bacteriophages



One teaspoon of productive soil can contain between 100 million and 1 billion bacteria. These living microorganisms recycle organic material, promoting soil fertility and supporting plant growth. By practicing conservation tillage, farmers can maintain biodiversity in their soil.

Soil Bacteria

Among the soil microorganisms, bacteria are most dominant group of organisms. All kinds of bacteria are found in soil. This is because all kinds of organic refuse are disposed on the soil

Many of the soil bacteria perform useful functions like decomposition of organic matter, conversion of soil constituents into useful materials, production of antibiotics in the soil and biogeochemical cycling of elements like Carbon, Nitrogen, Phosphorus, Iron, Sulphur and Manganese. The bacterial population of the soil exceed the population of all other groups of microorganisms in both number and variety.

Soil Actinomycetes

The actinomycetes population is present as many as millions per gram of soil. The most predominant genera present in the soil are *Nocardia, Streptomyces* and *Micromonospora*. Actinomycetes are capable of degrading many complex organic substances and therefore play an important role in building soil fertility. One of the most notable characteristics of the actinomycetes is their ability to produce antibiotics. Examples: Streptomycin, neomycin, erythromycin and tetracycline.

Soil Fungi

Next to bacterial population in soil, fungi dominates in all kinds of soil. It possess filamentous mycelium composed of individual hyphae. All environmental factors which influence the distribution of bacteria and actinomycetes also influence the fungal flora of soil. The quality and quantity of organic matter present in the soil have a direct influence on the fungal numbers in soil. Fungi are dominant in acidic soils because acidic environment is not supportive for the existence of either bacteria or actinomycetes.

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Soil microbes create humus: Humus is the dark organic matter in soil. The humus is formed when dead plant and animal matter are decayed by soil microorganisms. Humus has many nutrients that improve the fertility of soil, Nitrogen being the most important.

Humus helps soil to retain moisture, and encourages the formation of soil structure. Soil organisms promote plant growth.

Soil Algae

Soil algae are ubiquitous in nature wherever moisture and sunlight are available. They are visible to the unaided eye in the form of green scum on the surface of soils. Numerically, they are not as many as Fungi, Bacteria or Actinomycetes. Some of the common algae in Indian soil are *Chlorella, Chalmydomonas, Chlorochytrium, Chlorococcum* and *Oedogonium*.

Blue green algae, or *Cyanophyceae,* are responsible for Nitrogen fixation. The amount of Nitrogen they fix depends more on physiological and environmental factors rather than the organism's abilities. These factors include intensity of sunlight, concentration of inorganic and organic Nitrogen sources and ambient temperature and stability.

Soil Protozoa

Soil protozoa are unicellular. They are characterized by a cyst in their life cycle which can help the species to withstand adverse soil conditions. The protozoans prefer certain species of bacteria for their nutrition. Protozoa are abundant in the upper layer of the soil and their numbers are directly dependent on bacterial population.

HOTS

"Without fungi even death will be incomplete" - Pasteur

Justify the statement.

10.4.1 Factors Influencing Microbial Population in Soil

The major factors that influence the microbial community in soil are

- Moisture
- pH
- Temperature
- Gases
- Organic and inorganic fertilizer
- Organic matter of soil
- Types of vegetation and growth stages
- Ploughing
- Season
- Depth of soil

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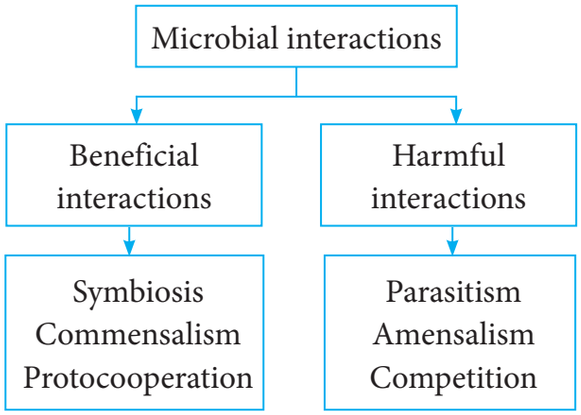
Some soil microbes produce a variety of substances that promote plant growth, including Auxins, Gibberellins and antibiotics.

10.5 Microbial Interactions

Microorganisms in soil interact with themselves and lead to beneficial and

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harmful relationships (Flowchart 10.1). Some of the interaction and interrelationship have been discussed in this connection in Table 10.2.



Flowchart 10.1: Microbial interactions

10.5.1 Beneficial Interactions

The beneficial interactions such as symbiosis (mutualism) and commensalism are found to operate among the soil inhabitants.

Symbiosis (mutualism)

Mutualism is an example of symbiotic relationship in which each organisms benefits from the association. One type of mutualistic association is involving the exchange of nutrients, between two species, a phenomenon called syntrophism. Many microorganisms synthesise vitamins and amino acids in excess of their nutritional requirements. Other have a requirement for one or more of these nutrients. Symbiosis is an obligatory relationship between two populations that benefit both the population. Both populations live together for mutual benefit.

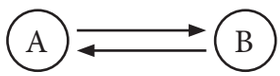


Table 10.2: Types of microbial interaction in soil

Interaction	Microorganisms A	Microorganisms B
Neutralism	No effect	No effect
Commensalism	+	No effect
Amensalism	No effect	-
Mutualism Synergism Protoco-operation Symbiosis	+	+
Competition	-	-
Parasitism	+	-
Predation	+	-
+ = positive effect – = negative effect		

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The relationship between algae and fungi that result in the formation of lichen is a classical example of mutualistic intermicrobial relationship (Figure 10.3).

Lichens are composed of primary producer, the phycosymbiont (algae) and a consumer the mycosymbiont (fungus)

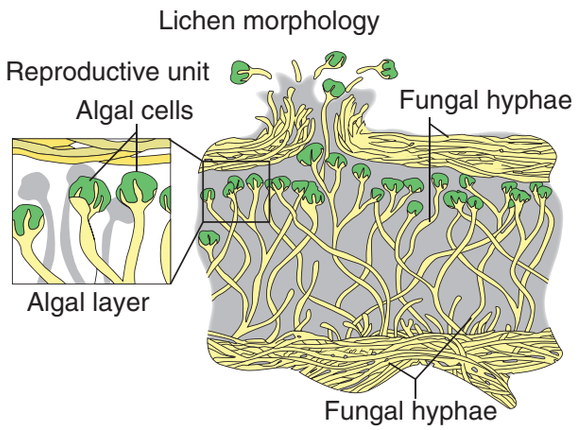
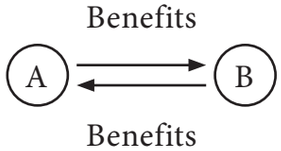


Figure 10.3: Lichen Morphology

Commensalism

In a commercial relationship between two microbial population, one population is benefited and other population remains unaffected. Commensalism is an unidirectional relationship between two population. The unaffected population does not benefit by the action of second population. For the receiving population, the benefit provided may be essential. In commensalism, the unaffected population modifies the habitat in such a way that another population is benefited.



For example: A population of facultative anaerobes utilizes oxygen and creates a habitat suitable for the growth of anaerobes. In soil, vitamin and growth factor producing organisms benefit vitamin and growth factors requiring organisms.

10.5.2 Harmful Microbial Interactions

Harmful microbial interaction is otherwise described as negative interaction or antagonistic interaction. Any inhibitory effect of an organism created by any means to the other organism is known as harmful interaction or antagonistic interaction and the phenomenon of this activity is called antagonism

Ammensalism

Ammensalism is the phenonmenon where one microbial species is affected by other species, where as other species is unaffected by first one. Ammensalism is accomplished by secretions of inhibitory substances such as antibiotics. Certain organisms may be of great practical importance, since they often produce antibiotics or other inhibitory substances, which affect the normal growth of other organisms. Antagonistic relationships are quite common in nature. For example: *Pseudomonas aeruginosa* is antagonistic towards *Aspergillus terreus* (Figure 10.4).

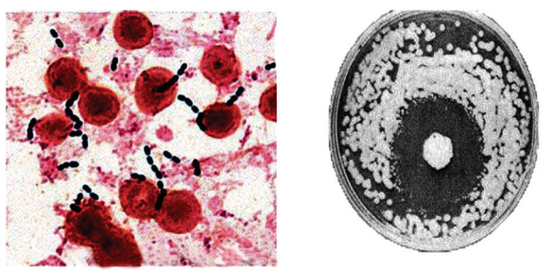


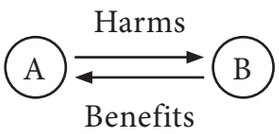
Figure 10.4: Microbial antagonism

Parasitism

This is a relationship in which one of the population benefits from the other and the host is usually harmed. Parasitism

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is one of the most complex microbial interactions. The line between parasitism and predation is difficult to define. The parasites feed on the cells, tissues or fluids of another organisms the host, which is harmed in this process.



The parasites depends on the host and lives in intimate physical and metabolic contact with the host. All types of plants and animals are susceptible to attack by microbial parasites.

10.6 Rhizosphere

In 1904, L.Hiltner for the first time coined the term "rhizosphere" to denote the area of intense microbiological activity that extends several millimeters from the root system of the growing plants.

The region which is adjacent to the root system is called rhizophere. The microbial population on and around root system is considerably higher than the root free soil or non rhizophere soil. This may be due the availability of nutrients from plants root in the form of root nodules, secretion, lysates, mucigel and sloughed off cells (Figure 10.5).

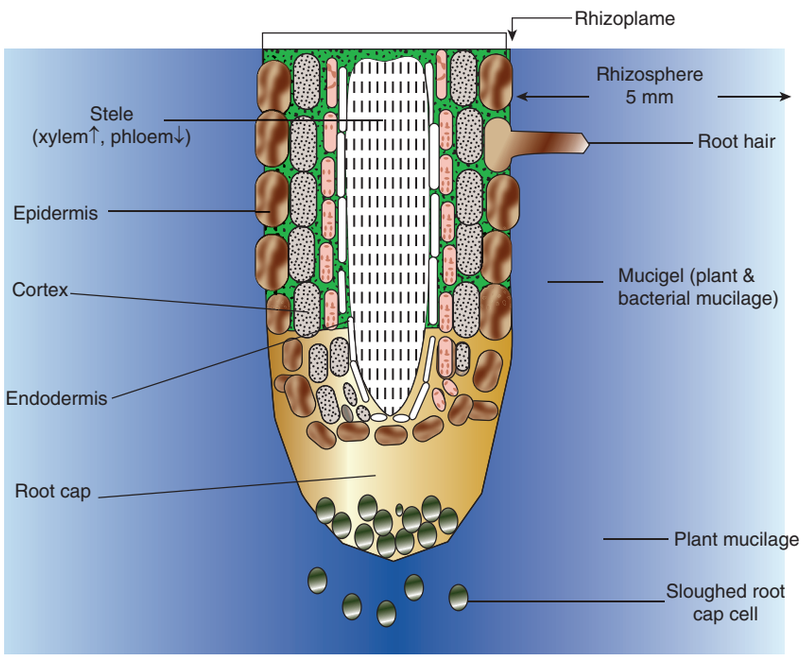


Figure 10.5: Root hair and Rhizosphere

The rhizospere region can be divided into two zones.

- Exorhizosphere
- Endorhizophere

However the root surface is termed as "rhizoplane".

Rhizophere Effect

The rhizophere is a zone of increased microbial community as well as microbial activities influenced by the root itself.

Greater rhizosphere effect is seen with bacteria (R: S values ranging from

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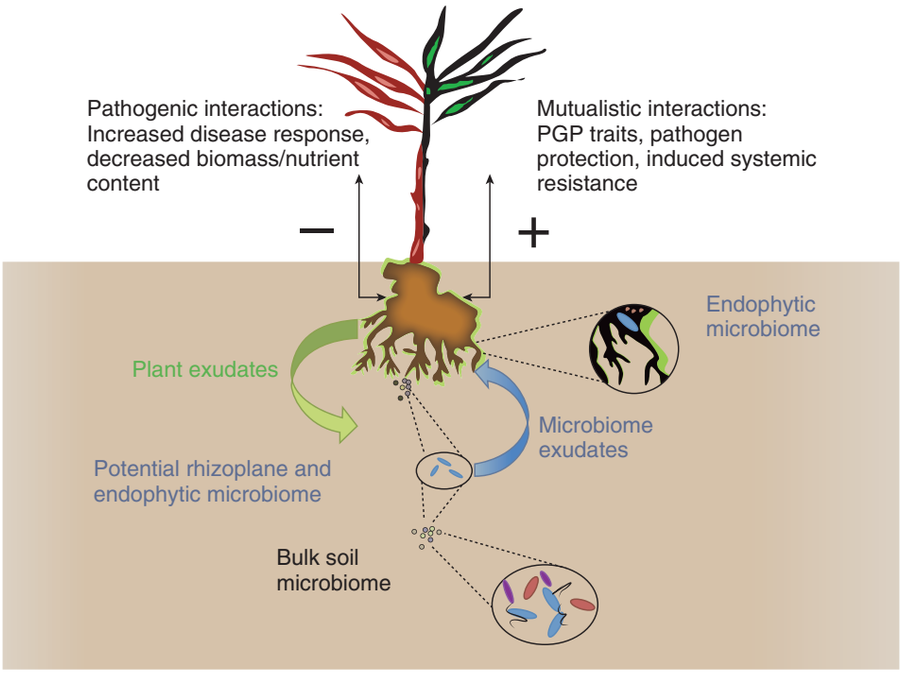


Figure 10.6: Effect of Rhizosphere in plant growth

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Bioleaching:

Soil microorganisms are very closely involved as catalytic agents in many geological processes. These include mineral formation, mineral degradation, sedimentation and geochemical cycling. In recent years, a new discipline of mineral science namely bio-hydrometallurgy or microbial mining (mining with microbes) is rapidly growing. Broadly speaking, bio-hydrometallurgy deals with the application of biotechnology in mining industry. In fact, microorganisms can be successfully used for the extraction of metals (Example: copper, zinc, cobalt, lead, uranium) from low grade ores. Mining with microbes is both economical and environmental friendly.

10 to 20 or sometimes more) than with actinomycetes or fungi (Figure 10.6). From the agronomic point of view, the abundance of Nitrogen fixing and Phosphate solublising bacteria in the rhizosphere of crop plants assumes a natural significance.

It has been reported that amino acid requiring bacteria exist in the rhizosphere in large numbers than in the root free soil. The rhizosphere effect improves the physiological conditions of the plant and ultimately result in higher yield.

10.7 Phyllosphere

The term "Phyllosphere" was coined by the Dutch Microbiologist Ruinen. The leaf surface has been termed as Phylloplane and the zone on leaves inhabited by the microorganisms as Phyllosphere (Figure 10.7). In forest vegetation, thick microbial epiphytic

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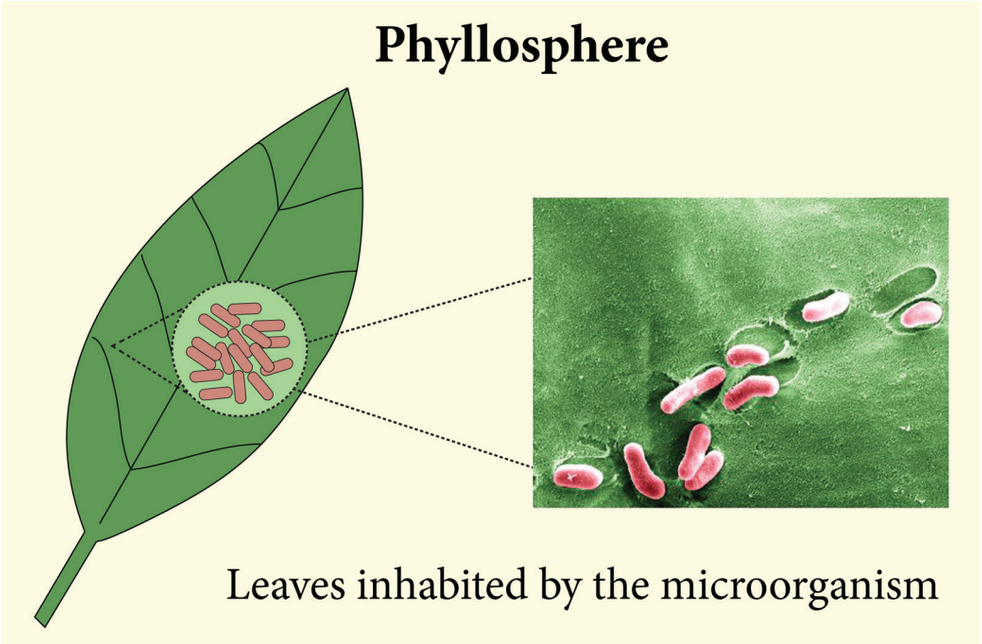


Figure 10.7: Microscopic appearance of Phyllosphere Bacteria

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PGPB can promote plant growth. The bacteria include those that are free-living, those that form specific symbiotic relationships with plants (Example: *Rhizobia* and *Frankia*), bacterial endophytes that can colonize some or a portion of a plant's interior tissues, and cyanobacteria (blue-green algae). PGPB may promote plant growth directly usually by either facilitating resource acquisition or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogenic agents on plant growth and development, that is, by acting as biocontrol bacteria. It is envisioned that in the not too distant future, plant growth-promoting bacteria (PGPB) will begin to replace the use of chemicals in Agriculture, Horticulture, Silviculture, and environmental cleanup strategies.

associations exist on leaves. The dominant and useful microorganisms on the leaf surfaces in the forest, vegetation happened to be Nitrogen fixing bacteria like *Beijerinckia* and *Azotobacter*.

Apart from Nitrogen fixing bacteria, other genera such as *Pseudomonas, Pseudobacterium, Phytomonas* are also encountered on the leaf surface. The age of plant, its leaf spread, morphology and maturity level and the atmospheric factors greatly influence the phyllosphere microflora.

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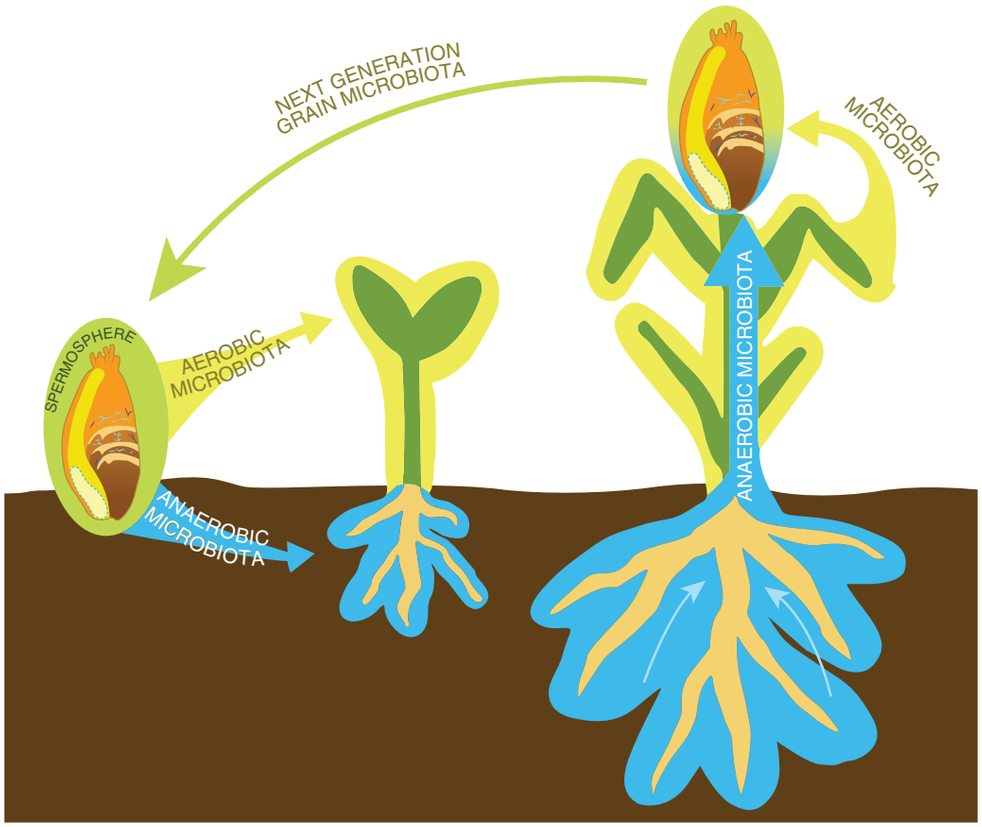


Figure 10.8: Spermosphere

10.8 Spermosphere

The region which is adjacent to the seed surface is termed as spermosphere (Figure 10.8). Healthy seeds carry specific bacterial flora in respect to number and species. There are several reports in the literature on the quantity and quality of microorganisms carried by the seeds of different plants species both externally and internally. When the seed is sown in soil, certain interactions between the seed borne microflora and the soil microorganisms take place under the influence of chemicals excluded by the germinating seed.

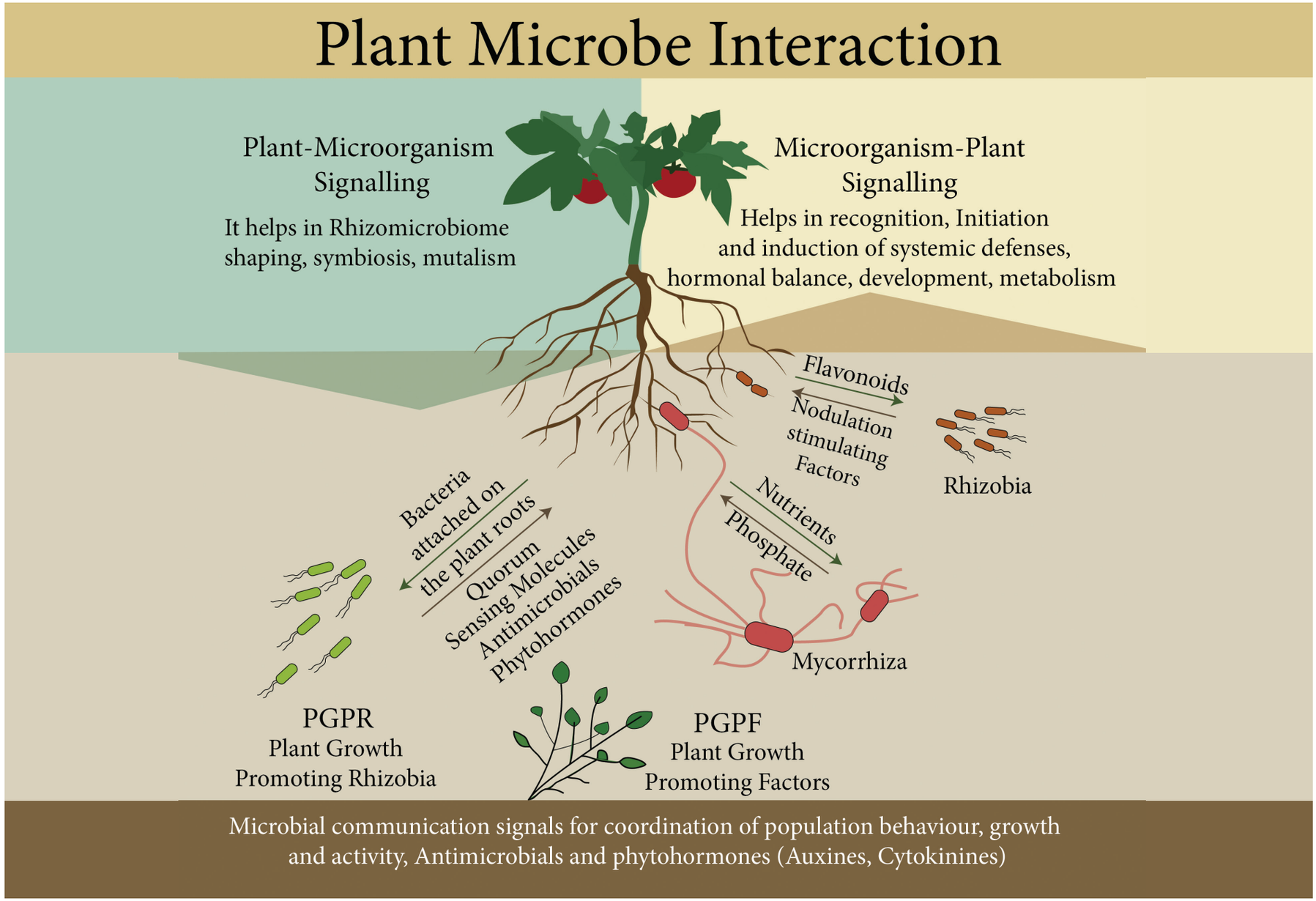
Summary

Soil is the outer most covering of the earth. Soil consists of living and non living components that contribute its fertility. There are five major components in the soil, that includes mineral matter, water, air organic matter and living organisms. The soil environment is unique in several ways. It consists of bacteria, fungi, actinomycetes, algae and protozoa. Several factors influences the moisture, pH, temperature, organic and inorganic matter of the soil.

Microorganisms in soil interact themselves and lead to both beneficial and harmful interactions. Beneficial interaction includes symbiosis and commensalism. Harmful microbial interaction includes parasitism. The region adjacent to the root system is called rhizosphere. Bacteria predominate in rhizophere. Soil and their growth is influenced by nutritional substances released from plant tissues.

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The leaf surface has been termed as phylloplane and the zone on leaves inhabited by the microorganisms is phyllosphere. The region, which is adjacent to the seed surface is termed as spermosphere.

Evaluation

Multiple choice questions

1. Example for soil algae _____
   a. *Anabaena*
   b. *Oscillatoria*
   c. *Nostoc*
   d. All the above
2. Lichens are example for
   a. Symbiosis
   b. Parasitism
   c. Commensalism
   d. All the above
3. The relationship between _____ and that result _____ in the formation of lichen
   a. Bacteria and virus
   b. Algae and bacteria
   c. Algae and fungi
   d. Virus and fungi
4. Harmful interaction is otherwise called as _____
   a. Mutualism
   b. Antagonism
   c. Commensalism
   d. Symbiosis
5. _____ first coined the term rhizophere
   a. L. Hiltner
   b. Ruinen
   c. Pasteur
   d. Koch
6. Leaf surface has been termed as _____.
   a. Rhizosplane b. Spermoplane
   c. Phylloplane d. All the above
7. Spermosphere is _____
   a. Leaf and microorganisms
   b. Root and microorganisms
   c. Seed and microorganisms
   d. All the above

Answer the following

1. What is soil?
2. Give examples for the soil bacteria?
3. What are the types of microbial interaction?
4. What is harmful microbial interaction?
5. Define rhizophere.
6. Define rhizoplane.
7. Define phyllosphere.
8. Define spermospere.
9. Explain parasitism.
10. Explain commensalism.
11. Give examples for the soil Fungi & Actinomycetes.
12. What are the components of soil?
13. Mention the different types of soil microorganism with help of chart.
14. Explain – factors influencing microbial population in soil.
15. Explain – microbial interaction.
16. Write about symbiosis or mutualism.
17. Describe rhizosphere.
18. Explain rhizophere effect.

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Chapter 11

Agricultural Microbiology

Chapter Outline

11.1 Biogeochemical Cycles

11.2 Biofertilizers

11.3 Biopesticides

LMDFB



The *Anabaena azollae-Azolla* association is a symbiotic relationship between a blue green alga and a rice plant. *Azolla* is a small, fast growing floating water fern. In the cavities of leaves of azolla, *Anabaena azollae* a blue green algae (a cyanobacterium) fixes nitrogen from the air.

Learning Objectives

After studying this chapter the student will be able,

- *To understand the various biogeo chemical cycle*
- *To know the nitrogen fixation process*
- *To infer about the biofertilizer*
- *To learn the role of biopesticides in agriculture*

Agricultural microbiology is a branch of microbiology which deals with the study of microorganisms that are involved in various agricultural processes taking place in soil, plants and agro industries.

Agriculturally important processes like the Biological nitrogen fixation, nutrient cycling of Carbon, Sulphur, Phosphorus and Nitrogen are microbe mediated and play a very significant role in replenishing the nutrient supply in soil. Certain microorganisms like bacteria, fungi and viruses are economically important since they cause plant diseases and are responsible for severe crop losses. The study that deals with plant diseases is called plant pathology.

There are microorganisms capable of producing plant hormones thereby enhancing the plant growth. Some micro organisms can be used as biopesticides as they have ability to kill insects that damage plants.

11.1 Biogeochemical Cycles

Biogeochemical cycling is defined as the movement and conversions of materials by biochemical activities throughout air, water and soil. All living organisms participate in biogeochemical cycling but

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microorganisms play a major role. This is because of their high enzymatic activity and their ubiquity.

Most macro elemental components of living organisms like Carbon, Nitrogen, Sulphur, Oxygen, Phosphorus and Hydrogen are cycled most intensely and other elements like Copper, Chromium, Iron, Zinc, Nickel are cycled less intensely.

11.1.1 Carbon Cycle

Carbon is a macro element present in all living cells. In microorganisms, they are present in all macromolecules like cell wall, cytoplasmic membrane, proteins and nucleic acids.

Reservoirs of Carbon:

Reservoirs are the storage places of nutrients that are present in nature. They store nutrients in large amounts for longer periods of time.

Atmospheric CO_2, dissolved carbon in oceans and freshwater, organic matter are actively cycled carbon reservoirs. Sediments and fossil fuels like coal, petroleum and natural gas are slowly cycled carbon reservoirs. Carbon is cycled between these reservoirs by the biochemical activities of micro organisms and other living things (Figure 11.1).

The different stages or processes involved in carbon cycle are

1. Photosynthesis
2. Decomposition
3. Methanogenesis

1. Photosynthesis

It is a process where atmospheric CO_2 is converted to organic carbon $(CH_2O)n$. This is carried out by higher plants, photosynthetic bacteria, cyanobacteria and algae using radiant energy from

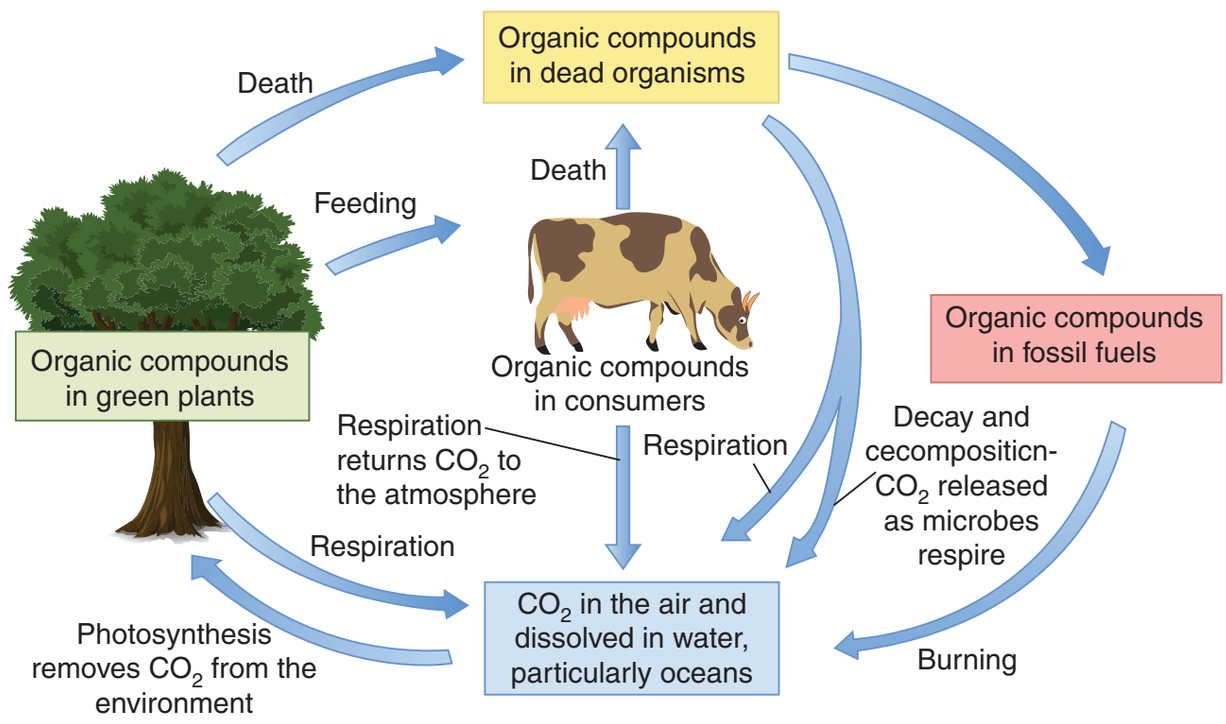


Figure 11.1: A simplified diagram of Carbon cycle is as follows

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the sun. This can be explained by the equation,

$$CO_2 + H_2O \rightarrow (CH_2O)_n + O_2$$

where $(CH_2O)_n$ represents the organic form of carbon (Example: Carbohydrates) which gets incorporated into the photosynthetic organisms. This organic carbon serves as food for herbivores and in turn for carnivores.

2. Decomposition

The organic matter fixed as a result of photosynthesis is eventually degraded by microorganisms to CO_2 during processes like respiration and decomposition. When aerobic and anaerobic organisms respire, CO_2 is released into the atmosphere. Much of the CO_2 is released when dead organisms decompose in the soil predominantly by the activities of soil microorganisms. Burning of fossil fuels also release CO_2 into the atmosphere.

3. Methanogenesis

It is an anaerobic process where CO_2 gets converted to CH_4 (methane) by strict anaerobes like methanogens (Example: *Methanobacterium*). Methanogens are a group of Archaebacteria found in anaerobic environments like swamps, marshes, rumen of ruminants, paddy fields and gut of termites.

$$H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$$

Methane is converted back to carbon dioxide by a process called **Methylotrophy**.

11.1.2 Nitrogen Cycle

The element Nitrogen (N) is a key constituent in microbial cell. Nitrogen is needed for the formation of proteins, amino acids, nucleotides and is present in a number of oxidation states inside the cell. Nitrogen is cycled between atmosphere, organic compounds in living things, soil and sediments.

The processes that are involved in Nitrogen cycle are

1. Nitrogen fixation
2. Nitrification
3. Ammonification
4. Denitrification

Nitrogen fixation

Nitrogen is present as N_2 ($N \equiv N$) in air (78% N_2). The triple bonded state of nitrogen makes it very stable and nitrogen in its gaseous state cannot be assimilated by plants or animals for their metabolism. Only few groups of prokaryotes are

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Fistulated cow:

Fistulated cows are very useful in studying rumen microorganisms and ruminant nutrition and are used to treat indigestion in cows. Fistula is a sampling port that allows access to the rumen. Rumen is the first and the largest chamber of the stomach of cows and other ruminant animals.



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capable of breaking the triple bond and use it for building up their proteins and aminoacids. The process of reduction of gaseous nitrogen (N_2) to ammonia (NH_4) is called Nitrogen fixation. This process is carried out by a group of prokaryotes called diazotrophs.

$$N_2+8H \rightarrow 2\ NH_3 + H_2$$

Cyanobacteria, *Rhizobium* and *Frankia* are some of the examples of **diazotrophs** that can fix atmospheric nitrogen. The fixed ammonia gets incorporated into proteins and amino acids, thus building up organic nitrogen.

$$NH_4 \xrightarrow{\text{assimilation}} \text{Proteins, amino acids}$$

Ammonification

The production of ammonia during the decomposition of organic nitrogen compounds, by micro organisms after the death of plants and animals is called **ammonification** (Figure 11.2). Much of the ammonia released by aerobic decomposition in soil is taken up rapidly by plants and micro organisms and is converted to amino acids.

Bacteria like *Bacillus, Clostridium, Pseudomonas* and fungi like *Aspergillus, Mucor* and *Penicillium* are few examples of micro organisms that can ammonify.

Nitrification

The oxidation of ammonia (NH_3) to nitrate (NO_3) is called **nitrification**. It is carried

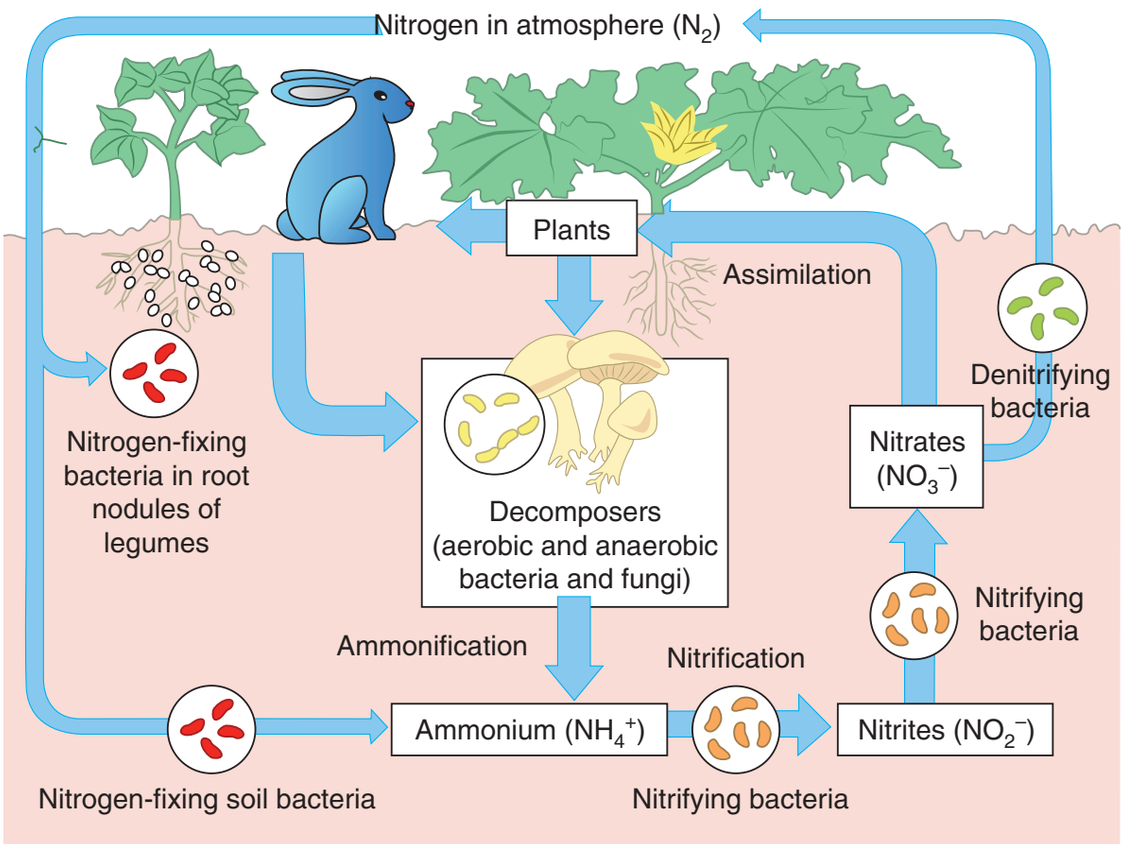


Figure 11.2: Simplified diagram of nitrogen cycle

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out by nitrifying bacteria. It is a two step process where ammonia is first converted to nitrite (NO_2) and then to nitrate (NO_3).

$$2NH_3 + 3O_2 \rightarrow 2HNO_2 + 2H_2O$$

The above given oxidation reaction is the first step that produces nitrite. This reaction provides energy and is carried out by *Nitrosomonas* and *Nitrosococcus*.

In the second step, the nitrite is oxidized to nitrate

$$2HNO_2 + O_2 \rightarrow HNO_3$$

This reaction is carried out by *Nitrobacter*.

Nitrates are readily assimilated by plants but are very water soluble and rapidly leached from soil.

Denitrification

The reduction of NO_3^- from soils by denitrifying bacteria to gaseous nitrogen is called **denitrification**. In this process, carried out by bacteria like *Pseudomonas, Thiobacillus denitrificans,* organic compounds serve as hydrogen donors and nitrates serve as electron acceptor.

$$NO_3 \rightarrow NO_2 \rightarrow N_2O \rightarrow N_2$$

Biological Nitrogen Fixation

One of the most significant biological process taking places on the Earth is biological nitrogen fixation (BNF). This fixation of atmospheric nitrogen carried out by few prokaryotes is cost efficient because industrial production of ammonia by Haber's Bosch process is very expensive.

Organisms carry out BNF in a free living state in soil or they can establish symbiotic association with other plants or micro organisms and fix N_2.

Organisms capable of BNF are

Free living	
Aerobic	*Azotobacter* Cyanobacteria
Anaerobic	*Clostridium*
Symbiotic	*Rhizobium* *Frankia.*

BNF by *Rhizobium* in leguminous plants

Leguminous plants belong to the family Leguminaceae and bear seeds in pods. Example: Black gram, Green peas, Soyabean, Subabul. The bacteria belonging to the genus *Rhizobium* which can exist in free living state in soil but can enter into symbiosis with legume plants and carry out nitrogen fixation.

Process of BNF

It consists of the following steps

1. Infection of legume roots by *Rhizobium*
2. Formation of root nodules
3. Reduction of $N \equiv N$ to NH_4 in root nodules

1. Infection of legume roots by *Rhizobium*

Rhizobium recognises and attaches to the root hairs of legume plant. It invades the root hairs and secretion of certain *nod* factors result in root hair curling typically called **Shepherd's crook symptom** which leads to the formation of infection thread. Infection thread is a cellulosic tube like structure through which *Rhizobium* moves into the cortex from root hairs.

2. Formation of root nodules

The invaded plant cells are stimulated to divide repeatedly thus forming a mass

TN_GOVT_XI_Micro_Biology_CH11.indd 166 16-02-2019 10.04.43 AM

of tissue on the roots which are called root nodules (Figure 11.3). Root nodules are fleshy light pink colored globose structures seen on the roots. The bacteria inside the root nodules transform into swollen, mishappened forms. These are called **bacteroids**. The bacteroids are capable of nitrogen fixation (Figure 11.4)

3. Reduction of N≡N to ammonia in root nodules

This biochemical process is catalysed by an enzyme called **Nitrogenase** present in bacteroids and happens under diminished O_2 levels. The O_2 levels in the nodules are controlled by an oxygen binding protein called **leghemoglobin**. This is a red, iron containing protein which can keep the nodule environment free of oxygen.

Nitrogenase

The enzyme nitrogenase is a complex enzyme consisting of 2 enzymes, dinitrogenase reductase and dinitrogenase. Electrons from organic compounds like pyruvate are passed on to dinitrogenase reductase first and then to dinitrogenase which in turn passes them to N≡N thus reducing it to NH_4. This reduction needs 16 ATP, ferredoxin and cytochromes.

$$N_2 + 8_e^- + 8_{H^+} \rightarrow 2NH_3 + H_2$$

$$N \equiv N + 4H \longrightarrow HN{=}NH + 2H \longrightarrow$$
$$H_2N\text{-}NH_2 + 2H \longrightarrow 2NH_3$$

HOTS

Will nitrogen fixation occur in the presence of air? What will be the fate of nitrogenase enzyme in aerobic condition?



Figure 11.3: Root nodules in the leguminous plant root

11.1.3 Phosphorus Cycle

The element phosphorus (P) is an essential macro element in all living organisms. They are found in nucleic acids and phosphate esters. It is an essential component of ATP and other high energy phosphates and phospholipids.

Reservoirs of Phosphorus

1. Phosphate rock like apatite, a large inert reservoir
2. Marine and aquatic sediments
3. Dissolved phosphates in soils and waters
4. Organic phosphates in dead and living organisms.

Phosphorous transformations mostly happen as inter conversion of inorganic to organic phosphate and insoluble form to soluble phosphates.

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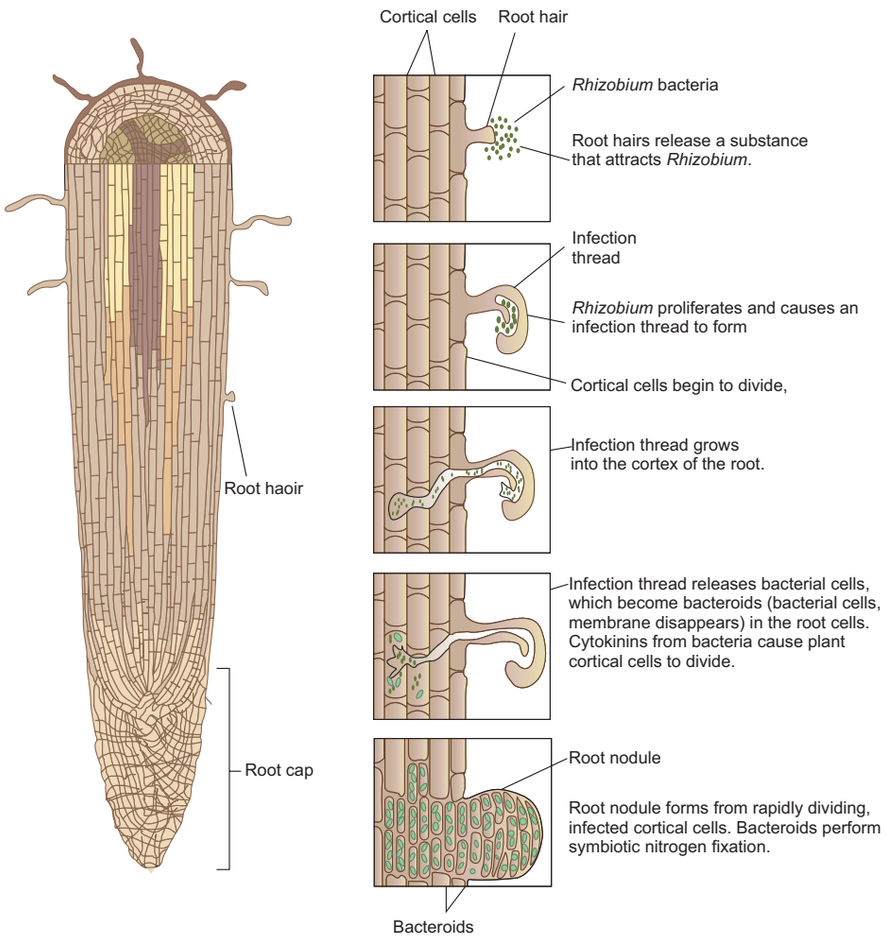


Figure 11.4: Showing stages of formation of root nodules on legume plant

Phosphate solubilizations

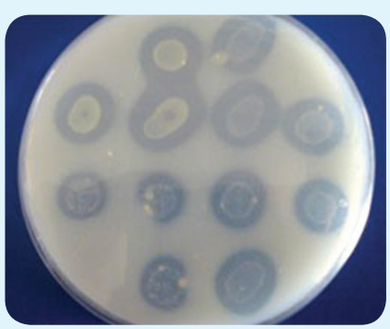
Most of phosphates occur in combination with Calcium, Iron, Magnesium and Aluminium (inorganic P) and thus are insoluble and unavailable to plants and micro organisms. Some micro organisms solubilize those insoluble phosphates by producing organic acids. Example: *Thiobacillus, Bacillus* thus enabling the plants to utilize it.

Phosphate assimilation

Plants and micro organisms can readily assimilate soluble forms of inorganic phosphates like $H_2PO_4^-$, HPO_4^{-2} and HPO_4^{-3} and incorporate them as organic forms of phosphates like ATP, nucleic acids.

Infobits

The ability of bacteria to solubilise phosphates can be tested in the laboratory by streaking the bacterial culture in Pikovaskaya agar which contains tricalcium phosphate. Positive cultures show clear halo around growth.



TN_GOVT_XI_Micro_Biology_CH11.indd 168 16-02-2019 10.04.44 AM

Phosphate mineralisation

Breakdown of organic phosphates to form soluble inorganic phosphates is called mineralisation. Organisms produce phosphatase enzymes and catalyse mineralisation. The mineralised phosphates can be utilized by plants (Figure 11.5).

$$\text{Phytic acid} \xrightarrow[\text{Phytase}]{} \text{Inositol}$$

11.1.4 Sulphur Cycle

Sulphur is present in sulphur containing aminoacids. The sulphur cycle involves oxidation – reduction reaction between Sulphate (SO_4), Elemental S and H_2S and hence there is change in the valence states of sulphur from -2 to +6.

The basic steps involved in sulphur cycle are

1. Sulphide/ sulphur oxidation
2. Sulphate reduction
3. Sulphur reduction
4. Organic sulphur compound oxidation or reduction
5. Desulfurylation

Sulphide/Sulphur oxidation ($H_2S \rightarrow S^0 \rightarrow SO_4^{2-}$)

It is carried out by prokaryotes under aerobic and anaerobic conditions. Under aerobic conditions, H_2S is spontaneously oxidized at neutral pH to elemental sulphur. Elemental sulphur is oxidized to suphates by chemolithotrophic bacteria like *Thiobacillus, Beggiatoa.*

If light is available, H_2S can be used as electron donor to carry out photosynthesis

HOTS

If there is no biogeochemical cycle in the ecosystem, What will happen to the earth?

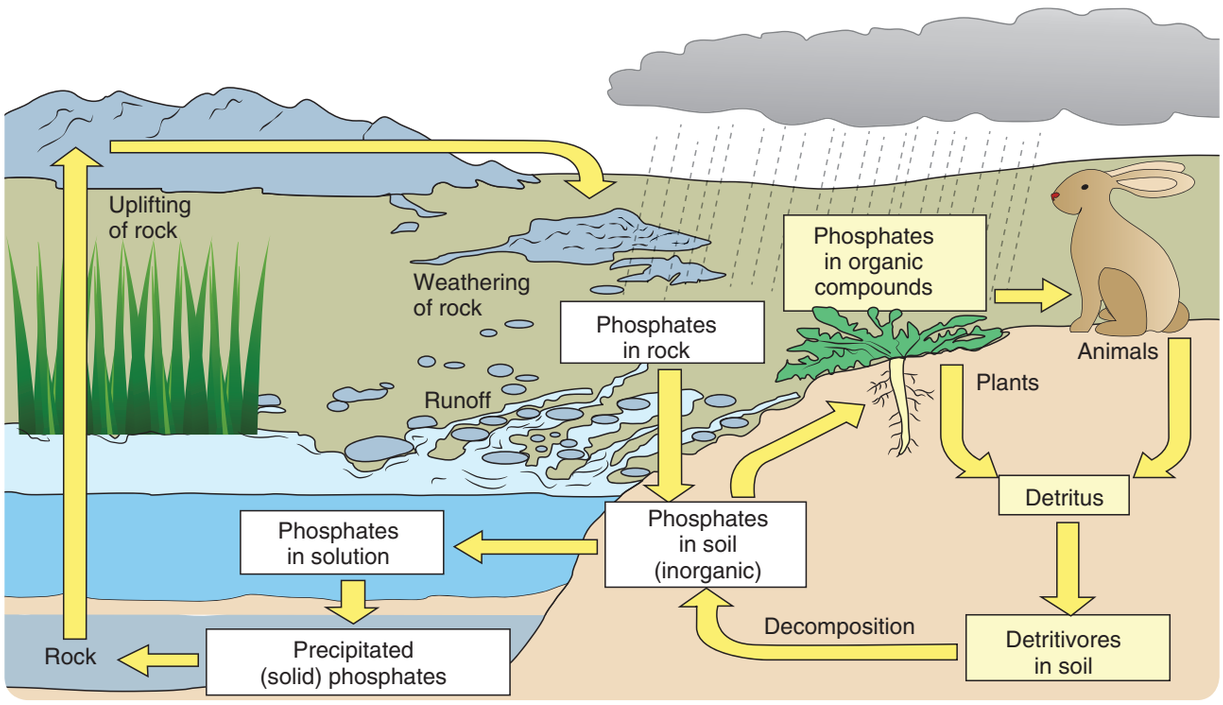


Figure 11.5: Phosphorus cycle

TN_GOVT_XI_Micro_Biology_CH11.indd 169 16-02-2019 10.04.44 AM

under anoxic conditions by phototrophic sulphur bacteria like *Chromatium* and *Chlorobium*.

Sulphate reduction

When sulphate is present in habitats, different groups of microorganisms are capable of carrying out sulphate reduction.

Beijerinck described the use of sulphate (SO_4^{2-}) as a terminal electron acceptor during anaerobic respiration to form sulphide (H_2S). This process is called **Dissimilatory Sulphate Reduction(DSR)**. The anerobic bacteria capable of carrying out DSR are *Desulfovibrio, Desulfococcus, Desulfotomaculum* (Figure 11.6) This reaction by sulphate reducers requires organic carbon sources like pyruvate or lactate. H_2S accumulated in such habitats by the action of sulphate reducers is toxic to aerobic organisms.

The reduction of sulphate to H_2S, for building up aminoacids and proteins is called as **assimilatory sulphate reduction**. The H_2S thus produced is immediately incorporated into organic compounds.

Sulphur reduction ($S^0 \rightarrow H_2S$):

The dissimilative sulphur reducing bacteria can reduce elemental sulphur to hydrogen sulphide. Example: *Desulfuromonas*, an obligate anaerobe. Under aerobic conditions, organisms like *Pseudomonas, Proteus* and *Salmonella* are also capable of performing this reaction.

Organic sulphur compounds reduction/oxidation

Organic sulphur compounds like dimethyl sulphide can be used as carbon and energy source for many microorganisms.

Desulfurylation

It is a process where organic sulphur compounds are used up by microorganisms for energy to produce H_2S.

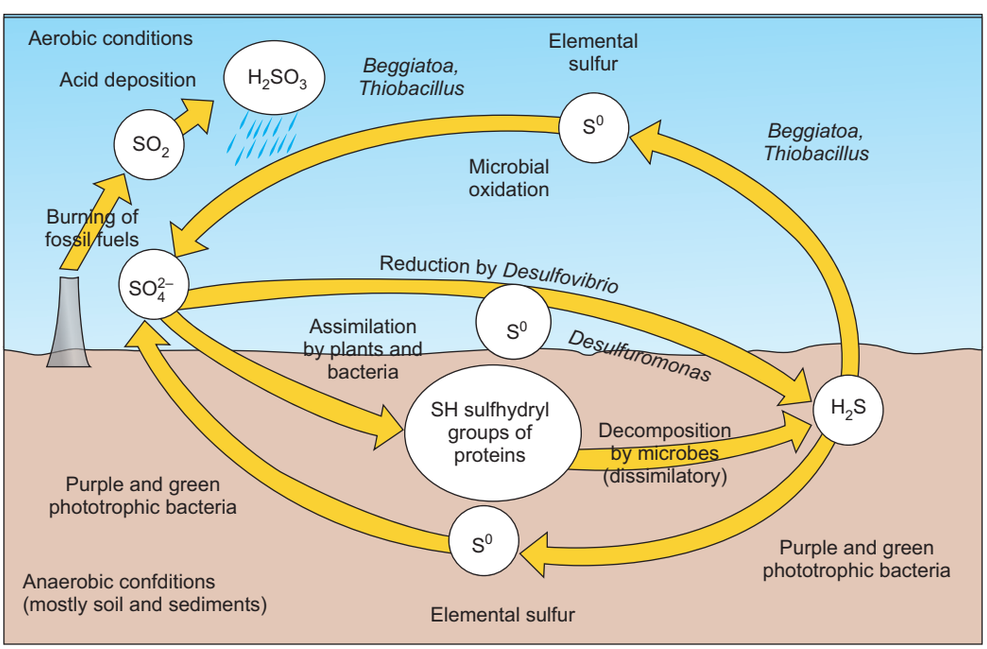


Figure 11.6: Sulphur cycle

TN_GOVT_XI_Micro_Biology_CH11.indd 170 16-02-2019 10.04.44 AM

11.2 Biofertilizers

In India, the availability and affordability of fossil fuel based chemical fertilizers at the farm level have been ensured only through imports and subsidies. Indiscriminate and imbalanced use of chemical fertilizers, especially urea, along with chemical pesticides and unavailability of organic manures has led to considerable reduction in soil health. Biofertilizers can act as a renewable supplement to chemical fertilizers and organic manures. They have the capacity to produce natural resistance in plants against pests and soil borne diseases, adding fertility to soil.

Nitrogen fixation by leguminous and other crops is reported to be 44 million metric tons per annum. The appropriate strain of *Rhizobium* can increase the crop yield up to 10-35%. Also, residual N is beneficial for the next crops grown in the same field.

It has been estimated that 40-250 kg N / hectare(ha) / year is fixed by different legume crops by the microbial activities of *Rhizobium*.

Definition

Biofertilizers are preparations containing beneficial micro organisms like N_2 fixers, PO_4 solubilizers in a viable static state intended for seed or soil application and designed to improve soil fertility.

Advantages

1. They reduce the need for chemical fertilizers.
2. They provide the plant with certain vitamins, plant growth promoting substances and increase the vigour of the plant.
3. It is cheap and cost effective.

Based on the nutrients that they provide, biofertilizers are of the following types

Nitrogenous biofertilizers-

- *Rhizobium,*
- *Azotobacter,*
- *Azospirillum*
- *Frankia*

Phosphate solubilisers

- *Bacillus*
- VAM

11.2.1 Rhizobium

Rhizobium – legume symbiosis is a well studied plant microbe interaction and *Rhizobium* is the most extensively used nitrogenous biofertilizer in India.

Rhizobium is a gram negative, non-spore forming aerobic bacillus inhabiting the soil in a free living state. The colonies of *Rhizobium* on YEMA (Yeast Extract Mannitol Agar) plate are gummy, pale white in colour (Figure 11.7). They can establish symbiotic relationship with leguminous plants and fix atmospheric nitrogen thereby greatly improving soil fertility.

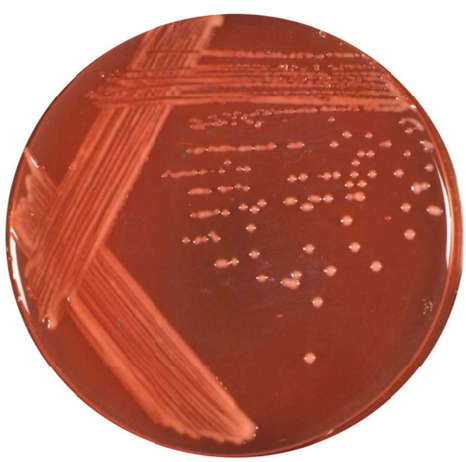
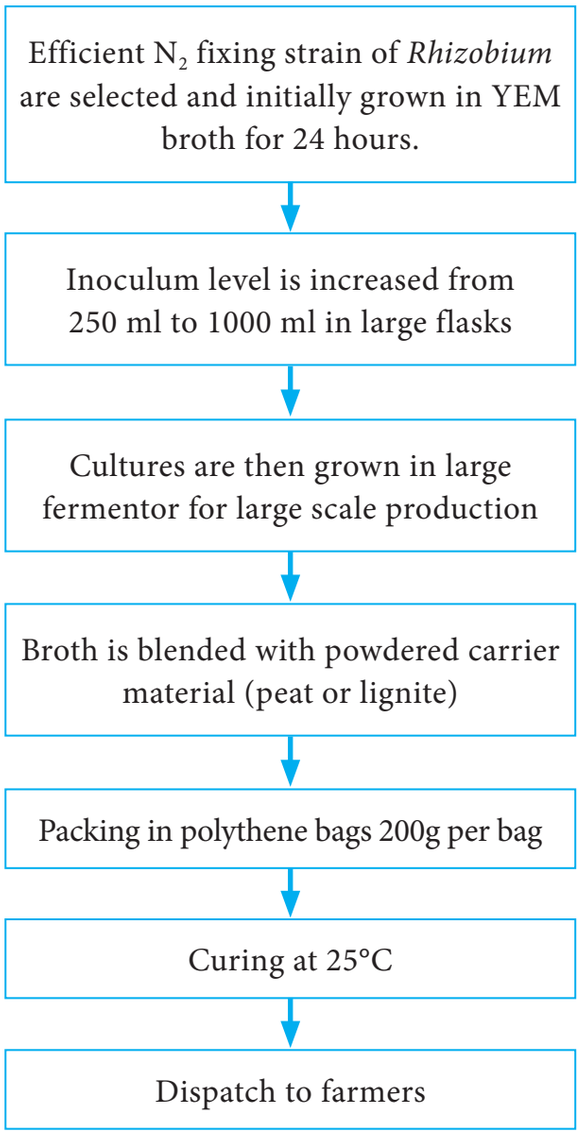


Figure 11.7: Pale pink mucoid colonies of *Rhizobium* on Yeast Extract Mannitol Agar plate

TN_GOVT_XI_Micro_Biology_CH11.indd 171 16-02-2019 10.04.46 AM

Mass production of *Rhizobium*

The flowchart explaining the mass production of *Rhizobium* biofertilizer is given below



Method of application of *Rhizobium* to plants

Carrier based *Rhizobium* inoculants are mixed with water to form slurry to which the seeds of plants are added (Figure 11.8). The coated seeds are dried in shade and used for sowing.

11.2.2 Phosphate Solubilizers

Several soil bacteria like *Pseudomonas* and *Bacillus* possess the ability to convert insoluble mineral phosphates into soluble form by secreting organic acids thereby making it available to plants.

For mass cultivation and inoculant preparation, the cultures are grown in Pikovaskaya broth for 7-18 days and mixed in suitable carrier like peat or lignite. After curing for a week, the inoculants are packed and made ready for use in a similar manner as *Rhizobium* inoculants.

11.2. 3 VAM

Mycorrhiza means fungus root. It describes the symbiotic association between plant and fungus. Vesicular Arbuscular Mycorrhiza (VAM) is an endomycorrhiza which is used as a fungal biofertilizer. They mobilize the soluble

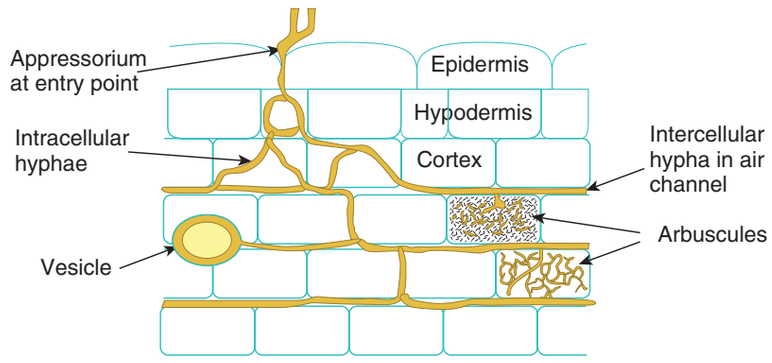


Figure 11.8: Showing the colonization of VAM fungi in root cells of plants

TN_GOVT_XI_Micro_Biology_CH11.indd 172 16-02-2019 10.04.46 AM

phosphates in the root zone of plants and satisfy the phosphorus nutrition of plants.

Morphology

VAM is an example of endomycorrhiza meaning, the storage organelles of phosphates like vesicles and arbuscles are seen intracellularly. Vesicle is a globose structure and arbuscle is a tree like branching structure present in the root cortical cells (Figure 11.9). VAM fungi are naturally most prevalent in angiosperms. gymnosperms, pteridophytes and bryophytes.



Figure 11.9: The fresh water fern *Azolla.*

Mass production

Root based inoculum is used for preparing VAM biofertilizer (Figure 11.10). The selected spores of VAM fungi are allowed to infect plants like onion, sorghum and other grasses. After 3-4 months, the roots along with the soil are macerated or pelleted with an inert material and packed in polythene pouches which can be used as biofertilizer.

11.2.4 Cyanobacteria / Blue green Algae

Blue green algae are single celled or filamentous prokaryote capable of nitrogen

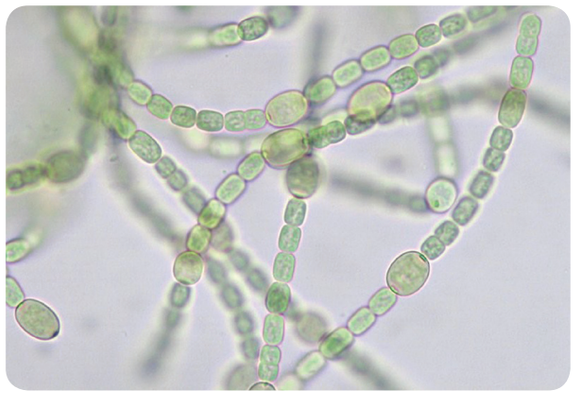


Figure 11.10: Microscopic view of *Anabaena azollae*

fixation and photosynthesis. Most of the filamentous forms have specialized large, thick walled cells called heterocysts which are sites of nitrogen fixation.

Example: *Nostoc*, *Anabaena* is examples for filamentous BGA. *Gleocapsa* is an example of unicellular BGA. Some of the filamentous forms do not possess heterocysts but still fix atmospheric nitrogen. Since they need standing water for their growth, BGA can effectively colonize paddy fields and enrich the soil with nitrogen.

Mass cultivation of BGA

Applying BGA to paddy fields can reduce the amount of chemical nitrogenous fertilizer applied for the growth of paddy crop. Therefore cultivation of BGA in large quantities is necessary. Mass cultivation of BGA has the following steps.

1. Isolation of BGA
2. Mass cultivation of BGA

Isolation of BGA

BGA can be isolated from soil or paddy fields. Appropriate dilutions from serially diluted algal sample are inoculated in the liquid flasks containing algal media

TN_GOVT_XI_Micro_Biology_CH11.indd 173 16-02-2019 10.04.47 AM

like BG-11 or Pringsheim's media. After several weeks of incubation at 28°C, the individual colonies are picked up, identified and stored. This can be used as starter culture for mass cultivation. Mass culture can be done in 2 ways.

Mass cultivation of BGA

1. Open air shallow culture:

Pits are prepared (6′ × 3′ × 9′)

↓

Filled with soil and 200g of superphosphate and water is added

↓

Starter culture is sprinkled over water

↓

1 week

↓

Algal scum is formed on the surface

↓

Water is allowed to dry and the dried up algal flakes are collected and stored in poly bags

The dried algal flakes around 10kg/ha can be applied in paddy fields after transplantation.

11.2.5 Azolla

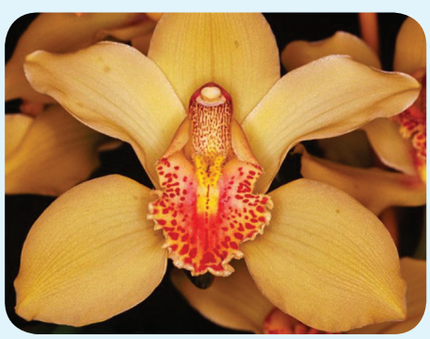
Azolla is a floating freshwater fern. The plant has a branched stem, deeply bilobed leaves which are arranged alternately on the stem and each leaf has a dorsal and ventral lobe (Figure 11.9). The dorsal lobe houses the cyanobacterial symbiont *Anabaena azollae* (Figure 11.10). The fern and the cyanobacteria exhibit symbiotic relationship in which *Anabaena* provides the fern with fixed nitrogen and fern provides niche for the cyanobacteria free from competition from other microorganisms.

Azolla can be used as a nitrogenous biofertilizer for paddy crop. When applied into the paddy fields, *Azolla* provides nitrogen nutrition to standing rice crop and can reduce the need for synthetic fertilizers.

Infobits

Mycorrhiza and orchid germination

In the early stages of their life cycle, all terrestrial orchids are non photosynthetic, totally lacking chlorophyll and relying on carbon(C) acquired from a fungal symbiont (Mycorrhiza) for growth until the production of the first green leaves above the ground, a nutritional strategy termed mycoheterotrophy. Around 200 species of orchids remain achlorophyllous throughout their lifetimes. Species such as *Galeola*, *Gastrodia*, *Corallorhiza*, *Rhizanthella* and many others continue to gain carbon from mycorrhizal fungi.



TN_GOVT_XI_Micro_Biology_CH11.indd 174 16-02-2019 10.04.47 AM

Mass multiplication of *Azolla*

Small plots (50-100 sq.m) or concrete tanks with standing water are inoculated with *Azolla*

↓

pH is adjusted to 8.0 with lime.

↓

Superphosphate is applied as a nutrient and carbofuran is applied to deter insects

↓

After 20 days, *Azolla* biomass can be harvested and used as biofertilizer for rice plantings

Method of application of *Azolla* in rice fields

Azolla is grown on the flooded rice fields prior to planting for 2-3weeks. Then water is drained and *Azolla* is incorporated into the soil followed by rice transplantation within a week's time.

HOTS

Why bio- fertilizer are preferred to chemical fertilizer?

11.3 Biopesticides

Pests are insects that damage crop plants and stored products. They feed on leaves and roots or suck the sap of the plants causing severe crop losses. Chemical pesticides sprayed on plants can be detrimental to ecosystem if the usage is prolonged and pests may develop resistance to the pesticides.

The term **biopesticides** refers to compounds that are used to manage agricultural pests by means of specific biological effects. It refers to products containing biocontrol agents like natural substances such as plants, certain minerals, animals, micro organisms including their genes or metabolites.

They are an important part of Integrated Pest Management (IPM) strategy in controlling the pest.

Advantages

They are less toxic to humans and environment and they do not leave harmful residues.

They affect only the target pest.

They cause long term suppression of pest populations since they persist in the environment.

Microbial biopesticides are of three kinds

1. Bacterial biopesticide
2. Fungal biopesticide
3. Viral biopesticide

11.3.1 Bacterial Biopesticide

Bacteria like *Bacillus thuringiensis, Bacillus papillae* and *Bacillus lentimorbus* have the potential to kill certain insect pests and are entomopathogenic.

Bacillus thuringiensis

It is a gram positive, spore forming, rod shaped soil bacterium. During sporulation, the bacterium produces insecticidal proteins as parasporal crystals. These are called ***delta endotoxin*** also called as ***Cry proteins***. ***Cry proteins*** are specifically

TN_GOVT_XI_Micro_Biology_CH11.indd 175 16-02-2019 10.04.47 AM

toxic to insects belonging to Lepidoptera, Coleoptera and other few insect orders.

Mode of action of Bt

The Bt cells sprayed on the leaves have to be ingested by the larval forms of the insects in order to exert its action. This is because the Bt toxin gets activated in the insect gut at a specific pH.

Process

Insect ingests the parasporal crystals containing Cry protein

↓

The crystals dissolve in the alkaline environment of the insect gut

↓

The solubilised inactive delta endotoxin are cleaved rendering them active

↓

The activated toxin binds to specific receptor of the midgut epithelium

↓

This leads to the insertion of toxin thereby developing pores in the epithelium

↓

Cell lysis and disruption of epithelium releases the cell contents leading to insect death.

Symptoms

- Larvae stops feeding
- Larvae becomes sluggish and static
- Water oozes out from the body
- Larvae dies and falls off the leaf

Various species of Bt are able to work against cotton boll worm, cabbage worm and gypsy moths.

Infobits

Photograph of a cotton plant showing opened and unopened bolls. BT cotton is a genetically modified cotton plant (GM crop) which has the gene for the crystal toxin integrated in its genome. Crystal toxin is expressed in plant parts which reduce the need for spraying pesticides. BT cotton is the only GM crop approved for commercial cultivation in India.

11.3.2 Fungal Biopesticides

These entomopathogenic fungi attack insects and cause diseases in insect body which lead to insect death. Two prominent fungi used as mycopesticide are

- *Beauveria bassiana* which causes white muscardine disease
- *Metarhizium anisopliae* which causes green muscardine disease

Mode of action of *Beauveria bassiana*

Beauveria bassiana, a filamentous fungus belongs to class Deuteromycetes also called imperfect fungi. It can be successfully used against Colarado potato beetle, (Figure 11.11) Codling moth and American boll worm.

This fungus invades the haemocoel of insects through spores. Once the spores attach to the cuticle, it germinates and the hyphae penetrates the insect cuticle (cuticle is the outer membrane of insects) Penetration is aided by formation of appresorium and penetration peg. The fungi

TN_GOVT_XI_Micro_Biology_CH11.indd 176 16-02-2019 10.04.47 AM

secrete chitinases, lipases and proteases which can dissolve the cuticle. The hyphae enter the haemolymph and proliferate and colonise the entire insect and release blastospores. Insect death occurs due to nutrient depletion of the haemolymph or by toxaemia by secretion of toxic metabolites.



Figure 11.11: Picture of insect infected with *Beauveria bassiana*



Biopesticides registered in India:

1. *Bacillus thuringiensis var. israelensis*
2. *Bacillus thuringiensis var. kurstaki*
3. *Bacillus thuringiensis var. galleriae*
4. *Bacillus sphaericus*
5. *Trichoderma viridae*
6. *Trichoderma harzianum*
7. *Pseudomonas fluorescens*

11.3.3 Viral Biopesticides

Viral insecticides are pathogens that attack insects and other arthropods. Viral pesticides are used to control Lepidopteran larvae like *Helicoverpa, Spodoptera* sp on Cotton, Corn, Sorghum, tomatoes. Baculoviruses are the commonly used viral biopesticide. They are extremely small and are composed of double stranded DNA. The genus Baculoviruses contains 3 subgroups.

- Nuclear Polyhedrosis viruses (NPVs)
- Granulosis viruses (GVs)
- Non occluded viruses

Mode of action of NPV



The virus enters the insect body via ingestion by insects and infects the midgut cells by membrane fusion. The NPV uncoat within the nucleus of cells and pass through the intestinal epithelium (Figure 11.12) and establish a systemic infection of the haemocoel.

Symptoms

Discoloration (larvae turns brown or yellow)

- Decomposition or softening of larvae
- Lethargy
- Infected larvae hang upside down twigs
- Larvae become swollen with fluid containing virus and eventually die turning black in color.

Mass production of NPV

NPV are mass produced in laboratory using suitable larval hosts. The fifth stage larvae are fed with food infected with NPV. After 4-5 days, the dead larvae are collected and macerated. The liquid is centrifuged and the pellet containing the viruses is suspended in sterile distilled water. This viral suspension can be used for spraying in the fields.

1Summary

Carbondioxide fixation and Biological Nitrogen Fixation are the most significant biological processes taking place on planet Earth. Methanogenesis is an anaerobic

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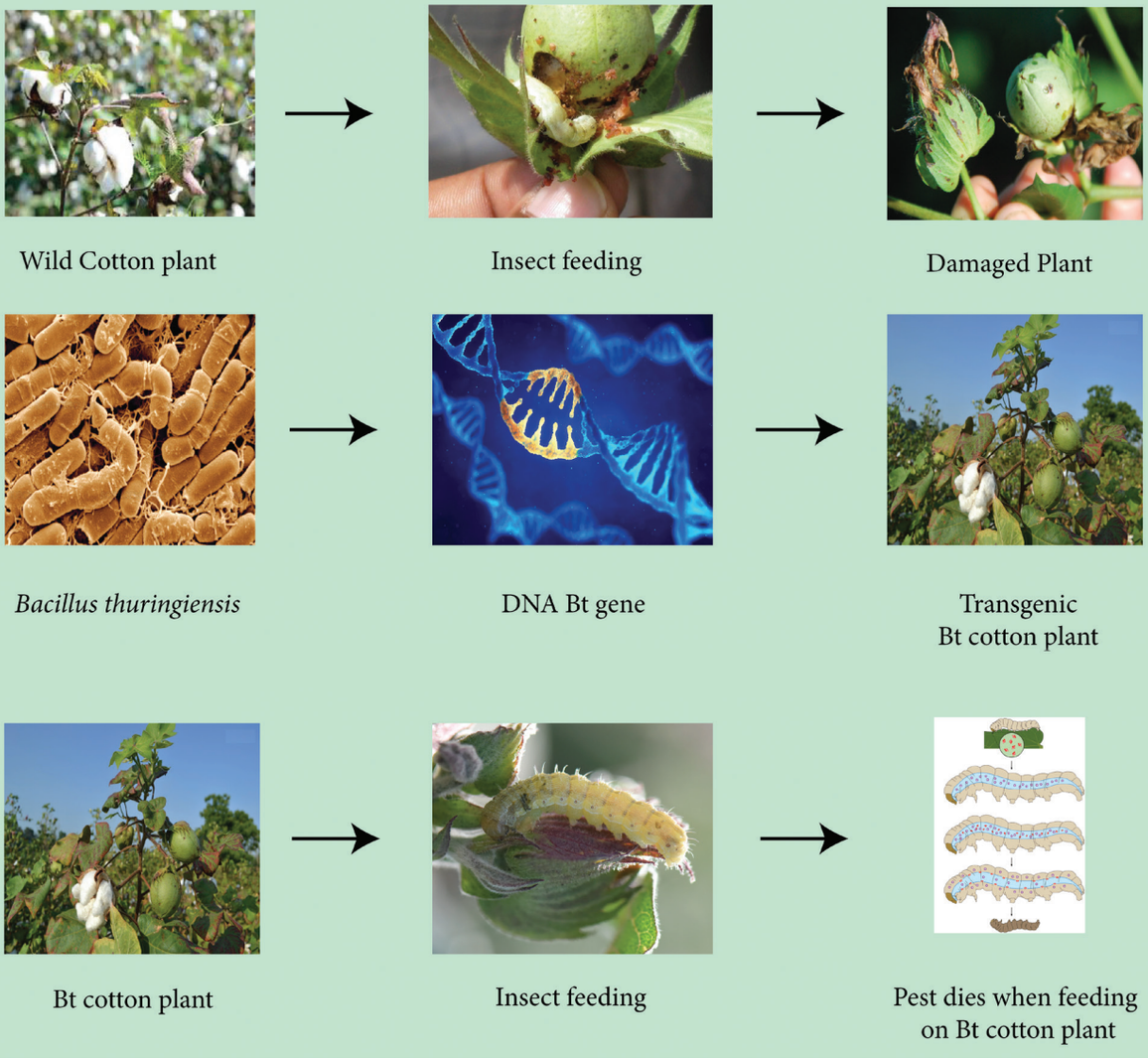
process converting CO_2 to CH_4. It is carried out by methanogens like *Methanobacterium* sp. Phosphorous transformations mostly happen as inter conversion of inorganic to organic phosphate and insoluble form to soluble phosphates.Purple and green sulphur bacteria store sulphur as granules as a result of which they appear yellow in colour.

Biofertilizers are preparations containing beneficial micro organisms like N_2 fixers, PO_4 solubilizers in a viable static state intended for seed or soil application and designed to improve soil fertility. *Azolla* and *Anabaena* share symbiotic relationship in which *Anabaena* provides fixed atmospheric nitrogen to *Azolla*.

Vesicles and arbuscles in VAM fungi are the storage organelles of polyphosphates.Blue Green algae are prokaryotes that can perform both photosynthesis and nitrogen fixation.

Biopesticides refer to compounds that are used to manage agricultural pests by means of specific biological effects. *Bacillus thuringiensis* produces crystal toxin which is detrimental to insects.

Bacterial Biopesticide - *Bacillus thuringiensis*



TN_GOVT_XI_Micro_Biology_CH11.indd 178 16-02-2019 10.04.49 AM

Evaluation

Multiple choice questions

1. The conversion of atmospheric nitrogen to ammonia by prokaryotes is called
   a. Biological Nitrogen Fixation
   b. Nitrification
   c. Ammonification
   d. Denitrification
2. The oxidation of sulphide is carried out by
   a. *Thiobacillus*
   b. Purple bacteria
   c. *Beggiatoa*
   d. Both a and b
3. Phosphate solubilisation by bacteria is mediated by the production of
   a. Organic acids
   b. Phosphatases
   c. Phosphoric acid
   d. Phytases
4. The process of production of CH_4 from CO_2 is called
   a. CO_2 fixation
   b. Methylotrophy
   c. Methanogenesis
   d. Photosynthesis
5. The reduction of sulphate for building up aminoacids and proteins is called
   a. Desulfurylation
   b. Assimilatory sulphate reduction
   c. Dissimilatory sulphate reduction
   d. Sulphur reduction
6. An example of nitrogenous biofertilizer
   a. *Bacillus*
   b. *Pseudomonas*
   c. *Rhizobium*
   d. VAM
7. The other name for Blue Green Algae is
   a. Green algae
   b. Brown algae
   c. Cyanobacteria
   d. Blue algae
8. The selective media for Rhizobium YEMA contains the sugar
   a. Maltose
   b. Mannitol
   c. Glucose
   d. Lactose
9. Solubilisation of inorganic phosphates by *Bacillus* is brought about by the
   a. Production of enzymes
   b. Production of organic acids
   c. Production of alkali
   d. Mineralisation
10. The mass cultivation of VAM is preferably done in
    a. Sorghum roots
    b. Rice roots
    c. Potato roots
    d. Cotton roots
11. _____ is an example of entomopathogenic fungi
    a. *Verticillium*
    b. *Beauveria bassiana*
    c. *Metarhizium anisopliae*
    d. All of the above
12. The toxic effect of *Bacillus thuringiensis* is due to
    a. Cry protein
    b. Delta endotoxin
    c. Parasporal crystals
    d. All of the above

TN_GOVT_XI_Micro_Biology_CH11.indd 179 16-02-2019 10.04.49 AM

13. The action by mycopesticide depends on the
    a. Ingestion of fungi by insects
    b. Penetration of cuticle by fungi
    c. Ingestion of leaves infected with fungi
    d. Ingestion of spores by fungi.
14. Crown gall is caused by *Agrobacterium* on
    a. Monocots
    b. Dicots only
    c. Monocots and dicots
    d. Ferns
15. Smut is a disease caused by
    a. *Puccinia sp*
    b. *Ustilago sp*
    c. *Verticillium*
    d. None of the above

Answer the following

1. What is the role of purple and green bacteria in sulphur cycle?
2. What is nitrogenase? Give its function.
3. Define biofertilizers.
4. What is VAM?
5. Define biopesticides.
6. What is a smut?
7. What is NPV?
8. List out Bacterial diseases of plants.
9. What is the end result of decomposition of organic matter in carbon cycle? Give the role of microorganisms with examples.
10. What is the function of leghemo-globin?
11. Give the method of application of *Azolla* in paddy fields.
12. Give the salient features of *Rhizobium*.
13. Explain the mass production of phosphate solubilisers.
14. What is *Azolla-Anabaena* symbiosis?
15. What is crystal toxin? Write short notes on BT cotton.
16. Explain the process of root nodule formation with appropriate diagram.
17. Give in detail the reactions involved in phosphorus/carbon/sulphur/nitrogen cycle.
18. Explain the mass production of VAM.
19. Give detailed account on *Bacillus thuringiensis*.

Student Activity

- Sow two groundnut seeds in a plastic cup/earthern pot. After one month, pull out the plant and observe the root system for nodules.
- Collect pictures of all organisms involved in sulphur cycle and prepare a collage showing its role.
- Prepare a chart work showing the biogeochemical cycles of carbon, nitrogen and phosphorus
- Collect pictures of various diseases of plants and prepare a chart.
- Prepare a model on the mode of action of BT.
- Collect diseased parts of plants and identify the symptoms of the disease.

TN_GOVT_XI_Micro_Biology_CH11.indd 180 16-02-2019 10.04.49 AM

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Chapter 12

Medical Microbiology

Chapter Outline

12.1 Microbial Infections of the Human Body

12.2 Skin and Wound Infections

12.3 Respiratory Tract Infections

12.4 Gastrointestinal Tract Infections

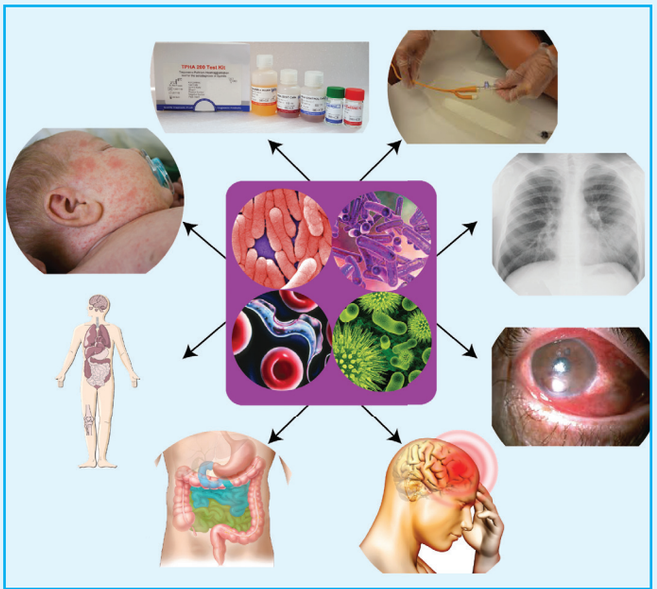
12.5 Ocular Infections

12.6 Urinary Tract Infections

12.7 Reproductive Tract Infections

12.8 Infections of the Nervous System

12.9 Systemic Infections



Medical Microbiology or Clinical Microbiology plays an important role by providing the necessary diagnostic testing, means of epidemiological detection, and future innovation required in an era of emerging and reemerging infectious diseases.

Learning Objectives

After studying this chapter the student will be able,

- *To describe the importance of medical microbiology.*
- *To understand the types and sources of infections.*
- *To know the types of infectious diseases and virulence factors of the pathogen.*
- *To tell the etiological agents of skin wound respiratory, gastro intestinal, ocular, urinary, reproductive, nervous system and systemic infections.*
- *To know the causative agents of varoius human diseases and their portal of entry.*

12.1 Microbial Infections of the Human Body

Medical microbiology is the branch of microbiology which deals with prevention, diagnosis and treatment of infectious diseases. There are four kinds of microorganisms that cause infectious diseases. They are bacteria, fungi, parasites and viruses. Any disease that spreads from one host to another, either directly or indirectly is said to be a communicable disease. Chicken pox, measles, genital herpes, typhoid fever and tuberculosis are examples of such diseases, that are easily spread from one person to another.

A non communicable disease does not spread from one host to another. For example, *Clostridium tetani,* a soil

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inhabitant, produces Tetanus when it is introduced into a wound or an abrasion. Tetanus is thus an infectious disease, but not communicable.

Infectious disease occurs when the infecting microorganism causes damage to the host. The term infection refers to the establishment of the microorganisms in the tissues resulting in injury or harmful effect to the host. Infection is a pathological condition due to the growth of microorganisms in a host. To initiate an infection, a pathogenic microbe enters the tissues of the body by a characterization route, the portal of entry.

12.1.1 Routes of Infections

There are various ways in which microorganisms enters into the host are explained below.

a. Contact

Infection may be acquired by contact which may be direct or indirect. Sexually transmitted diseases such as syphilis and gonorrhea spread by direct contact. Indirect contact may be through the agency of inanimate objects such as clothing, pencils or toys which may be contaminated by a pathogen from one person to another. Pencils shared by school children may act as fomites in the transmission of diphtheria, and face towels in trachoma.

b. Inhalation

Respiratory infections such as influenza and tuberculosis are transmitted by inhalation of the pathogen in droplet and droplet nuclei that are shed by the patients during sneezing, speaking or coughing. Common cold virus, Adenovirus is some of the virus producing respiratory infections.

c. Ingestion

Intestinal infections are generally acquired by the ingestion of food or drinks contaminated by pathogens. Infection transmitted by ingestion may be waterborne (cholera), food borne (typhoid) or fecal-oral route (dysentery).

d. Inoculation

Pathogens, in some instances, may be inoculated directly into the tissues of the host. Tetanus spores implanted in the depth of wounds, rabies virus deposited subcutaneously by dog bites, inoculation through unsterile syringes and surgical equipments are examples that enter through direct inoculation.

e. Congenital

Some pathogens are able to cross the placental barrier and infect the fetus in uterus. Bacteria like *Treponema pallidum*, viruses like *Rubella, Cytomegalovirus* parasite like *Toxoplasma gondi* are some of the organisms that enter through placenta and cause disease in the newborn.

12.1.2 Types of Infections

Infections may be classified in various ways. Initial infection with a parasite in a host is called a primary infection. Subsequent infections by the same parasite in the host are termed reinfections. When a new parasite sets up an infection in a host whose resistance is lowered by a preexisting infectious disease, this is termed secondary infection.

When in a patient already suffering from a disease, a new infection is setup

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from another host or other external sources it is termed cross infection. Cross infections occurring in hospitals are called nosocomial infections. Iatrogenic infection refers to physician induced infections resulting from investigative, therapeutic or other procedures.

Depending on whether the source of infection is from the host's own body or from external sources, infections are classified as **endogenous** or **exogenous**, respectively.

Endogenous infection

Endogenous infections are acquired from the host himself from the normal flora of the body.

Microorganisms are present in certain areas of the body in all human beings. They are called normal flora. The common areas are Nose, Mouth, Teeth, Throat, Intestine, Urethra, Vagina and Skin (Figure 12.1).

1. When the skin is breached normal flora enters the tissues.
2. When the urethral organisms ascend, they cause urinary tract infection
3. When a patient is treated with antibiotics, normal flora is eliminated and replaced by potential pathogens
4. When the intestine is perforated, normal flora enter the previously sterile body parts
5. Similarly when the pH of the vagina increases potential pathogens occupy the space.

However normal flora helps host against pathogen and benefits the host in many ways

- Normal flora of skin produces fatty acids which inhibit other species
- Intestinal bacteria secrete antibacterial substances (bacteriocins, colicins) and many metabolic products that prevent other species to survive.

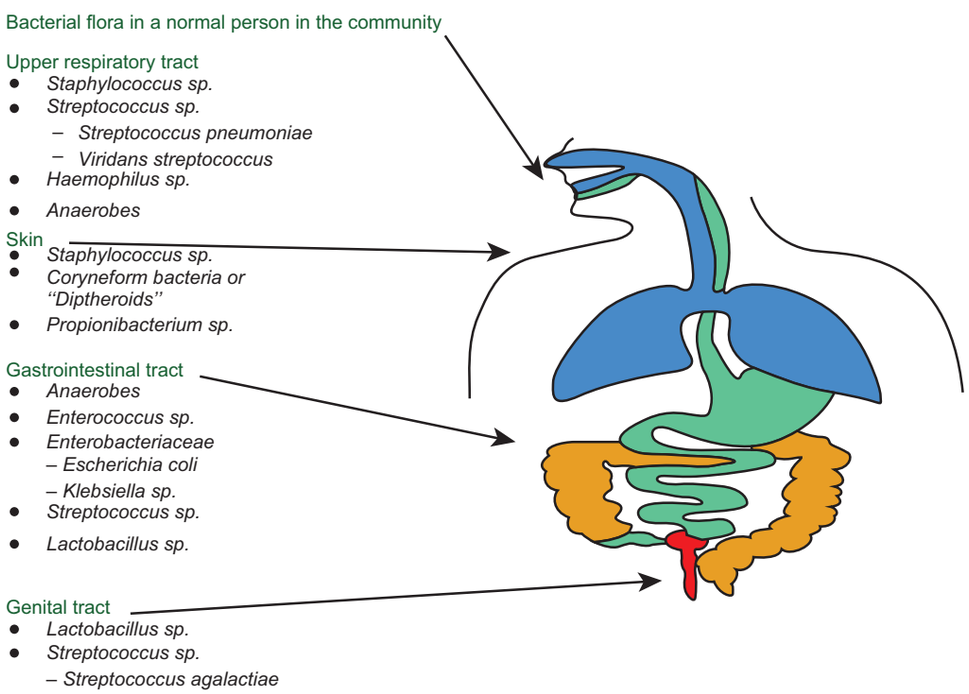


Figure 12.1: Microorganisms present as normal flora
(There are many organisms in a site. Only few are listed)

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- Because of their large numbers other species do not have space in the intestine
- Acidic environment created by vaginal Lactobacilli suppresses growth of other bacteria.

HOTS

How do normal flora help host against pathogenic microorganisms?

Exogenous sources of infections

Human beings: The commonest source of infections in human are from other human beings. The parasite may originate from a patient or a carrier. A patient is a person who harbours the pathogenic microorganism and suffers from ill effect because of it. A **healthy carrier** is the one who harbours the pathogens but has never suffered from the disease caused by the pathogen. A **convalescent carrier** is one who has recovered from the disease and continues to harbor the pathogen in his body (Figure 12.2).

Animals: Many pathogens are able to infect both human beings and animals. Infectious disease transmitted from animals to human beings are called zoonoses. Zoonotic diseases may be bacterial (Example: plague from rats) or viral (Example: rabies from dogs).

Insects: Blood sucking insects may transmit pathogens to human beings. The diseases so caused are called arthopod borne diseases. Insects such as mosquitoes, ticks, mites, flies, fleas and lice that transmit infections are called vectors.

Transmission may be mechanical or biological. Mechanical transmission is the passive transport of the pathogens on the insects feet or other body parts. Example: Houseflies can transfer the pathogens of Typhoid fever and Bacillary dysentery

Infobits

The story of typhoid Mary

The classic example of role of carriers in disease transmission is the story of Mary Mallon.

Mary Mallon was an Irish immigrant who worked as a cook in New York in the early twentieth century. Over seven years, from 1900 to 1907, Mallon worked for number of different households. Unknowingly spreading illness to the people who lived in each one. Later George Soper, tracked Mallon linked 22 cases of typhoid fever through her. He discovered that Mallon was a carrier for typhoid but was immune to it herself. Although active carriers had been recognized before, this was the first time that an asymptomatic carrier of infected had been identified. Epidemiologists were able to trace 51 cases of typhoid fever and three deaths directly to Mallon, who is remembered as "Typhoid Mary". She was forced to prison and then released under the conditions that she could no longer be a cook. She assumed a false name and began cooking again and of course, infecting numerous people. She was again prisoned where she died 26 years later of pneumonia.

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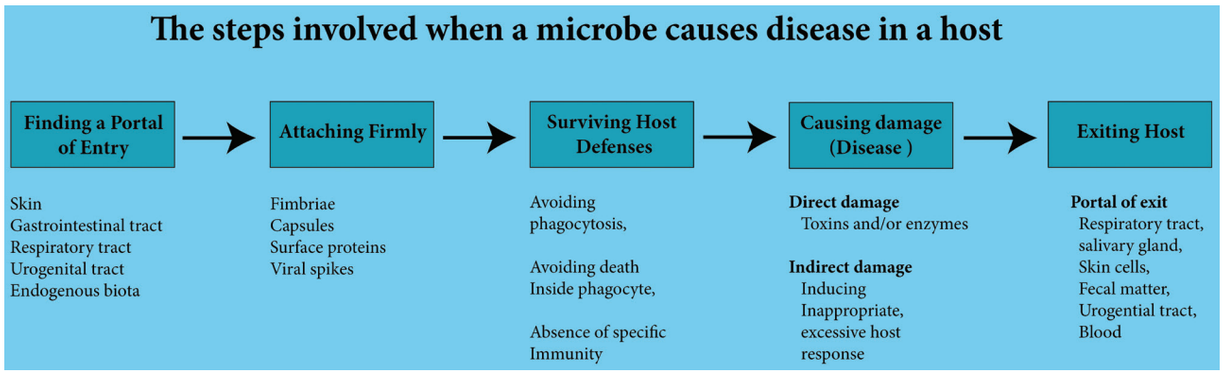


Figure 12.2: The steps involved when a microbe causes disease in a host

(shigellosis) from feces of infected people to food. Such vectors are called mechanical vectors.

Biological transmission is an active process and is more complex. The pathogens multiply in the body of the vectors often undergoing part of a developmental cycle in it. Such vectors are termed biological vectors. Example: *Aedes aegypti* mosquito transmitting dengue, *Anopheles* mosquito transmitting malaria.

Soil: Some pathogens are able to survive in the soil for very long periods. Spores of tetatus bacilli may remain viable in the soil for several decades and serve as the source of infection.

Water: Water may act as the source of infection due to contamination with pathogenic microorganisms. Example: Cholera causing *Vibrio cholerae*.

Food: Contaminated food materials may act as source of infection. The presence of pathogens in food may be due to external contamination. Example: Food contaminated by *Staphylococcus*.

12.1.3 Types of Infectious Diseases

Infectious diseases may be localised or generalised.

Localised infections: An infection that is restricted to a specific location or region within the body of the host is called localised infection.

Generalised infections: An infection that has spread to several regions or areas in the body of the host. This involves the spread of the infecting agent from the site of entry through tissue spaces or channels, along the lymphatics or through the bloodstream.

Circulation of bacteria in the blood is known as **Bacteremia**. **Septicemia** is the condition where bacteria circulate and multiply in the blood, form toxic products and cause high fever. **Pyemia** is a condition where pyogenic bacteria produce septicemia with multiple abscesses in the internal organs such as the spleen, liver and kidney.

Occurrence of a disease

To understand the full scope of a disease, we should know about its occurrence. Epidemiology involves in the study of the frequency and distribution of disease and other health related factors in defined populations. The incidence of a disease is the number of people in a population who develop a disease during a particular time period. The prevalence of a disease

TN_GOVT_XI_Micro_Biology_CH12.indd 185 16-02-2019 10.05.39 AM

is the total number of existing cases with respect to the entire population.

Depending on the spread of infectious disease in the community, they may be classified into different types.

- Endemic diseases are those which are constantly present in a particular area. Typhoid fever is endemic in most parts of India.
- Epidemic disease is one that spreads rapidly, involving many persons in an area at the same time. Example: Epidemic of Dengue in 2017.
- A pandemic is an epidemic that spreads through many areas of the world involving very large numbers of persons within a short period. Example: H1N1 Influenza outbreak in 2009. Ebola outbreak in 2014-2016 in West Africa was the largest in history and first ever epidemic, affecting multiple countries.
- If a particular disease occurs only occasionally, it is called a sporadic disease. The most commonly occurring sporadic diseases in India are Diphtheria and Hepatitis A and E.

Severity or duration of a disease

Another useful way of defining the scope of a disease is in terms of its severity or duration.

- An **acute** disease is one that develops rapidly but lasts for a short time.
- A **chronic** disease develops more slowly, and the body's reactions may be less severe, but the disease is likely to be continual or recurrent for long periods.
- A disease that is intermediate between acute and chronic is described as a subacute disease.

Facts about Fever:

Fever is as more healthful than harmful. An experiment with vertebrates shows that fever increases the rate of antibody synthesis. Increased temperatures stimulate the activities of T cells and increase the effectiveness of interferon. Fever appears to enhance phagocytosis. Fever almost never occurs as a single response; it is usually accompanied by chills. The explanation lies in the natural physiological interaction between the thermostat in the hypothalamus and the temperature of the blood. For example: If the thermostat has been set (by pyrogen) at 102°F but the blood temperature is 99°F, the muscles are stimulated to contract involuntary (shivering) as a means of producing heat. In addition, the vessels in the skin constrict, creating a sensation of cold and the piloerector muscles in the skin develops 'goose bumps'.



- A latent disease is one in which the causative agent remains inactive for a time but then becomes active to produce symptoms of the disease.

12.1.4 Interaction between Microbes and Host

Pathogen is a microorganism which causes disease.

Pathogenecity is the ability of a pathogen to produce disease.

Virulence is the degree of pathogenecity of a microorganism. Virulence is not generally attributable to a single property

TN_GOVT_XI_Micro_Biology_CH12.indd 186 16-02-2019 10.05.39 AM

but depends on several parameters related to the organism, the host and their interaction.

Microorganisms first enter the body, survive, multiply and elaborate many factors and produce the disease.

Adhesion: The initial event in the pathogenesis of many infections is the attachment of the bacteria to body surfaces. Adhesions may occur as organized structures, such as fimbriae and pili. Adhesions serve as virulence factors.

Capsule: It is an envelope or slime layer surrounding the cell wall of certain microorganisms. Capsule plays important roles in immune evasion as it inhibit's phagocytosis, as well as protecting the bacteria while outside the host.

Toxins: Toxins are specific chemical products of microbes, plants and some animals that are poisonous to other organisms. Toxigenicty is the power to produce toxins.

A toxin is named according to specific target of action: Neurotoxin acts on the nervous system. Enterotoxin acts on the intestine, Haemotoxin lyses red blood cells, and Nephrotoxins damages the kidneys.

A toxin molecule secreted by a living bacterial cell into the infected tissue is an **exotoxin**. A toxin that is not actively secreted but is shed from the outer membrane is an **endotoxin**. the difference between exotoxin and endotoxin were given in Table 12.1.

Production of enzymes

Some enzymes like proteases, DNAases and phospholipases are produced and they help in destruction of the cell structure and to hydrolyse host tissues.

Antigenic variation

Microorganisms evade the host immune responses by changing their surface antigens. Antigenic drift and antigenic shift are common in influenza viruses. The distinction between the commensal and the organism associated with disease.

12.1.5 Diagnostic Cycle

Specific diagnosis is important for better patient care, use of appropriate antibiotics

Table 12.1: Differences between endotoxin and exotoxin

Exotoxins	Endotoxins
Heat labile proteins, secreted by certain species of bacteria and diffuse readily into the surrounding medium	Heat stable polysaccharide proteins, lipid complex which form an integral part of the cellwall of Gram negative bacteria
Proteins with a strong specificity to a target cell and extremely powerful sometimes deadly	A Lipopolysaccharide (LPS), which is part of the outermembrane of gram negative cell walls
Highly immunogenic	Less immunogenic
Toxoids can be made by treating toxins with formalin	Toxoids cannot be made
Produced mainly by Gram positive bacteria but also by some Gram negative bacteria	Produced by Gram negative bacteria

TN_GOVT_XI_Micro_Biology_CH12.indd 187 16-02-2019 10.05.39 AM

and to initiate appropriate preventive measures. The diagnostic cycle begins when the clinician takes a microbiological sample and ends when a clinician receives the laboratory report and uses the information to manage the condition (Figure 12.3).

The steps in diagnostic cycle are

1. Clinical request and provision of clinical information.
2. Collection and transport of appropriate specimens.
3. Laboratory analysis.
4. Interpretation of microbiology report and use of the information.

Specimen Collection and transport: It is important to collect the specimen appropriately and protect it from contamination. Transport media are used that are compatible with the organism believed to be present in the clinical sample. Quality of patient specimens and their transport to the laboratory is important.

Infections and samples used

Respiratory tract infections: Nasal and bronchial washings, throat and nasal swabs, sputum.

Eye infections: Conjunctival swab or scraping.

Wound infections: Pus, skin scraping, wound swap.

Gastrointestinal infections: Stool, rectal swabs.

Genital infections: Vesicle fluid or swab.

Urinary tract infections: Urine.

Blood borne infections: Blood.

Nervous system infections: Cerebrospinalfluid (CSF).

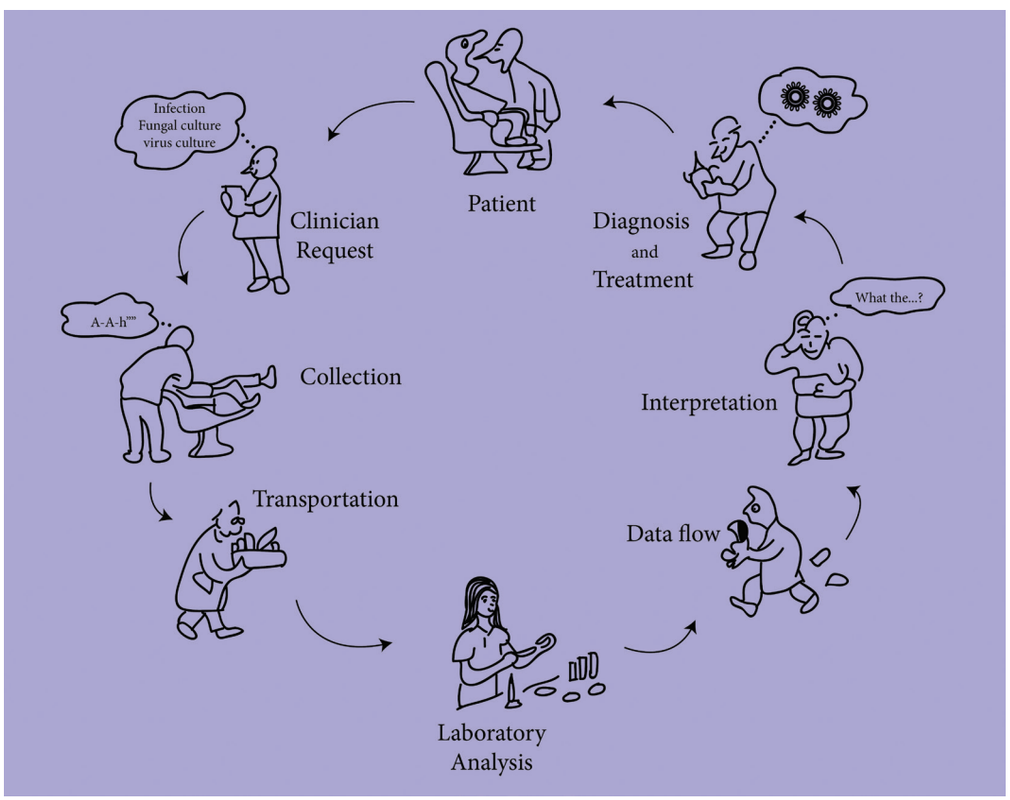


Figure 12.3: The steps in diagnostic cycle

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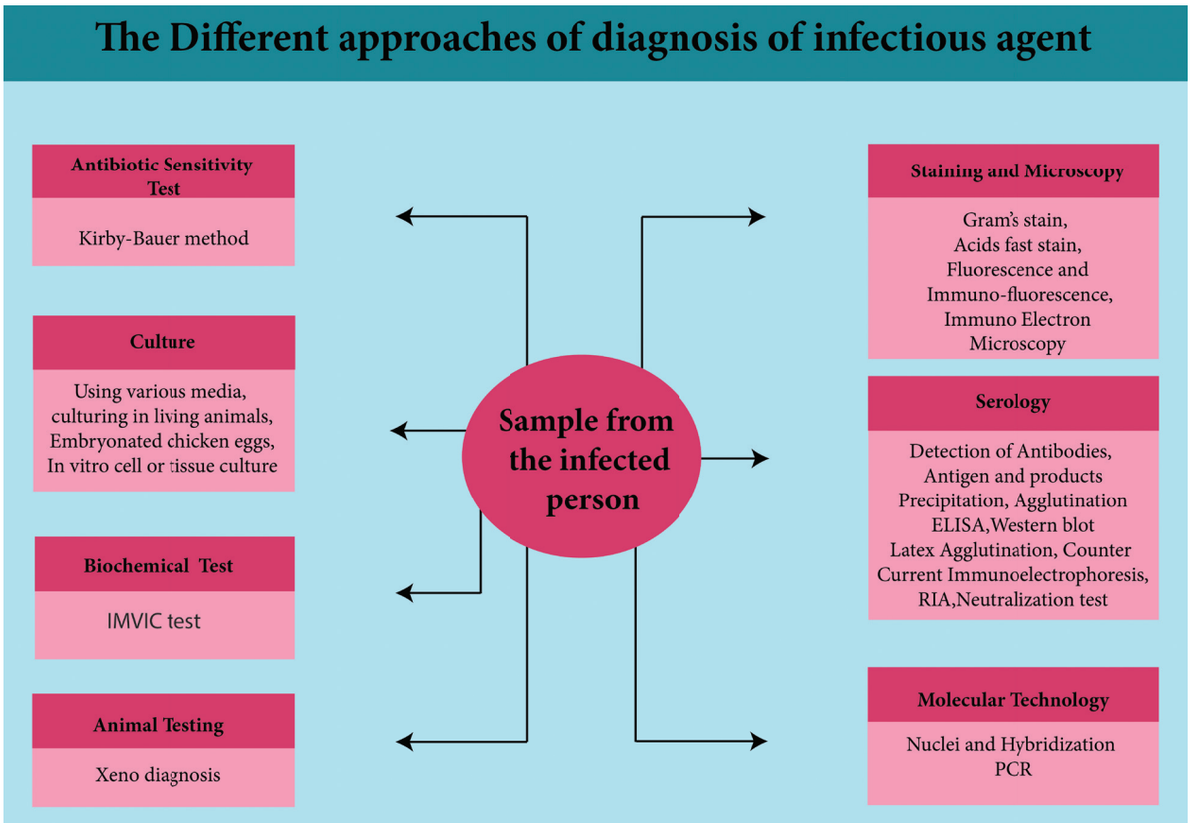


Figure 12.4: Different approaches of diagnosis

Laboratory diagnosis of infectious agents

Direct diagnosis: It is the demonstration of the presence of an infectious agent, antigen or nucleic acids

Indirect diagnosis: It is the demonstration of presence of antibodies to a particular infectious agent, cytopathic effects, haemagglutination, inclusion bodies and neutralization.

The different approaches for diagnosis or identification of infectious agents are shown in Figure 12.4.

12.2 Skin and Wound Infections

The skin, which covers and protects the body, is the body's first line of defense against pathogens. As a physical barrier, it is almost impossible for the pathogens to penetrate it. However, microorganisms can enter through skin breaks that are not readily apparent, and the larval forms of a few parasites can penetrate the intact skin. The skin has up to seven layers (Figure 12.5) of ectodermal tissue and guards the underlying tissues viz; muscles, bones, ligaments and internal organs. Nearly all human skin is covered with hair follicles. Because it interfaces with the environment, skin plays an important role in protecting the body against pathogens and excessive water loss. Its other functions are insulation, temperature regulation, sensation, synthesis of vitamin D, and the protection of vitamin B folates. Severely damaged skin will try to heal by forming scar tissue. This is often discolored and depigmented.

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12.2.1 Normal Microbiota of the Skin

The skin's normal microbiota contains relatively large numbers of Gram positive bacteria, such as *Staphylococci* and *Micrococci*. Bacteria in the skin tends to be grouped into small clumps. Vigorous washing can reduce their numbers but will not eliminate them. Microorganisms remaining in hair follicles and sweat glands after washing will soon reestablish the normal populations. Areas of the body with high moisture, such as armpits and between the legs, have higher populations of microorganisms. They metabolize secretions from the sweat glands and are the main contributors to body odour.

Also part of the skin's normal microbiota are Gram positive pleomorphic rods called diphtheroids. Some diphtheroids, such as *Propionibacterium acnes*, are typically anaerobic and inhabit hair follicles. These bacteria produce propionic acid, which helps maintain the low pH of skin, generally between 3 and 5

12.2.2 Wound Infection

Wound can be defined as any interruption of continuity of external or internal surfaces caused by violence

Wounds may occur following: surgery, trauma or injections

Wound infections may occur mainly after surgical procedures

Wound sepsis is the result of cross infection from human sources and from other outside sources.

Bacteria associated with wound infections

Many bacteria are associated with wound infection (Figure 12.6). The normal flora may also cause infection. The most common normal flora of the skin are: *Staphylococci*, and various *Streptococci*, *Sarcina* sp, anaerobic Diphtheroids, Gram negative rods and others.

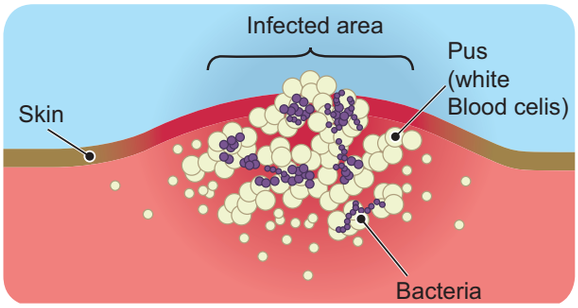


Figure 12.6: Bacterial infection on the skin

Post operative infections

Gasgangrene organisms like *Clostridium perfringens, Staphylococcus aureus* and *Clostridium tetani* may cause post operative infections.

HOTS

1. What are the possible infecting agent you could pick up when you are injured while playing on the ground? List them and name the diseases that they could cause.
2. What are the possible infectious agent that can infect you when you are injured by a rusted nail?

Route of entry

Wounds may occur following surgery, trauma or injections. Wound infections may occur mainly after surgical procedures. Wound sepsis is the result of cross infection from human sources and from other outside sources. Infections of skin are listed in Table 12.2.

TN_GOVT_XI_Micro_Biology_CH12.indd 190 16-02-2019 10.05.43 AM

Mechanisms of damage

1. Organisms enter through the skin, multiply there and produce the disease in the skin.
   For example, impetigo, abscess and cellulitis (Figure 12.7) are caused by *Staphylococcus aureus* and *Streptococcus pyogenes*.



Figure 12.7: Cellulitis

As soon as the organisms enter the skin they multiply and produce various toxins that kill the cells and produce cellulitis. Further damage leads to necrosis and ulcer formation (Figure 12.8).



Figure 12.8: Ulcer formation

2. Organisms multiply in the skin and produce disease in internal organs. For example some Group A *Streptococci* multiply in the skin and produce disease known as Acute Glomerulonephritis causing damage to the kidneys. Some times *Corynebacterium diphtheriae* may multiply in the skin and affect the heart due to the toxin

Table 12.2: Bacterial Infections of the skin

Disease	Pathogen	Signs and Symptoms	Transmission
Cellulitis	*Streptococcus pyogenes*	Localised inflammation of dermis and hypodermis; skin red, warm, and painful to the touch	Through cut or abrasion
Erysipelas	*Streptococcus pyogenes*	Inflamed, swollen patch of skin, often on face; may be suppurative	Through cut or abrasion
Impetigo	*Staphylococcus aureus, Streptococcus pyogenes*	Vesicles, pustules, and sometimes bullae around nose and mouth	Highly contagious, especially via contact
Wound infections	*Pseudomonas aeruginosa,* others	Formation of biofilm in or on wound	Exposure of wound to microbes in environment; poor wound hygiene

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3. Sometimes organism may multiply in the skin and produce the toxin which affect the Central Nervous System (CNS) and the effects seen. In the case of *Clostridium tetani* infection, convulsions and paralysis occur due to the production of a powerful toxin.

12.3 Respiratory Tract Infections

With every breath, we inhale several microorganisms and therefore the respiratory system is a major portal of entry for pathogens. In fact, respiratory system infections are the most common type of infections and among the most damaging. Some pathogens that enter via respiratory route can infect other parts of the body, such as skin incase of measles, mumps and rubella.

The upper respiratory system has several anatomical defenses against airborne pathogens. Coarse hairs in the nose, filter large dust particles from the air. The nose is lined with a mucous membrane that contains numerous mucous secreting cells and cilia. The upper portion of the throat also contains a ciliated mucous membrane. The mucous moistens inhaled air and traps dust and microorganisms. The cilia help to remove these particles by moving them towards the mouth for elimination.

12.3.1 Structure of Respiratory Tract

The structure of respiratory tract is divided into two main parts viz: upper respiratory tract (URT) and lower respiratory tract (LRT).

Upper respiratory tract includes mouth, nose, nasal cavity, sinuses, throat or pharynx, epiglottis and larynx.

Lower respiratory tract includes trachea, bronchi, bronchioles, lungs and alveoli (Figure 12.9).

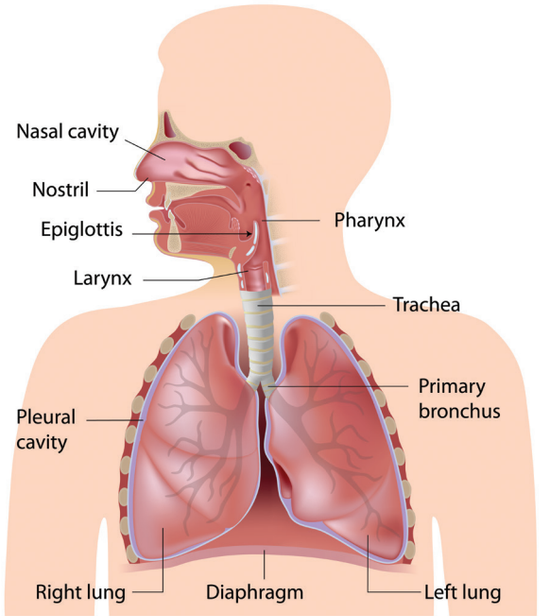


Figure 12.9: Structure of human respiratory tract

12.3.2 Normal Defenses against Infections

Respiratory tract infection are divided into upper respiratory tract (URT) tract infection and lower respiratory tract (LRT) infection. Infection of the respiratory tract are listed in the Table 12.3.

URT: Infections are Sinusitis, Pharyngitis Laryngitis and Epiglotitis

LRT: Infections are Trachiitis, Tracheo bronchitis, Bronchitis, Alveolitis and Pneumonia.

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Upper respiratory system		
Diseases	**Pathogen**	**Symptoms**
Bacterial diseases		
Epigottitis	*Haemophilus influenzae*	Inflammation of the epiglottis
Streptococcal pharyngitis (strep throat)	*Streptococci*, especially *Streptococcus pyogenes*	Inflamed mucous membranes of the throat;
Diphtheria	*Corynebacterium diphtheriae*	Bacterial exotoxin interferes with protein synthesis; damages heart, kidney, and other organs; membrane forms in throat; cutaneous form also occurs;
Otitis media	Several agents, especially *Staphylococcus aureus, Streptococcus pneumonia* and *Haemophilus influenza*	Accumulations of pus in middle ear build up painful pressure on eardrum
Viral diseases		
Common cold	Rhino virus	Familiar symptoms of coughing, sneezing, running nose.
Lower respiratory system		
Bacterial diseases		
Pertussis (whooping cough)	*Bordetella pertussis*	Cilia in upper respiratory tract inactivated, mucus accumulates, spasms of intense coughing to clear mucus;
Tuberculosis	*Mycobacterium tuberculosis*	Tubercle bacilli entering lungs survive phagocytosis, reproduce in macrophages; tubercles formed to isolate pathogen; defenses eventually fail, and infection becomes systemic;
Viral diseases		
Respiratory syncytial virus (RSV)	Respiratory syncytial virus	A serious respiratory disease of infants;
Fungal diseases		
Blastomycosis	*Blastomyces dermatitidis*	Abscesses; extensive tissue damage;
Bacterial pneumonia		
Pneumococcal pneumonia	*Streptococcus pneumonia*	Infected alveoli of lung fill with fluids; interferes with oxygen uptake
Haemophilus influenzae pneumonia	*Haemophilus influenzae*	Symptoms resemble pneumococcal pneumonia

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12.4 Gastrointestinal Tract Infections

Human systems function by the energy produced from the digested food molecules. The food is swallowed through mouth and digested in the gastro intestinal tract. The food we consumed should be free of contaminations. The contaminated food causes gastrointestinal infections.

Through contaminated food and water the pathogens are ingested and they enter the GIT. In the small intestine they initiate an infection. Many times the pathogens that cause intestinal infections multiply in the GIT and produce their pathogenic effect in the intestine itself. Example: Shigellosis, Cholera.

The gastrointestinal tract (GIT) or alimentary canal includes the mouth, pharynx, throat, oesophagus (food Tube lead to the stomach), stomach, small and large intestine. It also includes accessory structures salivary glands, liver, gall bladder and pancreas lying outside the GIT. Secretions of these organs enhance the digestion of food molecules (Figure 12.11).

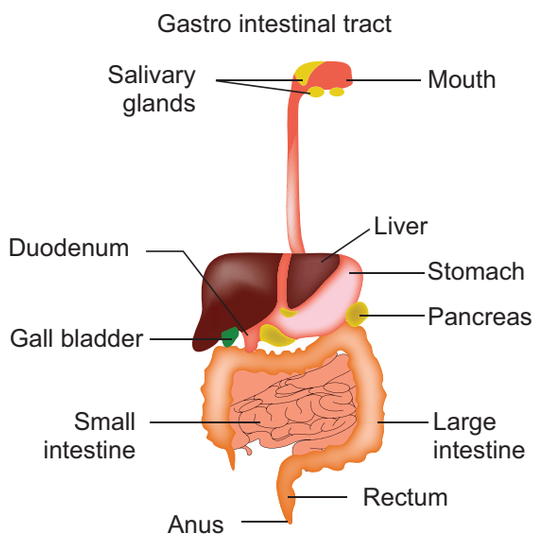


Figure 12.11: The structure of Gastro intestinal tract

Difference between infection and intoxication

Microbial diseases of digestive system are typically transmitted by a fecal oral route. Most such diseases result from the ingestion of food or water contaminated with pathogenic microorganisms or their toxins. These pathogens usually enter the food or water supply after being shed in the feces of people or animals infected with them.



- Each milliliter of saliva can contain millions of bacteria
- Stomach/small intestine has very few microorganisms because of hydrochloric acid present in the stomach.
- Large intestine harbours microbial population exceeding 100 billion of bacteria per gram of feces (40% fecal masses contain microbial cell material)
- Large intestine microbial population mainly contain anaerobes and facultative anaerobes.

After ingestion of pathogenic microorganisms, localization and multiplication of organisms takes place in the GIT and is called infection. Microorganisms may penetrate into intestinal mucosa and grow there or they may penetrate to other organs. Gastroenteritis is usually classified as either infection or intoxication. Food borne diseases can arise from either infection or intoxication. In both cases, bacterial toxins are typically responsible for producing disease signs and symptoms. In a food infection the microbial agent ingested

TN_GOVT_XI_Micro_Biology_CH12.indd 194 16-02-2019 10.05.45 AM

colonise in the gut and then produces toxins that damage host cells.

In case of food intoxication the toxins produced by bacteria in the food are ingested. Infection and intoxication differ in their onset of symptoms. Infections are characterized by a delay in the appearance of gastrointestinal disturbance until the pathogen increases in number or affects invaded tissue. Infection is correlated with onset of fever, one of the basic body's general responses to an infective organism. In case of intoxication, the symptoms are characterized by sudden appearance of gastrointestinal disturbances like cramping, nausea, vomiting or diarrhoea.

HOTS

What is likely to happen to a child who drinks contaminated water?

12.4.1 Microbial Flora of Gastrointestinal Tract

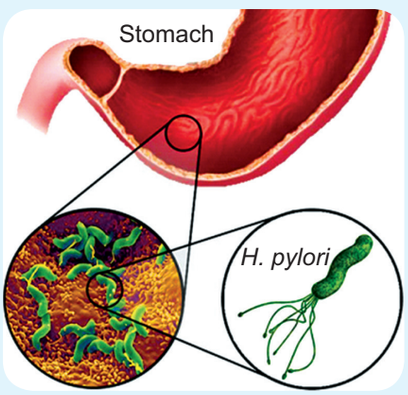
The stomach and gastrointestinal tract are not sterile and are colonized by the organisms that perform functions beneficial to the host, including the manufacture of essential vitamins. *Escherichia coli* found in the intestine help the body to produce vitamin K and *Bifodobacteria* can synthesizes vitamins such as vitamin B12, folate, and riboflavin. Humans cannot produce these vitamins. The normal flora changes according to the diet, age, cultural conditions and the use of antibiotics (Table 12.4).

12.4.2 Terms used in GIT Infections

Gastroenteritis: Inflammation of lining of stomach and intestine. It is a syndrome characterized by nausea, vomiting, diarrhea, abdominal discomfort.

DO YOU KNOW?

Stomach is acidic because of the presence of hydrochloric acid. So in this acidic condition organisms generally not survive except one bacterium *Helicobacter pylori.* This bacterium is the leading cause of stomach ulcers. This bacterium has maximum evidence of correlation with the development of stomach and intestinal cancer.



Diarrhea: Condition in which feces, are discharged from the bowels frequently and in a liquid form.

DO YOU KNOW?

Botulism is a special case of intoxication because, the ingestion of the preformed toxin affects the nervous system rather than GIT.

Infant Botulism is the infectious form of Botulism which results when spores of *Clostridium botulinum* swallowed colonise in the intestine. Botulism spores can be found in honey.

TN_GOVT_XI_Micro_Biology_CH12.indd 195 16-02-2019 10.05.45 AM

Table 12.4: Normal flora of human gastrointestinal tract

At human birth	Stomach and intestine are sterile
Breast fed babies	*Lactobacillus bifidus*
Bottled milk fed babies	Enteric bacteria, *Lactobacillus* bifidus, *Enterococci, Clostridium* sp
Small intestine	*Lactobacilli, Enterococcus faecalis, Escherichia coli*
Large intestine	Anaerobic bacteria, *Streptococci*, *Bacteroides, Bifidobacteriumbifidum*

Dysentery: Inflammatory disorder of the GIT associated with pus and blood in feces.

Gastritis: Inflammation of the stomach lining that results in swelling.

Enteritis: Inflammation of the intestinal mucosa

Colitis: Inflammation of the colon

Hepatitis: Inflammation of the liver

Enterocolitis: Inflamation involving the mucosa of both large and small intestine.

Peritonitis: Inflammation of peritoneum (it is the serous membrane that forms the lining of the abdominal cavity). Infections of digestive system are listed in Table 12.5.

Table 12.5: Diseases of the digestive system

Infection	Pathogen	Symptoms
Bacterial Diseases		
Staphylococcal food poisoning	*Staphylococcus aureus*	Nausea, vomiting, and diarrhea
Shigellosis (bacillary dysentery)	*Shigella sp-*	Tissue damage and dysentery
Salmonellosis	*salmonella enterica*	Nausea and diarrhea
Typhoid fever	*Salmonella typhi*	High fever, significant mortality
Cholera	*Vibrio Cholerae*	Diarrhea with large water loss
Yersinia gastroenteritis	*Yersinia enterocolitica*	Abdominal pain and diarrhea, usually mild; may be confused with appendicitis
Viral Diseases		
Mumps	*Mumps virus Paramyxoviridae*	Painful swelling of parotid glands
Viral gastroenteritis	*Rotavirus*	Vomiting, diarrhea for 1 week
Fungal Diseases		
Ergot poisoning	*Claviceps purpurea*	Restricted blood flow to limbs; hallucinogenic
Aflatoxin poisoning	*Aspergillus flavus*	Liver cirrhosis; liver cancer

TN_GOVT_XI_Micro_Biology_CH12.indd 196 16-02-2019 10.05.45 AM

12.5 Ocular Infections

A number of microorganisms cause infection when introduced into the mucosa of the eye. In general, bacterial eye infections can lead to inflammation, irritation, and discharge, but they vary in severity. Some are typically short-lived, and others are chronic and lead to permanent eye damage. Prevention requires limiting the exposure to contagious pathogens. When infections do occur, prompt treatment with antibiotics can often limit or prevent permanent damage.

The external surfaces of the eye viz. the conjunctiva and cornea are susceptible to infection. These are exposed to external world and are easily accessible to infective agents. Particularly the conjunctiva is susceptible because it is covered with eyelid that provides warm, moist and enclosed environment in which contaminating organisms can quickly establish a focus of infection. However, eyelid and tears protect the external surfaces of the eye, both mechanically and biologically.

Infection of Eyelid

Most common cause of eyelid infection is *Staphylococcus aureus*.

Infection involves lid margins and cause blepharitis.

When the eyelid glands or follicles are affected stye (sticky eye) is seen (Figure 12.14).

Conjunctivitis (inflammation of conjunctiva) Conjunctivitis or pink eye can be caused by many different kinds of viruses and bacteria.

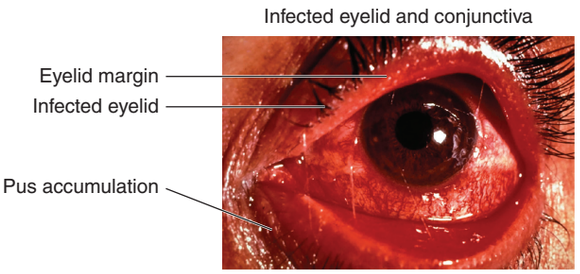


Figure 12.14: Infected eyelid and conjunctiva

Table 12.6: Bacterial infections of the eye

Disease	Pathogen	Signs and Symptoms	Transmission
Acute bacterial conjunctivitis	*Haemophilus influenza*	Inflammation of conjunctiva with purulent discharge	Exposure to secretions from infected individuals
Bacterial keratitis	*Staphylococcus epidermidis*, *Pseudomonas aeruginosa*	Redness and irritation of eye, blurred vision, sensitivity to light; progressive corneal scarring, which can lead to blindness	Exposure to pathogens on contaminated contact lenses
Neonatal conjunctivitis	*Chlamydia trachomatis*, *Neisseria gonorrhoeae*	Inflammation of conjunctiva, purulent discharge, scarring and perforation of cornea; may lead to blindness	Neonate exposed to pathogens in birth canal of mother with chlamydia or gonorrhea

TN_GOVT_XI_Micro_Biology_CH12.indd 197 16-02-2019 10.05.45 AM

Trachoma

Trachoma, or **granular conjunctivitis**, is a common cause of preventable blindness that is rare in the United States but widespread in developing countries, especially in Africa and Asia. The condition is caused by the same species that causes neonatal inclusion conjunctivitis in infants, *Chlamydia trachomatis*. *Chlamydia trachomatis* can be transmitted easily through fomites such as contaminated towels, bed linens, and clothing and also by direct contact with infected individuals. *Chlamydia trachomatis* can also spread by flies that transfer infected mucous containing *Chlamydia trachomatis* from one human to another. Infections of eye are listed in Table 12.6.

12.6 Urinary Tract Infections

The urinary system is composed of organs that regulate the chemical composition and the volume of the blood excrete mostly nitrogenous wastes products and water.

Infobits

Many of the bacteria which cause UTI's have developed resistance to antibiotics. Research has turned to probiotic (*Lactobacillus*) strain which stimulates immune function, lowers acidity levels in the urinary tract, and discourages the growth of UTI causing organisms.

The urinary system consists of two kidneys, two ureters, a single urinary bladder and a single urethra. Wastes are removed from the blood as it circulates through the kidneys (Figure 12.15).

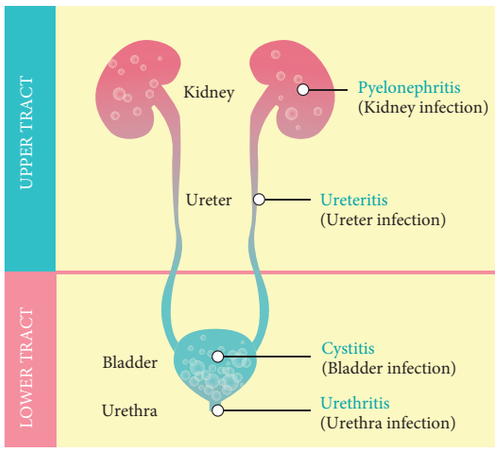


Figure 12.15: Structure of lower and upper urinary tract infection

Infections of the kidney, ureter and bladder constitute Urinary Tract Infections (UTI). When infection occur in the kidney and ureter it is called upper urinary tract infections and bladder downwards is called lower urinary tract infections. Urinary tract infection is common in females than males. The urinary system normally contains few microbes but it is subjected to opportunistic infections that can be quite troublesome. Almost all such infections are caused by bacteria although occasional infections by pathogens such as parasites, protozoa and fungi also occured. Microorganisms invloved in UTI are listed in Table 12.7.

Table 12.7: Microorganisms involved in UTI

Microorganisms	Examples
Bacteria (most common)	*Escherichi coli, Klebsiella, Enterobacter, Proteus*
Viruses	Adenovirus, Mumps
Fungi	*Trichomonas vaginalis, Schistosoma haematobium*
Parasites	*Candida*

TN_GOVT_XI_Micro_Biology_CH12.indd 198 16-02-2019 10.05.46 AM

12.6.1 Predisposing Factors for UTI

Urinary tract infection is common in females than in males. The urethra in females are shorter and wider and is less effective in preventing the bacteria entering the bladder. Sexual intercourse is a predisposing factor in females. High incidence is seen in pregnant women due to hormonal changes and impairment of urine flow due to pressure on urinary tract.



Obesity increases the risk of UTI's in men. A 2013 study examineed how obesity affected the chance of developing UTI and it was found that obese men were twice more likely to develop the UTI than obese women.

12.6.2 Urinary Tract Infection caused by *Escherichia coli*

Escherichia coli is the predominant cause of UTI.

It is a normal flora of the gut and can cause extra intestinal infections (UTI, Wound infection.) UTI (it can also be involved in other infections like wound infection peritonitis) UTI is common in (a) married women (b) elderly men with prostate enlargement.

Pathogenesis of cystitis in woman

Bladder infections can result from the downward migration of organisms from an infected kidney. But majority arise by ascent of pathogens from the rectum and vagina to the urethra meatus and bladder, leading to cystitis. If left untreated, the infection can further ascend to involve the kidneys (pyelonephritis) (Figure 12.16).

The rectum and vagina function as the reservoir of bacteria for sporadic infections

In men, the longer urethra is believed to protect against ascending infections.

When *Escherichia.coli* (and other Gram Negative rods) causes UTI, usually the number of organisms in freshly passed urine is more than 100,000 organisms/ml.

This is called "significant bacteriuria". Counts less than this is associated with contaminants from urethra or externalia. Infection of urinary tract are listed in Table 12.8.

Table 12.8: Microbial Diseases of the Urinary System

Disease	Pathogen	Symptoms
Bacterial Diseases of the Urinary system Cystitis (Urinary bladder infection)	*Escherichia* coli, *Staphylococcus saprophyticus*	Difficulty or pain in urination
Pyelonephritis (Kidney infection)	Primarily *Escherichia coli*	Fever; back or flank pain
Leptospirosis (Kidney infection)	*Leptospira interrogans*	Headaches, muscular aches, fever; kidney failure a possible complication

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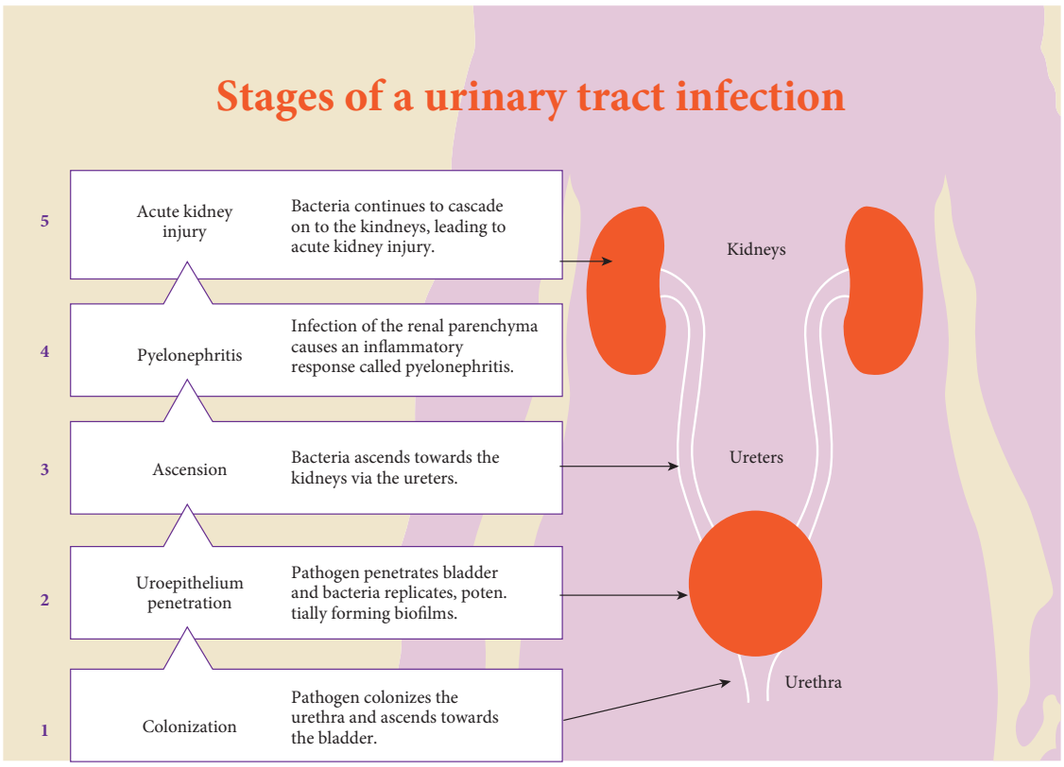


Figure 12.16: Various stages of a urinary tract infection

12.7 Reproductive Tract Infections

Reproductive tract infections are caused by organisms normally present in the reproductive or genital tract or introduced from the outside during sexual contact or medical procedures. It occur both in men and women. Based on mode of infection reproductive tract infections are classified into three types:

1. Sexually Transmitted Disease

It is caused through means of sexual contact. Examples: Chlamydia, Gonnorhea, Chancroid, and Acquired Immuno Deficiency Syndrome (AIDS).

2. Endogenous Infections

These are caused by the overgrowth of organisms normally present in the genital tract of healthy women. Example: Bacterial Vaginosis or Vulvo Vaginal Candidiasis.

3. Iatrogenic Infections

These infections are associated with improperly performed medical procedures such as unsafe abortion or poor delivery practices. The endogenous organisms in the vagina or sexually transmitted organisms in the cervix may be transferred during a transcervical procedure into the upper reproductive tract and cause serious infections of the uterus, fallopian tubes, and other pelvic organs.

In men reproductive tract infections transmitted by sexual contact are much more common than by endogenous or iatrogenic reproductive infections. In women reproductive infections spread through non sexual routes are usually more common.

TN_GOVT_XI_Micro_Biology_CH12.indd 200 16-02-2019 10.05.46 AM

12.7.1 Mode of Transmission

Reproductive tract infections are caused by pathogenic bacteria, parasite, virus. It is mainly caused by pathogens entering into the body through the mucous membranes during unprotected vaginal, oral, anal intercourse with an infected partner. In developing countries bacterial infections like Gonorrhoea, Chlamydia, Syphilis, Bacterial Vaginosis, Lymphogranuloma Venereum, Trichomoniasis, Chancroid, and viral infections caused by Human Papilloma Virus, Hepatitis B Virus, Herpes Simplex Virus, Human Immunodeficiency Virus are very common.

12.7.2 Normal Flora of Reproductive Tract

Mycobacterium smegmatis, a harmless commensal found in the smegma of the genitalia of both men and women. In nomal men aerobic and anaerobic bacteria, lactobacilli, alpha haemolytic *Streptococci, Chalmydia trachomatis* and *Ureaplasma urealyticum* may also be present.

The adult female genital tract has a very complex microflora. The character of the population changes with the variation of the menstrual cycle. Mostly the predominant bacteria are acid tolerant *Lactobacilli*. Glycogen is accumulated in the vaginal wall due to ovarian hormonal activity. The breakdown of glycogen by the lactic acid bacteria (Doderlien's bacillus) leads to the formation of acidic pH (4.4-4.5). This acidic nature prevents the vagina from bacterial vaginosis and yeast infections. However before puberty and after menopause there is no glycogen formation. The normal flora during this period contain normal skin microorganisms. The vaginal pH is mild alkaline. The normal vaginal flora often includes *Listeria,* anaerobic *Streptococci, Mycoplasma, Gardnerella vaginalis, Neisseria, Spirochetes, Candida, Staphylococcus epidermidis.*

12.7.3 Pathogenesis

After the entry of pathogenic organisms, with sufficient incubation time, symptoms are clearly manifested in the affected individual. The most common symptoms include unusual vaginal discharge, penile discharge, pelvic pain, itching, abnormal or heavy vaginal bleeding, rashes, warts, lesions, burning or pain during urination. However most of the infections are asymptomatic, which act as a effective control of reproductive tract infections. Diseases of reproductive system are listed in Table 12.9.

Infobits

Tamilnadu has AIDS testing centres at all district head quarters with more than 55 Anti Retroviral Therapy(ART) centres and 750 (ICTC)-Integrated (voluntary) and confidential counselling and testing centres under the national AIDS control programme at district level government hospitals and medical colleges across the state.

TN_GOVT_XI_Micro_Biology_CH12.indd 201 16-02-2019 10.05.46 AM

Table 12.9: Microbial diseases of the reproductive system

Disease	Pathogen	Symptoms
Bacterial Diseases		
Gonorrhea	*Neisseria gonorrhoeae*	Painful urination, discharge of pus in males, abnormal vaginal discharge in females
Nongonococcal urethritis (NGU)	*Chlamydia trachomatis* or other bacteria, including *Mycoplasma hominis* and *Urea plasma urealyticum*	Painful urination and watery discharge, Chronic abdominal pain in females
Syphilis	*Treponema pallidum*	Initial sore at site of infection, later skin rashes and mild fever; final stages may be severe lesions, damage to cardiovascular and nervous systems.
Lymphogranuloma venereum (LGV)	*Chlamydia trachomatis*	Swelling in lymph nodes in groin
Viral Diseases		
Genital Herpes	*Herpes simplex* virus type 2; HSV type 1	Painful vesicles in genital area
Genital warts	Human papilloma viruses	Warts in genital area
AIDS	Human Immunodeficiency virus (HIV)	loss of appetite, weight loss, persistent cough, attack on T cells (immunocompromise), easily prone to fungal and other bacterial pathogens as secondary opportunistic infections.
Fungal Diseases		
Candidiasis	*Candida albicans*	Severe vaginal itching, yeasty odor, yellow discharge
Protozoan Diseases		
Trichomoniasis	*Trichomonas vaginalis*	Vaginal itching, greenish yellow discharge

TN_GOVT_XI_Micro_Biology_CH12.indd 202 16-02-2019 10.05.46 AM

12.8 Infections of the Nervous System

Some of the most devastating infectious diseases are those that affect the nervous system, especially the brain and the spinal cord. Damage to these areas can lead to deafness, blindness, learning disabilities, paralysis and death. Microbial infections of CNS are infrequent but often have serious consequences. In pre antibiotic times, they were almost always fatal. An infection of CNS can be life threatening condition, especially for children with weakened immune system. These infections need quick diagnosis and immediate treatment by an infectious disease specialist. Bacteria, Fungi and viruses are the most common causes of CNS infections.

12.8.1 Structure of Nervous System

The human nervous system is organized into two divisions: The Central Nervous System (CNS) and Peripheral Nervous System (PNS). The Central Nervous System (CNS) consists of brain and spinal cord. It controls most functions of the body and mind. The peripheral nervous system (PNS) consists of all the nerves that branch off from the brain and spinal cord. These peripheral nerves are the lines of communication between the CNS, the various parts of the body and the external environment (Figure 12.19).

Brain and spinal cord are covered by three layers of membranes called meninges. These layers are the outermost dura mater, the middle arachnoid mater, and the innermost pia mater. Between the pia mater and arachnoid membranes is a space called the subarachnoid space, in which there is cerebrospinal fluid (CSF) circulating.

12.8.2 Barriers of CNS

Dyes such as Trypan blue injected into the systemic circulation stain virtually all tissues, with the exception of the brain and spinal cord. This blood brain

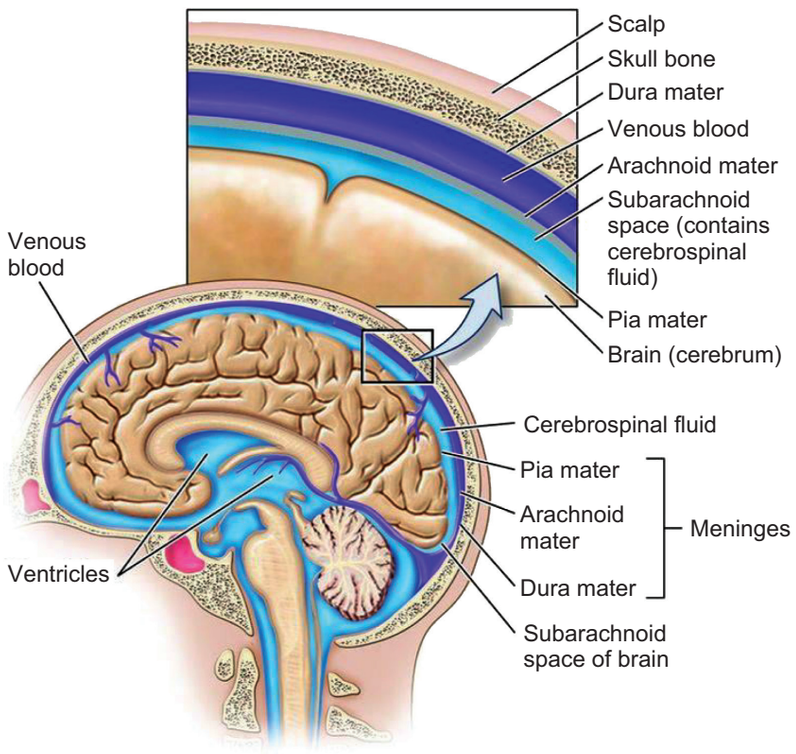


Figure 12.19: Structure of central nervous system

TN_GOVT_XI_Micro_Biology_CH12.indd 203 16-02-2019 10.05.46 AM

barrier excludes most macromolecules, microorganisms, immunocompetent cells and antibodies. Even pathogens that are circulating in the bloodstream usually cannot enter the brain and spinal cord because of blood brain barrier. Certain capillaries permit some substances to pass from the blood into the brain but restricts others. These capillaries are less permeable than others within the body and are therefore more selective in passing materials (Figure 12.20). The blood brain barrier (Figure 12.21) is due to the cellular configuration of cerebral capillaries, the choroid plexus and arachnoid cells. It acts as a natural barrier that prevents the invasion of microorganisms into the brain. If this is breached organisms enter the brain. The blood CSF barrier (Figure 12.22) (also calle brain CSF barrier) consists of endothelium with fenestrations, and tightly joined choroid plexus epithelial cells. It acts as a natural barrier that prevents the invasion of microorganisms into the meninges.

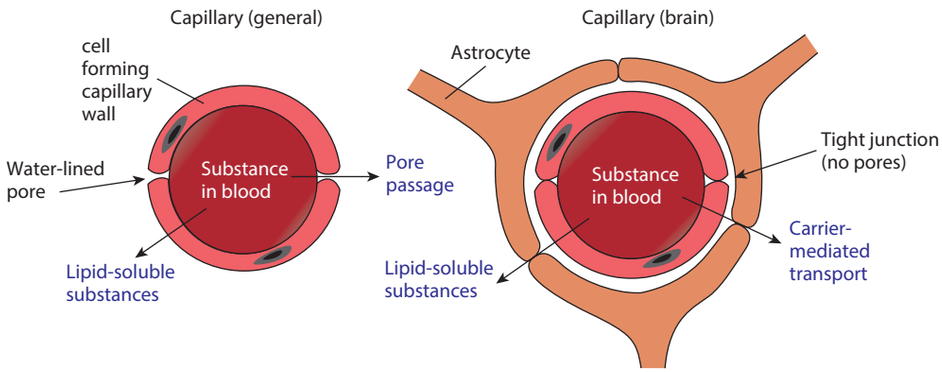


Figure 12.20: Capillaries of brain

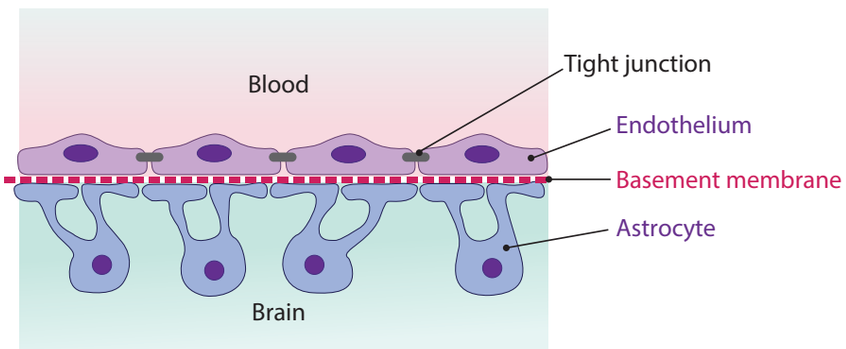


Figure 12.21: Blood brain barrier

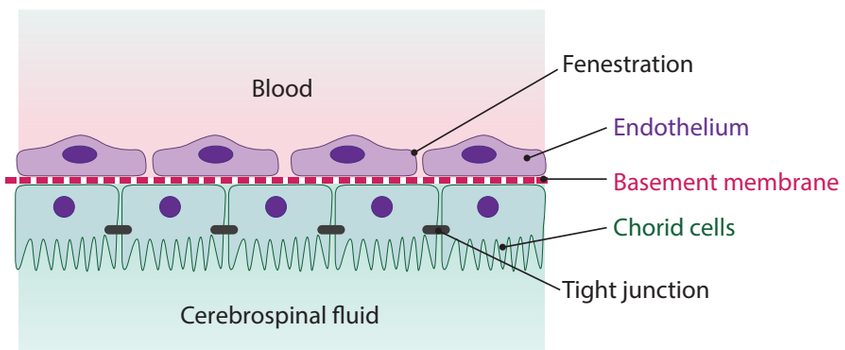


Figure 12.22: Blood CSF barrier

TN_GOVT_XI_Micro_Biology_CH12.indd 204 16-02-2019 10.05.46 AM

12.8.3 Routes through which microorganisms enter nervous system

- Skull or bone fractures
- Medical procedures
- Peripheral nerves
- Blood or lymph

12.8.4 Clinical Manifestations of Nervous System Infections

Some of the symptoms of nervous System infections are headache, fever, stiff neck, focal signs, seizures, confusion, weakness, hallucinations, stupor, coma, abnormal behavior and sleep disorder

12.8.5 Infections of Nervous System

- Meningitis is an inflammation of the meninges (membrane covering the brain). Meningitis is a diffuse infection caused by a variety of different agents.
- Encephalitis is defined as inflammation of the brain. Unlike an abscess, which is a localised area of bacterial or fungal growth, Encephalitis is usually due to viruses that produce more widespread intracellular infections.
- Brain abcess is a focus of purulent infection and is usually due to bacteria. Brain abscesses develop from either a contiguous focus of infection (such as the ears, the sinuses, or the teeth) or hematogenous spread from a distant focus (such as the lungs or heart, particularly with chronic purulent pulmonary disease, subacute bacterial endocarditis, or cyanotic congenital heart disease). In many cases the source is undetectable.

Etiological agents of Meningitis

This can be caused by a wide range of microorganisms and can be classified as pyogenic and non pyogenic meningitis. In pyogenic meningitis infiltration of pus cells (neutrophils) will be seen. In Non pyogenic or aseptic meningitis infiltration of lymphocytes may be seen. Diseases of nervous system are listed in Table 12.10.



Drugs cannot cross the blood brain barrier unless they are lipid soluble. Glucose and many amino acids are not lipid soluble, but they can cross the barrier through special transport systems. The lipid soluble antibiotic Chloramphenicol enters the brain readily. Penicillin is only slightly lipid soluble, but, if it is taken in very large doses, enough may cross the barrier to be effective. Inflammations of the brain tend to alter the blood brain barrier in such a way as to allow antibiotics to cross that would not be able to cross if there were no infection.

Antibodies found in the normal CNS are derived from the serum and are present at low levels compared to serum levels. There are a few phagocytic cells and complement is also largely excluded. CSF is especially vulnerable because it lacks many of the defenses found in the blood, such as phagocytic cells. It is not easy for the microorganisms to enter CNS but it hampers their clearance once it is penetrated.

TN_GOVT_XI_Micro_Biology_CH12.indd 205 16-02-2019 10.05.46 AM

Table 12.10: Microbial diseases of the Nervous system

Diseases	Pathogen	Portal of Entry	Method of Transmission
Bacterial Diseases			
Haemophilus influenzae meningitis	*Haemophilus. Influenzae*	Respiratory tract	Endogenous infection; aerosols
Meningococcal meningitis	*Neisseria meningitidis*	Respiratory tract	Aerosols
Pneumococcal meningitis	*Streptococcus pneumoniae*	Respiratory tract	Aerosols
Tetanus	*Clostridium tetani*	Skin	Puncture wound
Botulism	*Clostridium botulinum*	Mouth	Food borne intoxication
Viral Diseases			
Poliomyelitis	Poliovirus	Mouth	Ingesting contaminated water (fecal oral route)
Rabies	Lyssavirus, including rabies virus	Skin	Animal bite
Fungal Diseases			
Cryptococcosis	*Cryptococcus neoformans*	Respiratory route	Inhaling soil contaminate with spores
Protozoan Diseases			
African trypanosomiasis	*Trypanosoma brucei Rhodesiense, Trypanosoma brucei gambiense*	Skin	Tsetse fly

12.9 Systemic Infections

An infection that is in the bloodstream is called a systemic infection. Systemic diseases such as flu and typhoid affect the entire body. Bacteria can enter the circulatory and lymphatic systems through acute infections or breaches of the skin barrier or mucosa. Breaches may occur through fairly common occurrences, such as insect bites or small wounds. Even the act of tooth brushing, which can cause small ruptures in the gums may introduce bacteria in to the circulatory system. In most cases, the Bacteremia result from such common exposure is transient and remains below the threshold of detection. In severe cases, bacteremia can lead to septicemia with dangerous complication such as Toxemia sepsis and Septic shock. In these situations, it is often the immune response to the infection that result in the clinical signs and symptoms rather than microbes themselves.

Summary

In the branch of medical microbiology we discussed about prevention, diagnosis and

TN_GOVT_XI_Micro_Biology_CH12.indd 206 16-02-2019 10.05.46 AM

treatment of infectious diseases. Infections are acquired through contact, inhalation, ingestion, inoculation and congenital. Sources of infections are endogenous and exogenous in origin. Normal flora are organisms present in certain areas of the body. Infectious diseases may be generalised or localised. Based on the occurrence of infectious diseases the infection may be epidemic, endemic, or sporadic. There are various virulence factors which are responsibility for the pathogenicity.

Skin is the first line of defence against pathogen. Normal uninterrupted skin provides protection against "invasion by bacteria". Many exogenous and endogenous factors are responsible for wound infections. The mechanism of damage may be in the skin or some cases its spreads to the internal organs and CNS system.

Respiratory system of both lower and upper is the major path for entry of pathogens. The infections of upper respiratory tract are sinusitis, pharyngitis, laryngitis and epiglottitis. The infection of lower respiratory tract are trachitis, tracheobronchitis, bronchitis, and alveolitis.

Gastrointestinal tract infections are infections of the digestive system. The food borne infection and food intoxication are the common cause of gastroenteritis. The gut flora and natural defence mechanism by defensins bacteriocins, globet cells, IgA antibodies protect the individual against pathogenic infection. Diarrhea, dysentery, vomitting are the common symptoms of GIT. Oral rehydration therapy, proper hygiene to be manifested to reduce the risk of gastroenteritis.

The external structure and parts of the eye are easily susceptible to infections. The eyelids, tears, lysozyme, IgA are the natural defence against infections. Conjunctivitis and Trachoma are the common eye diseases. Proper diagnosis and treatment should be suggested.

Uninary tract infections are more common in females than in males. There are many predisposing factors making female prone to the infections. The predominant causative agents in urinary tract infection is *Escherichia.coli*. The number of organisms in freshly passed urine is more than 100,000 organisms/ml. It is called significant bacteriuria.

The infections spread through reproductive tract by direct contact is called sexually transmitted disease. Mostly these infections are asymptomatic in women.

Nervous system infection affect brain and spinal cord. They are of two types meningitis and encephalatis. An infection that is in the blood stream is called systemic infections. Systemic diseases like flu and typhoid affect the entire body.

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Modes of Infection



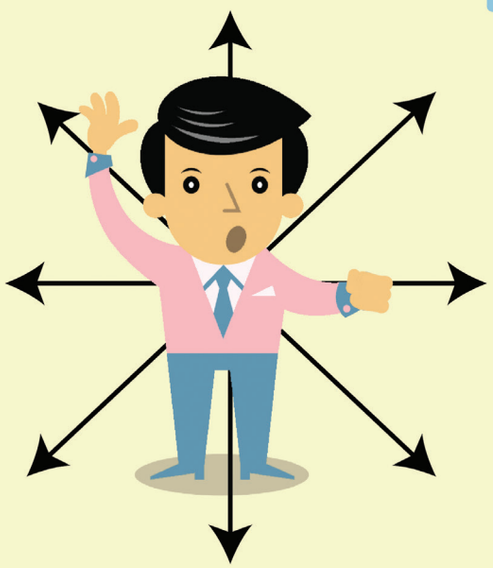
Inoculation



Indirect contact



Infected Needle





Direct contact



Dog bite



Food and Drinks



Congenital



Sneezing

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ICT CORNER

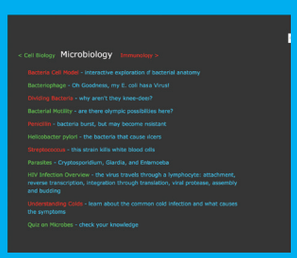
Respiratory Tract Infections

Know the myths of cold

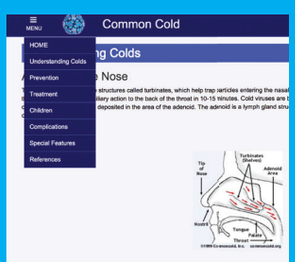


STEPS:

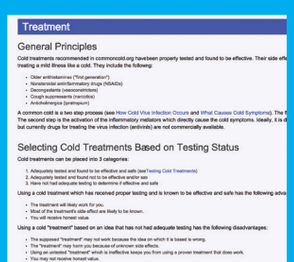
- Use the link or scan the QR code given below. "Cells Alive" home page will open. You can select any topic you wish. For example click "understanding colds"
- "Understanding Colds" page will open. You can go through anatomy of the nose, CAT scan view etc..
- At the top left of the page click on "Menu" and select "Treatments" and analyze.
- Also select "Special features" and go through the topic. Also you can select how penicillin kills bacteria in the "Cells Alive" page, and know the action of penicillin against bacteria.



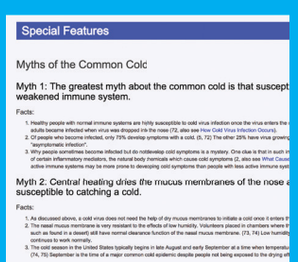
Step1



Step2



Step3



Step4

URL:

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

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Evaluation

Multiple choice questions

1. Syphilis is _____ disease
   a. Sexually transmitted disease
   b. Respiratory tract disease
   c. Gastro tract disease
   d. Urinary tract disease
2. _____ is the person who harbours the pathogenic microorganisms and suffers from till effect because of it?
   a. Carrier
   b. Healthy carrier
   c. Patient
   d. All the above
3. Circulation of bacteria in the blood is known as _____
   a. Septicimia
   b. Pyemia
   c. Bacterimia
   d. None of the above
4. From the skull down to the brain, select the arrangement of layers of meninges from the following:
   a. Dura mater/Arachnoid mater/Pia mater *
   b. Arachnoid mater/Dura mater/Pia mater
   c. Pia mater/Arachnoid mater/Dura mater
   d. Dura mater/Pia mater/Arachnoid mater
5. Cerebrospinal fluid (CSF) is present in which of the following?
   a. Perivascular spaces
   b. Sub arachnoid space *
   c. Between skull and dura mater
   d. Sub dural space
6. _____ antibody gives first line defense against respiratory tract infections.
   a. IgM
   b. IgA
   c. IgD
   d. IgE
7. The nose is lined with _____ membrane.
   a. Mucous
   b. Epithelial
   c. Secretion
   d. None of these
8. _____ nature of stomach act as a natural defense mechanism.
   a. Acidic
   b. Neutral
   c. Alkaline
   d. None of the above
9. Traveller"s diarrhea is caused by
   a. *Escherichia coli*
   b. *Staphylococcus aureus*
   c. *Vibrio cholerae*
   d. All the above
10. _____ is the predominant cause of UTI?
    a. *Staphylocous aureus*
    b. *Escherichia coli*
    c. *Salmonella*
    d. *Streptococcus pyogenes*
11. _____ fungi involved in urinary tract infection?
    a. *Klebsiella*
    b. *Candida sp*
    c. *Penicillium*
    d. *Escherichia coli*

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12. During the breakdown of glycogen by lactobacilli in the vagina, makes vaginal pH as _____.
    a. Acidic
    b. Neutral
    c. Alkaline
    d. None of the above

Answer the following

1. Define congenital infection?
2. What is meant by nosocomial infection?
3. Define the term bacteremia, septicemia pyremia?
4. Explain mode of transfer of infection?
5. Define a wound.
6. What are the causes of wound?
7. Name two types of CNS infections.
8. Give the names of the etiologic agents of wound infection.
9. Describe microbial disease of upper respiratory tract infection?
10. State the difference between dysentery and diarrhoea?
11. What is the difference between food borne infection and intoxication?
12. Give the normal flora of the gastrointestinal tract of humans?
13. What is called significant bacteriuria?
14. Explain the predisposing factors for urinary tract infection?
15. Define iatrogenic infection.
16. Explain the role of lactobacilli in the prevention of bacterial vaginosis.
17. Give detailed study of various bacterial, fungal and viral infectious diseases of reproductive tract infection.

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Student Activity (1)

1. Get information from your parents/neighbor about types of diseases one gets due to contamination. Example: If you drink contaminated water, you get diarrhoea.

No	After certain activity	Getting a disease	Preventive method to advocate
1	Contaminate drinking water	Diarrhoea	Don't drink or Boil, cool and drink
2			
3			
4			
5			

2. Give a list of organisms present as normal flora of the skin (include other than that is given in the text book).

3. Prepare model of respiratory tract with innovations.

   Prepare a list of URT infections with the etiologic agents and prevention.
   Observe a chronic smoker. He coughs very often. List out the reasons for his cough.
   collect information from nearby neighbors kids (10). How many of them are immunized DTP vaccinated?

No	Kid's name	DOB	Immunized on	Where corporation or pvt
1				
2				
3				

4. Student is asked to prepare a model of GIT with their innovations.
   See for example:
   What all the organisms that can be transmitted through the fly contaminated food. Give a list.
5. (1) Write an assignment on Madras eye (conjunctivitis due to viruses)
   (2) Write Dos and Don's when a dust particle comes into your eye.
6. 1) Draw the structure of urinary tract in a chart board using your innovation. Label the parts (make a poster presentation material with flow of urine from kidney to urethra).
7. Prepare a chart showing all sexually transmitted diseases.

   Collect the disease photographs from the net.
8. Write a chart showing differences between pyogenic and aseptic meningitis.

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Chapter 13

Immunology

BXSCF

Chapter Outline

13.1 Historical Background

13.2 Organs of the Immune System

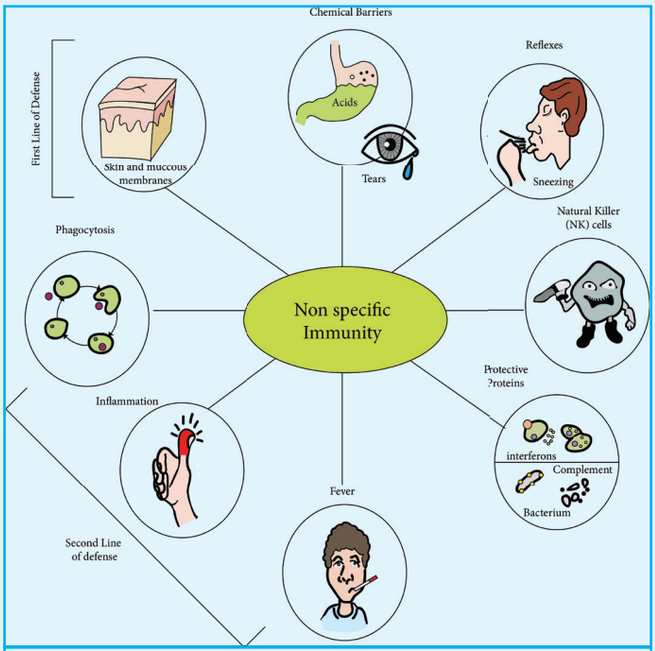
13.3 Cells of Immune System

13.4 Immunity

13.5 Antigens

13.6 Antibodies

13.7 Antigen– Antibody Reactions



Non specific immunity or Innate immunity has four types of defense barriers namely anatomical barriers, chemical barriers, phagocytic barriers and inflammatory barriers.

Learning Objectives

After studying this chapter the student will be able,

- *To gain knowledge on the history of immunology and Know the Nobel prize winners in immunology.*
- *To know the structure and functions of primary and secondary lymphoid organs.*
- *To know the cells of immune system and understand the role of granulocytes, mast cells, macrophages, dendritic cells and lymphocytes.*
- *To define immunity and Differentiate between innate immunity and acquired immunity.*
- *To understand the properties of antigen.*
- *To describe the basic structure and function of immunoglobulin (antibodies).*
- *To explain the mechanism of antigen-antibody interactions and their applications in clinical laboratory.*

13.1 Historical Background

Immunology is the study of immunity to diseases. Immunology began as a branch of Microbiology. Its origin is usually attributed to Edward Jenner who introduced variolation in 1796.

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The success of vaccination enabled the World Health Organization to announce in 1979 that small pox had been eradicated. Late in 19th century Robert Koch proved that infectious diseases are caused by microorganisms. The discoveries of Koch stimulated the extension of Jenner's strategy of vaccination to other diseases.

Pasteur used attenuated culture and called it vaccine (Latin vacca, cow) in honour of Edward Jenner. Table 13.1 and Figure 13.1 list and shows the scientist who contributed to the field of immunology.

Table 13.1: Scientists and their contributions to immunology

Year	Name of the Scientists	Contributions to immunology
1796	Edward Jenner	Discovered that cowpox or vaccinia, induced protection against human small pox
Discovery of humoral and cellular immunity		
1890	Von Behring and Kitasto (von Behring earned the Nobel Prize in medicine in 1901)	Gave the first insights into the mechanism of immunity
1930's	Kabat	Showed that gamma - globulin (now immunoglobulin) a fraction of serum exhibited the active component of immunity
1883	Elie Metchnikoff	He observed that certain white blood cells, which he termed **phagocytes**, were able to ingest microorganisms and other foreign material
1903	Sir Almoth Wright	Reported that antibodies could aid in the process of phagocytosis. Wright called these antibodies '**opsonins**'
1996	Claman, Chaperon and Triplett	Discovered the presence and cooperation of B cells and T cells

(Continued)

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Year	Name of the Scientists	Contributions to immunology
Specificity of immune response		
Around 1900	Jules Bordet	Demonstrated that nonpathogenic substances, such as red blood cells from other species, could also serve as antigens
	Karl Landsteiner	Showed that injecting an animal with almost any non-self, organic chemical could induce production of antibodies that would bind specifically to the chemical
Molecular immunology		
1959	Porter	Separated fragments of immunoglobulin
	Edelman	Heavy and light chains of antibodies were separated by him
1965	Putnam, Hirschmann and Craig	Discovered constant and variable regions of immunoglobulin
1979	Kung et al.	Described the first monoclonal antibody identifying a T cell subset
1982-83	Allison et al and Haskins et al.	Isolated T cell receptor
Immunogenetics and Genetic Engineering		
1936	Gorer	Discovered the major histocompatibility antigens
1968	McDevitt and Tyan	Showed that immune response genes were linked to the genes of the major histocompatibility complex (MHC)
1974	Doherty and Zinkernagel	Reported that recognition of antigen by T cells was restricted by MHC molecules
1978	Tonegawa et al.	Demonstration of immunoglobulin gene rearrangement

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Infobits

Nobel Prizes for immunologic research

Year	Recipient	Country	Research
1908	Elie Metchnikoff Paul Ehrlich	Russia Germany	Role of phagocytosis (Metchnikoff) and antitoxins (Ehrlich) in immunity
1913	Charles Richet	France	Anaphylaxis
1919	Jules Bordet	Belgium	Complement-mediated bacteriolysis
1930	Karl Landsteiner	United States	Discovery of human blood groups
1972	Rodney R. Porter Gerald M. Edelman	Great Britain United States	Chemical structure of antibodies
1977	Rosalyn R. Yalow	United States	Development of radioimmunoassay
1980	George Snell Jean Dausset Baruj Benacerraf	United States France United States	Major histocompatibility complex
1984	Cesar Milstein George E. Kohler	Britain Germany	Technological advances in the development of monoclonal antibodies
1991	E. Donnall Thomas Joseph Murray	United States United States	Transplantation immunology
2002	Sydney Brenner H. Robert Horvitz J. E. Sulston	South Africa United States Great Britain	Genetic regulation of organ development and cell death (apoptosis)
2008	Harald zurHausen Françoise Barré-Sinoussi Luc Montagnier	Germany France France	Role of HPV in causing cervical cancer (Hausen) and the discovery of HIV (Barré-Sinoussi and Montagnier)
2011	Jules Hoff man Bruce Beutler Ralph Steinman	France United States United States	Discovery of activating principles of innate immunity (Hoff man and Beutler) and role of dendritic cells in adaptive immunity (Steinman)

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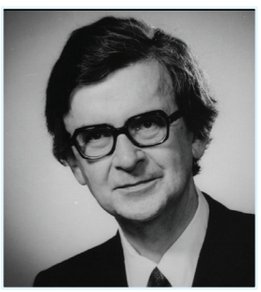

Elie Metchnikoff


karl Landsteiner


Emil von Behring


Paul Ehrlich


Robert Koch


Niels K.Jerne


Jules J.B.V.Bordet


Max Theiler


Rodney R.Porter

Figure 13.1: Notable Scientists who contributed to the development of Immunology

13.2 Organs of the Immune System

The immune system consists of structurally varied organs that are distributed throughout the body. Based on function, the organs can be divided into primary and secondary lymphoid organs (Figure 13.2). The primary lymphoid organs are responsible for providing the appropriate microenvironments for the development and maturation of antigen sensitive B and T cells. The thymus is the primary lymphoid organ for development of T cells and the bone marrow is the primary lymphoid organ for development of B cells. The secondary lymphoid organs serve as sites where lymphocytes interact with antigen and undergo proliferation and differentiation into antigen specific effector cells. The spleen, lymph nodes and mucosal associated lymphoid tissues (MALT) are secondary lymphoid organs. These are discussed in more detail below.

13.2.1 Primary Lymphoid Organs

a. Thymus

The thymus is a highly organized lymphoid organ located above the heart. The thymus consists of two lobes. Each

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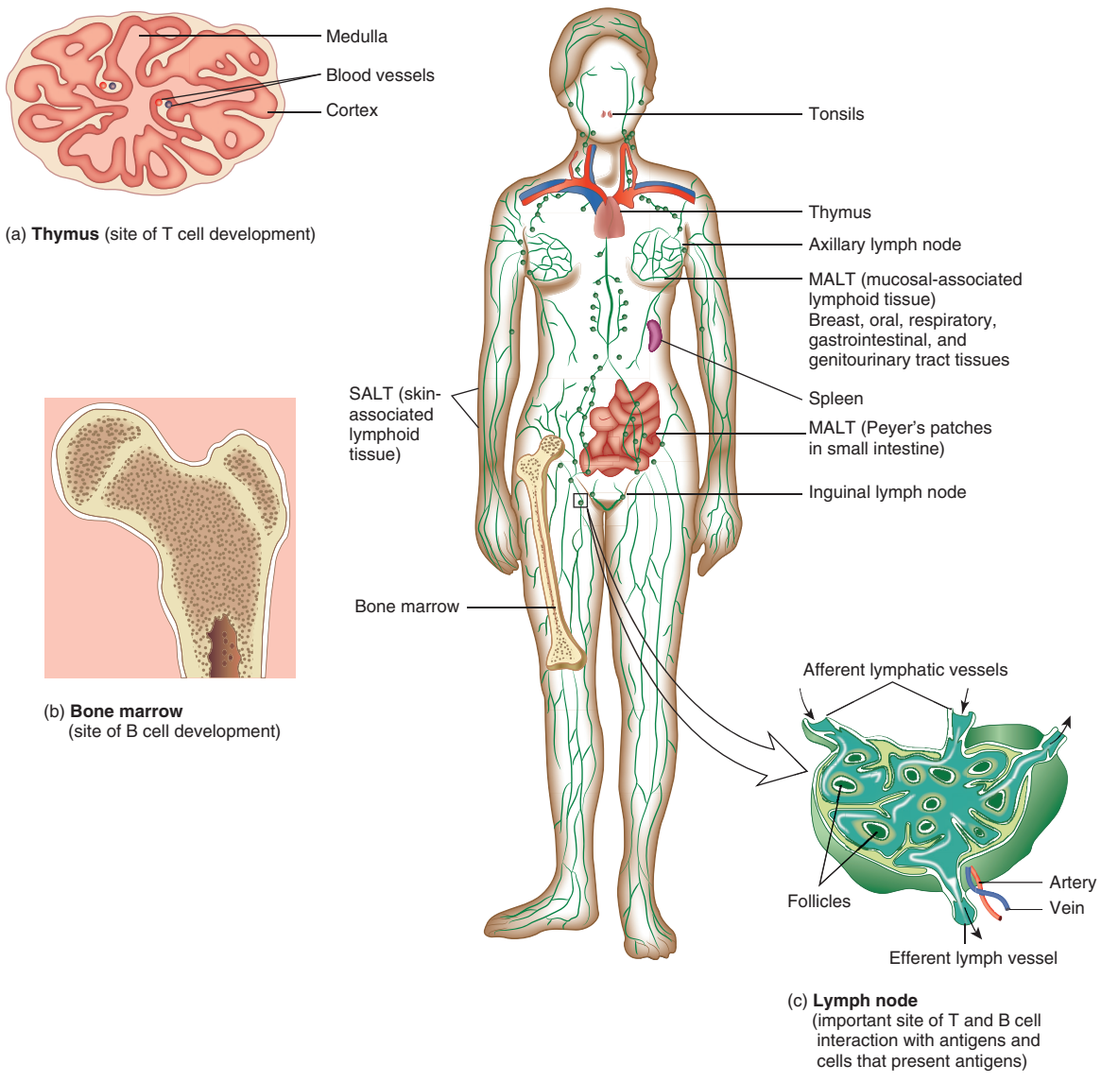


Figure 13.2: The distribution of Lymphoid tissues in the body

lobe is surrounded by a capsule and is divided into several lobules by strands of connective tissue called trabeculae. Each lobule contains an outer cortex and an inner medulla. The cortex contains many dividing immature lymphocytes. The medulla consists of reticular and epithelial cells with fewer lymphocytes and isolated Hassall's corpuscles (Figure 13.3). The primary function of the thymus is the production of mature T cells. Precursor cells from the bone marrow migrate into the outer cortex where they proliferate. As they mature, about 98% die. This is due to a process known as thymic selection in which T cells that recognize host (self) antigens are destroyed. The remaining 2% move into the medulla of the thymus, become mature T cells and subsequently enter the blood stream. These T cells recognize non host (non self) antigens.

b. Bone marrow

In mammals, the bone marrow (Figure 13.4) is the site of B cell maturation. Stromal cells within the bone marrow interact directly with the B cells and secrete various cytokines that are required for B cell development. Like

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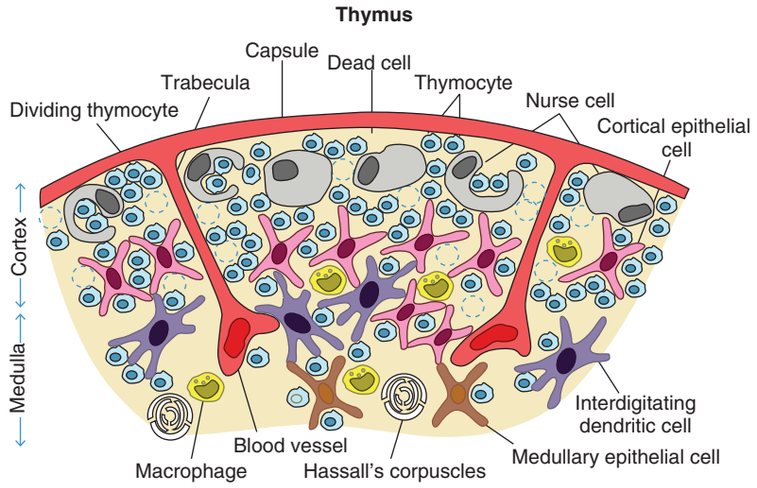


Figure 13.3: Diagrammatic Cross-section of a portion of the thymus

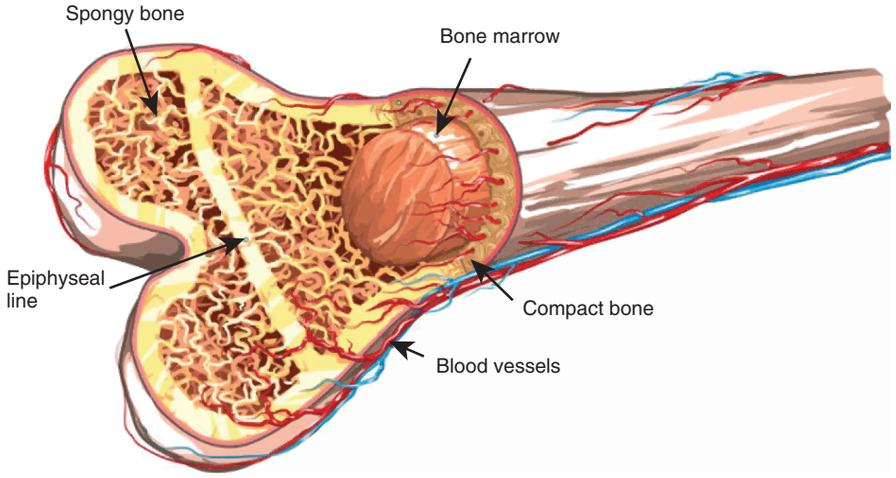


Figure 13.4: Structure of Bone marrow

thymic selection during T cell maturation, a selection process within the bone marrow eliminates non functioning B cells and those bearing self reactive antigen receptors. In birds, undifferentiated lymphocytes move from the bone marrow to the Bursa of Fabricius, where B cell mature; this is where B cells were first identified and how they came to be known as " B" (for bursa) cells.

13.2.2 Secondary Lymphoid Organs

a. Spleen

The spleen is the most highly organized secondary lymphoid organ. The spleen is a fist sized organ just behind the stomach. It collects and disposes of aged red blood cells. Its organization is shown schematically in Figure 13.5. The bulk of the spleen is composed of red pulp which is the site of red blood cell disposal. The spleen is not supplied by lymphatic vessels. The lymphocytes surround the arterioles entering the spleen, forming areas of white pulp. The inner region of white pulp is

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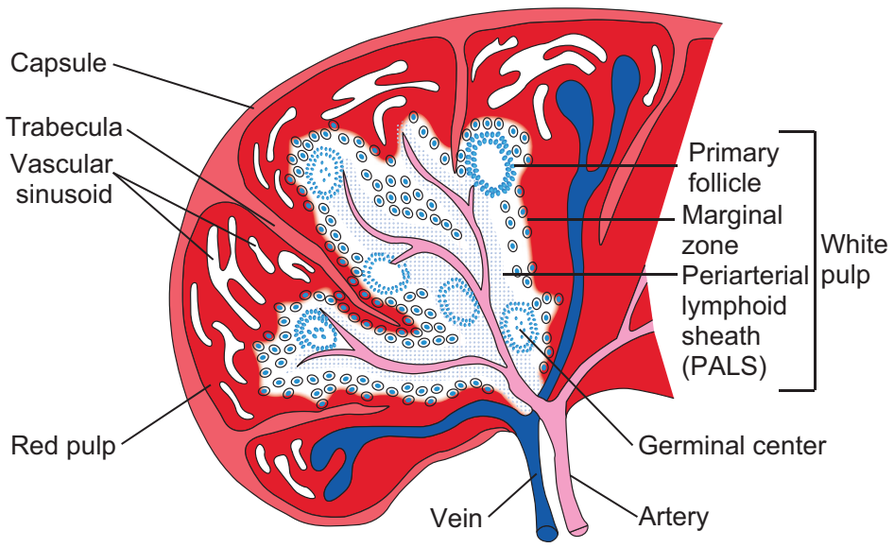


Figure 13.5: Structure of Spleen

divided into a Periarteriolar Lymphoid Sheath (PALS) containing mainly T cells. The spleen filters the blood and traps blood borne microorganisms and antigens. Once trapped by splenic macrophages or dendritic cells, the pathogen is phagocytosed, killed and digested.

b. Lymph nodes

The lymph nodes are encapsulated round structures located at the junction of major lymphatic vessels. Lymph node is morphologically divided into three regions: the cortex, the paracortex and the medulla (Figure 13.6). The outer most layer, the

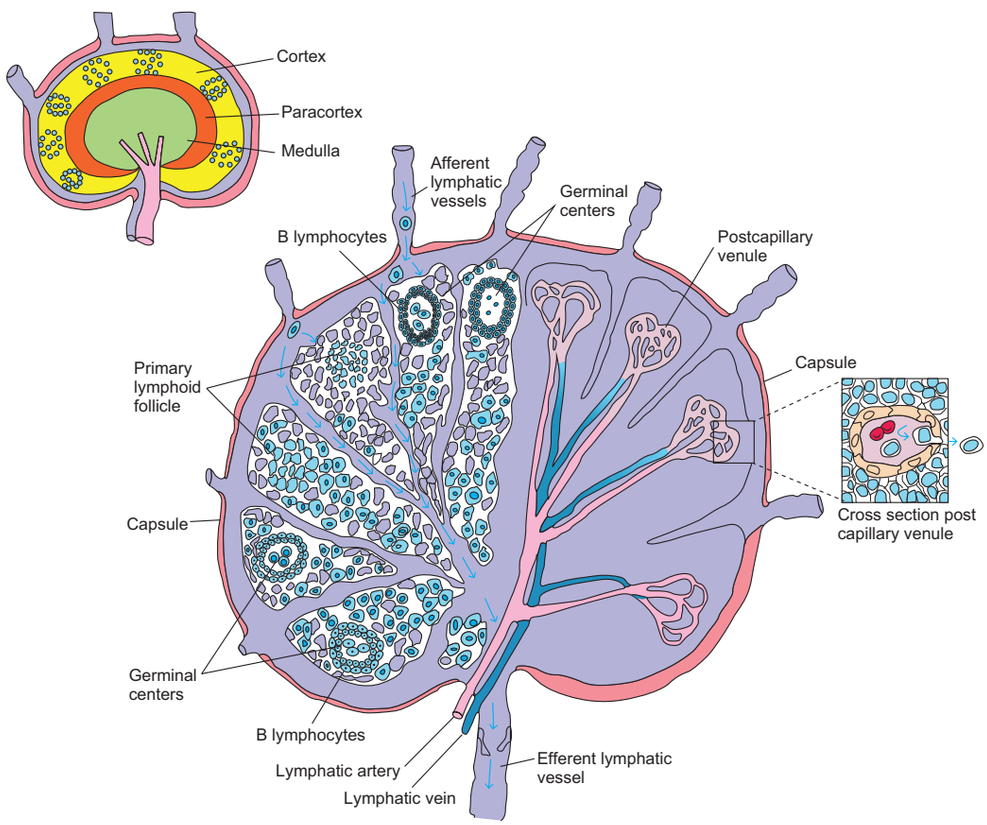


Figure 13.6: Structure of Lymph node

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cortex contains lymphocytes (mostly B cells), macrophages and follicular dendritic cells arranged in primary follicles. After antigenic challenge, the primary follicles enlarge into secondary follicles, each containing a germinal centre. Beneath the cortex is the paracortex which is populated largely by T lymphocytes and also interdigitating dendritic cells thought to have migrated from tissues to the node. These interdigitating dentritic cells express high levels of class II MHC molecules, which are necessary for presenting antigen to T helper (TH) cells. Lymph nodes are specialized to trap antigen from regional tissue spaces. As antigen is carried into a lymph node by the lymph, it is trapped, processed and presented together with class II MHC molecules by interdigitating dendritic cells in the paracortex, resulting in the activation of TH cells. Activated TH cells release cytokines needed for B cell activation. Thus lymph nodes represent one environment where B cells differentiate into memory cells and antibody – secreting plasma cells.

HOTS

What happens when thymus is removed from the human body?

c. MALT and SALT

The specialized lymphoid tissue in mucus membranes is called mucosal associated lymphoid tissue (MALT). There are several

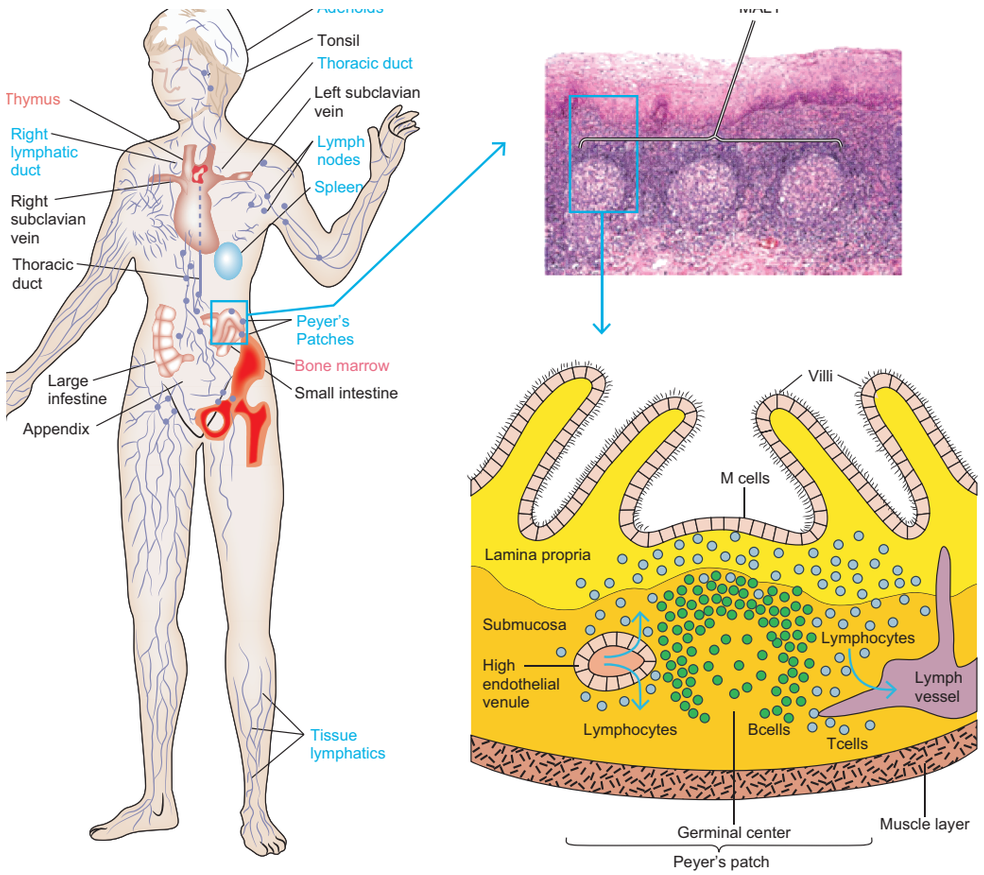


Figure 13.7: Malt Mucosa Associated Lymphoid Tissue (MALT)

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(a) The Peyer's patch is a representative of the extensive MALT system that is found in the intestine. (b) A stained tissue cross-section of Peyer's patch lymphoid nodules in the intestinal submucosa is schematically diagrammed in (c). The intestinal lamina propria contains loose clusters of lymphoid cells and diffuse follicles.

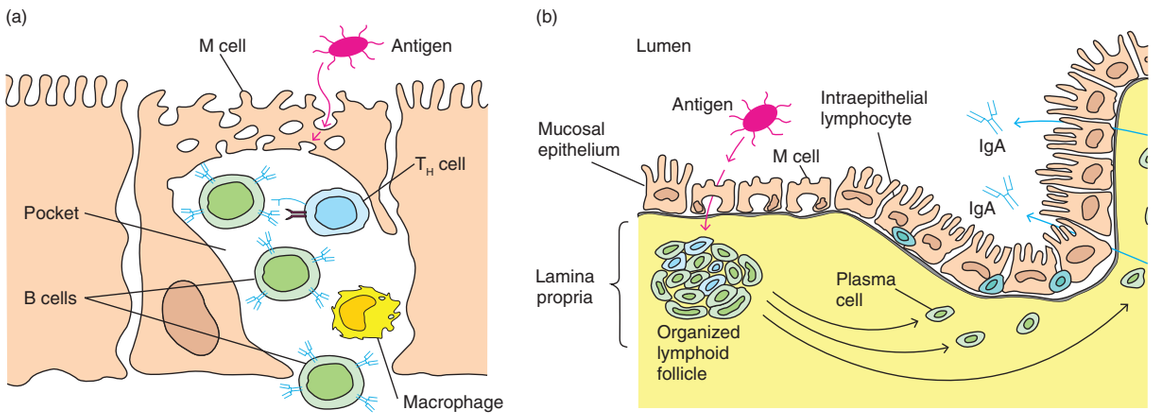


Figure 13.8: Structure of M cells and production of IgA:

(a) M cells, situated in mucous membranes, endocytose antigen from the lumen of the digestive, respiratory, and urogenital tracts. The antigen is transported across the cell and released into the large basolateral pocket. (b) Antigen transported across the epithelial layer by M cells at an inductive site activates B cells in the underlying lymphoid follicles. The activated B cells differentiate into IgA-producing plasma cells, which migrate along the lamina propria, the layer under the mucosa. The outer mucosal epithelial layer contains intraepithelial lymphocytes, of which many are T cells.

types of MALT. The system most studied is the gut associated lymphoid tissue (GALT). GALT include the tonsils, adenoids, and appendix and specialized structures called peyer's patches (Figure 13.7) in the small intestine, which collect antigen from the epithelial surfaces of the gastrointestinal tract. In peyer's patches, the antigen is collected by specialized epithelial cells called M cells (Figure 13.8). The lymphocytes form a follicle consisting of a large central dome of B lymphocytes surrounded by small numbers of T lymphocytes. Similar but more diffusely organized aggregates of lymphocytes protect the respiratory epithelium, where they are known as bronchial- associated lymphoid tissue (BALT).

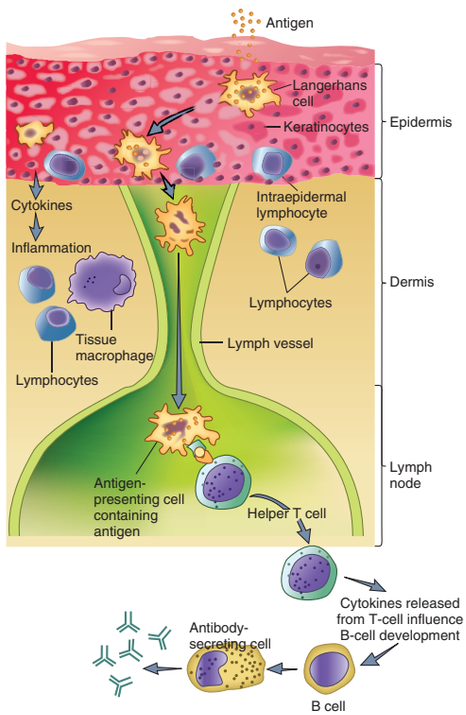


Figure 13.9: SALT

Despite the skin's defenses, at times pathogenic microorganisms gain access to the tissue under the skin surface. Here, they encounter a specialized set of cells called the skin associated lymphoid tissue (SALT) (Figure 13.9). The major function of SALT is to confine microbial invaders to the area immediately underlying the epidermis and to prevent them from gaining access to the blood stream. One type of SALT is the langerhans cell, a specialized myeloid cell that can phagocytose antigens.

13.3 Cells of the Immune System

All blood cells arise from a type of cell called the hematopoietic stem cell(HSC). Stem cells are cells that can differentiate into other cell types. They are self renewing and they maintain their population level by cell division. This chapter describes the formation of blood cells and the properties of the various cells of the immune system.

13.3.1 Hematopoiesis

Hematopoiesis is the formation and development of blood cells of all types. In humans, hematopoiesis begins in the yolk sac in the first weeks of embryonic development. As gestation continues, the site of hematopoiesis gradually shifts to the bone marrow such that it becomes the principle site at the time of birth.

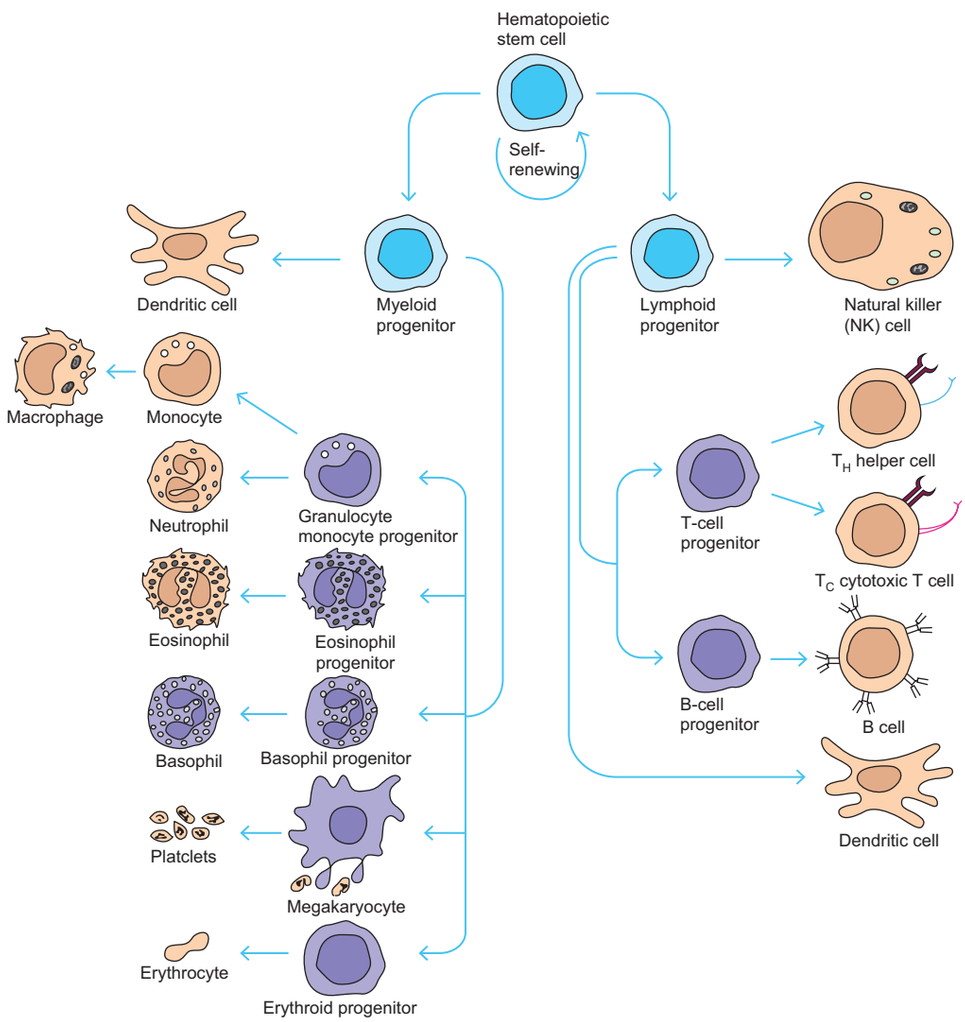


Figure 13.10: Hematopoiesis

As hematopoietic stem cells can give rise to all of the different types of blood cells, they are often known as pluripotent stem cells. (Figure 13.10)

The myeloid progenitor gives rise to erythrocytes, neutrophils, eosinophils, basophils, monocytes, mast cells and platelets. The common lymphoid progenitor gives rise to B lymphocytes, T lymphocytes and natural killer (NK) cells.

13.3.2 Types of Leukocytes

The cells responsible for both innate immunity and acquired immunity are the leukocytes (Greek leukos, white and kytos cell). The average adult has approximately 7400 leukocytes (white blood cells) per cubic millimeter of blood (Table 13.2). The average value shifts substantially during an immune response. In defending the host against pathogenic microorganisms, leukocytes cooperate with each other first to recognize the pathogen as an invader and then to destroy it. The different types of leukocytes are now briefly described.

a. Granulocytes

Granulocytes have irregularly shaped nuclei with two or five lobes. Their cytoplasm has granules that contain reactive substances that kill microorganisms and enhance inflammation. Three types of granulocytes exist: basophils, eosinophils, and neutrophils. Because of the many lobed (3-5) nuclei, neutrophils are also called polymorphonuclear neutrophils or PMNS (Figure 13.11).

HOTS

Heard about stem cell treatment!

Why do we need stem cells bank?

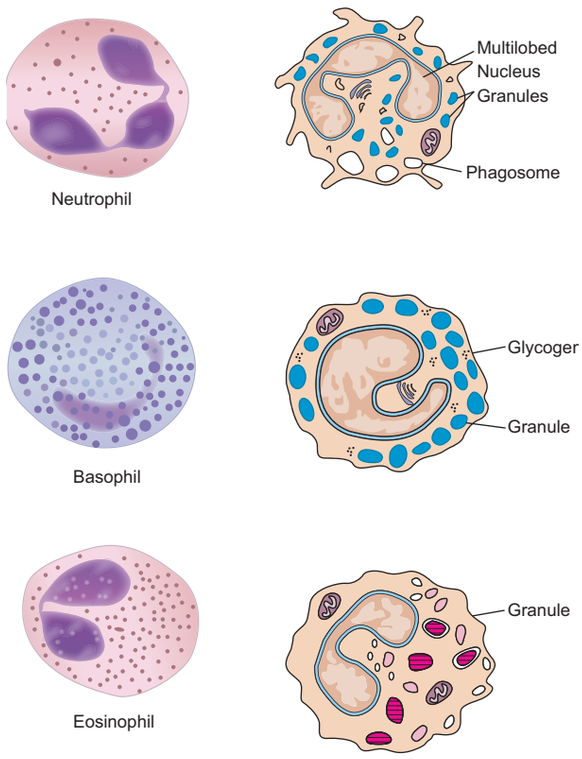


Figure 13.11: Structure of granulocytes

Table 13.2: Normal Adult Blood Count

Cell type	Cells/ mm3	% WBC
Red blood cells	50,00,000	–
Platelets	2,50,000	–
Leukocytes	7,400	100
Neutrophil	4320	60
Lymphocytes	2160	30
Monocytes	430	6
Eosinophils	215	3
Basophils	70	1

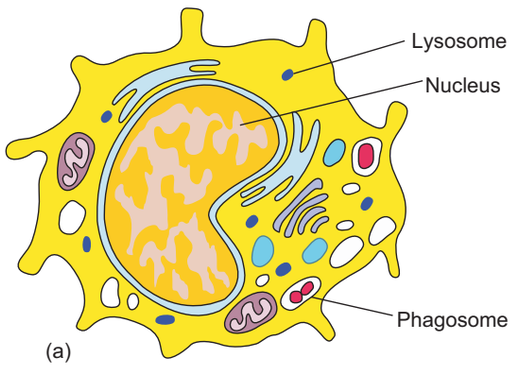
b. Mast cells

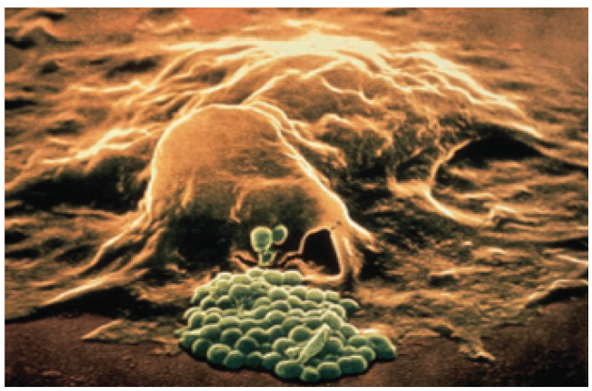
Mast cells are bone marrow derived cells that differentiate in the blood and connective tissue.

c. Monocytes and Macrophages

Monocytes are mononuclear leukocytes. They are produced in the bone marrow and enter the blood, circulate for about eight

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(b)

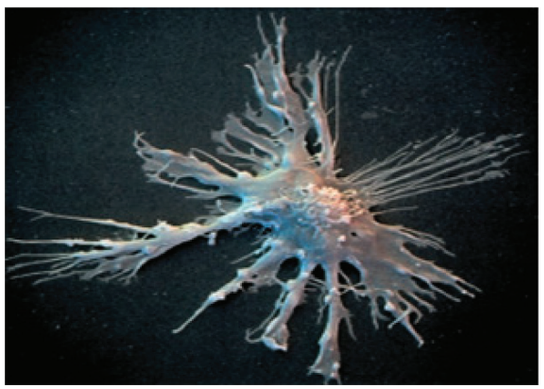

(c)

Figure 13.12: (a) Structure of Monocytes (b) Phagocytosis by a Macrophage (c) Dendritic Cell

hours, enlarge, migrate to the tissues and mature into macrophages or dendritic cells (Figure 13.12 a).

Macrophages are derived from monocytes and are classified as mononuclear phagocytic leukocytes. These microbial molecules are examples of pathogen associated molecular patterns (PAMPs) (Figure 13.12 c).

PAMPs enable macrophages to distinguish between potentially harmful microbes and other host molecules. After the pathogen is recognized, the macrophages', pattern recognition receptors (Example: Toll like receptors) bind the pathogen and phagocytose it. Macrophages also have receptors for antibodies and complement proteins. Both antibody and complement proteins can coat microorganisms and enhance their phagocytosis. This enhancement is termed opsonization. Macrophages spread throughout the body and take up residence in specific tissues. Macrophages serve different functions in different tissues and are named according to their tissue location.

- Alveolar macrophages in the lung
- Histiocytes in connective tissue
- Kupffer cells in the liver
- Mesangial cells in the kidney
- Microglial cells in the brain
- Osteoclasts in bone

d. Dendritic cells

Dendritic cells are not a single cell type. They are a heterogeneous group of cells so named because of their Dendron (neuron) like appendages (Figure 13.12d). They arise

TN_GOVT_XI_Micro_Biology_CH13.indd 225 16-02-2019 10.36.09 AM

from various hematopoietic cell lineages. Most dendritic cells are tissue bound, where they play an important role in bridging innate immunity and acquired immunity.

Dendritic cells can be classified by their location:

- Langerhans cells found in the skin and mucus membranes
- Interstitial dendritic cells which populate most organs (heart, lungs, liver, kidney, gastrointestinal tract)
- Interdigitating cells present in T cell areas of secondary lymphoid tissue and the thymic medulla.
- Circulating dendritic cells in the blood and lymph.

e. Lymphocytes

Lymphocytes are the major cells of the specific immunity. Lymphocytes can be divided into three populations: T cells, B cells, and NK (natural killer) cells. Clusters of differentiation are group of monoclonal antibodies that identify the same cell surface molecule. The cell surface molecule is designated CD (cluster of differentiation followed by a number (CD1, CD2).

i) B Lymphocytes

B lymphocytes mature within the bone marrow. When they leave bone marrow, each expresses a unique antigen binding receptor on its membrane. The B cell receptor is a membrane bound antibody molecule (Figure 13.13a). When a naive B cell, first encounters the antigen that matches its membrane bound antibody, the binding of the antigen to the antibody causes the cell to divide rapidly. Its progeny differentiate into memory B cells and effector B cells called plasma cells

Memory B cells have a longer life span than native cells. They express the same membrane bound antibody as their parent naive B cell. Plasma cells do not express membrane bound antibody. Plasma cells secrete large quantities of antibodies. Secreted antibodies are the major effector molecules of humoral immunity.

ii) T Lymphocytes

T lymphocytes also arise in the bone marrow. T cells then migrate to the thymus to mature. During its maturation within thymus, the T cells express a unique antigen binding molecule called the T cell receptor (Figure 13.13b) on its membrane. Unlike membrane bound antibodies on B cells, which can recognize antigen alone, T cell receptor can recognize only antigen that is bound to MHC molecules. There are two major types of MHC molecules. Class I MHC molecules are expressed by all nucleated cells. Class II MHC molecules are expressed only by antigen presenting cells. When a naive T cell encounters antigen combined with an MHC molecule on a cell the T cell proliferates and differentiates into memory T cell and various effector T cells.

There are two subpopulations of T cells: T helper (TH) and T cytotoxic (TC) cells. Although a third type of T cells called a T suppressor (T_S) cell, has been postulated, recent evidence suggests that it may not be distinct from the TH and TC subpopulations. T cells displaying CD4 function as TH cells whereas; those displaying CD8 function as TC cells (Figure 13.14).

After a TH cell recognizes and interacts with an antigen-MHC class II molecule complex, the cell is activated. It becomes an effector cell that secretes cytokines. The secreted cytokines activate B cells, TC cells,

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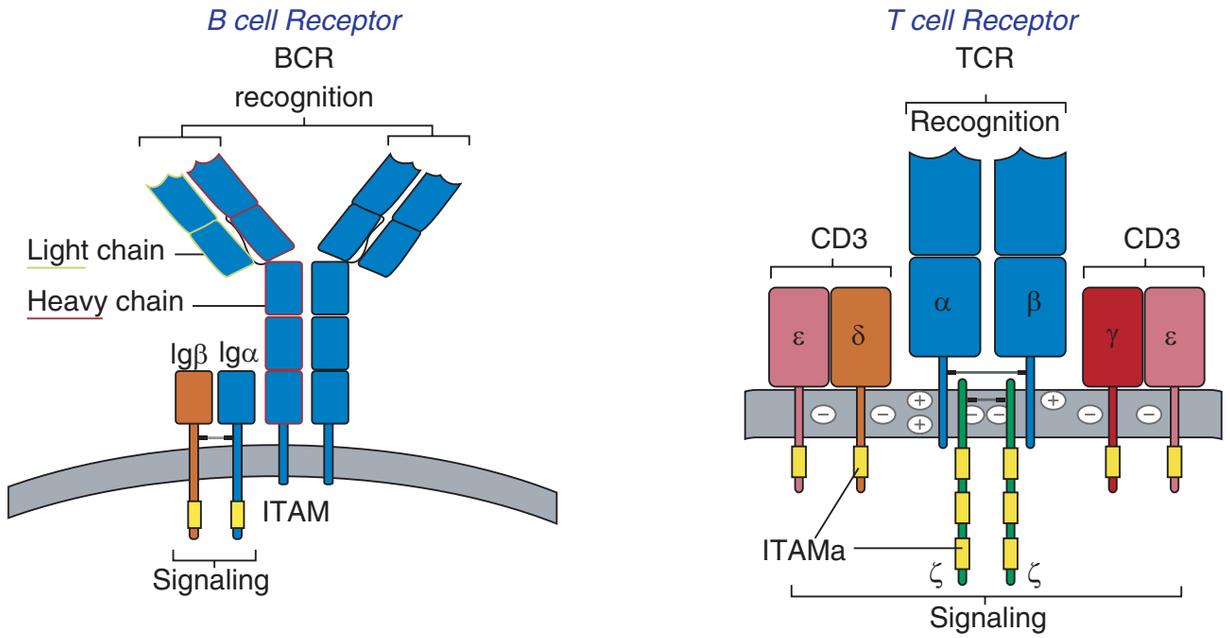


Figure 13.13: (a) B cell receptor. (b) T cell receptor

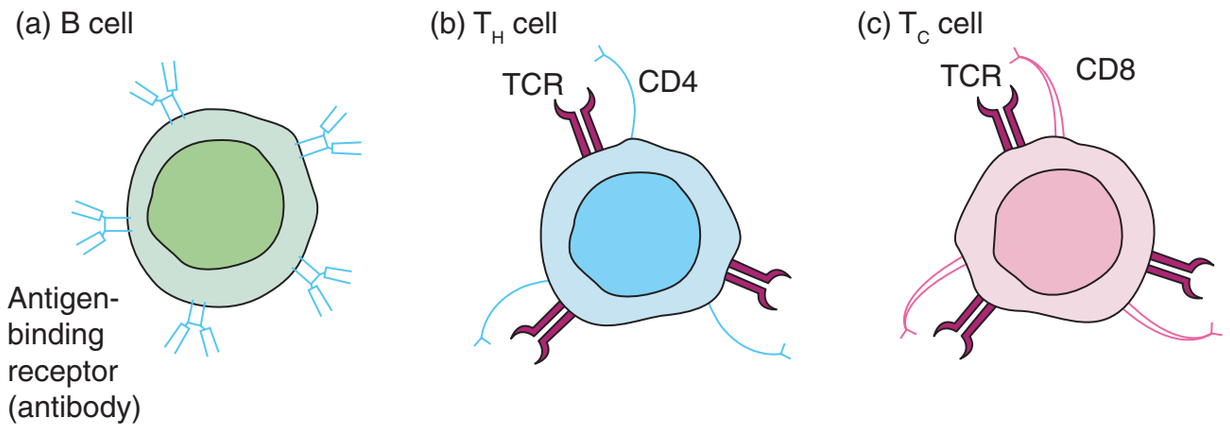


Figure 13.14: Distinctive membrane molecules on lymphocytes

macrophages and various other cells that participate in the immune response.

Under the influence of TH derived cytokines, a TC cell that recognizes an antigen-MHC class I molecule complex proliferates and differentiates into a cytotoxic T lymphocyte (CTL). Cells that display foreign antigen complexed with a class I MHC molecule are called altered self cells. CTL destroy virus infected cells and tumor cells.

iii) Natural killer (NK) Cells (Null cells)

NK cells are a small population of large, non phagocytic granular lymphocytes that play an important role in innate immunity. The major NK cell function is to destroy cancer cells and cells infected with microorganisms. They recognize their targets in one of two ways. They can bind to antibodies that coat infected or cancer cells. Thus the antibody bridges the two cell types. This process is called antibody dependent cell mediated cytotoxicity (ADCC) (Figure 13.15) The second way that NK cells recognize infected cells and cancer cells relies on the presence of specialized proteins on the surface of

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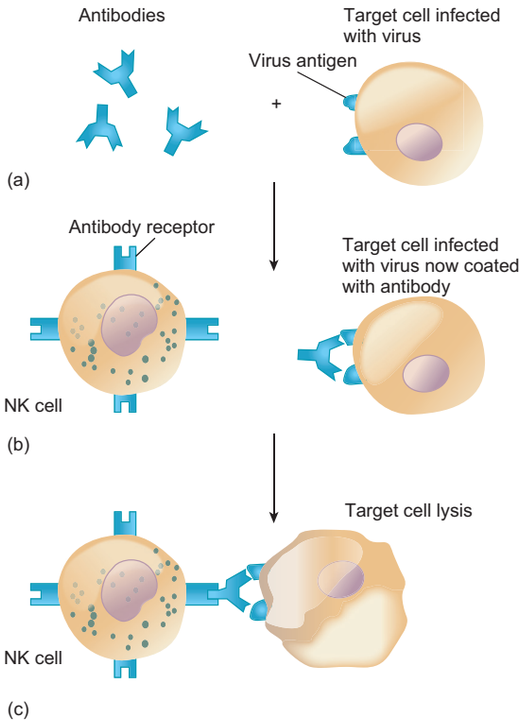


Figure 13.15: Antibody-Dependent Cell-Mediated Cytotoxicity

all nucleated host cells known as class II MHC molecules. If a hosts cell loses this MHC protein, as when some viruses or cancers overtake the cell, the NK cells kill it by releasing pore forming proteins and cytotoxic enzymes called granzymes (Figure 13.16).

13.4 Immunity

To establish an infection, an invading microorganism must first overcome many surface barriers, such as skin, degradative enzymes and mucus. These surface barriers have either direct antimicrobial activity or inhibit attachment of the microorganism to the host. Any microorganism that penetrates these barriers encounters two levels of resistance: nonspecific resistance mechanisms and the specific immune response.

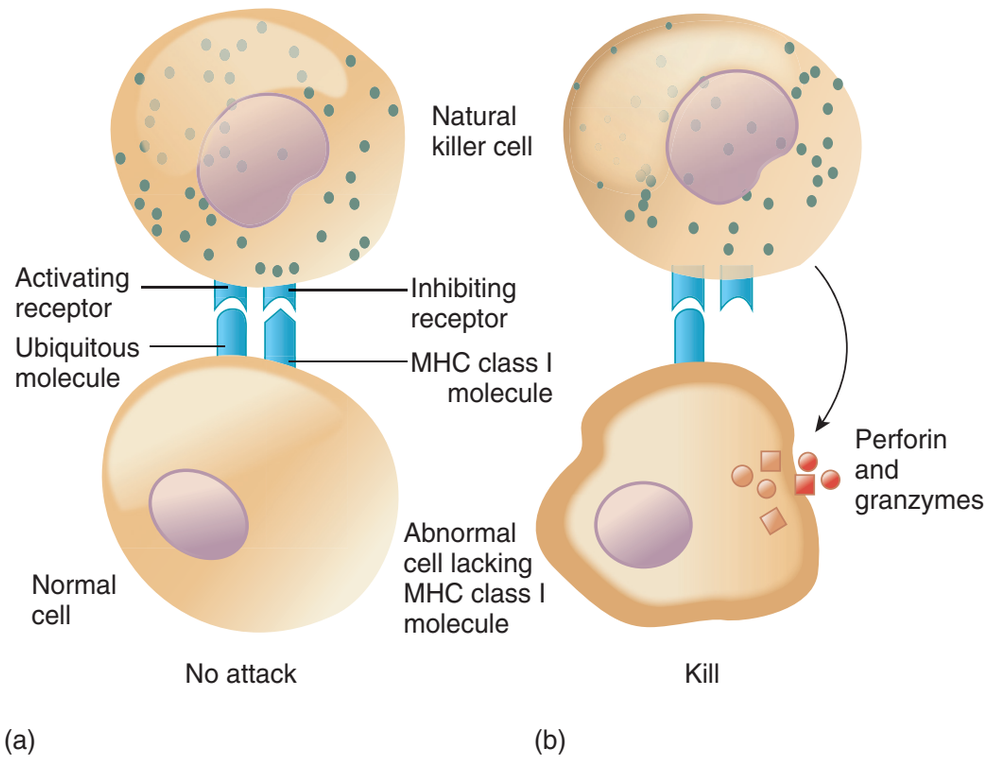


Figure 13.16: The system used by natural killer cells to recognize normal cells and abnormal cells that lack the Major Histocompatibility Complex Class I surface molecule

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13.4.1 Types of Immunity

The term immunity (Latin immunis, free of burden) refers to the general ability of a host to resist infection or disease. There are two interdependent components of the immune response to invading microorganisms and foreign material. They are non-specific immune response or innate immunity or natural immunity and specific immune response or acquired immunity or adaptive immunity.

I. Innate immunity

Innate immunity refers to those general defence mechanisms that are inherited as part of the innate structure and function of each animal (such as skin, mucus and lysozyme). Innate immunity is the first line of defence against any microorganism or foreign material encountered by the vertebrate host. Innate immunity defends against foreign invaders equally and lacks immunological memory.

II. Acquired immunity

Acquired immunity refers to the type of specific immunity that develops after exposure to a suitable antigen (Figure 13.17). The effectiveness of acquired immunity increases on repeated exposure to foreign

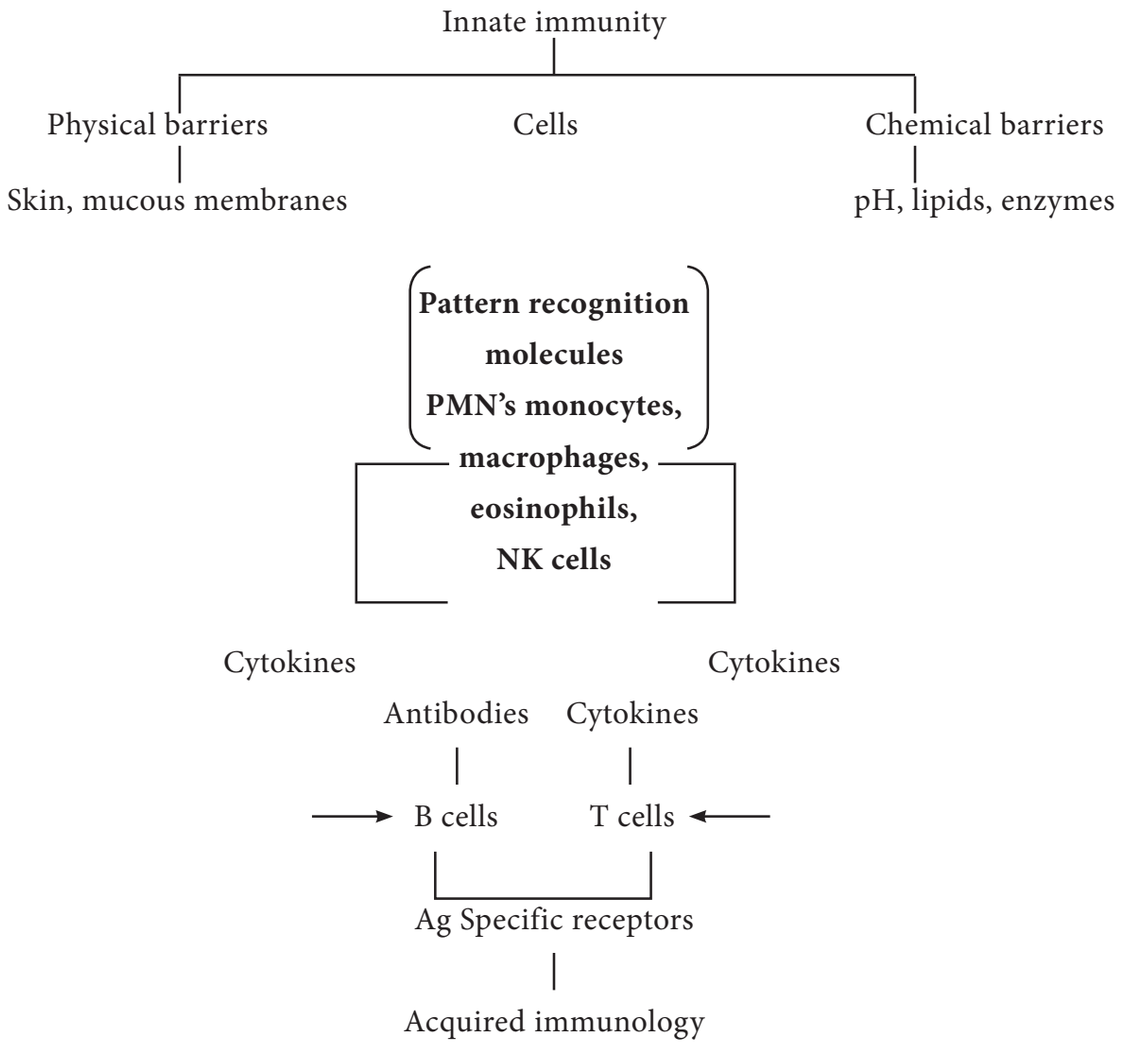


Figure 13.17: The interrelationship between innate and acquired immunity

TN_GOVT_XI_Micro_Biology_CH13.indd 229 16-02-2019 10.36.10 AM

agents such as viruses, bacteria or toxins. So acquired immunity has memory. The innate immunity and acquired immunity work together to eliminate pathogenic microorganisms and other foreign agents. Although innate systems predominate immediately upon initial exposure to foreign substances, multiple bridges occur between innate and acquired immune system components.

13.4.2 Mechanisms of Innate Immunity

A potential microbial pathogen invading a human host immediately confronts a vast array of nonspecific defence mechanisms. Many direct factors (nutrition, physiology, fever, age, genetics) and equally as many indirect factors (personal hygiene, socioeconomic status, living conditions) influence all host microbe relationships. In addition to these direct and indirect factors, a vertebrate host has the following four non specific defence mechanisms.

A. Physical barriers
B. Chemical mediators
C. Phagocytosis
D. Inflammation

A. Physical barriers

i) Skin

Intact skin contributes greatly to host resistance. It forms a very effective mechanical barrier to microbial invasion. Its outer layer consists of thick, closely packed cells called keratinocytes, The skin is slightly acidic (around pH 5-6) due to skin oil, secretion from sweat glands and organic acids produced by commensal *Staphylococci*. It also contains a high concentration of sodium chloride and is subject to periodic drying.

ii) Mucous membranes

The mucous membranes of the eye (conjunctiva), the respiratory, digestive and urogenital systems withstand microbial invasion. One antibacterial substance in these secretions is lysozyme, an enzyme that lyses bacteria. Mucous secretions possess the iron binding protein, lactoferrin. Lactoferrin sequesters iron from the plasma reducing the amount of iron available to invading microbial pathogens and prevents their ability to multiply. Mucous membranes produce lactoperoxidase, an enzyme that catalyzes the production of superoxide radicals, reactive oxygen intermediate that is toxic to many microorganisms.

iii) Respiratory system

Microbes smaller than 10µm pass through the nasal cavity and are trapped by the mucociliary blanket and the trapped microbes are transported by ciliary action that moves them away from lungs. Coughing and sneezing reflexes clear the respiratory system of microorganisms by expelling air forcefully from the lungs through the mouth and nose, respectively. Salivation also washes microorganisms from the mouth and nasopharyngeal areas into the stomach.

iv) Gastrointestinal tract

Most microorganisms that reach the stomach are killed by gastric juice. (pH 2-3). However, organisms embedded in food particles are protected from gastric juice and reach the small intestine. The mucous membranes of the intestinal tract contain paneth cells. These cells produce lysozyme and cryptins (toxic for bacteria).

v) Genitourinary tract

Under normal circumstances, the kidneys, ureters and urinary bladder of mammals

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are sterile. Urine within the urinary bladder is also sterile. In addition to removing microbes by flushing action, urine kills some bacteria due to its low pH and the presence of urea and other metabolic end products (uric acid, hippuric acid, indican, fatty acids, mucin, and enzymes). The acidic environment (pH 3-5) of the vagina is unfavorable to most microbes.

vi) Eye

The conjunctiva is specialized mucus secreting epithelial membrane that lines the interior surface of each eyelid and the exposed surface of the eye ball. It is kept moist by the continuous flushing action of tears. Tears contain large amounts of lysozyme, lactoferrin, and antibody and thus provide chemical as well as physical protection (Figure 13.18).

B. Chemical mediators

- **Antimicrobial peptides**

They are low molecular weight proteins that exhibit broad spectrum antimicrobial activity toward bacteria.

i) Cationic peptides

Cationic peptides are found in humans. There are three generic classes of cationic peptides that have the ability to damage bacterial plasma membrane. Classes of Cationic Peptides are Cathelicidins, Defensins and Histatin

ii) Bacteriocins

Bacteriocins are produced by gram negative and gram positive bacteria. For example, Escherichia coli synthesize bacteriocins called colicins. Colicins causes cell lysis.

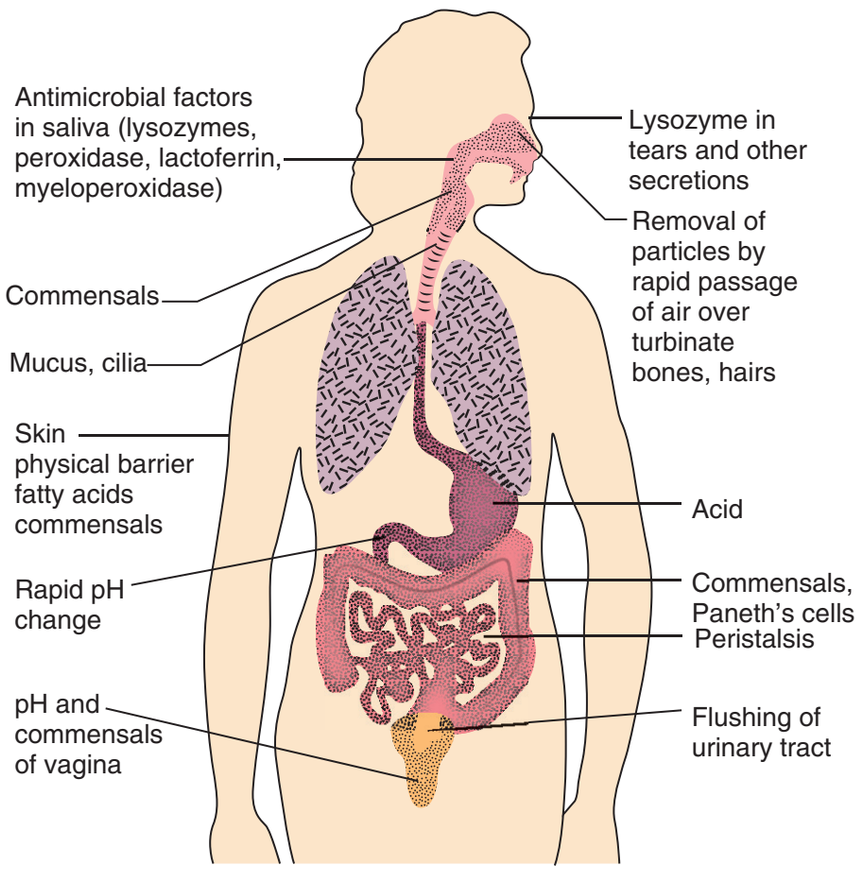


Figure 13.18: Physical Barriers

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- **Cytokines**

Cytokines are proteins made by cells that affect the behavior of other cells. When released from mononuclear phagocytes, they are called monokines. When released from T lymphocytes they are called lymphokines. When released from leukocytes they are called interleukins. Cytokines are required for regulation of both the nonspecific and specific immune responses. Interferons (IFNS) are a group of cytokines produced by virus infected cells. Several classes of interferons are recognized. IFN γ is synthesized by virus infected leukocytes, antigen stimulated T cells and natural killer cells. IFN α / β is derived from virus infected fibroblasts. Interferons prevent viral replication and assembly, thereby limiting viral infection.

Another group of noteworthy cytokines are endogenous pyrogens which elicit fever in the host. Examples of endogenous pyrogens include interleukin – 1, Interleukin – 6 and tissue necrosis factor. All are produced by host macrophages in response to pathogens.

- **Complement system**

The complement system is a part of the immune system, consists of a series of proteins that interact with one another in a highly regulated manner, in order to eliminate pathogens. Complements are soluble proteins and glycoproteins mostly produced by hepatocytes. More than 20 types of complements are present in serum found circulating normally in human body in inactive forms (called as zymogens or proenzymes). Complement activation is triggered by an antibody when it is bound to the antigen. It can also be triggered by some components of innate immunity. Thus the complement system works in both innate and acquired immunity.

Functions of complements

Some major functions of complements are:

- Opsonization and phagocytosis
- Cell lysis
- Chemotaxis
- Activation of mast cells and basophils and enhancement of inflammation
- Production of antibodies
- Immune clearance and inflammation by attracting macrophages and neutrophils.

C. Phagocytosis

i. Phagocytosis is the ingestion by phagocytic cells of invading foreign particles such as bacteria. After ingestion, the foreign particle is entrapped in a phagocytic vacuole (phagosome), which fuses with lysosomes forming the phagolysosome. The lysosomes release their powerful lytic enzymes which digest the particle. (Figure 13.19). Phagocytosis is conducted by blood monocytes, neutrophils and tissue macrophages. Phagocytosis may be enhanced by a variety of factors collectively referred to as opsonins which consist of antibodies and various serum components of complement.

ii. Phagocytic cells use two basic mechanisms for the recognition of microorganisms. Opsonin dependent and opsonin independent

iii. Phagocytes use pathogen recognition receptors to detect pathogen associated molecular patterns on microorganisms. Toll like receptors are a distinct class of pathogen recognition receptors.

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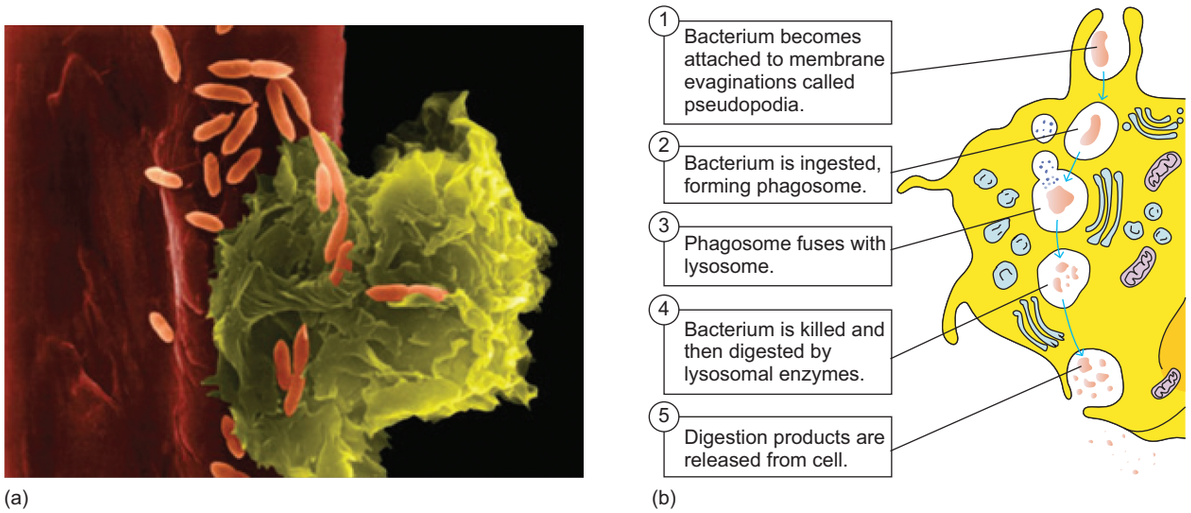


Figure 13.20: (a) Scanning electron micrograph of alveolar macrophage phagocytosis of E. coli bacteria on the outer surface of a blood vessel in the lung pleural cavity. (b) Steps in the phagocytosis of a bacterium.

D. Inflammation

Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as inflammatory response. Inflammation can either be acute or chronic. The gross features were described over 2000 years ago and are still known as the cardinal signs of inflammation: redness (rubor), warmth (calor), pain (dolor), swelling (tumor), and loss of function (functiolaesa)

The cardinal signs of inflammation reflect the three major events of an inflammatory response.

1. Vasodilation (an increase in the diameter of blood vessels) of nearby capillaries occurs as the vessels that carry blood away from the affected area constrict. This results in engorgement of the capillary network. The engorged capillaries are responsible for tissue redness (erythema) and an increase in temperature.
2. An increase in capillary permeability facilitates an influx of fluid and cells from the engorged capillaries into the tissue. The fluid that accumulates (exudate) has much higher protein content. Accumulation of exudate contributes to tissue swelling (edema)

Infobits

Reactive Nitrogen Species: Highly cytotoxic antimicrobial compounds formed by the combination of nitric oxide and superoxide anion within phagocytes such as neutrophils and macrophages.

Reactive Oxygen Species (ROS): Highly reactive compounds such as superoxide anion O_2, hydroxyl radicals (OH)(OH^-), hydrogen peroxide (H_2O_2), and hypochlorous acid (HClO) that are formed from oxygen under many conditions in cells and tissues, including microbe-activated innate responses of phagocytic cells; have anti-microbial activity.

TN_GOVT_XI_Micro_Biology_CH13.indd 233 16-02-2019 10.36.11 AM

3. Influx of phagocytes from the capillaries into the tissues is facilitated by increased capillary permeability. As phagocytic cells accumulate at the site and begin to phagocytoses bacteria, they release lytic enzymes, which can damage nearby healthy cells. The accumulation of dead cells, digested material and fluid forms substances called pus.

13.4.3 Acquired Immunity

Lower animal forms possess so called innate or non-specific immune mechanisms such as phagocytosis of bacteria by specialized cells. Higher animals have evolved an adaptive or acquired immune response. This acquired immune response provides a flexible, specific and more effective reaction to different infections.

- **Definition of Acquired (Adaptive) Immunity**

Acquired (adaptive)immunity refers to the type of specific immunity that a host develops after exposure to a suitable antigen.

- **Important features of acquired immunity**

This is the immunity one develops throughout life time. Adaptive or acquired immunity has four important features namely (1) Memory (2) Specificity (3) diversity and (4) discrimination between self and non self.

1) Memory

We rarely suffer twice from diseases such as measles, mumps, chicken pox, whooping cough and so on. The first contact with an infectious organism clearly imprints some memory so that the body is effectively prepared to repel any later invasion by that organism.

2) Specificity

The establishment of immunity by one organism does not provide protection against another unrelated organism. After an attack of measles we are immune to further infection but are susceptible to polio or mumps viruses. Thus the body can differentiate specifically between the two organisms.

3) Diversity

The immune system is able to generate an enormous diversity of molecules such as cellular receptors and soluble proteins, including antibodies that recognize trillions of different foreign substances.

4) Discrimination between self and nonself

The specific immune system almost responds selectively to non self and produces specific responses against the stimulus. This is possible because host cells express a unique protein on their surface, making them as residents of that host or as self. Thus the introduction of materials lacking that unique self marker results in their attack by the host.

13.4.4 Humoral and Cellular Immunity

Two branches or arms of specific immunity are recognized: humoral (antibody mediated) immunity and cellular (cell mediated) immunity (Figure 13.20).

Humoral (antibody mediated) immunity

The antigen specific arm of the humoral immunity consists of the B cells. Each B cell expresses a unique antigen binding receptor on its membrane. The B cell receptor (BCR) is membrane bound antibody molecule. When a naive B cell first encounters the antigen that matches its membrane bound

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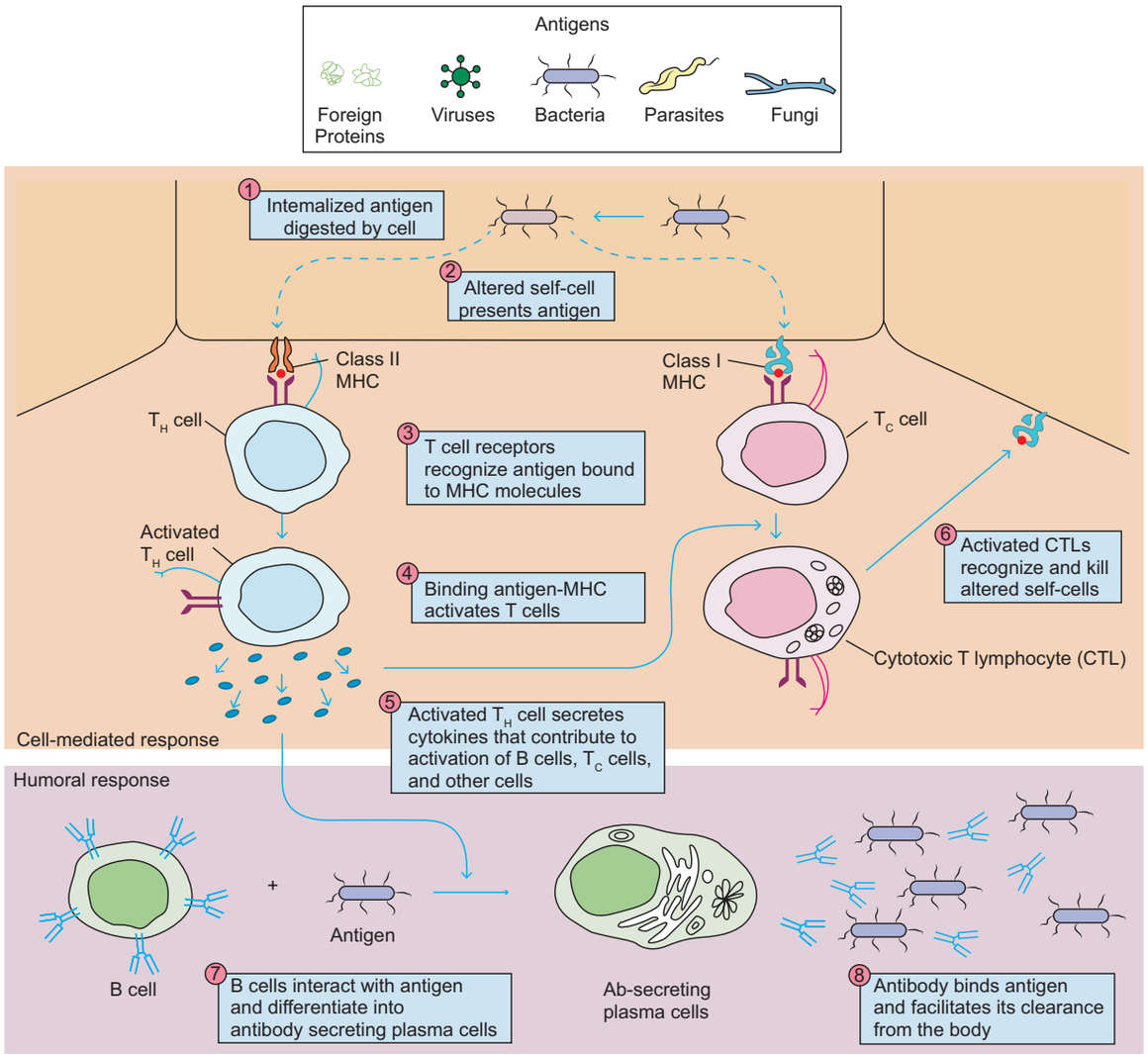


Figure 13.21: Overview of the humoral and cell-mediated branches of the immune system. In the humoral response, B cells interact with antigen and then differentiate into antibody-secreting plasma cells. The secreted antibody binds to the antigen and facilitates its clearance from the body. In the cell-mediated response, various subpopulations of T cells recognize antigen presented on self-cells. T_H cells respond to antigen by producing cytokines. T_C cells respond to antigen by developing into cytotoxic T lymphocytes (CTLs), which mediate killing of altered self-cells (Example: virus-infected cells).



Passive Immuno-therapy: Treatment of an infectious disease by administration of previously generated antibodies specific for the infectious pathogen.

antibody, the binding of the antigen to the antibody causes the cell to divide rapidly. Its progeny differentiate into memory B cells and antibody secreting plasma cells. A single plasma cell can secrete more than 2000 molecules of antibody per second. Circulating antibodies bind to

TN_GOVT_XI_Micro_Biology_CH13.indd 235 16-02-2019 10.36.11 AM

microorganisms, toxins and extracellular viruses, neutralizing them or tagging them for destruction by phagocytes and other mechanisms.

The cellular (cell mediated) immunity consists of the T cells. Each T cell expresses antigen receptors called T cell receptors (TCRS). Unlike membrane bound antibody on B cells, which can recognize antigen alone, T cell receptors can recognize only antigen that is bound to MHC molecules. There are two major types of MHC molecules. Class I MHC molecules are expressed by all nucleated cells. Class II MHC molecules are expressed only by antigen presenting cells such as dendritic cells, macrophages and B cells. When a naive T cell encounters antigen combined with an MHC molecule on a cell, the T cell proliferates and differentiates into memory T cells and various effector T cells (helper T cells, cytotoxic T cells and regulatory T cells). Specific kinds of T cells directly attack target cells infected with viruses or parasites, transplanted cells or organs and cancer cells. T cells can induce target cell suicide (apoptosis), lyse targets cells, or release chemicals (cytokines) that enhance specific immunity and non specific defences such as phagocytosis and inflammation.

13.4.5 Types of Specific Immunity

Specific immunity can be acquired by natural means actively through infection or passively through receipt of preformed antibodies as through colostrum. Specific immunity can be acquired by artificial means actively through immunization or passively through receipt of preformed antibodies as with antisera.

13.5 Antigens

Substances capable of inducing a specific immune response are called antigens. The molecular properties of antigens and the way in which these properties ultimately contribute to immune activation are central to our understanding of the immune system.

13.5.1 Immunogenicity Versus Antigenicity

Two properties are exhibited by antigens; they are immunogenicity and antigenicity. Immunogenicity is the ability of an antigen to induce a humoral and / or cell mediated immune response.

B cells + antigen ⟶ effector B cells (Plasma cells) + memory B cells

T cells + antigen ⟶ effector T cells (T_C, T_H cells) + memory T cells

Although a substance that induces a specific immune response is usually called an antigen, it is more appropriately called an immunogen. Antigenicity is the ability of an antigen to combine specifically with the final products of the above responses. (antibodies and/or cell surface receptors). All immunogens are antigens but all antigens are not immunogens. Some small molecules called haptens are antigenic but incapable, by themselves, of inducing a specific immune response. In other words haptens lack immunogenicity. Examples of haptens are dinitrophenol, penicillin and m-amino benzene sulphonate.

13.5.2 Factors that Influence Immunogenicity

Immunogenicity is not an intrinsic property of an antigen but rather depends on a number of properties of the particular biological system that the antigen encounters. The

TN_GOVT_XI_Micro_Biology_CH13.indd 236 16-02-2019 10.36.11 AM

factors that influence immunogenicity can be divided under two categories.

1. Contribution of the immunogen to immunogenicity
2. Contribution of the biological system to immunogenicity

1. Contribution of the immunogen to immunogenicity

Immunogenicity is determined in part, by the following four properties of the immunogen.

A. Foreignness

The immune system normally discriminates between self and non self, so that only molecules that are foreign to the host are immunogenic. For example, albumin isolated from the serum of a rabbit and injected back into the same or another rabbit will not induce an immune response but the same protein when injected into other vertebrate species (rat) will induce an immune response.

B. Molecular size

There is a correlation between the size of a macromolecule and its immunogenicity. The best immunogens tend to have molecular mass approaching 100,000 daltons (Da). Generally, substances with a molecular mass less than 5000-10000 Da are poor immunogens; however a few substances with a molecular mass less than 1000 Da have proven to be immunogenic.

C. Chemical composition and complexity

Proteins are the most potent immunogens with polysaccharides ranking second. In contrast, lipids and nucleic acids of an infectious agent generally do not serve as immunogens unless they are complexed with proteins or polysaccharides (examples- lipoprotein or nucleo - protein). For example, attachment of tyrosine chains to the weakly immunogenic protein gelatin markedly enhances its immunogenicity.

D. Susceptibility to antigen processing and presentation

The development of both humoral and cell mediated immune responses requires interaction of T cells with antigen that has been processed and presented together with MHC (Major Histocompatibility Complex) molecules. To TH cells, the antigen must be presented with class II MHC molecules on an antigen presenting cell; to T_C Cells the antigen must be presented with class I MHC molecule on an altered self cell. 2. Contribution of the biological system to immunogenicity

Even if a macromolecule has the properties that contribute to immunogenicity, its ability to induce an immune response will depend on the following properties of the biological system that the antigen encounters.

A. Genetic constitution of the host animal

The genetic constitution (genotype) of an immunized animal plays an important role in determining whether a given substance will stimulate an immune response. Genetic control of immune responsiveness is largely made by genes mapping within the MHC

B. Immunogen dosage and route of administration

Whether an immunogen will induce an immune response also depends on the dose and mode of administration. A quantity of an immunogen that has no effect when injected intravenously may evoke a good antibody

TN_GOVT_XI_Micro_Biology_CH13.indd 237 16-02-2019 10.36.11 AM

response when injected subcutaneously, particularly if it is accompanied by an adjuvant.

C. Adjuvants

The response an immunogen is often enhanced if it is administered as a mixture with adjuvants. Adjuvants are substances that enhance the immunogenicity of an antigen. Example: Freund's incomplete antigen, Freund's complete antigen, Mycobacterium tuberculosis, Aluminum potassium sulphate (alum) and Bacterial lipopolysaccharide (LPS).

13.5.3 Epitopes



Immune cells do not interact with or recognize an entire immunogen molecule instead; lymphocytes recognize discrete sites on the macromolecule called epitopes or antigenic determinants. Epitopes are the immunologically active regions of an immunogen that bind to antigen specific membrane receptors on lymphocytes or to secreted antibodies. Antigenic epitopes may consist of a single epitope or have varying number of the same epitope on the same molecule (Example: polysaccharides).

13.5.4 Haptens and the Study of Antigenicity

The pioneering work of Karl Landsteiner in the 1920s and 1930s created a simple, chemically defined system for studying the binding of an individual antibody to a unique epitope on a complex protein antigen. Landsteiner employed various haptens(small organic molecules that are antigenic but not immunogenic). Chemical coupling of a hapten to a large protein called a carrier, yields an immunogenic hapten-carrier conjugate.

13.5.5 Cross-Reactivity

When two antigens possess structurally similar antigenic determinants, the antibodies obtained to one of these antigens tend to react with the other antigen. These reactions are called cross reactions.

Infobits

Penicillin Allergy: New antigens are produced by altering epitopes. This can be done by conjugating haptens to the molecule. A classic example in human medicine is the **allergic response** of some persons to penicillin. A derivative of penicillin, **penicilloic acid** acting as a hapten, can couple with body protein and elicit an immune response that can be harmful, even life threatening, thus excluding this antibiotic from use in certain individuals.

13.6 Antibodies

The first real chemical information regarding the structure of antibodies was provided by Tiselius and Kabat in the early 1940s. They demonstrated that the gamma globulin fraction of serum proteins that migrated most slowly in electrophoresis contained most of the serum antibodies. This section deals with the structural and biological properties of antibodies (immunoglobulins).

Definition of antibodies

Antibodies are glycoproteins present in serum gamma globulins produced by

TN_GOVT_XI_Micro_Biology_CH13.indd 238 16-02-2019 10.36.11 AM

B-lymphocytes (B cells) or Plasma cells in response to exposure to antigen. Antibodies are also known as immunoglobulins. They react specially with that antigen in vivo or in vitro and are hence a part of the adaptive immune response specifically, humoral immunity.

13.6.1 Structure of an Immunoglobulin

1. Basic unit

The basic structural unit (monomer) of an immunoglobulin molecule consists of four polypeptide chains linked covalently by disulfide bonds (Figure 13.21). The four-chain structure is composed of two identical light (L) and two identical heavy (H) polypeptide chains. Every immunoglobulin can be represented by the general formula $(H_2L_2)_n$.

a) Light chains

Light Chains have a molecular weight of approximately 25000 Da and are composed of about 220 amino acids. Light chains are common to all immunoglobulin classes and are of two types – kappa (κ) or lambda (λ) - based on their structural differences. A given immunoglobulin molecule may contain either identical κ or λ chains but never both.

b) Heavy chains

Heavy chains have a molecular weight of approximately twice that of light chains (57000-70000 Da) and twice the number of amino acids (about 440). Five antigenically distinct isotypes of heavy chains are recognized-gamma (γ), alpha (α), mu (μ), delta (δ) and epsilon (ε) – based on structural differences in the carboxy terminal portion of heavy chains. The heavy chains isotypes form the basis of five classes of immunoglobulin molecules – IgG (contains γ chain), IgA (contains α chain),

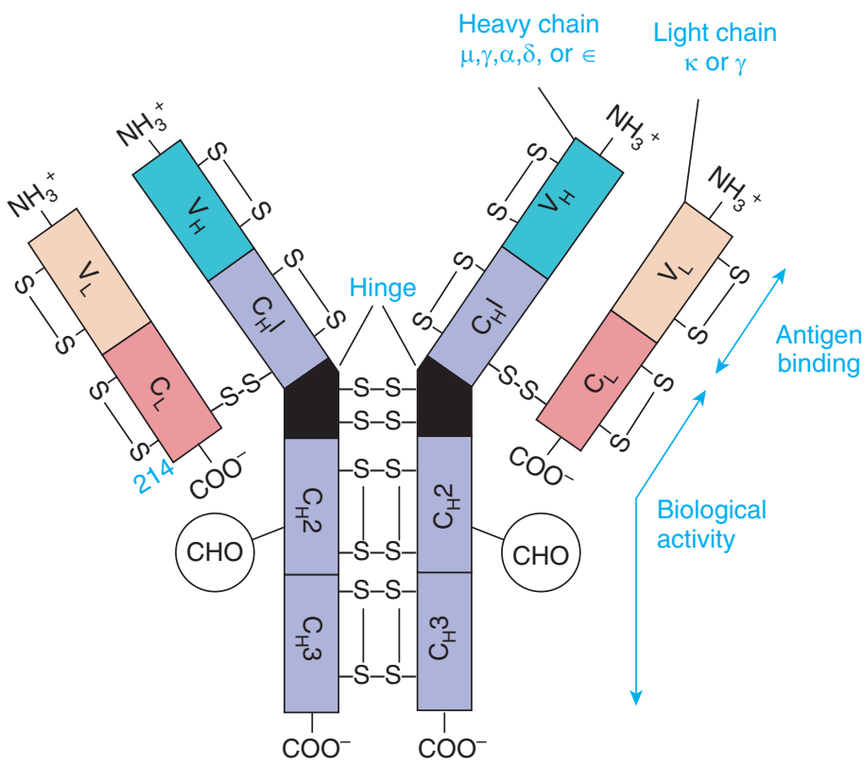


Figure 13.25: Structure of Immunoglobulin

IgM (contains μ chain), IgD (contains δ chain) and IgE (contains ε chain). Five heavy chain classes of immunoglobulin can be easily remembered as GAMDE. Heavy chain classes are again subdivided into subclasses of molecules.

i. Four known subclasses of the γ chain exist – γ1, γ2, γ3 and γ4 - which yield IgG1, IgG2, IgG3 and IgG4.

ii. Two subclasses of the α chain are known – α1 and α2 - which yield IgA1 and IgA2.

iii. Two subclasses of the μ chain are known – μ1 and μ2 - which yield IgM1 and IgM2.

iv. No subclasses of the δ and ε (IgD and IgE) are known.

2. Disulfide bonds

Disulfide bonds hold together the four polypeptide chains in normal immunoglobulin molecules and are of two types namely interchain bonds and intrachain bonds.

3. Regions

Each heavy and light chain consists of two segments, the variable region and the constant region. The variable (V) region shows a wide variation in amino acid sequence in the amino terminal portion of the molecule.

4. Domains

Each immunoglobulin chain consists of a series of globular regions enclosed by disulphide bonds. Each heavy chain consists of four or five domains - one in the variable region (VH) and three or four in the constant region (CH1, CH2, CH3, and CH4). Each light chain consists of two domains – one in the variable region (VL)and one in the constant region (CL).

5. Fragments.

Proteolytic (peptide bond -splitting) enzymes such as papain and pepsin are used to degrade immunoglobulin molecules into definable fragments to facilitate study of their structure Figure (13.22).

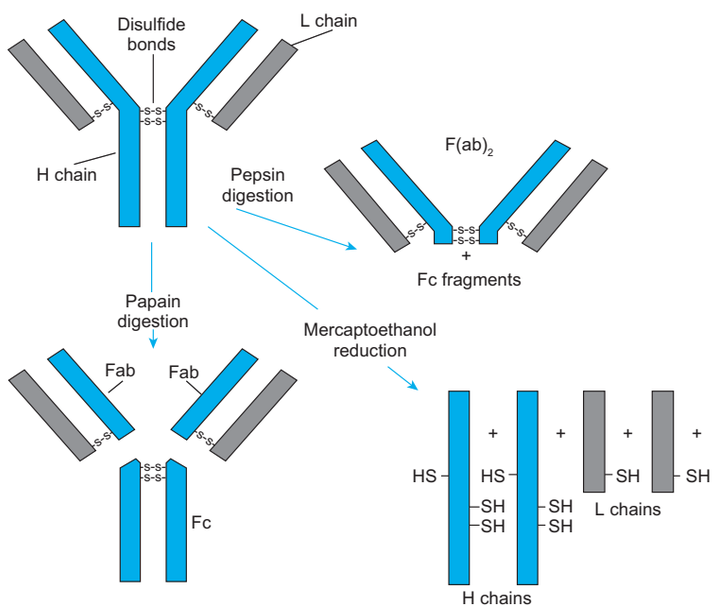


Figure 13.22: Prototype structure of IgG, showing chain structure and interchain disulfide bonds

6. Hinge region

Hinge region is the portion of heavy chain between the CH1 and CH2 domains. It is highly flexible and allows for movement of the Fab arms in relation to each other. The S values (sedimentation coefficient that is expressed in Svedberg units(s)) of immunoglobulins range from 7S- 19S.

13.6.2 Immunoglobulin Function

There are three major effector functions that enable antibodies to remove antigens and kill pathogens. Opsonization promotes antigen phagocytosis by macrophages and neutrophils. Complement activation by IgM and IgG can activate a pathway that leads to the generation of a collection of proteins that can perforate cell membranes. Antibody-dependent cell-mediated cytotoxicity (ADCC) can cause NK cell mediated death of target cells when antibody bound to the target cells associates with Fc receptors of natural killer (NK) cells.

HOTS

Which antibody protects the new born for few months against infections?

13.6.3 Properties and Activities of Immunoglobulin Classes

Each immunoglobulin class differs in its general properties, distribution in the body and interaction with other components of the host defensive systems.

i) IgG

1. IgG is the major immunoglobulin in human serum, accounting for 80% of the immunoglobulin pool.
2. It is present in blood plasma and tissue fluids. It has a monomeric structure.
3. IgG class acts against bacteria and viruses by opsonizing the invaders and neutralizing toxins and viruses.
4. IgG molecules are capable of fixing complement, except for IgG4.
5. It is the major antibody in the secondary immune response and it has half life of 23 days.
6. IgG is the only immunoglobulin molecule able to cross the placenta and provides natural immunity in utero and to the neonate at birth.

ii) IgA

It is present in the serum and in various bodily secretions and thus takes two forms – serum IgA and secretory IgA (sIgA)

A) Serum IgA

1. It accounts for about 12% of serum immunoglobulin.
2. In humans, over 80% of serum IgA exists in a monomeric form and the remaining existing as polymers in the form of dimers, trimers or tetramers. In polymeric IgA, the monomeric units are linked by disulphide bonds and joining (J) chain.
3. Serum IgA fixes complement via the alternative pathway. It has a half life of 5 days.

B) Secretory IgA

1. Secretory IgA is the primary immunoglobulin of mucosal associated lymphoid tissue (MALT). It is also found in saliva, tears, and breast milk.

TN_GOVT_XI_Micro_Biology_CH13.indd 241 16-02-2019 10.36.11 AM

2. It consists of two monomeric units plus J chain and secretory component (Figure 13.29).
3. The dominant subclass of sIgA is sIgA2 which is unique for its absence of a covalent bond between the light and heavy chains. In this subclass, light chains are linked by disulphide bonds.
4. It has a half life of 5-6 days. It is responsible for local immunity.
5. The sIgA molecules protect mucosal surfaces by reacting with the surface of potential pathogens and interfering with their adherence and colonization. It also plays a role in the alternative complement pathway.

iii) IgM

1. IgM accounts for about 5-10% of the serum immunoglobulin pool.
2. It has a pentameric structure consisting of five monomeric units linked by J chain and disulphide bonds at the Fc fragment (Figure 13.23).
3. It is the predominant antibody in the primary immune response to most antigens and the predominant antibody produced by the fetus.
4. It is the first immunoglobulin made during B cell maturation and individual IgM monomers are expressed on B cells, serving as the antibody component of the B cell receptor (BCR).
5. IgM tends to remain in the bloodstream, where it agglutinates (clumps) bacteria, activates complement by the classical pathway and enhances the ingestion of pathogens by phagocytic cells.

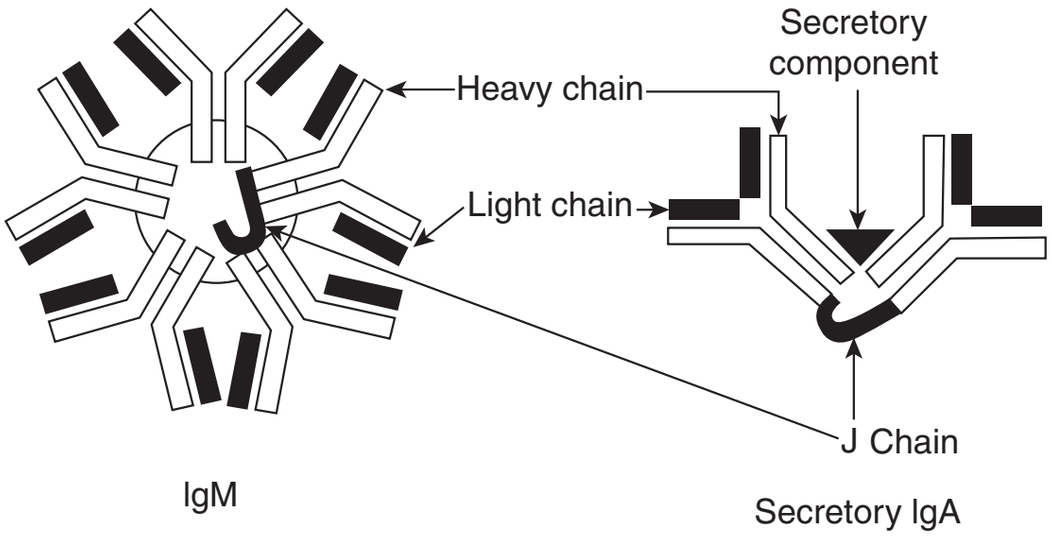


Figure 13.27: Structural models of IgM and secretory IgA. IgM has a pentameric structure linked by J chain at the Fc fragment. Secretory IgA has a dimeric structure plus J chain plus secretory component and is shown in the dominant IgA2 subclass, which is unique for its absence of a convalent bond between the light and heavy chains. Light chains are linked by disulfide bonds.

TN_GOVT_XI_Micro_Biology_CH13.indd 242 16-02-2019 10.36.11 AM

6. It has a half life of approximately 5 days.

iv) IgD

1. IgD accounts for about less than 1% of the total immunoglobulin pool.
2. One unique structural feature is the presence of only a single H-H inter chain bond along with two H-L interchain bonds.
3. It has a monomeric structure similar to that of IgG.
4. IgD antibodies are abundant in combination with IgM on the surface of B cells and thus are part of the B cell receptor complex. Therefore their function is to signal the B cell to start antibody production upon initial antigen binding.
5. It has a half life of 2-3 days.

v) IgE

1. IgE accounts for only 0.004% of serum immunoglobulin. It has a monomeric structure. It is also called reagin or reaginic antibody.
2. The skin sensitizing and anaphylactic antibodies belong to this class.
3. The Fc portion of IgE can bind to Fc receptors specific for IgE that are found on mast cells, eosinophils and basophils. Thus these cells can become coated with IgE molecules. When two cell- bound IgE molecules are cross linked by binding to the same antigen, the cells degranulate. This degranulation releases histamine and other mediators of inflammation.
4. IgE also stimulates production of an excessive number of eosinophils in the blood (eosinophilia) and increased rate of movement of the intestinal contents (gut hypermotility) which aid in the elimination of helminthic parasites. IgE has a half life of 2-3 days.



Polyclonal Antibody: A mixture of antibodies produced by a variety of B-cell clones that have recognized the same antigen. Although all of the antibodies react with the immunizing antigen, they differ from each other in amino acid sequence.

Breast Milk: Breast milk is uniquely suited to the human infant's nutritional needs and is a live substance with unparalleled immunological and anti-inflammatory properties that protect against a host of illnesses and diseases for both mothers and children. All five classes of immunoglobulins have been found in human milk, but by far the most abundant type is IgA, specifically the form known as secretory IgA.

Antitetanus Serum: Antitetanus serum, also known as tetanus immune globulin (TIG) is made up of antibodies against the tetanus toxin. It is used to prevent tetanus in those who have a wound that is at high risk and have not been fully vaccinated with tetanus toxoid. It is also used to treat tetanus along with antibiotics and muscle relaxants. It is given by injection into a muscle.

TN_GOVT_XI_Micro_Biology_CH13.indd 243 16-02-2019 10.36.11 AM

Infobits

i) Isotype

Isotypic determinants are constant region determinants that collectively define each heavy chain class and subclass and each light chain type and subtype within a species. Each isotype is encoded by a separate constant region gene and all members of a species carry the same constant region genes. Within a species, each normal individual will express all isotypes in the serum. Different species inherit different constant region genes and therefore express different isotypes. Therefore, when an antibody from one species is injected into another species, the isotypic determinant will be recognized as foreign, inducing an antibody response to the isotypic determinants on the foreign antibody.

ii) Allotype

Although all members of a species inherit the same set of isotype genes, multiple alleles exist for some of the genes. These alleles encode subtle aminoacid differences, called **allotypicdeterminnats**that occur in some.

The unique amino acid sequence of the VH and VL domains of a given antibody can function not only as an antigenic binding site but also as a set of antigenic determinants. Therefore, the idiotypic determinants are generated by the conformation of the heavy and light chain variable regions. Each individual determinant is called an idiotope and the sum of the individual idiotopes is the idiotype. Anti-idiotype antibody is produced by injecting antibodies that have minimal variation in their isotypes and allotypes, so that the idiotypic difference can be recognized.

13.6.4 Antigenic Determinants on Immunoglobulins

Since antibodies are glycoproteins, they can themselves function as potent immunogens to induce an antibody response. Such anti-Ig antibodies are powerful tools for the study of B cell development and humoral immune response. The antigenic determinants or epitopes, on immunoglobulin molecules fall into three major categories: isotypic, allotypic and idiotypicdeteminants, which are located in characteristic portions of the molecule.

13.7 Antigen – Antibody Reactions

Antigen and antibody combine with each other specifically and in an observable manner. The exquisite specificity of antigen-antibody interactions has led to the development of a variety of immunological assays. These assays can be used to detect the presence of either antibody or antigen. These assays are also helpful in diagnosing diseases, monitoring epidemiological surveys and identifying molecules of biological or medical interest. Antigen-antibody reactions in vitro are known as serological reactions.

TN_GOVT_XI_Micro_Biology_CH13.indd 244 16-02-2019 10.36.12 AM

13.7.1 Three stages of Antigen – Antibody Reactions

a) Primary stage

The reactions between antigen and antibody occur in three stages. The primary stage is the initial interaction between the two without any visible effects. This reaction is rapid and obeys the general laws of physical chemistry and thermodynamics. The reaction is reversible. The combination between antigen and antibody is effected by the weaker intermolecular forces such as electrostatic forces, hydrogen bonds, Van der Waals forces and hydrophobic forces.

b) Secondary stage

The primary stage is followed by the secondary stage leading to demonstrable events such as precipitation, agglutination, lysis of cells, killing of live antigens, neutralization of motile organisms, complement fixation and enhancement of phagocytosis.

c) Tertiary stage

Some antigen-antibody reactions occurring in vivo initiate chain reactions that lead to neutralization or destruction of injurious antigens or to tissue damage. These are the tertiary reactions and include humoral immunity against infectious diseases as well as clinical allergy and other immunological diseases.

13.7.2 General Features of Antigen – Antibody Reactions

Antigen-antibody reactions have the following general characteristics:

1. The antigen-antibody reaction is specific. An antigen combines only with its homologous antibody and vice versa.
2. An entire molecule reacts and not fragments.
3. There is no denaturation of the antigen or the antibody during the reaction.
4. The combination occurs at the surface.
5. TBoth antigen and antibody participate in the formation of agglutinates or precipitates.

13.7.3 Measurement of Antigen and Antibody

Many methods are available for the measurement of antigens and antibodies participating in the primary, secondary and tertiary reactions. Measurement may be in terms of mass (Example: mg Nitrogen) or more commonly as units or titre. The antibody titre of a serum is the highest dilution of the serum which gives an observable reaction with the antigen in the particular test. The titre of a serum is influenced by the nature and quantity of the antigen and the type and conditions of the test. Antigens may also be titrated against sera.

The various tests used for detection of antigen and antibodies are given below:

1. Precipitation tests
2. Agglutintion tests
3. Complement Fixation test
4. Immunofluorescence
5. Radio immuno assay
6. Enzyme linked immuno sorbent assay

TN_GOVT_XI_Micro_Biology_CH13.indd 245 16-02-2019 10.36.12 AM

7. Western Blotting technique
8. Neutralization test

1. Precipitation reactions

When a soluble antigen combines with its antibody in the presence of electrolytes (NaCl) at a suitable temperature and pH, the antigen-antibody complex, forms an insoluble (visible) precipitate and this reaction is called precipitation. When instead of sedimenting, the precipitate remains suspended as floccules, the reaction is known as flocculation.

- **Applications of precipitation reactions**

The following types of precipitation tests are in common use:

a) Ring test

Examples of ring precipitation test are the C- reactive protein test, Ascoli's thermoprecipitin and the grouping of streptococci by the Lancefield technique.

b) Slide test

When a drop of antigen and a drop of antiserum are placed on a slide and mixed by shaking, floccules appear. The VDRL test for syphilis is an example of slide flocculation.

c) Tube test

A quantitative tube flocculation test is used for the standardization of toxins and toxoids.
Precipitation reaction in gels

There are several advantages in allowing precipitation to occur in a gel rather than in a liquid medium. The reaction is visible as a distinct band of precipitation, which is stable and can be stained for preservation, if necessary. Imunodiffusion is usually performed in 1% agarose gel. Different modifications of the test are available.

- Single Diffusion in One Dimension (Oudin Procedure)
- Double Diffusion in One Dimensions (Oakley-Fulthorpe Procedure)
- Single Diffusion in Two Dimensions (Mancini Procedure)
- Double Diffusion in Two Dimensions (Ouchterlony Procedure)

Immunoelectrophoresis

Immunoelectrophoresis was devised by Grabar and Williams (1953). This method consists of two steps. The first step is agarose electrophoresis of the antigen. Rectangular trough is then cut into the agarose gel parallel to the direction of the electric field and is filled with the antiserum. By diffusion, lines of precipitation develop with each of the separated compounds (Figure 13.24). This method is used to detect normal and abnormal serum proteins.

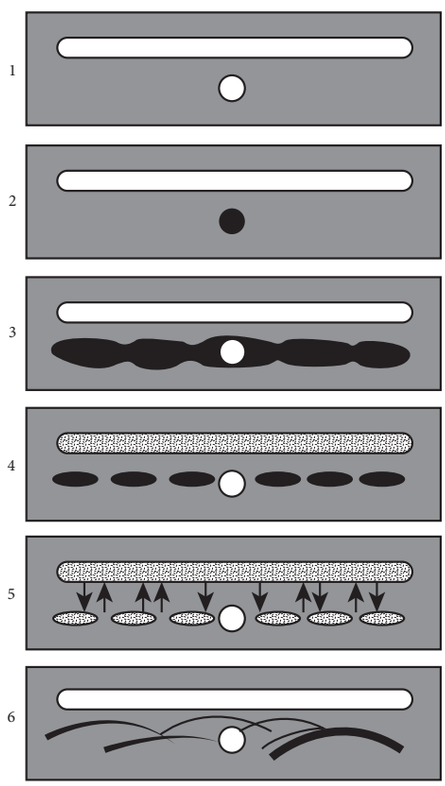


Figure 13.28: Immunoelectrophoresis

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1. Semisolid agar layered on the glass slide. A well for antigen and a trough for antiserum cut out of agar.
2. Antigen well filled with human serum.
3. Serum separated by electrophoresis.
4. Antiserum trough filled with antiserum to whole human serum.
5. Serum and antiserum allowed to diffuse into agar.
6. Precipitin lines form for individual serum proteins
   - Counterimmunoelectrophoresis
   - Rocket Electrophoresis

2. Agglutination reactions

When a particulate antigen is mixed with its antibody in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated, and the reaction is called agglutination.

ADirect agglutination test

In the direct technique, a cell or insoluble particulate antigen is agglutinated directly by antibody. An example is the agglutination of group A erythrocytes by anti-A sera.

Indirect (Passive) agglutination test

Passive agglutination refers to agglutination of antigen coated cells or inert particles (bentonite or latex particles) which are passive carriers of soluble antigens. An example is the latex agglutination for detection of rheumatoid factor.

- **Applications of agglutination reactions**

a) Slide agglutination

Slide agglutination is a routine test for the identification of many bacterial isolates from clinical specimens. It is also the method used for blood grouping and cross matching.

b) Tube agglutination

This is a standard quantitative method for measurement of antibodies. Widal test done for typhoid and Weil Felix test done for rickettsial infections are examples of Tube agglutination.

Latex agglutination test

Here latex particles are used as passive carriers for adsorbed soluble antigens. The most widespread application of latex agglutination has been in the detection of rheumatoid factor. Latex agglutination tests are also employed in the clinical laboratory for detection of HBs Ag, ASO (Antistreptolysin O) and CRP (Carbohydrate Reactive Protein)

Coombs test (antiglobulin test)

This test was devised by Coombs, Mourant and Race (1945) for the detection of anti-Rh antibodies that do not agglutinate Rh-positive red blood cells in saline. The Coombs test may be of the direct or the indirect type.

Applications of coombs test

1. Erythrocyte typing in blood banks.
2. The evaluation of hemolytic disease of the newborn.
3. The diagnosis of autoimmune hemolytic anemia.

TN_GOVT_XI_Micro_Biology_CH13.indd 247 16-02-2019 10.36.12 AM

Summary

Immunology began as a study of the response of the whole animal to infection. Over the years, it has become progressively more basic, passing through phases of emphasis on serology, cellular immunology, molecular immunology and immunogenetics.

The thymus and bone marrow are the primary lymphoid organs. The primary lymphoid organs provide sites for the development and maturation of B and T lymphocytes.

The secondary lymphoid organs function to capture antigen and provide sites where lymphocytes interact with that antigen and undergo clonal proliferation and differentiation into effector cells. The lymphatic system drains the tissue spaces and interconnects many organized lymphoid tissues. The spleen, lymphnodes and mucosal associated tissues (GALT and SALT) are secondary lymphoid organs. Lymph nodes are specialized to trap antigen from regional tissue spaces, whereas the spleen traps blood- borne antigens.

The cells that participate in the immune response are white blood cells or leukocytes. All of the white blood cells develop from a common pluripotent stem cell in hematopoiesis. Lymphocytes are the central cells of the immune system and are responsible for acquired immunity. The other types of white blood cells play ancillary roles such as engulfing and destroying microorganisms, presenting antigens and secreting cytokines. Basophills and mast cells are non phagocytic granulocytes that play a role in allergic responses. Eosinophils are motile phag ocytic cells. Their phagocytic role is less important than that of neutrophils. They play a role in the defense against parasitic organisms. Macrophages and neutrophils are the accessory cells of the immune system that phagocytose and degrade antigens. Dendritic cells are antigen presenting cells. They play an important role in T_H cell activation by processing and presenting antigen bound to class II MHC molecules. Lymphocytes can be subdivided into B lymphocytes, T lymphocytes and null cells (NK cells). The two major subpopulations of T lymphocytes are T helper (T_H) cells and T cytotoxic (T_C) cells.

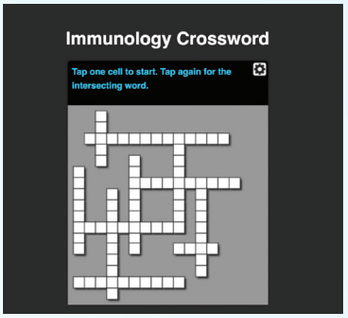
Antibodies are a group of glycoproteins present in the blood tissue fluids and mucous membranes of vertebrates. All immunoglobulins have a basic structure composed of four polypeptide chains (two lights and two heavy) connected to each other by disulphide bonds. In any given antibody molecule, the constant region contains one of five basic heavy chain sequences (γ, α, μ, δ and ε) and one of two basic light chain sequences (k or γ). There are three major effector functions of immunoglobulins are Opsonization, Complement activation and Antibody-dependent cell-mediated cytotoxicity (ADCC).

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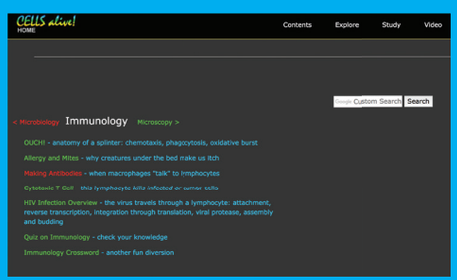
Immunology

How are we protected from microbes?

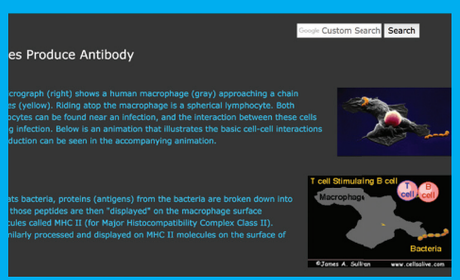


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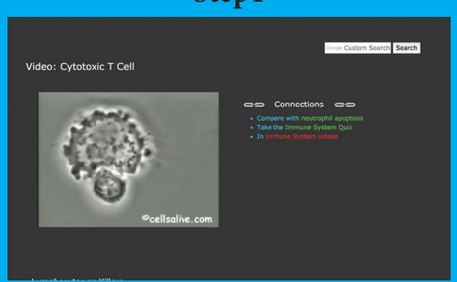
- Use the link or Scan the QR code given below. “Cells Alive-Immunology” will open. You can select any topic you wish. For example click “Making Antibodies”
- ‘Making Antibodies’ page will open. You can go through How ‘Lymphocytes Produce Antibody’, ‘Antigen Processing’, etc….
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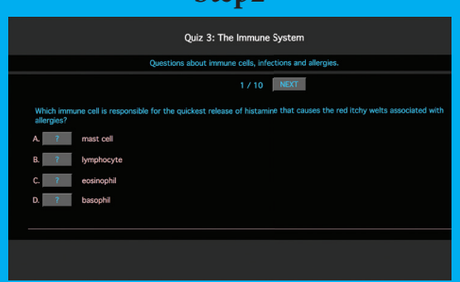
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Step2



Step3



Step4

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Evaluation

RMI2S

Multiple choice questions

1. Who coined the term vaccine?
   a. Jenner
   b. Pasteur
   c. Koch
   d. Roux

2. Who advanced the idea that immunity was primarily due to white blood cells?
   a. Metchinikoff
   b. Ehrlich
   c. Wright
   d. Kitasato

3. Which of the following does apply uniquely to secondary lymphoid organs?
   a. Presence of precursor B and T cells.
   b. Circulation of lymphocytes.
   c. Terminal differentiation.
   d. Cellular proliferation.

4. Which of the following is the major function of the lymphoid system?
   a. Acquired immunity.
   b. Innate immunity.
   c. Inflammation.
   d. Phagocytosis.

5. Lymph nodes taken from neonatally thymectomized mice have unusually few cells in the
   a. Paracortex
   b. Cortex
   c. Medulla
   d. Thymus

6. The myeloid progenitor gives rise to
   a. Erythrocytes, neutrophils, eosinophils, basophils, monocytes, mast cells and platelets.
   b. Erythrocytes, eosinophils, basophils, monocytes, mast cells, platelets and B lymphocytes.
   c. Erythrocytes, eosinophils, basophils, monocytes, mast cells, platelets and T lymphocytes.
   d. Erythrocytes, eosinophils, neutrophils, basophils, monocytes, mast cells and NK cells.

7. Which of the following is a correct statement about NK cells?
   a. They kill target cells by phagocytosis and intracellular digestion.
   b. They proliferate in response to antigen.
   c. They kill target cells in an extracellular fashion.
   d. They are a subset of polymorphonuclear cells.

8. Which of the following cells play an important role in the development of allergies?
   a. Neutrophils
   b. Mast cells
   c. Monocytes
   d. Dendritic cells

9. All of the following will act as opsonins except
   a. Complement
   b. Antibody
   c. Acute – phase proteins
   d. Lactoferrin

10. Which of the following is not the important feature of acquired immunity?
    a. Phgocytosis
    b. Memory.
    c. Specificity
    d. Discrimination between self and non-self

11. Cell mediated immunity is brought about by

TN_GOVT_XI_Micro_Biology_CH13.indd 250 16-02-2019 10.36.13 AM

a. B cells b. T cells
c. NK cells d. Null cells

12. Vaccines induce immunity that is
    a. Naturally acquired active immunity.
    b. Naturally acquired passive immunity.
    c. Artificially acquired passive immunity.
    d. Artificially acquired active immunity.

13. Haptens
    a. Require carrier molecules to be immunogenic.
    b. Interact with specific antibody, even if the haptens are monovalent.
    c. Cannot stimulate immune responses without carriers.
    d. All of the above.

14. The protection against small pox virus infection afforded by prior infection with cowpox represents
    a. Antigenic specificity
    b. Antigenic cross reactivity.
    c. Innate immunity.
    d. Passive protection.

15. An adjuvant is a substance that
    a. Enhances the immunogenicity of haptens.
    b. Increases the chemical complexity of the immunogen.
    c. Enhances the immune response to the immunogen.
    d. Enhances the immunologic cross – reactivity.

16. Antigenic sites with which antibodies react are called
    a. Immunogens b. Carriers
    c. Epitopes d. haptens

17. Basic structural unit of an immunoglobulin molecule includes
    a. Identical λ light chains only.
    b. One constant and three variable regions.
    c. Two identical heavy and two identical light chains.
    d. A total of five domains.

18. J chain is a glycopeptides chain associated with which of the following immunoglobulins?
    a. IgA b. IgG c. IgD d. IgE

19. Primary interactions between antigens and antibodies involve all of the following except which?
    a. Van der Waals forces
    b. Hydrophobic forces
    c. Electrostatic forces
    d. Covalent bonds

20. When instead of the antigen, the antibody is adsorbed to carrier particles in test for estimation of antigen, this technique is known as
    a. Indirect agglutination
    b. Direct agglutination
    c. Reverse passive agglutination
    d. Hemagglutination inhibition

Answer the following

1. What is immunology?
2. Define the term vaccination.
3. What are M cells?

TN_GOVT_XI_Micro_Biology_CH13.indd 251 16-02-2019 10.36.13 AM

4. What is the role of primary lymphoid organs and secondary lymphoid organs?
5. Define hematopoiesis.
6. What are pluripotent stem cells?
7. What is acquired immunity?
8. What is immunological memory?
9. Define the term active/passive immunity.
10. What is immunogenicity?
11. Define the term immunogen.
12. What are haptens?
13. What is antigenicity?
14. Define epitopes.
15. Define the term antibodies.
16. What is opsonization?
17. What is immunity/complement?
18. What is precipitation/agglutination?
19. Write short notes on eosinophils/ neutrophils.
20. Give a short account of natural killer cells.
21. How do intact mucous membranes resist microbial invasion of the host?
22. Briefly explain the various stages involved in phagocytosis.
23. Write short notes on interferons.
24. Write short notes on primary immune response/secondary immune response?
25. How do adjuvants function?
26. Explain immunoglobulin structure and function?
27. Describe briefly the structure and function of thymus.
28. Describe the structure and function of spleen/lymph node.
29. Describe the characteristics of macrophages
30. Write the characteristics of B/T cells.
31. Briely explain the three major events in the inflammatory response.
32. Briefly explain humoral immunity.
33. Write an account of cell mediated immunity.
34. Mention the properties of IgM.
35. List out the general features of antigen-antibody reactions.

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Chapter 14

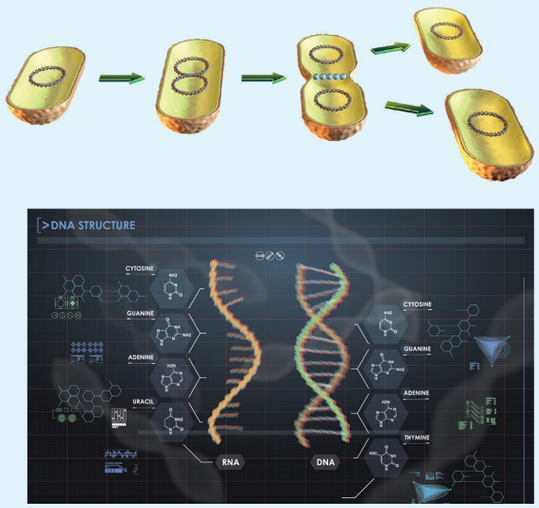
Microbial Genetics

Chapter Outline

14.1 Genetic Information is Stored in DNA

14.2 Structure of DNA

14.3 DNA Replication



Microbial genetics provides powerful tools for deciphering the regulation, as well as the functional and pathway organization of cellular processes. The genetic study of microbes has played a highly significant role in the developments of Molecular Biology, Recombinant DNA Technology and in the preparation of useful products. Microbial Genetics makes microbes beautiful, beneficial and bountiful.

Learning Objectives

After studying this chapter the student will be able,

- To *review the historical discoveries that led to establishing DNA as the genetic material.*
- *To identify the role of genetic material.*
- *To recognize the contributions of Griffith, Avery, MacLeod, and McCarty, and Hershey and Chase.*
- *To explain the structure of DNA.*
- *To recognize the contributions of Chargaff, Rosalind Franklin, Maurice Wilkins, Watson and Crick.*
- *To describe the Watson and Crick model of DNA.*
- *To compare structure of DNA and RNA.*
- *To know Meselson and Stahl's experiment.*
- *To explain the steps of replication.*
- *To know the enzymes and their roles involved in DNA replication.*

14.1 Genetic Information is Stored in DNA

Microorganisms are diverse in nature. A particular bacterium can be identified based on certain characteristics. When a bacterial cell grows and divides, it gives rise to cells with similar characteristics. Have you ever pondered as to why some of the characteristics of progeny cells are similar and a few dissimilar?

In the middle of the 19th century it was assumed that there was some particle present somewhere in the cell which

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was the controlling factor to carry the characteristics from one generation to another.

Genetics, a branch of science aims to understand the working of the controlling factor. The factor governing the transfer of information is now very well known as Gene. Gene can be defined as a unit of heredity which is transferred from parent to progeny.

Although there were experiments to prove the inheritance pattern due to gene, there was no real understanding of the molecular nature of the gene. Work of Frederick Griffith introduced the transforming principle which was further confirmed as Deoxyribonucleic acid (DNA) by experiments of Avery, Mac Leod and Mac Carty in 1944 followed by Hershey and Chase in 1952.

14.1.1 Frederick Griffith's Experiment

In 1928, British bacteriologist Frederick Griffith (Figure 14.1) was trying to develop a vaccine against pneumonia. In his experiments Griffith used two related strains of *Streptococcus pneumoniae* (Figure 14.2).



Figure 14.1: Frederick Griffith

1. Rough strain (R) – avirulent, non-capsulated strain, forming rough colonies on culture media.
2. Smooth strain (S) – virulent, capsulated strain (resists phagocytosis), forming smooth colonies on culture media.

Griffith injected live smooth strain into mice which caused disease and killed it. When he injected live rough strain into mice, it did not cause disease and mice remained alive. He heat killed the smooth strain and injected into mice, the mice remained alive. But the experiment gave surprising results when a mixture of harmless live rough strain and heat-killed smooth strain was injected into mice. Griffith observed that the mice developed pneumonia and died. Further, when he analysed the blood sample from dead mouse, he found that it contained live smooth strain. This accidental discovery made Griffith to conclude that the rough strain changed (transformed) into smooth strain by taking up a substance which he called a "transforming principle" from heat killed smooth bacteria. This phenomenon is called "Bacterial Transformation" Griffith's experiment is summarized in Figure 14.3.

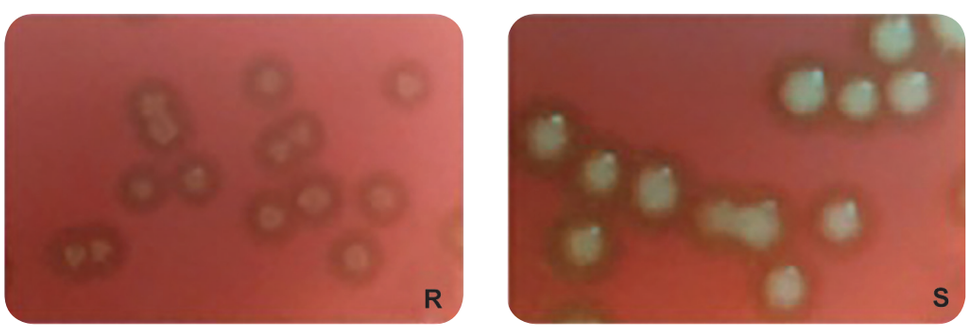


Figure 14.2: Rough and Smooth colonies of *Streptococcus pneumoniae*

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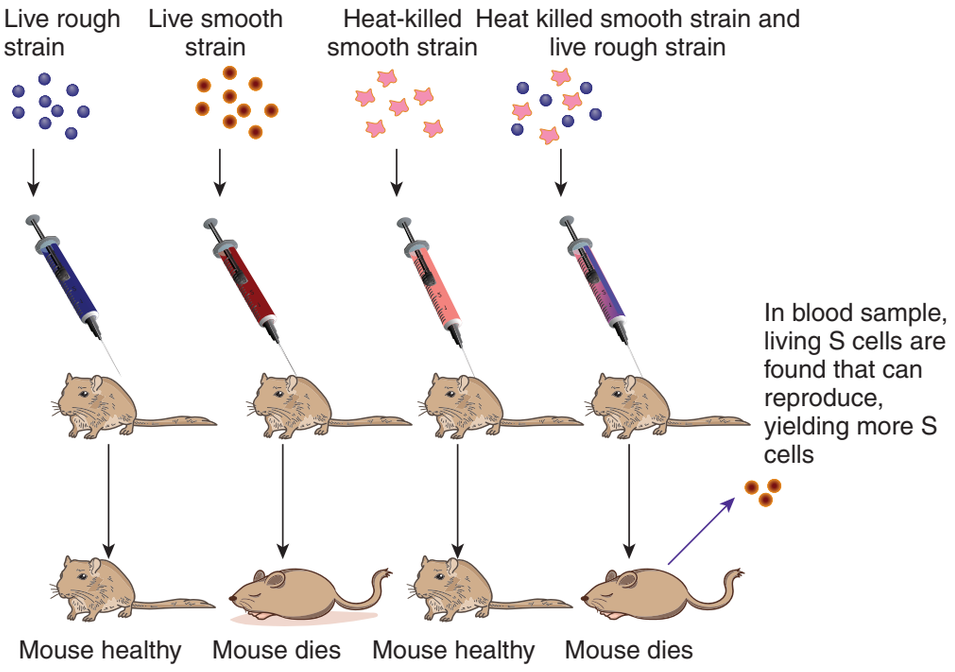


Figure 14.3: Summary of Griffith's experiment

HOTS

What did Griffith expect to happen to mouse when he injected it with live rough strain and heat killed smooth strain

14.1.2 Oswald T. Avery, Colin Mac Leod and Maclyn Mc Carty's Experiment



Figure 14.4: Avery *et al.,*

Griffith's experimental results led to the curiosity to explore the transforming principle. Avery and his colleagues (Figure 14.4) used the extracts of heat-killed smooth bacteria and treated it with enzyme protease, RNase, DNase to eliminate proteins, RNA and DNA respectively. Each of the treated extracts were mixed with live rough bacteria and injected into mice. The mice injected with a mixture of DNase treated extract and live rough strain did not die. This partially proved that DNA was responsible for changing the rough strain of *Streptococcus pneumoniae* bacteria into smooth bacteria. Avery *et al.,* experiment is summarized in Figure 14.5. Later Hershey and Chase's experiment on T2 bacteriophage confirmed that genetic information is present in DNA.

These important early experiments and many other lines of evidence have shown that DNA bears the genetic information of living cells and it is responsible for transfer of characteristics from one generation to another. This is true in all organisms, the notable exceptions being RNA viruses

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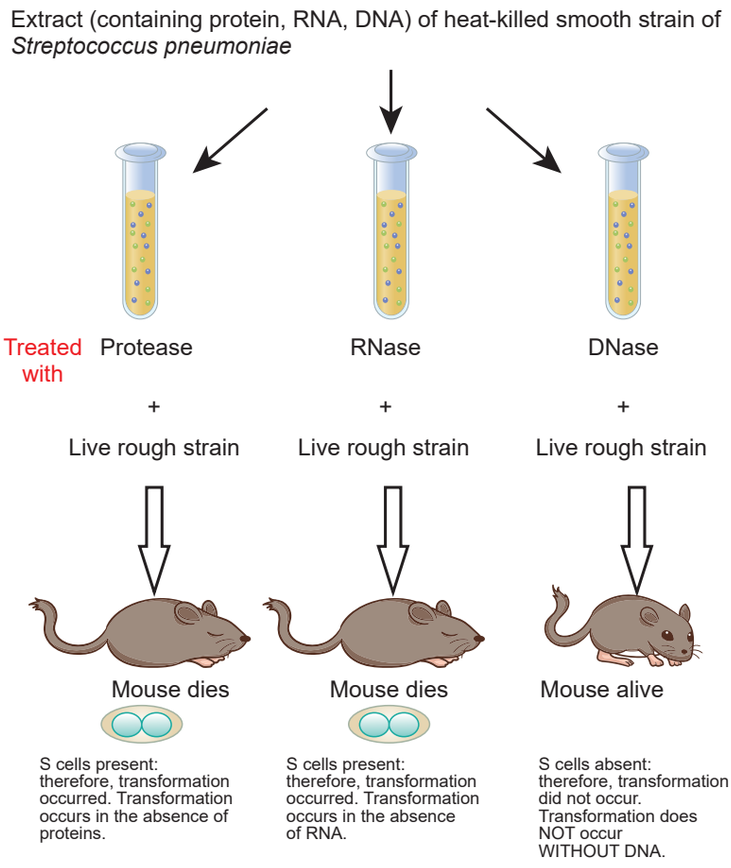


Figure 14.5: Avery, Mc Cleod and Mac Carty's experiment

Infobits

Bacterial transformation

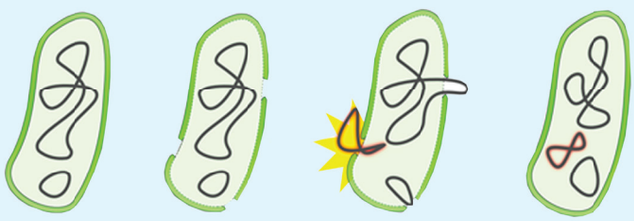
Getting a plasmid into a bacterium

Here is an E.coli. bacterium in natural state. (Notice how bacterial DNA is circular)

Extreme cold causes pores (small holes) to appear in the bacterial membrane.

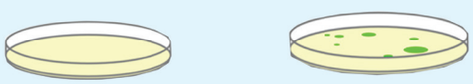
Small DNA molecules like plasmid can move through these holes.

When the bacteria are heated again, some of them end up with plasmid inside them. These are the transformed bacteria.



We can filter out the untransformed bacteria (the ones that got no plasmid) by growing all the bacteria in an antibiotic containing medium.

Untransformed bacteria are killed by the antibiotic in the medium. (They don't have the plasmid with the antibiotic resistance gene.)

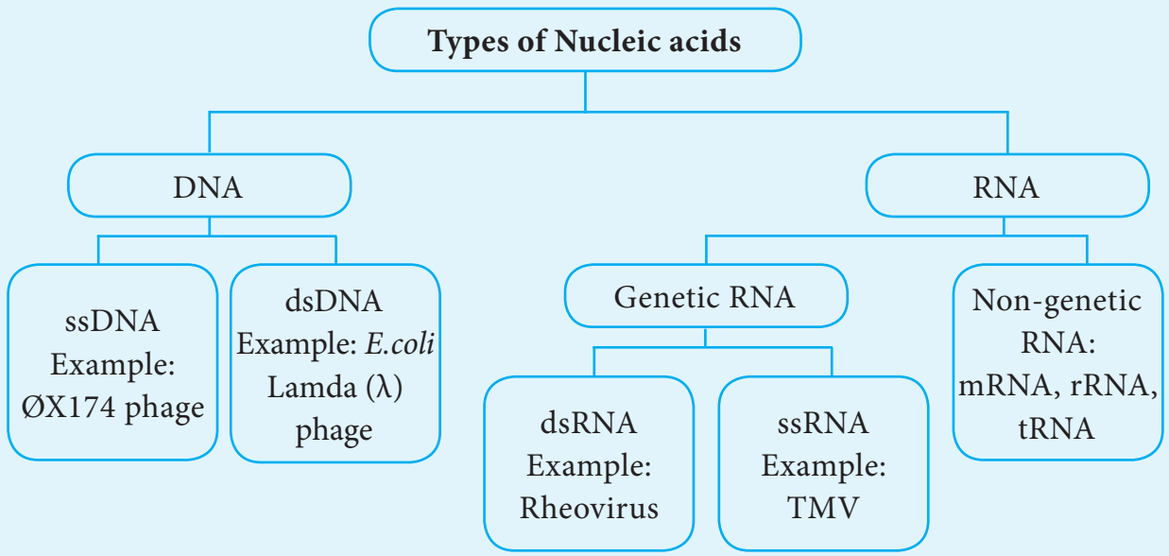


The transformed bacteria grow. Now we can pick them off the plate and grow more if we want.

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The entire genetic content of a cell is known as its genome and the study of genomes is genomics. In eukaryotic cells, but not in prokaryotes, DNA forms a complex with histone proteins to form chromatin, the substance of eukaryotic chromosomes. A chromosome may contain tens of thousands of genes. Many genes contain the information to make protein product. DNA controls all of the cellular activities by turning the genes "on" or "off."



which store genetic information in RNA. The understanding of DNA's role in heredity has led to variety of practical applications including forensic analysis, paternity testing and genetic screening.

14.2 DNA Structure

- DNA is a polymer of simple monomeric units, the nucleotides (Figure 14.6).
- Each nucleotide is made up of three components:
  1. Nitrogenous base
  2. Sugar
  3. Phosphate group
- Nucleotide without phosphate group is known as nucleoside.
- The sugar present in DNA is deoxyribose sugar.
- The nitrogenous bases present in DNA are
  - * Purines – Adenine (A), Guanine (G)
  - * Pyrimidines – Thymine(T), Cytosine (C)
- The nucleotide as a unit is formed by
  - * Glycosidic bond between nitrogenous base and sugar,
  - * Ester bond between phosphate group and sugar
- Each of the nucleotides is bonded by a phosphodiester bond to form a polynucleotide chain (strand) (Figure 14.7a).

TN_GOVT_XI_Micro_Biology_CH14.indd 257 16-02-2019 10.37.51 AM

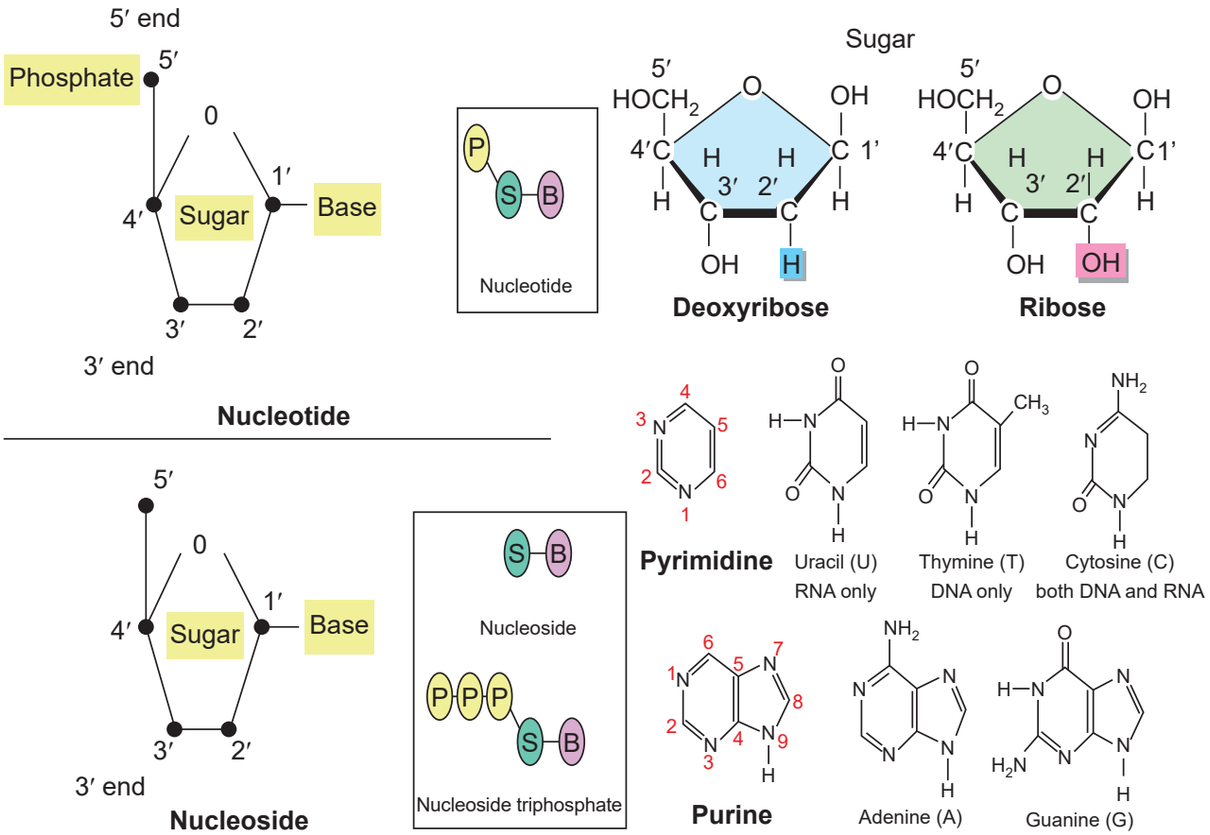


Figure 14.6: Structure of nucleotide, nucleoside, deoxyribose, ribose and nitrogenous bases

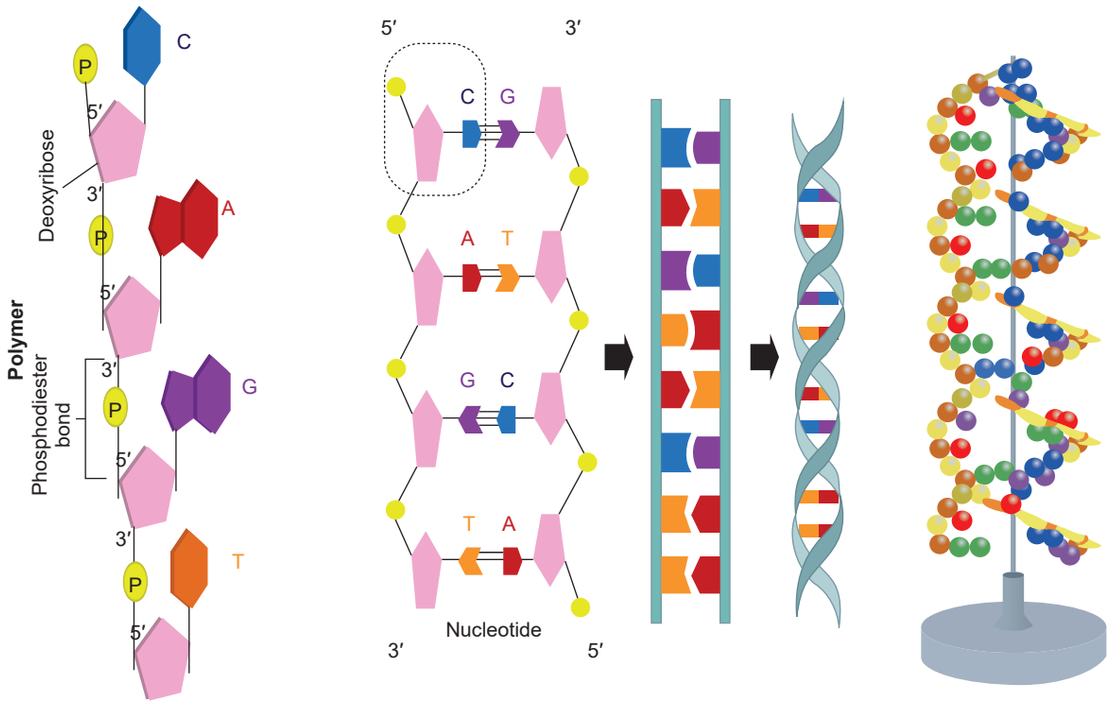
- Two polynucleotide chains join together through hydrogen bonds between nitrogenous bases, to form double stranded DNA (Figure 14.7b).
- Two hydrogen bonds exists between adenine and thymine and three hydrogen bonds between guanine and cytosine.
- DNA is coiled in the form of a double helix, in which both the strands of DNA coil around an axis (Figure 14.7d & e).
- The further coiling of this axis upon itself produces DNA supercoiling an important property of DNA structure.

All DNA, whether large or small, possess the same sugar phosphate backbone. What distinguishes one DNA from another is the length of the polymer and distribution of four bases along the backbone. The variety of sequences that can be made from the four nitrogenous bases is limitless, as is the number of melodies possible with a few musical notes. RNA differs from DNA by having a ribose sugar instead of deoxyribose and nitrogenous base Uracil instead of Thymine.

14.2.1 Watson And Crick Model of DNA Double Helix

In the early 1950's, Rosalind Franklin and Maurice Wilkins used the powerful method of X-ray diffraction to shed more light on the structure of DNA. From the X-ray Diffraction pattern it was deduced that DNA molecules are helical. In 1953 Watson and Crick (Figure 14.8) postulated a three dimensional model of DNA structure based on Franklin's X-ray crystallographic studies. In recognition of their work leading to

TN_GOVT_XI_Micro_Biology_CH14.indd 258 16-02-2019 10.37.52 AM



a) DNA polynucleotide chain | b) Two DNA strands bonded by hydrogen bonds | c) DNA ladder | d) DNA double helix | e) DNA around a central axis

Figure 14.7: Structure of a single polynucleotide chain of DNA, hydrogen bonding between two DNA strands, Double helix around axis

the double helix model, Nobel prize was awarded in 1962 to Watson, Crick and Wilkins. According to Watson and Crick model (Figure 14.9),

- The DNA consists of two helical polynucleotide chains wound around the same axis to form a right handed helix.
- The Purine and Pyrimidine bases of both strands are stacked inside the double helix.
- Each nitrogenous base of one strand is paired in the same plane with a base of the other strand.
- According to Watson and Crick rule Adenine base pairs with Thymine and Guanine base pairs with Cytosine.
- Two hydrogen bonds are present between A and T (symbolised as A=T)

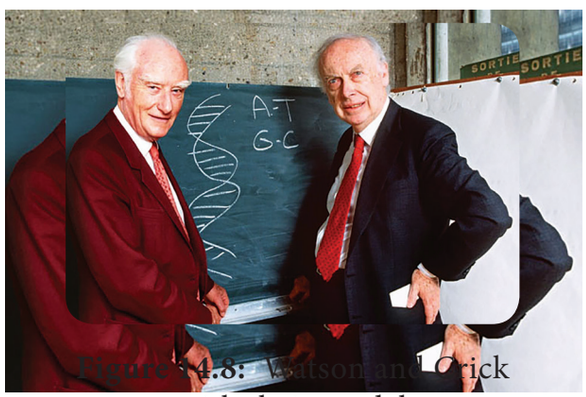


Figure 14.8: Watson and Crick with their model

and three hydrogen bonds are present between G and C (G ≡ C).

- The hydrogen bonds provide chemical stability essential to hold the two chains together.
- The specific A equal to T and G equal to C base pairing is the basis for the complementarity concept. This complementarity concept is very

TN_GOVT_XI_Micro_Biology_CH14.indd 259 16-02-2019 10.37.52 AM

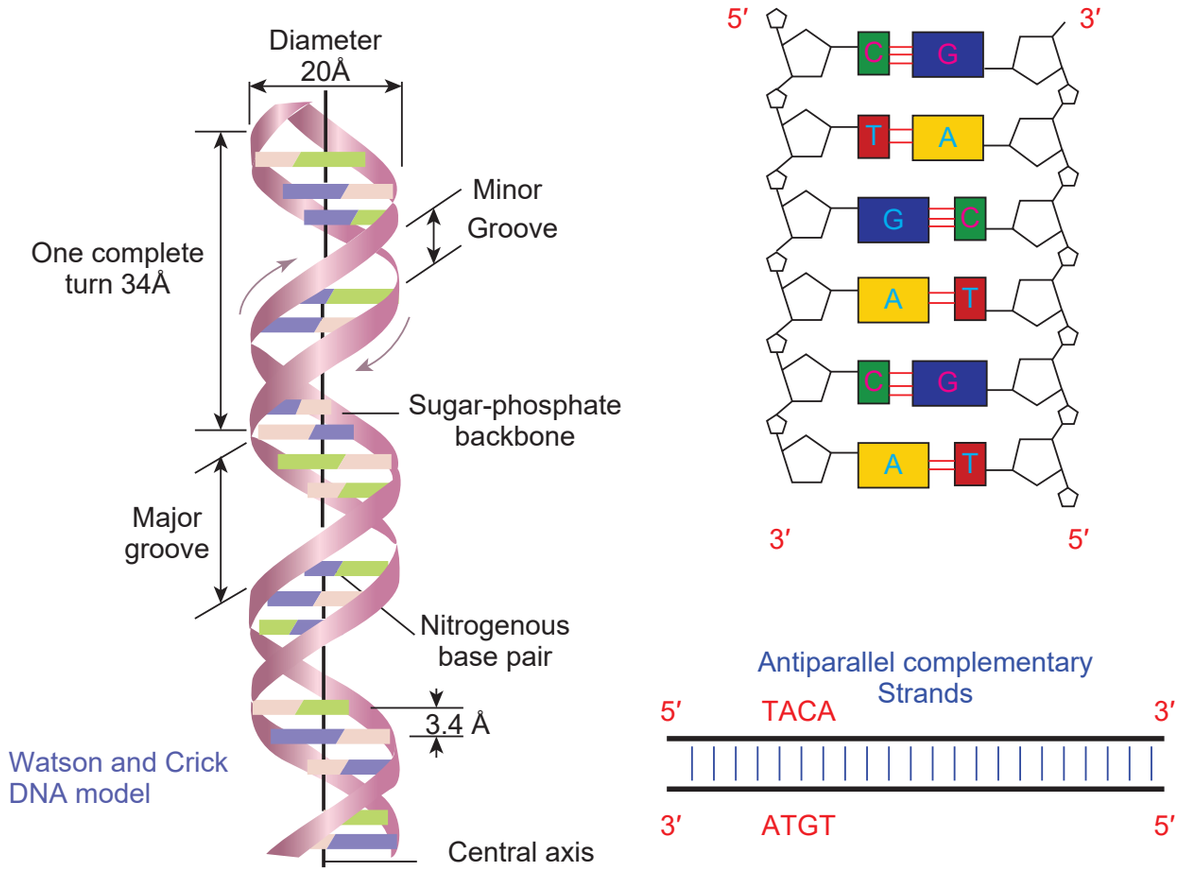


Figure 14.9: Watson and Crick DNA model, Antiparallel dsDNA

important in the process of DNA replication and gene expression.

- The pairing of two strands creates a major groove and minor groove on the surface of the duplex.
- The two strands are antiparallel, that is their 5′, 3′ phosphodiester bonds run in opposite directions.
- The vertically stacked bases are 3.4 A° apart.
- Each complete turn of double helix contain base pairs, which is 34 A° units long.

14.2.2 Erwin Chargaff's Rule

In the late 1940s Erwin Chargaff and his colleagues found that the four nucleotide bases of DNA occur in different ratios in the DNA of different organisms. Erwin Chargaff measured the quantity of the bases in DNA and noticed that the number of Adenine is equal to the number of Thymine and the number of Guanine is equal to the number of Cytosine residues. Hence the sum of Purine residues equal to the sum of the Pyrimidine residues.

Quantitatively A=T or A/T = 1
C=G or C/G = 1
A+G = T+C

HOTS

If percentage of adenine in one of the DNA strand is 20. Can you determine the percentage of other bases. If yes how?

TN_GOVT_XI_Micro_Biology_CH14.indd 260 16-02-2019 10.37.52 AM

14.2.3 Alternative Forms of DNA

DNA is a remarkably flexible molecule. Considerable rotation is possible around a number of bonds in sugar-phosphate backbone and thermal fluctuations can produce bending, stretching and unpairing of the strands. Watson and Crick model of DNA is called as B-DNA or B-form. However DNA can exist in A or Z form. In 1979 Alexander Rich discovered Z form (Figure 14.10). Recently, several alternative forms of DNA have been discovered C-form, D-form and E-form. The B-form of DNA is the most stable structure and is therefore the standard point of reference in any study of the properties of DNA (Table 14.1).

Table 14.1: Properties of different forms of DNA

	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~ 26A°	~ 20 A°	~ 18 A°
Base pairs per helical turn	11	10	12
Distance between adjacent bases	2.6 A°	3.4 A°	3.7 A°



Bacterial genome size

- Bacterial genomes are typically expressed in Mb
- The length of Bacterial genomes are typically in the mm range and therefore 1000X bigger than the typical bacterial size.
- The mass of Bacterial genomes are typically in 10^{-3} pg(picogram) range.

Conversions

1 Kb = 10^3 bp (base pairs)

1 Mb = 10^6 bp

1 Gb = 10^9 bp

1 bp ≈ 0.33nm

1 kb ≈ 0.33μm

1 Mb ≈ 0.33mm

1 Gb ≈ 0.33m

1 pg = 10^{-12} g

1pg = 978 Mb

Number of base pairs = mass in pg × (0.978 × 10^9)

1 kb ≈ 10^{-6} pg

1 Mb ≈ 10^{-3} pg

1 Gb ≈ 1 pg

	Bacteria	Virus, organelles	Eukaryotes
Genomes	Small(Mb)	Tiny (Kb)	Large (Gb)
Gene Density	High	High	Low
Example	*E.coli* 5000 genes	Bacteriophages 10-100 genes	*Homo sapiens* 25000 genes

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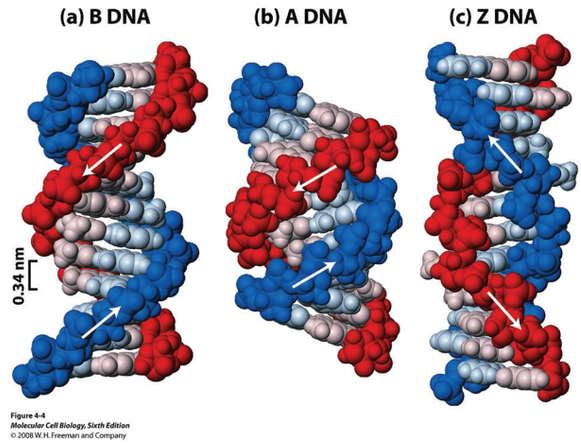


Figure 14.10: Forms of DNA

HOTS

Write the base sequence of complementary DNA and RNA strand for the following.

5′GCGCAATATTTCT3′

14.3 DNA Replication

DNA is a marvelous device for the stable storage of genetic information. DNA replication is a process in which copies of DNA molecules are faithfully made. Here double stranded DNA molecule is copied to produce two identical dsDNA molecules. Replication is an essential process because whenever a cell divides, the two new daughter cells must contain the same genetic information in DNA as parent cell. DNA replication occurs during the S (DNA synthesis) phase and precedes cell division.

Watson and Crick proposed the hypothesis of semiconservative replication. According to them each DNA strand serves as a template for the synthesis of a new strand, producing two new DNA molecules each with one old strand and one new strand. (Figure 14.11).

Infobits

Max Delbruck suggested that there could be three possible ways in which DNA could replicate.

Semiconservative – DNA replication that produce two copies of double stranded DNA (dsDNA) each containing one old strand and one new strand.

Conservative – DNA replication that produces two daughter ds DNAs, one of which consists of two original strands whereas the other daughter DNA consists of two newly synthesised strands.

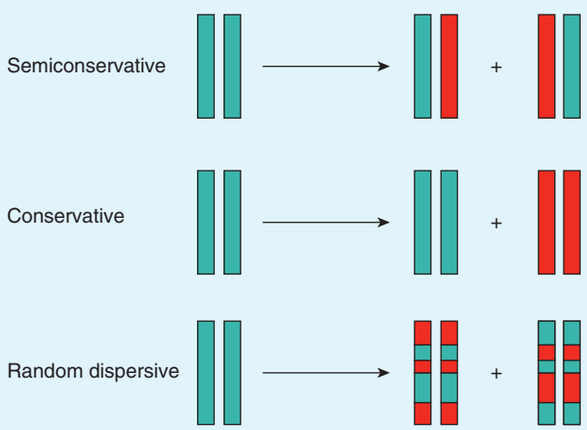


Figure 14.11: Semiconservative, conservative and dispersive replication

Dispersive – DNA replication in which the original dsDNA undergoes fragmentation, the fragments synthesize complementary structure both of which assemble to form two replicas.

TN_GOVT_XI_Micro_Biology_CH14.indd 262 16-02-2019 10.37.53 AM

14.3.1 Meselson and Stahl's Experiment

Mathew Meselson and Franklin Stahl in 1957 gave experimental evidence for semiconservative replication process.



Steps

1. *E.coli* cells were grown for many generations in a medium containing radioactive isotope (heavy isotope) of nitrogen source ^{15}N.
2. After many generations all nitrogen containing molecules in *E.coli* cells, including nitrogen bases of DNA contained ^{15}N.
3. The cells labeled with ^{15}N were then transferred to a medium containing only ^{14}N (light isotope). Hence all subsequent DNA synthesised during replication contained ^{14}N.
4. Cell samples were removed at periodic time intervals from the growth medium.
5. From each of the above samples, DNA was isolated and subjected to density gradient centrifugation (Caesium chloride (CsCl) centrifugation).

Expected results

- The heavy isotope ^{15}N containing DNA, will reach equilibrium in a gradient point closer to the bottom of the tube.
- ^{14}N containing DNA will reach equilibrium at a gradient point closer to the surface of the tube.

Observed Results

- After first generation the isolated DNA occupied an intermediate density band.
- After second generation two bands were observed, one at intermediate density and the other at lighter density corresponding to the ^{14}N position in the gradient.

These experimental results and other experiments repeated by Meselson and Stahl with prokaryotes suggested that semiconservative mechanism of replication is universal (Figure 14.12).

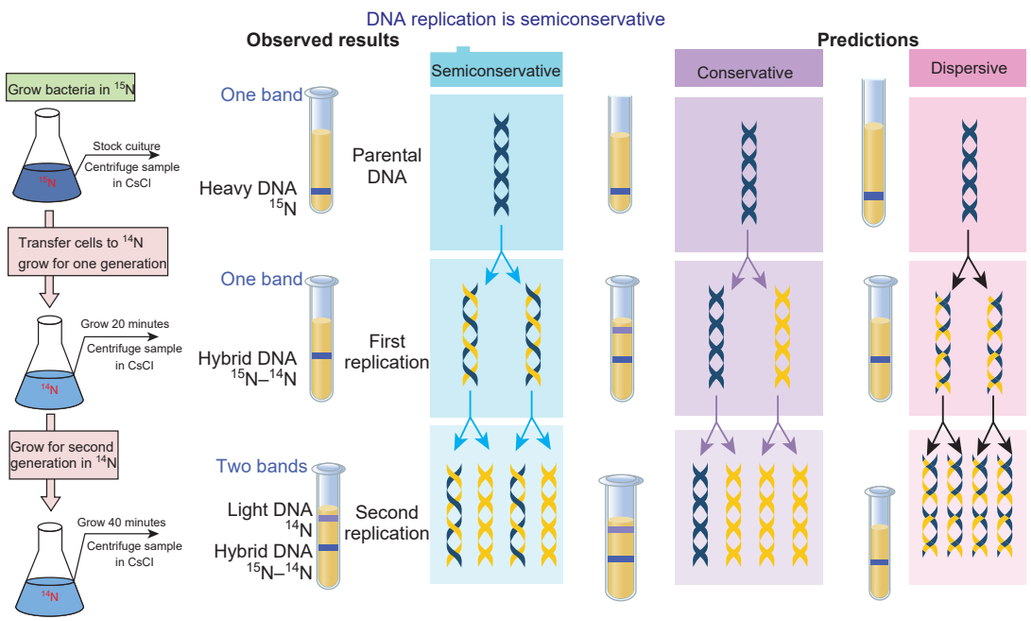


Figure 14.12: Meselson's and Stahl's experiment

TN_GOVT_XI_Micro_Biology_CH14.indd 263 16-02-2019 10.37.53 AM

14.3.2 Enzymes Involved in DNA Replication

DNA replication in *E.coli* requires many enzymes and proteins, each performing a specific task. The entire complex is called the DNA replicase system or replisome. The major enzymes and proteins involved with their functions are tabulated in Table 14.2.

Table 14.2: Enzymes involved in DNA replication

Enzyme	Function
Helicase	Unwinds DNA
DNA gyrase	Relieves stress created by unwinding
SSB protein	Binds to single stranded DNA and stabilises it
Primase	Synthesis of RNA primer
DNA pol I	Excision of primers and filling of gaps with nucleotides
DNA pol III	New strand elongation
DNA ligase	Joins the nick

Infobits

- First in vitro synthesis of DNA with a template was carried out by Kornberg in 1959.
- First in vitro synthesis of DNA without template was carried out by H.G. Khorana in 1965.

14.3.3 Events in DNA Replication in *E.coli*

1. Initiation
2. Elongation
3. Termination

DNA replication is depicted in Figure 14.14.

Initiation

- DNA replication is initiated at replication origin known as oriC (245 base pairs in *E.coli*).
- Dna A protein molecules bind to the origin of replication.
- Helicase (DnaB) denatures the DNA helix by breaking the hydrogen bonds between base pairs.
- Many molecules of SSB (single stranded binding) proteins bind cooperatively to single stranded DNA, stabilizing the separated strands and preventing renaturation.
- Gyrase (topoisomerase) releases the topological stress produced by helicase.
- RNA primers are synthesised by primase.

The separated polynucleotide strands are used as templates for synthesis of complementary strands. The area of the DNA opened by helicase for DNA synthesis is referred to as the replication fork. At the replication fork there are four strands of DNA, two are conserved and two are newly synthesised. Replication may occur in either a unidirectional or bidirectional manner (Figure 14.13) from each origin. Bidirectional replication can be explained as two replication forks moving in opposite directions around the circular chromosome. Both forks move along the double helix away from the origin of replication in opposite directions and around the circular chromosome.

Elongation

- DNA synthesis proceeds in a 5′→3′ direction(read as 5 prime to 3 prime).
- One strand is synthesised continuously and is known as leading strand.

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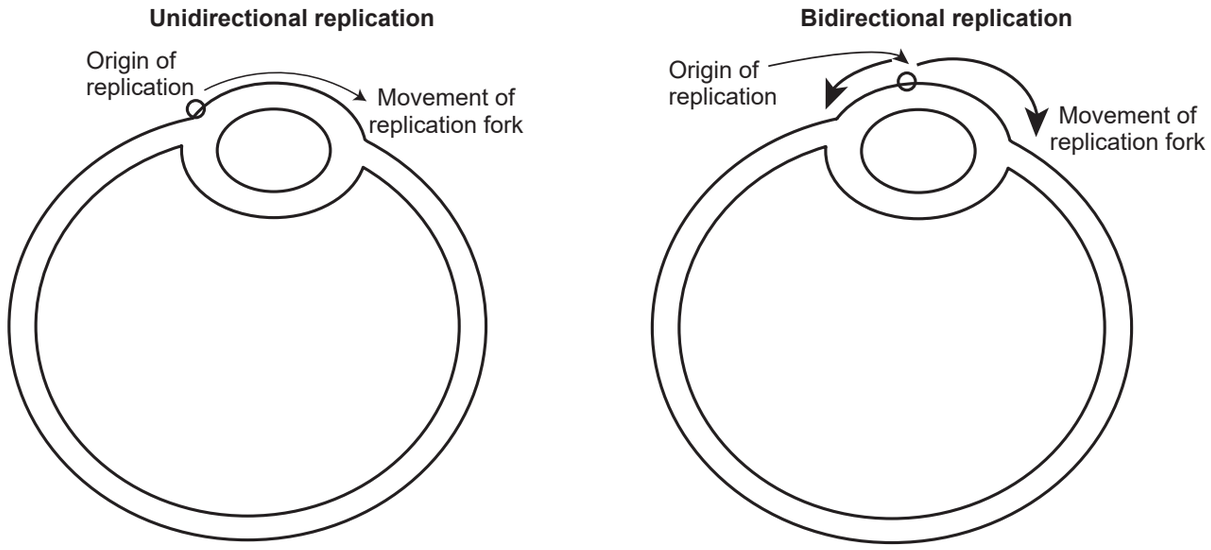


Figure 14.13: Unidirectional and bidirectional replication

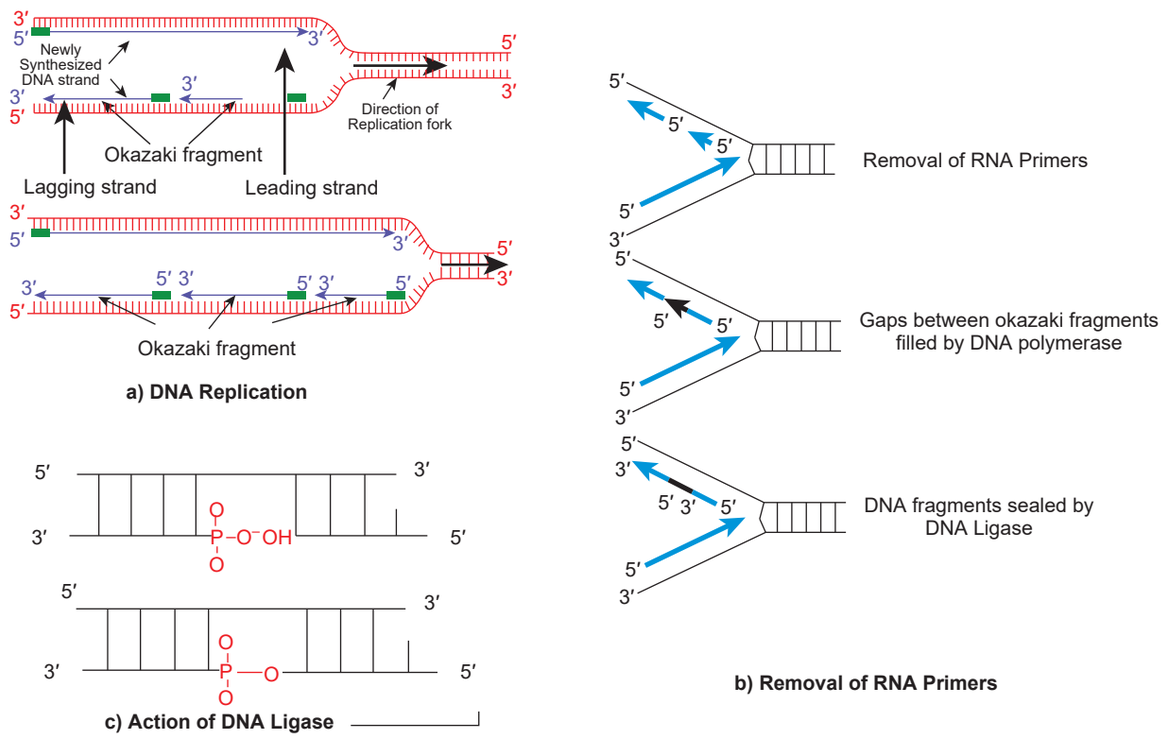


Figure 14.14: a) DNA replication b) Removal of RNA primers c) Action of Ligase and

- Other strand is synthesised discontinuously and is known as lagging strand.
- The enzymes that are able to synthesise new DNA strands on a template strand are called DNA polymerases. Kornberg was awarded Nobel Prize for discovering DNA polymerase in 1956.
- There are three known enzymes in *Escherichia.coli* viz., DNA polymerase I, II and III.
- All of the known DNA polymerases can extend a deoxyribonucleotide

TN_GOVT_XI_Micro_Biology_CH14.indd 265 16-02-2019 10.37.53 AM

chain from a free 3′OH end, but none can initiate synthesis.

- Synthesis of DNA requires nucleoside triphosphates or nucleotides - deoxyadenosinetriphosphate (dATP), deoxythymidinetriphosphate (dTTP), deoxycytidinetriphosphate (dCTP), deoxyguanosinetriphosphate (dGTP). When a nucleoside triphosphate bonds to sugar in a growing DNA strand, it loses two phosphates.
- RNA primer synthesised during initiation is removed and replaced with DNA by DNA polymerase I
- Sealing of the nick by DNA ligase which catalyses the formation of phosphodiester bond between a 3′ hydroxyl end of one DNA fragment and 5′ phosphate at the end of another strand.

The elongation phase of replication includes two distinct but related operations

- Leading Strand Synthesis
- Lagging Strand Synthesis

Leading strand synthesis begins with the synthesis of short (10 to 60 nucleotide long) RNA primer at the replication origin. Deoxyribonucleotides are added to this primer by DNA polymerase III. Leading strand synthesis then proceeds continuosly, keeping pace with the unwinding of DNA at the replication fork. The continuous strand or leading strand is one in which 5′→3′ synthesis proceeds in the same direction as replication fork movement.

Lagging strand or discontinuous strand is one in which 5′→3′ DNA synthesis proceeds in the direction opposite to the direction of fork movement. This strand is synthesised as short fragments, known as Okazaki fragments named after R. Okazaki. Okazaki fragments range in length from a few hundred to a few thousand nucleotides depending on the cell types. Each okazaki fragment must be initiated by the action of primase. Once an Okazaki fragment has been completed its RNA primer is removed and replaced with DNA by DNA polymerase I and the nick is sealed by DNA ligase.

The fidelity of DNA replication is maintained by (1) base selection by the polymerase, (2) a 3′→5′ proofreading exonuclease activity that is part of most DNA polymerases, and (3) specific repair systems for mismatches left behind after replication.

Infobits

RNA dependent DNA polymerases, also called reverse transcriptases, were first discovered in retroviruses, which convert their RNA genomes into double-stranded DNA as part of their life cycle. These enzymes transcribe the viral RNA into DNA, a process that can be used experimentally to form complementary DNA.

Termination

Eventually, the two replication forks of the circular *E.coli* chromosome meet at a terminus region called Ter (for terminus). The Ter sequence function as binding sites for protein Tus (Termination utilization substance). The Tus-Ter complex can arrest a replication fork from only one direction. When either replication fork encounters a functional Tus-Ter complex, it halts. The other fork halts when it meets the first (arrested) fork (Figure 14.15).

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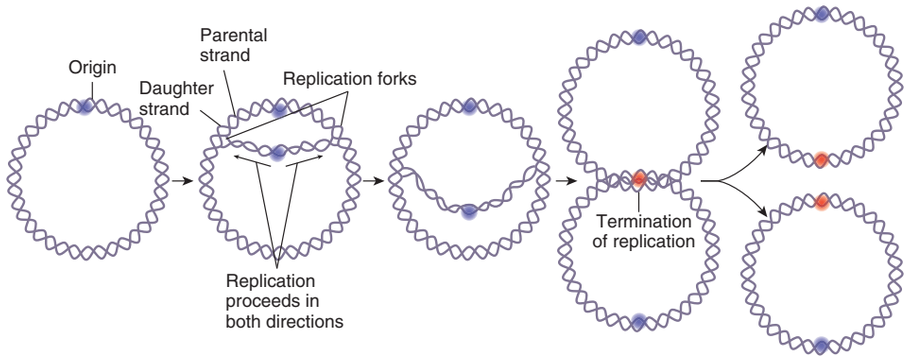


Figure 14.15: Termination of replication in a circular DNA

14.3.4 Eukaryotic DNA Replication

Replication in Eukaryotic cells is more complex. The DNA molecules in Eukaryotes are considerably larger than those in bacteria and are organized into complex nucleoprotein structures (chromatin). The essential features of DNA replication are same in eukaryotes and prokaryotes. However, some interesting variations do occur. Initiation of replication in all eukaryotes requires a multisubunit protein. Multiple origins of replication are probably a universal feature in eukaryotic cells. Like bacteria,

Infobits

DNA molecules exists in circular form in prokaryotic microorganisms, viruses and in organelles of eukaryotic organisms. However not all DNA molecules are circular. The chromosomes of eukaryotic organisms and of many viruses consists of linear DNA molecules. There are three general methods of replication of DNA molecule

1. Theta (θ) mode
2. Sigma (σ) mode
3. Linear mode



The two essential functions of genetic material are replication and expression. Genetic material must replicate accurately so that progeny inherit all of the specific genetic determinants (the genotype) of the parental organism. A gene is a DNA sequence that encodes a protein, rRNA, or tRNA molecule (gene product).

Gene expression usually involves transcription of DNA into messenger RNA and translation of mRNA into protein. Genetic information encoded in DNA is expressed by synthesis of specific RNAs and proteins, and information flows from DNA to RNA to protein.

Expression of specific genetic material under a particular set of growth conditions determines the observable characteristics (phenotype) of the organism. Bacteria have few structural or developmental features that can be observed easily, but they have a vast array of biochemical capabilities and patterns of susceptibility to antimicrobial agents or bacteriophages. These latter characteristics are often selected as the inherited traits to be analyzed in studies of bacterial genetics.

TN_GOVT_XI_Micro_Biology_CH14.indd 267 16-02-2019 10.37.53 AM

eukaryotes have several types of DNA polymerases (Example: DNA polymerase α [alpha] and DNA polymerase δ [delta]). Some have been linked to particular functions, such as the replication of mitochondrial DNA. The termination of replication on linear eukaryotic chromosomes involves the synthesis of special structures called telomeres at the ends of chromosome.

HOTS

What will happen if single stranded binding proteins are not present during replication of DNA?

Summary

Many lines of evidence show that DNA bears genetic information. Frederick Griffith showed transformation of bacteria. Avery, Mac Leod, Mc Carty experiment and further experiment by Hershey Chase confirmed that DNA is the transforming principle.

There are two types of nucleic acid: RNA and DNA. Nucleic acids are polymers of nucleotides, joined together by phosphodiester linkages between the 5′hydroxyl group of one pentose and the 3′hydroxyl group of the next. The nucleotides in RNA contain ribose, and the common pyrimidine bases are uracil and cytosine. In DNA, the nucleotides contain deoxyribose sugar, and the common pyrimidine bases are thymine and cytosine. The primary purines are adenine and guanine in both RNA and DNA.

Erwin Chargaff rules states that A = T and G = C. Watson and Crick postulated that DNA consists of two antiparallel chains in a right-handed double-helical arrangement. Complementary base pairs, A = T and G ≡ C, are formed by hydrogen bonding within the helix. The basepairs are stacked perpendicular to the long axis of the double helix. DNA can exist in several structural forms. Two variations of the Watson-Crick form, or B-DNA, are A-DNA and Z-DNA.

Replication of DNA occurs with very high fidelity and at a designated time in the cell cycle. Replication is semiconservative, each strand acting as template for a new daughter strand. It is carried out in three phases: initiation, elongation, and termination.

The reaction starts at the origin and usually proceeds bidirectionally. DNA is synthesized in the 5′→3′ direction by DNA polymerases. At the replication fork, the leading strand is synthesized continuously in the same direction as replication fork movement; the lagging strand is synthesised discontinuously as Okazaki fragments, which are subsequently ligated by DNA ligases.

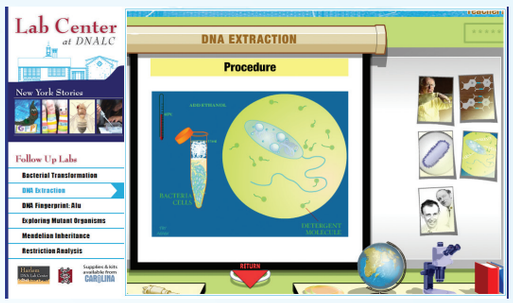
Most cells have several DNA polymerases. In *E. coli,* DNA polymerase III is the primary replication enzyme. Replication of the *E. coli* chromosome involves many enzymes and protein factors. Replication is similar in eukaryotic cells, but eukaryotic chromosomes have many replication origins.

TN_GOVT_XI_Micro_Biology_CH14.indd 268 16-02-2019 10.37.53 AM

ICT CORNER

Bacterial DNA extraction

Lets separate DNA from bacteria

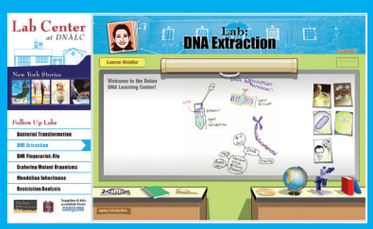


STEPS:

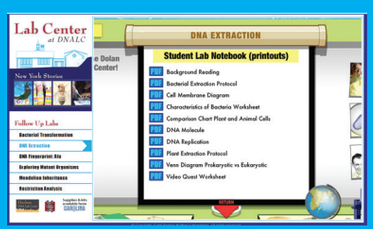
- Scan the QR code
- Click DNA extraction on the left tab
- Select student lab notebook and click open Bacterial Extraction Protocol
- Press return and read students protocol on the left table
- Click producer and follow the steps

OBSERVATIONS :

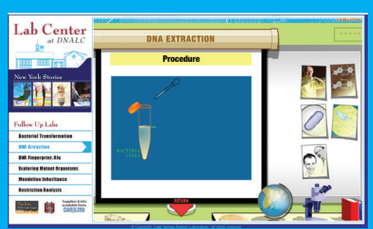
- Select base pair interactions at the right side and join nitrogenous base pairs as in DNA.



Step1



Step2



Step3

URL:

http://labcenter.dnalc.org/labs/dnaextraction/dnaextractiond.html

TN_GOVT_XI_Micro_Biology_CH14.indd 269 16-02-2019 10.37.55 AM

Evaluation

Multiple choice questions

1. The genetic material of virus is
   a. DNA
   b. RNA
   c. a or b
   d. None
2. _____ is used to denature RNA
   a. DNase
   b. Protease
   c. Nuclease
   d. RNase
3. In DNA molecule, the sugars
   a. Bond to nitrogenous bases by hydrogen bonds
   b. Bond to nitrogenous bases by glycosidic bonds
   c. Bond to phosphate by hydrogen bonds
   d. Bond to phosphate by glycosidic bonds
4. Which of the following is not present in DNA
   a. Adenine
   b. Guanine
   c. Uracil
   d. None
5. A nucleoside contains
   a. Sugar
   b. Nitrogenous base
   c. Both a and b
   d. Only b
6. Glycosidic bond is present between
   a. Phosphate and sugar
   b. Sugar and nitrogenous base
   c. Nitrogenous bases
   d. All the above
7. The bond between adenine and thymine in a DNA double helix is
   a. Hydrogen
   b. Double hydrogen
   c. Vander Waal's
   d. Triple hydrogen
8. Watson and Crick DNA model is
   a. A form
   b. B form
   c. Z form
   d. D form
9. In the first generation of Meselson and Stahl's experiment, the results showed a hybrid band of DNA containing both ^{14}N and ^{15}N. Which of the following is the best interpretation of these results?
   a. The results are consistent with semi-conservative replication
   b. The results support conservative replication
   c. The results support both semi conservative and conservative replication
   d. Neither dispersive nor conservative replication can take place.
10. A form of DNA - A is
    a. Left Handed helix with 20 nucleotide pairs per turn
    b. Right handed helix with 10 nucleotide pairs per turn
    c. Right handed helix with 12 nucleotide pairs per turn
    d. Left handed helix with 11 nucleotide pairs per turn
11. DNA polymerase is required for the synthesis of
    a. RNA from DNA
    b. DNA from RNA
    c. DNA from DNA
    d. RNA from RNA
12. Okazaki segments are
    a. Segment of a chain of nucleotides

TN_GOVT_XI_Micro_Biology_CH14.indd 270 16-02-2019 10.37.55 AM

removed during replication of DNA

b. Segment of a chain of nucleotides formed during replication of DNA

c. Segments of gene which undergo recombination

d. Segments of DNA capable of replication

13. DNA replication is aided by
    a. DNA Polymerase only
    b. Both DNA polymerase and primase only
    c. DNA ligase only
    d. RNA Polymerase.

14. The semi-conservative mode of DNA replication was proved by
    a. Beadle and Tatum
    b. Meselson and Stahl
    c. Watson and Crick
    d. H.G Khorana

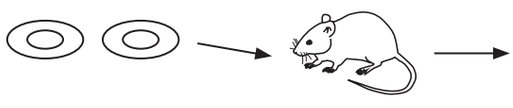
15. Enzymes involved in unwinding of DNA at replication are
    a. Ligases
    b. Helicases
    c. Endonucleases
    d. DNA Polymerases

16. DNA replication is semiconservative because the _____ strand will become half of the _____molecule
    a. RNA, DNA
    b. Template, finished
    c. Sense, mRNA
    d. Condon, anticodon

17. In DNA adenine is complementary base for _____ and cytosine is the complementary for _____
    a. Guanine, thymine
    b. Uracil, guanine
    c. Thymine, guanine
    d. Thymine, uracil

Answer the following

1. Define gene
2. DNA is not always the genetic material, what are the exceptions?
3. Define Nucleotide.
4. List any two difference between DNA and RNA
5. In what sense are the two strands of DNA antiparallel.
6. What is a nucleoside?
7. Depict Erwin Chargaff rule by an equation.
8. Give examples of nitrogenous bases.
9. Draw the structure of Deoxyribose.
10. State Watson and Crick rule.
11. List 2 characteristics of Z DNA
12. Define replication.
13. What is a template DNA?
14. Explain **semiconservative** replication.
15. List major events in replication
16. Write two main events during initiation of replication.
17. What is the role of topoisomerase?
18. What do you understand by leading strand/lagging strand?
19. What is RNA primer?
20. Name two enzymes that make primers for DNA synthesis
21. What is the origin of replication?
22. Label the following diagram of Griffith.



23. Point out the mistake in the following scheme:

    Extracts of smooth strain + R**Nase**

    ⟶ Mouse ⟶ Alive

24. Differentiate between right handed and left handed DNA forms with any three salient features.

TN_GOVT_XI_Micro_Biology_CH14.indd 271 16-02-2019 10.37.55 AM

25. What are the types of DNA polymerases present in *E.coli*? Write their functions.
26. Explain replication fork.
27. Explain bidirectional replication.
28. Explain continuous replication
29. Define okazaki fragments.
30. Why are RNA primers required
31. Describe what is meant by the antiparallel arrangement of DNA
32. Why is one strand of DNA synthesised discontinuously?
33. How is the faithfulness of DNA replication maintained? Write the name of the enzymes with its function.
34. Outline the experiment of Griffith.
35. Relate the experiments of Griffith and Avery.
36. Discuss Avery, Mc Cleod's experiment.
37. What was the motive of Avery and colleagues for conducting the experiment.
38. Differentiate between R and S strains of *Streptococcus pneumoniae*.
39. Describe the various characteristics of the Watson and Crick double helix model for DNA
40. Discuss the various bonds present in DNA double helix
41. Discuss various forms of DNA double helix structure.
42. Diagramatically explain the DNA double helix structure
43. Draw a four base pair segment of a DNA molecule, including each nucleotide and associated bonds involved in the maintenance of the double helix.
44. Diagrammatically represent the results of Meselson and Stahls experiment.
45. Explain how Meselson's and Stahl ruled out dispersive model of replication.
46. Tabulate the enzymes involved in DNA replication with their functions
47. Explain Elongation of DNA during replication

Student Activity

1. Fun with beads – students will understand the concept of polymer – polynucleotide and different sequences of DNA by preparing a chain of 20 beads of four different colours.
2. Prepare a model of DNA
3. Supercoiling of DNA – students will hold the ends of the rubber band and twist it. The two ends will be joined to feel the stress of coiling relieved due to supercoiling.
4. On paper replicate the following segment of DNA

   5′ATCGGCTACGTTCAC3′

   3′TAGCCGATGCAAGTG5′

   Show the direction of replication of the new strands and explain what the lagging and leading strands are? Explain how this is semiconservative replication. Are the new strands identical to the original segment DNA?

TN_GOVT_XI_Micro_Biology_CH14.indd 272 16-02-2019 10.37.55 AM

Glossary

1. Acute disease: A disease in which symptoms develop rapidly but lasts for only a short time.
2. Aseptic techniques: Laboratory techniques to minimize contamination.
3. Assimilation: The absorption and digestion of nutrients by any biological system.
4. Axenic: Pure cultures of micro organisms, which are not contaminated by any foreign organisms.
5. Base Stacking: Stacking implies vertical interactions between bases as they sit on top of one another.
6. Bio-augmentation: The use of pollutant acclimated microbes or genetically engineered microbes for bioremediation.
7. Coagulation: The action or process of a liquid, especially blood, changing to a solid or semi-solid state.
8. Coal-tar dyes: Liquid produced by distilling coal containing benzene naphthalene, phenols, aniline and many other organic chemical.
9. Coliforms: Aerobic or facultatively anaerobic, Gram negative, non endospore forming, rod shaped bacteria that ferment lactose with acid and gas formation within 48 hours at 350 °C.
10. Colony: A Colony is defined as a visible mass of microorganism all originating from a single mother cell.
11. Cover slip: A small, thin piece of glass used to cover and protect a specimen on a microscope slide.
12. Denaturation of DNA: Separation or unwinding of dsDNA strands into single strands.
13. Denature: To deprive something of its natural character and properties.
14. Depyrogenation: Removal of pyrogens from solutions mostly from injectable pharmaceuticals.
15. Dermatomycosis: A fungal infection of skin.
16. Diatomaceous earth: A soft, crumbly, porous sedimentary deposit formed from the fossil remains of diatoms.
17. DNA amplification: The production of multiple copies of a sequence of DNA.
18. Electromagnetic spectrum: The range of wavelengths or frequencies over which electromagnetic radiation extends.
19. Exudate: Low molecular weight metabolites that enter the soil from plant roots.
20. Flake: A small flat thin piece of which has broken away or been peeled from a larger piece.
21. Fluoresence: The property of absorbing light of short wave length and emitting light of longer wave length.
22. Folliculitis: An infection of hair follicles, often occurring as pimples.
23. Fulminating: A condition that develops quickly and rapidly increases in severity.
24. Furuncle: A pus filled, painful infection of a hair follicle.
25. Gene: A unit of heredity which is transferred from parent to progeny.
26. Genetic code: The mRNA codons and the amino acids they encode.
27. Genetics: The study of heredity and variation of inherited characteristics.
28. Genome: One complete copy of the genetic information in cell.

TN_GOVT_XI_Micro_Biology_Glossary.indd 273 16-02-2019 10.38.29 AM

29. Genomics: Study of genes and their functions.
30. Genotype: The genetic makeup of an organisms.
31. Gestation: The development of something over a period of time.
32. Horizontal gene transfer: The transfer of genes between two organisms in the same generation.
33. Hypotonic environment: Environment with higher water concentration and less solutes.
34. Immunodiffusion test: A test consisting of precipitation reactions carried out in an agar gel medium.
35. Immunoelectrophoresis: The identification of proteins by electrophoretic separation followed by serological testing.
36. Inoculation loop: They are made of platinum or nichrome wire. They are used to make smears.
37. Inoculum: The material used to introduce an organism into a certain medium for growth or culture medium in which microorganisms are implanted.
38. In vivo: Process taking place in a living organisms.
39. Ionizing radiation: Radiation consisting of particles, X-rays, or gamma rays with sufficient energy to cause ionization in the medium through which it passes.
40. Latent infection: A condition in which a pathogen remains in the host for long periods without producing disease.
41. Lymph: A colourless fluid containing white blood cells, which bathes the tissues and drains through the lymphatic system into the bloodstream.
42. Lysis: Destruction of a cell by the rupture of the plasma membrane, resulting in a loss of cytoplasm.
43. Lysozyme: An enzyme capable of hydrolyzing bacterial cell walls.
44. MHC: Major histocompatibility complex – The genes that code for histocompatibility antigens; also known as human leucocyte antigens.
45. Microaerophile: An organism that grows best in an environment with less molecular oxygen (O2) than is normally found in air.
46. Molasses: It is a viscous product resulting from refining sugar cane or sugar beets into sugar.
47. Monomer–A small molecule that collectively combines to form polymers.
48. Mucigel: Mucilage or complex polysaccharide forming a layer around plant roots.
49. Neutralism: A lack of interaction between two organisms in the same ecosystem.
50. Nick: It is discontinuity in a dsDNA molecule where there is no phosphodiester bond between adjacent nucleotides of one strand.
51. Normal microbiota: The microrganisms that colonize a host without causing disease; also called normal flora.
52. Occupational health: The branch of medicine dealing with the prevention and treatment of job related injuries and illnesses.
53. Osmotic lysis: Rupture of the plasma membrane resulting from movement of water into the cell.

TN_GOVT_XI_Micro_Biology_Glossary.indd 274 16-02-2019 10.38.30 AM

54. Oxidation: The removal of electrons from a molecule.
55. Oxidation reduction: A coupled reaction in which one substance is oxidized and one is reduced also called redox reaction.
56. PCR: Polymerase chain reaction, a technique using DNA polymerase to make multiple copies of a DNA template in vitro.
57. Plasmolysis: Loss of water from a cell in a hypertonic environment.
58. Polynucleotide: Chain of nucleotides.
59. Prevalence: The fraction of a population having a specific disease at a given time.
60. Progeny: Offspring, decendant of a cell.
61. Protein sequencing: The practical process of determining the amino acid sequence of all or part of a protein or peptide.
62. Protocooperation: An association of mutual benefit to two or more species but without the cooperation or without being obligatory for their existence or the performance of some function.
63. Pustule: A small pus filled elevation of skin.
64. Renaturation/Annealing: Process in which ssDNA or ssRNA pair to form double stranded DNA.
65. Salmon-GAL (6 chloro 3- indolyl –β – D galactopyranoside): It is a chromogenic substrate capable of detecting LacZ gene encoded β galactosidase.
66. Semi-transparent: Partially admitting the passage of light through its substance.
67. Serological methods: Methods for identifying microorganisms based on its reactions with antibodies.
68. Smear: A thin spread of bacterial suspension from a clinical spemicen or from a culture on a glass slide.
69. Spectrophotometer: An apparatus for measuring the intensity of light in a part of the spectrum, especially as transmitted or emitted by particular substances.
70. Stab culture: A long straight wire dipped in culture is punctured into a solid medium usually to see the motility.
71. Topological stress: stress created due to over winding or repeated interwinding of DNA during replication.
72. Topography: the arrangement of the natural and artificial physical feature of an area.
73. Toxigenic: (especially of a bacterium) producing a toxin or toxic effect.
74. Turbo blower: It is a fan that blows the air.
75. Vaccine: A preparation of killed, inactivated, or attenuated microorganisms or toxoids to induce artificial immunity.
76. Vacuoles: A space or vesicle within the cytoplasm of a cell enclosed by a membrane and typically containing fluid.
77. Vegetative cells: A bacterial cell growing actively under favorable conditions.
78. Virulence: The degree of a pathogenicity of a pathogenic microorganism.

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Chapter - 2

Working of compound microscope

https://youtu.be/cmzWDkOYTjM

Chapter – 3

Gram Staining

https://youtu.be/L9bats-vGDY

Endospore Staining

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Chapter – 4

Quick review of sterilization

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Streak Plate

http://youtu.be/NDMNGnxCZ1Q

Bacterial colony description

https://youtu.be/gH--8YWdyyk

Chapter – 6

Photosynthesis

https://youtu.be/1Dn_zdAZN0I

Chapter – 7

Bacterial flagellum

https://youtu.be/PIOfMifowP4

Chapter – 8

Classification of microbes

https://youtu.be/W2nNIRUs6Wo

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https://youtu.be/yCMDHd44ekQ

Chapter – 9

Can microbes clean up oil

https://youtu.be/a_HWLFzgQiM

Composting

https://youtu.be/VNgFXvL9ZH8

Chapter – 10

soil horizons types

https://youtu.be/OEvLuucpYw8

Chapter – 11

Nitrogen fixation

https://www.youtube.com/watch?v=qzh7ZzJQJ84

Late blight of potato

https://youtu.be/2Y77KEYuw_g

Chapter – 12

Bacterial meninigitis

https://youtu.be/HhWjA1xq3Ig

Chapter – 13

Agglutination Reaction

https://www.youtube.com/watch?v=3W67OH3v2lU

Coomb's Test

https://www.youtube.com/watch?v=sUHsX3xrlFM

Chapter – 14

Structure of DNA

https://youtu.be/F5JazhVvlm4

Difference between prokaryotic and eukaryotic DNA

https://youtu.be/0CoZT6hYemk

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Elegant Maplitho paper.

Printed by offset at:

TN_GOVT_XI_Micro_Biology_Acknowledgement.indd 308 16-02-2019 10.40.16 AM