NATIONAL OPEN UNIVERSITY OF NIGERIA

NSC 106

Medical Mircobiology and Parasitology

Module 1

NSC 106 Medical Microbiology and Parasitology) Module 1

Course Developer/Writer
Prof. A. Aboderin, Obafemi Awolowo University, Ile-Ife
Dr. O.O. Irinoye, Obafemi Awolowo University, Ile-Ife
Mrs. E.J.-Shehu, National Open University of Nigeria

Content Editor
Mrs. E.M. Joseph-Shehu, National Open University of Nigeria

Course Coordinator
Adeolu Ejidokun, National Open University of Nigeria

Programme Leader
Dr. Jane-Francis Agbu, National Open University of Nigeria

Credits of cover-photo: Mr. Gbenga Aiyejumo, National Open University of Nigeria.

National Open University of Nigeria - 91, Cadastral Zone, Nnamdi Azikiwe Express Way, Jabi, Abuja, Nigeria

www.nou.edu.ng centralinfo@nou.edu.ng
oer.nou.edu.ng oerunit@noun.edu.ng OER repository
Published in 2021 by the National Open University of Nigeria

Module 1

Microorganisms are everywhere and they can be beneficial as well as harmful to human. For instance, eschelicial coli that are found in the lower alimentary canal are harmless at this site (normal body flora) and harmful at another site like vagina (causing infection). This module deals with composition of the microbial world, historical aspects of microbiology, the relevance and scope of microbiology, microscopy and specimen preparation and finally a brief survey of microbes as friends and foes

Module Objectives

At the end of this module, you should be able to:

- describe microorganisms
- discuss the relevance and scope of microbiology.

Unit 1 Composition of the Microbial World

1.0 Introduction

Microbiology is the study of microorganisms. These are organisms too small to be seen clearly by the unaided eyes. Microorganisms include bacteria, fungi, algae, protozoa and entities at the borderline of life that are called viruses. The cell is the fundamental unit of life. Most microorganisms are unicellular, in unicellular organisms all the life processes are performed by a single cell. However, some are multicellular, having more than one cell. This unit examines the definition of microbiology, types of microbial cells, the different groups of microorganisms and the domains in which they are placed and why viruses are not placed in any of the domains.

2.0 Objectives

At the end of this unit, you should be able to:

- define the term microbiology
- list the groups of organisms classified as microorganisms
- distinguish between prokaryotic and eukaryotic cells
- explain the distribution of microorganisms into domains
- state the characteristics of the microorganisms in each domains
- state the characteristics of viruses.

3.0 Main Content

3.1 Microorganisms

Microorganisms are organisms too small to be seen clearly by the unaided eyes. They are very small life forms so small that individual microorganisms cannot be seen without magnification. They include fungi, bacteria, algae, protozoa and viruses. Some microorganisms however, like the eukaryotic microorganisms are visible without magnification. Examples are bread moulds and filamentous algae.

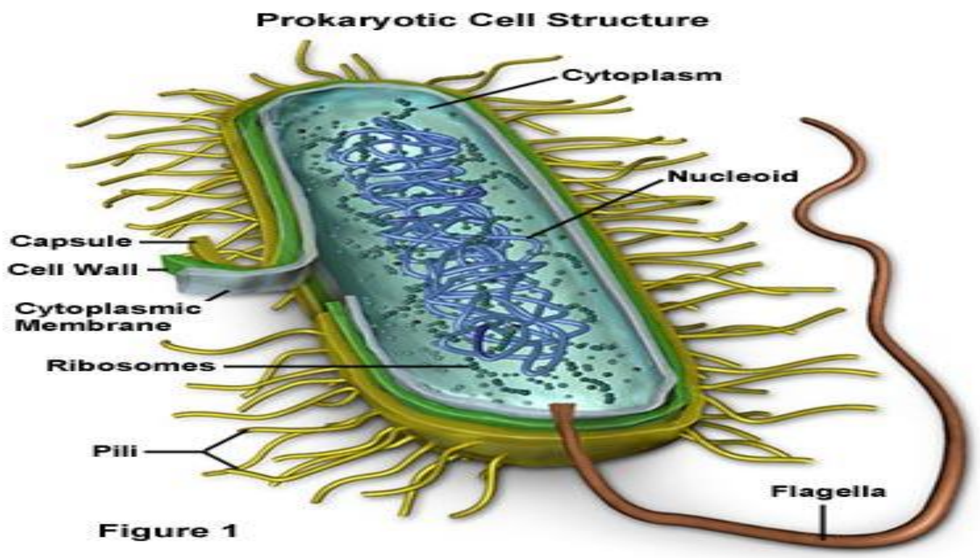
3.2 Microorganisms are Cells

The cell is the fundamental unit of life; a single cell is an entity isolated from other cells. Two fundamental different types of cells exist among microorganisms; which are prokaryotic and eukaryotic.

- Prokaryotes: These microbial cells lack membrane-bound nucleus and organelles.
- Eukaryotes: Possess a membrane-bound nucleus and organelles.

Differences between Prokaryotic and Eukaryotic Cells.

Properties	Prokaryotic cell	Eukaryotic cell
Nuclear membrane	Absent	Present
Chromosome	Single, closed, circular, dsDNA	Multiple linear
Ribosomes	70s(50s + 30s)	80s(60s + 40s)
Mitochondrion	Absent	Present
Examples	Bacteria	Animals, plants, fungi, algae, protozoa



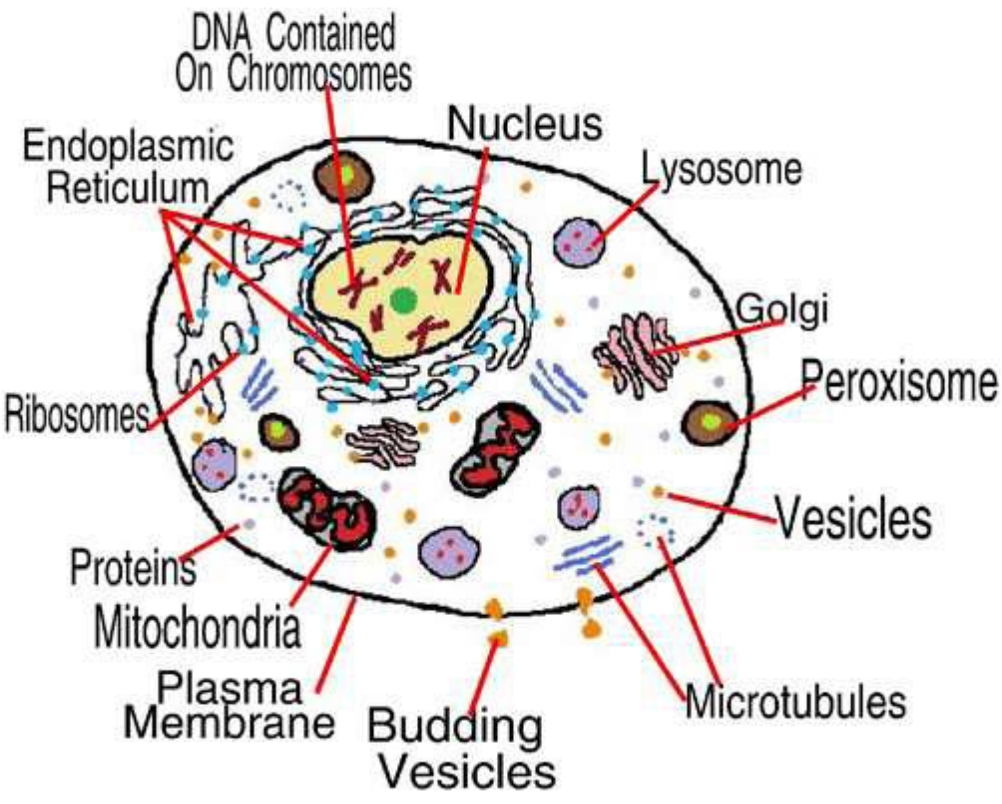


Fig. 1.2: A Eukaryotic Cell
Source: http://www.the-simplehomeschool.com/imagefiles/labeled_cell.gif)

3.3 Classification Systems for Microorganisms

The Five Kingdom System of Classification

Based on cell type and mode of nutrition, there was an establishment of the five kingdom system of classifying organisms in which we have:

- Monera
- Protista
- Fungi
- Planta
- Animalia.

Microorganisms except for viruses, which are acellular and have their own classification system, were placed in the first three kingdoms.

The Three Domains System of Classification

Presently, through advances in cell biology, biochemistry and genetics, microorganisms are now placed into three domains, each of which comprises of various kingdoms.

The domains are:

1. Bacteria (prokaryotic – "true bacteria")
2. Archaea (prokaryotic – "ancient bacteria")
3. Eucarya (eukaryotic)

Domain Bacteria

- They are prokaryotic.
- They are single celled organisms.
- They lack membrane bound nucleus and organelles.
- Most have cell wall that contains peptidoglycan.
- They are found in the soil, water and air and on other living organisms.
- Some are harmful while others are beneficial to man.

Domain Archaea

- They were formerly known as archaeobacteria.
- They are prokaryotic.
- They are single celled organisms.
- They lack membrane bound nucleus and organelles.
- They lack peptidoglycan in their cell walls.
- They have unique membrane lipids.
- Some have unusual metabolic characteristics, e.g. methanogens which generate methane gas.
- Many are found in extreme environments.

Domain Archaea is distinguished from Bacteria based upon:

- Differences in ribosomal RNA sequences.
- The absence of cell wall peptidoglycan.
- The presence of unique membrane lipids

Domain Eucarya

- The major groups of microorganism in this domain are protists and fungi.
- Protists
- These groups of microorganisms are unicellular algae, protozoa, slime moulds and water moulds.

Algae

- They are simple organisms.
- Mostly unicellular.
- They are photosynthetic together with cyanobacteria.
- They produce about 75% of the plant's oxygen.
- Commonly found in aquatic environment.
- They are primary producers in food chains in aquatic habitat.

Protozoa

- They are unicellular.
- Eukaryotic organisms and animal like.
- They are usually motile.
- Some are free living while some are pathogenic.

Slime Moulds

They are protists which have different forms at different stages of their life cycles. At a stage they are like protozoa and at another stage like fungi.

Water Moulds

These are found on the surface of fresh water and moist soils. They feed on decaying vegetation such as logs and mulch.

Fungi

- These are microorganisms that range from unicellular forms like yeasts to moulds and mushrooms which are multicellular with thread like structures called hyphae.
- They absorb nutrients from their environments.
- Many play beneficial roles while others cause diseases in plants, animals and human.

Viruses

- They are acellular entities (non cellular).

- They lack the fundamental structure of living cell but only carry out functions of living organisms when in living cells.
- They are the smallest of all the microorganisms (10,000 smaller than a typical bacterium).
- They can only be seen by the electron microscope.
- They cause many diseases of plants, animals and humans.
- Entities are not placed in any of the domain but are classified on a separate system.
- They cause many diseases of plants, animals and humans

4.0 Conclusion

You have learnt about the following microorganisms and the classification systems for microorganisms.

5.0 Summary

In this unit, you have learnt about the following microorganisms and the classification systems for microorganisms.

6.0 Self-Assessment Exercise

1. Give three examples of diseases caused by each classification of microorganism.
2. Define the term, microorganism (LO1)
3. Compare and contrast prokaryotic and eukaryotic cells (LO3)
4. List the three domains under which microorganisms are classified (LO4)
5. List three characteristics each of the following domains: a. Bacteria b. Archaea Fungi (LO5).
6. What are the differences between bacteria and archeae? (LO5)
7. Why are viruses not placed in any of the domains? (LO6)

7.0 References/ Further Reading

Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book. Inc.

Medigan, M.T. et al. (2009). *Brock Biology of Microorganisms*. (12thed.). Pearson Education Inc.

Pelczar, M.J., Chan, E.C.S. & Krieg, R.N. (2001). *Microbiology*. (5th ed.). McGraw-Hill.

Willey, J.M., Sherwood, L.M & Woolverton, C.J. (2008). *Microbiology*. (7th ed.). Boston Bur Bridge, IL: McGraw-Hill Higher Education.

Unit 2 Historical Aspects of Microbiology

1.0 Introduction

The history of microbiology is the story of men and women who developed a technique, a tool or a concept that was generally adopted in the studying of microorganisms. It is also the history of events and metamorphosis of microbiology as a science. In this unit, we will be studying the stages in the development of the science of microbiology, some early scientists and their contributions to the field of microbiology.

2.0 Objectives

At the end of this unit, you should be able to:

- explain how microorganisms were discovered
- discuss the concept of spontaneous generation and the experiments that were performed to disprove the concept
- discuss koch's postulate and how they are used to establish a link between a suspected microorganisms and the disease
- explain development of microbiology in this century
- explain the era of molecular biology.

3.0 Main Content

3.1 Discovery of Microorganisms

The advent of the microscope permitted the studying of microorganisms. The first microscopes were simple ground glass lenses that magnified images of previously unseen microorganisms. Among the first to observe this previously unseen and invisible microbial world were Robert Hooke and Anthony Van Leeuwenhoek.

1. Robert Hooke (1635-1703), an English mathematician and natural historian.

- He coined the term "cells" to describe the "little boxes" he observed in examining cork slices with a compound microscope.
- He was the first to make a known description of microorganisms.
- He made microscopic observation and the earliest description of many fungi.
- Various species of fungi were clearly identified in his drawing and recorded in his book Micrographia.

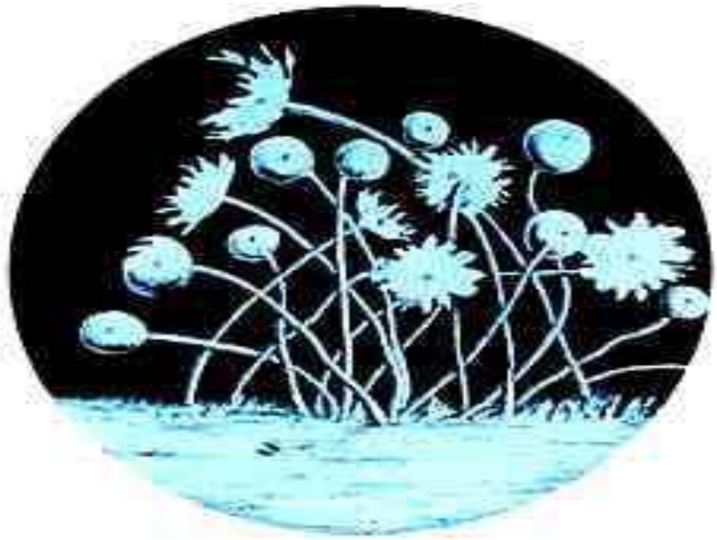
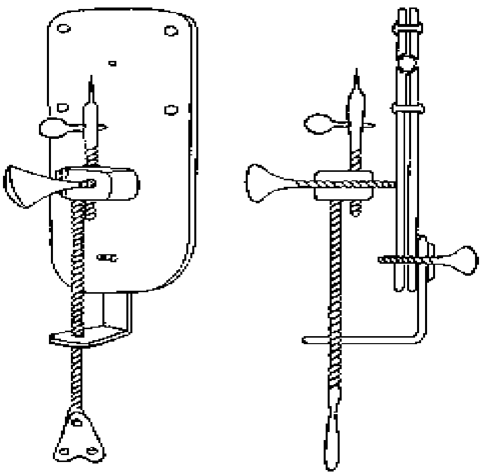


Fig.1: Robert Hooke's detailed Diagram of Fungi made in 1667

Source: Microorganisms in our World by Atlas R. M. (1995**)**

2. Anthony Van Leeuwenhoek (1632-1723) lived in Delft, Holland.

- He is known as the father of bacteriology and the first person to publish extensive and accurate observations of microorganisms
- He was a draper and an amateur microscope builder.
- He learned lens grinding as a hobby and made over 100 simple microscopes each capable of magnifying an image about 300 times.
- By using simple microscopes, he observed microscopic organisms which he called 'animalcules'.
- He discovered bacteria in 1676 while studying pepper water infusion and reported his observations in a series of letters to Royal Society of London which published them in 1684 in English translation.
- He made sketches of the different shapes of bacteria.



(a) (b)

Fig. 2.: (a) Antony Leeuwenhoek (1632-1723) Holding one of his Microscopes

(b) Leeuwenhoek's Microscopes and some of the Sketches of Bacteria from Human Mouth
Source: Microorganisms in our World by Atlas R. M. (1995)

After Van Leeuwenhoek's death, the study of microbiology did not develop rapidly because microscopes were rare and interest in microorganisms was not high. Scientists then were debating the theory of spontaneous generation.

3.2 The Spontaneous Generation Conflict

The concept spontaneous generation states that living organisms could develop from non-living matter. The proponents of the concept of spontaneous generation claim that living organisms could develop from non living or decomposing matter.

1. Francesco Redi (1626-1697) challenged this concept by showing that maggots on decaying meat came from fly eggs deposited on the meat, and not from the meat itself.

- He carried out a series of experiments on decaying meat and its ability to produce maggot spontaneously.
- He placed meat in three different containers, one was not covered, and the second was covered with fine gauze to exclude flies.
- Flies laid eggs on the uncovered meat and maggots developed.
- The two other meats did not produce maggots.
- Spontaneously, flies were attracted to the gauze-covered container and laid their eggs on the gauze, these later produced maggots.
- Hence, it become evident that the generation of maggots resulted from the presence of fly eggs and that meat (a non-living matter) did not spontaneously generate maggots as previously believed.

2. Louis Jablot (1670) conducted an experiment in which he divided a hay infusion that had been boiled into two containers: a heated container that was closed to the air and a heated container that was freely open to the air. Only the open vessel developed microorganisms. This further helped to disprove abiogenesis.

3. John Needham (1713-1781) showed that mutton broth boiled in flasks and then sealed could still develop microorganisms, which supported the theory of spontaneous generation.

4. Lazzaro Spallanzani (1729-1799) showed that flasks sealed and then boiled had no growth of microorganisms, and he proposed that air carried germs to the culture medium. He also commented that external air might be needed to support the growth of animals already in the medium. The latter concept was appealing to supporters of spontaneous generation.

5. Louis Pasteur (1822-1895) was a Professor of Chemistry. He devised a series of swan necked flasks known as Pasteur-flasks, filled the flasks with broth and heated the broth

to sterilization. After cooling, the flasks were opened to the air, but bends on the neck of the flasks prevented microorganisms from falling on the broth and contaminating it rather the microorganisms fell on the neck of the bottle. Pasteur proved that no growth occurred because dust and germs were trapped on the wall of the curved necks. If the neck were broken, growth will occur. By these experiments he disproved and defeated the theory of spontaneous generation.

Fig. 3 shows the defeat of spontaneous generation.

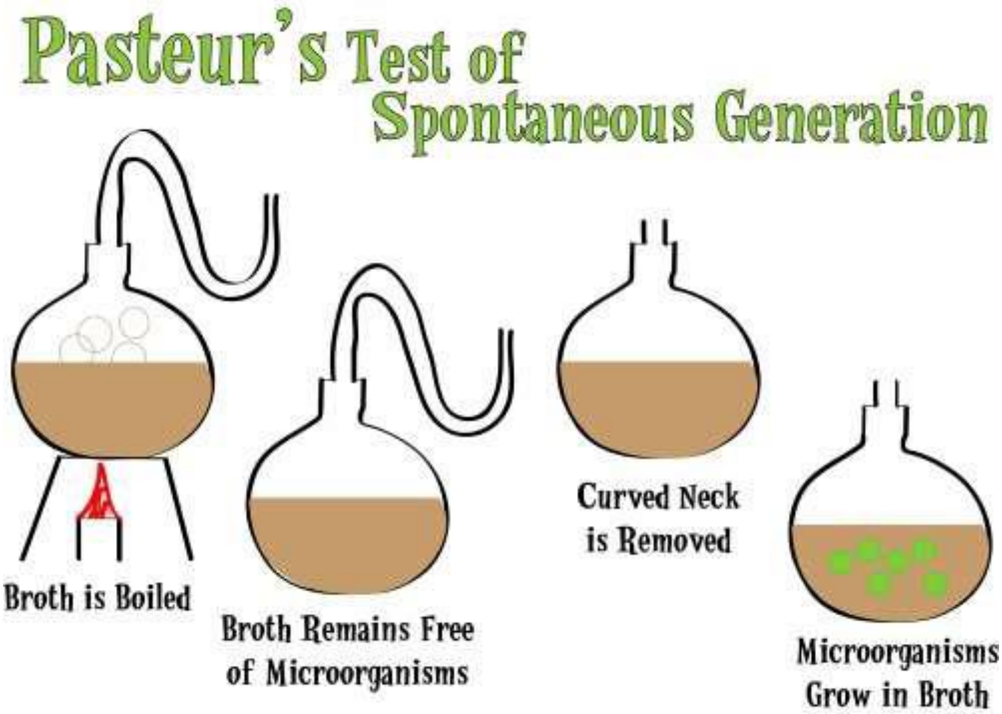


Fig. 3: The Defeat of Spontaneous Generation - Pasteur's Experiment with the Swan- Necked Bottles

Source: Amoebamike.Wordpress.Com

Apart from the defeat of the concept of spontaneous generation:

- Pasteur's work led to an effective sterilisation method which involve holding juices and milk at 62.8OC (145OF) for 30 minutes known as Pasteurisation.
- He discovered that alcoholic fermentation was catalysed by Living Yeast Cells.
- He developed vaccines for the diseases anthrax, fowl cholera and rabies between 1880 and 1890.
- As a result of his research on rabies, he became a legend and the French government built the Pasteur Institute in Paris in 1888. It was originally established as a clinical centre for treating rabies, but is now a major biomedical research centre for antiserum and vaccine production.
- He postulated the Germ Theory of Disease which states that microorganisms are the cause of infectious diseases.
- Pasteur's work ushered in the Golden Age of Microbiology.

3.3 The Recognition of the Role of Microorganisms in Disease

1. Joseph Lister (1872-1912) developed a system of surgery designed to prevent microorganisms from entering wounds. He implemented the use of sterile surgical instrument, and used carbolic acid (phenol) during surgery and on wound dressings.

2. Robert Koch (1843-1910) was a German physician. He was the first to directly prove the role of microorganisms in causing diseases. He established the relationship between Bacillus anthracis and the disease it causes, anthrax. Using mice as experimental animals, he demonstrated that when a small amount of blood from a diseased mouse was injected into a healthy mouse, the healthy mouse quickly developed anthrax.

From this work he developed Koch's postulates which are:

- The suspected disease-causing organism should be present in all cases of the disease and absent from healthy animals.
- The suspected organism must be cultivated in a pure culture away from the animal body.
- The isolated organism must cause the disease when inoculated into a healthy susceptible animal.
- The organism must be re-isolated from these experimental animals and culture again in the laboratory after which it should still be the same as the original organism.

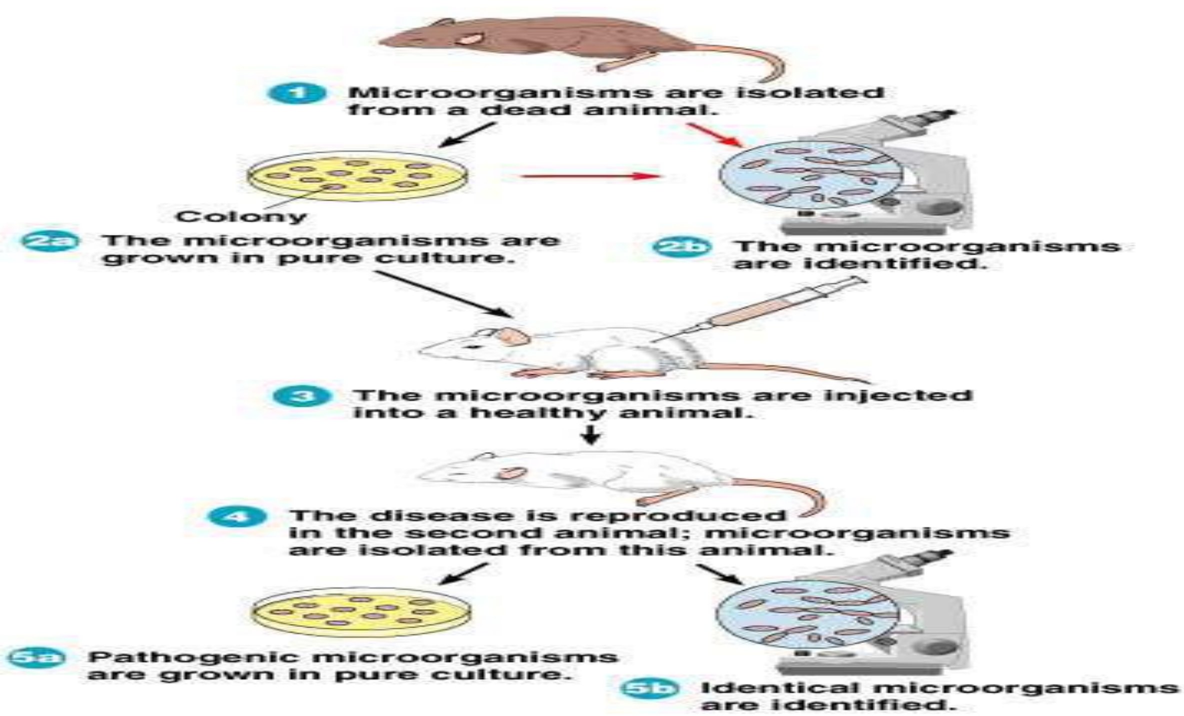


Fig. 4: Diagrammatic Illustration of Koch's Postulate

Using these principles, Koch discovered causative organisms of anthrax (1876), tuberculosis (1882) and cholera (1883).

- He was the first to grow bacteria on solid culture media to get pure culture; hence he developed the pure culture concept and developed different solid media.
- Koch's discovery of solid culture media and pure culture concept supplied the most needed tools for the development of microbiology as a field of science.
- For his contribution on tuberculosis, he was awarded the 1905 Nobel Prize for Physiology or Medicine.

Today, "Molecular Koch's postulates" have been established in light of advances in the molecular biology of pathogenic microbes.

3. Edward Jenner (ca. 1798) used a vaccination procedure to protect individuals from smallpox.

4. Emil Von Behring (1854-1917) and Shibasaburo Kitasato (1852-1931) induced the formation of diphtheria tetanus antitoxins in rabbits which were effectively used to treat humans, thus demonstrating humoral immunity.

3.4 The Development of Microbiology in this Century

Microbiology established a closer relationship with other disciplines during the 1940s because of its association with genetics and biochemistry.

1. George W. Beadle and Edward L. Tatum (ca. 1941) studied the relationship between genes and enzymes using the bread mould, Neurospora.
2. Salvadore Lurai and Max Delbruck (ca. 1943) showed that mutations were pontaneous and not directed by the environment.
3. Oswald T. Avery, Colin M. Mcleod, and Maclyn McCarty (1944) provided evidence that deoxyribonucleic acid (DNA) was the genetic material and carried genetic information during transformation.

3.5 Era of Molecular Microbiology

This Began in the 1970s with:

- Advancement in the knowledge of bacterial physiology, biochemistry and genetics.
- Genetic manipulation which involves the transfer of DNA from one organism into another or a bacterium and the proteins encoded by the DNA harvested led to the development of the field of Biotechnology.
- DNA sequencing revealed the phylogenetic (evolutionary) relationships among bacteria which led to revolutionary new concepts in microbial systematic.
- In 1990s, DNA sequencing gave birth to the field of genomics.

4.0 Conclusion

In this unit, you have learnt about the discovery of microorganisms; the spontaneous generation conflict; the recognition of the role of microorganisms in disease; the development of microbiology in this century and the era of molecular microbiology.

5.0 Summary

In this unit you have been exposed to the following:

- The discovery of microorganisms
- The spontaneous generation conflict
- The recognition of the role of microorganisms in disease
- The development of microbiology in this century
- Era of molecular microbiology.

6.0 Self-Assessment Exercise

Activity: Observe a decay meat under the microscope and discuss your findings in the discussion forum

Answer the following questions:

1. Write short note on Robert Hooke and Anthony Van Leeuwenhoek contribution to the discovery of microorganisms (LO1).
2. State the concept of spontaneous generation (LO2).
3. Discuss the experiments that were performed to disprove the concept of spontaneous generation (LO2).
4. Explain the steps involved in using Koch's postulate to establish the link between a suspected microorganism and a disease (LO3).
5. Explain development of microbiology in this century (LO4).
6. Explain the era of molecular biology (LO5).

7.0 References/ Further Reading

Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book. Inc.

Medigan, M.T. *et al.* (2009). *Brock Biology of Microorganisms*. (12th ed.). Pearson Education Inc.

Pelczar, M.J., Chan, E.C.S. & Krieg, R.N. (2001). (5th ed.). *Microbiology*. McGraw-Hill.

Willey, J.M., Sherwood, L.M & Woolverton, C.J. (2008). *Microbiology*. (7th ed.). Boston Bur Bridge, IL: McGraw-Hill Higher Education. amoebamike.wordpress.com.

Unit 3 The Relevance And Scope Of Microbiology

1.0 Introduction

Modern microbiology is a large discipline with different specialized areas. This is because the entire ecosystem depends on the activities of microorganisms and microorganisms influence human society in countless ways. Microbiology has a great impact on medicine, agriculture, food science, ecology, genetics, biochemistry and other fields. In this unit, we shall examine the different aspects of microbiology and their relevance to human life.

2.0 Objectives

At the end of this unit, you should be able to:

- explain the two branches of microbiology
- discuss the different areas of study in basic and applied microbiology.

3.0 Main Content

The main branches of Microbiology are Basic and Applied. Both branches intertwine and are complementary to each other.

3.1 The Basic Aspects of Microbiology

The basic branch of microbiology is concerned with the study of the biology of microorganisms. Fields of study here include:

Bacteriology: This is the study of bacteria.

Mycology: The study of fungi such as yeasts, molds, and mushrooms.

Algology: The study of algae.

Protozoology: The study of protozoa; a branch of protozoology called parasitology deals exclusively with the parasite or disease producing protozoa and other parasitic micro and macro organisms.

Microbial Cytology: Studies the structures of microbial cells.

Microbial Physiology: Studies of the nutrients that microorganisms require for metabolism and growth and the products that they make from nutrients.

Microbial Genetics: Focuses on the nature of genetic information in microorganisms in microorganisms and how it regulates the development and functions of cells and organisms.

Microbial Ecology: The study of microorganisms in their natural environment. It also studies the global and local contribution to nutrient cycling. In addition, it employs microorganisms in bioremediation to reduce pollution.

Microbial Taxonomy: This is the study of the classification of microorganisms or the grouping of microorganisms.

Biochemistry: This deals with the discovery of microbial enzymes and the chemical reactions they carry out.

3.2 The Applied Aspects of Microbiology

The applied aspect of microbiology deal with practical application of microorganisms to solve problems related to diseases, water and waste water treatment, food spoilage and food production. The various fields of study in applied microbiology include:

1. Medical Microbiology: Studies of the causative agents of diseases, diagnostic procedures for identification of the causative agents and preventive measures.
2. Agricultural Microbiology: This is the study of microbial processes in the soil to promote plant growth. It involves the study of soil microorganisms which has led to the discovery of antibiotics and other important chemicals. It also deals with the methods of combating plant and animal diseases caused by microbes, methods of using microbes to increase soil fertility and crop yields. Currently, much work is being done on using bacterial and viral insect pathogens to substitute chemical pesticides.
3. Industrial Microbiology: This is the large scale growth of microorganisms for the production of medicinal products such as antibiotics and vaccines; fermented beverages; industrial chemicals; production of hormones and proteins by genetically engineered microorganism.
4. Aquatic and Marine Microbiology: Aquatic and Marine Microbiology deals with microbial processes in lakes, rivers, and the oceans. It also examines issues that concern water purification, microbiology examination and biological degradation of waste.
5. Public Health Microbiology: This is closely related to medical microbiology. It deals with the identification and the control of the spread of communicable diseases. It involves monitoring of community food establishments and waste supplies so as to keep them safe and free from infectious agents.
6. Immunology: Deals with how the immune system protects the body from pathogens and the response of infectious agents. It also involves practical health problem such as the nature and treatment of allergies auto-immune diseases like rheumatoid arthritis.
7. Food and Diary Microbiology: Deals with the use of microbes to make foods such as cheese, yoghurt, wine and beer. It also deals with the methods of preventing microbial spoilage of food and the transmission of food-borne diseases such as Botulism and Salmonellosis. Microorganisms are also used as single cell protein, which is an important source of protein or nutrients to livestock and humans.
8. Aeromicrobiology: Advances thought in the dissemination of diseases in the air, contamination and spoilage.
9. Exomicrobiology: Exploration for life in outer space.
10. Geochemical Microbiology: Coal, mineral and gas formation; prospecting for deposits of coal, oil and gas and recovery of minerals from low-grade ores.

3.3 The Future of Microbiology

There are many promising areas of microbiological research and their potential practical impacts in the future. These areas include combating new and re-emerging human diseases such as Hiv/Aids, Sars, Tuberculosis, Poliomyelitis, etc. For this combat to be effective there would be need for the production of new drugs and vaccines. The use of molecular biology and recombinant DNA technology will be applied to give solutions to these problems.

Microorganisms would be needed for environmental bioremediation of pollutants which is on the increase globally. Much work will also be needed to be done on microorganisms living in extreme environments such as to advance the development of new antimicrobial agents, industrial processes and bioremediation. Analyses of genome and its activities will advance the field of bioinformatics and help to investigate biological problems.

4.0 Conclusion

In this unit, we discussed modern microbiology as a large discipline with many different specialised areas. It is subdivided into two main areas of research (basic and applied). The basic area of research in microbiology deals with the biology of microorganisms and includes fields such as bacteriology, mycology, microbial ecology. The applied aspect of microbiology deals with the practical application of microorganisms to solve various human problems related to diseases, water and waste treatment, food production and spoilage, etc. The field of microbiology will be faced with many important future challenges such as finding new ways to new and reemerging diseases, reduced environmental pollution and investigating biological problems.

5.0 Summary

In this unit, you have learnt about the following:

- The Basic Aspects of Microbiology
- The Applied Aspects of Microbiology
- The Future of Microbiology.

6.0 Self-Assessment Exercise

Activity

Discuss the relevance of microbiology to nursing practice and list five basic areas of research in microbiology and state what each area entails. Share your submission in you group forum.

1. List the fields of microbiology that deal with the following:

a. Metabolism (LO2)
b. Enzymology (LO2)
c. Nucleic acid and protein synthesis (LO2)
d. Microorganisms in the natural environment (LO2)
e. Microbial classification (LO2)
f. Microbial cell structure (LO2)

2. Explain what the field of medical microbiology entails (LO1)
3. State the importance of microbiology in five different fields of human endeavours (LO1)

7.0 References /Further Reading

Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book. Inc.

Medigan, M.T *et al.* (2009). *Brock Biology of Microorganisms*. (12th ed.). Pearson Education Inc.

Pelczar, M.J., Chan, E.C.S & Krieg, R.N. (2001). *Microbiology*. (5th ed.). McGraw-Hill,

Willey, J.M., Sherwood, L.M & Woolverton, C.J. (2008). *Microbiology*. (7th ed.). Boston Bur Bridge, IL: McGraw-Hill Higher Education.

Unit 4 Microscope and Specimen Preparation

1.0 Introduction

Microbiology is the study of organisms too small to be seen distinctly with the unaided eyes. The nature of this discipline makes the microscope of crucial importance because the study of microorganisms is impossible without the microscope. Microscopes provide magnification which enables us to see microorganisms and study their structures. The magnification attained by microscopes range from x100 to x400,000 in addition there are different types of microscopes and many techniques have been developed by which specimens of microorganisms can be prepared for examination. This unit examines the different types of microscopes, how the microscopes work and how specimens are prepared for examination.

2.0 Objectives

At the end of this unit, you should be able to:

- define the term microscope
- state the two categories of microscope
- describe the bright field microscope
- explain the resolving power
- describe methods of preparing and staining specimens
- describe the scanning electron microscope and the transmission electron microscope.

3.0 Main Content

The Microscope: A microscope is an instrument for producing enlarged images of objects too small to be seen unaided. There are two types of microscopes: Light (optical) and electron depending on the principle on which magnification is done.

3.1 The Light Microscope

This is a type of microscope in which magnification is obtained by a system of optical lenses using light waves. It includes:

- Bright field microscope
- Dark field microscope
- Fluorescence microscope
- Phase contract microscope.

Modern microscopes are compound microscopes. That is, the magnified image formed by the objective lens is further enlarged by one or more additional lenses. Most undergraduate students of microbiology perform most of their examinations with the bright field microscope which is the most widely used instrument for routine microscopic work. The other types of microscope are used for special purposes or research investigation.

The Bright Field Microscope

- The ordinary microscope is called a bright field microscope because it forms a dark image against a brighter background.
- The microscope consists of a sturdy metal body or stand made up of a base and an arm to which the remaining parts are attached.
- A light source, either a mirror or an electric illuminator, is located at the base.
- Two focusing knobs, the fine and coarse adjustment knobs are located on the arm and can move either the stage or the nose piece to focus the image.
- The stage is positioned about halfway up the arm and hold microscope slides by slide clips or a mechanical stage clip.
- There is a substage condenser mounted within or beneath the stage which focuses a core of light on the slide.
- The upper part of arm of the microscope holds the body assembly to which a nose piece and one or more eyepieces or ocular lenses are attached.
- Most advanced microscopes have eyepieces for both eyes and are called binocular microscopes.
- The nose piece holds three to five objective lenses of different magnifying power and is easily rotated to position any objective.
- The image you see when viewing a specimen is focused by the objective and ocular lenses working together.
- Light from the specimen which has been illuminated is focused by the objective lens creating an enlarged image within the microscope. The ocular lens further magnifies this primary image.
- The total magnification is calculated by multiplying the objective and eye piece magnification together; e.g. if a 45x objective is used with a 10x eyepiece, the overall magnification of the specimen will be 450x.

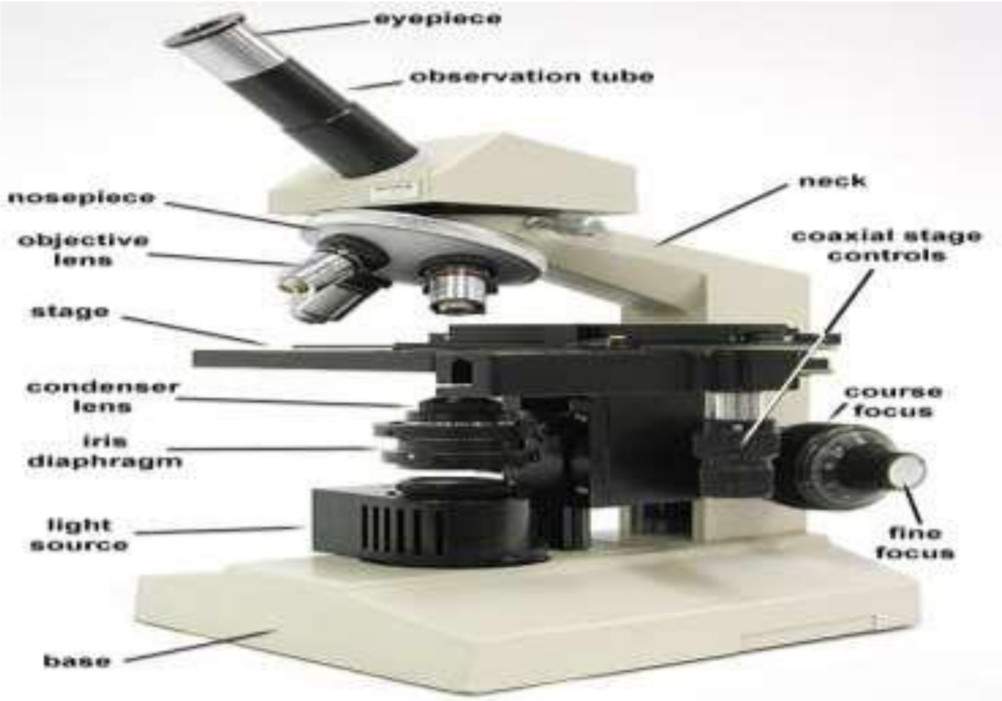


Fig. 1: The Bright Field Microscope
Source:http://biology.unm.edu/ccouncil/Biology_203/Images/Microscopes/microscope6.jpeg

The Dark-Field Microscope

- The dark field microscope is used to observe living unstained cells and organisms as a result of change in the way they are illuminated.
- A hollow core of light is focused on the specimen in such a way that unreflected and unrefracted rays do not enter the objective only light that has been reflected or refracted by the image forms an image.
- The field surrounding the specimen appears black while the object itself is brightly illuminated.
- The dark field microscope is useful in revealing many internal structures in larger eukaryotic microorganisms.
- It is also used in the examination of unstained microorganisms suspended in fluids, e.g. wet mount and hanging drop preparation.

The Phase-Contrast Microscope

- This type of microscope converts slight differences in refractive index and cell density into easily detected variations in light intensity and is used to view living cells.
- The background formed by the undeviated light is bright while the unstained objects appear dark and well defined.
- This microscope is very useful for studying microbial motility, determining the shape of living cells and detecting some bacterial components such as endospores and inclusion bodies. It is also used in studying eukaryotes.

The Fluorescent Microscope

- This type of microscope exposes a specimen to ultraviolet, violet or blue light and forms an image of the object with resulting fluorescent light.
- The most commonly used fluorescence microscope light is epifluorescence microscope which is also called incident light or reflected light microscope.
- Epifluoresence microscope employs an objective lens that also acts as a condenser. A mercury vapor arc lamp or other source produces an intense beam of light that passes through an exciter filler. The exciter filler transmits on the desired wavelength of excitation light
- The excitation light is directed down the microscope by a speed minor called the dichromatic minor.
- This minor reflects light of shorter wavelength but allows light of longer wavelength to pass through.
- The excitation light continues down through the objective lens to specimen stained with spaced dye molecules called fluorochromes.

Microscope Resolution

Resolution is the ability of a lens to separate or distinguish between small objects that are close together, i.e. the microscope must produce a clear image and not just a magnified one. It is also known as the resolving power. Resolution is described mathematically by an equation in the 1870s by Ernest Abbe, a German physicist. The Abbe equation states that the minimal distance (d) between two objects that reveal them as separate entities depends on the wavelength of light (□) used to illuminate the specimen and on the numerical aperture of the lens (nsin□) which is the ability of the lens to gather light.

$$d = \frac{0.5\square}{n\sin\square}$$

As d becomes smaller, the resolution increases and finer details can be discerned in a specimen; d becomes smaller as the wavelength of light used decreases and as the numerical aperture (NA) increases. Hence, the greatest resolution is obtained using a lens with the largest NA and light with the shortest wavelength.

The relationship between NA and resolution can be expressed as follows:

$$d = \frac{\square\square\square\square\square.}{2NA}$$

where d = resolution and □□= wavelength of light. Using the values 1.3 for NA and 0.5□□m, the wavelength of green light, for > □, resolution can be calculated as

$$d = \frac{0.55}{2 \times 1.30} = 0.21\square\square m.$$

From these calculations, we may conclude that the smallest details that can be seen by the light microscope are those having dimensions of approximately 0.2 □m.

Preparation for Light-Microscope Examination

There are two general methods used for preparing specimens for light microscope examination.

- The organisms are suspended in a liquid (the wet-mount or the hanging drop technique), and
- The organism is dried fixed and stained before observing under the microscope.

The wet mount or hanging drop technique
The technique permits examination of organisms in a normal living condition. A wet mount is made by placing a drop of fluid containing the organisms on a glass slide and covering the drop with a cover slip. Petroleum jelly may be used to provide a seal between the slide and covers slip after which the slide is viewed under the microscope.

This method is desirable because:

- It prevents distortion of the morphology of spiral bacteria when they are stained and dried.
- It reveals whether organisms are motile or not.
- Some cell inclusion bodies are easily observed.
- Spore formation and germination may also be observed in living cells.

Fixed, Stained Smears of Microorganisms
These are frequently used for the observation of the morphological characteristics of bacteria. The procedure makes the cell more clearly visible, and differences between cells of different species and within the same species can be demonstrated. The essential steps in this procedure are:

- Preparation of the film or smear
- Fixation and
- Application of one or more staining solution.

Fixation
Fixation is the process by which the internal and external structures of cells and microorganisms are preserved and fixed in position. It inactivates enzymes that might disrupt cell morphology and tough cell structures so that they do not change during staining and observation. A microorganism usually is killed and attached firmly to the microscope slide during fixation.

There are two fundamentally different types of fixation.
Heat Fixation: Is routinely used to observe prokaryotes. Typically, a film of cells (a smear) is gently heated as a slide is passed through a flame. Heat fixation preserves overall morphology but not structures within cells.

Chemical Fixation: Is used to protect fine cellular sub-structure and the morphology of larger, more delicate micro organisms. Chemical fixatives penetrate cells and react with cellular components, usually proteins and lipids, to render them inactive, insoluble, and immobile. Common fixative mixtures contain such components as ethanol, acetic acid, mercuric chloride, formaldehyde, and glutaraldehyde.

Staining of Specimens
Although living microorganisms can be directly examined with the light microscope, they often must be fixed and stained to increase visibility, accentuate specific morphological features, and preserve them for future study.

Types of Staining
Simple Staining: This is a kind of staining in which a single stain or dye is used. Basic dyes such as crystal violet, methylene blue, and carbolfuchsin are used in simple staining to determine the size, shape and arrangement of prokaryotic acids.

Differential Staining: These are staining procedures that make visible the differences between bacterial cells or part of a bacterial cell. It usually involves more than one dye used for staining.

Gram Staining: The Gram stain was developed in 1884 by the Danish physician Christian Gram. It is the most widely used differential staining procedure.

The steps involved are as follows:

- The smear is stained with the crystal violet (which is the primary stain).
- This followed by treatment with iodine functioning as a mordant.
- The smear is decolourised by washing with ethanol or acetone.
- The smear is counterstained with a simple dye safranin.

Bacteria stained by the gram stain method fell into two groups:
1. Gram positive bacteria which retain the crystal violet and appear deep violet in colour and Gram negative bacteria which, lose the crystal violet and are counterstained with safranin appear red in colour.
2. Acid Fast Staining: This is another differential staining procedure commonly used to identify mycobacterium tuberculosis and mycobacterium leprae, the pathogens responsible for tuberculosis and leprosy respectively.

These bacteria have cell walls with high lipid content in particular, mycolic acid which prevents dye from readily binding to the cells. In the acid fast staining procedure, the red stain and carbol fuchsin is used as primary stain; next acid-alcohol is used as a decolouriser. The acid-alcohol will remove the red stain form bacteria such as Escherichia coli which the acid fast mycobacteria will remain red.

3.2 Electron Microscope

This type of microscope uses a beam of electron in place of light waves to produce the image. There are two types:

1. Scanning electron microscope
2. Transmission electron microscope.

The Transmission Electron Microscope

Electron microscopes use a beam of electrons to illuminate and create magnified images of specimens. Electrons replace light as the illuminating beam. They can be focused, much as light is in a light microscope, but their wavelength is around 0.005mm approximately 1000,000 times shorter than that of visible light. Therefore, electron microscopes have a practical resolution roughly 1,000 times better than the light microscope, with many electron microscopes point closer than 0.5nm can be distinguished, and the useful magnification is well over 100,000x. In transmission electron microscope, the electron beam is transmitted through the specimen.

The Scanning Electron Microscope

The scanning electron microscope produces an image from electron released from atoms on an object's surface. It has been used to examine the surfaces of microorganisms in great detail. Many SEM has a resolution of 7nm or less.

4.0 Conclusion

In this unit, we learnt that electron microscope uses a beam of electron in place of light waves to produce the image of an object. The ordinary compound microscope is called the bright field microscope because if forms a dark image against a bright background. In the bright field microscope which is a compound the primary image is formed by an objective lens and enlarged by the eye piece or ocular lens to form the final image. We also discussed that Electron microscopes use a beam of electrons to illuminate and create magnified images of specimens.

5.0 Summary

In this unit, you have learnt about the following:

- The light microscope
- Electron microscope.

6.0 Self-Assessment Exercise

Activity: Prepare two specimens for light microscope examination.

Answer the following questions

1. Define microscope (LO1).
2. With the aid of a well label diagram describe a bright field microscope (LO3)
3. What is microscope resolution? (LO4).
4. List the stages involved in preparing a specimen for observation under the light microscope (LO5).
5. What is the basic difference between a transmission electron microscope and a scanning electron microscope? (LO2).
6. Define the resolving power (LO4).
7. List 5 parts of a light microscope and state the function of each (LO3).

7.0 References /Further Reading

Atlas, R.M. (1995). *Microorganisms in our World*. Mosby Year Book. Inc.

Medigan, M.T., *et al*. (2009). *Brock Biology of Microorganisms*. (12th ed.). Pearson Education Inc.

Pelczar, M.J., Chan, E.C.S. & Krieg, R.N. (2001). *Microbiology*. (5th ed.).McGraw-Hill.

Prescott, Harley & Kleins. *Microbiology*. (7th ed.). Boston Bur Bridge, IL: McGraw-Hill Higher Education.

http://biology.unm.edu/ccouncil/Biology_203/Images/Microscopes/microscope6.jpeg.

Unit 5 A Brief Survey of Microbes as Friends and Foes

1.0 Introduction

Microorganisms occur in large numbers of most natural environments and bring about many changes. Some are desirable and others are undesirable. Microorganisms affect the wellbeing of people in many ways. Many are beneficial to man and can be called 'friends' while some are harmful and can be regarded as 'foes' to man. The beneficial impact of microorganisms ranges from the production of goods and pharmaceutical products, to enhancement of soil fertility, environmental cleanup while their harmful effect can be seen in their ability to cause disease in man, animals and plants as well as their usage in biological warfare. However, there are more species of microorganisms that perform friendly and beneficial functions than those that harm other living organisms. This unit gives us a brief survey of microorganisms as friends and foes.

2.0 Objectives

At the end of this unit, you will be able to:

- explain the different ways in which microorganisms can act as friends to man
- explain ways in which microorganisms can act as foes to man.

3.0 Main Content

3.1 Microorganisms as Friends

Microorganisms have found application in various aspects of life. They are useful in food industries to produce many food substances, in medicine to produce vaccines and antibiotics, in environmental protection, and in agriculture to optimize yield. Few of these various aspects will be discussed in this material.

Microorganisms and Food Production

- Many microorganisms are used to produce many of the foods and beverages we consume. Microbially-produced food products have properties that are very different from those of the starting materials. Most of these food products are produced by fermentation.
- Fermentation is the chemical transformation of organic compounds carried out by microorganisms and their enzymes. In industrial fermentation, raw materials (substrate) are converted by microorganisms in a controlled favourable environment (created in a fermentor) to form a desired end product substance.
- The accumulation of fermentation products such as ethanol and lactic acid produces characteristic flavours and other desirable properties in food substances.
- Pickles and some sausages are also produced by fermentation processes.
- Microorganisms are used to produce fermented dairy products such as cheese, yoghurt and acidophilus milk.

- They are also used to produce alcoholic beverages such as beer by conversion of sugar to alcohol and carbon dioxide.
- Wine fermented from fruits using yeast strains Saccharomyces cerevisiae and bread is also produced by using yeasts.
- Microorganisms can also be used as direct source of food known as single cell protein. Various species of yeasts, algae are grown as single cell protein and use as animal feeds thus helping to meet the world food needs.

Production of Pharmaceuticals

Microorganisms are used to produce different pharmaceuticals such as antibiotics, steroids vitamins, hormones, etc. Antibiotics are microbially produced substances or substances synthetically derived from natural sources that inhibit or kill microorganisms, Steroids regulate various aspects of human metabolisms and are produced by organisms such Rhizopus nigricans.

Vaccines are produced using microorganisms with the antigenic properties to elicit a primary immune response; they are used to prevent many once deadly diseases such as polio, small pox, tuberculosis, measles, diphtheria and whooping cough.

Vitamins

Vitamins are essential animal nutritional factors; some vitamins are produced by microbial fermentation, e.g. Vitamin B12 by Streptomyces, B12 by Pseudomonas denitrificans and Propionibacterium shermanni. Riboflavin produced by various species of Clostridium and Ashbya gossypii. Human insulin and human growth hormone are produced by genetically engineered bacteria.

Production of Organic Acids

Various organic acids are produced by microorganisms examples are:

1. Gluconic Acid: used as a pharmaceutical to supply calcium to the body by several fungi including penicillium and aspergillus species. Citric acid produced by Aspergillus niger and used as a food additive especially in the production of soft drinks.

2. Lactic Acid by different lactic acid bacteria for example, Lactobacillus delbrueckii, lactic and is used in foods as preservatives, in leather production for deliming hides and in the textile industry for fabric treatment, plastics making in baking powders.

Hygiene

- Hygiene is the avoidance of infection and food spoilage by eliminating microorganisms from the surrounding.
- Our knowledge of how disease causing microorganisms spread has permitted us to reduce the incidence of many diseases. Also improved sanitation practices have helped to reduce the incidence of diseases.
- Microorganisms from the surroundings can be totally removed by methods such as sterilisation or reduced to acceptable levels using methods such as disinfection and antisepsis. In food preparation, microbes are reduced to acceptable levels using methods such as pasteurisation, addition of vinegar. While complete sterility is achieved by autoclaving or irradiation.

Useful in the Study of Science

Microbes are essential tools in biotechnology, biochemistry, genetics molecular biology and genomics. Examples are the yeasts (Saccharomyces cerevisiae) and fission yeast (Shizosaccharomyces pombe) which are model organisms in science. They can easily be

grown rapidly in large quantities and are easily manipulated.

Biotechnology uses genetic engineering which is the artificial manipulation of genes and gene products.

Genes from any source can be manipulated and modified using microorganisms and their enzymes as molecular tools, e.g. human insulin, a hormone which is very low in people with diabetes is produced by genetically engineered bacteria into which human genes have been inserted.

Microorganisms and the Environment

- Microorganisms can be used to clean up pollution created by human activities in a process called bioremediation.
- Pollutants such as pesticides, spilled oil solvents which could pose human health hazard are degraded to nontoxic substances by microorganisms.
- Microorganisms are used to degrade wastes and pollutants so as to maintain and restore environmental quality.

3.2 Microorganisms as Foes

Microorganism can act as foes to man and other living organisms by causing diseases and by their usage as biological weapons.

Microorganisms as Disease Agents

Microbial diseases are still the major cause of death in many developing countries. Microorganisms cause different diseases in man such as:

- AIDS (Acquired Immune Deficiency Syndrome) caused by the Human Immuno Deficiency Virus (HIV).
- Tuberculosis caused by a bacterium, mycobacterium tuberculosis.
- Cholera caused by a bacteria Vibrio cholera.
- Malaria caused by four species of the protozoa called plasmodium transmitted by the female anopheles mosquito.
- Other emerging diseases include: bird flu and swine flu.

Microorganisms as Agents of Warfare and Terrorism

Biological warfare is also known as germ warfare. It is the use of pathogens such as viruses, bacteria, or the toxins produced by them as biological weapons or agents of warfare. A biological weapon may be used to kill, incapacitate or seriously impair a person, group of people or even an entire population. It can be used as a military technique by nations during wars. There are four kinds of biological warfare agents, bacteria, viruses, fungi and rickettsiaes. They are living organisms that reproduce with their host victims who then become contagious with a deadly if weakening multiple effects. Toxins on the other hand do not reproduce in the victims but within a short incubation period (usually with a few hours) kill the victims.

4.0 Conclusion

In this unit, we have discussed Microorganisms in details. Their usefulness as well as their potential disadvantages were also treated.

5.0 Summary

In this unit, you have learnt about the following:

- Microorganisms as Friends
- Microorganisms as Foes.

6.0 Self-Assessment Exercise

Activity: mention at least five microorganisms different from the ones discussed in this text can cause diseases in man.

Answer the following questions

1. Explain the role of microorganisms in food production (lo1).
2. Explain the role of microorganisms' production of pharmaceuticals (lo1).
3. Describe the various organic acids produced by microorganisms (lo1).
4. Outline the relevant of microorganism in hygiene (lo1).
5. Explain the usefulness of microorganisms in the study of science (lo1).
6. What are the relevant of microorganisms to the environment (lo1).
7. Explain two ways in microorganisms are harmful to man (lo2).

7.0 References/Further Reading

Atlas, R.M. (1995). *Microorganisms in Our World*. Mosby Year Book. Inc.

Gray, N. F. (2004). *Biologica l of Waste Water Treatment*. Imperial College Press.

Medigan, M.T., *et al*. (2009). *Brock Biology of Microorganisms*. (12th ed.). Pearson Education Inc.

Pelczar, M.J., Chan, E.C.S. & Krieg, R.N. (2001). *Microbiology*. (5th ed.). McGraw-Hill.

Pimental, D. (2007). *Food, Energy and Society*. CRC Press.

Prescott, H. & Kleins. *Microbiology*. (7th ed.). Boston Bur Bridge, IL: McGraw-Hill Higher Education.