COURSE GUIDE

PHS 217
GENERAL MICROBIOLOGY

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INTRODUCTION

General Microbiology is a first semester course. It is a three -credit unit compulsory course which all students offering Bachelor of Science in Biology must take.

Microbiology is a branch of biology which involves the study of microorganisms. Microorganisms can be defined as living organisms which cannot be seen by the unaided eyes. These organisms include bacteria, fungi, algae, protozoa, viruses, etc.

Microorganisms are numerous in nature and have some characteristics which make them ideal specimens for the study of numerous fundamental like processes which occur at the cellular level in all living organisms. In microbiology, study of microorganisms is done extensively by observing their life processes while they are actively metabolising.

Microorganisms have a wider range of physiological and biochemical potentials than all other organisms combined. Some are able to utilize atmospheric nitrogen for the synthesis of proteins and other complex organic nitrogen compounds. The study of microorganisms is applicable to all aspects of human endeavour including: medicine, food, agriculture, conserving human and animal reaction, combating diseases and used also as biological weapons. Some organisms are friends (beneficial) while others can be regarded as foes (harmful) to human beings.

WHAT YOU WILL LEARN IN THIS COURSE

In this course, you have the course units and a course guide. The course guide will tell you what the course is all about. It is a general overview of the course materials you will be using and how to use those materials. It also helps you to allocate the appropriate time to each unit so that you can successfully complete the course within the stipulated time limit. The course guide also helps you to know how to go about your Tutor-Marked Assignment which will form part of your overall assessment at the end of the course. Also, there will be regular tutorial classes that are related to this course, where you can interact with your facilitator and other students. This course exposes you to microbiology, a very important and interesting field in biology.

COURSE AIMS

The course aims to give you an understanding of microbiology which is an important branch of biology.

COURSE OBJECTIVES

To achieve the aim set above, there are objectives. Each unit has a set of objectives presented at the beginning of the unit. These objectives will give you what to concentrate/focus on while studying the unit. Please read the objectives before studying the unit and during your study to check your progress.

The comprehensive objectives of the course are given below. By the end of the course, you should be able to:

- identify the different components of the microbial world
- explain the historical aspects, relevance and scope of microbiology
- explain the general characteristics of the different groups of microorganisms
- describe microbial growth and reproduction and methods of controlling microbial growth
- give a systemic classification of bacteria, fungi, viruses, etc.
- explain the causes of microbial variation and hereditary
- describe some biogeochemical cycles in nature.

WORKING THROUGH THIS COURSE

To successfully complete this course, you are required to read each study unit, textbooks and other materials provided by the National Open University of Nigeria. Reading the referenced materials can also be of great assistance. Each unit has self- assessment.

Exercises which you are advised to do. At certain periods during the course, you will be required to submit your assignment for the purpose of assessment. There will be a final examination at the end of the course. The course should take you about 17 weeks to complete. This course guide will provide you with all the components of the course how to go about studying and how you should allocate your time to each unit so as to finish on time and successfully.

THE COURSE MATERIALS

The main components of the course are:

1. The Study Guide
2. Study Units
3. Reference/Further Reading
4. Assignments
5. Presentation Schedule

STUDY

The study units in this course are given below:

Module 1 Introduction to Microbiology

Unit 1 Composition of the Microbial World
Unit 2 Historical Aspects of Microbiology
Unit 3 The Relevance and Scope of Microbiology

Module 2 General Characteristics of Microorganisms

Unit 1 Microscopy and Specimen Preparation
Unit 2 A Brief Survey of Microbes as Friends and Foes
Unit 3 General Characteristics of Bacteria
Unit 4 General Characteristics of Fungi

Module 3 General Characteristics of Microorganisms Contd

Unit 1 General Characteristics of Viruses
Unit 2 General Characteristics of Algae
Unit 3 General Characteristics of Protozoa

Module 4 Microbial Growth, Reproduction and Control

Unit 1 Microbial Growth
Unit 2 Measurement of Microbial Growth and Factors that Influence Microbial Growth
Unit 3 Physical Methods of Controlling Microbial Growth
Unit 4 Chemical Methods of Controlling Microbial Growth

Module 5 Systematic Classification of Microorganisms

Unit 1 Introduction to Systemic Classification of Microorganisms
Unit 2 Systematic Classification of Bacteria
Unit 3 Systematic Classification of Fungi
Unit 4 Systematic Classification of Algae

Module 6 Systematic classificationcontd/Microbial Genetics and Biogeochemical Cycling of Elements

Unit 1 Systematic Classification of Protozoa
Unit 2 Mechanisms of Genetic Variation and Hereditary
Unit 3 Biogeochemical Cycling of Elements

In module one, unit one deals with the historical aspects of microbiology, the second unit focuses on the meaning of microbiology, microorganisms as *cells*, and the different groups of microorganisms, and the domains in which they are placed. The third unit focuses on the relevance and scope of microbiology. The module 2 unit 1 focuses on the use of difference microscopes to study microorganisms while unit 2 is a brief survey of microorganisms as friends and foes. Module 3 continued with the general characteristics of microorganisms. While in module 4, units 1 and 2 discusse microbial growth and reproduction,unit 3 deas with measurement of microbial growth and factors that influence microbial growth. Module 5 deals with systematic classification of microorganisms. In module 6, unit one continues with classification while unit 2 is on microbial variation and hereditary while unit3 focuses on biogeochemical cycling of nutrients in nature. Each unit will take a week or two. Lectures will include an introduction, objectives, reading materials, self -assessment exercises, conclusion, summary, tutor-marked assignments (TMAs), references and other reading resources. There are activities related to the lecture in each unit which will help your progress and comprehension of the unit. You are required to work on these exercises which together with the TMAs will enable you to achieve the objectives of each unit.

PRESENTATION SCHEDULE

There is a timetable prepared for the early and timely completion and submissions of your TMAs as well as attending the tutorial classes. You are required to submit all your assignments by the stipulated date and time. Avoid falling behind the schedule time.

ASSESSMENT

There are three aspects to the assessment of this course. The first one is the self-assessment exercises. The second is the tutor-marked assignments and the third is the written examination or the examination to be taken at the end of the course. Do the exercises or activities in the unit by applying the information and knowledge you acquired during the course. The tutor-marked assignments must be submitted to your facilitator for formal assessment in accordance with the deadlines stated

in the presentation schedule and the assignment file. The work submitted to your tutor for assessment will account for 30% of your total course work. At the end of this course, you have to sit for a final or end of course examination of about a three hour duration which will account for 70%of your total course mark.

TUTOR-MARKED ASSIGNMENT

This is the continuous assessment component of this course and it accounts for 30% of the total score. You will be given 4 TMAs by your facilitator to answer. Three of which must be answered before you are allowed to sit for the end of course examination. These answered assignments must be returned to your facilitator. You are expected to complete the assignments by using the information and material in your reading references and study units. Reading and researching into the references will give you a deeper understanding of the subject.

1. Make sure that each assignment reaches your facilitator on or before the deadline given in the presentation schedule and assignment file. If for any reason you are not able to complete your assignment, make sure you contact your facilitator before the assignment is due to discuss the possibility of an extension. Request for extension will not be granted after the due date unless there in exceptional circumstances.
2. Make sure you revise the whole course content before sitting for the examination. The self-assessment exercises and TMAs will be useful for this purposes and if you have any comments pleased o before the examination. The end of course examination covers information from all parts of the course.

FINAL EXAMINATION AND GRADING

The end of Course of Examination for Introduction to General Microbiology will be for about 2 hours and it has a value of 70 percent of the total course work. The examination will consist of questions, which will reflect the type of self-testing, practice exercise and tutor-marked assignment problem you have previously encountered. All areas of the course will be assessed.

You are advised to use the time between finishing the last unit and sitting for the examination to revise the whole course . You might find it useful to review your self-tests, TMAs and comments on them before the examination. The end of Course examination covers information from all parts of the Course .

COURSE MARKING SCHEME

Assignment Marks

Assignments 1 – 4 Four assignments, best three marks of the four count at 10% each- 30% of course marks.

End of course examination 70% of overall course marks.

Total 100% of course materials.

FACILITATORS/TUTORS AND TUTORIALS

Sixteen hours are provided for tutorials for this course. You will be notified of the dates, times and location for these tutorial classes. As soon as you are allocated a tutorial group, the name and phone number of your facilitator will be given to you. These are the duties of your facilitator:

He or she will mark and comment on your assignment.

He will monitor your progress and provide any necessary assistance you need.

He or she will mark your TMAs and return to you as soon as possible. Do not delay to contact your facilitator by telephone or e-mail for necessary assistance if:

you do not understand any part of the study in the course material.

you have difficulty with the self -assessment activities.

you have a problem or question with an assignment or with the grading of the assignment. It is important and necessary you attend the tutorial classes because this is the only chance to have face to face contact with your facilitator and to ask questions which will be answer instantly. It is also a period where you can point out any problem encountered in the course of your study.

SUMMARY

General Microbiology is a course that introduces you to the microbial world around us.

Biology is a field very important to your welfare or well -being in both positive and negative ways. Microorganisms are cellular and a cellular

(viruses) entities which are capable of life processes found in plants and animals. On completion of this course, you will have an understanding of basic knowledge of microorganisms, the history of men and women who contributed to this field of study by their discoveries during their research works. You also learn the general characteristics of microorganisms, microbial growth and reproduction, how the microorganisms are classified or placed in different groups, theme chanisms of variation and hereditary in microorganisms and the role of microorganisms in cycling elements in our environments. In addition, you will be able to answer the following questions:

What is microbiology?

What are microorganisms?

Identify the different groups of microorganisms and their general characteristics.

Explain the relevance and scope of microbiology.

Differentiate between phyletic classification and phylogenetic classification.

What are point mutations?

What are frame shift mutations?

Describe the four phases of the growth curve in a closed system. The list of questions you are expected to answer is not limited to the above list. Finally, you are expected to apply the knowledge you have acquired during this course to your practical life. I believe you will agree with me that microbiology is a very interesting field of biology with a wide application to life. I wish you success in this course.

MAIN COURSE

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MODULE 1 INTRODUCTION TO MICROBIOLOGY

UNIT 1 COMPOSITION OF THE MICROBIAL WORLD

CONTENTS

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; they are prokaryotic and eukaryotic.

i. **Prokaryotes**
These microbial cells lack membrane-bound nucleus and organelles.
ii. **Eukaryotes**
Possess a membrane-bound nucleus and organelles.
iii. **Differences between Prokaryotic and Eukaryotic Cells**
There are notable differences between prokaryotic and eukaryoticcells. These are shown in detail in table 1.

Table 1: Differences between Prokaryotic and Eukaryotic Cells

Features	**Prokaryotic cell**	**Eukaryoticcell**
Plasma membrane	Present	Present
Internal organelles (membrane bound)	Absent	Present
Genetic (hereditary)molecule	DNA as a single circular bacterialchromosome not enclosed in a nucleus	DNA as multiple linear Chromosomes enclosed within a nucleus
Site of energy(ATP) Generation	Cytoplasm and, in some cases, plasma membrane or Photosynthesis membrane	Cytoplasm and mitochondria or chloroplasts.

Source: Atlas *et al*., (1995)

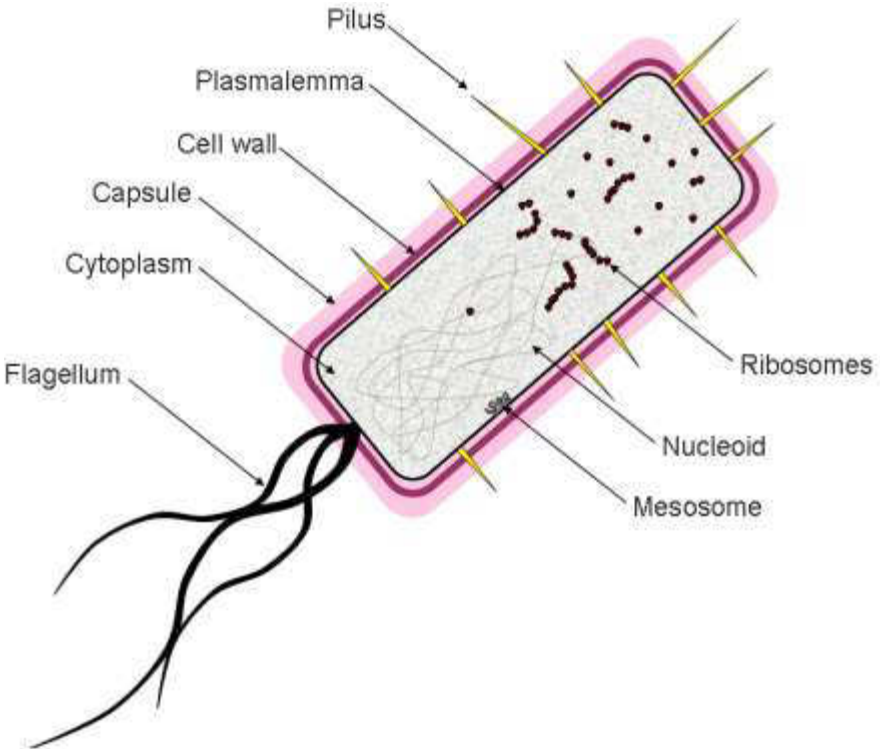


Fig. 1(a): A Prokaryotic Cell
Source: www.fluwiki.info/pmwiki. php?n=Science.Glossary

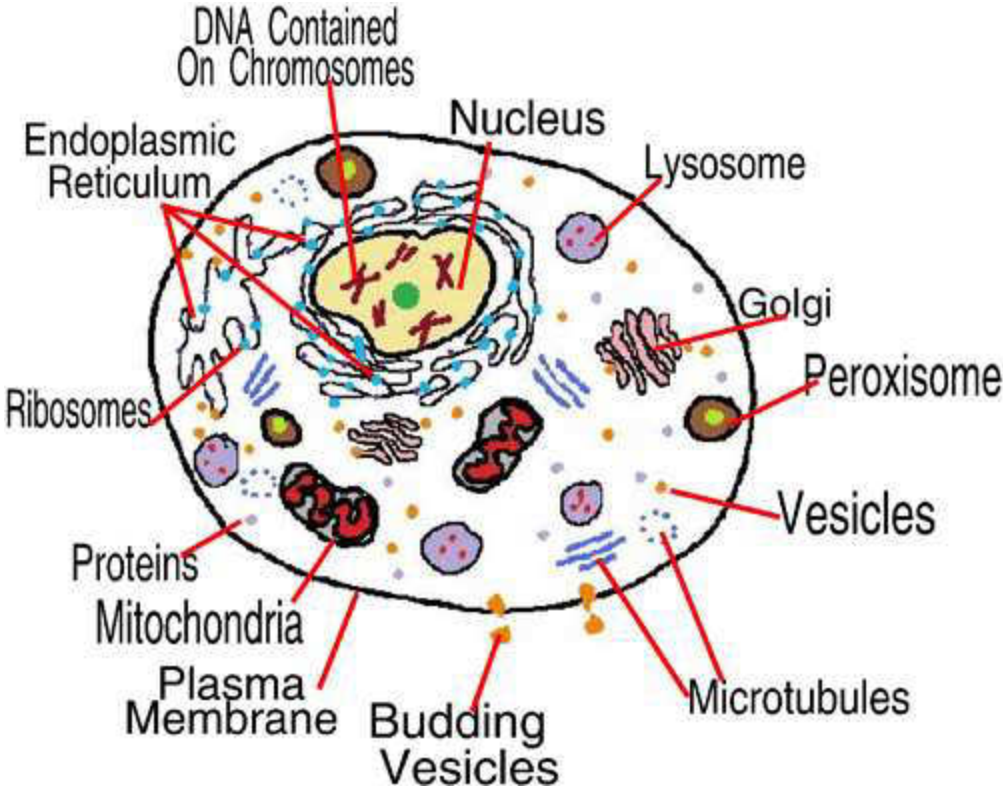


Fig. 1(b): A Eukaryotic Cell

SELF-ASSESSMENT EXERCISE

i. Define the term, microorganism.
ii. Compare and contrast prokaryotic and eukaryotic cells.

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:
1. Monera
2. Protista
3. Fungi
4. Planta
5. Animalia

Microorganisms except for viruses, which are cellular 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)

3.4 Domain Bacteria

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

3.5 Domain Archaea

1. They were formerly known as archaeobacteria.
2. They are prokaryotic.
3. They are single celled organisms.

4. They lack membrane bound nucleus and organelles.
5. They lack peptidoglycan in their cell walls.
6. They have unique membrane lipids.
7. Some have unusual metabolic characteristics, e.g. methanogens which generatemethane gas.
8. Many are found in extreme environments.

Domain archaea is distinguished from bacteria based upon

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

SELF-ASSESSMENT EXERCISE

i. List the three domains under which microorganisms are classified.
ii. List three characteristics each of the following domains:
    a. Bacteria
    b. Archaea
    c. Fungi

3.6 Domain Eucarya

The major groups of microorganism in this domain are protists andfungi.

Protists

These groups of microorganisms are unicellular algae, protozoa, slimemoulds and water moulds.

Algae

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

Protozoa

1. They are unicellular.
2. Eukaryotic organisms and animal like.
3. They are usually motile.

4. 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

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

3.7 Viruses

1. They are acellular entities (non- cellular).
2. They lack the fundamental structure of living cell but only carryout functions of living organisms when in living cells.
3. They are the smallest of all the microorganisms (10,000 smaller than a typical bacterium).
4. They can only be seen by the electron microscope.
5. They cause many diseases of plants, animals and humans.
6. 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

Microbiology is the study of microorganisms, most of which are unicellular while some are multicellular. Presently, they are classified under three domains. Viruses are classified under a separate system because they function only as living things when present in living organisms.

5.0 SUMMARY

In this unit, we have learnt that:

- microbiology is the study of microorganisms
- microorganisms are organisms too small to be seen with the unaided eyes
- microorganisms include: bacteria, fungi, algae, protozoa and viruses
- microorganisms may be prokaryotic which lack a membrane bound nucleus oreukaryotic which have a membrane boundnucleus but undifferentiated tissues
- microorganisms are grouped into three domains: bacteria, Archaea and eucarya
- the domain bacteria and archaea are simple and prokaryotic microorganisms. While the domain eucarya consists of the protists and fungi which are eukaryotic microbes
- viruses are acellular entities and are not placed in any of the domain but are classified on a separate system.

6.0 TUTOR-MARKED ASSIGNMENT

1. Define the term microbiology.
2. Distinguish between a prokaryotic cell and eukaryotic cell.
3. What are the differences between bacteria and archeae?
4. Why are viruses not placed in any of the domains?

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*. (5thed.).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

CONTENTS

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
- describe Koch's postulate and how they are used to establish a link between a suspected microorganisms and the disease
- state the discovery of microbial effect on organic and inorganic matters
- explain development of microbiology in this century
- explain the era of molecular biology.

3.0 MAIN CONTENT

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.

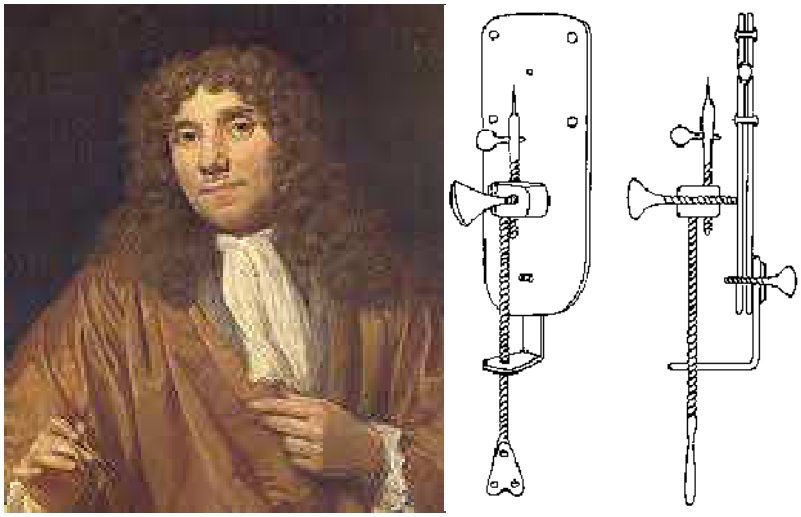
1. Robert Hooke (1635-1703), an English mathematician and natural historian.
    - He coined the term "cells" to describe the "little boxes" he observed in examining cork slices with a compound microscope.
    - He was the first to make a known description of microorganisms.
    - He made microscopic 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 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 300times.

- 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.
- He was the first person to publish extensive and accurate observations of microorganisms.
- He is known as the father of bacteriology.



a. **b.**

Fig. 2: (a) Antony Leeuwenhoek (1632-1723) Holding one of his Microscopes
(b) Leeuwenhoek’s Microscopes and some of theSketches 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.

3.1 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 on- 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: 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 a biogenesis.

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 sterilisation. 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. Figure 3 shows the defeat of spontaneous generation.

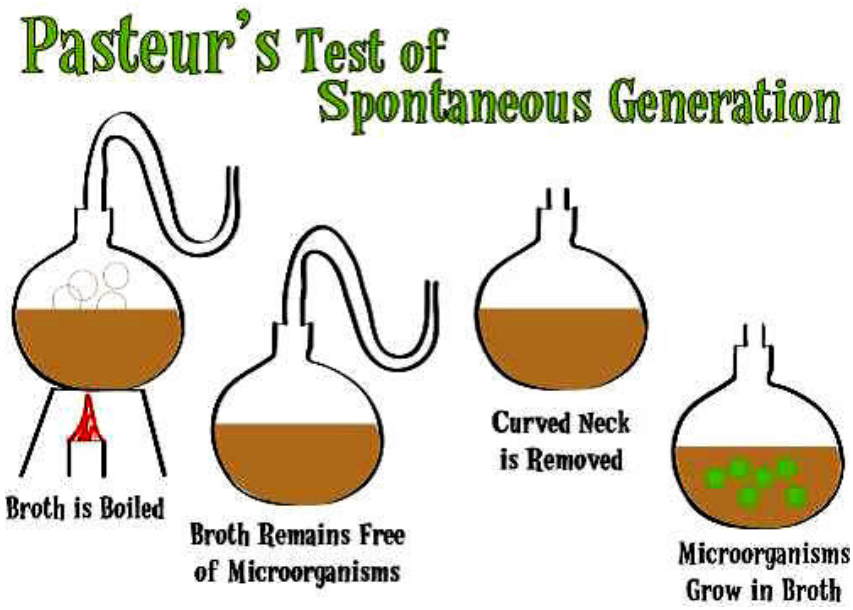


Fig. 3: The Defeat of Spontaneous Generation - Pasteur's Experiment with the Swan- Necked Bottles

Source: Amoebamike.wordpress.com

Apart from the defeat of the concept of spontaneous generation:

- Pasteur's work led to an effective sterilization method which involve holding juices and milk at 62.8OC (145OF) for 30 minutes known as Pasteurization.
- He discovered that alcoholic fermentation was catalyzed 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.

3.2 The Recognition of the Role of Microorganisms in Disease

1. **Agostino Bassi (1773-1856)** showed that a silkworm disease was caused by a fungus.
2. **M. J. Berkerley (ca. 1845)** demonstrated that the great potatoblight of Ireland was caused by a fungus.
3. **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.

4. **Robert Koch (1843-1910):** Robert Koch 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 small amount of blood from a diseased mouse was injected into healthy mouse, the healthy mouse quickly developed anthrax. From this work he developed Koch's postulates.

5. **Koch's postulates are:**

- The suspected disease-causing organism should be present in allcases of the disease and absent from healthy animals.
- The suspected organism must be cultivated in a pureculture away from the animalbody.
- The isolated organism must cause the disease wheninoculated into a healthy susceptible animal.
- The organism must be re-isolated from these experimentalanimals and culture again in the laboratory after which itshould still be the same as the original organism.
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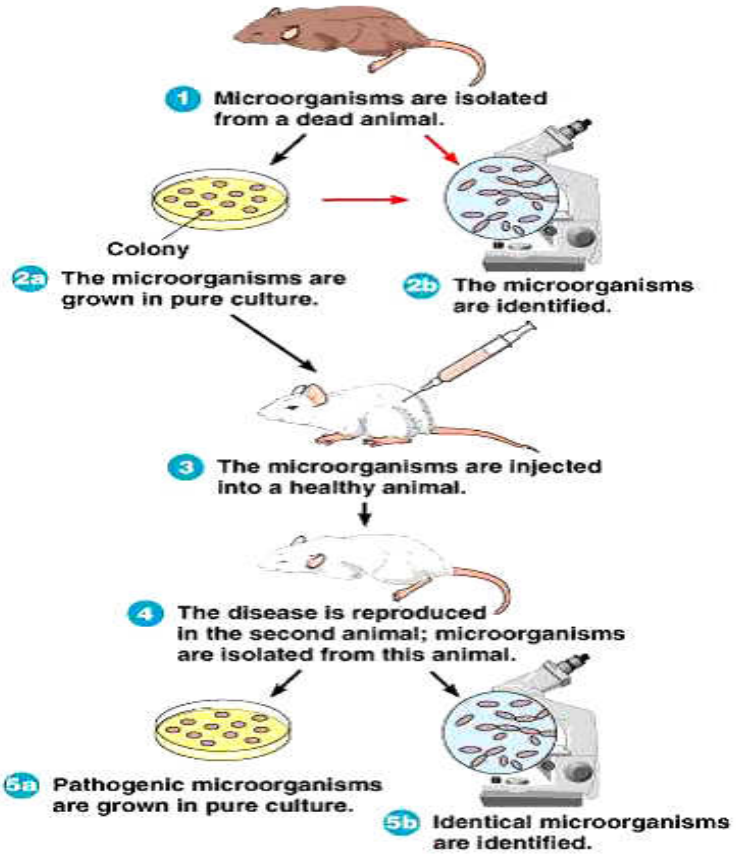

Fig. 4: Diagrammatic Illustration of Koch's Postulate

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 1905Nobel Prize for Physiology or Medicine.

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

6. Edward Jenner (ca. 1798) used a vaccination procedure to protect individuals from smallpox.
7. 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.

3.3 The Discovery of Microbial Effects on Organic andInorganic Matter

1. **Martinus Beijerinck (1851-1931)**
   - Martinus Beijerinck was a Professor at the Delftm Polytechnic.
   - He isolated the first pure culture of many soil and aquatic microorganisms, including sulphate reducing and sulfur oxidizing bacteria, nitrogen fixing root nodule bacteria.
   - He described the first virus and the basic principles ofvirology.

2. **Sergei Winogradsky (1856-1953)**
   - He proposed the concept of chemo-lithotrophy, (the oxidation of inorganic matter). He worked with soil bacteria and discovered they could oxidise iron, sulphur and ammonia to obtain energy. He also studied anaerobic nitrogen fixation and cellulose decomposition. He published many scientific papers and a major monograph,*Microbiologic du sol* (Soil Microbiology).
   - **Beijerinck and Winogradsky** pioneered the use of enrichment cultures and selective media.

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 spontaneous 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

Began in the 1970s.

- 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

The study and development of microbiology as a field in science became possible due to the works and contributions of men who discovered microorganisms, disproved the theories of spontaneous generation and established the relationship between microorganisms and disease among other things.

5.0 SUMMARY

- **Robert Hooke (1635-1703) and Anthony Van Leeuwenhoek(1632-1723)** contributed to the discovery of microorganisms through the use of microscope.
- Experiment by **Francesco Redi** and others disproved the theory of spontaneous generation.

- **Louis Pasteur** defeated the theory of spontaneous generation.
- **Robert Koch** developed postulate to establish relationship between a suspected microorganism and disease.
- **Serge Winogradsky** and **Martinus Beijerinck** discovered microbial effect on organic and inorganic matter both of them pioneered the use of enrichment culture and selective media.
- **George Beadle** and others contributed to the development of microbiology.
- In the twentieth century, era of molecular microbiology began inthe 1970s and led to the field of biotechnology.
- In the 1990s, DNA sequencing gave birth to the field of genomics.

6.0 TUTOR-MARKED ASSIGNMENT

1. Explain Anthony Van Leeuwenhoek contribution to the discovery of microorganisms.
2. (a) State the concept of spontaneous generation.
   (b) Explain the steps involved in using Koch's postulate to establish the link between a suspected microorganism and a disease.

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UNIT 3 THE RELEVANCE AND SCOPE OF MICROBIOLOGY

CONTENTS

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:

- define microbiology
- state the two branches of microbiology
- differentiate areas of study in basic and applied microbiology.

3.0 MAIN CONTENT

Main Branches of Microbiology

Microbiology has two main branches:
1. Basic
2. 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:

1. **Bacteriology**: This is the study of bacteria.
2. **Mycology**: The study of fungi such as yeasts, moulds, and mushrooms.
3. **Algology**: The study of algae.
4. **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 macroorganisms.
5. **Microbial Cytology**: Studies the structures of microbial cells.
6. **Microbial Physiology**: Studies of the nutrients that microorganisms require for metabolism and growth and the products that they make from nutrients.
7. **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.
8. **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.
9. **Microbial Taxonomy:** This is the study of the classification of microorganisms or the grouping of microorganisms.
10. **Biochemistry**: This deals with the discovery of microbial enzymes and the chemical reactions they carry out.

SELF-ASSESSMENT EXERCISE

List 5 basic areas of research in microbiology and state what each area entails.

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 wastewater 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. **Aero-microbiology**: Advances thought in the dissemination of diseases in the air, contamination and spoilage.

9. **Exo-microbiology**: 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.

SELF-ASSESSMENT EXERCISE

State the importance of microbiology in five different fields of human endeavours.

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 InTechnology 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

Microbiology is one of the most rewarding professions because it givesits practitioners the opportunity to be able to be in contact with all other natural sciences and thus contribute in many different ways to the betterment of human life. One indicator of the relevance and importance of microbiology is reflected in the number of Nobel Prizewinners in science - one third of all awardees are microbiologists or investigators using a microbial model.

5.0 SUMMARY

- Modern microbiology is a large discipline with many different specialised areas. Microbiology 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, etc.
- 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.

6.0 TUTOR-MARKED ASSIGNMENT

1. List the fields of microbiology that deal with the following:
   a. Metabolism
   b. Enzymology
   c. Nucleic Acid and Protein Synthesis
   d. Microorganisms in the Natural Environment
   e. Microbial Classification
   f. Microbial Cell Structure
2. Explain what the field of agricultural microbiology entails.

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MODULE 2 GENERAL CHARACTERISTICS OF MICROORGANISMS

UNIT 1 MICROSCOPE AND SPECIMEN PREPARATION

CONTENTS

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 micro organisms 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 x100to 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; and
- 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.

Types of Microscopes

Microscopes are of two types:

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:

1. Bright Field Microscope
2. Dark Field Microscope
3. Fluorescence Microscope
4. 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.

3.1.1 The Bright Field Microscope

1. The ordinary microscope is called a bright field microscope because it forms a dark image against a brighter background.
2. 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.
3. A light source, either a mirror or an electric illuminator, is located at the base.

4. Two focusing knobs, the fine and coarse adjustment knobs are located on the arm and can move either the stage or the nosepiece to focus the image.
5. The stage is positioned about halfway up the arm and hold microscope slides by slide clips or a mechanical stage clip.
6. There is a sub-stage condenser mounted within or beneath the stage which focuses a core of light on the slide.
7. 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.
8. Most advanced microscopes have eyepieces for both eyes and are called binocular microscopes.
9. The nose piece holds three to five objective lenses of different magnifying power and is easily rotated to position any objective.
10. The image you see when viewing a specimen is focused by the objective and ocular lenses working together.
11. 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.
12. 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.

3.1.2 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.

3.1.3 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 isused 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.

3.1.4 The Fluorescent Microscope

This type of microscope exposes a specimen to ultraviolet, violet or bluelight 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 orreflected 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 anexciter filler. The exciter filler transmits on the desired wavelength ofexcitation lightThe excitation light is directed down the microscope by a speed minorcalled the dichromatic minor. This minor reflects light of shorterwavelength but allows light of longer wavelength to pass through. Theexcitation light continues down through the objective lens to specimenstained with spaced dye molecules called fluorochromes.

3.2 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 byErnest Abbe, a German physicist. The Abbe equation states that the minimal distance (d) between two objects that reveal them as separateentities 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 = 0.5□□nsin□□as d becomes smaller, the resolution increases and finer details can bediscerned in a specimen; d becomes smaller as the wavelength of lightused 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 = □□.

2NAwhere d = resolution and □□= wavelength of light. Using the values 1.3for NA and 0.5□m, the wavelength of green light, for >□, resolution can be calculated asd = 0.55 = 0.21□m.2 x 1.30From 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.

SELF-ASSESSMENT EXERCISE

i. Define the resolving power.
ii. List 5 parts of a light microscope and state the function of each.

3.3 Preparation for Light-Microscope Examination

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

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

3.3.1 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.

3.3.2 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.

3.3.3 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. Amicroorganism usually is killed and attached firmly to the microscopeslide during fixation.

There are two fundamentally different types of fixation.

1. **Heat Fixation:** Is routinely used to observe prokaryotes. Typically, a film of cells (a smear) is gently heated as a slide ispassed through a flame. Heat fixation preserves overallmorphology but not structures within cells.
2. **Chemical Fixation:** Is used to protect fine cellular sub-structureand the morphology of larger, more delicate micro - organisms.Chemical fixatives penetrate cells and react with cellularcomponents, usually proteins and lipids, to render them inactive,insoluble, and immobile. Common fixative mixtures contain suchcomponents as ethanol, acetic acid, mercuric chloride,formaldehyde, and glutaraldehyde.

3.4 Staining of Specimens

Although living microorganisms can be directly examined with the lightmicroscope, 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 carbolfuchs in are used in simple staining to determine thesize,shape and arrangement of prokaryotic acids.

Differential staining

These are staining procedures that make visible the differencesbetween bacterial cells or part of a bacterial cell. It usuallyinvolves more than one dye used for staining.

Gram staining

The Gram stain was developed in 1884 by the Danish physicianChristian Gram. It is the most widely used differential staining procedure.

The steps involved are as follows:

1. The smear is stained with the crystal violet (which is the primarystain).
2. This followed by treatment with iodine functioning as a mordant.
3. The smear is decolourised by washing with ethanol or acetone.
4. The smear is counterstained with a simple dye safranin.
   Bacteria stained by the Gram stain method fell into two groups:

Gram positive bacteria which retain the crystal violet and appear deep violet in colour and Gram negative bacteria which, lose thecrystal violet and are counterstained with safranin appear red in colour.

Acid fast staining

This is another differential staining procedure commonly used toidentify *Mycobacterium tuberculosis* and *Mycobacterium leprae*, thepathogens 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 carbolfuchsin isused as primary stain; next acid-alcohol is used as a decolouriser. Theacid-alcohol will remove the red stain form bacteria such as *Escherichiacoli* which the acid fast mycobacteria will remain red.

3.5 Electron Microscope

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

3.5.1 The Transmission Electron Microscope

Electron microscopes use a beam of electrons to illuminate and createmagnified images of specimens. Electrons replace light as theilluminating beam. They can be focused, much as light is in a lightmicroscope, but their wavelength is around 0.005mm approximately1000,000 times shorter than that of visible light. Therefore, electron microscopes have a practical resolution roughly 1,000 times better thanthe light microscope, with many electron microscopes point closer than0.5nm can be distinguished, and the useful magnification is well over100,000x. In transmission electron microscope, the electron beam istransmitted through the specimen.

3.5.2 The Scanning Electron Microscope

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

SELF-ASSESSMENT EXERCISE

Differentiate between the transmission electron microscope andscanning electron microscope.

4.0 CONCLUSION

Scientific observation is an important part of scientific study.Microbiology as a field of science would not have developed without thenecessary instruments such as the microscope and the methods used forobserving the microorganism.

5.0 SUMMARY

In light microscope, magnification is obtained by a system of opticallenses using light waves. Many types of light microscopes have beendeveloped. They include bright fields, dark field, phase contrast andfluorescence microscope.

Electron microscope uses a beam of electron in place of lightwaves to produce the image of an object.

The ordinary compound microscope is called the bright fieldmicroscope because if forms a dark image against a brightbackground.

In the bright field microscope which is a compound the primaryimage is formed by an objective lens and enlarged by the eyepiece or ocular lens to form the final image.
The dark field microscope uses only refracted light to form animage and objects glow against a black background.

The dark field microscope is useful in revealing many internalstructures in larger eukaryotic microorganism.

The phase-contrast microscope converts slight differences inrefractive index and cell density into easily detected variations inlight intensity and is used to view living cells, for studyingmicrobial motility and detecting some bacteria components suchas endspores.

The fluorescent microscope exposes a specimen to ultraviolet,violet or blue light and forms an image of the object withresulting fluorescent light.

Two general methods for preparing specimens for lightmicroscope examination are the wet mount or the hanging droptechnique and the dried fixed stained technique.

A wet mount is made by placing a drop of fluid containing theorganisms on a glass slide and covering the drop with a cover slipbefore viewing under the microscope.
Fixation is a process by which the internal and external structuresof cells and microorganisms are preserved and fixed in a position.It involves preparation of the smear, fixing with heat or chemicaland application of one or more staining solutions.

Electron microscopes use a beam of electrons to illuminate andcreate magnified images of specimens.

Simple staining is a kind of staining in which a single stain or dyesuch as methylene and crystal violet is used.

Differential staining involves the use of more than one stain ordye is used to make visible the differences between bacterial cellsor part of a bacterial cell examples are the Gram stain.

6.0 TUTOR-MARKED ASSIGNMENT

(1a) What is microscope resolution?
(b)List the stages involved in preparing a specimen for observation under the light microscope.
(2a) Describe the bright field microscope.
(b) What is the basic difference between a transmission electron microscope and a scanning electron microscope?

7.0 REFERENCES/FURTHER READING

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UNIT 2 A BRIEF SURVEY OF MICROBES AS FRIENDS AND FOES

CONTENTS

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 well- being 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 should 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

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 optimise yield.

3.1 Microorganisms and Food Production

Many microorganisms are used to produce many of the foods andbeverages we consume. Microbially-produced food products have properties that are very different from those of the startingmaterials. 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 convertedby 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 andlactic 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 beerby conversion of sugar to alcohol and carbon dioxide.

Wine fermented from fruits using yeast strains *Saccharomycescerevisiae*and bread is also produced by using yeasts. Microorganisms can also be used as direct source of food knownas single cell protein. Various species of yeasts, algae are grownas single

cell protein and use as animal feeds thus helping to meetthe world food needs.

3.2 Production of Pharmaceuticals

Microorganisms are used to produce different pharmaceuticals such asantibiotics, steroids vitamins, hormones, etc. Antibiotics are microbially produced substances or substances synthetically derived from natural sources that inhibit or kill microorganisms, Steroids regulate variousaspects of human metabolisms and are produced by organisms such as *Rhizopusnigricans*.Vaccines are produced using microorganisms with the antigenicproperties to elicit a primary immune response; they are used to preventmany once deadly diseases such as polio, small pox, tuberculosis,measles, diphtheria and whooping cough.

3.3 Vitamins

Vitamins are essential animal nutritional factors; some vitamins areproduced by microbial fermentation, e.g. Vitamin B12 by *Streptomyces*,B12 by *Pseudomonas denitrificans* and *Propionibacteriumshermanni*.Riboflavin produced by various species of *Clostridium* and *Ashbyagossypii*. Human insulin and human growth hormone are produced by geneticallyengineered bacteria.

3.4 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 *Aspergillusniger*and used as a food additive especially in the production of soft drinks.
2. Gibberellic acid: a plant hormone is formed by the fungus.*Gibberellafujikuroi*. It is used as growth promoting substancesto stimulate plant growth flowering and seed germination.
3. 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.

3.5 Hygiene

1. Hygiene is the avoidance of infection and food spoilage by eliminating microorganisms from the surrounding.
2. 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.
3. 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.

3.6 Energy Production

Microorganisms play major roles in energy production. Microbes areused in fermentation to produce ethanol and in biogas reactions toproduce methane using various forms of agricultural and urban wastes.Microbial production of synthetic fuels acts as an alternative fuelresources to petroleum.The microbial production of ethanol from sugarcane or cornstarch hasbecome an important source of a valuable fuel, particularly in areas ofthe world that have abundant supplies of plant residues such as Brazil and is becoming popular in the United States. The bacteria *Zymomonasmobilis*and *Thermoanaerobacterethanolicus* and different yeast strains are used for product of ethanol.Methane (natural gas) is produced by methanogenic bacteria is another important natural renewable energy sources.Methane can be used for the generation of mechanical, electrical andheat energy.

3.7 Useful in the Study of Science

Microbes are essential tools in biotechnology, biochemistry, geneticsmolecular biology and genomics. Examples are the yeasts(*Saccharomyces cerevisiae*) and fission yeast (*Shizosaccharomycespombe*) 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 artificialmanipulation of genes and gene products.
Genes from any source can be manipulated and modified usingmicroorganisms and their enzymes as molecular tools, e.g. humaninsulin, a hormone which is very low in people with diabetes isproduced by genetically engineered bacteria into which human genes have been inserted.

3.8 Recovery of Metals from their Ores

Microorganisms are used to recover metals from their ores by theprocess of bioleaching. Bioleaching uses microorganisms to alter thephysical or chemical properties of a metallic ore so that the metal can beextracted. The bacteria *Thiobacillusferoxidans*recover copper anduranium from their ores.

3.9 Microorganisms and Agriculture

Agriculture depends in many ways on the activities ofmicroorganisms. Microorganisms help in nitrogen fixation usedby plants for growth.

In terrestrial habitats, the microbial fixation of atmosphericnitrogen is carried out by free living bacteria such as *Rhizobium*and *Bradyrhizobium*living in symbiotic association with plants.

Legumes live in close association with bacteria that formstructures called nodules on their roots.
These bacteria in the root nodules of the legumes, convertatmospheric N2 fixed into Nitrogen (NH3) that plants use forgrowth and eradicates the need for chemical fertilisers.

Microorganisms in the rumen of ruminant animals such as cattleand sheep also help in the digestion of cellulose present in grasseson which they feed on.

Microorganisms help in the cycling of nutrients such as carbon,nitrogen and sulphur which are needed to maintain ecologicalbalance.
Microorganisms are also used as biological control agents.
Fungi, bacteria and viruses can be used as bioinsecticides orbiospesticides, e.g. *Bacillus thuringensis*.

Microbial activities in soil and water convert these elements toforms that are readily assimilated by plants.

3.10 Microorganisms and the Environment

a. Microorganisms can be used to clean up pollution created by human activities in a process called bioremediation.

b. Pollutants such as pesticides, spilled oil solvents which could pose human health hazard are degraded to nontoxic substances by microorganisms.

c. Microorganisms are used to degrade wastes and pollutants so asto maintain and restore environmental quality.

3.11 Sewage Treatment

Microorganisms are also used in sewage treatment. Specially culturedmicrobes are used in the biological treatment of sewage and industrialwaste effluent in a process known as bioaugmentation. These microbeshelp to get rid of waste materials which could have accumulated in theenvironment.

SELF-ASSESSMENT EXERCISE

i. State the beneficial role of microorganisms in:
a. Agriculture
b. Environmental Protein
c. Food Production.

3.12 Microorganisms as Foes

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

3.13 Microorganisms as Disease Agents

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

AIDS (Acquired Immune Deficiency Syndrome) caused by theHuman Immuno Deficiency Virus (HIV).
Tuberculosis caused by a bacterium, *Mycobacteriumtuberculosis.*
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 are also agents of diseases of plants and animals ofagriculturalimportance. These microbial diseases of plants and animalscause major economic losses in the agriculture industry and to theworld.

3.14 Microorganisms as Agents of Warfare and Terrorism Use in Biological Warfare

Biological warfare is also known as germ warfare. It is the use ofpathogens such as viruses, bacteria, or the toxins produced by them asbiological weapons or agents of warfare.A biological weapon may be used to kill, incapacitate or seriouslyimpair a person, group of people or

even an entire population. It can beused 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 hostvictims 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.

SELF-ASSESSMENT EXERCISE

Explain ways in which microorganism can act as foes to man.

4.0 CONCLUSION

Through the activities of microorganism we have received manybenefits such as production of foods with improved qualities, improvedagricultural crop yields, maintained environmental quality and decreasethe incidence of diseases. However, some microorganisms are still lifethreatening and harmful to man.

5.0 SUMMARY

Microorganisms can act as friends or foes to man.
Microorganisms are friends, and useful to man in several ways.
Microorganisms are used in the production of foods such asbreed, beer, wine, dairy products such as cheese, yoghurt, and insingle cell protein.
Microorganisms are used in the production of pharmaceuticalssuch as antibiotics, vaccines and steroids.
Microorganisms are used in production of fuels like ethanol andmethane used for energy.
Microorganisms are used in environmental clean-up.

Microorganisms are important in agriculture.
Microorganisms are useful in the study of science.
Microorganisms are used to produce different organic acids suchas lactic acid, and gluconic acid.
Microorganisms are used to maintain hygiene.
Microorganisms also cause diseases of animals and plants.
Microorganisms act as foes by causing diseases such as AIDS,and tuberculosis in man.
Microorganisms are used as agents of warfare or terrorism.

6.0 TUTOR-MARKED ASSIGNMENT

1. List two fuels produced by microorganisms.
2. Give the name of one bacterium used to recover copper from it sore.
3. Explain two ways in microorganisms are harmful to man.

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UNIT 3 GENERAL CHARACTERISTICS OFBACTERIA

CONTENTS

1.0 INTRODUCTION

Bacteria are characterised based on the cell shape, size and structure cell arrangement, occurrence of special structures and developmental forms, staining reactions and motility and flagella arrangement. They are also characterised by the cell wall component, Gram stain reaction, cellularrespiration and mode of nutrition. This unit examines the general characteristics of bacteria, shapes and forms of bacteria, structures external and internal in bacteria among other things.

2.0 OBJECTIVES

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

- describe the general characteristics of basic bacteria
- identify and name the general shapes and forms of bacteria
- describe the external and internal structures of bacteria
- explain the significance of the cell wall structure and composition
- explain the modes of nutrition and energy source in bacteria
- explain the modes of cellular respiration in bacteria
- explain the modes of reproduction in bacteria.

3.0 MAIN CONTENT

General Characteristics of Microorganism

General characteristics of bacteria:

1. They are prokaryotic
2. They are simplest of all microbial cells
3. Bacteria are single celled organisms
4. They have distinctive cell wall which contain peptidoglycan
5. They are measured in unit called micrometer
6. Bacteria lack a true nucleus but have a region called the nucleroidregion, i.e. DNA is free floating
7. They may have additional DNA called a plasmid
8. Their reproduction is by binary fission
9. They are extremely diverse and numerous in soils and waters.

3.1 Size, Shape and Arrangement of Bacterial Cell

3.1.1 Size

Bacteria are very small, 0.5 to 1.0μm in diameter. Because of their small size, they have high surface area/volume ratio which results in a high growth and metabolism rate. No circulatory mechanism is needed for nutrients taken in because the mass of cell substance to be nourished is very close to the surface. Examinations of a microbial cell require the use of a high power microscope usually of about 1,000 diameters.

3.1.2 Shape and Arrangement

The shape of a bacterium is governed by its rigid cell wall which gives ita definite shape.

Typical shapes of bacteria are:

Cocci (Singular: Coccus), e.g. *Staphylococcus*
Bacilli (rods) (Singular: rod, bacillus), e.g. *Bacillus subtilis*
Vibrios (Singular: *Vibrio*)
Spirilla (Singular: Sprillum)
Spirochaetes (Singular: *Spirochaete*), e.g. *Treponemapallidum*
Some species of bacteria are pleomorphic, i.e. they are able to change their forms especially when grown on artificial media.

1. **Cocci**: They are round, oval or spherical in diametercharacteristic arrangement when multiplying is based on arrangement of cells, they are called:

Diplococci: cocci in pairs, e.g. meningococci andgonococci.
Streptococci cocci in chains.
Staphylococci: cocci in irregular clusters (like a bunch ofgrapes).
Tetracocci: cocci in a group of four cells.
Sarcinae: cocci in regular clusters.

2. **Bacilli (Rod)**: These are stick like bacteria with rounded, square,tapered or swollen ends. They measure 1-10μm in length by 0.3-1.0μm in width.

   Bacilli are not arranged in patterns as complex as cocci. Mostoccur singly. Other arrangements are:
   Diplobacilli: Rods in pairs.
   Streptobacilli: Rods in chains.
   Trichomes: Similar to chains but have larger area ofcontact between adjacent cells.
   Mass together, e.g. *Mycobacterium leprae.*
   Palisade arrangement cells are lined side by side likematchsticks and at angles to each other like Chineselecters, e.g. *Corynebacteriumdiptheriae*.

3. Vibrios: These are small slightly curved rods,or comma shaped 3-4μm in length by 0.5μm in width. Most are motile with a singleflagellum at one end, e.g. *Vibrio cholerae*.

4. Spirilla: These are helical bacteria, small, regularly coiled, rigid,organisms measuring 3-4μm in length. Each coil measures about 1μm, e.g. *Spirillum minus.*

5. Spirochaetes: They are helical, (complete twist), flexible, coiled organisms, can twist and contort their shapes. Spirochaeters are divided into three main groups.
   Treponemes: Tiny and delicate with regular tight coils,measuring 6-15μm by 0.2μm in width, e.g. *Treponemapallidum*and *Treponemapertenue.*
   Borreliae: Large spirochaetes with irregular open coils 10-20μm in length by 0.5μm in width, e.g. *Borella.,duttoni*and *Borreliavinceti*.
   Leptospires: Tiny spirochaetes with many tightly packedcoils that are difficult to distinguish; 6-20μm in length by0.1μM in width and have hooked ends, e.g. *Leptospira interrigans.*

In addition to the common bacterial shapes, many others also occur in different shapes, which include: pear shaped cells, e.g. *Pasteuri*
lobed spheres, e.g. *Sulfolobus*

rods with squared ends, e.g. *Bacillus anthracis*
disk arranged stacks of coins, e.g. *Caryophanon*
rods with helically sculptured surfaces, e.g. *Seliberia* and many others. The shape of a cell affects its survival and activity in the environment.

SELF-ASSESSMENT EXERCISE

Describe the different shapes of bacteria.

3.2 Bacterial Structures

Examination of a bacterial cell will reveal several components andstructures. Some are external to the cell wall while others are internal tothe cell wall.

3.2.1 Structure External to the Cell Wall

1. Flagella (Singular: Flagellum): These are hair like, helicalappendages that protrude through the cell wall, 0.01 – 0.02μm indiameter and simple in structure. Based on their location on thecell, flagella may be polar or lateral.

I **Polar**: At one or both ends of bacterium.
Ii **Lateral**: Along the sides of the bacterium.
A flagellum is composed of three parts:
i A based body associated with the cytoplasmic, membraneand cell wall.
ii A short hook and a helical filament which is usuallyseveral times as long as the cell.
iii A flagellum grows at the tip rather than at the base.

Types of Flagella

Monotrichous: A single polar flagellum. Many thatappears and functions as monopolar or bipolar flagellaconsist of bundles of 2 to 50 single units (polytrichous).

Lophotrichous: A cluster of polar flagella.

Amphitrichous: Flagella, either single or clusters at bothcell poles.

Peritrichous: Cell surrounded by lateral flagella.

Function of Flagella
Bacteria propel themselves by rotating their helical flagella.

2. Pili (Singular: Pilus): They are also called fimbriae. They arehollow, non-helical filamentous appendages that are thinner,shorter and more numerous than flagella: long, thin, straightthreads 3-25µm in diameter and 12µm in length. They do notfunction in motility since they are found on non-motile andmotile species. Several functions are associated with different types of pili.F pilus (Sex pilus) serves as the path of entry of genetic materialduring bacterial mating. Some play major role in human infection by allowing pathogenic bacteria to attach to the epithehal cellslining the respiratory, intestinal or genitourinary tracts, thisprevents the bacteria from being washed away by the flow ofmucous or body fluids and permits infections to be established.

3. Capsules: This is a viscous substance forming a covering layeror envelope around the cell wall of some bacteria. Capsules canbe categorised into three based on their visualisation by lightmicroscope using special staining methods.If the covering layer can be visualised by light microscope usingspecial staining methods, it is termedcapsule.

Microcapsule: If the layer is too thin to be seen by lightmicroscope.

Slime: If it is so abundant that many cells are embedded ina common matrix.

Most bacterial capsules consist of polysaccharides which can behomopolysaccharides or heteropolysaccharides.

Homopolysaccharides: Capsule made up of/composed ofa single kind of sugar usually synthesised outside the cellby exocellular enzymes, e.g. glucan (a polymer of glucose)from sucrose by *S. mutans*.

Heteropolysaccharides: Composed of several kinds ofsugars.A few capsules are polypeptide, e.g. *Bacillus anthracis*has acapsule made up of a polymer of glutamic acid.

Functions

They may provide protection against temporary drying bybinding water molecules.

They may block attachment of bacteriophages.They may be antiphagocytuc, i.e. they may inhibit the engulfmentof pathogenic bacteria by white blood cells. Hence contribute toinvasive or infective ability (*virulence*).Promote attachment of bacteria to surfaces.

4. Sheaths: Some bacterial species form chains or trichomesenclosed by a hollow tube called sheaths. These sheaths consist of a heteropolysaccharides containing glucose, glucuronic acid,galactose and fucose.

Functions
In a few bacteria, they facilitate moderate change of position.Sheaths enable individual cells to stay associated in cell colonies.

5. Prosthecae and Stalks

Prosthecae: They are semi rigid extensions of the cell wall andcytoplasmic membrane and have a diameter less than that of thecell. Found in some aerobic bacteria from fresh water and marineenvironment.

Functions
Increase surface area of the cell for nutrient absorption.Some have adhesive substances that aid attachment to surfaces.

Stalks: They are non-living ribbon -like or tubular appendagesexcreted by some bacterial cells, e.g. found in *Gallionella*or*Planctomyces*.

Functions

They aid in attachment of the cells to surfaces.

SELF-ASSESSMENT EXERCISE

List four different structures external to the cell wall of bacteria andstate one function of each.

3.2.2 The Cell Wall

It is a very rigid structure that gives shape to the cells. It also preventsthe cell from expanding and eventually bursting of uptake of water sincemost bacteria live in hypotonic environment (i.e. environments having alower osmotic pressure than exists within the bacterial cells). Cell wallsare essential for bacterial growth and division.

Structure and Chemical Composition of the Cell Wall

The cell wall of bacteria is made up of peptidoglycan (sometimes called Murein). Peptidoglycan is found only in prokaryotes. It is an insoluble,porous, cross-linked polymer. Peptidoglycan differs in composition andstructure from one species to another but it is basically a

polymer of Nacetyglucosamine,N-acetylmuramie acid, L-alanine, D-alanine, Dglutamateand adiamino acid.Bacteria are classified based on differences in the composition of cellwall. This is determined by the Gram stain technique. Gram stain identifies bacteria as Gram positive or Gram negative. The Gram stain isnamed after Christian Gram, a Danish physician who invented it in1884.Gram positive bacteria stained purple whereas Gram negative bacteriastain pink or red by the Gram stain technique.The difference is the reaction to the Gram staining technique is due to the presence of a single 20 to 80μm thick homogenous layer of peptidoglycan (Murein) which is present in the all wall of Gram positivebacteria on the other hand, the Gram negative cell is more complex, ithas a 2 to 7μm peptidoglycan layer covered by 7 to 8μm thick outerlayer of lipopolysaccharides (LPS). These LPS are toxic substanceswhich make Gram negative organisms more harmful than Gram positiveorganisms.

3.2.3 Structures Internal to the Cell

1. **Cytoplasmic Membrane**

This lies immediately beneath the cell wall.

It is approximately 7.5μm (0.0075μm) thick andcomposed primarily of phospholipids (20 to 30 percent)and protein (60 to 70 percent).

It serves as a barrier to most water soluble molecules.

It contains various enzymes involved in respiration, andmetabolism and in synthesis of capsular and cell wallcomponent.

Proteins are also synthesised in the cytoplasm.

2. **Protoplast**

A protoplast is the portion of a bacterial, all made up of the cytplasmic membrane and the cell material bounded by it.

3. **The Cytoplasm**

This is the cell material bounded by the cytoplasmic membraneand it may be dividedinto:

i The cytoplasmic area, granular in appearance and rich inthe macromolecular RNA-protein bodies calledRibosomes on which proteins are synthesised.

ii The chromatin area rich in DNA and

iii The fluid portion with dissolved substances.

4. **Nuclear Material**

Unlike eucaryotic cells bacterial cells do not have a distinctmembrane enclosed nucleus but they have an area near the centreof the cell that is regarded as the nuclear structure, the DNA ofthe cell is confined to this area. The DNA is circular and bearsthe genes of the cell.

5. **Spores and Cysts**

Certain bacteria produce spores either within the cells(endospores) or external to the cell (exospores). The spore ismetabolically dormant form which under appropriate condition can germinate to form a vegetative cell. Endospores areextremely resistant to desiccation, staining, disinfectingchemicals, radiation and heat.Cysts are also dormant, thick walled desiccation resistant formsthat can germinate also under favourable conditioning.

3.3 Nutrition

The nutrition requirements of bacteria vary widely.Based on their source of energy, they are classified as:

I Phototrophs: These are bacteria that use light energy as their energy sources.
Ii Chemotrophs: They obtain their energy by oxidizing inorganic ororganic – chemical compounds. Based on the source of carbon which is the major source of nutrient for all cells bacteria can be classified as:

Heterotrophs: These are bacteria that derive carbon frompreformed organic nutrients such as sugar or carbohydrate.

Autotrophs: They derive carbon from inorganic sources such ascarbon dioxide.

3.4 Cellular Respiration

Based on whether they need oxygen to survive or not, bacteria may be:

I **aerobic or strict aerobes**: these require oxygen, e.g. *Bacilluscereus.*
Ii **anaerobic bacteria or strict anaerobes**: they cannot tolerateoxygen, e.g. *Clostridium* spp.

Iii **facultative anaerobes**: These are generally aerobes but have the capacity to grow in the absence of oxygen, e.g. *Staphylococcus*spp.

3.5 Reproduction

Bacteria reproduce mainly by asexual method which most of the time istransverse binary fission. This is a process in which a bacterial celldivides to give two daughter cells after developing a transverse septum(cross wall).

SELF-ASSESSMENT EXERCISE

Differentiate between Gram positive bacteria and Gram negativebacteria.

4.0 CONCLUSION

Different types of bacteria differ from one another not only in theirshapes and forms but also in their chemical characteristics, modes ofcellular respiration, nutrition and reproduction.

5.0 SUMMARY

Bacteria are prokaryotic single celled organisms that lackmembrane-bound organelles. Bacteria are very small, with sizesranging from 0.5 to 1.0nm in diameter.

The typical shapes of bacteria are Cocci, Bacilli (Rod) Vibrio,Spirila and Spirochaetes. Some bacteria are pleomorphic.

Structures external to bacterial cell wall include flagella, pili,capsules, sheaths, prosthecae and stalks.

Flagella are hair-like, helical appendages that protrude throughthe cell wall used for locomotion. May be polar or lateral basedon location on the cell.

Types of flagella are Monotrichous, Lophotrichous,Amphitrichous, Peritrichous.

A capsule is a viscous substance forming a covers layer orenvelope around the cell wall of some bacteria. Capsules act asprotection against drying, bacteriophages and engulfment ofpathogenic bacteria by white blood cells.

The cell wall is rigid structure made up of peptidoglycan thatgives shape to bacterial cells. Bacteria are classified based ondifferences in the composition of cell wall as determined by theGram stain techniques.

Gram-positive bacteria stained purple while Gram-negativebacteria stain pink or red by Gram stain technique.

Structures internal to the bacterial cell include cytoplasmicmembrane, protoplast, the cytoplasm, the nuclear material, sporesand cysts.

Based on source of energy bacteria can be classified asphototrophs and chemotrophs.

Based on source of carbon for nutrition, bacteria can be classifiedas heterotrophs and chemotrophs.

Based on oxygen requirement, bacteria are classified as aerobic,anaerobic and facultative anaerobe.

Bacteria reproduce asexually by transverse binary fission.

Bacteria are very small, simple prokaryotic cells that havedefined shapes and organelles which perform definite functions.They are capable of independent living and existence.

6.0 TUTOR-MARKED ASSIGNMENT

1. Describe the structure and functions of a flagellum.
2. State the difference types of bacteria based on their mode of cellular respiration.
3. Differentiate between Gram positive and Gram negative bacteria cell walls.

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UNIT 4 GENERAL CHARACTERISTICS OF FUNGI

CONTENTS

1.0 INTRODUCTION

Fungi are eukaryotic spore bearing organisms that lack chlorophyll and generally reproduce both sexually and asexually. They are of great practical and scientific importance. One of the reasons for this is that many fungi are of microscopic cellular dimensions. Fungi have a diversity of morphological appearances depending on the species. Fungi comprise the molds, mushrooms and yeasts. Molds are filamentous and multicellular while yeasts are unicellular. They are widely distributed and found wherever moisture is present. They are of great importance toman in both beneficial and harmful ways. This unit examines the general characteristics of fungi, the distribution, morphology, nutrition and reproduction of fungi.

2.0 OBJECTIVES

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

- define a fungus
- state the general characteristics of fungi
- describe the structure of a yeast
- analyse the structure of a mold
- explain the mode of nutrition in fungi

- d**istinguish** the methods of asexual reproduction and sexualreproduction in fungi.

3.0 MAIN CONTENT

Definition of Fungi

Fungi are eukaryotic spore bearing organisms that lack chlorophyll andgenerally reproduce both sexually and asexually.

3.1 Distinguishing Characteristics of Fungi

They are large, diverse and widespread group of organisms, the molds, mushrooms and yeasts.

1. Fungi are Eucaryotic. They are members of the domain Eucarya.
2. They contain a membrane-enclosed nucleus and several otherorganelles.
3. They have no chlorophyll.
4. They are chemo organotrophic organisms.
5. The body of the fungi is called thallus.
6. The thallus may consist of a single cell as found in yeasts.
7. The thallus may consist of filaments, 5 to 10μm across which are commonly branched as found in molds.
8. The yeast cell or mold filament is surrounded by a true cell wall(exception is the slime mould which have a thallus consisting of anaked amoeboid mass of protoplasm).
9. Some fungi are dimorphic, that is they exist in two forms. Somepathogenic fungi of humans and other animals have a unicellularand yeast like form in their host but when growing saprobically in soil or on a laboratory medium they have a filamentous mold form.
10. Habitat distribution of fungi is diverse. Some are aquatic, living primarily in fresh water and a few marine fungi are terrestrial. They inhabit soil and dead plant. Some are parasitic, inhabiting and infecting living hosts either plants or animals. Some form beneficial relationships with other organisms as mycorrhizae.
11. The study of fungi is known as mycology.

3.2 Structure and Forms of Fungi

The body or vegetative structure of a fungus is called a thallus (pluralthalli). It varies in complexity and size ranging from the single cellmicroscopic yeasts to multicellular moulds and mushrooms. The fungalcell is usually enclosed in a cell wall of chitin.

3.2.1 Yeasts

They are unicellular fungi that have a single nucleus.

They are commonly egg-shaped but some are elongated and somespherical. Yeasts have no flagella or other organelles oflocomotion.

They possess most of the other eukaryotic organelles.

Yeast cells are larger than most bacteria. Yeasts varyconsiderably in size ranging from 1 to 5μm in width and from 5to 30μm or more in length.

They reproduce asexually by budding and traverse division orsexually through spore formation.

3.2.2 Molds

The thallus of a mold consists of long branched threadlike filaments ofcells called hyphae. These hyphae form a mycelium which is a tangledmass or tissue like aggregation of hyphae.

Hyphae

1. Each hypha is about 5 to 10μm wide. Hyphae are composed of anouter tube like wall surrounding a cavity the *Lumen* which is filled or lined by protoplasm. Between the protoplasm and the wall is the *plasmalemma*, a double layer membrane which surrounds the protoplasm.

2. The hyphal wall consists of micro fibrils composed of hemicelluloses or chitin. True cellulose occurs only in the wallsof lower fungi.
3. Wall matrix material in which the micro fibrils are embedded consists of proteins, lipids and other substances. Growth of a hypha is distal near the tip.

The Mycelium

1. The mycelium is a complex of several filaments called hyphae(singular, hypha). New hyphae generally arise from a germinated spore. The germinated spore puts out a germ tube or tubes which elongate to form hyphae. These hyphae form a tangled mass or tissue like aggregation.

In some fungi, protoplasm streams through hyphae uninterrupted by cross walls, these hyphae are called coenocytic or aseptate.The hyphae

of others have cross walls called septa (*s. septum*) witheither single pore or multiple pores that enables cytoplasmic streaming.These hyphae are termed septate. Summarily, hyphae can be said to occur in three forms:

I Nonseptate or coenocytic; such hyphae have no septa.
Ii Septate with uninucleate cells.
Iii Septate with multinucleate cells. Each cell has more than one nucleus in each compartment.

SELF-ASSESSMENT EXERCISE

i. What are the major differences between a mold and yeast?
ii. State five general characteristics of fungi.

3.3 Nutrition and Metabolism

Most fungi are saprobes, securing their nutrients from dead organicmatters. They release hydrolytic exo-enzymes that digest external substrates and absorb the soluble products.

They are also chemoorganoheterotrophs, i.e. they use organiccompounds as a source of carbon, electrons and energy.Fungi are usually aerobic; however, some yeasts are facultativelyanaerobic and can obtain their energy by fermentation. Obligatelyanaerobic fungi are found in the rumen of cattle.

3.4 Reproduction

Reproduction in fungi can either be asexual or sexual.

3.4.1 Asexual Reproduction

Asexual reproduction is a type of reproduction involving only oneparent that produces genetically identical offspring by budding or by thedivision of a single cell or the entire organism into two or more parts.Asexual reproduction, also called somatic or vegetative reproduction isaccomplished in several ways and does not involve the fusion/union of nuclei, sex cells or sex organs. It may be accomplished by:

fission of somatic cells yielding two similar daughter cells budding each bud a small outgrowth of the parent cell developsinto a new individual
fragmentation or disjointing of the hyphal cells each fragment becoming a new organism spore formation.

There are several types of asexual spores each with a name.

a. **Sporangiospores**: These are single-celled spores formed within sacs called sporangia (singular: sporangium) at the end of specialhyphae called sporangiospores).
b. **There are two types of sporangiospores**: Aplanospores which are non-motile and zoospores which are motile. Motility is due tothe presence of flagella.
c. Condiospores or conidia (singular, conidium). These are formedat the tip or side of a hypha. Single celled conidia are called microconidia while large multicelled conidia are called macroconidia.
d. **Oidia (singular oidium) or arthrosopores**: These are single celled spores formed by disjointing of hyphal cells.
e. **Chlamydospores**: These are thick walled single celled spores which are highly resistant to adverse conditions. They are found from cells of the vegetative hypha.
f. **Blastospores**: These are spores formed by budding.

Fig. 3: Different types of Asexual Spores

3.4.2 Sexual Reproduction

Sexual reproduction is a type of reproduction in which two parents giverise to offspring that have unique combinations of genes inherited fromthe gametes of the two parents.

It is carried out by fusion of the compatible nuclei of two parent cells.The process of sexual reproduction begins with the joining of two cellsand fusion of their protoplast (plasmogamy) thus enabling the twohaploid nuclei of two mating types to fuse together (karyogamy) to forma diploid nucleus. This is followed by meiosis, which again reduces thenumber of chromosomes to the haploid number.The sex organelles of fungi if present are called gametangia. They mayform differentiated sex cells called gametes or may contain instead oneor more gamete nuclei. If the male and female gametangia are morphologically different, the male gametangium is called theantheridium (plural antheridia) and the female gamentangium is calledthe Oogonium (Oogonia).

Methods of sexual reproduction include:

I Gametic copulation: This is the fusion of naked gametes, one or both of which are motile.
ii Gamete-gametangial copulation: Two gametangia came intocontact but do not fuse; the male nucleus migrate through a poreor fertilization to be into the female gamentangium.
Iii Gametangial copulation: Two gamentangia or their protoplastfuse and give rise to a zygote that develops into a resting spore.

iv Somatic copulation: Fusion of somatic or vegetative cells.
V Spermatisation: Union of a special male structure called aspermatium (plural spermatia) with a female receptive structure.The spermatum empties its content into the female duringplasmogamy.Sexual spores are produced by the fusion of two nuclei. Examples are:

I **Ascospores**: These are single-celled spores produced in a saccalled an ascus. There are usually eight ascospores in each ascus.
Ii **Basidiospore**: These are single celled spores borne on a clubshapedstructure called a basidium.
Iii **Zygospores**: These are large thick walled spores formed when the tips of two sexually compatible hyphae or gametagia fuse together.
Iv **Oospores**: These are formed with a special female structure, theoogonium. Fertilization of the eggs or oospheres by the malegametes formed in an antheridium give rise to oospores.

SELF-ASSESSMENT EXERCISE

Describe sexual reproduction as it occurs in fungi.

3.5 Physiology

Fungi are better able to withstand certain extreme environmentsthan other microorganisms. They can tolerate more acidicconditions than other microbes. Some types of yeasts arefacultative; they can grow under both aerobic and anaerobic
conditions. Molds and many types of yeast are usually aerobicmicroorganisms.

Fungi grow over a wide range of temperature. The optimum temperature for most saprobic species is 22 to 300C, while pathogenic fungi have a higher temperature optimum of 30 to370C.

Some fungi will grow at or near 00C and thus can cause spoilageof meat and/or vegetables in cold storage.

3.6 Importance of Fungi

About 90,000 fungal species have been described according to literature. However, some estimates suggest that 1.5 million species may exist. Fungi are important to humans in both beneficial and harmful ways.

Beneficially, fungi act as decomposers. They degrade complex organic materials in the environment and release simple organic and inorganic molecules like carbon, nitrogen, phosphorus needed by other living organisms.

Moulds and yeasts are used in many industrial processes involving fermentation to produce beer, wine and bread, cheesesoy-sauce, organic acids and many antibiotics.

They are important research tools in the study of fundamental processes such as cytology, genetics, biochemistry and microbiology.

On the other hand, fungi cause many diseases of plants, animals and humans. About 20 new human fungal pathogens are documented each year.

4.0 CONCLUSION

Fungi are eukaryotic, spore bearing organisms that lack chlorophyll. They are diverse in distribution, reproduce sexually and asexually. Fungi are of high importance to man in both beneficially and harmful ways.

5.0 SUMMARY

Fungi are eukaryotic spore bearing organisms that lackchlorophyll and reproduce both asexually and sexually.

Fungi are widespread in environment and found wherever watersuitable organic nutrients and an appropriate temperature occur.

Habitats of fungi are diverse. Many are terrestrial some areaquatic and are parasite in living hosts.

The body or vegetative structure of a fungus is called thallus.

Fungi may be grouped into molds or yeasts based on the development of the thallus.

Yeasts are unicellular fungi that have a single nucleus and reproduce either asexually by budding or asexually by sporeformation.

A mold is made up of long branched thread-like filament calledhyphae that form a tangled mass called mycelium.

Some fungi are saprophytes and grow best in moist dark habitats.

They are usually aerobic but some types of yeasts are facultative.

Asexual reproduction occurs in fungi by the production of specific types of spores which are easily dispersed.

Sexual reproduction occurs by the fusion of hyphae or cells of different mating types.

6.0 TUTOR-MARKED ASSIGNMENT

1. Describe the structure of a mold.
2. Describe each of the following types of asexual fungal spores:
   a. Sporangiospore
   b. Conidiospore and
   c. Blastospor

7.0 REFERENCES/FURTHER READING

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MODULE 3 GENERAL CHARACTERISTICS OF MICROORGANISMS CONTD.

UNIT 1 GENERAL CHARACTERISTICS OF VIRUSES

CONTENTS

1.0 INTRODUCTION

Viruses are a cellular entities. They are genetic elements that cannot replicate independently of a living cell called the host cell. Viruses have extracellular forms which enable them to exist outside the host for long periods. But to multiply, they have to enter a cell in which they can replicate causing infection. Viruses are the most numerous microorganisms on earth and infect all types of cellular organisms. The study of viruses is known as virology. This unit examines the general characteristics of viruses, their structures, genomes, symmetry, replication in hosts and purification.

2.0 OBJECTIVES

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

- define the term virus
- state the general characteristics of virus
- describe the structure of a typical virus particle
- explain the symmetry of capids
- explain the process of viral replication in susceptible host
- state various methods of cutting viruses

- state the various methods of virus purification.

3.0 MAIN CONTENT

Definition

Viruses are simple a cellular entities that can only reproduce within living cells.

3.1 General Characteristics of Viruses

i. They are the smallest microorganisms. They range in size from10 to 400□m in diameter and can only be viewed under an electron microscope.
ii. They are a cellular, i.e. not cellular and non living.
iii. They only reproduce when present within living cells.
iv. They are infectious agents.
v. A complex virus particle or virion consists of one or more molecules of DNA or RNA enclosed in a coat of protein.
vi. Viruses can exist in two phases: extracellular and intracellular.
vii. The extracellular phase known as virion possesses few if any enzymes and cannot reproduce independent of living cells. It is metabolically inert and does not carry out respiration.
viii. In the intracellular phase, viruses exist primarily as replicating nucleic acids in the host cells that induce host metabolism to synthesise virion components which are later released.

Viruses differ from living cells in three ways:

i. They have simple a cellular organisation.
ii. The presence of either DNA or RNA but not both in almost all virions.
iii. They do not have the ability to reproduce independent of cells and carry out cell division as procaryotes and eukaryotes do.

3.2 Virion Size

Virions range in size from about 10 to 400μm in diameter. The smallest viruses are a little larger than ribosomes whereas the pox viruses which include vaccinia are about the same size as the smallest bacteria and can be seen in the light microscope. Most viruses however, are too small to be visible in the light microscope and must be viewed with scanning and transmission electron microscope.

3.3 The Structure of Viruses

A virus is made up of a central genetic nucleic acid molecule surrounded by a protein coat called a capsid. The combination of both is called the nucleocapsid. The capsid surrounds and protects the viral nucleic acid. The capsid also gives the virus a characteristic shape and help to establish the specificity of the virus for a particular host cells. Capsids are large macromolecular structures that self assemble from many copies of one or a few types of proteins. The protein used to build the capsids is called protomers. The simplest virus is a naked virus (nucleocapsid) consisting of a geometric capsid assembled around a nucleic acid. Onthe other hand, we can have a virus made up of a nucleocapsid surrounded by a flexible membrane called an envelope. This type of virus is called an envelope virus.The various morphology types of viruses results from the combination of a particular type of capsid symmetry with the presence or absence of an envelope which is a lipid layer external to the nucleocapsid.

3.4 Viral Genomes

All cells contain double stranded DNA genomes. By contrast, viruses have either DNA or RNA genomes (one group of viruses does use both DNA and RNA as their genetic material but at different stages of the replication cycle). Hence, we have RNA viruses or DNA viruses. Virus genomes can be classified based on whether the nucleic acid in the virion is DNA or RNA and further subdivided to whether the nucleicacid is single or double stranded. Linear or circular, some viral genomesare circular but most are linear.

We can have single stranded DNA, double stranded DNA, single stranded RNA and double stranded RNA. All four types are found in animal viruses. Most plant viruses have single stranded RNA genomesand most bacteria viruses contain double stranded RNA.

3.5 Capsids Symmetry

There are three types of capsid symmetry: helical, icosahedral and complex.

1. **Helical Capsids**

They are shaped like hollow tubes with protein walls. The tobacco mosaic virus is an example of this virus. In this virus, the self assembly of protomer in a helical or spiral arrangement produces a long rigid tube, 15 to 18 nm in diameter by 300nmlong.The capsid encloses an RNA

genome, which is wound in a spiral and lies within a groove formed by the protein molecule. The size of a helical capsid is influenced by both its protomers and nucleicacid enclosed within the capsid.

2. **Icosahedral Capsid**

The icosahedral is a regular polyhedron with 20 equilateral triangular faces and 12 vertices and is roughly spherical in shape. It is one of the nature's favourite shapes. A few genes sometimes only one can code for protein that self-assemble to form the capsid. These capsids are constructed from ringo-krob-shapedinto caller capsomers each usually made up of five or six protomers. Pentamers (pentons) have five subunits hexamers (hexons) have six.

3. Viruses with Capsids of Complex Symmetry (Complex viruses) Some virons are more complex than the helical and icosahedralcapsid being composed of several parts, each with separateshapes and symmetries. The most complex viruses in terms of structures are some of the bacterial viruses which possessicosahedral heads plus helical tails. In some bacterial viruses such as bacteriophage T4 of *Escherichia coli* the tail itself has a complex structure. The complete T4 tail has 20 different proteins and the T4 head has several more protein.

SELF-ASSESSMENT EXERCISE

i. How does a virus differ from a cell?
ii. What is the difference between a naked virus and an enveloped virus?

3.6 Virus Reproduction

Viruses need a host cell in which to reproduce; hence the first step in the life cycle of a virus is attached to a host. This is followed by entry of either the nucleocapsid or the viral nucleicacid into the host. If the nucleocapsid enters uncoating of the genomeusually occurs before further steps can occur. Once free in the cytoplasm, genes encoded by the viral genome are expressed, i.e. the viral genes are transcribed and translated. This allows the virus to control the host cell's biosynthetic machinery so that new virions can be made. The viral genome is then replicated and viral proteins are synthesised. New virions are constructed by self assembly of coat proteins with the nucleic acids and finally, the matured virions are released from the host. Summarily, the steps involved in viral replication or reproduction are:

- attachment of the virion to a susceptible host
- penetration or entry of the virion or its nucleic acid into the host

- synthesis of virus nucleic acid and protein by cell metabolism as directed by the virus
- assembly of capsids and packaging of viral genomes into new virions
- release of mature virions from the cell.
- However, there is great variation in the details of virus reproduction for individual virus species.

3.7 The Cultivation of Viruses

Because viruses are unable to reproduce independent of living cells, they cannot be cultured in the same way as prokaryotic and eukaryotic microorganisms. Animal viruses are cultivated by inoculating suitable host animals or embryonated egg – fertilised chicken eggs incubated about 6 to 8 days after laying. More recently, animal viruses have been grown in tissue (cell) culture on monolayers of animal cells. Bacterial and Archea viruses are cultivated in either broth or agarcultures of young, actively growing cells. Plant viruses are cultivated in a variety of ways which include plant tissue cultures, cultures of separated cells, or cultures of protoplasts (cells lacking cell wall) and growing of the viruses in whole plants.

3.8 Virus Purification and Assay

Viral purification and Assays are necessary so as to accurately study virus structure, reproduction and other aspects of their biology.

Virus Purification

This involves getting or isolating the viral particle in its pure state, purification makes use of several virus properties. Four of the most widely used methods to isolate and purify viruses are:

- differential and density gradient centrifugation. This is often used in the initial purification steps to separate virus particles from host cells.
- precipitation of viruses particles.
- denaturation of contaminants.
- enzymatic digestion of host cells constituents.

Virus Assays

The quantity of viruses in a sample can be determined either directly by counting particle numbers using the electron microscope or indirectly by measurement of an observable effect of the virus using techniques such as the hemagglutination as say.

SELF-ASSESSMENT EXERCISE

i. Explain how viruses are cultivated in different hosts.
ii. Give the four major approaches by which viruses may be purified.

4.0 CONCLUSION

It can be seen clearly that viruses are a complex, diverse and fascinating group of microorganisms, uniquely different from other groups of microorganisms which are cellular.

5.0 SUMMARY

Viruses are simple a cellular entities that can only reproduce within living cells.

A virus is made up of a central genetic nucleic acid molecule which could be DNA or RNA surrounded by a protein called capsid.
Capsids are large macromolecular structures that self assemble from many copies of one or a few types of proteins.

There are three types of capsids: symmetry helical, icosahedraland complex.

Viruses range in size from 10 to 400 NM in diameter and can only be viewed with scanning and transmission electron microscope.
Viral nucleic acid can either be single stranded or double stranded DNA or RNA.
Some viral geromes are circular while some are linear.

Virus reproduction can be divided into five steps:

i attachment
ii entry into host
iii synthesis of viral nucleic acid and proteins
iv self assembly of virions and
v release from host.

Viruses are cultivated using tissue cultures, embryonated eggs, bacterial culture and other living hosts.

Viral purification involves getting the viral particle in its purestate and involves techniques such as differential and gradient centrifugation, precipitation, denaturation or digestion of contaminants.

Virus particles can be counted directly with the transmission electron microscope or indirectly by the hemagglutination assay.

6.0 TUTOR-MARKED ASSIGNMENT

i. Explain the processes involved in viral replication or virus reproduction.
ii. Write briefly on viral genomes.
iii. Define the following terms:
   a. virus
   b. nucleocapsid
   c. helical symmetry
   d. viral purification.

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UNIT 2 GENERAL CHARACTERISTICS OF ALGAE

CONTENTS

1.0 INTRODUCTION

Algae (singular, alga) are unicellular microorganisms that have chlorophyll and are photosynthetic. Algae are heterogeneous and range from microscopic unicellular forms to macroscopic seaweeds. They are different from green plants due to their simple reproductive structure for sexual reproduction. Many live in aquatic environments but many also thrive as subterranean algae. Algae are of great importance to biologist because single algal cells are complete organisms capable of photosynthesis and the synthesis of other compounds which constitute the cell. The study of algae is known as phycology. This unit examines the general characteristics, the distribution, the morphology and importance of algae.

2.0 OBJECTIVES

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

- define the term algae
- state the general characteristics of algae
- describe the general structure of algae with a typical example
- draw a well labeled diagram of algae
- state the different habitats of algae
- explain the methods of reproduction in algae
- state the economic importance of algae.

3.0 MAIN CONTENT

General Characteristics of Algae

Algae are eukaryotic microorganisms.

They are photosynthetic microorganisms.
Chlorophyll and other pigments are found in membrane bound organelles known as chloroplasts. Algae contain a discretenucleus. Other inclusions are starch grains, oil droplets and vacuoles. They contain chlorophyll and utilise light energy to generate their chemical energy.

They have a wide range of sizes and shapes. Many species occuras single cells that may be spherical, rod shaped, club-shaped orspindle-shaped. Others are multicellular and appear in every conceivable form, shape and degree of complexity.

In most species the cell wall is thin and rigid cell walls of diatoms are impregnated with silica making them thick and very rigid.

The motile algae such as euglena have flexible cell membrane called periplasts.

They are also able to produce oxygen from water.

3.1 Occurrence and Distribution

Algae are found in many places on earth. They occur in great abundance in the ocean, seas, salt lakes, fresh water lakes ponds and streams. Many are found in damp soil, on rocks, stones and tree barks. Some are found on plants and animals. Small aquatic forms make up a large part of the free-floating microscopic life in water called plankton which is the principal food for aquatic animals including such large ones as whales. Plankton is generally considered to be composed of both algae and microscopic animal forms. Phytoplankton is made up of plants, i.e. algal forms and zooplankton is composed of animal organisms. Algae are found where there are sufficient light, moisture and simple nutrients to sustain them. Some species of algae grow on the snow and ice of Polar Regions and mountain peaks, sometimes occurring in such abundance that the landscape becomes coloured by the red pigments in their cells.

At the other extreme, some algae grow in the hot springs at temperatures as high as 550C. Some freshwater algae have adapted their metabolism to the high salt concentration found in the brine lakes of the arid South-Western United States. Some algae are adapted to moist soil, the bark of

trees and the surface of rocks, which the algae degrade. The decomposition products are made available for soil building and enrichment. Algae are often a problem in water supplies because they produce undesirable taste and odour.

Heavy algal growth may form blankets or mats which interfere with the use of some natural waters for recreational purposes. These algal mats may act as barriers to the penetration of oxygen into the water; they prevent photosynthesis by excluding light from deeper water and this may cause fish and other marine animal to suffocate. On the other hand, when dispersed in natural waters, algae increase the oxygen concentration through photosynthesis. Heavy growth of some algae reduces hardness of water and removes slats which are the cause of blackishness. Some algae are endophytic; that is, they are not free-living but live in other organisms. Such algae are widespread in protozoa, molluscs, sponges and corals.

3.2 Morphology

Algae have a wide range of sizes and shapes. Many are unicellular and may be spherical, rod shaped, club shaped or spindle shaped. Others are multicellular and appear in every conceivable forms, shape and degree of complexity including membranous colonies, filaments grouped, singly or in clusters with individual strands which may be branched or unbranched tubes. Algal cells are eukaryotic. Most are thin and rigid cell walls; however, the cell walls of diatoms are impregnated with silica threads which make them thick and very rigid. Algae have a discrete nucleus, starch grains on droplets and vacuoles: chlorophyll and other pigments are found in membrane-bound organelles known as chloroplasts. The chloroplast ultra-structure and type of pigment presents in algae are used for their classification, e.g. green algae, red algae, yellow green algae, the golden algae, etc. A very common green alga is spirogyra; a filamentous alga found on the scum that cover ponds are slow moving water.

3.3 Motility

The motile algae also called the swimming algae have flagella occurring singly or in clusters at the anterior or posterior ends of the cells. Some algae have no means of locomotion and are carried by tides, waves and currents. Some attach themselves to the substrate in the body of water where they live and are occasionally broken loose by currents which move them to new locations. In some forms, only the zoospores, the asexual reproductive cells are motile.

3.4 Reproduction

Algae may reproduce either asexually or sexually. Some species are limited to one of these processes. However, they have complicated lifecycles involving both asexual and sexual means of reproduction.

3.4.1 Asexual Reproduction

Asexual reproduction processes in algae include:

- purely vegetative binary fission.
- production of unicellular spores, many of which, especially in the aquatic forms have flagella and are motile, these are called zoospores.

In terrestrial types of algae, non-motile spores or aplanospores are formed; however, some aplanospores can develop into zoospores.

3.4.2 Sexual Reproduction

All forms of sexual reproduction are found among the algae. In this processes there is a fusion (conjugation) of sex cells called gametes to form a zygote. If the gametes are identical, i.e., there is no visible sex differentiation. The fusion process is called isogamous. However, if two gametes are different, the process is called heterogamous. In higher algae, the sex cells are differentiated into male and female.The female egg cell (ovum) is large and non motile, while the malegametes (sperm cell) is small and are actively motile. This type of sexual reproduction is called zoogamy.

3.5 Biological and Economic Importance of Algae

1. Algae as Primary Producers

Algae form the base or beginning of most aquatic food chains because of their photosynthetic activities and are therefore called primary producers of organic matter.

2. Commercial Product from Algae

Many product of economic value are derived from algal cell walls. Three of these, agar, alginic acid and carrageenan, are extracted from the walls of algae. Another, diatomaceous earth, is composed of millions upon millions of diatom glass walls deposited over time on either fresh water or the ocean. All three compounds are used either make gels or to make solution viscous. Carrageenan has been used as a stabiliser or

emulsifierin foods such as ice cream and other milk products. It is also used as a binder in toothpaste or in pharmaceutical products, as well as an agent in ulcer therapy. Carrageenan is also useful as a finishing compound in the textile and paper industries, as a thickening agent in sharing creams and lotions and in the soapindustry. Agar is well known as solidifying agent in the preparation of microbiological media. It is obtained from red algae. Species of *Gelidium* and *Gracelaria* are used extensively. It is also important in the food industry when it is valuable in the manufacture of processed cheese, mayonnaise, pudding, jellies, baking products and canned goods. In the pharmaceutical industry, agar can be used as a carrier for a drug. Lotions and ointments can contain some agar. About 50 percent of the ice cream in the U.S. contains alginates which provide a smooth consistency and eliminate ice crystal formation. Alginate is also incorporated into cheeses and bakery products, especially frostings. Other industrial application includes paper manufacturing, the printing of fabrics and paint thickening.

Diatomaceous earth is used primarily for filters or filter aids. It is especially suitable because it is not chemically reactive, is not readily compacted or compressed during use and is available in many grades.

3. Algae as Food

Many species of algae (mostly red and brown algae) are used as food in the Far East. Of the red algae are of the most important in porphyria; it is used as a food in Japan where it is called “mori”and is usually processed into dried sheets.

4.0 CONCLUSION

Algae are of great and general interest to all biologists because single algal cells are complete organisms capable of photosynthesis and the synthesis of a multitude of other compounds which constitute the cell.

5.0 SUMMARY

The algae are photosynthetic eukaryotic microorganisms.

Algae are found in many places on earth including ocean, lakes, ponds, streams, moist soils, rocks, tree barks, ice and hot springs.

Algae may be unicellular or multicellular, have cell wall, a discrete nucleus starch grains, oil droplets, vacuoles chlorophyll and other pigments.

Algae reproduce asexually by binary fission and sexually by

fusion of gametes to form a zygote.
Algae are primary producers in most aquatic food chains.
Commercial products such as agar alginic acid and carrageenanare derived from algae.

6.0 TUTOR-MARKED ASSIGNMENT

i. State five characteristics of algae.
ii. Discuss the various uses of algae that make the commercially important.

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UNIT 3 GENERAL CHARACTERISTICS OFPROTOZOA

CONTENTS

1.0 INTRODUCTION

Protozoa are unicellular, non-photosynthetic eukaryotic organisms. They are distinguished from other eukaryotic protists by their ability to move at some stage of their life cycle and by their lack of cell walls. Some protozoa are free living while some are parasitic. Some protozoa form colonies, in a colony, the individual cells are joined by cytoplasmic thread or embedded in a common matrix, hence colonies of protozoa are essentially a cluster of independent cells. The study of protozoa is called Protozoology. This unit examines the general characteristics of protozoa, the morphology, occurrence, reproduction and economic importance of protozoa.

2.0 OBJECTIVES

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

- define the term, protozoa
- state the general characteristics of protozoa
- state the places where protozoa are found
- describe some intracellular structures of protozoa.

3.0 MAIN CONTENT

General Characteristics of Protozoa

- They are unicellular, non-photosynthetic microorganisms.
- They are predominantly microscopic in size.
- They occur generally as single cells.
- They lack cell walls.
- They have ability to move at some stages of their life cycle. Many are motile.
- The majority of protozoa are between 5 and 250μm in diameter.
- They occur in colonies with each colony having independent individual cells.
- Protozoa may be divided into free-living forms and those living on or in other organisms.

3.1 Occurrence/Distribution of Protozoa

Protozoa are found in all moist habitats. They are common in the sea, in soil and freshwater. Free-living protozoa have even been found in the Polar Regions and at very high altitudes. Parasitic protozoa may be found in association with most animal groups. Most protozoa survive dry conditions by the formation of a resistant cyst or dormant stage.

3.2 Ecology of Protozoa

From the ecological standpoint, protozoa may be divided into free-living forms and those living on or in other organisms. The latter group is referred to as the symbiotic protozoa. Some of the symbiotic ones are parasitic and may cause disease. Others such as those found in the gut of the termite are beneficial to the host (live in a mutualistic association).

3.2.1 Free-Living Protozoa

Free-living protozoa are found in a variety of habitats. The factors which influence the distribution and number of free-living protozoa in a habitat

are: moisture, temperature, light, available nutrients, and other physical and chemical conditions.

3.2.2 Symbiotic Protozoa

This is a type of co-existence between protozoa and other organisms which differ in many ways and include:

1. Commensalism: In which the host is neither injured nor benefitted but the commensal (protozoa) is benefitted, e.g. the protozoa living in the lumen of the alimentary tract.
2. Mutualism in which some flagellates are present in the gut of termites and help to digest the woody materials eaten by termite to a form which can be used by the host cells. If deprived of these flagellates, the termite dies, if the flagellates are removed from the termite gut, they also die.
3. Some protozoa are parasites, they live at the expense of other organisms, and an example is *Plasmodium* which is a parasite of man and causes malaria in man.

3.3 Morphology of Protozoa

The size and shape of these organisms show considerable variable. Like all eukaryotic cells, the protozoan cell also consists of cytoplasm, separated from the surrounding medium by a special cell envelope, and the nucleus or nuclei.

3.3.1 The Cytoplasm

The cytoplasm is a more or less homogeneous substance consisting of globular protein molecules loosely linked together to form a three-dimensional molecular framework. Embedded within it are the various structures that give protozoan cells their characteristic appearance. Submicroscopic protein fibrils (fibrillar bundles, myonemes, and microtubules) are groups of parallel fibrils in the cytoplasm. Protozoan contractility is probably due to these fibrils. In several forms of protozoa, pigments are diffused throughout the cytoplasm. These are numerous. They can be green, brown, blue, purple
or rose.

In the majority of protozoa, the cytoplasm is differentiated into the ectoplasm and the endoplasm. The ectoplasm is more gel-like and the endoplasm is more voluminous and fluids, but the change from one layer to another is gradual. Structures are predominantly found in the endoplasm. Like other eukaryotic cells, protozoa have membrane systems in the cytoplasm. They form a more or less continuous network

of canals and lacunae giving rise to the endoplasmic recticulum of the cell. Other structures in the cytoplasm include ribosomes, Golgi complexes ordictyosomes (piles of membranous sacs) mitochondria, kinetosomes or blepharoplasts (intracytoplasmic basal bodies of cilia or flagella), food vacuoles, contractile vacuoles, and nuclei.

3.3.2 Nucleus

The protozoan cell has at least one eukaryotic nucleus. Many protozoa, however, have multiple nuclei (e.g. almost all ciliates throughout the greater part of the life cycle). The protozoan nuclei are of various forms, sizes, and structures. In several species, each individual organism has two similar nuclei. In the ciliates two dissimilar nuclei, one large (macronucleus) and one small (micronucleus) are present. Themacronucleus controls the metabolic activities and regeneration processes; the micronucleus is concerned with reproductive activity.

3.3.3 Cysts

Many protozoa form resistant cysts at certain times of their life cycle. As indicated before, these cysts are able to survive adverse environment conditions such as desiccation, low nutrient supply, and even anaerobiosis. In parasitic protozoa, the developmental stages are often transmitted from host to host within a cyst. Other kinds of cysts (e.g.

- reproductive) are also known.
- Cysts have four basic functions:
- protect against unfavourable conditions
- serve as site of multiplication
- assist in attachment to surfaces such as hosts
- transmission stage from host to host.
- Asexual reproduction in some ciliates and flagellates is associated with cyst formation. Sexual reproduction of sporozoa invariably results in acyst.

3.3.4 Locomotory Organelles

Protozoa may move by three types of specialised organelles:pseudopodia, flagella and cilia. In addition, a few protozoa without such organelles can carry out a gliding movement by body flexion.

1. **Pseudopodia**
A pseudopodium is a temporary projection of part of the cytoplasm of those protozoa which do not have a rigid pellicle. Pseudopodia are

therefore characteristic of the amoebas (sarcodina). These organelles are also used for capturing food substances.

2. **Flagella**

The flagellum is an extremely fine filamentous extension of the cell. As a rule, the number of flagella present in an individual protozoan varies from one to eight; one or two is the most frequent number. A flagellum is composed of two parts; anelastic filament called an axoneme and the contractile cytoplasmic sheath that surrounds the axoneme.

3. **Cilia**

1. They are fine and short threadlike extensions from the cell.
2. In addition to their locomotory function, also aid in the ingestion of food and serve often as a tactile organelle. They may be uniform in length, or may be of different lengths depending on their location. Generally, cilia are arranged in longitudinal, oblique, or spiral rows, inserted either on the ridges or in the furrows.

3.3.5 Feeding Structure

Food-gathering structures in the protozoa are diverse and range from the pseudopodia of amoebas through the tentacular feeding tubes of sectarians to the well-developed “mouths” of many ciliates. Amoebas gather food by means of pseudopodia engulfment. In ciliates the cytostome is the actual opening through which food is ingested.

An oral groove is an indentation in the pellicle of certain ciliates. It guides food toward the cytostome and acts as a concentrating device. The addition of membranelles to the oral groove makes it a peristome.

Nutrition

1. Nutrition in protozoa is heterotrophic.
2. They obtain cellular energy from organic substances such as proteins.
3. Protozoa engulf and ingest their food sources.

3.4 Two Examples of Protozoan

3.4.1 Amoeba

3.4.2 Paramecium

Fig. 1b: A Paramecium

3.5 Reproduction of Protozoa

Protozoa general multiply by asexual reproduction. Many protozoa are able to carry out both asexual and sexual processes. Some parasitic forms may have an asexual phase in one host and a sexual phase in another host.

3.5.1 Asexual Reproduction

Asexual reproduction occurs by simple cell division, which can be equalor unequal – the daughter cells are of equal or unequal sizes,respectively. If two daughter cells are formed, then the process is calledbinary fission. If many daughter cells are formed, it is called multiplefission. Budding is a variation of unequal cell division.

1. **Binary Fission**

The simplest form of binary fission is found in the amoebas. The pseudopodia are withdrawn before the nucleus divides. After the nucleus divides, the organism elongates and constricts in the centre in order to form two daughter cells.

2. **Multiple Fission**

In multiple fission, a single mother (parental) cell divides to form many daughter (filial) cells. Division is usually preceded by formation of multiple nuclei within the mother cell, which then cleaves rapidly to form a corresponding number of daughter cells.

Multiple fission is not as widespread as binary fission but it often takes place in addition to the latter process. In ciliates and flagellates, this type of fission is found in relatively few species.

3. **Budding**

In protozoology it is often used to describe the varied processes by which sessile protozoa produce motile offspring. That is, the mother cell remains sessile and releases one or more swarming daughter cells. The

swarmer differs from the parent cell not only in a lower degree of differentiation but also in the possession of special locomotor organelles. Some form of budding is found in all sessile ciliates and is used to disseminate the species while the mother cell remains in situ.

3.5.2 Sexual Reproduction

Various types of sexual reproduction have been observed amongprotozoa. Sexual fusion of two gametes (syngamy or gametogamy) occurs in various groups of protozoa.

They include:

Conjugation, which is generally a temporary union of two individuals for the purpose of exchanging nuclear material, is asexual process found exclusively in the ciliates. After exchange of nuclei, the conjugants separate and each of them gives rise to its respective progency by fission or budding. When the gametes (which develop from trophozoites) are morphologically alike, they are called isogametes. When they are unlike in morphology(as well as physiology), they are anisogametes and can be either rmicrogametes or macrogametes.

3.6 Economic Importance of Protozoa

Protozoa are important links in the food chain of communities inaquatic environment where they act as primary consumers.

They are used in biological treatment of sewage or industrial effluents.
Some protozoa cause disease in mammals including man.
They are important research organisms for biologists and chemists.

4.0 CONCLUSION

Protozoa are eukaryotic heterototrophic microorganisms that are classified on the basis of morphological characteristics.

5.0 SUMMARY

- Protozoa are unicellular non-photosynthetic microorganisms that lack cell wall and have ability to move at some stages of their life.
- Protozoa are normally found in moist habitats.
- Protozoa may be free living found in various habitats or symbiotic found in co-existing with other organisms.

- The size and shape of protozoa vary considerable and the cell consists of the cytoplasm (separated from the surrounding medium by an envelope) and the nucleus or nuclei.
  Locomotory organelles in protozoa include pseudopodia, cilia and flagella.
- Reproduction in protozoa is mainly asexual, however many are able to carry out both asexual and sexual reproduction.
- Methods of asexual reproduction in protozoa include binary fission, multiple fission and budding.
- Sexual reproduction in protozoa involves the fusion of two gametes.
- Protozoa are primary consumer in food chain, in aquatic environment. They are used to degrade biological and industrial effluents. They also cause disease of man and other animals.

6.0 TUTOR-MARKED ASSIGNMENT

1. State five general characteristics of protozoa.
2. Draw a well labeled diagram of amoeba.
3. Write on three locomotory organelles in protozoa.

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MODULE 4 MICROBIAL GROWTH, REPRODUCTIONAND CONTROL

UNIT 1 MICROBIAL GROWTH

CONTENTS

1.0 INTRODUCTION

Microbial growth is defined as an increase in the number of cells. Amicrobial cell has a lifespan and a species is maintained only as a result of continued growth of its population. Growth is the ultimate process in the life of a cell – one cell becoming two and subsequently leading to an increase in the number in a population of microorganisms.

In microbiology, growth is synonymous to reproduction. This unit examines the term growth, binary fission, the mode of cell division in prokaryotic cells, stages in the growth curve and the mathematics of growth.

2.0 OBJECTIVES

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

- define the term growth
- explain the process of a binary fission in a prokaryotic cell

- draw and explain the microbial growth curve of micro organisms in a batch culture
- explain the mathematics of growth
- describe a continuous culture.

3.0 MAIN CONTENT

Definition of Growth

Growth is defined as an increase in the number of cells in a population of microorganisms. It is an increase in cellular constituents leading to arise in cell number when microorganisms reproduce by processes like binary fission or budding.

3.1 The Prokaryotic Cell Cycle

A prokaryotic cell cycle is the complete sequence of events from the formation of a new cell through the next division. Most prokaryotes reproduce by binary fission, budding or fragmentation.

3.2 Binary Fission

Binary fission is a form of asexual reproduction process. In which a single cell divides into two cells after developing a transverse septum(cross wall).Binary fission is a simple type of cell division and the processes involved are: the cell elongates, replicates its chromosomes and separates the newly formed DNA molecules so that there is a chromosome in each half of the cell. A septum is formed at mid cell; divide the parent cell into two progeny cells and each having its own chromosome and a copy or complement of other cellular constituents.

3.2.1 Stages in Binary Fission

3.2.2 Chromosome Replication and Partitioning

Most prokaryotic chromosomes are circular. Each circular chromosome has a single site at which replication starts called the origin of replication, or simply the origin. Replication is completed at the terminus which is located directly opposite the origin. Early in the cell cycle, the origin and terminus move to mid-cell and a group of proteins needed for DNA replication proceeds in both directions from the origin and the parent DNA synthesis assemble to form the replisome at the origin. DNA replication proceeds in both directions from the origin and the parent DNA is thought to spool through the replisome, which remains relatively stationary. As progeny chromosomes are synthesized,

the two newly formed origins move toward opposite ends of the cell, and the rest of the chromosome follows in an orderly fashion.

3.2.3 Cytokinesis

Septation is the process of forming a cross wall between two daughter cells. Cytokinesis, a term that has traditionally been used to describe the formation of two eukaryotic daughter cells, is used to describe the process in procaryotes as well. Septation is divided into several steps:
Selection of site where the septum will be formed.

Assembly of a specialised structure called the Z ray, which divides the cell in two by construction.

Linkages of the Z ring to the plasma membrane and perhaps components of the cell walls. Assembly of the cell wall-synthesizing machinery.
Construction of the Z ring and septum formation.

The Z protein, a tubulin homologue found in most bacterial and many archaea forms the Z ray. Fts Z, like tubulin, polymerizes to form filaments. Once the Z-rings form, the rest of the division machinery is constructed. First one or more anchoring protein links the Z ring to the cell membrane. Then the cell wall – synthesizing machinery is assembled. The final steps in division involve construction of the Z ring, accompanied by in vagination of the cell membrane and synthesis of the septal wall.

SELF-ASSESSMENT EXERCISE

i. Define the term growth.
ii. What is binary fission?
iii. Outline the stages in binary fission.

3.3 The Growth Curve

This is a curve that describes the entire growth cycle of a microorganism. It is the growth of microorganism reproducing by binary fission, plotted as the logarithm of the number of viable cells versus the incubation time.

Stationery phase, Exponential (log)phase ,Death phase ,Lag phase Time

When microorganisms are cultivated in liquid medium they are usually grown in a batch culture or closed system. That is, they are incubated in a closed culture vessel with a single batch of medium. The growth curve

has four phases: *the lag phase, the exponential phase,the stationary phase and the death phase.*

3.3.1 Lag Phase

This is a phase in which there is no increase in the cell number of a microbial population freshly introduced into a fresh culture medium. In this phase, there is no cell division and growth. However, the cell is metabolically active synthesising new components.
Lag phase before cell division begins may be necessary for these reasons:

i The cell may be old and depleted of ATP, essential cofactors and ribosome which the cell synthesises at this phase or stage.
ii The new medium may be different from the one the microorganism was growing in previously; the cells synthesise new enzymes to be used in the new medium.
iii The cell is acclimatising to a new environment.
iv The cells may be injured and require time to recover.

Log Number of Viable cells

The period of the lag phase varies depending on the condition of the microorganisms and the nature of the medium. The phase may be long, if:

The inoculum is from an old culture or one that has been refrigerated.
If the new medium is chemically different from the old one from which the microorganism was taken. However, if a young, actively growing culture is transferred to a fresh medium of the same composition, the lag phase will be short or absent.

3.3.2 Exponential Phase

This is also known as the log phase. It is a period in which the microorganisms are growing and dividing at the maximal rate possible given their genetic potential, the nature of the medium and the conditions under which they are growing. The rate of growth is constant during this phase because the microorganisms are dividing and doubling in number at regular interval. It is during this period that the generation time of the organism is determined. The log of the number of cells plotted against time results in a straight line. In this phase, the population is most nearly uniform in terms of chemical composition of cells, metabolic activity and other physiological characteristics exponential phase vary among different bacteriae.The exponential growth is balanced growth this is because cellular constituents are

manufactured at constant rates relative to each other .Unbalanced growth results if the nutrient level and other environmental conditions change. Unbalanced growth is growth during which the rate of synthesis of cell components vary relative to each other until a new balanced state is reached. This is observed in two types of experiments (1) shift up and(2) shift down. The shift up occurs when a culture of microorganism is transferred from a nutritionally poor medium to a richer one while the shift down occurs when a culture of microorganism is transferred from a rich medium to a poor one.

3.3.3 The Stationary Phase

This is a phase in which population growth ceases and the growth curve becomes horizontal. In this phase, the total number of viable microorganisms remains constant. This may result from a balance between cell division and cell death or the population may simply cease to divide but remain metabolically active. Factors responsible for stationary phase when a required nutrient is exhausted are:

i Nutrient limitation: If an essential nutrient has been used up, e.g.O2 or carbon source and becomes unavailable to the microorganisms, population growth will cease.

ii Accumulation of toxic waste products: e.g. *Streptococci* can produce too much lactic acid and other organic acids from sugar fermentation that their medium becomes acidic and growth is inhibited.

iii When a critical population level has been reached: This will prevent further dividing and doubling of the cells/when physical conditions do not permit a further increase in population size. Once the stationery growth phase is reached, there is no further net increase in bacterial cell numbers.

3.3.4 Death Phase

This is a phase in which the number of viable cells begins to decline. During this phase, the number of living cells decreases because the rate of cell death exceeds the rate of new cell formation. The depletion of essential nutrients and the accumulation of laboratory products such as acids contribute to the death rate.

3.4 The Mathematics of Growth

During the exponential phase each microorganism is dividing at constant interval which means the population will double in number during a specific length of time called the generation time. One cell divides to

produce two cells. Thus, if we start with a single bacterium, the increase in population is by geometric progression:1 2 22 23 24 25 …2n
Where n= the number of generations. Each succeeding generation, assuming no cell death, doubles the population. The total population Nat the end of a given time period would be expectedly:

N=1 X 2n …….Eq. (1)
However, under practical conditions, the number of bacteria no inoculated at time zero is not 1 but more likely several thousand, so the formula now becomes:
N = N0 X 2n …….Eq. (2)
Where N = total population at the end of given time period. Solving equation (2) for n, we have:
log10N=log10N0 + nlog102
n=log10N-log10N0
log102 …….Eq. (3)
N0= Initial population at a given time
n= the number of generation.
If we now substitute the value of Log102, which is 0.301, in the above equation,
n = log10N-log10N0
0.301
n = 3.3 (log10N-log10N0)
…….Eq. (4)
Thus, using equation (4), we can calculate the number of generations that have taken place, provided we know the initial population and the population after the growth has occurred. The generation time g (the time required for the population to double)can be determined from the number of generations n that occur in a particular time interval t.

Using equation 4 for n, the generation time can be calculated by the formula.

g = t = t
n 3.3 (log10N – log 10N0) ..…….Eq. (5)
The growth rate R is the number of generations per hours) and it is the reciprocal of the generation time (g). It also the slope of straight n obtained when the log number of cells is plotted against the time.

Hence we have: R = 3.3 log10N – log10 Not

SELF-ASSESSMENT EXERCISE

i. In what phase of the growth curve are cells dividing in a regular and orderly process?
ii. Why do cells enter the stationery phase?

3.5 The Continuous Culture of Microorganism

A continuous culture is an open culture. The continuous culture vessel maintains a constant volume to which fresh medium is added at a constant rate and an equal volume of spent culture medium is remove the same rate. Once such a system is in equilibrium the chemostat volume, cell number and nutrient status remain constant and the system is said to be in a steady state.

Two major types of continuous culture system commonly used are:

i chemostats and
ii turbid stats.

The chemostat: A chemostat is a device in which a liquid medium is continuously fed into the bacteria culture. It is an apparatus designed to permit the growth of bacterial cultures at controlled rates. A chemostatis constructed so that sterile medium is fed into the culture vessel at the same rate as the spent media containing microorganisms is removed. The culture medium for a chemostat possesses an essential nutrient in limiting quantities. Hence, the growth rate is determined by the rate at which new medium is fed into the growth chamber and the final cell density depends on the concentration of the limiting nutrients. The rate of nutrient exchange is expressed as the dilution rate (D), the rate at which medium flows through the culture vessel relative to the vessel volume, where f is the flow rate (ml/hr) and V is the vessel volume (ml)
D= fv

Both the microbial population and generation time are related to the dilution rate. The generation time decreases as the dilution rate increase, while the microbial population density remains unchanged over a wide range of dilution rates at very low dilution rates, an increase in D causes a rise in both cell density and the growth rate and as the dilution rate increases, the amount of nutrients and resulting cell density rise because energy is available for both maintenance and reproduction. Continuous Culture Systems are very useful because they provide a constant supply of cells in exponential phase and growing at known rate. They also make possible the study of microbial growth at very low nutrients levels concentration close to those present in natural environments. These are useful for research in many areas. Continuous systems are also used in food and industrial microbiology.

4.0 CONCLUSION

Growth is the ultimate process in the life of a cell. Microbial cells have a finite lifespan. And a species is maintained only as a result of continued growth of its population.

5.0 SUMMARY

Growth is an increase in the number of cell in a population.

Most procaryotes reproduce by binary fission, a process in which the cell elongates and the chromosome is replicated and segregates to opposite poles of the cell prior to the formation of a septrum, which divides the cell into two progeny cells.

Two overlapping pathways function during the procaryotic cell cycle: the pathway for chromosome replication and segregation and the pathway for septrum formation, both are complex and poorly understood. The partitioning of the progeny chromosomes may involve homologues of eucaryotic cytoskeletal proteins.

When microorganisms are grown in a closed system or batch culture, the resulting growth curve usually has four phases; the lag, exponential or log, stationery, and death phases.

In the exponential phase, the population number of cells undergoing binary fission doubles at a constant interval called the doubling or generation time. The mean growth rate constant (k)is the reciprocal of the generation time.

Exponential growth is balance growth; cell components are synthesised at constant rates relative to one another. Changes in culture conditions (e.g. in shift-up and shift-down experiments)lead to unbalanced growth. A portion of the available nutrients is used to supply maintenance energy.

Microorganisms can be grown in an open system in which nutrients are constantly provided and wastes removed.
A continuous culture system is an open system that can maintain a microbial population in the log phase. There are two types of these systems: chemostats and turbidostats.

The chemostat is a continuous device in which a liquid medium is continuously fed into the bacteria culture.

6.0 TUTOR-MARKED ASSIGNMENT

1. Describe the four phases of the growth curve in a closed system.
2. Define the terms (i) generation time and (ii) mean growth rate.
3. During log-phase growth of a bacterial culture, a sample is taken at 8.00 a.m. and found to contain 1,000 cells per milliliter. A second sample is taken at 5.54 p.m. and is found to contain1,000,000 cells per milliliters. What is the generation time in an hour?

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UNIT 2 MEASUREMENT OF MICROBIAL GROWTHAND FACTORS THAT INFLUENCE MICROBIAL GROWTH

CONTENTS

1.0 INTRODUCTION

Measurement of microbial growth helps to determine growth rate and generation time. Population growth is measured by following changes in cell number and cell mass; this is because growth leads to increase in both. Also, there are different factors that affect the growth rate of microorganisms. This unit examines different methods of measurement growth and factors affecting microbial growth.

2.0 OBJECTIVES

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

- describe the methods of measuring total sum number of microorganisms
- describe the methods of counting viable cells of microorganisms
- describe the methods of measuring cells mass of microorganisms
- state the advantage and disadvantages of the difference methods of measuring microbial growth

- explain how different environmental factors affect microbial growth.

3.0 MAIN CONTENT

Measurement of Microbial Growth

Population growth is measured by following changes in the number of cells or changes in the level of some cellular components. This could be done by measuring the total cell number or by measuring the cell mass.

3.1 Measurement of Total Cell Number

The total number of microbial cells can be achieved by using direct count methods.

3.1.1 Direct Count Methods

Bacteria, microorganisms can be enumerated by direct counting procedure using:

Special counting chambers such as hemocytometer and Petroffitausser chamber can be employed to determine the number of bacteria. These chambers are ruled with squares of a know area and are constructed in such a way that a film of liquid of known depth can be introduced between the slide and the cover slips, and viewed under the microscope. Consequently, the volume of the sample overtyping each square is known. These specially designed slides have chambers of known depth with an etched grid on the chamber bottom. The number of microorganism in a sample can be calculated by taking into account the chambers volume and any sample dilution required.

Advantages

I Using a counting chamber is easy, inexpensive, and quick.
ii It gives information about the size and morphology of microorganism.

Disadvantages

i. The microbial population must be fairly large for accuracy because it involves sampling a small volume. Prokaryotes are more easily counted in these chambers if they are stained or when a phase contrast or fluorescence microscope is used.
ii. Large microorganisms such as protists and yeast can be directly counted using electronic chambers such as the coulter counter

and the flow cytometer. The microbial suspension is forced through a small hole or orifice in the coulter chamber. An electrical current flow through the hole and electrodes placed on both sides of the orifice measure its electrical resistance. Each time a microbial cell passes through the orifice, electrical resistance increases or the conductivity drops and the cell is counted. The coulter counter gives accurate results with larger cells and is extensively used in hospital laboratories to count red and white blood cells.

iii. The Membrane Filter Technique The number of bacterial in aquatic sample can be determined from direct counts after the bacteria have been trapped on special membrane filters such as a nitrocellulose 0.2 μM pore size filter or a black polycarbonate membrane filter. The sample is first filtered through the membrane after which the bacterial are stained with a fluorescent dye such as acridine orange or the DNA stain DAPI and observed under the microscope. The stained cells are easily observed against the black background of the membrane filter and can be counted when viewed under the microscope. The disadvantage of this method is that it does not distinguish between dead cells and live cells.

3.1.2 Viable Counting Methods

These methods involve plating serial dilutions of a suspension of microorganisms unto a suitable solid growth medium and after a period of incubation (in which single cells multiply to form visible colonies)the number of colonies are counted or enumerated. These methods are referred to as viable counting methods because they count only those cells that are alive and able to reproduce. Two commonly used methods are the spread plate technique and the pour plate technique.

i In the spread plate technique, the suspension sample is spread over the surface of an agar plate containing growth nutrients while in the pour plate technique; the suspension is mixed with the agar while it is still in the liquid state and poured into the plate. The plates are incubated to allow the organisms to grow and form colonies. It is assumed that each colony arises from an individual bacterial cell. By counting the number of colonies formed the colony forming units (CFUs) and taking account of the dilution factors, the concentration of bacteria in the original can be calculated. Two or three plates of the same sample are counted to determine the viable number of bacteria. Countable plates are those having between 30 to 300 colonies. Less than 30 colonies is not acceptable for statistical reasons and more than 300 colonies are likely

to produce colonies too close to distinguish as individual CFUs such samples are noted as TNTC (too numerous to count).

ii The membrane filter technique is another method that can be used for viable counts of bacteria. The sample is poured through the filter which traps bacteria. The filter is then placed on an agar medium or a pad soaked with liquid media and incubated until each cell forms a separate colony. A colony count gives the number of microorganisms in the filtered sampled and special media can be used selectively for specific microorganisms.

Advantages of Viable Counts Methods or Plating Techniques

i. They are simple technique sensitive.
ii. Widely used for viable counts of bacteria and other microorganisms in samples of food, water and soil.

Disadvantages or Limitation

i. It is selective.
ii. The nature of the growth medium and the incubation condition determine which bacteria can be grown and counted.
iii. Sometimes, cells are viable but not culturable. The viable plate count technique is selective because no one combination of media allows the growth of all types of bacteria.

3.1.3 Most Probable Number (MPN) Technique

Most probable number is a statistical method based on probability theory. In this method, multiple serial dilutions are performed to reach a point of extinction. The point of extinction is the dilution level at which no single cell is deposited into one or more multiple tubes. A criterion such as development of cloudiness or turbidity or gas production is established for indicating whether a particular dilution contains bacteria.

The pattern of positive and negative test results are then used to estimate the concentration of bacteria in the original sample by comparing the observed pattern of result with a table of statistical probabilities for obtaining those results.

SELF-ASSESSMENT EXERCISE

Briefly describe methods by which total number of microbial cells can be measured.

3.2 Measurement of Cell Mass

Techniques for measuring changes in cell mass can be used to measure growth of microorganisms. They include:

3.2.1 Determination of Microbial Dry Weight

Cells growing in liquid medium are collected by centrifugation, washed, dried in an oven and weighed. This is a useful technique for measuring growth of filamentous fungi; however, it is time consuming and not very sensitive.

3.2.2 Spectrophotometry

This method depends on the fact that microbial cells scatter light that strikes them because microbial cells in a population are of roughly constant size; the amount of scattering is directly proportioned to the biomass of cell present and indirectly related to cell number. When the concentration reaches about 10 million cells (107) per ml, the medium appears slightly cloudy or turbid. Further increase in concentration result in greater turbidity and less light is transmitted through the medium. The extent of light scattering can be measured by a spectrophotometer and is almost linearly related to cell concentration at low absorbance level. If the amount of a substance in each cell is constant, the total quantity of the cell constituted is directly related to the total microbial cell mass.

3.4 Factors Influencing Microbial Growth

The rate of microbial growth and death are greatly influenced by environmental factors or parameters.

Some environmental conditions favour rapid microbial growth while others do not permit bacterial reproduction.

Understanding the influence of environmental factors on microorganisms helps in the control of microbial growth and the study of ecological distribution of microorganisms.

These factors include: temperature, solute, and water activity, pH, oxygen level, pressure, radiation. The growth and activities of microorganisms are greatly affected by physical and chemical state of the environment. The four key factors are temperature, pH, water and availability.

SELF-ASSESSMENT EXERCISE

Briefly describe methods by which the total number of microbial cells can be measured.

3.3.1 Temperature

Temperature is the most important factor affecting the growth and survival of microorganism. At either too cold or too hot temperature, microorganisms will not be able to grow and may even die. For every microorganism, there is a minimum temperature below which growth is not possible, an optimum temperature at which growth is most rapid and a maximum temperature above which growth is not possible.

These three temperatures are called the cardinal temperature and are characteristics for any given microorganism.

The Temperature Classes of Microorganisms
Microorganisms can be placed in five classes based on their temperature for growth:

i Psychrophiles: These are organisms that grow well at 0ºC and have an optimum growth temperature of 150C or lower. The maximum is around 100C. They are readily isolated from arctic and antartic habitat, many psychrophiles are found in the ocean.

ii Psychrotrophs or Facultative Psychrophiles: These organisms can grow at 0ºC to 7ºC even though they have optima between 20ºCand 30ºC and maxima at about 35ºC. These organisms are the major causes of spoilage of refrigerated food.

iii Mesophiles: These are microorganism with growth optima around 20ºC to 45ºC. They often have temperature minimum of15ºC to 20ºC. Their maximum is about 45ºC or lower. Examples of this group of microorganisms are the human pathogens because of their environment the body is fairly constant 37ºC

iv Thermophiles: These microorganisms can grow at temperature of 55ºC or higher. Their growth minimum is usually around 45ºCand they often have optima between 55ºC and 60ºC. Majority of thermophiles are prokaryotes although a few photosyntheticprotists and fungi are thermophiles. These organisms flourish in many habitats including compost, heating hay stacks, hot waterline and hot spring.

v Extreme Thermophiles or Hyperthermophiles: These are prokaryotes that have growth optima between 80ºC and 113ºC.They do not grow well below 55ºC. *Pyrococcus abyss* and

Pyrodictum are example of marine hyperthermophiles found in hot areas of the seafloor.

3.3.2 Oxygen Concentration

The importance of oxygen to the growth of an organism correlate with its metabolism especially with the processes it uses to conserve the energy supplied by its source. Based on the ability to grow in the presence or absent of oxygen. Microorganisms are classified as:

i Aerobes: These are organism that able to grow in the presence of atmospheric oxygen.
ii Anerobic: They grow in the absence of atmospheric oxygen
iii Facultative: these are organism that do not require oxygen for growth but grow better in its presence.
Iv Aerotolrant Anaerobe: These are not dependent on oxygen. They grow equal whether oxygen is present or absent, e.g. *Enterococusfaccalis*.
v Strick or Obligate Anaerobe: They do not tolerate oxygen at all and due to its presence, e.g. *Clostridium pasteurianum*.
vi Microaerophile: These organisms are damaged by normal atmospheric level of oxygen (20%) and require O2 level below the range of 2 to 10% for growth, e.g. *Campylobacter*.

A microbial group may show more than one type of relationship to oxygen (O2). A type is found among the prokaryotes and protozoa.
Fungi are normally aerobic but a few species particularly among the yeasts are facultative anaerobes. Photosynthetic protists are almost always obligate aerobes.

3.3.3 pH or Acidity

pH is a measure of the hydrogen ion activity of a solution and is defined as the negative logarithms of the hydrogen concentration (expressed in terms of molarity).
$pH = -\log(H+) = \log(1/(H+))$

The pH scale extends from ph 0.0 to ph 14 and each pH unit represents at enfold change in hydrogen ion concentration. Based on pH growth range and pH growth optimum we have the following group of organisms.

i Acidophiles: They have their growth optimum between ph 0 and5.5.
ii Neutrophiles: Growth optimum between 5.5 and 8.0.
iii Alkalophiles: Growth optimum between 8.0 and 11.5.Extreme alkalophiles have growth optima at pH 10 or higher. In general,

different microbe groups have characteristics pH preference. Most bacteria and protists are neurophiles. Most fungi are acidophiles (pH between 4 and 6). Photosynthetic protist also favours slight acidity.Many archaea are acidophiles.

3.3.4 Solute and Water Activity

This is the ability of a microorganism to grow over a wide range of water activity or osmotic concentration. Selectively permeable plasmamembrane separates microorganisms from their environment; hence they are affected by changes in the osmotic concentration of their surroundings. If a microorganism is placed in hypotonic solution (one with a lower osmotic concentration) water will enter the cell and cause it to burst if nothing is done to prevent it. On the other hand, if a microorganism is placed in hypertonic solution (one with a higher osmotic concentration) water will flow out of the cell .In microbes that have cell walls (i.e. most prokaryotes, fungi and algae),the membrane shrinks away from the cell wall - a process called plasmolysis. The amount of water activity actually available to microorganism is expressed in terms of water activity (aw). Some microbes are adapted to extreme hypertonic environment. In microbes that have cell wall (i.e. most prokaryotes, fungi and algae)the membrane shrinks away from the cell wall by a process called plasmolysis. Some organisms are adapted to extreme hypertonic environment. Microorganisms usually have a specific requirement for NaCl in addition to growing optimally at the water activity of sea water such organism are called halophiles. Halophiles grow optimally in the presence of NaCl or other salt at a concentration above 0.2M.Most microorganisms are unable to cope with environment of very low water activity and either die or become dehydrated and dormant undersuch condition. Halotolerant organisms can tolerate some reduction inthe aw of their environment but grow best in the absence of the added solute. By contrast, some organisms thrive and indeed require low water activity for growth. Organisms able to live in environments high in sugar as a solute are called Osmophiles and those able to grow in very dry environments(made dry by lack of water rather than by dissolved solute) are called xerophiles. Radiation Sunlight is the major source of radiation on the earth. It includes visible light, ultraviolet (UV) radiation, infrared ray, and radio waves. Visible light is a most conspicuous and important aspect of our environment. Most life is dependent on the ability of photosynthetic organisms to trap the light energy of the sun. Most forms of electromagnetic radiation are very harmful to microorganisms. This is particularly true of ionizing radiation, radiation of very short wavelength and high energy, which can cause atoms to lose electrons (ionize). Two major forms of ionizing radiation are:

i X -rays, which are artificially produced, and
ii Gamma rays which are emitted during radioisotope decay. Low level of ionization will produce mutation and may indirectly resultin death, whereas higher levels are directly lethal. Ultraviolet (UV)radiation can kill all kinds of microorganism due to its short wavelength(approximately from 10-400nm) and high energy the most effectively absorbed by DNA.

SELF-ASSESSMENT EXERCISE

Briefly describe the five classes of microorganisms based on temperature.

4.0 CONCLUSION

The growth of microorganisms is greatly affected by the chemical and physical nature of their environment. An understanding of the factors that influence microbial growth aids ecological distribution of microorganisms.

5.0 SUMMARY

Microbial population can be counted directly with counting chambers, electronic counters, or fluorescence microscope. Viable counting techniques such as the spread plate, the pour plate, or the membrane filter can be employed.

Most bacterial, photosynthetic protists, and fungi have rigid cell walls and are hypertonic to the habitat because of solutes such as amino acids, polyols, and potassium ions. The amount of water actually available to microorganism is expressed in terms of the water activity (aw).

Each species of microorganism has an optimum pH for growth and can be classified as an acidophile, neutrophile, or alkalophile.
Microorganisms can alter the pH of their surroundings, and mosculture media must be buffered to stabilise the pH.

Microorganisms have distinct temperature ranges for growth with minima, maxima, and optima – the cardinal temperatures. These ranges are determined by the effects of temperature on the ratesof catalysis, protein denaturation, and membrane disruption.

There are five major classes of microorganisms with respect to temperature preferences: (1) psychrophiles (2) facultative psychrophiles or psychrotrophs, (3) mesophiles, (4) thermophiles, and (5) hyperthermophiles.

Microorganisms can be placed into at least five different categories based on their response to the presence of O2: obligateaerobes, facultative anaerobes, aerotolerant anaerobes, strict orobligate anaerobes, and microaerophiles.

Most deep-sea microorganisms are barotolerant, but some are barophilic and require high pressure for optimal growth.

High-energy or short-wavelength radiation harms organisms in several ways ionizing radiation. X-rays and gamma rays – ionizes molecules and destroys DNA and other cell components. Ultraviolent (UV) radiation induces the formation of thymine dimmers and strand breaks in DNA.

6.0 TUTOR-MARKED ASSIGNMENT

1. What are the advantages and disadvantages of the viable plate count method?
2. What are cardinal temperatures?
3. Describe the types of oxygen relationship seen in microorganism.

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UNIT 3 PHYSICAL METHODS OF CONTROLLINGMICROBIALGROWTH (STERILISATION)

CONTENTS

1.0 INTRODUCTION

Most microorganisms are beneficial to human beings; however, many have undesirable consequences. They cause food spoilage and many diseases to plants, animal and human. To control and inhibit the growth and activities of harmful microorganisms which are capable of causing diseases and contaminating water, food and substances used. This unit examines the various frequently employed physical methods of sterilisation which are: heat, filtration and radiation. Different sterilisation techniques such as heat, radiation are used which totally remove microorganisms from an object or habitat.

2.0 OBJECTIVES

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

- define and explain the term sterilisation
- explain the use of heat sterilisation to control microbial growth
- describe the use of various filters to sterilise liquid substances
- explain the use of radiation for sterilisation.

3.0 MAIN CONTENT

Definition of Sterilisation

Sterilisation is the process by which all living cells, spores and a cellular entities (e.g.) viruses, viroids and prions) are either destroyed or removed from an object or habitat.

It can also be defined as the killing or removal of all viable organisms within a growth medium. A sterile object is totally free of viable microorganisms, spores and other infectious agents.

3.1 The Pattern of Microbial Death

Microbial population death is exponential or logarithmic, meaning the population will be reduced by the same fraction at constant interval. If the logarithm of the population remaining is plotted against the time of exposure to the agent, a straight-line plot will result. A bacterium is often defined as dead if it is not viable and does not grow and reproduce when inoculated into a culture medium that normally supports its growth.

3.2 Heat Sterilisation

The most common sterilisation method used for controlling and destroying microbial growth is the use of heat.

Heat can kill microorganisms by denaturing the enzymes which prevent them from multiplying.

At temperature exceeding the maximal growth temperature, the death rate exceeds the growth rate.

Factors that determine the effectiveness of heat sterilization include the temperature and duration of the heat treatment, and whether the heat is moist or dry

3.2.1 Measuring Heat Sterilisation

All microorganisms have a maximum growth temperature beyond which viability decreases. Viability is lost because at very high temperatures most macromolecules lose structure and function, a process called denaturation. The effectiveness of heat as a sterilant is measured by the time required for a tenfold reduction in the viability of a microbial population at a given temperature. This is the decimal reduction time or

D. The time and temperature, therefore, must be adjusted to achieve sterilisation for each specific set of condition. In addition, the type of heat is also important. Moist heat has been observed to possess better penetrating power than dry heat and, at a given temperature, produces a faster reduction in the number of living organisms. The determination of a decimal reduction time requires a large number of viable count measurements. An easier way to characterise the heat sensitivity of an organism is to measure the thermal death point i.e. the time it takes to kill all cells at a given temperature. Thermal death point(TDP) is the lowest temperature required to kill all the microorganisms in a liquid suspension in 10 minutes. Heat is the most widely applicable and effective agent for killing microorganisms and also the most economical and easily controlled. To determine the thermal death time, samples of a cell suspension are heated for different times, mixed with aculture medium and incubated. If all the cells have been killed, no growth is observed in the incubated samples. The thermal death time depends on the size of the population tested; a longer time is required to kill all cells in a large population than in a small one.

3.2.2 Moist Heat Sterilisation

Moist heat readily kills viruses, bacteria and fungi. It kills by degrading nucleic acids and by denaturing enzymes and other essential proteins. Exposure of microorganisms to boiling water will destroy vegetable cells and eukaryotic spores. However, this temperature will not kill bacterial endospores. In order to destroy bacterial endospores moist heat sterilisation above1000C using saturated steam under pressure is done. Steam sterilization is carried out with an autoclave.

The Autoclave

The autoclave is a sealed heating device that allows the entrance of steam under pressure. The killing of heat-resistant endospores requires heating at temperatures above 1000C (the boiling point of water at normal atmospheric pressure). This is accomplished by applying steam under pressure at a temperature of 1210C.The materials to be sterilised are placed in a chamber and the chamber is sealed. Steam is transferred from a jacket into the chamber forcing out all the air. The steam is held in the chamber for 15 minutes at 1210C and then vented from the chamber. If an object being sterilised is bulky, heat transfer to the interior is retarded and the total heating time must be extended to ensure that the entire object is at 1210C for 10-15 minutes. Extended times are also required when large volumes of liquid are being autoclaved because large volumes take longer time to reach sterilisation temperatures. It must be noted that it is not the pressure inside the autoclave that kills the

microorganisms but the high temperature that can be achieved when steam is applied under pressure.

3.2.3 Dry Heat Sterilisation

This is a method of heat sterilisation in which the objects or materials are sterilised in the absence of water. Some items are sterilised by incineration, for example; inoculating loops used in the laboratory during the culturing of bacteria can be sterilised in a small bench top incinerator. The use of an oven at a temperature of 150 to 160oC for 2 to 3 hours can also be used to sterilise glass wares such as pipettes, and test tubes in Laboratories. Dry heat kills the microorganisms by the oxidation of the cell constituents and denaturation of proteins. This method of sterilisation has some definite advantages, it does not corrode glassware and metal instruments as moist heat does and it can be used to sterilise powders, oils and other similar items. However, it is very slow and less effective than moist heat. For example, the spores of *Clostridium botulinum* the organism that causes botulism are killed in 5minutes at 1210C by moist heat but only after two hours at 1600C with dry heat.

SELF-ASSESSMENT EXERCISE

i. Define the term sterilisation
ii. Explain the use of moist heat for sterilisation.

3.3 Filter Sterilisation/Filtration

1. Filtration is a method that accomplishes decontamination and even sterilisation.
2. Heat-sensitive liquids and gases are sterilised by the use of filtration method.
3. The liquid or gas is passed through a filter, a device with pores too small for the passage of microorganisms, but large enough to allow the passage of the liquid or gas. The selection of filters for sterilisation must account for the size range of the contaminants to be excluded.
4. Some microbial cells are greater than 10 μm in diameter, and the smallest bacteria are less than 0.3 μm in diameter. Rather than directly destroy the contaminating microorganism the filters simply removes them.

5 There are two types of filters:
a Depth Filters
b Membrane Filters.

Depth Filters

This is made up of fibrous or granular materials that have been bonded into a thick layer filled with twisting channels of small diameters. They can also be made of diatomaceous earth, unglazed porcelain, asbestos, bromosilicates and other similar materials.

The solution containing microorganisms is sucked through the thick layer of the fibrous material under vacuum and microbial cells are removed by physical screening or entrapment and by adsorption to the surface of the filter material. They are used for the filter sterilisation of air in industrial processes. In the home, the filter is used in forced air heating and cooling systems is a simple depth filter, designed to trap particulate matter such as dust, spores, and allergens. Depth filters are important for bio-safety applications. For example, manipulation of cell cultures, microbial cultures, and growth media require that contamination of both the operator and the experimental materials are minimal. These operations can be efficiently performed in a biological safety cabinet with airflow, both in and out of the cabinet, directed through a depth filter called a HEPA filter or high-efficiency particulate air filter. A typical HEPA-Filter is a single sheet of borosilicate (glass) fibers that has been treated with a water-repellant binder. It removes 0.3 μM test particles with an efficiency of at least99.97%; they thus effectively remove both small and large particles, including most microorganisms, from the airstreams.

Membrane Filters

Membrane filters are the most common type of filters used for liquid sterilisation in the microbiology laboratory.

They are porous membrane, a little over 0.1mm thick, made of cellulose acetate, cellulose nitrate polycarbonate, polyvinylidenefluoride and other synthetic materials.

Membranes with pores about 0.2μM in diameter are used to remove most vegetative cells but not viruses from solutions ranging in volume from 1ml to many litres.

The membrane is held in a special holder and is often preceded by depth filter to remove larger particles that may clog the membrane filter.
It differs from the depth filter because it functions more like asieve and trapping particles on the filter surface. About 80-85%of the membrane surface area consists of open pores. The porosity provides for a relatively high fluid flow rate.

The solution to be sterilised is forced through the filter with a vacuum or with pressure from a syringe, peristaltic pump and collected in a previously sterilised container.

They are used to sterilise pharmaceutical ophthalmic solutions, culture media, oils, antibiotics and other heat sensitive solutions □□Air can also be sterilised by filtration. Examples are surgical masks and cotton plugs on culture vessels that let air in but keep microorganisms out.

SELF-ASSESSMENT EXERCISE

Explain the use of membrane filter for sterilisation.

3.4 Radiation

Many forms of electromagnetic radiation are very harmful to microorganisms. Microwaves, ultraviolet (UV) radiation, X-rays, gamma rays (Y-rays) and electrons can effectively reduce microbial growth if applied in the proper close.

3.4.1 Ionising Radiation

Ionising radiation is a form of radiation which has very short wavelength and high energy, which can cause atoms to lose electrons(ionize). Two major forms of ionising radiation are:

i X-rays (short wavelength of 10-3 to 102 nanometers) which are artificially produced.

ii Gamma rays (short wavelength of 10-3 to 10-1 nanometer) which are emitted during radioisotope decay. Low levels of doses of Radiation will produce mutation in the microorganisms which may indirectly lead to the death of microorganisms. In cases where large doses or high levels of these radiation are used the microorganisms are directly and instantly killed. Ionising radiation can be used to sterilise items. Gamma and X-radiation have high penetrating power and are able to kill microorganisms by inducing or forming toxic free radicals (ions) viruses and other microorganisms are inactivated by exposure to ionizing radiation. Ionising radiations are used to pasteurise or sterilise products;e.g. most commercially produced disposables and plastic petri dishes are sterilised by exposure to gamma rays.

3.4.2 Ultraviolet (UV) Radiation

These are radiation of short wavelength (from 10 to 400μm) and high energy.

The most lethal UV radiation has a wavelength of 260μm, the wavelength mostly absorbed by DNA.

The primary mechanism of UV damage is the formation of thymine dimmers in DNA.

Two adjacent thymines in a DNA strand are covalently joined to inhibit DNA replication and function.

Ultraviolet (UV) radiation can kill all kinds of microorganisms especially microorganisms on or near the surface of clear solution exposure to ultraviolet rays can be used to maintain sterility of surfaces such as bench tops in laboratories and hospitals.

Radiation is currently used for sterilisation and decontamination in the medical supplies and food industries. In the U.S. for example, the Food and Drug Administration has approved the use of radiation for sterilisation of such diverse items as surgical supplies, disposable lab ware, drugs, and even tissue grafts. However, because of the costs and hazards associated with radiation equipment, this type of sterilisation is limited to large industrial applications or specialized facilities. The use of radiation sterilisation practice has not been readily accepted in some countries because of fears of possible radioactive contamination, alteration in nutritional value, production of toxic or carcinogenic products, and perceived “off” tastes in irradiated food.

SELF-ASSESSMENT EXERCISE

Why is ionising radiation more effect than ultraviolet radiation for sterilisation of food products?

4.0 CONCLUSION

Different methods of sterilisation are used to sterilise different types of objects and to make them free of viable microorganism spores and other infectious objects.

5.0 SUMMARY

Sterilisation is the process by which all living cells, spores and cellular entities (viruses) are either completely destroyed or removed from an object or habitat.

Physical methods frequently used to sterilise are heat, low temperature, filtration and radiation.
Heat sterilisation is used to kill all microorganisms in a sample. It may be moist or dry. Moist heat sterilisation involves the useof autoclave.
Autoclaving involves using steam under pressure at a temperature of 1210C for 15 minutes.

Dry heat involves sterilising objects at a higher temperature, in the absence of water and for a longer exposure time.

The use of oven at a temperature of 1600C to 1700C for 2 to 3hours to sterilise glasswares and the use of incinerator are examples of dry heat sterilisation.

Microorganisms can be effectively removed by filtration with either depth filters or membrane filters.
Radiation of short wavelength or high energy ionising and ultraviolet radiation can be used to sterilise objects.

6.0 TUTOR-MARKED ASSIGNMENT

1. In a tabular form, give physical or chemical agents that should be best used for the sterilization of the following items:
   a. Glass pipettes
   b. Nutrient agar
   c. Antibiotic solution
   d. Interior of a biological safety cabinet
   e. Package of plastic Petri dishes
   f. Water
2. Explain the difference types of radiation that are destructive to microorganisms.

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UNIT 4 CHEMICAL METHODS OF CONTROLLING MICROBIAL GROWTH (DISINFECTION)

CONTENTS

1.0 INTRODUCTION

A large number of chemical compounds have the ability to inhibit the growth and metabolism of microorganisms or to kill them. They are called antimicrobial agents. These different chemicals are available for use as disinfectants and each has its own characteristics and mode of action, advantages and disadvantages. This unit examines the term disinfection, the characteristics of an ideal disinfectant and different chemical groups used as disinfectants.

2.0 OBJECTIVES

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

- define the term disinfection
- state the characteristics of an ideal disinfectant
- list different chemicals that can be used as disinfectants
- explain the use of different chemical as disinfection agents.

3.0 MAIN CONTENT

Definition of Disinfection

Disinfection is the killing, inhibition or removal of organisms that may be capable of causing diseases.

It is the process of destroying infectious agents.
Disinfectants are antimicrobial agents, usually chemicals, used to carry out disinfection; they are normally used on an in animate object, e.g. disinfection may not lead to the total removal of microorganisms because viable spores and a few microorganisms may remain.

Antimicrobial bleach (sodium hypochlorite) solution, for example, is a disinfectant used to clean and disinfects food preparation areas.

3.1 Antimicrobial Agents

An antimicrobial agent is a natural or synthetic chemical that kills or inhibits the growth of microorganisms. Agents that kill organisms are called -cidal agents, with a prefix indicating the type of microorganism skilled. Thus, they are called bactericidal, fungicidal and viricidal agents because they kill bacteria, fungi and viruses, respectively. Agents that donot kill but only inhibit growth are called - static agents. These include bacteriostatic, fungistatic and viristatic compounds.

3.2 Characteristics of an Ideal Antimicrobial Agentor Disinfectant

i. It should have a broad spectrum of antimicrobial activity i.e. it must be effective against a wide range of infectious agents such as gram positive and Gram negative bacteria, acid fast bacteria, bacterial endospores, fungi and viruses.
ii. It must be active even at low concentration.
iii. It must be active in the presence of organic matter.
iv. Non-toxicity to human and other animals. It should be toxic tothe infectious agent.
v. It must be non-corroding and non-staining.
vi. It must be stable upon storage.
vii. Odourless or with pleasant smell.
viii. It must be soluble in water and lipids for proper penetration of microorganisms.
ix. It must be uniform in composition so that active ingredients are present in each application.
x. It must have a low surface tension so as to penetrate cracks in surfaces.

xi. It must be readily available.
xii. It must be relatively inexpensive.

3.3 Factors for the Selection of a Chemical Agent

The major factors that need to be considered in the process of selecting the most appropriate chemical agent for a specific practical application are:

1. The nature of the material to be treated: e.g. a chemical agent used to disinfect contaminated utensils might be quite unsatisfactory for application to the skin.
2. Types of microorganisms: Chemical agents are not all equally effective against bacteria, fungi, viruses, and other microorganisms. Spores are more resistant than vegetative cells. Differences exist between Gram-positive and Gram-negative bacteria.
3. Environmental condition: Such as temperature, pH, time, concentration, and presence of extraneous organic materials, may all have a bearing on the rate and efficiency of antimicrobial action.

SELF-ASSESSMENT EXERCISE

What is a disinfectant?

3.4 Major Groups of Chemical Antimicrobial Agents

Phenol and phenolic compounds
Alcohols
Halogens
Heavy metals and their compounds
Dyes
Detergents
Quaternary ammonium compounds
Aldehydes
Gaseous agents

3.4.1 Phenolics

Phenol is also known as carbolic acid and is the oldest recognised disinfectant.

Phenol (Carbolic acid) was the first widely used antiseptic and disinfectant. In 1867, Joseph Lister employed it to reduce risk of infection during surgery.

The mode of the Action of phenol is by disrupting plasma membranes; inactivate enzymes and denaturing proteins of microorganism.

They are stable when heated or dried and retain their activity in the presence of organic material. It is used for disinfection of hospital floors and walls.

Examples of phenol and phenolics (phenol derivatives) are cresols, xylenols and orthophel/phenols which are used as disinfectants in laboratories and hospitals.

Advantage

They are effective against microbial agents of tuberculosis, effective in the presence of organic material and remain on surface long after application.

Disadvantage

They have a disagreeable odour and can cause skin irritation.

3.4.2 Alcohols

Alcohols are widely used as disinfectants and antiseptic. They are bactericidal and fungicidal but not sporicidal. Some viruses are also destroyed by alcohols. Two most popular alcohols germicides are ethanol and isopropanol usually used at 70 to 80% concentration. Isopropanol has the highest bactericidal activity and is the most widely used. They act by denaturing proteins and by dissolving membrane lipids and acting as a dehydratingagent. 10-15 minutes soaking a thermometer in alcohol is sufficient to disinfect the thermometer.

3.4.3 Halogens

A halogen is any of the five elements in group VIIA of the periodic table. They are fluorine, chlorine, bromine, iodine and astatine and are effective microbial elements widely used as disinfectants. The halogens iodine and chlorine are important antimicrobial agents.

Chlorine

Chlorine is the usual disinfectant for municipal water supplies and swimming pools. It is also used in diary and food industries. Various forms of chlorine are used for disinfection. It may be applied as chlorine gas, sodium hypochlorite (bleach) or calcium hypochlorite. All yield hypochlorous acid (HCLO) followed byatomic oxygen. It causes oxidation of cellular materials, destruction of vegetative bacteria and fungi by disrupting

membranes and inactivating enzymes but does not destroy spores. The germicidal action of chlorine is based on the formation of hypochlorites when it is added to water. HA releases an active form of death of almost all microorganisms occur within 30minutes of use.

Chlorine is also an excellent disinfectant for individual use. Small quantities of drinking water can be disinfected with halozone tablets. This tablet (parasulfoen dichloramido benzoic acid)slowly releases chlorite when added to water and disinfects it in
about 30 minutes. It is frequently used by campers lacking accessto uncontaminated drinking water.

Chlorine is an effective disinfectant because it is inexpensive, effective and easy to employ.

It is commonly used in disinfecting and deodorising most houses.
Food processing plants and restaurants also use calcium and sodium hypochlorite solution to disinfect utensils.

Hypochlorite is used in hospital to disinfect rooms, surfaces and non-surgical instruments. It used on a consumable product such as drinking water, their concentration must be reduced before product is consumed. The germicidal action of chlorine is based on the formation of hypochlorous acid when it is added to water. Hypochlorous acid releases an active form of oxygen that reacts with cellular biochemical.

Chlorine forms condensed into liquids is widely used for disinfection and is the standard treatment for disinfecting drinking water in many communities.

It is also used to disinfect effluents from sewage treatment plants to minimise the spread of pathogenic microorganisms. It is also used to disinfect swimming pools. A residual chlorine level of0.5mg/l will achieve control of microbial population and prevent the multiplication of pathogens in swimming pools. Such levels are harmless to human tissues. Usually, commercial forms of sodium or calcium hypochlorite contain 5.25% chlorine. These need to be diluted ten times to achieve the forms used for disinfection in homes and swimming pools. The commercial types are Chlorox, JIK, Parazone, etc. The powdered form used in swimming pools and water treatment plants is known as HTH(commercially).

3.4.4 Heavy Metals and their Compounds

Heavy metals such mercury, silver, zinc, copper and arsenic are used as germicides. For example, copper sulphate is an effective algicide in lakes and swimming pools.

They act by combining with proteins and inactivating them. They may also precipitate cell proteins.

1% of Silver nitrate is a solution added to the eyes of infants to prevent ophthalmic gonorrhea.

3.4.5 Quaternary Ammonium Compounds (Detergents)

These are detergents that have antimicrobial activity.

Detergents are organic cleaning agents that are amphipathic, having both polar hydrophilic and non-polar hydrophobic components.
They act by disrupting microbial membrane and by denaturing proteins.
If the detergents are electrically charged, they are termed ionic.

Anionic (negatively charged) detergents are only mildly bactericidal and are used as laundry detergents to remove soil and debris. They also reduce number of microorganisms associated with the item being washed.

Cationic (positively charged) detergents are highly bactericidal, i.e. they kill bacteria. They are effective against *Staphylococcus* and various viruses.

Advantages
They are stable, non-toxic not inactivated by hard water and soap. Cationic detergents are used as disinfectant for food utensils and small instruments.

3.4.6 Aldehydes

Formaldehyde and glutaraldehyde are useful for disinfection. They are highly reactive molecules that combine with nucleic acid and proteins and inactive them. They are sporicidal and can be used as chemical sterilant.

Formaldehyde is usually dissolved in water or alcohol before use. A 2% buffered solution of glutaraldehyde is an effective disinfectant. It is less irritating than formaldehyde and is used to disinfect hospitals and laboratory equipment. Glutaraldehyde

usually disinfects objects within minutes but may require as long as 12 hours to destroy all spores.

SELF-ASSESSMENT EXERCISE

List five groups of chemical antimicrobial agents and explain their uses.

3.5 Sterilising Gases

Gases such as ethylene oxide gas are used to sterilise heat sensitive items such as disposable plastic Petri dishes, syringes, heart lung machines components, sutures and catheters.

3.5.1 Ethylene Oxide (EtO)

This is both microbicidal and sporicidal and kills by combining with cell proteins. Sterilisation is carried out in a special ethylene oxide steriliser, very much resembling an autoclave in appearance, that control the EtO concentration, temperature, and humidity. Because pure EtO is explosive, it is usually mixed with either CO2 or dichlorodifluoromethane. The ethylene oxide concentration, humidity and temperature influence the rate of sterilisation. A clean object can besterilised if treated for 5 to 8 hours at 40 to 50% and the EtOconcentration at 700mg/litre. Extensive aeration of the sterilized material is necessary to remove residual EtO because it is very toxic.

3.5.2 Betapropiolactone (BPL)

This is occasionally employed as a sterilising gas. In the liquid form it has been used to sterilise vaccines and sera. BPL decomposes to an inactive form after several hours and is therefore not as difficult to eliminate as EtO. It also destroys microorganisms more readily than ethylene oxides but does not penetrate materials well. It may be carcinogenic. For these reasons, BPL has not been used as extensively asEtO.

3.5.3 Vaporised Hydrogen Peroxide

This can be used to decontaminate biological safety cabinets, operating rooms and other large facilities. These systems introduce vapourised hydrogen peroxide (H2O2) into the enclosure for some time, depending on the size of the enclosure and material within. Hydrogen peroxide is toxic and kills a wide variety of microorganisms. However, during the course of the decontamination process, it breaks down to H2O and oxygen, both of which are harmless. Other advantages of these systems are that they can be used at a wide range of temperatures (4 to 80oC) and they do not damage most materials.

4.0 CONCLUSION

Many different chemicals are available for use as disinfectants and each has its own advantages and disadvantages. In selecting an agent, it is important to keep in mind the characteristics of a desirable disinfectant.

5.0 SUMMARY

Chemical agents are usually used as disinfectants.
Characteristics of an ideal disinfectant include broad spectrum antimicrobial activity, stability, non-toxicity to humans, non conodingand non-staining, easy penetration no odour or pleasantodour among others.

Phenolics are used as disinfectants in hospitals and laboratories. They act by denaturing proteins, disrupting cell membranes and inactivating enzymes of microorganisms.

Halogens (Chlorine and iodine) kill microorganising by oxidizing cellular constituents.

Chlorine is used to disinfect municipal water supply and swimming pools.

The germicidal action of chlorine is based on the formation of hypochlorous acid when it is added to water.

Alcohols are the most effective and most used agents forsterilisation and disinfectant.

Aldehydes such as formaldehyde and glutaraldehyde can sterilize as well as disinfect because they kill spores.

Cationic detergents are used as disinfectants antiseptic; they disrupt the membrane and denature proteins of microorganisms.
Ethylene oxide gas is use to sterilise heat sensitive materials likedisposable plastic Petri dishes.

60 TUTOR-MARKED ASSIGNMENT

1. List five characteristics of ideal antimicrobial agents.
2. Explain the use of chlorine as a disinfectant.

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MODULE 5 SYSTEMIC CLASSIFICATION OF MICROORGANISMS

UNIT 1 INTRODUCTIONTO SYSTEMIC CLASSIFICATION OF MICROORGANISMS.

CONTENTS

1.0 INTRODUCTION

In order to make sense of the diversity of organisms, it is necessary to group similar organisms together and organise these groups in a non overlapping hierarchical arrangement. One of the tools needed to perform this grouping is a reliable classification method. The Swedish botanist Carl von Linne or Carolus Linnaeus, as he is often called, developed the first natural classification based largely on anatomical characteristics in the middle of the eighteenth century. The term systematic is often used for taxonomy. However, many taxonomists define systemics in more general terms as “the scientific study of organisms with the ultimate objective of characterising and arranging them in an orderly manner.” Any study of the nature of organisms, when the knowledge gained is used in taxonomy, is a part of systematics. Thus, systematics encompasses discipline such as morphology, ecology, epidemiology, biochemistry, molecular biology and physiology. This unit examines the definition of terms in systematic classification of microorganisms, methods used to classify microorganisms, taxonomicranks and different characteristics used in classifying microorganisms.

2.0 OBJECTIVES

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

- define the following terms: taxonomy, nomenclature, classification, identification and systemics
- explain the different methods that have been used in classification
- explain the different taxonomy ranks
- explain the different characteristics used in classification and identification of microorganisms.

3.0 MAIN CONTENT

Definition of Terms

Taxonomy is defined as the science of biological classification. In a broader sense, it consists of three separate but interrelated parts: classification, nomenclature, and identification.

Classification – This is the organisation of organisms into progressively more inclusive groups on the basis of either phenotypic similarity or evolutionary relationship. It is the arrangement of organisms into groups called taxa based on mutual similarity.

Nomenclature – This is the branch of taxonomy concerned with the assignment of giving names to taxonomic groups in agreement with published rules.

Identification – This is the practical side of taxonomy, the process of determining if a particular isolate belongs to a recognised taxon.

Systematics - The term systematics is often used for taxonomy. Itis the scientific study of organisms with the ultimate objective of characterising and arranging them in an orderly manner. In practice, the determination of the genes and species of a newly discovered prokaryote is based on polyphasic taxonomy. This approach includes phylogenetic phenotypic and genotypic features.

3.1 The Polyphase Taxonomy

The polyphasic approach to taxonomy uses three kinds of methods: phenotypic, genotypic and phylogenetic for the identification and description of bacteria. Phenotypic analysis examines them morphological, metabolic, physiological and chemical characteristics of the organisms. Genotypic analyses consider comparative aspects of cells

at the level of the genome. These two kinds of analyses group organisms on the basis of similarities. They are complemented by phylogenetic analysis which places organisms in a framework of evolutionary relationships. The habitat and ecology of the organisms is also used in polyphasic taxonomy.

3.2 Methods of Classification

Phenotypic classification: This is the grouping of microorganisms together based on the mutual similarity of the phenotypic characteristics. This classification system succeeded in bringing order to biological diversity and classified the function of morphological structures. Organisms sharing many characteristics make up a single group or taxon.

Phylogenetic classification: Compares organisms on the basis of evolutionary relationships. The term phylogeny refers to the evolutionary development of species. This method is restricted because of lack of good fossil records.

Genotypic classification: Compares the genetic similarity between organisms' individual genes or whole genomes can be compared.
Numeric taxonomy: This is grouping of taxonomic units intotaxa on the basis of their character state by numerical methods. Information about the properties of organisms is converted into a form suitable for numerical analysis and they are compared by means of a computer. The resulting classification is based on general similarity as judged by comparison of many characteristics each given equal weight. The result of numerical taxonomic analysis is often summarised with a thread like diagram called a dendogram.

3.3 Taxonomic Ranks

The classification of microbes involves placing them within hierarchical taxonomic levels. Microbes in each level or rank share a common set of specific features. The highest rank is the domain, and all prokaryotes belong to either the Bacteria or the Archaea. Within each domain, each microbe is assigned (in descending order) to a phylum, class, order, family, genus and species. The basic taxonomic group in microbial taxonomy is the species. A prokaryotic species is a collection of strains that share many stable properties and differ significantly from other groups of strains. A strain consists of the descendants of a single, pure microbial culture. Each species is assigned to a genus, the next rank in the taxonomic hierarchy. A genus is a well-defined group of one or more species that is clearly separate from other genera. Taxonomy groups of

higher rank than genus are listed below: Family: A group of similar genera Order: A group of similar families Class A group of similar orders Phylum: A group of similar classes

Domain: A group of similar phylaMicrobiologists name microorganisms by using the binomial system of Linneaeus. The Latinised, italicised name consists of two parts. The first part, which is capitalised, is the generic name, and the second is the uncapitalised species name (e.g. *Escherichia coli*). Often, the name will be shortened by abbreviating the genus name with a single capital letter, for example: *E. coli*, *S. aureus* etc. The species name is stable; however, a generic name can change if the organism is assigned to another genus because of new information. For example, some members of the genus *Streptrococcus* were placed into two new genera. *Enterococcus* and *Lactococcus* based on rRNA analysisand other characteristics. Bergey's manual of systematic Bacteriology contains the currentlyaccepted system of prokaryotic taxonomy.

Domain *Bacteria*
Phylum *Proterobacteria*
Class *α-proterobacteria β-proterobacteria □-proterobacteria □-proterobacteria □-*
proterobacteria
Order *Chromatiales Thiotricales Legionalles Pseudomonadeoles Vibrionales*
Enterobacteriales Pasteurellales
Family *Enterobacteriacae*
Genus *Enterobacter Escherishia Klebsiella Proteus Salmonella SerattiaShigella Yersinia*
Species *Siboytii S, dysenteriae S.flexneri S. Sonnei*

3.4 Techniques for Determining Microbial Taxonomy and Phylogeny

Many different approaches are used in classifying and identifying microorganisms. For clarity, these have been divided into two groups: classical and molecular.

SELF-ASSESSMENT EXERCISE

i. Define the following terms:
   a. Taxonomy
   b. Classification
   c. Nomenclature

d. Identification and Systematics

3.4.1 Classical Characteristics

Classical approaches to taxonomy make use of morphological, physiological, biochemical, ecological and genetic characteristics. They are quite useful in routine identification and may provide phylogenetic information as well.

1. Morphological Characteristics

Morphological features are important in microbial taxonomy for many reasons. One, morphology is easy to study and analyse, particularly in eukaryotic microorganisms and the more complex prokaryotes. In addition, morphological comparisons are valuable because structural features depend on the expression of many genes, are usually genetically stable. Thus, morphological similarity is often a good indication of phylogenetic relatedness. The transmission and scanning electron microscopes, with their greater resolution, have immensely aided the study of all microbial groups.

Table 2: Some Morphological Features Used in Classification and Identification

Feature Microbial Groups
Cell shape All major groups
Cell size All major groups
Colonial morphology All major groups
Ultrastructural characteristics All major groups
Staining behaviour Bacteria, some fungi
Cilia and flagella All major groups
Mechanism of motility Gliding bacteria, spirochetes
Endospore shape & location Endospore-forming bacteria
Spore morphology & location Bacteria, protists, fungi
Cellular inclusions All major groups
Colour All major groups
Used in classifying and identifying at least some bacteria, fungi and protists.

2. Physiological and Metabolic Characteristics

Physiological and metabolic characteristics are very useful because they are directly related to the nature and activity of microbial enzymes and transport proteins. Since proteins are gene products, analysis of these characteristics provides an indirect comparison of microbial genomes.

Some physiological and metabolic characteristics used in classification and identification are:

i. Carbon and nitrogen sources
ii. Cell wall constituents
iii. Energy sources
iv. Fermentation products
v. General nutritional type
vi. Growth temperature optimum range
vii. Lumniscence
viii. Mechanisms of energy conversion
ix. Motility
x. Osmotic tolerance
xi. Oxygen relationships
xii. pH optimum growth range photosynthetic pigments
xiii. Salt requirements and tolerance
xiv. Secondary metabolites formed
xv. Sensitivity to metabolic inhibitors and antibiotics
xvi. Storage inclusions.

3. Ecological Characteristics

The ability of micro-organisms to colonise a specific environment is of taxonomic value. Some microbes may be very similar in many other respects but inhabit different ecological niches, suggesting that they may not be as closely related as first suspected. Some examples of taxonomically important ecological properties are life cycle patterns, the nature of symbiotic relationships; the ability to cause disease in a particular host; and habitat preferences, such as requirements for temperature, pH,oxygen, and osmotic concentration. Many growth requirements are considered physiological characteristics as well.

4. **Genetic Analysis**

Although prokaryotes do not reproduce sexually, the study of chromosomal gene exchange through transformation, conjugation and transduction is sometimes useful in their classification. Transformation can occur between different prokaryotic species but only rarely between genera. The demonstration of transformation between two strains provides evidence of a close relationship since transformation cannot occur unless the genomes are fairly similar. Transformation studies have been carried out with several genera: *Bacillus, Micrococcus, Haemophilus, Rhizobium* and others. Despite transformation's usefulness, its results are sometimes hard to interpret because anabsence of transformation may result from factors other than major differences in DNA sequence. Plasmids are important taxonomically because they can

confound the analysis of phenotype traits. Most microbial genera carry plasmids and some plasmids are passed from one microbe to another with relative ease. When such plasmids encode a phenotypic trait (or traits) that is being used to develop a taxonomic scheme the investigator may assume that the trait is encoded by chromosomal genes.

3.4.2 Molecular Characteristics

Microorganisms have left no fossil record unlike evolutionary biologist studying plants and animals that have drawn from a rich fossil record to assemble a history of morphological changes. In this case, molecular approaches serve to supplement this data, so molecular analysis is the only feasible means of collecting a large and accurate data set from a number of microbes.

1. Nucleic Acid Base Composition or G.C. Ratios G+C ratio data are valuable in at least two ways. First, they can confirm a taxonomic scheme developed using other data. Second, G+C content appears to be useful in characterizing prokaryoticgenera because the variation within a genus is usually less than10% even though the content may vary greatly between genera. The GC ratio is the percentage of guanine plus cytosine in an organism's genomic DNA. GC ratios vary over a wide range, with values as low as 20% and as high as nearly 80% among Bacteria and Archaea, a range that is some-what broader than for eukaryotes. It is generally considered that if two organisms GC ratios differ by more than 5%, they will share two DNAsequences in common and are therefore unlikely to be closely related.
2. Nucleic Acid Hybridisation: The similarity between genomes can be compared more directly by use of nucleic acid hybridisation studies.
3. Nucleic Acid Sequencing: The method uses rRNA from small ribosomal sub units (165 and185 rRNAs from prokaryotes and eukaryotes, respectively) have become the molecules of choice for inferring microbial phylogenetic and making taxonomic assignments at the genus level. The small subunit rRNAs (SSU rRNAs) are almost ideal for studies of microbial evolution and relatedness because they play the same roles in all microorganisms. In addition, because the ribosome is absolutely necessary for survival and the SSUrRNA are part of the complex ribosomal structure.
4. Genomic Fingerprinting: A group of techniques called genomic fingerprinting can also be used to classify microbial and help determine phylogenetic relationships. Unlike the molecular analysis so far discussed, genomic fingerprinting does not involve

nucleotide sequencing. Instead, it employs the capacity of restriction endo nucleases to recognise specific nucleotide sequences.

5. Amino Acid Sequencing: The amino acid sequences of proteins directly reflect mRNA sequences and therefore represent the genes coding for their synthesis.
6. The Major Division of Life: The division of all living organisms into three domains – Archaea, Bacteria and Eucarya – has become widely accepted among microbiologists.
7. DNA-DNA Hybridisation: When two organisms share many highly similar (or identicalgene,) their DNAs would be expected to hybridise to one anotherin approximate proportion to the similarities in their gene sequences. To this way, measurement of DNA-DNAhybridisation between the genomes of two organisms provides a rough index of their similarity to each other. DNA-DNA hybridisation therefore is useful for differentiating between organisms as a complement to SSU, RNA gene sequencing.

SELF-ASSESSMENT EXERCISE

Explain the use of morphological, ecological and molecular characteristics in the classification of microorganisms.

4.0 CONCLUSION

Systemic classification is the scientific study of organisms, their organisation and arrangement in orderly manner into groups based on their classical and molecular characteristics. This classification is important for the study of the diversity of microorganisms in nature.

5.0 SUMMARY

Taxonomy is the science of biological classification, is composed of three parts: classification, nomenclature and identification.

A polyphasic approach is used to classify microorganisms. It makes use of genetic, phenotypic and phylogenetic analysis.

Methods of classification of microorganisms include phenotypic, phylogenetic, genotypic and numerical taxonomy.
Taxonomy ranks are arranged in a non overlapping hierarchy.
The taxonomic ranks are species, genus, family, order, class, phylum and domain.
Microorganisms are named according to the binomial system.

The classical approach to determining microbial taxonomy and phylogeny include morphological, physiological, metabolic, ecological and genetic characteristics.
Molecular techniques used in classification include nucleic acid base composition, nucleic acid hybridisation, nucleic acid, sequencing genomic finger printing and amino acid sequencing.

6.0 TUTOR-MARKED ASSIGNMENT

1. Define the following terms:

a. Species
b. genus
c. Order
d. class
e. family
f. domain,
g. phylum.

2. Arrange the listed terms in their hierarchical taxonomic levels.
3. Differentiate between phonetic classification and phylogeneticclassification.

7.0 REFERENCES/FURTHER READING

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UNIT 2 SYSTEMATIC CLASSIFICATION OFBACTERIA

CONTENTS

1.0 INTRODUCTION

The most widely used reference for bacteria classification is Bergey's Manual of Systematic Bacteriology, which is divided into four volumes. Divisions within Bergey's manual are based on characteristics such as:

Gram, reaction, cell, shape, cell arrangement, oxygen requirements, motility, metabolic properties. Bacteria are also classified according to the international agreed rules by the international committee on systematic bacteriology. This unit examines the systematic classification of bacteria, the major groups of bacteria and main characteristics of each group.

2.0 OBJECTIVES

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

- identify the major groups of bacteria
- state the major characteristics which set each group apart from others
- explain important genera within the appropriate group.

3.0 MAIN CONTENT

3.1 Bergey's Manual of Systematic Bacteriology - Volume 1

Ordinary Gram Negative Bacteria Bergey's Manual of Systematic Bacteriology, Volume 1 is made up of the ordinary Gram negative chemoheterotrophic bacteria. Many of which have clinical, industrial or agricultural importance. They include:

3.1.1 The Spirochetes

These bacteria have helical shapes. They have the ability to twist and contract their shape (they are flexible). There is presence of a special kind of flagella termed periplasmic flagella or axial fibrils which may be more than one. They are so thin they cannot be easily seen in light microscope. When gram stained, dark field microscope is used to visualise these organisms, they are Gram negative bacteria. Many spirochetes are human pathogens.

Important genera

Treponema: causes syphilis and yaws
Borrelia: causes lymes diseases
Leptospira: causes fever, liver and kidney damages

3.1.2 Aerobic/motile, Helical/Vibrioid Bacteria

They are Gram negative bacteria. The cells are rigid and range from vibrioid (having less than one tumor twist) to helical. They swim by means of polar flagella. They are aerobic or microaerophilic. Most are harmless saprobes and occur in soil, fresh water or marine environment but a few are parasitic and pathogenic for human animals and other bacteria.

Important Genera

Spirillum - adapted to low concentration of organic matter
Azospirillum - associated with plant roots, nitrogen fixer important to agriculture.
Campylobater- a food poisoning bacteria
Bdellovibrio - Predator on bacteria.

3.1.3 Non Motile Gram Negative Curved Bacteria

These bacteria have rigid cell that are curved to various degrees forming coil, helical spirals and sometime ring, they are not motile. They occur mainly in soil, fresh water and marine environment.

3.1.4 Gram Negative, Aerobic Rods and Cocci

This group of bacteria forms one of the largest and most diverse group of bacteria. They are straight or slightly curved rods, some are cocci. They are a strictly respiratory type of metabolism: Many industrially, medical, and environmentally important bacteria habitats include soil, water, animal parasites.

Important Genera

a. *Pseudomonas*-Opportunistic infections in burns. Aerobic motile with rods with polar flagella. Many may synthesise a yellow green-pigment that fluoresces under UV light. Are resistant to many chemicals and antibiotics.

b. *Legionella* - Legionnaire's Disease, fastidiuos organisms found in many environments.
c. *Neisseria* - STD mostly of humans and animals
d. *Brucella* - Obligate intracellular parasites
e. *Bordetella* - Whooping and kernel coughs
f. *Francisella* - Tularemia in rabbits, require cysteine.
g. *Rhizobium* - A nitrogen-fixing soil bacterium
h. *Agrobacterium* - Used to introduce DNA into plants.

3.1.5 Facultatively Anaerobic, Gram-Negative Rods

Many important pathogens, oxidase and a requirement for organic Habitats include soil, plants, and animals, respiratory and intestinal tracts Many in this group known as 'enterics' found in human intestine)

Important genera: (inhabit intestine of animals) bulky dysentery
Escherichia, Salmonella, Shigella, Klebsiella, Yersina, Vibrio,Haemophilus, Gardnerella, Pasteurella, Proteus, Seratia

3.1.6 Aerobic, Gram-Negative Rods

Straight, curved and helical rods. They are rigid. They are obligately anaerobic (cannot live in pressure of O2)

Habitats: Mostly in intestinal traits, some in mouth and genital trait andsome of the most common organism in the intestine.

Important genera:

Bacteriodes
Fusobacterium
Leptotrichia

3.1.7 Dissimilatory Sulphate-Reducing or Sulfur - Reducing

Bacteria

They have a Gram-negative cell wall. They are found in anaerobic sediments; reduce oxidised forms of sulphur to H2S. Dissimilation = nutrition not assimilated but rather
excreted. Occur in mud, fresh water, marine or brackish environments.
Important genera: *Desulfovibrio* – vibroid or helical cells.*Desulfococcus*

SELF-ASSESSMENT EXERCISE
In what way does spirochete differ from other bacteria?

3.2 Bergey's Manual of Systematic Bacteriology - Volume 2

3.2.1 Ordinary Gram- Positive Bacteria

Bergey's Manual of Systematic Bacteriology, Volume 2 is made up ofthe ordinary gram positive chemoheterotrophic bacteria, many of whichhave clinical or industrial or agricultural importance. They are:

a. Gram-Positive Cocci. Aerobic/Facultatively Anaerobic Cocci
They possess cytochrome.

They are able to respire with oxygen.Some can also obtain energy under anaerobic conditions by fermentation. Members are placed in two families.
Deinococcaceae and Micrococcaceae.

Important genera

1. *Micrococcus* aerobes or facultative anaerobes that form irregular clusters by dividing in two or more planes.
2. *Streptococcus* aerotolerant anaerobes that obtain energy from fermenting sugars to lactic acid form chains by dividing in one or two planes lack catalase can cause extensive tissue destruction by the release of enzymes that degrade fibrin.
3. *Staphylococcus* common human pathogen responsible for skin abscesses or boils will tolerate and grow in high salt concentrations will rapidly develop antibiotic resistance.
4. *Peptococcus* obligate anaerobes lack both catalase and enzymesto ferment lactic acid form pairs or irregular clusters
   can cause many infections.
5. *Peptostreptococcus*

b Aerotolerant Fermentative Cocci

They do not have cytochromes. They have only a fermentative type of metabolism and do not respire yet they can grow anaerobically or aerobically. The cells are arranged in pairs, chains or tetrads. Some representative genera include Streptococcus. Leuconostocand Pediococcus.

c Anaerobic Gram-Positive Cocci

These cocci have a fermentative type of metabolism, cells inclusters, tetrads, short or long chains.

3.2.2 Endospore Forming Gram-Positive Bacteria

Most are rod shaped but some are cocci. Majority are gram-positive but a few species stain gram-negative. Motility if present is by means of peritrichous flagella.

Some genera included in the group are:

Aerobic/Facultatively Anaerobic spore forming Rods and Cocci.
These groups are rod and cocci in shape.

Two genera found in the group are Bacillus and Sporosarcina.
Bacillus. They are rod shaped bacteria.
May form exocellular enzymes that hydrolyse proteins and complex polysaccharides.

They form endospores which are heat resistant. They areharmless saprobes found in soil, fresh water or sea water. Examples include *B. subtilis, B. cereus* and *B. thuringiensis*.

Sporosarcina: They are cocci in shape and arranged in tetrads orcubical packets of eight cells. They are widely distributed infertile soil.

3.2.3 Anaerobic Spore forming Rods

They have a fermentative type of metabolism. They are widelydistributed in soil, in marine and fresh water anaerobic sediments. Theyinclude *Clostridium* and *Desulfotomaculum*.

3.2.4 Non-Spore Forming Gram Positive Rods of RegularShape

They are short or long rods in shape.The group is made of harmless saprobes.

Some are parasitic organisms.

Important Genera

1. Lactobacillus
(a) foods and cheeses.
2. Listeria
(a) infection of brain and its membranes, will damagefetus.
3. Erysipelothrix
(a) red sores in human.

3.2.5 Non-Spore Forming Gram-Positive of Irregular Shape

This group contains a heterogenous variety of bacteria.
The few common features are. Straight to slightly curved rods.

1. May be aerobic or facultatively anaerobic. Some examples of the genera in the group include *Corynebacterium*, *Arthrobacter, Brevibacterium*, *Micrococcus* and *Cellulomonas. Corynebacterium*: This genus contains rod shaped cells which are pleomorphic and frequently exhibit club-shaped swellings and a palisade arrangement.

They may be saprobes, pathogens of human and or animals as well as plant pathogens. An example is *C. diptheriae* which causes diphtheria in humans, *C. sepedonicum* which causes ring rot of potatoes.

2. Aerobic/Facultatively Anaerobic Branched Filamentous Rods. The bacteria of this group form colonies which at first are microscopic in size (microcolonies) and contain branched filamentous cells. As the colonies develop to macroscopic size many of the cells become diphtheroid (like caryobacteria) orcocci in shape. Examples include *Agromyces* and *Arachnia*

3. Anaerobic Non-filamentous or Filamentous .The organisms are either anaerobes or if facultatively anaerobicare preferentially anaerobic. Two of the genera in this group are *Propionbacterium* and *Actinomyces*. They are differentiated by their morphology and by their fermentation end products as determined by gas chromatography.

Important genera

1. Corynebacterium
(a) can cause diphtheria.
2. Propionibacterium: anaerobic, causes acne
3. Eubacterium
4. Actinomyces:
(a) branching filamentous soil microbes.

3.2.6 Mycobacterium

They are aerobic bacteria. Their cell walls contain large amounts oflipids. They are made up of a single genus *Mycobacterium* which areslightly curved or straight rods that may show branching. They are acid fast.Some are saprophytes, e.g. *M. phlei* While some are pathogens, e.g. *M.tuberculosis.*

3.2.7 Nocardioforms

They are aerobic bacteria that produce a substrate mycelium i.e. a mat of branching hyphae formed under the surface of the agar medium.

Important genera

Nocardia: pulmonary nocardiosis
Some pathogens.

SELF-ASSESSMENT EXERCISE

i. List the group of bacteria placed in Bergey's Manual of Systematic Bacteriology Volume II.
ii. Differentiate between *Staphylococcus* and *Streptococcus*.

3.3 Bacteria with Unusual Properties

The organisms in this volume have unusual properties which are quite different from those in volumes one and two. The anoxygenic phototrophs can be divided into two major groups based on their pigmentation purple bacteria and green bacteria. They occur in anaerobic fresh water or marine environment. They may also occur beneath the surface of shallow aquatic environments rich in organic matter such as stagnant ponds and ditches.

Anoxygenic Phototropic Bacteria

They belong to the order Rhodospirillales. They are Gram-negative and capable of carrying out photolithothrophic or photoorganotrophic type of metabolism. They contain bacteriochlorophyll. Also present in their cells are various water-insoluble carotenoid pigments which can also trap or absorb light energy and transmit it to the bacteriochlorophyll. The anoxygenic bacteria grow phototrophically only under anaerobic conditions and are incapable of forming O2 because they possess only photosystems.

Oxygenic Phototrophic Bacteria

They are bacteria that contain chlorophyll. They can use light as an energy source and evolve O2 in a manner similar to that of green plants. The group include: the Cyanobacteria (blue-green algae).

Gliding, Fruiting Bacteria

Gram-negative, non-phototrophic bacteria.
They lack flagella; yet can glide across solid surfaces. They have a complex life cycle in which the cells swarm together in masses and form fruiting bodies. The fruiting bodies contain myxo-spores which are shorter and thicker than the vegetative cells.

They are found in surface layers of soil, compost, manure, rotting wood and animal dung. Constituent genera include *Stigmatella Chondromyes*. They are aerobic or micro aerophilic organisms. They live in soil or water.

Gliding, Non-fruiting Bacteria

Gram-negative non-phototrophic rods, filaments or multicellulartrichomes that glide across solid surfaces: fruiting bodies are not produced.

Examples of organisms include *Cytophaga, Flexibacter or Vitreoscilla Beggiotoa, Simonsiella,Saprospira* and *Thiothrix*.

Sheathed Bacteria

Gram-negative non-phototrophic bacteria that form an external sheath that covers the chain or trichomes.They inhabit fresh water of marine environments. Among the genera included in this group are *Sphaerotilus, Leptothrix*, etc.

Budding and/or Appendaged Bacteria

They are Gram-negative non-phototrophic bacteria that reproduce asymmetrically by budding and or form prosthecea or stalks (Nonliving ribbon-like or tubular appendages that are excreted by the cell).The organisms range from aerobic to microaerophilic to facultativelyanaerobic. Examples include *Hyphomicrobium Ancalomicrobium, Caulobacter.*

Chemolithotrophic Bacteria

Gram-negative non-phototrophic bacteria that obtain energy for carbondioxide fixation from the oxidation of ammonia, nitrite, reduced sulfur compounds or ferrous iron. Examples of families in this group are the nitrifying bacteria, the sulfurmetabolizing bacteria and the Siderocapsaceae. Many of these organisms are found in the soil, fresh water and marine environments.

Archaeobacteria

Gram-positive or Gram-negative that are phylogenetically distinct from eubacteria; some produce methane gas; some require unusually high level of NaCl for growth: others are distinguished by their ability to grow at a low pH and a high temperature. Three main categories of archaeobacteria recognised are the methanogens the red extreme halophiles, and the thermo-acidophiles.

SELF-ASSESSMENT EXERCISE

List the major group of organism in volume 3 and state the characteristics of each.

3.4 Bergey Manual of Systematic Bacteriology - Volume 4

Gram-positive filamentous bacteria of complex morphology. The organisms in volume IV are aerobic gram positive bacteria which form

structures such as mycelium of filamentous hyphae and asexual spores as found in microscopic eukaryotic fungi and have different cell types of cell walls based on amino acid and sugar composition.

Filamentous bacteria that divide in more than one plane
The hyphae divide not only transversely but also longitudinally to produce cluster or packet of cells or spore; cell-wall type iii; soilorganisms, animal pathogens, and symbiotic nitrogen-fixers arerepresented. The three genera present in this group are *Geodermatophilus*, *Dermatophilus* and *Frankia*.

Filamentous bacteria that form true sporangia
Harmless soil and water organism whose hyphae divide in a single plane; the spores are formed within special sacs; cell-wall type ii or iii A good example of a genus in this division is *Actinoplanes*.

Streptomyces and related genera

The hyphae divide in a single plane; long chain of conidiospore are formed at the tips of sporogenic hyphae; the organism are mainly harmless soil organism that are noted for production of antibiotics; a few are human or plant pathogen; cell-wall type.Several genera in this group include *Streptroverticullium*,*Actinopycnidium*, *Actinosporangium* but the most popular of these genera is *Streptomyces.*

Additional Filamentous Bacteria Having Uncertain Taxonomic Placement

The taxonome placement of the bacteria of this heterogeneous group is not yet agreed upon because many of the organisms have unusual and striking morphological or physiological characteristics such as:

(i) The extreme halophilism exhibited by *Actinopolyspora.*
(ii) Formation of heat resistant spores by *Thermoactinomyces* and other unusual properties. A heterogenic collection of organism whose relationship to the major groups of gram-positive filamentous bacteria is not yet agreed upon; some have remarkable morphological or physiological properties; a few organisms are pathogenic for humans; the cell-wall type vary.

4.0 CONCLUSION

Division within Bergey's Manual of Systematic Bacteriology Volumes I-IV is based on characteristics such as gram reaction, cell shape, cell arrangement, oxygen requirement motility and metabolic properties.

5.0 SUMMARY

The Bergey's Manual of Systematic Bacteriology Volume 1-IV is as reference for the classification of bacteria.

Division is based on characteristics such as Gram Stain reaction, cell shape, oxygen requirement and other properties.

Bergey's Manual Volume 1 is made up of ordinary Gramnegative bacteria. They include: the spirochetes which have helical shapes, flexible with periplasmic flagella. Important genera include *Treponema* and *Leptospira* Aerobic, motile helical or *vibriod* bacteria with rigid cells and vibriod or helical in shape with polar flagella. Important genera are *Spirillum* and *Azospirillum*.

Non-motile Gram negative curved bacteria have rigid cell that are curved, they occur in soil, fresh water and marine environment.

Gram negative aerobic rods and cocci are straight or slightly curved rods; they include *Pseudomonas*, *Nisseria* and other genera.
Other gram-negative organisms in this group include facultatively anaerobic Gram-negative rods, Aerobic Gram-negative rods, and dissimilatory sulphate-reducing bacteria

Bergey's Manual of Systematic Bacteriology Volume 2 is made of ordinary Gram positive bacteria among which are Aerobic/facultatively anaerobic cocci.

Aerotolerant fermentative cocci, Anaerobic Gram positive, cocci.
Endospore forming gram positive bacteria of regular and irregular shapes and *Mycobacterium*.

Bergey's Manual of Systematic Bacteriology – Volume 3 is made up bacteria of unusual properties among which are Anoxygenic Phototropic Bacteria, Oxygenic Phototropic Bacteria, Gliding fruiting Bacteria, Gliding Non-fruiting Bacteria Sheathed Bacteria and Building and unappendaged Bacteria.

Volume IV of Bergey's Manual of Systematic Bacteriology is made up of gram positive bacteria of complex morphology which include filamentous bacteria that divide in more than one plane,filamentous bacteria that form true sporangia, *Streptomyces* and related genera and filamentous bacteria having uncertain taxonomic placement.

6.0 TUTOR-MARKED ASSIGNMENT

1. What genera of gram-negative bacteria are associated with plants as nitrogen fixers?
2. Write on systematic classification of Gram-positive cocci.

7.0 REFERENCES/FURTHER READING

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UNIT 3 SYSTEMATIC CLASSIFICATION OF FUNGI

CONTENTS

1.0 INTRODUCTION

The classification of fungi like that of bacteria is described mainly for practical application but it also bears some relation to phylogenic consideration. The nomenclature is binomial, with a generic and specific name (e.g. *Aspergillus niger*). Species are collected in genera, families(suffice-acae), families in order (suffix ales), and order in class (suffixmycetes). Sequence analysis of 185-RNA and certain protein – coding

- gene has shown that the fungi comprises a monophyletic group with either subdivisions. Four of these subdivisions, the *Chytridiomycetes,Zygomycota, Ascomycota* and *Basidomycota* have been recognised asseparate groups for some time. The other four – *Uredinomycetes,Ustilaginomycetes, Glomeromycota* and *Microsporidia* have been proposed recently as separate groups. This unit examines the systematic classification of fungi.

2.0 OBJECTIVES

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

- identify the different divisions of fungi
- state the characteristics of each division of fungi and
- state the mode of reproduction of each division of fungi.

3.0 MAIN CONTENT

Chytridiomycetes or Chytrids

Chytridiomycetes or chytrids are the earliest and the simplest group of fungi. They are unique among fungi because they produce motilezoospore with a single posterior whiplash flagellum. The cell wall is made of chitin. Some exist as single cell while other form colonies with hyphae. Some are free living and found living on plant or animal matter in fresh water, mud or soil. Others are parasitic and infect aquatic plant and animal including insects. They display a variety of life cycles involving both asexual and sexual reproduction. Sexual reproduction usually involves production of sporangiospores from sporagia. Based on zoospore morphology, the orders with the Chytridiomycetes include the Blastocladiales, Monoblepharidales, Neocallimasticales, Spizellomycetales and the Chytridiales.

3.1 Zygomycota

The *Zygomycota* are made of fungi called *Zygomycetes*. They are commonly found in soil and on decaying plant materials. All are multinucleate (*coenocytic*). Asexual spores develop in sporangia at the tip of aerial *hypae* and are usually dispersed by wind. Sexual reproduction produces tough, thick walled zygotes called zygospores that can remain dormant when the environment is too harsh for growth of the fungus. The bread mould *Rhizopus stolonifer* is very common member of this division, the fungus grows on moist, carbohydrate-rich foods such as bread and vegetables.

SELF-ASSESSMENT EXERCISE

What feature of Zygomycetes gives this group its name?

3.2 Ascomycota

Members of this group are called Ascomycetes, commonly known as sacfungi. These ascomycetes are large and highly diverse groups of fungi ranging from single-celled species such as yeast (Saccharomyces) to species that are filamentous like *Neurospora*.

The ascomycetes derived their names from the production of asci(singular, ascus) cells in which two haploid nuclei from different mating types come together and fuse, forming a diploid nucleus that undergoesmeiosis to form haploid ascopores. Asexual reproduction in ascomycetes is by the production of conidiaformed at the tip of

specialised hyphae called conidiospore, an example of fungi in this group is yeast. Cell division in *Saccharomyces cerevisiae*(yeast) occurs by budding. During the budding process, a new cell forms as a small outgrowth of the old cell, the bud gradually enlarges and separates from the parent cell. Sexual reproduction also involves ascus formation with each ascus usually bearing eight haploid ascospores.

3.3 Basidiomycota

The Basidiomycota include the Basidiomycetes, commonly known as club fungi. Examples include jellyfungi, puffballs, toadstools and mushrooms. They are named for their characteristic structure cell, the basidium, which is involved. A basidium is produced at the tip of hyphae and is normally club shaped. Two or more basidio spores are produced by the basidium and basidia may be held within fruiting bodies called basidiocarps. The basidiomycetes affect humans in many ways. Most are saprobes that decompose plant debris, e.g. cellulose and lignin, e.g. *Polyporus squamosus*. Some are used as food, e.g. the mushroom. *Agaricus campestris* while some like *Crytococcus, Neoformans* are important human and animal pathogens.

3.4 Glomeromycota

The glomeromycetes are a relatively small group of fungi with major ecological importance. Only about 160 species of glomeromycetes are currently known. All known species of glomeromycetes form endomycorrhizae, also called arbuscular mycorrhizae with the roots of herbaceous plants. They aid the plant acquisition of materials from the soil. They produce only asexually and are mostly coenocytic in their morphology. There is mutualistic relationship between the fungus, the fungus help protect the plant from stress and deliver soil nutrients to the plant which in turn provides carbohydrates to the fungi.

3.5 Microsporidea

These are tiny (2-5μm), unicellular parasite of animals and protists. They have been considered protists and are sometime cited as such. Molecular analysis of ribosomal RNA and specific protein such as α-and β-tubulin shows that they are most closely related to fungi. However, unlike fungi, they lack mitochondria, peroxisomes and centrio ls. They are obligate parasites that infect insects, fish and human, in particular they infect immunosuppressed individuals such as those with HIV/AIDS; an example is *Enterocystozoa spp* which causes diarrhea and pneumonia. It reproduces asexually by spore formation.

3.6 Uredinomycetes and Ustilaginomycetes

Both the uredinomycetes and the ustilaginomycetes include plant pathogens causing rust and smuts. Some uredinomycetes include human pathogens. They are often considered basidomycota. Both unlike the basidomycota, they do not form large basidocarps instead small basidiaarise from hyphae at the surface of the host plant. The hyphae grow either intracellular or extracellularly in the plant tissue. A good example is *Ustilago maydis*, a common corn pathogen that causes smuts.

SELF-ASSESSMENT EXERCISE
State the role of arbuscular mycorrhizae on plants.

4.0 CONCLUSION

Systematic classification of fungi is based on sequence analyses of 185rRNA and some protein coding genes and on the characteristics of the sexual spores and fruiting bodies present during sexual reproduction.

5.0 SUMMARY

Sequence analyses of 185r RNA and some protein-coding genes show that the fungi comprise a monophyletic group with eight subdivisions. Chytridiomycetes are the earliest and simplest group of fungi. They produce motile spores, some are saprobic while some are parasites.
Zygomycetes are coenocytic (multinucleate) saprobic. They reproduce asexually by spore formation and sexually by fusion of two gametangia to form a zygospore, an example is the bread mould *Rhizopus stolonifer*.
As comycota are known as sac shaped reproductive structure called an ascus. Asexual reproduction occurs by conidia production while sexual reproduction occurs by fusion of gametes.

Basidiomycota are the club fungi. They are named after their basidium which carries 4 basidiospores. Some are saprobes, while some are pathogens of human and animals.

Glomeromycota form endomycorrhizae with plants roots. They help to increase nutrient intake in the plants.

Microsporidia are still sometimes considered as protists but molecular analysis has shown they are most closely related to fungi. They lack mitochondria, centrioles and peroxisomes. They are usually pathogens to insect and those with compromised immunity.

Uredinomycetes and Ustilaginomycetes are often considered basidiomycetes but they do not form basidiocarp.

6.0 TUTOR-MARKED ASSIGNMENT

1. What are chytridiomycetes? How do they differ from other fungi?
2. In what structure are the basidiospores formed?

7.0 REFERENCES/FURTHER READING

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

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UNIT 4 SYSTEMATIC CLASSIFICATION OF ALGAE

CONTENTS

1.0 INTRODUCTION

Algae are generally classified on the basis of the following characteristics: nature and properties of pigment chemistry of reserve food products or assimilatory products of photosynthesis type and number, insertion (point of attachment), and morphology of flagella chemistry and physiological features of cell walls morphological characteristics of cells and thalli life history, reproductive structures and method of reproduction. This unit examines the systemic classification of algae and the characteristics of the major groups of algae.

2.0 OBJECTIVES

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

- identify the major groups of algae
- state the types of pigment present in each group of algae
- state the major characteristics of each group of algae
- state the mode of reproduction of each group of algae.

3.0 MAIN CONTENT

3.1 Rhodophycophyta

The Rhodophycophyta, or red algae, are marine forms found in the warmer seas and oceans, but some grow in colder water as well as in fresh water. Most red algae grow in the subtidal (submerged) zone, only a few being able to survive desiccation or exposure. Some species deposit upon their surfaces lime from seawater; ultimately this results in deposition of lime in the ocean and plays a part in the formation of algal reefs. They range from unicellular to multicellular. They are photosynthetic and contain chlorophyll A. Their chloroplast lack chlorophyll B and contain phycobili proteins. The major light-harvesting pigments of the cyanobacteria, the reddish colour of red algae resultsfrom phycoerythrin, an accessory pigment that masks the green colourof chlorophyll. And example is *Gelidium* from which agar is made,*Polysiphonia* and *Chondrus crispus*.

3.2 Xanthophycophyta - The Yellow-Green Algae

These yellow-green algae were once classified with the green algae. However, their pale green or yellow-green colouration indicates that they have a unique group of pigments. They are found more frequently in temperate regions in freshwater and marine habitats, as well as on and in soil. Xanthophytes exist as single cell colonies, and as both branched and unbranched filaments. Motile genera are not common, although, some reproduce asexually by motile reproductive cells (zoospores).Flagella are of unequal lengths.

The xanthophyte walls are typically of cellulose and pectin. The cellular storage product is an oil or (a branched glucan) chrysolaminarin. *Vaucheria*, the water felt, is a well-known member of this division and is widely distributed on moist soil and in both quiet and rapidly flowing water. Both freshwater and marine species are known. Zoospores are formed singly in terminal sporangia in asexual reproduction.

3.3 Chrysophycophyta – The Golden Algae

Species of *Chrysophycophyta* are predominantly flagellates; some are amoeboid, with pseudopodia extensions of the protoplasm. The naked amoeboid forms can ingest particulate food with the pseudopodia. Nonmotile coccoid and filamentous forms are also included in the division. The chrysophycophyta differ from the green algae in the nature of their pigments, in storing reserved food as oil or chrysolaminarin rather than

as starch, and in their frequent incorporation of silica. Most forms are unicellular, but some form colonies. Their characteristic colour is due to the marking of their cholorophyll by brown pigments. Reproduction is commonly asexual (binary fission) but occasionally is ogamous. Anexample is *Ochromonus.*

SELF-ASSESSMENT EXERCISE
What are the major characteristics of the Crysophycophyta?

3.4 Phaeophycophyta – The Brown Algae

These algae are multicellular and contain a brown pigment which gives them their characteristic colour and common name of brown algae, or brown seaweeds. Nearly all are marine dwelling and most frequently, found in the cool ocean waters. They are structurally quite complex, and some – the kelps – are large, the individual plants reaching a length of several hundred feet. Many have holdfasts; and some have air bladders, which give them buoyancy. They reproduce asexually by zoospores and sexually by is ogamy and heterogamy. This group includes algae used in commerce, such as the many varieties of kelp. They are used as food for humans, other animals and fish.

3.5 Bacillariophycophyta – The Diatoms

Members of this group are the diatoms, they are found in both fresh and salt water and in moist soil. They are abundant in cold waters. Diatoms are the most plentiful form of plankton in the Arctic. The thousands of species diatoms provide an ever present and abundant food supply for aquatic animals. Diatoms are unicellular, colonial, or filamentous and occur in a wide variety of shapes. Each cell has a single prominent nucleus which is massive and ribbon-like, or smaller lens-like, plastids. They produce shells (cell walls) containing silica, some of which are very beautiful. Shells of diatoms are called **frustules**. Deposits of these shells resulting from centuries of growth are called **diatomite** or **diatomaceous earth.**

3.6 Euglenophycophyta – The Euglenoids

They are unicellular organisms and they are actively motile by means of flagella. They reproduce by cell division. Of particular interest is the genus Euglena, which is representative of a group designated as animals by some zoologists but as plants by many botanists. Euglena is widely distributed and occurs in soil as well as in water, where it often forms a variety film or bloom. The Euglena is not rigid; it is pliable. There is no cell wall containing cellulose. The outer membrane is an organised

periplast. An anterior “gullete” is present even though, no food is ingested through it. Certain species develop a prominent stigma or red eyespot. Contractile vacuoles and fibrils are also present in the cell. All these are animal attributes. On the other hand, the organism carries out photosynthesis. A few types can even ingest particulate food through transient openings adjacent to the gullet. Reproduction is by longitudinal binary fission. Dormant cysts are formed by all types.

3.7 *Chlorophycophyta* – The Green Algae

Members of this large and diverse group of organisms, called **greenalgae**, are principally freshwater species. They are also found in seawater, and many of them are terrestrial. The cells of the chlorophycophyta have a well-defined nucleus and usually, a cell wall, and the chlorophyll pigments are in chloroplasts, as in higher plants. The majority of green algae contain one chloroplast per cell. It may belaminate, cup-shaped, or reticulate. The chloroplasts also often containdense regions called **pyrenoids**, on which surface starch granules are formed. Food reserves are stored as starch, a product of photosynthesis. They bear chloroplasts containing chlorophyll A and B giving them their characteristic green colour. There are many single-celled forms and many colonial types of greenalgae. Many unicellular green algae are motile by flagella action. Colonial types occur as spheres, filaments, or plates. Some species have special structures called holdfasts, which anchor them to submerged objects or aquatic plants. An example is *Chlamydomonas.Chlamydomonas* is considered a typical green algae. It is a typical unicellular, motile, green alga and is widely distributed in soils and freshwater. It varies from 3 to 29 m in common forms and is motile except during cell division. Motility is by means of two flagella. Each cell has one nucleus and a single large chloroplast that in most species is cup-shaped, although in some, it may be star-shaped or layered. The cell wall contains cellulose; in many species, an external gelatinous layer is also present. There is some evidence that the red eyespot or stigma in the chloroplast is the site of light perception. In addition to motile unicellular algae like *Chlamydomonas*, other, nonmotile unicellular green algae are widely distributed. One of the most important of these is *Chlorella*, which has served usefully as a tool in many investigations on photosynthesis and supplemental food supply.

Volvox is a colonial green algae which may form water blooms. Its colonies are visible to the naked eye. Each colony contains from 500 to thousands of cells arranged at the surface of a watery colloidal matrix. The individual cells are biflagellate and are morphologically similar to that of chlamydomonas.

Desmids are one of the most interesting green algae found in a wide variety of attractive shapes and designs. Each cell is made up of two symmetrical halves with one or more chloroplasts.

Ulethrix is a filamentous form found in flowing streams, attached to wings or stones by holdfasts at the base of the filament. A very common green alga is *Spirogyra*, a filamentous form seen in the scums that cover ponds and slow-moving water. It is of interest because of its common occurrence and its possession of unusual chloroplasts, which are arranged spirally.

3.8 Cryptophycophyta – The Cryptomonads

The cryptomonads are a small group of biflagellate organisms. They have two unequal flagella, which arise from the base of a groove; both are of the tinsel type, with stiff hairs. The cells are slipper-shaped, flattened into a dorsal-ventral plane, and occur singly. Some forms have a cellulose wall while others are naked, being surrounded only by a plasmalemma with a thin granular material on the outside. There are one or two plastids, with or without pyrenoids, per cell. Food reserve is stored as true starch as well as oil. Asexual reproduction is either by means of longitudinal cell division or the formation of zoospores or cysts. An example is the genus *Cryptomonas*.

3.9 Pyrrophycophyta – The Dinoflagellates

This division includes the dinoflagellates, a diverse group of biflagellated unicellular organisms. The dinoflagellates are so named because of their twirling motion rather than their morphology. These organisms constitute an important component of marine, brackish, and fresh bodies of water. This is another group that has both plant-like and animal-like characteristics. The cells are typically flattened and have a transverse constriction, the girdle, usually around the cell equator. Distinguishing feature of dinoflagellates are that the flagella are insertedin the girdle and that the flagella are arranged with one encircling thecell and one trailing. Hairs project from the flagellar surface. Manydinoflagellates are covered only by a plasmalemma. In some forms, there is a wall made of cellulose. Still, others have a series of celluloseplates within the plasmalemma. These are termed thecal plates and dinoflagellates with them are said to be armored. Dinoflagellates are important constituents of planktons. They are best known as the organisms that produce “red tides”, or blooms in which concentration of cells may be so great as to colour large areas of the ocean red, brown, oryellow. Such an organism is *Gonyaulax*. Other

marine dinoflagellatessuch as *Noctiluca* are luminescent. Asexual reproduction takes place by division of the cell.

SELF-ASSESSMENT EXERCISE

What are the characteristics of the Chlorophycophyta?

4.0 CONCLUSION

It can be seen clearly from the different major classes or groups of algae that classification of algae were based on their characteristic coloured pigments and cell morphologies.

5.0 SUMMARY

The Rhodophycophyta – The red algae are found in aquatic habitats. They reproduce asexually by non-motile spores and sexually by the union of well differentiated non-motile male and female germ cells Examples are *Gelidium* and *Polysiphonia*.

The Xanthophycophyta: The yellow green algae are single cells, colonies or filamentous algae that have pale green or yellow green pigmentation and are found in temperate region fresh water and marine habitats. Reproduce asexually by cell division and production of motilespores.

The Phaeophycophyta: The brown algae are multicellular with brown pigments found most times in marines and reproduce asexually byzoospores and sexually both is ogamously and heterogamously.

Bacillariophycophyta: The diatoms are found in both fresh and saltwater and in moist soils. They occur in a wide variety of shapes and serve as food for aquatic animals.
Euglenophycophyta: are unicellular organisms that move by means of flagella and reproduce by binary cell division a good example is *Euglena* with animal like and plant like characteristics.
Chlorophycophyta: The green algae may be unicellular, colonies orfilament are found in fresh water, see water and terrestrial habitats. They have chlorophyll and other pigments in chloroplasts and have food reserve stored as starch and reproduce by zoospores, fission and other asexual methods.
Cryptophycophyta: The *Cryptomonas* are biflagellate organisms withtwo unequal flagella food reserved is stored as a true starch as well a soil. Reproduction is either by means of longitudinal cell division or

the formation of zoospores or cysts. Sexual reproduction has been confirmed in the genus *Cryptomonas*.

Pyrrophycophyta: The dinoflagellates are a diverse group of biflagellated unicellular organisms with animal-like and plant-like characteristics found in aquatic habitats where they produce "redtides" or blooms which colour large areas of the ocean red, brown or yellow. Asexual reproduction is by division of the cell. Examples are *Gonyaulax* and *Noctiluca*.

6.0 TUTOR-MARKED ASSIGNMENT

1. What are the characteristics Euglena shares with:
a. animals?
b. plants?
2. Write a short note on the Chlorophycophyta.

7.0 REFERENCES/FURTHER READING

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MODULE 6 SYSTEMATIC CLASSIFICATION CONTD/MICROBIAL GENETICS AND BIOGEOCHEMICAL CYCLING OF ELEMENTS

UNIT 1 SYSTEMATIC CLASSIFICATION OFPROTOZOA

CONTENTS

1.0 INTRODUCTION

Traditionally, the protozoa are grouped together based on general similarities: they lack cell walls, they are colourless and motile, they exhibit a wide range of morphologies and inhabit many different kinds of habitat, and they play major roles in human society and health. The different forms of protozoa have been grouped together not because they are all related in an evolutionary way, but simply for convenience. Theold classification schemes of protozoa were based primarily onorganelles of locomotion. The major groups are called Phyla (singular,phylum). This unit determines the systematic classification of protozoa and different phyla in which they were placed.

2.0 OBJECTIVES

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

- identify the major phyla of protozoa
- state the characteristics of each phylum
- differentiate between different phyla; and
- list some species found in each group or phyla.

3.0 MAIN CONTENT

3.1 The Flagellates (Subphylum Mastigophorea)

These protozoa are conventionally divided into two groups: The plant like forms (class Phytomastigophorea, the phytoflagellates) and the animal-like forms (class Zoomastigophorea, the zooflagellates). Plant like protozoa usually contain green or yellow chloroplasts as well as flagella and are photosynthetic. The zooflagellates have no chlorophyll and are heterotrophic. All members have one or more flagella. Some have pseudopodia. Asexual reproduction occurs by longitudinal binary fission. A form of multiple fission takes place in some organisms. Encystment is common but sexual reproduction is not. These organisms are considered as algae by some biologists. Since the zooflagellates have no chlorophyll, they must obtain nutrition heterotrophically. All members of this group have one or more flagella;some members are capable of forming pseudopodia.

3.2 The Zooflagellates (Class Zoomastigophorea)

The Choanoflagellates (order Choanoflagellida) are distinctive in that they are either stalked or embedded in jelly, and each cell has a thintransparent collar that encircles a single flagellum. The collar functions as a food catching device. Organisms in the order Kinetoplastida are grouped together because of the presence of a kinetoplast (an extra nuclear region of DNA associated with the mitochondrion). The single mitochondrion itself is extensive, traversing the length of the body as single tube, hoop or network of branching tubes. One or two flagella may be present; if there are two, one is either trailing free or attached to the body, with undulating membranes occurring in some cases.

3.3 The Amoebas (Subphylum Sarcodina)Structure

Amoebas get their name from the Greek word “amoibe”,meaning “change” because their shapes are constantly changing. A typical example is *Amoeba proteus*.

Amoebas are composed of protoplasm differentiated into a cell membrane, cytoplasm and a nucleus. The cytoplasm shows granules as well as vacuoles containing food, wastes, water, and possibly gases. The outer membrane is selective and permits the passage of certain soluble nutrients into the cell and waste materials out of the cell. Solid food is ingested with the help of pseudopodia. The nucleus functions in reproduction, metabolism and the transmission of hereditary characteristics.

Amoeba reacts to various physical and chemical stimuli in their surroundings. This is an irritability response which is at least superficially analogous to responses of higher organisms to their environment.

Nutrition of Amoeba
Uses pseudopodia to capture its food.
Reproduction – amoeba is asexually binary fission.

3.4 The Sporozoa (Phylum Apicomplexa)

They are in constant motion. They move by sending out portions of their bodies in one direction which the whole body follows. They use pseudopodia to capture food. Reproduction is asexually by binary fission. Some have the ability of encysting in unfavourable condition. Most are free living, some are saprophytic; however, one species *Entamoeba histolytica* causes amoebic dysentery in man. All sporozoa are parasitic for one or more animal species. Adult forms have no organs of motility but all are probably motile by gliding at one stage of their life cycle. They cannot engulf solid particles, but feed on the host' cells or body fluids.

Many have complicated life cycles, certain stages of which may occur inone host and other stages in a different host. They all produce spores at some time in their life history. Their life cycles exhibit an alternation of generations of sexual and asexual forms, such that the intermediate host usually harbours the asexual forms and the final host, the sexual forms. Sometimes, humans serve as hosts to both forms. Toxoplasmosis and malaria are the major human diseases caused by sporozoa. Malaria is caused by *Plasmodium* asporozoa which infect the liver and red blood cells.

3.5 The Ciliates (Phylum Ciliophora)

The ciliates are protozoa with cilia for locomotion. Common examples of the ciliated protozoa are included in the genus *Paramecium*, found in freshwater ponds and lakes where adequate food supplies exist.

Structure/Morphology
Paramecium

Paramecium moves rapidly by rhythmic beating of the cilia.
Nutrition: *Paramecium* takes in food through a fixed cytostoma at the base of the gullet.

Excretion is through the contractile vacuole.

Reproduction – Reproduce asexually by binary fission conjugation may also occur.

Paramecia are microscopic, some, however, are just barely visible to the unaided eye. The outer layer of the cell is less flexible than the outer membrane of the amoeba, and the interior is composed of semi fluid, granular protoplasm containing nuclei and vacuoles of several kinds. Paramecia are easily distinguished by their characteristic shape, which has been likened to that of a slipper. The anterior (front) end of the cell is rounded, and the posterior (rear) end is slightly pointed. The entire cell is covered with hundreds of short hair-like projections called cilia, which are the organs of locomotion and also serve to direct food into the cytosome.

3.6 Other Ciliated Protozoa

The ciliated protozoa are represented by many forms other than the paramecia. *Colpoda* is a common freshwater genus. The genus *Didinium* lives on a diet of paramecia, which are captured by a special structure and swallowed whole. The genus *Stentor* comprises largecone-shaped protozoa that move about freely but attach to some object by a tapered lower end while feeding.

4.0 CONCLUSION

Classification in protozoa is by the use of general characteristics and not as a result of evolution.

5.0 SUMMARY

Phytomastigophora are divided into two groups. The plant like forms class Phytoflagellates which have chlorophyll and flagella forms, class Zoomastigophora which have no chlorophyll and are heterotrophic.
The amoebae (subphylum Sarcodina) are constantly changing their shapes and composed of protoplasm differentiated into a cell membrane cytoplasm and a nucleus and feed with the help of the pseudopodia.

The sporozoa (phylum Apicomplexes) are parasitic for one ormore animal species. They feed by the use of pseudopodia and reproduce asexually by binary fission.

The ciliates (phylum Ciliophora): The ciliates are protozoa with cilia for locomotion.

Other ciliates include Colpodia and the genera *Didinium* and thegenus *Stentor*.

6.0 TUTOR-MARKED ASSIGNMENT

1. State the two groups of flagellates.
2. Describe the structure of paramecium.

7.0 REFERENCES/FURTHER READING

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

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UNIT 2 MECHANISMS OF GENETIC VARIATIONAND HEREDITARY

CONTENTS

1.0 INTRODUCTION

Each living organism resembles its ancestors in most of its characters. The maintenance of specific properties, that is, the constancy of characters over generations is called heredity. Genetics is the study of inheritance (heredity) and the variability of the characteristics of an organism. Inheritance exacts transmission of genetic information from parents to their progeny (offspring).Deoxyribonucleic acid (DNA) is the chemical substance responsible for hereditary in all cells because it beams the genetic information. Each genetic character can be assigned to a gene which carries the information. Microorganisms are capable of transmitting genetic information from generation to generation with great accuracy. (This unit examines the causes of variation in microorganisms). Variability or variation of the inherited characteristics occurs or alters as a result of change in the genetic makeup of a cell or in environmental conditions.

Variation in microorganisms takes place by mutation. Recombination and gene transfer.

2.0 OBJECTIVES

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

- define the term genetic variation
- define mutation.

3.0 MAIN CONTENT

Genetic Variation

This is changes in or of a gene which leads to a loss of the enzymes or to the production of an altered enzymes, hence, to recognisable changes in the hereditary character.

Genetic variation in bacteria can take place by:
Mutation gene transfer or recombination.

3.1 Mutation

Mutation can be defined as a change in the nucleotide sequence of DNA. Mutation can involve the addition, deletion or substitution of nucleotides. These changes in the nucleotides are stable and heritable and are passed down from one generation to the next. Mutation introduces genetic variation among organisms. A mutant is a strain of any cell or virus carrying a change in the nucleotide sequence. A mutant is different from its parent strain in:

i Genotype: The nucleotide sequence of the genome.
ii The Phenotype: The observable property of the mutant in the altered phenotype is called a mutant phenotype. The strain isolated originally from nature is called the wild type strain. Mutation can occur spontaneously or induced under the influence of external agents (mutagens).

3.1.1 Spontaneous Mutation

This is mutation that occurs without exposure to external agents or any known mutagenic treatment. It occurs at a fairly constant frequency in a particular organism, one per 106 to 1010 in a population derived from a single bacterium. It may result from errors in DNA replication or from the action of mobile genetic molecules called transposons.

Mutation arising from error in DNA replication can be:

Transition Mutation: This is the substitution of a purine for another purine (A or G) or one pyrimidine for another pyrimidine(C or T) that lead to a stable alteration of the nucleotide sequence. This type of mutation is relatively common.

Transversion Mutation: This is a mutation in which a purine is substited for a pyrimidine or pyrimidine for a purine. This is rarer due to the steric problems of pairing purines with purines and pyrimidine with pyrimidine. Both Transition and Transversion mutations are types of base substitution in point mutation.

Mutations as result of lesions on DNA which result in apurinesites, apyrimidinic sites, oxidation of DNA.
Mutation as a result of the insertion of DNA segments into genes. Insertion usually inactivates genes. They are caused by movement of insertion sequences and transpons.

3.1.2 Induced Mutation

This is mutation caused by external agent (mutagens), which may be physical or chemical agents.

1. **Physical Agents**: Physical agents include ultraviolet (UV) light, raising radiation, visible light and heat. Ionizing radiation (e.g. Xrays) can break both single and double strand DNA. The frequency of mutation is greatly increased at a temperature of37oC and above.
2. **Chemical Agent**: There are three main types of mutagenic chemicals.
(i) **Base analogs**: These are chemicals structurally similar to normal DNA bases and can be substituted for them during DNA replication. These compounds exhibit based pairing properties different from the bases they replace and eventually cause a stable mutation. An example: 5-bromouracil (5-BU) an analog of thymine.

(ii) **DNA Modifying Agents**: These chemicals react chemically with DNA. They change a base structure and alter its base pairing characteristics. Examples are Methylnitrosoguanidine and Hydroxylamine.
(iii) **Intercalating Agents**: These are chemicals with flat molecules that can intercalate (slip in) between bases pairs in the central stack of the DNA helix. They include single nucleotide pair insertion and deletions. Examples are acridine such as proflavin and acridine orange.

3.2 Types of Mutation

Two common types of mutation are: point mutation and frame shift mutation.

1. Point Mutations

Point mutations occur as a result of the substitution of one nucleotide for another in the specific nucleotide sequence of agene or defined as change in only one base pair. Point mutations in protein – coding genes can affect protein structure and are named according to if and how they change the encoded protein. The most common type of point mutations are:

(i) **Silent mutation**: Change the nucleotide sequence of acodon but do not change the amino acid encoded by that codon.
(ii) **Missence Mutations**: This involves a single base substitution that changes a codon for one amino acid into acodon for another.
(iii) **Nonsense Mutation**: This causes the early termination of translation and therefore results in a shortened polypeptide. They are called nonsense mutation because they convert a sense codon to a non-sense or stop code.

2. Frame Shift Mutations

These mutations result form an addition or loss of one or more nucleotides in a gene and are termed insertion or deletion mutations respectively. This results in a shift or the reading frame. Frame shift mutations usually are very harmful and yield mutantphenotypes resulting from the synthesis of non-functional protein. The effect of mutation can be described at the protein level and in terms of traits or other easily observed phenotypes. A mutation from wild type to a mutant form is called a forward mutation; however, a reversion mutation occurs when second mutual at the same site as the original mutation which restore the wild type phenotype. If the second mutation is at a different site than the original mutation is called suppressor mutation.

SELF-ASSESSMENT EXERCISE

i. What are point mutations?
ii. What are frame shift mutations?

3.3 Genetic Recombination

This is the formation of a new genotype by reassortment of genes following an exchange of genetic material between two different chromosomes which have similar gene at corresponding sites and are from different individuals. Progeny or offspring from recombination have combination of genes different from those that are present in the parents. In bacteria, genetic recombination's result from three types of gene transfer, they are:

1. **Conjugation**: This is the transfer of genes between cells that are in physical contact with one another.
2. **Transduction**: This is the transfer of genes from one cell to another by a bacteriophage.
3. **Transformation**: This is the transfer of cell free or naked DNA from one cell to another.

3.4 Mechanism of Recombination

Inside the recipient cell, the donor DNA fragment is positioned alongside the recipient DNA in such a way that homologous genes are adjacent. Enzymes act on the recipient DNA, causing nic and excision ofa fragment. The donor is then integrated into the recipient chromosome in place of the excised DNA. The recipient becomes the combination cell because its chromosomes contain DNA of both the donor and the recipient cell.

3.4.1 Conjugation

This is a mechanism of genetic transfer that involves cell to cell contact. It is plasmid encoded mechanism i.e. it is controlled by gene carried by certain plasmid (such as F plasmid). The process of conjugation involves a donor cell which contains conjugative plasmid and a recipient cell which does not.

F Plasmid

The F plasmid (F stands for fertility) is a circular DNA molecule of99159 bp. It is an extra chromosome DNA that encodes the necessary information necessary for conjugation. The F plasmid has a large regionof DNA, the extra region containing genes that encode transfer functions. Many genes in the extra region are involved in meeting pair formation and most of these have to do with the synthesis of a surface structure the sex pilus (plural, pili).Only donor cells produce these pili. Pili allow specific pairing to take place between the donor and recipient cells. The pilus makes specific contact with a receptor on the recipient

cell and is retracted by disassembling its subunit. This pulls the two cells together, making the donor and recipient cells remain in contact by binding proteins located in the outer membrane of each cell DNA is the transfer from donor to recipient cells through this conjugation junction.

Mechanisms of DNA Transfer

During conjugation DNA transfer is triggered by cell to cell contact by which one strand of the circular plasmid is mixed and is transferred to the recipient. The nicking enzyme required to initiate the process that is encoded by the of the F plasmid DNA synthesis by the rolling circle mechanisms replaces the transferred strand in the donor while a complementary DNA strand is being made in the recipient. At the end of the process, both donor and recipient cells possess complete plasmid. For transfer of the F plasmid, if an F-containing donor cell (designated as F+) mates with a recipient cell lacking the plasmid (designated as F-)the result is two F+ cells.

3.4.2 Transformation

This is a genetic transfer process by which free DNA is incorporated into a recipient cell and brings about genetic change. Several prokaryotes are naturally transformed including certain species of Gramnegative and Gram positive bacteria and some species of Archaea because the DNA of prokaryotes is present in the cell as a large single molecule, when the cell is gently lysed, the DNA pours out and breaks easily into fragments containing genes which are released into the surrounding environment. If a fragment contacts a competent cell, a cell that is able to take up DNA and be transformed, the DNA can be bound to the cell and taken inside. Competency for transformation is a complex phenomenon and is dependent on several conditions such as stage of growth. Naturalt ransformation has been discovered so far in certain genera including S*treptococcus, Bacillus, Acinectobacter, Azobacter, Helicobacter* and *Pseudomonas*. Gene transfer by this in process occurs in soil and aquatic environment.

3.4.3 Transduction

This is a mechanism of genetic transfer in which a bacterial virus(bacteriophage) transfers DNA from one cell to another. Virus can transfer the host genes in two ways:

i generalised transduction and
ii specialised transduction

1. **Generalised Transduction**

DNA derived from virtually any portion of the host genome is packaged inside the mature union in place of the virus genome. Any gene on the donor chromosome can be transferred to the recipient since they carry any of the host chromosome, they are called generalised transduction. When a bacteria cell is infected with a phage, the lytic cycle is initiated. During the lytic infection, the enzymes responsible for packaging viral DNA into the bacteriophage sometimes package host DNA accidentally. The resulting union is called a transducing particle. On lysis of the host cells, the transducing particles are released along with normal union (that is those containing the virus genome), hence the lysate is used to infect a population of recipient cells.Most of the cells become infected with normal virus. However, a small portion of the population receives transducing particles that inject the DNA they packaged from the previous host bacterium. These transducing particles undergo genetic recombination with the DNA of the new host. Generalised transduction allows the transfer of any gene from one bacterium to another at a low frequency.

2**. Specialised Transduction**

In specialised transduction, DNA from a specific region of the host chromosome is integrated directly into the virus genome usually replacing some of the virus genes. This occurs only in a certain temperate viruses. Specialised transduction allow sextremely efficient transfer but is selective and transfers only a small region of the bacteria chromosome. In the first case of specialised transduction to be discovered, gelatose genes were translated by phage lambda of *E. Coli*.When lambda lysogenizes a host cell, phage genomes are integrated into the host DNA at a specific site. The region in which lambda integrates in the *E. Coli* chromosome is next to the cluster of the gene that encode the enzyme for galactose utilisation. After insertion, viral DNA replication is under control of bacterial host chromosome. Upon induction, the viral DNA separates from the host DNA by a process that is reverse of integration. The phenotype of microorganisms can be affected in various ways:

i Morphological Mutations: Changes in the micro organism colonial or cellular morphology of the microorganisms.
ii Lethal Mutation: Resulting in the death of the microorganism.
iii Biochemical Mutation: Causing a change in the biochemistry of the cell.

3.4.4 Mutation Detection involves

i. Visual Observation
ii. Replace Plating Technique.

4.0 CONCLUSION

Microbial variation can arise as a result of mutations which can occur spontaneous or induced by physical and chemical changes. Mutation leads to changes in protein structure and function which in turn alter the phenotype of an organism. This results in the organism (mutant) being different from the one found originally in nature (the wild type).

5.0 SUMMARY

Mutation is a stable heritable change in the nucleotide sequence of a DNA molecule.

Mutation can be spontaneous or induced.
Spontaneous mutation can arise from replication errors(transition, transversion, addition and deletion of nucleotide)from DNA lesions and from insertion of DNA segments and transpons.
Induced mutation can be from physical and chemical agents called mutagens.
Physical agents, of mutation include ultraviolet rays, ionisin grays, radiation, light, heat, etc.
Chemical agents of mutation are base analogs, intercalating agents and DNA modifying agents.

Main types of mutation are point mutation and frame shift mutation.
Mutations are recognised when they cause a change from the normal wild type phenotype. A mutant phenotype can be restored to wild type by either reversion or suppressor mutations.
Some major types of mutations categorised based on the effect on phenotypes are morphological, lethal and biochemical.
Replace plating can be used for detection and isolating mutants.
Amen test is used to screen for mutagens and potential carcinogens.

6.0 TUTOR-MARKED ASSIGNMENT

1. What is mutation?
2. List four ways in which spontaneous mutation might arise.
3. List three methods of recombination in a prokaryotic cell.
4. Explain the mechanism of conjugation in bacteria.

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UNIT 3 BIOGEOCHEMICAL CYCLING OFELEMENTS

CONTENTS

1.0 INTRODUCTION

Life on earth would not be possible without microbes. Soil microorganisms serve as biogeochemical agents for the conversion of complex organic compounds into simple inorganic compounds or into their constituents' elements. The overall process is called Mineralisation. Biogeochemical cycling refers to the biological and chemical processes that elements such as carbon, nitrogen, sulfur, iron and magnesium undergo during microbial metabolism. This conversion of complex organic compounds into inorganic compounds or elements provides for the continuity of elements (or their compounds) as nutrients for plants and animals including man. This unit examines the biogeochemical cycles of elements such as carbon, sulfur, nitrogen and phosphorous.

2.0 OBJECTIVES

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

- define biogeochemical cycling
- state the features of biogeochemical cycles
- describe the carbon cycle
- describe the sulphur cycle
- describe the phosphorus cycle.

3.0 MAIN CONTENT

Biogeochemical Cycling

Biogeochemical cycling is the movement of materials via biochemical reactions through biospheres. The biosphere is that portion of the earth and its atmosphere in which living organisms occur. The activities of microorganisms within the biosphere have a direct impact on the quality of human life. Microorganisms are especially important in recycling materials. The metabolism carried out by microorganisms often transfers materials from one place to another. Changes in the chemical forms of various elements can lead to the physical movement of materials. Sometimes causing transfer between the atmosphere (air) hydrosphere (water) and lithosphere (land).Biogeochemical cycling also refers to the biological and chemical processes that elements such as carbon, nitrogen, sulfur, iron and magnesium undergo during microbial metabolism. It can also be defined as cyclical path that elements take as they flow through living (biotic) and non-living (abiotic) components of the ecosystem. These cycles are important because a fixed and limited amount of the elements that make up living cells exists on earth and in the atmosphere. Thus in order for an ecosystem to maintain and sustainits characteristics and life forms elements must continuously be recycled. For example, if the organic carbon that animals use as an energy source and exhale as carbon dioxide (CO_2) were not eventually converted back to an organic form we would run out of organic carbon to build cells. Elements involved in the biogeochemical cycles are used for three general purposes.

(i) Biomass Production

In biomass production, the element transferred (e.g. N, C, etc) Is incorporated into cell materials for example, all organisms require nitrogen to produce amino acids, hence plants and many prokaryotes assimilates nitrogen by incorporating ammonia(NH_3) to synthesise the amino acid glutamate. Animals cannot incorporate ammonia instead require amino acids in their diets. Some prokaryotes can reduce atmosphere nitrogen to form ammonia, which can then be incorporated into cellular material.

(ii) Energy Source

A reduced form of the element is used to generate energy in form of ATP. For example reduced carbon compounds such as lipids and amino acids are used as energy source by heterotrophs. Chemolitrophs can use reduced inorganic molecules such as hydrogen sulfide (H_2S) ammonia (NH_3) and Hydrogen gas.

(iii) **Terminal Electron Acceptor i.e. Carbohydrate Oxidised to CO2** Electrons from the energy source are transferred to an oxidized form of the element during respiration, this reduces the terminal electron acceptor in aerobic conditions, O2 is used as a terminal electron acceptor. In anaerobic conditions, some prokaryotes can use nitrate, sulphate (SO4), and carbon dioxide (CO2) as terminal electron acceptors. Global climate change, temperature precipitation and stability of ecosystem are all dependent on biogeochemical cycles.

3.1 Peculiar Features of Biogeochemical Cycles

Elements required are in five forms and mostly from non-living reservoir in the atmosphere. They can also be gotten from sedimentary rock or water.

The elements go in cycle and are always free in inorganic state in a biotic environment and when needed in biotic environment, they are turned to organic state. They can be combined with other elements.

The recycling of these elements maintains a necessary balance of nutrient and they are maintained throughout.
The cycles (biogeochemical) are complex and they involve the activity of producers, consumers and decomposers.
All organisms participate directly in recycling by removing, adding or altering nutrients. Microorganisms are noted for metabolic conversion especially in changing some elements from one nutritional form to another.
The total turnover rate of element is most rapid in atmosphere and lowest in sedimentary rocks.

SELF-ASSESSMENT EXERCISE
i. What is biogeochemical cycle of elements?
ii. State three features of biogeochemical cycles.

3.2 Carbon Cycle

Carbon is a very important element as it makes up organic matter which is a part of all life. Carbon follows a certain route on earth called the CARBON CYCLE.

The carbon cycle primarily involves the transfer of carbon dioxide and organic carbon between the atmosphere where carbon occurs principally as inorganic CO2 and the hydrosphere and lithosphere which contain

varying concentrations of organic and inorganic compounds. The carbon cycle begins with carbon fixation, which is the conversion ofCO2 to organic matter. Plants are thought of as the principal CO2 fixing organisms but at least half of the carbon on earth is fixed by microbes; particularly marine photosynthetic prokaryotes and protists. Cyanobacteria such as *Prochlorococcus* and *Synechococcus* are involved in carbon fixation using energy from sunlight. Chemolithoautotrophic microorganisms such as *Thiobacillus* and *Beggiatoa* also fix CO2 into organic matter while metabolizing compounds such as H2S for energy. Once carbon is fixed into organic compounds, the next stage in the cycle involves its transfer from population to population within the biological community, supporting the growth of a variety of heterotrophic organisms, i.e. heterotrophs such as animals and protozoa that eat autotrophs and may in turn be eaten by other animals. Hence, they acquire organic carbon to build biomass and to oxidize to gain energy. Decomposers use the remains of primary producers and consumers for the same purposes. The respiratory and fermentative metabolism of heterotrophic organisms returns inorganic carbon dioxide to the atmosphere completing the carbon cycle. When plants and animals die, these organic compounds are decomposed by bacteria and fungi and during decomposition the organic compounds are oxidised and CO2 is returned to the cycle. Carbon is stored in rocks such as limestone (CaCO3) and is dissolved as carbonate ions (CO3) in oceans. Vast deposits of fossil organic matter exist in the form of fossil fuel such as coal and petroleum. Burning these fuel releases CO2 resulting in an increased amount of CO2 in the atmosphere. Alternatively, inorganic CO2 and organic carbon can be reduced anaerobically to methane ((CH4). Methane is produced by Archaea in anoxic habitats. Archaea such as *Methanobrevibacter* in the gut oftermites also contribute to methane production. The carbon cycle has come under intense scrutiny in the last decade. This is because CO2 levels in the atmosphere have risen from their preindustrial concentration of about 280□mol per mol to 376□mol permol in 2003. This represents an increase of about one-third and CO2levelscontinue to rise. Like CO2, methane is also a greenhouse gas and its atmospheric concentration is likewise increasing about 1% per year, from 0.7 to 1.7ppm (volume) since the early 1700s. These changes are clearly the results of the combustion of fossil fuels and altered land use. The term greenhouse gas describes the ability of these gases to trap heat within earth's atmosphere, leading to a documented increase in the planet's mean temperature. Indeed, over the past 100 years, earth's average temperature has increased by 0.6% and continues to rise at arapid rate, i.e. global warming of the earth.

3.3 Nitrogen Cycle

Nitrogen is an essential component of DNA, RNA and proteins, which are the building blocks of life, hence all organisms require nitrogen to live and grow. Nitrogen, the most abundant substance in the atmosphere or air (almost80%) is not directly useable by most organisms. This is because the strong triple bond between the nitrogen atoms in the molecule makes it relatively inert. Only a few bacteria are able to utilise nitrogen directly. For plants and animals to be able to use N2, N2 gas must first be converted to more chemically available forms such as ammonium(NH4+), Nitrate (NO3-1), (NO2-1) and organic nitrogen containing compounds such as amino acids and proteins. Microorganisms also are able to utilise these other forms of nitrogen mentioned above. The conversion of nitrogen compounds primarily by microorganisms changes the oxidation states of nitrogenous compounds and establishes anitrogen cycle. Three processes carried out by microorganisms are critical in the nitrogen cycle. They are:

1. nitrogen fixation
2. nitrification and
3. denitrification.

3.3.1 Nitrogen Fixation

This is strictly a bacterial process in which molecular nitrogen is converted to ammonium ion and it is the only naturally occurring process that makes nitrogen available to living organisms. It is carried out by a few bacteria that have a nitrogenase enzymes system. This process brings nitrogen from the atmosphere to the hydrosphere and lithosphere. Because plants depend on the availability of nitrogen for growth, microbial metabolism of nitrogen containing compounds has a dramatic impact on agricultural productivity. Plants and animals rely entirely on the fixed forms of nitrogen (such as ammonium and nitrateions) provided by bacterial nitrogen fixation. Nitrogen fixation involves two types of bacteria or microorganisms).

1. **Free Living Nitrogen Fixing Bacteria**
These bacteria are found in high numbers or concentration in therhizosphere (the region where the soil and roots make contacts).In aquatic environment, blue green alga is an important free-living nitrogen fixer. Trichodesmium fix nitrogen aerobically while free-living anaerobes such as members of the genus *Clostridium* fix nitrogen anaerobically.

2. **Symbiotic Nitrogen Fixing Bacteria**

Members of the genera *Rhizobium*, *Bradyrhizobium* and othersare important nitrogen fixing bacteria. They play an important role in plant growth for crop production. These bacteria live in association with leguminous plants. The bacteria invade the root cells to form nodules, receives carbohydrate and a favourable environment from their host plants in exchange for the nitrogen they fix. However, other bacterial symbiotics fix nitrogen, for example the *Actinomycete Frankia* fixes nitrogen while colonising many type of woody shrub. The heterocystouscyanobacterium *Anabaenea* fixes nitrogen when in association with the water fern *Azolla*.The product of nitrogen fixation is ammonia (NH3). It is immediately incorporated into amino N-atoms and eventually as amino acids. The nitrogenase enzymes are very sensitive to oxygen and must be protected from oxidizing effects. The nitrogen cycle continues with the degradation of these molecules into ammonium (NH4+) within mixed assemblages of microbes *Rhizobium* and *Bradyrhizobium* species generally exhibits rates of nitrogen fixation that are two or three times higher than these accompanied by free nitrogen fixing bacteria.

3.3.2 Nitrification

This is a process carried out by chemolithotrophic bacteria whichconvert ammonium ions to nitrate (NO3-) ions. It is a two step process whereby ammonium ion is first oxidised to nitrite (NO2-) which is then oxidised to nitrate. First step NH4+ NO2-Ammonium ion Nitrite ion Second step NO2- NO3-Nitrite ion Nitrate ion Bacteria of the genera *Nitrosomonas* and *Nitrosococcus* play importantrole in the first step. *Nitrobacter* and related *Chemolithotrophic* bacteria carry out the second step. In addition *Nitrosomonas eutropha* has beenfound to oxidise ammonium ion anaerobically to nitrite oxide (N.O)using nitrogen dioxide (NO2) as an acceptor in a denitrification related reaction.

Genera of Nitrification Bacteria
Genus Converts Habitat
Nitrosomonas Ammonia to nitrite Soil, freshwater marine
Nitrosospira Ammonia to nitrite Soils
Nitrosococcus Ammonia to nitrite Soils, freshwater marine
Nitrosotobus Ammonia to nitrite Soils
Nitrobacter Nitrite to nitrate Soils, freshwater marine
Nitrospira Nitrite to nitrate Marine
Nitrococcus Nitrite to nitrate Marine
The production of nitrate is important because it can be reduced and incorporated into organic nitrogen. This process is known as assimilatory nitrate reduction (i.e. the use of nitrate as a source of

organic nitrogen is an example of assimilatory reduction) because assimilatory reduction of nitrate sometimes accumulates a transient intermediate. Alternatively, some microorganisms use nitrate as a terminal electron acceptor during anaerobic respiration this is a form of dissimilatory reduction.

3.3.3 Denitrification

This is a process in which nitrate is removed from the ecosystem and returned to the atmosphere as dinitrogen gas (N2) through a series of reactions. Denitrification leads to the return or loss of nitrogen to the atmosphere as nitrogen gas. Through this process, oxidised forms of nitrogen such as nitrate and nitrite are converted to dinitrogen (N2) and to a lesser extent, nitrous oxide gas. Denitrification is an anaerobic process carried out by denitrifying bacteria which convert nitrate to dinitrogen in the following sequence:NO3- NO2- NO N2O N2Nitrate Nitrite Nitric Nitrous Nitrogen Ion ion oxide oxide gas Nitric oxide and nitrous oxide are both environmentally important gases.N2O is an important Greenhouse gas contributing to global climate change while nitric oxide contributes to smog. Once converted to dinitrogen nitrogen is unlikely to be reconverted to a biologically available form because it is a gas and is rapidly lost to the atmosphere. Denitrification is the only nitrogen transformation that removes nitrogen from ecosystem (essentially irreversibly). Denitrification is a form of dissimilatory reduction. Finally, nitrate can be transformed to ammoniain dissimilatory reduction by a variety of bacteria, including *Geobacter,Metallireducens, Desulfovibro spp* and *Clostridium spp.* Other spcapable of transforming NO3 to N2 are *Achomobacter, Agrobacterium,Alkaligenes, Bacillus, Chromobacterium, Flavobacterium,Hypnomicrobium, Pseudomonas Thiobacillus* and *Vibrio*. From agricultural standpoint, it is an undesirable process because it leads toloss of nitrogen from the soil, hence a decline in nutrients for plant growth.

3.3.4 Ammonification

Ammonification is the decomposition process that converts organic nitrogen into ammonia (NH3). A wide variety of organisms, including aerobic and anaerobic bacteria as well as fungi can degrade protein, this they do through the action of extracellular proteolytic enzymes that break down protein into short peptides or amino acids. After transport ofthe breakdown products into the cell, releasing ammonium, the decomposer will assimilate much of this compound to create biomass.

3.4 Sulphur Cycle

Sulphur can exist in several oxidation states within organic and inorganic compounds. Oxidation-Reduction reactions mediated by microorganisms change the oxidation state of sulphur within various compounds establishing the sulphur cycle. Microorganisms are capable of removing sulphur from organic compounds under aerobic conditions, the removal of sulphur(desulfurization) of organic compounds results in the formation of sulphate, whereas under anaerobic conditions hydrogen sulphide is normally produced from the mineralisation of organic sulphur compounds. Hydrogen sulphide may also be formed by sulphatereducing bacteria that utilise sulphate as the terminal electron acceptor during anaerobic respiration. Hydrogen sulphide reacts with metals. Being negatively charged, it complexes or reacts easily with cations in the environment such as iron, aluminum and calcium. These compounds are relatively insoluble and most available to plants and microbes between pH 6 and 7 under these conditions. These organisms readily and rapidly convert phosphate to its organic form so that it becomes available to animals. The microbial transformation of phosphorusfeatures the transformation of simple orthophosphate (PO4-) which bears phosphorus in the +5 valence state to more complex forms. These include the polyphosphates seen in metachromatic granules as well as more familiar macromolecules.

3.5 The Phosphorus Cycle

Biogeochemical cycling of phosphorus is important because all living cells require phosphorus for nucleic acids, lipids and some polysaccharides. However, most environmental phosphorus is present in low concentration, locked within the earth's crust; hence it is the nutrient that limits growth. Unlike the Carbon and Nitrogen cycles, the phosphorus cycle has no gaseous component. Phosphorus is derived solely from the weathering of phosphate – containing rocks, hence in soil. Phosphorus exists in both inorganic and organic forms. Organic phosphorus is found in biomass, humus and other organic form. The phosphorus in these organic materials is recycled by microbial activity.
Inorganic phosphorus, on the other hand is negatively charged, so it complexes or reacts easily with cations in the environment such as iron, aluminum and calcium. These compounds are relatively insoluble and most available to plants and microbes between pH 6 and 7.Under these conditions these organisms readily and rapidly convert phosphate to its organic form so that it becomes available to animals. The microbial transformation of phosphorus features the transformation of simple orthophosphate (PO4-) which bears phosphorus in the +5 valence state to more complex forms. These include the polyphosphates seen in metachromatic granules as well as more familiar macromolecules.

SELF-ASSESSMENT EXERCISE

i. Define the following terms
a. Nitrogen Fixation
b. Nitrification
c. Denitrification
d. Ammonification

4.0 CONCLUSION

Microorganisms in the course of their growth and metabolism interact with each other to cycle nutrients such as carbon, sulphur and phosphorus. The nutrient cycling called biogeochemical cycling of elements involves both biological and chemical processes and is of global importance.

5.0 SUMMARY

Biogeochemical cycling of elements is the movement of materials via biochemical reactions through biospheres.

It also refers to the biological and chemical processes that elements such as carbon nitrogen and sulfur undergo during microbial metabolism.
Elements involved in biogeochemical cycles are used for three general purposes which are: (i) Biomass Production (ii) Energy Source and (iii) Terminal Electron Acceptor.

The carbon cycle primarily involves the transfer of carbondioxide and organic carbon between the atmosphere where it occurs as principally as inorganic CO2 and the hydrosphere and tithosphere which contain varying concentration of organic and inorganic compounds.

The first step in carbon cycle is carbon fixation which is the conversion of CO2 to organic matter by organisms such as *Cyanobacteria*.

The second stage is the transfer of the fixed carbon from population to population within the biological community.

The respiratory and fermentative metabolism of heterotrophic organisms returns inorganic carbon dioxide to the atmosphere.
Decomposition of organic compounds such as plants and animals return CO2 to the cycle.

Carbon is stored in rocks such as limestones (CaCO3) and is dissolved as carbonate ions (CO3) in oceans.

Burning of fuels such as coal and petroleum releases carbondioxide to the atmosphere.

Nitrogen is an essential compound of DNA, RNA and proteins which are the building blocks of life.

Atmospheric Nitrogen is not directly useable by most organisms but has to be converted to stable organic forms such as ammonium and nitrates.
Three processes carried out by microorganisms in the nitrogen cycle are nitrogen fixation, nitrification and denitrification.
Nitrogen fixation is strictly a bacterial process in which molecule nitrogen is converted to ammonium ion by a few bacteria that have a nitrogenase enzyme system.

Nitrogen fixation is carried out by free-living nitrogen fixingbacteria such as *Azobacter, Cyanobacteria* and *Clostridium* foundin the rhizosphere of plant. Symbiotic nitrogen fixing bacteria such as *Rhizobium* and*Bradyrhizobium* which in symbiotic association with leguminousplants help fix nitrogen in soil while *Cyanobacterium, Anabaenea*fix nitrogen when in association with the water fern.
Nitrification is carried out by chemolithotropic bacteria whichconvert ammonium ions to nitrate (NO3) ions in two steps.

Nitrosomonas and *Nitrosospira* convert ammonia to nitrite while*Nitrococcus* and *Nitrobacter* convert nitrite to nitrate.

Denitrification converts nitrate to dinitrogen.
Ammonification is the decomposition process that convertsorganic nitrogen to ammonia (NH3).

Oxidation – Reduction reactions mediated by microorganismschange the oxidation states of sulphur with in various compoundsestablishing the sulphur cycle.

Biogeochemical cycling of phosphorus is important because allliving cells require phosphorus for nucleic acids, lipids and somepolysaccharides.

The phosphorus cycle has no gaseous state and phosphorus isderived solely from weathering phosphate containing rocks andorganic phosphorus in biomass humus and other organic forms.

6.0 TUTOR-MARKED ASSIGNMENT

1. a, (i) State three uses of biogeochemical cycles.
(ii) State specific examples of microorganisms.

b Explain the role of symbiotic nitrogen fixing bacteria in biological nitrogen fixation in agricultural soils.

c) Outline the steps involved in nitrification.

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