COURSE GUIDE

ESM 236
ENVIRONMENTAL SCIENCE MICROBIOLOGY

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Published by:
National Open University of Nigeria

Printed 2013

ISBN: 978-058-508-4

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INTRODUCTION

Environmental Microbiology is a second semester course. It is a two-unit degree course in Environmental Microbiology that will eventually lead to a Bachelor of Science Degree in Environmental Science.

Environmental Microbiology is a special field in the study of microbes and the environment. The course delves on the influence of these microbes on the environment to the extent of making it either habitable or inhabitable. It is therefore not possible to discuss the environment without paying attention to its existing microbes.

Environmental Microbiology has several questions to answer. Imagine that you are in a ship where the toilet is a rack of irons over an open space, the excreta goes directly into the water, then one can imagine fishes feeding on the excreta, where the fish fail to catch the faeces, it flows with the water and yet some people swim in the same water, eat the fish, and carry the same water home to drink. No doubt life is endangered.

Secondly, assuming that you need to visit a toilet at the airport and you find out that all the toilets need to be flushed before use with water, the micro-air must have been polluted with the smell of unflushed faeces. Life is again in danger due to this microbial of the air. For one to drink the water in question and to breathe in the air in this environment, there must be a need for sterilisation and disinfection of the water and air. These conditions pose health hazards. There are questions and answers to be found in Environmental Science Microbiology. What are these microbes? Where do they come from? How can the harmful ones be combated? And how can man make the best use of the beneficial ones? These are some of the questions to be answered in environmental science microbiology. The purpose of studying environmental science microbiology is to understand the behaviour of micro-organisms in various environments supporting or endangering life.

WHAT YOU WILL LEARN IN THIS COURSE

The course consists of modules, units and a course guide. The course guide briefs you about the course; what materials are needed for the course and how to work with these materials. Additionally, it tells you more about the course, time allocation to the course, and amount of time to be spent on each unit and module in order to complete the course. It gives you guidelines in respect of the Tutor -Marked Assignments (TMAs) which will be made available in the assignment

file. There will be regular tutorial classes that are related to the course. It is advisable to attend these tutorial sessions. The course will prepare you for the challenges you will encounter in the field of Environmental Science Microbiology.

COURSE AIMS

The aim of the course is simple. It is to acquaint the students with the various environments inhabited by microbes; whether beneficial or deleterious to life in the various environments to enable the students apply such education in the day to day existence of life.

COURSE OBJECTIVES

To achieve the set goal, the course has a set of objectives. Each unit has a set of objectives inserted at the beginning of every unit. Students are advised to read the stated objectives properly before embarking on the study of every unit. It is necessary to refer to the objectives from time to time during the course study to check progress made. It is also important, to confirm the progress achieved at the end of each unit. The theme of this course is to extend the role of microbiology as a subject in the biosphere where the organisms live and life exists. The course therefore cuts across all areas of biological sciences. The course discusses the involvement of microbe on health problems, pharmaceutics, environmental hazards, dentistry etc and how such problems can be avoided. It also meets the needs of students interested in health sciences, environmental microbiology and related disciplines. The course branches on topics related to health and diseases. It discusses the several ways by which microbes affect life and the environment. It is important that student should anticipate what is to be learnt from each unit and make connections to the concept previously learnt that have relevance to every new unit. Below are the comprehensive objectives of the course as a whole. Meeting these objectives will enable you to achieve the aims of the course.

At the end of the course the students should be able to:

- define and describe ecology and how energy flows in the ecosystem
- explain the importance of recycling of water and carbon
- describe other biogeochemical cycles and the role of micro-organisms in the cycles
- enumerate the types of micro-organisms found in soil, air and water and their roles in recycling and as pathogens

- define water pollution and water borne diseases, pathogens and how they affect life
- list and describe the techniques of isolating micro-organisms to obtain them in pure culture for preservation
- catalogue the differences between fresh water and marine water and the micro-organisms found in them
- discuss the antigen and antibody reactions, their properties, structures and classes
- enumerate the sterilisation and disinfection procedures for combating the pathogens and contaminating micro-organisms.

WORKING THROUGH THE COURSE

To complete the course, the student is required to read each study unit, read the textbooks and read other materials provided by the National Open University of Nigeria. Each unit contains self-assessment exercises and at certain points in the course, the students are expected to submit assignments for assessment purposes. At the end of the course, there is a final examination. The course should take the student about 17 weeks to complete. Below are the list of all components of the course, what has to be done and how time should be allocated to each unit in order to complete the course in time and successfully. This course entails that the students should spend a lot of time to read. It is advisable that students should seize the opportunity of attending the tutorial sessions where they have the opportunity of comparing their knowledge with those of others.

THE COURSE MATERIALS

The main components of the course are:

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

STUDY UNITS

The study units in this course are as follows:

Module 1 Environments and Micro-organisms

Unit 1 Environment and Ecology of Micro-organisms
Unit 2 Biogeochemical Cycles
Unit 3 Characteristics of Micro-organisms

Unit 4 Isolation of Micro-organisms

Module 2 Structure, Reproduction and Importance of Micro-organisms

Unit 1 Structure of Micro-organisms: The Fungi
Unit 2 Reproduction in Micro-organisms: The Fungi
Unit 3 Importance of Micro-organisms: The Fungi
Unit 4 Structure and Reproduction: The Bacteria
Unit 5 Importance of Micro-organisms: The Bacteria
Unit 6 Structure and Reproduction: The Viruses
Unit 7 Importance of Micro-organisms: The Viruses
Unit 8 Structure, Reproduction and Importance of Micro-organism: The Algae

Module 3 Interactions and Control of Micro-organisms

Unit 1 Antigens and Antibodies
Unit 2 Sterilisation
Unit 3 Disinfection

The first unit discusses the environments and the composition of micro- organisms present in the air, water and soil. The second unit focuses on the recycling of nutrient elements through the biogeochemical cycles. The third deals with the characteristics of the micro-organisms. The fourth is on the techniques used for isolating micro-organisms and obtaining their pure cultures for preservation. Module 2 unit one delves on the structure of fungi. Units two, three, four, five, six, seven and eight, focus on the structure, reproduction and importance of the micro-organisms including fungi, bacteria, algae and viruses. Unit one of module 3 discusses the interaction between the host and the pathogenic microbes, highlighting the structures and properties of antigens and antibodies. Units two and three are basically on the control of both the pathogens and the superficial contaminants emphasising the differences between sterilisation and disinfection.

PRESENTATION SCHEDULE

Your course material has important dates for the early and timely completion and submission of your TMAs and attending tutorials. Remember to submit all your assignments by the stipulated time and date and guide against falling behind in your work.

ASSESSMENT

There are three aspects of assessment. It is made up of self-assessment exercises, the tutor- marked assignment and the written examination /end of course examination. You are advised to do the exercises. In tackling the assignments, you are expected to apply information, knowledge and techniques gathered during the course. The TMAs must be submitted for formal assessment in the accordance with the deadline stated in the presentation schedule and the assignment file. The best three TMAs out of the four TMAs for assessment count for 30% of your total course work. At the end of the course, you will need to sit for a final or end of the course examination of about three hour duration. This examination will account for 70% of your total course mark.

TUTOR- MARKED ASSIGNMENT (TMA)

The TMA is a continuous assessment component of your course, it accounts for 30% of the total score. You will be given four TMAs to answer. Three of these must be answered before you are allowed to sit for the end of course examination. The TMAs would be given to you as at when due and returned after you have done the assignments.

Assignment questions for the units in this course are contained in the assignment file. You will be able to complete your assignment from the information and material contained in your readings, references and study units. However, it is desirable in all degree level of education to demonstrate that you have read and researched more into your references, which will give you a wider view point and may provide you with a deeper understanding of the subject. Make sure that each assignment is done on or before the deadline given in the presentation schedule and assignment file. If for any reasons you cannot complete the work on time, contact the necessary office before the deadline given in the presentation schedule and assignment file. Extension will not be granted after the due date unless there are exceptional circumstances.

FINAL EXAMINATION AND GRADING

The end of course examination for environmental science microbiology may be about 3 hrs and it has a score of 70% of the total course work. The examination will consist of questions, which will reflect the type of self-testing, practice exercises and tutor-marked assignment problems that you have previously encountered. All areas of the course will be assessed. You need 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-test, 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 counts 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

There are 8 minimum hours of tutorials provided in support of this course. You will be notified of the dates, times and location of these tutorials as well as the name and phone number of your facilitator, as soon as you are allocated a tutorial group. Your facilitator will mark and comment on your assignments, keep a close watch on your progress and any difficulties you might face and provide assistance to you during the course. They will be marked by your tutor and returned to you as soon as possible. Do not delay to contact your facilitator by telephone if you need assistance. The following might be circumstances in which you find assistance necessary, hence you would have to contact your facilitator if:

- you do not understand any part of the study assigned readings.
- you have difficulty with self-tests
- you have a question or problem with an assignment or with grading assignment.

You should endeavour to attend the tutorials. This is the only chance to have face to face contact with your course facilitator and ask questions which are answered instantly. You can raise any problem encountered in the course of your study. To gain much benefit from course tutorials prepare a question list before attending them. You will learn a lot from participating actively in discussions.

SUMMARY

Environmental Science Microbiology is a course or discipline concerned with the role of microbes in the environments in relation to life that exists in the various sections of the biosphere. Upon completing this course, you will be equipped with the basics of the

environmental science and the micro-organisms interacting with the life in the various environments. In addition, you will be able to answer the following questions:

- What is ecology and how does energy flow in the ecosystem?
- What are biogeochemical cycles?
- Why is the recycling of nutrients important and how are water and carbon recycled?
- What other biogeochemical cycles exist and what roles the micro-organisms play in them?
- What kind of micro-organisms are found in air, soil and water?
- How can these organisms be trapped?
- What are the roles of these organisms in biogeochemical cycles?
- How do water pollution and water borne pathogens affect humans?
- How do fresh water and marine environments and their micro-organisms differ?
- How are these micro-organisms isolated and obtained in pure cultures for preservation.
- How are the micro-organisms preserved?
- How do the sterilisation and disinfection methods differ and how are they used in combating contaminants and pathogens in the environment?
- Of course, the list of questions that you can answer is unlimited to the above list. To gain the most from this course, you should endeavour to apply the principles you have learnt to your understanding of the Environmental Science Microbiology. I wish you success in the course and I hope you will find it both interesting and useful.

MAIN COURSE

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MODULE 1 ENVIRONMENTS AND MICRO-ORGANISMS

UNIT 1 ENVIRONMENT AND ECOLOGY OF MICRO-ORGANISMS

CONTENTS

1.0 INTRODUCTION

It is pertinent to understand and be cautious about the environment in which life exists. This is because the environment is be deviled with several macro-organisms and micro-organisms, some of which are deleterious to human existence while others are beneficial to life. This unit will therefore examine and define the different types of environment in which life exists and catalogue the different micro-organisms encountered and the role they play in the survival of life that exist in them.

2.0 OBJECTIVES

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

- define the biosphere
- distinguish the various environments
- explain the ecology in which life exists and the flow of energy in the ecosystem
- describe the type of micro-organisms that can be found in the different environments (air, water and soil)
- explain the role of these micro-organisms.

3.0 MAIN CONTENT

3.1 Definitions of Microbiology and Environment

Microbiology is the science that studies organisms that are usually too small to be seen with the naked eye.

The environment is that part of the earth's crust in which life exists. It consists of the biosphere, aerosphere, hydrosphere and lithosphere.

The biosphere is the region of the earth inhabited by living organisms, the plants and animals including micro-organisms. Micro-organisms are found in the air (aerosphere) we breathe; in the water (hydrosphere) we drink and in the soil (lithosphere) where crops are grown for the food we eat.

Many microbes are beneficial but some are harmful by causing diseases, deterioration and biodegradation. Human beings and micro-organisms are the major inhabitants of the environment. For human beings to control the diseases and other hazards caused by micro-organisms and to take advantage of the beneficial effects of these micro-organisms in the environment, it is necessary to know and understand the roles of this micro -organisms in the various environments.

3.2 Ecology of Micro-organisms

3.2.1 Ecology and Ecosystems

Ecology is the study of the relationship among organisms and their environment. These relationships include interactions of organisms with the physical features – the **abiotic** factors of the environment and interactions of organisms with one another- the **biotic** factors of the environment. An ecosystem comprises all the organisms in a given area together with the surrounding, abiotic and biotic factors.

Ecosystems are organised into various biological levels. The *biosphere* is the region of the earth inhabited by living organisms. It consists of the *hydrosphere* (earths water supply), the *lithosphere* (the soil and rock that include the earth crusts and the atmosphere, the gaseous envelope surrounding the earth.

A terrestrial ecosystem such as a desert, tundra, grassland or tropical rain forest, is characterised by a particular climate, soil type and organisms. The hydrosphere is divided into fresh water and marine ecosystems.

The organisms within an ecosystem live in **communities**: An ecological community consists of all kinds of organisms that are present in a given environment.

3.2.2 Indigenous and Non-Indigenous Micro-organisms

Micro- organisms can be categorised as **indigenous** or **non-indigenous** to an environment.

The indigenous or native organisms are always found in a given environment. They are able to adapt to normal seasonal change or changes in the quantity of available nutrients in the environment e.g. S*pirillum pollutants* is indigenous to stagnant water (Jacquelyn, 2002). Various species of S*treptomyces* are indigenous to soil and *Escherichia coli* is indigenous to the digestive tract. Regardless of variations in the environment, an environment will always continue to support the life of an indigenous organism.

Non-indigenous organisms are temporary inhabitants of an environment, they become numerous when growth conditions are favourable for them and disappear when conditions become unfavourable.

Communities are made up of populations, groups of organisms of the same species in general, community composed of many populations of organisms are more stable than those composed of only a few populations- that is only few different species.

The basic unit of the **population** is the individual organism. Organisms occupy a particular habitat and niche. The **habitat** is the physical location of the organism. Micro- organisms often occupy a micro-environment; a habitat in which the oxygen, nutrients and light are stable, including the environment immediately surrounding the microbe. A particle of soil could be the **micro-environment** of a bacterium. The

environment is more important to the bacterium than the more extensive micro-environment. An organism's niche is the role it plays in the ecosystem that is its use of the abiotic and biotic factors in its environment.

3.3 The Flow of Energy in the Ecosystem

Energy is essential in life and radiant energy from the sun is the ultimate source of energy for nearly all organisms in any ecosystem. The chemolithotropic bacteria that extract energy from inorganic compounds are exceptions.

3.3.1 Micro-organisms as Producers, Consumers and Decomposers

Organisms called **producers** are **autotrophs**. They capture energy from the sun. They use this energy and various nutrients in the soil and water to synthesise the substances they need to grow and to support their other activities. Energy stored in the bodies of the producers is transferred through an ecosystem when consumers who are heterotrophs obtain nutrients by eating the producers or other consumers.

Decomposers obtain energy by digesting dead bodies or wastes of producers and consumers. The decomposers release substances that producers can use to produce nutrients. The flow of energy and circulation of nutrients in an ecosystem are shown in Figure 1.1 below.

The producers include photosynthetic organisms e.g. cyanobacteria, and eukaryotic algae. Although green plants are primary producers on land, autotrophic micro-organisms fill the role in the water bodies. The **consumers** include heterotrophic bacteria, protozoa and microscopic fungi. The **decomposers as micro-organisms** play a greater role in the decomposition of dead organic substances than larger organisms.

SELF-ASSESSMENT EXERCISE 1

Describe in detail the role of the producers, consumers and decomposers in the recycling of nutrients in the soil.

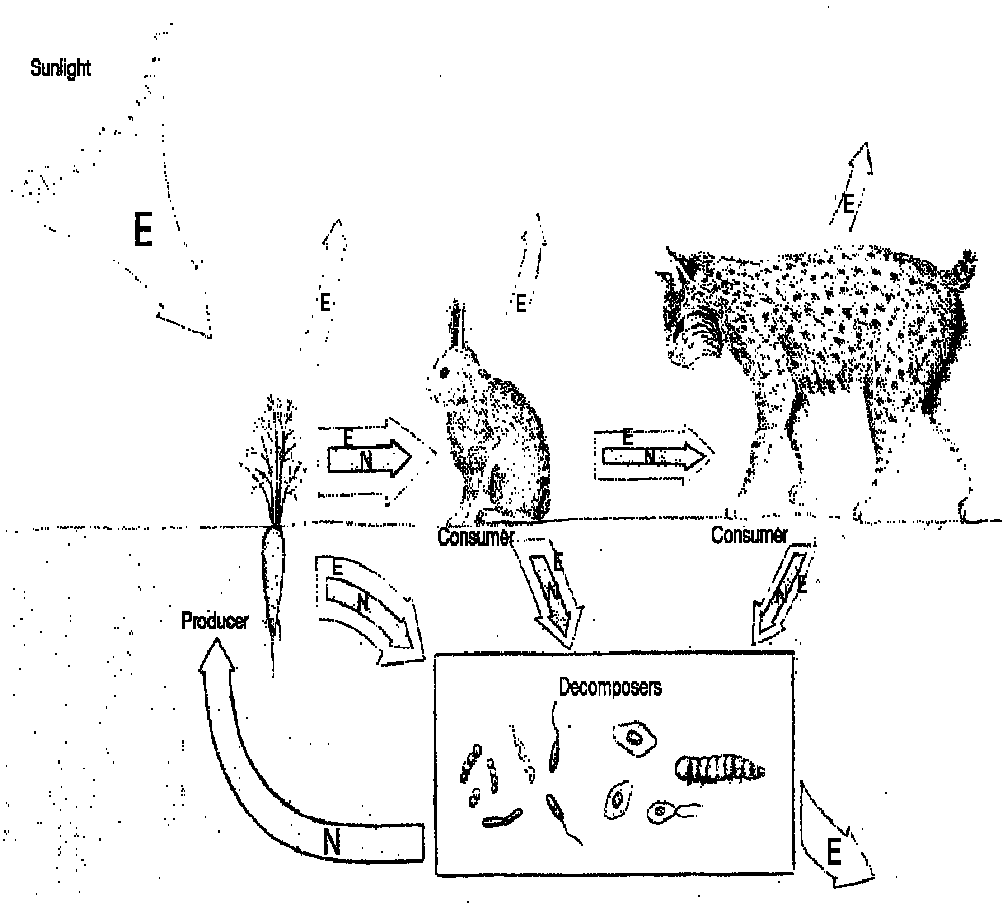

Fig 1.1: The Flow of Energy (E) and Nutrients (N) in Ecosystems Energy flow through the system (it is obtained continuously)

Source: Jacquelyn, 2002

3.4 Microbes in the Air

Micro-organisms do not grow in air because the air lacks the required nutrients needed for their growth. The spores of these microbes are carried freely in air. Vegetative cells can be carried on dust particles and water droplets in the air. The spores and vegetative cells are examples of propagules. The type and number of air-borne propagules of micro organisms varies from one environment to the other. Large numbers of different kinds of micro-organisms are present in air where humans are crowded together and in building where ventilation is poor.

The most common organisms found in air are the fungal spores. By far the most numerous and the most predominant are those of *Aspergillus* and yeast to some extent. Bacteria commonly found in air are those of aerobic spore formers e.g. *Bacillus subtilis* and non-spore formers like M*icrococcus* and S*acrcina sp.* Viruses have also been isolated from the

air. While coughing and sneezing, infected humans can release pathogens along with water droplets.

Micro-organisms in the air can be determined by the following methods:

a. This can be done by exposing glass slides covered with grease
b. Food left over in the kitchen for 2 or 3 days will reveal the presence of spores of *Aspergillus* or Mucor on decayed food materials e.g. bread, garri, paste or even on maize cobs
c. The use of spore traps such as Hirst spore trap and Kramer Collins spore-traps.

3.5 Microbes in the Soil

Soil as an ecosystem is a mixture of mineral particles and organic matter. It is the habitat for hordes of decomposers, detrivores and carnivores, as well as roots of green plants which are the producers in the soil ecosystems. In addition to producing nutrients for its inhabitants, soil is their source of air, heat and water. Many of the stages in cycling of matter also occur in the soil. The soil is full of macroscopic and microscopic organisms because it receives animal wastes and organic matter from dead organisms. Micro -organisms act as decomposers to break down the organic matter into simple nutrients that can be used by plant and the microbes. Soil micro- organisms are thus extremely important in recycling substances in an ecosystem e.g. through Nitrogen cycle, Carbon cycle, Sulphur cycle, Phosphorous cycle and other cycles.

All major groups of micro-organisms are present in the soil. These include bacteria, fungi, viruses, algae and protozoa. However bacteria are more common than any other micro- organisms (Jacquelyn, 2002). Among the bacteria in the soil are autotrophs, heterotrophs, aerobes and anaerobes and depending on the soil temperature, mesophiles and thermophiles can also be found. In addition to nitrogen fixing bacteria, nitrifying and denitrifying bacteria are also found. The soil contains some bacteria that digest special substances such as cellulose, protein, pectin, butyric acid and urea. Soil fungi are mostly moulds. Both mycelia and spores are present mainly in the top soil, the aerobic surface layer of the soil.

Fungi serve two functions in the soil, they decomposes plant tissues such as cellulose and lignin. Their mycelia form networks around the soil particles giving the soil crumble texture. In addition to moulds, yeasts are abundant in the soil in which grapes and other fruits are grown.

Small numbers of **cyanobacteria**, **algae, protists** and **viruses** are found in most soil. Soil viruses infect mostly bacteria but a few infect plants.

3.5.1 Soil Pathogens

Soil pathogens are primarily plant pathogens. A few soil pathogens affect humans and other animals e.g. the Geophillic dermatophytes. The main bacterial pathogen found in the soil is *Clostridium spp* e.g. *C. tetani that* causes tetanus and *C.botulism* causes botulism in man. Grazing animals contact anthrax from spores of B*acillus anthracis* in the soil. Most micro-organisms that infect warm-blooded animals exist as spores because soil temperature is usually too high to maintain vegetative cells of these pathogens.

3.6 Microbes and Water Bodies

Water covers70% of earth surface and is also present in varying amounts in the atmosphere (Lim, 1998). It is an essential component of all cells and is a requirement for life. The water composition of a cell varies from 45-95%. There are 2 types of water namely the **salt or ocean water** and **fresh water**. Salt or ocean water and fresh water organisms are quite different.

3.6.1 The Water Cycle

Water has many roles in living organisms and life is impossible without it. The total mass of water vapour in the atmosphere is equivalent to an ocean rainfall of 2.5cm a year over the earth's surface. The average rainfall is 90cm a year, so water in the atmosphere is cycled 90/2.5=3.6 times in a year.

Rain does not fall evenly throughout the world. The amount falling on land determines to a large extent the abundance and distribution of terrestrial plants. Two processes are involved in the cycling of water. (Figure 1.2)

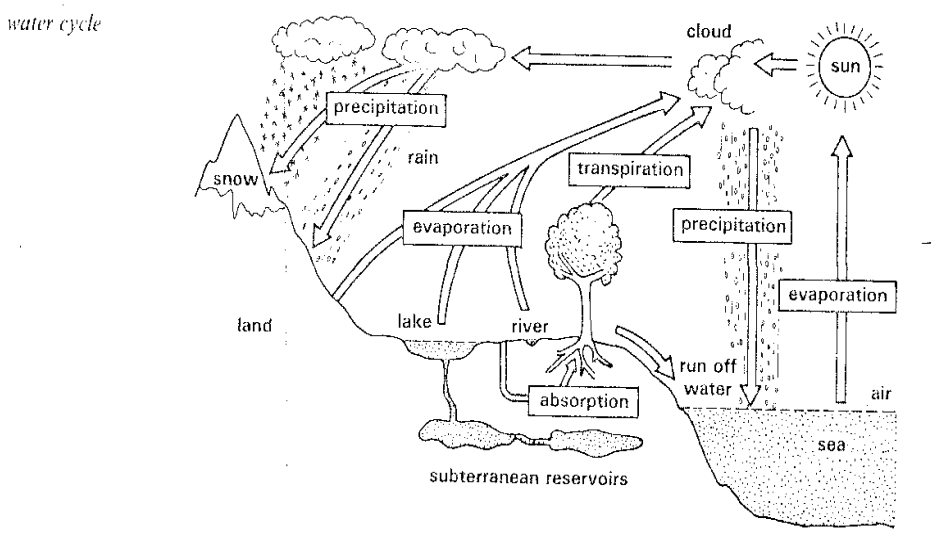

Fig.1.2: The Water Cycle
Source: Simpkins and Williams, 1986

(a) **Evaporation:** The earth's atmosphere has a lower potential than living organisms and aquatic habitats. It is the driest of environments. Consequently, water evaporates into the air (Figure 1.2). Most **evaporation** occurs from the oceans, and about $1500x10^6m^3$ of water vapours occurs each day. This compares with the approximately $200x10^6m^3$ each day from the land, roughly half from the soil and half from vegetation. Water vapour is less dense than air, so it rises to the upper atmosphere where the temperature is lower. Here it condenses, forming over the oceans, where evaporation is greatest. Clouds sometimes evaporate when they absorb heat from the sun. Alternatively, they are blown elsewhere, often over land where water precipitates from them.

(b) **Precipitation:** Water precipitates from clouds as rain, hail or snow (Figure 1.2). The mean daily **precipitation** overland is about $100x10^6m^3$ more than that lost by evaporation. The difference is because this amount of water runs off the land into the oceans in streams and rivers everyday. The total precipitation of water over the earth is, however, the same as the amount evaporated, so the mean water vapour content of the atmosphere remains constant. It is important to realise that evaporation and precipitation are uneven in different parts of the world. It explains why some terrestrial habitats are constantly wet; whereas others are arid. In many areas, precipitation is also seasonal and wet spells alternate with dry ones. The rate at which water is recycled causes the rapid removal of a variety of atmospheric pollutants. The cleaning of a haze after a shower is because dust, soot, smoke and other particles suspended in the air have been washed out. Rain also dissolves other atmospheric pollutants such as sulphurdioxide. The cloud cover in the atmosphere affects the

earth's **heat balance.** Rain clouds reflect or absorb about 20% of the radiation from the sun, mainly infra-red rays. Clouds reflect heat radiating from the earth back to the earth surface.

3.6.2 Salt Water and Microbes

The major types of salt water consist of oceans, seas, estuaries and salt water lakes. The bacterial populations in estuaries consist of *Pseudomonas , Flavobacterium* and *Vibrio* as well as enteric organisms. Most of the bacteria found in water runoff from animals and fowl fiscal matter is deposited on the ground. Sometimes overflow sewage system contributes to these higher levels of bacteria in water. The quantity of bacteria in water depends on the salinity, temperature, dissolved oxygen and pH. Certain pathogens are also present in marine environment including V*ibrio, e.g.V.parahaemolyticus* and hepatitis virus.

3.6.3 Fresh Water and Microbes

Fresh water habitats e.g. rivers, streams, swamps, marshes and lakes contain a wide variety of micro -organisms. Most rivers and lakes are fed from springs. Ground water and spring water usually have low levels of nutrients and micro -organisms because of filtration effect of soil. The bacteria inhabiting this habitat include *Pseudomonas,Seratia, Flavobacterium, Chromobacterium,* and *Achromobacter.* Flowing water in close contact with the soil may contain large numbers of soil bacteria like *Bacillus, Actinomyces* and *Streptomyces.*

Fungi like *Polyphagus,Penicillum* and *Aspergillus* and algae (*Microcystis* and *Nostoc*) are also common occurrence in fresh water.
Rivers also receive a high concentration of bacteria and agricultural chemicals through surface run off water from adjoining soil during heavy rains and irrigation. Rivers can also be heavily polluted with sewage bacteria especially, *E.coli, Enterococcus feacalis, Proteus vulgaris* and *Clostridum species.*

Most lakes are surrounded by rooted vegetation in large **litoral** zone (zone near to the shore). Light penetrates the shallow litoral zone and the open water **limnetic zone (**but is unable to reach the **profundal zone** (Figure.1.3). This zone is where the deep water in a lake is beyond the depth of effective light penetration.

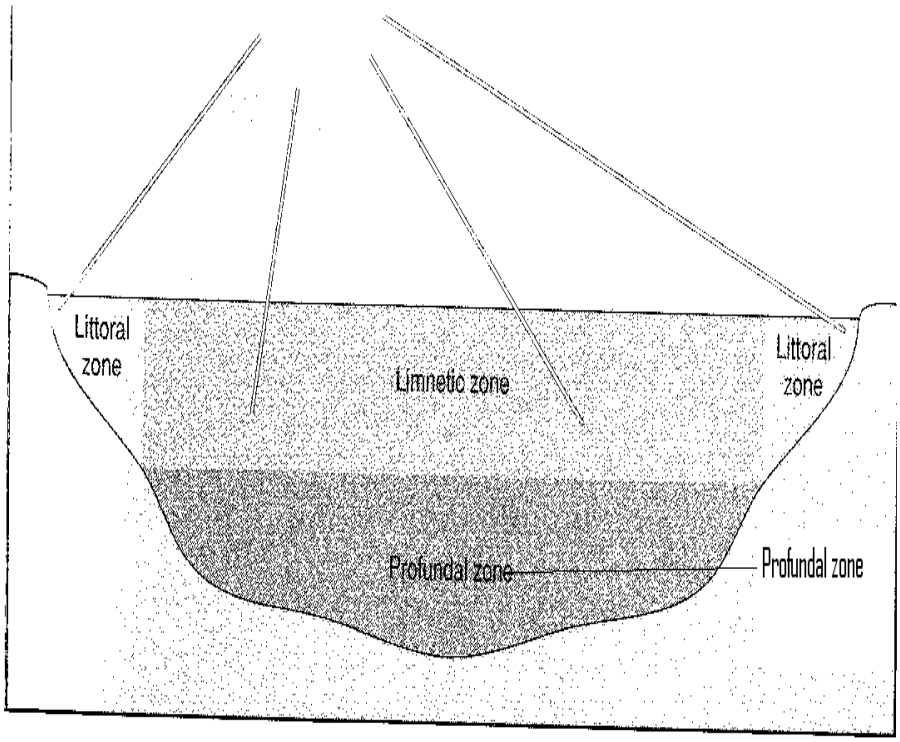

Fig.1.3: Light Penetration Zone of Fresh Water
Source: Lim, 1998

Lakes are stratified by differences in temperature caused by sunlight penetration. As lake vegetation and animal decompose, these organic matters provide a source of nutrients:

Lakes that have very high concentration of nutrients are termed **Eutrophic** and develop large active communities. Such lakes usually have low concentration of oxygen because of extensive microbial decomposition of organic matter.

Lakes that receive small amount of nutrient are termed **oligotrophic.** Some species of bacteria are adapted to this low level of nutrients concentration and are inhibited by higher level of nutrient concentration. The stalked bacterium *Caulobacter* grow well in environment containing 1% or more of organic material. This is because this bacterium has an extension of cytoplasm called *prosthesa* that provides it with a greater surface area for absorption of nutrients from the environment.

In conclusion, the micro flora of a lake is determined by the lakes nutrient content, thermal stratifications and light compensation level.

Cyanobacteria are abundant in the littoral and limnetic zone but are found in fewer numbers in the profondal zone.

Cholorbium,Rhodopseudomonas,andChromatiumspp
asphotoautotrophic bacteria are found at the lower depths where light is still available but oxygen tensions are reduced. These bacteria are anaerobes. At these depths, they use reduced organic and inorganic substances as electron Q donors. Large numbers of heterotrophs occur just below the zone of maximum photosynthetic activity and colourless S-bacteria, *Thiospira, Thiothrix* and *Thioploca* and *Sulphate* reducing organism, *Desulfovibrio* are found in the deeper, low oxygen layers of lakes.

Chemolithotrophic bacteria such as *Nitrosomonas, Nictrobacter* and *Thiobacillus* are also found in fresh water bodies and contribute significantly to the cycling of nitrogen, sulphur and other inorganic substances. The micro-organisms in rivers, lakes, streams, swamps and other fresh water environments are important source of food for other aquatic organisms.

SEIF-ASSESSMENT EXERCISE 2

List the major groups of bacteria that can be found in the soil and discuss their roles in the environment.

4.0 CONCLUSION

The biosphere (soil, water and air environments) harbour micro-organisms including fungi, bacteria, algae and viruses which play many vital roles in the existence of life.

5.0 SUMMARY

In this unit we have learnt that:

- The environment is made up of the biosphere, hydrosphere, aerosphere and lithosphere
- Many living organisms including microbes are found in these environments
- Ecology is the study of the relationship among organisms and the environment
- There are ecosystems and communities with their indigenous and non-indigenous organisms
- There is energy flow achieved through the various activities of different organisms in the ecosystems
- Microbes exist as spores in the air
- Microbes can be determined by a number of methods such as spore traps

- Nutrients levels determine the type and population of microbes in fresh and marine water bodies. Factors generally control the composition and varieties of microbes in the various environments.

6.0 TUTOR-MARKED ASSIGNMENT

i. What is ecology?
ii. Describe some important features of an ecosystem.
iii. Describe with appropriate diagram how energy flows through an ecosystem.
iv. Environments low in nutrients are referred to as
    a. Eutrophic
    b. Littoral
    c. Limnetic
    d. Oligotrophic.
v. Which of the following bacteria is anaerobic?
    a. Bacillus
    b. Clostridium
    c. Pseudomonas
    d. Streptomyces.

7.0 REFERENCES/FURTHER READING

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UNIT 2 BIOGEOCHEMICAL CYCLES

CONTENTS

1.0 INTRODUCTION

In the previous unit, we have learnt that microbes are found in the environment. A number of them through their microbial activities contribute to the survival of the environment. Thus some bacteria and fungi to some extent engage in the recycling of nutrients in the soil that affect the atmospheric composition of the air. This unit will cover those activities of micro-organisms that affect life especially within the various cycles called biogeochemical cycles.

Biogeochemical cycles: describes the movements of chemical elements through the biological and geographical components of the biosphere. The atmosphere (the gaseous mass surrounding the earth), lithosphere (the earth crusts) and hydrosphere (water) comprise the geographical portion of these cycles. The biological part consists of the living organisms which are classified as producers, consumers and decomposers (unit1). Micro-organisms are indispensable in the recycling process this is because they are not only producers and consumers but also have unique role as biological decomposers. The biogeochemical cycles are: nitrogen, sulphur, carbon, phosphorus and oxygen cycles.

2.0 OBJECTIVES

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

- identify the biogeochemical cycles
- describe the various biogeochemical cycles
- describe the roles of micro-organisms in the biosphere
- explain the importance of these cycles on the survival of plants and animals.

3.0 MAIN CONTENT

3.1 Micro-organisms as Decomposers of Dead Organic Matter

Bacteria and fungi break down decaying materials into simpler compounds or elements that other organisms within the ecosystem use for metabolism. Therefore these bacteria and fungi are called decomposers (unit1). The conversion of organic matter to minerals and other inorganic materials during decomposition is called *mineralisation.* It occurs as a result of the microbial decomposition of dead plants and animals which provides continuity to the recycling elements. Six major elements (carbon, nitrogen, sulphur, phosphorous, oxygen and hydrogen) make up 97% of the dry weight of a bacterium *Escherichia coli* and are required in large amount for growth (Table 2.1). These elements are involved in the recycling of nutrients by the various biogeochemical cycles.

Table 2.1: Percentage of Dry Weight of Cell Mass of *Escherichia coli*

Element	% Dry Weight
Carbon	50.0
Oxygen	20.0
Nitrogen	12.0
Hydrogen	10.0
Phosphorus	4.0
Sulphur	1.0
Potassium	< 1.0
Sodium	< 1.0
Calcium	< 0.5
Magnesium	< 0.5
Other elements	< 0.5

Source: Lim, 1998

3.1.1 The Nitrogen Cycle

This cycle (Figure 2.1) goes through several processes namely: ammonification, nitrification and denitrification.

(a) **Ammonification:** Dead plants and animals, animal excreta and plant debris are decomposed to yield ammonia gas. The ammonifying bacteria e.g. species of *Clostridium* and *Azotobacter* bring about the reduction of nitrogen to yield ammonia $N2+3H_2 = 2NH_3$.

(b) **Nitrification:** This is the action of nitrifying bacteria which bring about the oxidation of ammonia to nitrites e.g. *Nitrosomonas* and *Nitrococcus.*

$2NH_3 + 3O_2 = 2HNO_2+2H_2O$

The nitrite is further oxidised by *Nitrobacter* and *Nitrocystis* to nitrate.

$2HNO_2+2O_2 = 2HNO_3$

The energy obtained from these reactions is used by bacteria to synthesis carbohydrate using carbon dioxide and water. These nitrifying bacteria are therefore chemosynthetic and are referred to as chemolitrophs. The nitrates so produced are absorbed by plants for their use.

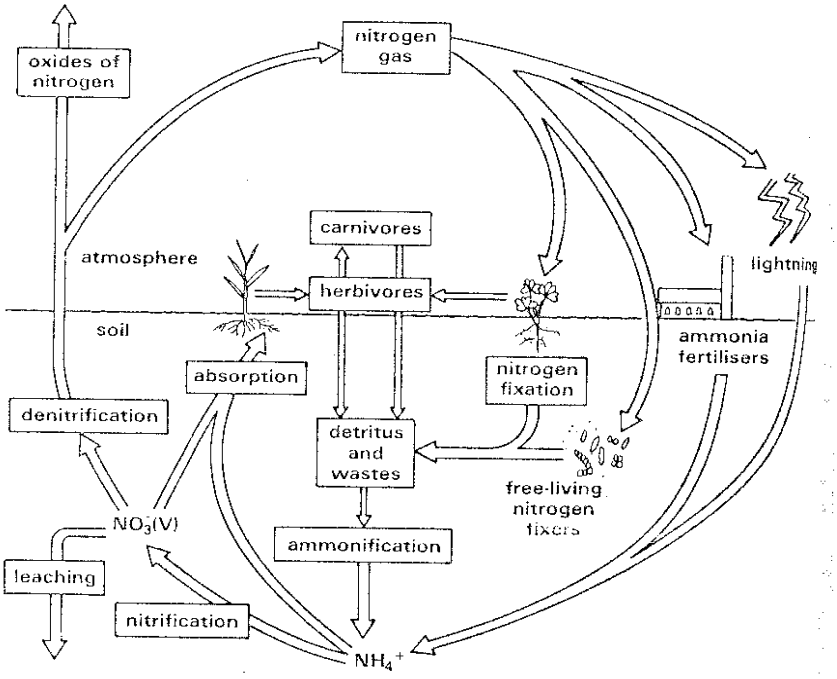

Fig 2.1: **Nitrogen Cycle**
Source: Simpkins and Williams, 1986

However, some bacteria such as *Rhizobium radicicola* and *Bacillus radicicola are* living mutually in the root nodules of leguminous plants –also contribute in making nitrogen available to the plants and in turn the bacteria obtain nutrients from the plants. They are found in root-nodules of species of *Vigna* and *Centosema.*

Rain, lightening and thunder also play a role in nitrogen cycle in the sense that oxides of nitrogen are formed during electrical sparks and these are brought into the soil by rain. The ammonia which also escapes into the air during decomposition is returned into the soil by rain. These are converted by the appropriate bacteria and fungi to release nitrates to the plants. Above all, nitrogen fixing bacteria must produce a nitrogen fixing enzyme called nitrogenease enzyme, a reducing agent that supplies hydrogen as well as energy from adenosine triphosphate (ATP) of the bacteria. In aerobic environments, the nitrogen fixers must have a mechanism to protect the oxygen- sensitive nitrogenase from inactivation.

(c) **Denitrification:** Under anaerobic conditions dentrification occurs. It is the process by which nitrates are reduced to nitrous oxide (N_2O) or nitrogen gas. This process can be accomplished by *Thiobacillus denitrificans, Micrococcus denitrificans, Bacterium denitificans.* and species of *Serratia* and *Achromobacter.*

$HNO_3+H_2 = HNO_2+H_20$
$HNO_2+H_2 = NH_3+O_2$
$2NH_3 = N_2+3H_2$

Denitrification is detrimental to soil fertility, since it ends up removing nitrates from the soil; nevertheless, denitrification is an important component of the nitrogen cycle providing a continuous flow of nitrogen in the ecosystem and maintains the atmospheric nitrogen composition constant. Microbial soil activities can benefit farmers ploughing fields. Their activities create an aerobic environment that favours ammonification and nitrification, there by maximising the amount of ammonium ions and nitrate in the soil.

3.1.2 The Sulphur Cycle

Sulphur is abundant and widely distributed in nature. Reservoir of sulphur includes fossil fuels, elemental sulphur deposits, rocks and minerals. Naturally, sulphur occurs in three different and common oxidation states: elemental sulphur (S), inorganic sulphides and sulphates. Transformation among these states that occurs anaerobically and aerobically make up the sulphur cycle (Figure 2.2).

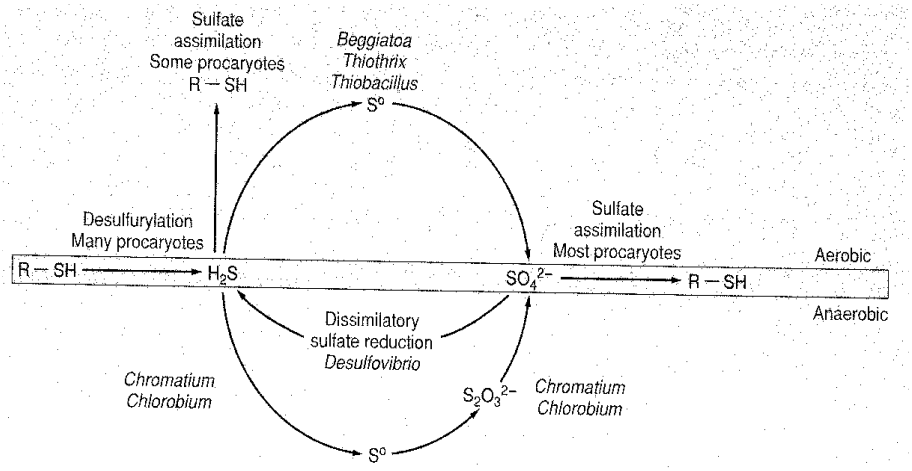

Fig.2.2: The Sulphur Cycle
Source: Lim, 1998

Some chemolithotrophic bacteria – the green S-bacteria, purple sulphur bacteria and colourless S- bacteria obtain energy from elemental sulphur and sulphides.

The Green Sulphur Bacteria

They use carbon dioxide to reduce hydrogen sulphide to sulphur to obtain carbohydrates; thus making sulphur available to the soil.

$2H_2S+CO_2 = CH_2O+H_2O++2S$

The Purple Sulphur Bacteria

Anaerobic environment in the absence of hydrogen sulphide use elemental sulphur to reduce carbon dioxide for the production of carbohydrates. *Chlorobium* and *Chlorotium* are examples.

$2S+8H_2O+3CO_2 = 2H_2SO_4+ 3CH_20 +3H_2O$

The Colourless Sulphur-Bacteria

These are bacteria that oxidise the H_2S to Sulphur which is stored in bacterial cells as granules and the energy released is used to assimilate carbon dioxide. The sulphur can later be used as reserve oxidisable substrate when H_2S is not available. The colourless sulphur bacteria include species of *Beggiatoa, Thiothrix, Thiospirilopsis, Thiospira* and *Thiobacillus.Thiobacillus thio-oxidans* is a well known oxidiser of sulphur to sulphate. They are all aerobic. The sulphates produced by aerobic and anaerobic oxidations are either assimilated into cellular proteins and other chemical compounds or reduced to hydrogen sulphide.

Dissimilatory Sulphate reduction to H_2S commonly occurs during the degradation of amino acids. Some bacteria use sulphate as terminal electron acceptor. **Desulfovibrio** has been used in bioremediation programmes to treat wastes and mine waste. **Desulfovibrio** neutralises the acid water and precipitates our metals in addition to removing the sulphates.

A large portion of the sulphur in the biosphere is found in fossil fuels. When fossil fuels are burnt, the sulphur is oxidised to sulphur dioxide which combines with water to form sulphorous acid (H_2SO_3). This results in acid rain and causes accelerated corrosion and is a health hazard particularly for those with respiratory problems. To avoid this, pollution standards have included restrictions on the sulphur dioxide emissions.
Sulphur is however an essential component of living cells because it occurs in amino acids (e.g. cystene, cystine and methionine), co-enzymes (thiamine, biotin and co-enzyme A).

Plants and other micro-organisms are able to use elemental sulphur. Micro-organisms especially the phototrophs and chemolithotrophs are capable of converting sulphur from one form to another and these are indispensable in sulphur-recycling.

SELF-ASSESSMENT EXERCISE1

Describe in details the nitrogen cycle. Explain the importance of this to life and the environment.

3.1.3 The Carbon Cycle

The most important single element in the Biosphere is carbon. It is the backbone of organic compounds and constitutes approximately 40-50 percent of the dry weight of living tissues. There are more compounds made of carbon than all other elements combined. Most of the carbon on earth is stored in the form of fossil fuels including coal, peat, oil and natural gas. The remaining carbon is found as part of living or decaying organisms, atmospheric carbon dioxide or dissolved form of carbon dioxide as bicarbonates and carbonates. The cycling of carbon in the environment is directly linked with the flow of energy, so carbon cycling is critically important to all forms of life.

The carbon cycle involves three groups of organisms: producers, consumers and decomposers. Because organic compounds also serve as food and energy sources, this cycle parallels food web and patterns of energy flow in an ecosystem and often involves the same or similar metabolic pathways and organisms (Figure 2.3).

Carbon transformation occurs via the carbon cycle, which revolves around fixing the atmospheric carbon dioxide. Organisms known as primary producers including not only photosynthetic organisms but also plants, algae, cyanobacteria, chemolithotrophs and also chemoorganotrophs fix atmospheric carbon dioxide into organic forms of carbon (organic carbon compounds).

Consumers obtain the carbon compounds by eating the producers, other consumers or the remains of either the consumers or producers.

The ultimate source of all carbon is carbon dioxide; it is a raw material for photosynthesis and is also a waste product of tissue respiration.

- $6H_20+6CO_2 = C_6H_{12}O_6 + 6\ O_2$ (photosynthesis)
- $C_6H_{12}O_6+6O_2 = 6H_20 + 6CO_2$ + Energy(tissue respiration)

It is formed from the decay of organic matter and from the combustion of carbonaceous fuels and yet this odourless, colourless, inert gas forms 0.032% of the air (atmosphere).

With the finite quantity of atmospheric CO_2 and its rapid rate of consumption by atmospheric bacteria, algae and plants, with the amount of carbon dioxide available, carbon dioxide would soon become exhausted without extensive transformation of the element-carbon.
The carbon that is fixed into organic matter is eventually returned to the air (atmosphere) in gaseous carbon dioxide in one of three ways:

1) Animals, plants and micro-organisms evolve carbon dioxide during respiration.
2) Plants are consumed by heterotrophic organisms and a significant portion of the carbon is oxidised as food by herbivores and eventually carnivores (**consumers**) or
3) Upon the death of the animals and plants, the dead tissue undergo decomposition by degrading micro-organisms(**decomposers**) which recycle the carbon into atmosphere in the form of $C0_2$. These series of events completes the carbon cycle.

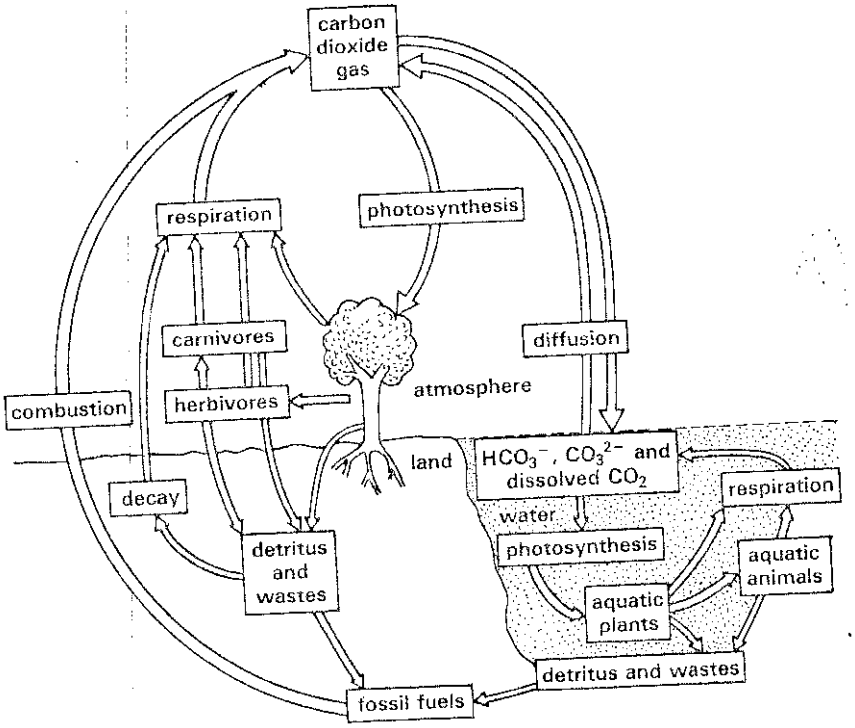

Fig.2.3: The Carbon Cycle
Source: Simpkins and Williams, 1986

3.1.4 The Phosphorus Cycle

The phosphorus cycle involves the movement of phosphorus among inorganic and organic forms (Figure 2.4). Soil micro-organisms are active in the phosphorus cycle in at least two important ways:

1) They break down organic phosphates from decomposing organisms to inorganic phosphates
2) They convert inorganic phosphate to orthophosphate, a water soluble nutrients used by both plant and micro- organisms (Figure 2.4).

These functions are particularly important because phosphorus is often the limiting nutrient in many environments (Jacquelyn 2002).

Phosphorus is an important element in living cells. It is found in cells not as a free uncombined element but as organic phosphate complexes or phosphate ions.

It forms the backbone of nucleic acids and is an integral part of the high energy bond Adenosine-Tri-phosphate (ATP), phosphoenolpyruvate, acetyl phosphate and other high energy compounds.

Plasma membranes contain phosphorus in the form of phospholipids and many co- enzymes NAD (Nicotinamide dinucleotide – a co-enzyme that carries hydrogen atoms and electrons) and FAD (Flavin adenine dinucleotide, a co-enzyme that carries hydrogen atoms and electrons) have phosphorus as part of their structures.

The element is generally associated with rocks or minerals as insoluble salts of calcium, iron, magnesium and aluminum.

Although phosphorus is abundant in the ecosystem, it is a limiting factor for the growth of many prokaryotic and eukaryotic organisms because much of phosphorus is bound in insoluble salts. This is true of and evident in aquatic environments, where the insoluble phosphates are salts precipitate (Figure 2.4) into the sediments resulting in a reduction of algae biomass.

Micro-organisms solubilise phosphate salts as part of the **phosphorus cycle** and make the phosphorus available for themselves and other organisms. The solubilisation of phosphate salts usually occurs by the action of organic acids produced during microbial metabolism. Solubilised phosphates are then assimilated in the vicinity. Upon death and decay of the micro-organism, the phosphates once more enter the environment and are recycled.

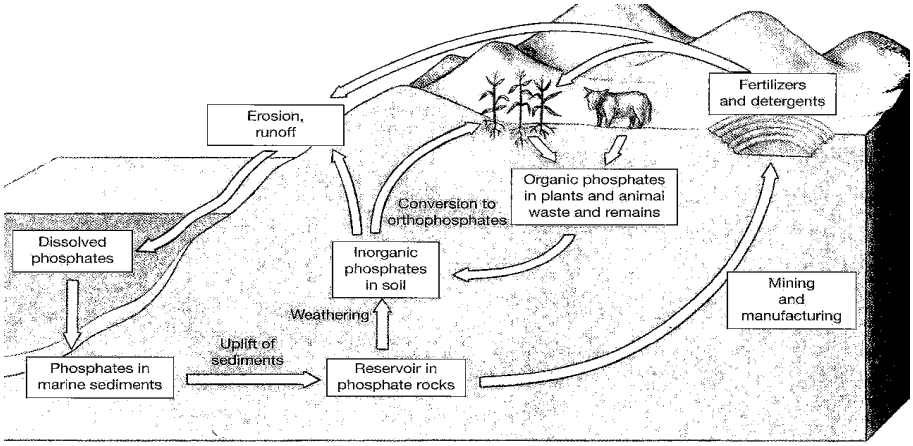

Fig 2.4: The Phosphorus Cycle
Source: Jacquelyn 2002

3.1.5 The Oxygen Cycle

This important element is taken into the animal's body for oxidation. A waste product CO_2 is given off in the process e.g. tissue respiration $C_6H_{12}O_6+6O_2 = 6CO_2+6H_2O+Energy$. (tissue respiration)

The carbon dioxide is taken up by the green plants; the carbon becomes part of the green plants as carbohydrates or starch of the plant especially during photosynthesis, while the oxygen is released into the atmosphere ready to be used again in respiration. Oxygen ion goes through this series of chances endlessly without loosing its properties (Figure 2.5) $6CO_2+6H_2O=C_6H_{12}O_6+6O_2$.

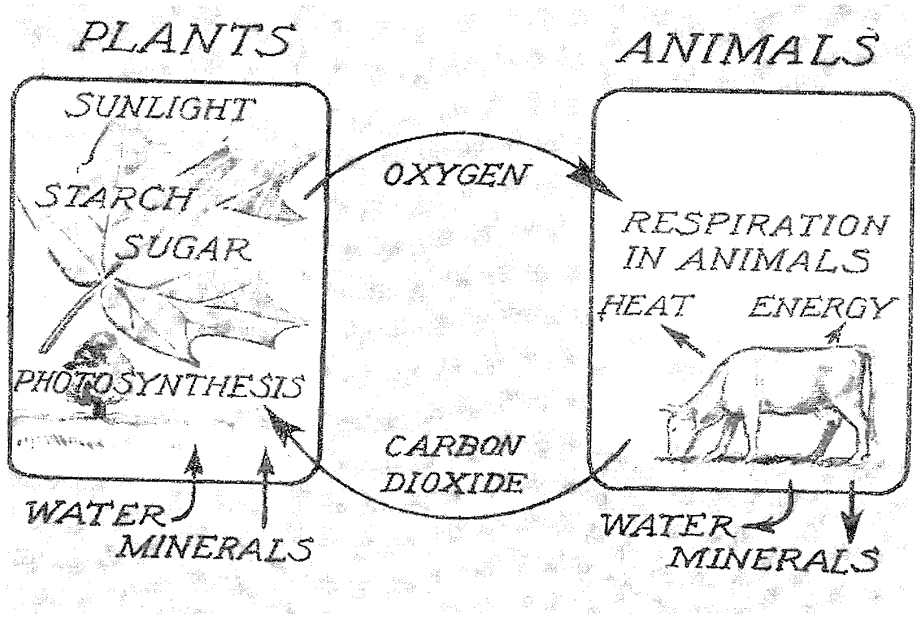

Fig. 2.5: The Oxygen Cycle
Source: Dodge, 1964

Again some bacteria e.g. the **aerobic bacteria** rely on the presence of oxygen for some of their metabolic activities- while others the **anaerobic bacteria** go through their metabolic process in the absence of oxygen**.** Thus oxygen cycle helps to maintain the constant supply of oxygen necessary for life survival and maintains the oxygen composition of the atmosphere (18%). It can be concluded that oxygen cycle is mostly the exchange of oxygen and carbon dioxide between plants and animals.

SELF-ASSESSMENT EXERCISE 2

Describe in details the phosphorus cycle. What is the importance of this cycle to life?

4.0 CONCLUSION

It is very clear from the foregoing that there is a relationship between microbes and the higher organisms in the recycling of nutrients and raw materials for their mutual co-existence in the environment.

5.0 SUMMARY

The presence of micro and macro- organisms in the environments (water, air and soil) has made it possible to make nutrients and necessary gases available in the environment through the recycling of these preformed organic and inorganic compounds and gases have continued to support life in the ecosystem and made gaseous content of the air constant.

6.0 TUTOR-MARKED ASSIGNMENT

i. Which group of the micro-organisms contributes to the sulphur cycle?
ii. How do herbivores and carnivores contribute to the carbon cycle?
iii. What is the ultimate source of all carbon?
iv. Identify the three stages of the nitrogen cycle.
v. Why is phosphorus a limiting factor, How is it made available?
vi. ─ is primarily responsible for denitrification.
vii. ─ and ─ are primarily responsible for nitrogen fixation.
viii. Why are algae and cyanobacteria likely to exist in the soil?

7.0 REFERENCES/FURTHER READING

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UNIT 3 CHARACTERISTICS OF MICRO-ORGANISMS

CONTENTS

1.0 INTRODUCTION

To understand and appreciate micro-organisms, it is imperative that a good knowledge of their characteristics will aid biologist in studying and identifying the micro-organisms and will also enable the biologist in separating the organisms into their various groups. Even though these micro-organisms were once placed under the same class Thallopyta, there are distinct characteristics between them. This is the subject of our discussion in this unit.

2.0 OBJECTIVES

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

- discuss the characteristics of viruses, fungi, bacteria and algae
- compare and contrast viruses and micro-organisms (fungi, bacteria and algae)
- differentiate between prokaryotes and eukaryotes
- classify each of these micro-organisms into the appropriate groups.

3.0 MAIN CONTENT

3.1 Characteristics of Viruses

3.1.1 Definition of a Virus

Virus is a Latin word which means poison or toxin.

Kunkle in 1947 in distinguishing virus diseases from fungal diseases came to the conclusion that viruses are the most efficient pathogen because:

- Viruses get themselves into the cells by the finest and most efficient hypodermic syringes known to man, that is the proboscis of insects.
- Viruses escape quarantine screening very simply in masked carries hosts.
- When the plant being attacked becomes immune, they mutate to produce a more virulent strain that can successfully invade the plant.
- Viruses are invisible, non microscopic except with electron microscope.
- Bowden in 1964 describes viruses as “submicroscopic”, infective entities that multiply only intracellularly and are potentially pathogenic.
- He associated viruses with three characteristics (i) invisibility (ii) pathogenicity and (iii) ability to multiply only intracellularly.
- Because some viruses could pass through bacterial filters, they are described as filterable virus.
- They are host specific i.e. each type of virus can infect and parasitise only a limited range of host cells called host ranges.
- They identity their specific host by a lock and key system .i.e. fit between proteins and the outside of the virus specified receptor molecules on the surface of cells.
- Some viruses can infect a broad host ranges e.g. the rabies virus can infect rodents, dogs, and humans.
- Viruses that parasitise bacteria e.g. *Escherichia coli* are called phages.
- The phages parasitise only the bacterium *E.coli*. The influenza virus only infects the lining of human upper respiratory tract ignoring other tissues.
- The AIDs virus binds to specific receptor on certain types of white blood cells passes through bacterial filters,
- Chester in 1974 described viruses as one of the most interesting, mysterious and elusive plant pathogens. They are contagious,

transmissible and are capable of causing some of the most destructive diseases not only on plants but also on animals and man.

- Viruses are non-motile but are carried by insects' vectors.
- Viruses lack metabolic capabilities possessed by bacteria and fungi but depend on their hosts not only for substances but also for the mechanism that synthesise their substances.

Viruses exist in various sizes and shapes (Figure 3.1)

(A) (B) (C) (D)

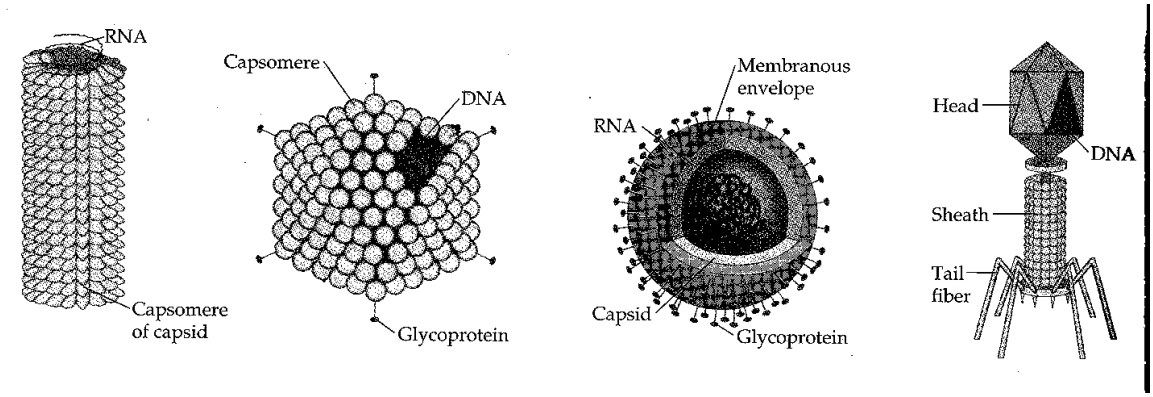

Fig.3.1: Viral Shapes
Source: Campbell, 1999

(A) Tobacco mosaic virus has a helical capsid with overall shape of a rigid rod.
(B) Adenovirus has a polyhedral capsid with a protein spike at each vertex. Some adenoviruses cause respiratory infections in humans.
(C) Influenza virus has an outer viral envelope studded with glycoprotein spikes.
(D) A phage (bacterial virus).

3.1.2 Viruses can either be Living or Non- Living

They are living particles because they have the ability to:

- multiply within appropriate cell, a process resembling reproduction
- to undergo mutation.

They are non- living particles because they have no:

- metabolic system
- intrinsic motility
- ability to respond to stimuli
- can not be cultured outside the host cell i.e. they are obligate parasite. they live in the host cells intracellularly.

3.1.3 Deleterious Effects of Viruses

- Man is affected by many virus diseases, e.g. smallpox, common cold, epidemic influenza, mumps, measles, poliomyelitis, rabies e t c.
- Animals are also affected by viruses e.g. foot and mouth diseases of cattle, sheep and goats. Cattle plague, (rinderpest), rabies of cats, dogs, sheep and goats, Newcastle disease of pigeons, ducks, turkeys, fowl-pox, cow pox and psittacosis of birds.
- Domesticated honey bees are affected by fowl brood and caterpillar wilt, fishes are said to have virus also and even bacteria are affected by virus (bacteriophage). As cited by Bowden in 1964, Gandy and Hollings in 1962 reported the die-back of mushrooms.
- Viruses have no protein synthesing apparatus for manufacturing of ribosome, transfer RNA, mRNA. A virus therefore depends on its host cell for its energy and for translating its genomes into proteins, tRNA, mRNA a and rRNA e.g. they infect bacteria cells to give rise to what is known as a bacteriophage e.g. *Escherichia coli* and T2 even phage.

3.1.4 Comparison of Viruses with other Micro-organisms

A virus particle is not a micro-organism because:

- An organism consists of one or more cells that operate by following the instruction of their genomes. A virus on the other hand is an independent genome (nucleic acid) enclosed in a protective covering (capsid) that allows it to survive outside the host cell and to invade functioning cells which becomes its host.
- The virus takes the form of a particle called virion each of which consists of a nucleic acid (NA) genome encloses in a capsid. The virion contains only one kind of nucleic acid either DNA or RNA.
- Viruses reproduce solely by using the information in the one nucleic acid while the organisms including parasites reproduce through integrated actions of all their constituents.

- Viruses do not grow as cells do by enlarging and dividing nor do they reproduce as organisms do. Either asexually or sexually. Instead virus infected cells synthesise new virions in much the same way a factory manufactures products.
- Viral genomes do not contain an apparatus for generating energy.

3.1.5 Transitory and Persistent Viruses

Viruses can be transitory if:

a. It does not persist in its vector i.e. the infectivity decreases with time
b. It has a short retention period
c. It lacks a demonstrable latent period in the vector and
d. Infectivity is lost after the insect molts.

Viruses can be non- transitory or persistent if:

a. It persists in its vector
b. It has a long retention period
c. It has a latent period vector and
d. It does not loose infectivity after insect molting.

3.2 General Characteristics of Bacteria

- Bacteria are prokaryotic unicellular organisms.
- Bacteria are larger than virus ranging from 0.5-1.5$_{um}$ in diameter. They can be seen with the aid of a light microscope i.e. they are microscopic.
- They have all the characteristics common to other organisms. They are cellular, posses comparable metabolism and are microscopic especially with the oil immersion(x100) objective.
- Some bacteria cells are flagellated. The flagella vary from one to many which may be positioned on any part of the bacterial cell. The location of the flagella could be group characteristics.
- They are either Gram negative or Gram positive.
- Bacteria are micro-organisms without a true nucleus (prokaryotes) or plastids and packing any sexual reproduction process.
- Bacteria reproduces asexually by binary fission.
- Bacteria are saprophytes, parasites or autotrophs.
- Bacteria can be cocci, rod-like, spiral. Bacteria can also be single cells or in chains.
- Some bacteria are naked i.e. without cell wall.

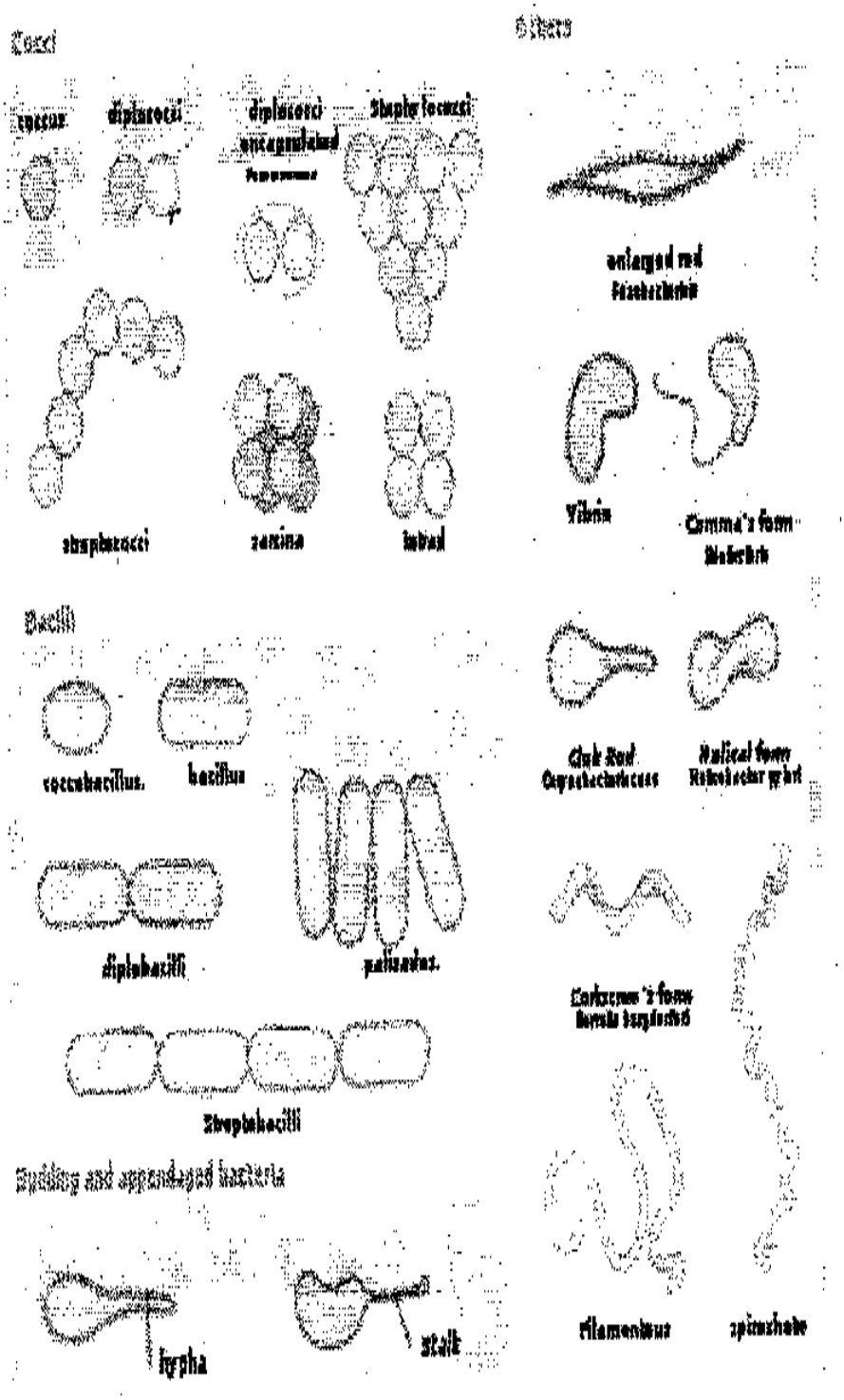

Fig.3.2: Morphology of Bacteria
Source: Pandey and Trivedi 2006

3.3 Characteristics of Fungi

Fungi are plants with thread like non- photosynthetic threads called hypha (ae). These were initially classified among the *Thallophyta* (Lowson,1962) a class under the non-flowering, non-seed producing but spore bearing plants. However by 1969, the fungi was raised to the level of a kingdom (Campbell, 1996).The non-photosynthetic thread (hyphae) are either septate with cross-walls (non-coenocytic Figure 3.3a) or without crosswalls (co-enocytic Figure 3.3b).

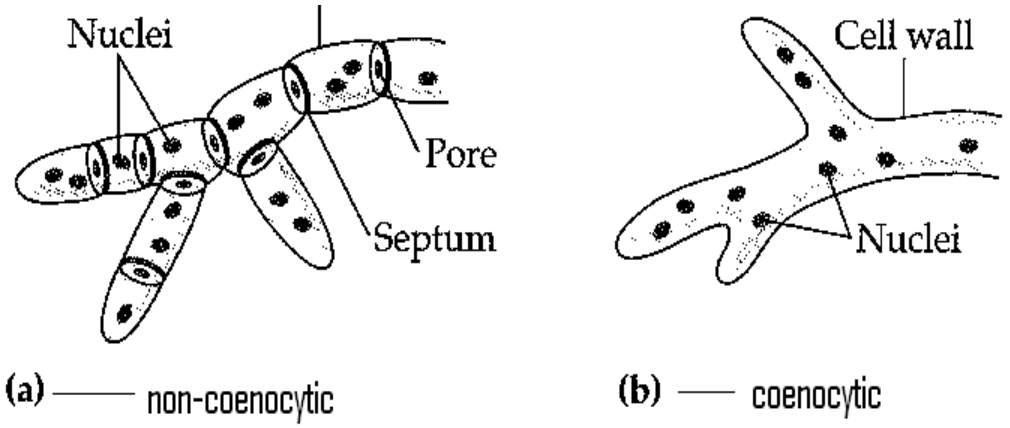

Fig.3.3: **(a) Non-coenocytic (b) Coenocytic Fungal Hyphae**
Source: Campbell, 1996

A collection of these hyphae are referred to as mycelium. Fungal hyphae are bounded by a cell wall made up of glucans and chitin. The eukaryote contains a nucleus with a nuclear membrane enclosing the chromosomes. Also present are mitochondria and endoplasmic reticulum. These characteristics differentiate fungi from bacteria.

In the diagram below (Figure 3.4) are specialised hyphae called haustoria that parasitise the host cell from outside, separated from the host cell's cytoplasm by plasma membrane of the plant cell.

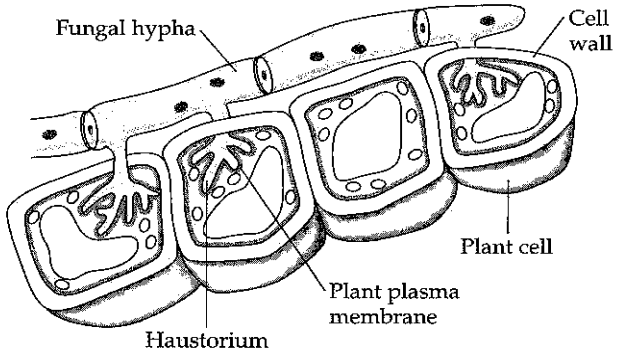

Fig. 3.4: **Specialised hyphae called haustoria**
Source: Campbell, 1996

The fungal kingdom is divided into two divisions. The *Myxomycota* (false fungi) and the *Eucomycota* (true fungi). The *Eucomycota* is divided into five sub-divisions based on their reproductive structures.

These are *Hemiascomycota, Mastigomycota, Zygomycota, Ascomycota, Basidiomycota* and *Deuteromycota*. These five sub-divisions vary from being unicellular as in *saccharomyces* to multicellular as in the advanced fungi e.g. *Ascomycota, Basidiomycota* and *Deuteromycota*, these are fungi with the septate hyphae and heterothallic method of reproduction. The primitive fungi are the coenocytic non-septate fungi including the *Mastigomycota* and *Zygomycota*.

Generally, Fungi are known to have the following characteristics: They

- are chlorophyll less, eukaryotic unicellular or multicellular plants.
- are heterotrophs, acquiring their nutrients by absorption, consisting of sabrobic decomposers, parasitic species and mutualistic forms.
- The body or vegetative structure of a fungus is called a thallus varying in complexity and size ranging from the unicellular yeast to multicellular molds.
- reproduce asexually by dispersing different types of spores and sexual reproduction can be homothallic, heterothallic or by conjugation.
- have a tremendous ecological impact as molds, yeasts, lichens, saprophytes mycorrhizas and parasites.
- without fungi and bacteria as decomposers, biological communities would be deprived of the essential recycling of chemical elements.
- are important decomposers of wood, food and other useful objects.
- cause diseases in plants (parasitic) and animals (dermatophytic).

SELF-ASSESSMENT EXERCISE 1

How will you differentiate the bacteria from the fungi based on their characteristics?

3.4 Characteristics of the Algae

- Algae were formally member of the class Thallophyta of the old plant kingdom.
- They are filamentous, thread like, photosynthetic plants ranging from unicellular as in *Chlamydomonas sp*.

- They are of various colours ranging from green, blue-green, red, brown or golden.
- They are the eukaryotic-with distinct nucleus,
- They could be heterotrophs, photoautotrophic except the prokaryotic cyanobacteria (blue-green algae in a symbiotic relations).
- Some are flagellated while others are not.
- They are aquatic either fresh water or as marine phytoplanktons.
- Algae form the bases of aquatic food webs that support enormous abundance and diversity of life.
- All algae posses' chlorophyll, the primary pigments that trap wavelengths of light to which chlorophyll is not as sensitive.
- These pigments include other forms of chlorophyll (green) carotenoids (yellow/orange, xantophylls (brown) and Phycobilins (red or blue varieties).
- The mixture of pigments chloroplasts lend characteristic colour related to these algae.

SELF-ASSESSMENT EXERCISE 2

Compare and contrast viruses with micro-organisms.

4.0 CONCLUSION

Fungi, bacteria and algae are micro-organisms while viruses are not.
Fungi and algae are eukaryotes while bacteria are prokaryotes.
Fungi are non-photosynthetic micro-organisms while algae are photosynthetic.
Some viruses are pathogens of plants and animals.

5.0 SUMMARY

In this unit, you have learnt that:

- fungi have thread like structures called hyphae which may be septate or non-septate they are also multicellular and eukaryotic
- bacteria are unicellular micro-organisms and prokaryotic
- algae are unicellular, multicellular or filamentous photosynthetic eukaryotic organisms
- viruses are called particles or virions that rely on the host for all their life activities. They live intracellularly and they rely on their vectors for their movement.

6.0 TUTOR-MARKED ASSIGNMENT

i. Mention one unique characteristic that separates the fungus from an alga.
ii. Mention one characteristic that distinguishes a fungus from a bacterium.
iii. The following are micro-organisms except
   a. fungi
   b. bacteria
   c. viruses
   d. algae
iv. The following are eukaryotes except
   a Fungi
   b Bacteria
   c Algae
   d plantae

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UNIT 4 ISOLATION OF MICRO-ORGANISMS

CONTENTS

1.0 INTRODUCTION

When a micro-organism grows in the laboratory medium, it is referred to as a culture. Different organisms growing on the same kind of medium may appear quite different, thus knowledge of the appearance or the cultural characteristics of a species is useful for the recognition of certain types of organism and may also serve as an aid in the

identification of species. However, organisms must be obtained in a pure culture before the cultural morphological characteristics of a species can be determined (Jacquelyn, 1996). A pure culture consists of a population of cells all derived from a single parent cell. Micro-organisms inhabit various parts of the body e.g. the oral cavity, intestinal tract and the skin.

They are present in large numbers e.g. a single sneeze may contain several thousands of bacterial cells. One gramme of feaces may contain millions of bacteria. The biosphere (air, water and soil) contain quite a large number of micro-organisms. One gramme of soil may contain several thousands of bacteria, fungi, algae and protozoa.

To determine the characteristics of isolated micro-organisms, it is imperative that the pure cultures must be obtained in the laboratory. This unit will discuss a number of techniques involved in the isolation of micro-organisms and growing them in pure cultures for use and for storage.

2.0 OBJECTIVES

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

- describe the methods of isolation of micro-organisms in pure cultures
- discuss the advantages of each technique
- describe the various methods for the maintenance and preservation of pure cultures.

3.0 MAIN CONTENT

3.1 Isolation of Viruses

3.1.1 Sources for Isolation

Virus isolates can be obtained from various parts of the plant or animals or even humans that are infected. This is referred to as the donor organism. In the case of plants, younger leaf of the materials contains a higher concentration of virus particles than older woody tissues.

3.1.2 Homogenisation (Grinding or Blending)

After selecting the infected leaf, root or tissue, the isolate is extracted by grinding the tissue in a chilled mortar and pistle or by using some power driven homogeniser (blender).

Whatever the means of grinding the tissue, however, various metabolites and cellular debris are released together with the virus particles. Some of these compounds may inactivate the virus or inhibit infectivity if allowed to stay. It is also important to blend the leaf tissue in a suitable buffer or other solution at a low temperature (0°C) to minimise loss of virus infectivity e.g. phosphate buffer is recommended (Fulton, 1964). Infected tissue is usually ground in 0.1m potassium phosphate buffer at a pH of 7.0-7.5. If the sap is particularly acidic, buffer solution of pH 8.0-8.5 is recommended but excessive alkaline may also inactivate the virus. The addition of a reducing agent in the phosphate solution may help prevent oxidation and hence loss of virus activity.

A solution of 1% dipotassium hydrogen phosphate (K_2HP0_4) containing 1% sodium sulphide is excellent for isolating and transmitting many viruses.

To isolate viruses from some hosts strong reducing agents e.g. 0.1% **thioglycollic acid** (synoname captoacetic acid, $CH_2(SH)COOH$) may be added to the phosphate solution, whilst chelating agents such as 1-2% sodium EDTA(Ethylene diamine tetra-acetic acid) may need to be added to prevent oxidation of polyphenols. Another acid may be added to reduce the activity of ribonuclease enzyme which is powered by clay called **bentonine**. (Fraenkael-conrat *et al.,* 1961).

The addition of polyvinyl pyrrodidone (PVP) to the extraction buffer is also frequently used to protect virus against phenols in the hosts' e.g. Rosaceae species. The synthetic polymer or a complex, which efficiently binds the tannins to prevent them form inactivating the virus (Matthew 1981).

Once the infected tissues have been homogenised, the sap inoculums may be used directly or filtered through a piece of cotton-gauze to remove the larger particles or cellular debris.

If the isolate is not used immediately, or if larger number of test plants is to be inoculated, the isolate should be kept cool in an ice-bucket at 0°C until applied to the host. For pathogenicity tests, the donor host or various species of Nicotiana (tobacco), *Chemopodium*, and *phaseolus vulgaris* have been found to be highly susceptible to infection by a wide range of plant viruses and are often suitable for pathological assays of viruses.

3.1.3 Isolation, Cultivation and Identification of Animal Viruses

The fact that viruses cannot multiply outside a living host cell complicates their isolation, detection, enumeration and identification. It is necessary to provide viruses with living cells instead of a family simple chemical medium. Living plants and animals are difficult to maintain, and disease causing viruses that grow only in higher primates and human hosts cause additional complications. However, viruses that use bacterial cells as a host (bacteriophage) are rather easily grown on bacterial cultures. This is why our knowledge and understanding of viral multiplication has come from bacteriophages.

3.1.4 Growth of Bacteriophages in the Laboratory

Bacteriophage can be grown either in suspension of bacteria in liquid media or in bacterial cultures on solid media. The use of solid media makes possible the plague method for detecting and counting viruses. A sample of bacteriophage is mixed with a host of bacteria and melted agar. The agar containing bacteriophage and host bacteria is then poured into a Petri plate containing a hardened layer of agar growth medium. The virus-bacteria mixture solidifies into a thin top layer that contains a layer of bacteria approximately one-cell thick.

Each virus infects a bacterium, multiplies and releases several hundreds of new viruses. These newly produced viruses infect other bacteria within the immediate vicinity and more new viruses are produced. Following several virus multiplication cycles, all the bacteria in the area surrounding the original virus are destroyed. This process produces a number of cleanings or plagues (Figure 4.1) visible against a lawn of bacterial growth on the surface of the agar.

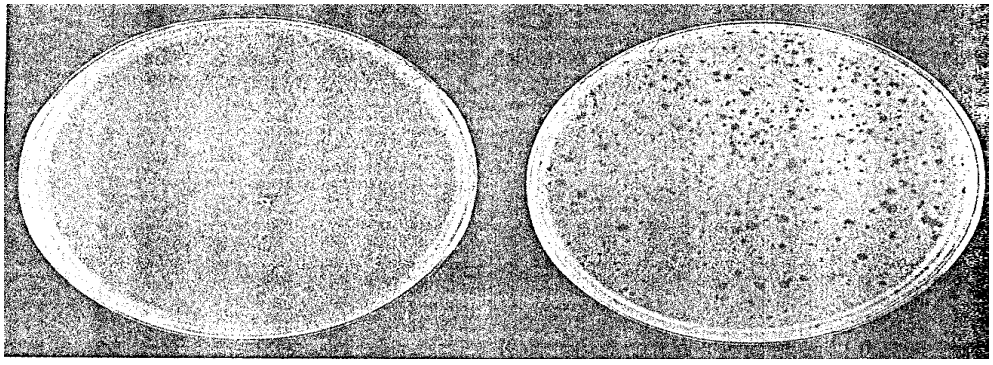

Fig. 4.1: Viral Plague formed by Bacteriophage. In the Petri plate left, clear viral plagues of varying sizes have been formed by the bacteriophage on a lawn of E.coli. For comparison, the Petri plate on the right contains culture without phage.
Source: Tortora *et al.,* 1992

While the plagues form, uninfected bacteria elsewhere in the Petri-plate multiply rapidly and produce a turbid background. Each plague corresponds to a single virus in the initial suspension. Because a single plague can arise from more than one virion, and because some virions may not be infectious, the concentrations of viral suspensions measured by the number of plagues are usually grown in terms of plague-forming units (pfu).

3.1.5 Growth of Animal Viruses in the Laboratory

In Living Animals

Some animal viruses can be cultured only in living animals such as mice, rabbits, and guinea pigs. More experiment to study immune system response to viral infections must also be performed in virally infected animals. Animal inoculation may be used as a diagnostic procedure for identifying and isolating virus from a clinical specimen. After the animal is inoculated with the specimen, the animal is observed for signs of diseases or is killed so that infected tissues can be examined for the virus. Some human viruses can not be grown in animals, or can be grown but do not cause disease. The lack of animal models in AIDS has slowed our understanding of the disease process and prevented experimentation with drugs that inhibit growth of the virus in vivo. Chimpanzee can be infected with one strain of human immunodeficiency virus (HIV), but since they do not show symptoms of the disease, they can not be used to study the effects of viral growth and diseases treatment. AIDS vaccines are presently being tested in humans that it can take years to determine the effectiveness of these vaccines. In

1990, a way to infect mice with engineering to produce human t-cells and human gamma globulin was initiated.

In Embryonated Eggs

A hole is drilled in the shell of embryonated egg, and a viral suspension or suspected virus-containing a tissue is injected into the fluid of the egg (Figure 4.2). There are several membranes and the virus is injected into the proper location in the egg. Viral growth is signalised by the death of the embryo, by embryo cell damage or by the formation on the membrane of the egg of typical pocks or lesion that result from viral growth. This method was once the most widely used method of viral isolation and growth and it is still used to grow viruses for some vaccines.

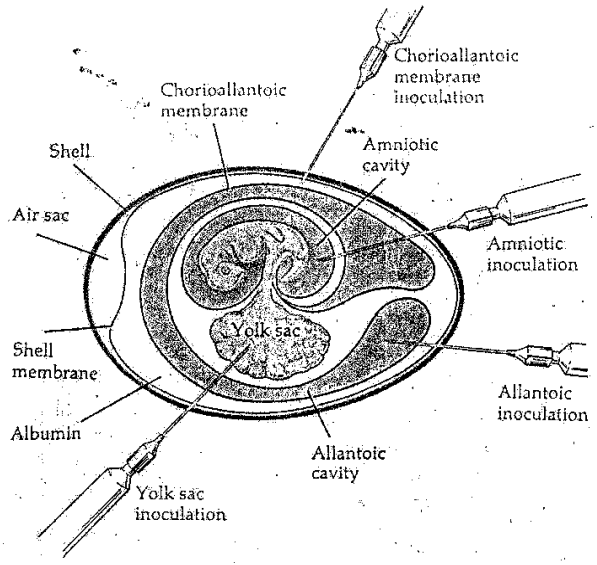

Fig.4.2: Inoculation of Embryonated Eggs. The injection site determines the membrane on which the viruses will grow.

Source: Tortora *et al.,* 1992

In Cell Culture: Cell cultures sometimes called tissue cultures have replaced embryonated eggs as growth media for many viruses. Cell cultures consist of cells grown in culture media in the laboratory. Because these cultures are generally rather homogenous collections of cells and can be propagated and handled much like bacterial cultures, they are more convenient to work with than whole animals or embryonated eggs. Cell cultures are usually started by treatment of a slice of animal tissue with enzymes that separate the individual cells (Figure 4.3). These cells are suspended in a solution that provides the osmotic pressure, nutrients and growth factors needed for the cell to grow. The normal cells tend to adhere to the glass or plastic container and reproduce to form a monolayer. Transformed (or cancerous) cells do

not form a single layer. Viruses infecting such a monolayer sometimes cause the cells of the monolayer to deteriorate as they multiply. This tissue deterioration is called Cytopathic effect (CPE) as shown in (Figure 4.4).

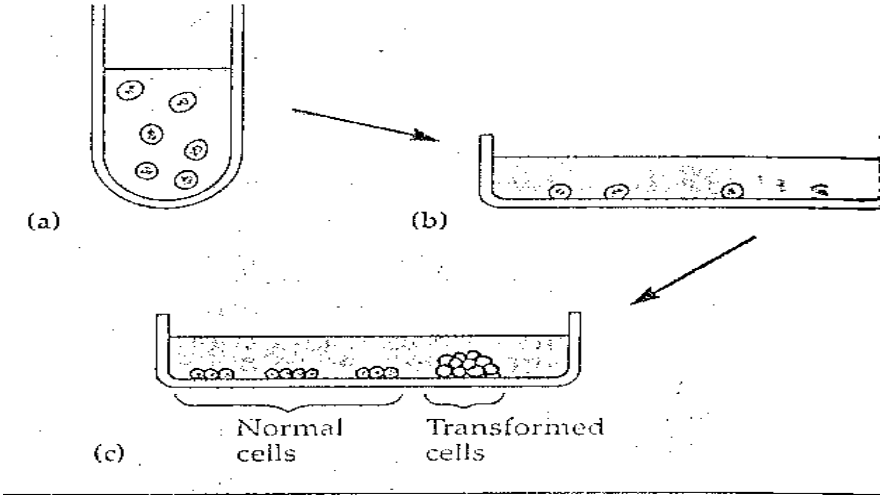

Fig.4.3: `Cell Cultures

(a) A tissue is treated with enzymes to separate the cells. (b) Cells are suspended in culture medium. (c) Normal cells or primary cells lines grow a mono layer across glass or plastic containers. Transformed cells or continuous cell cultures do not grow on monolayer.

Source: Tortora *et al,* 1992

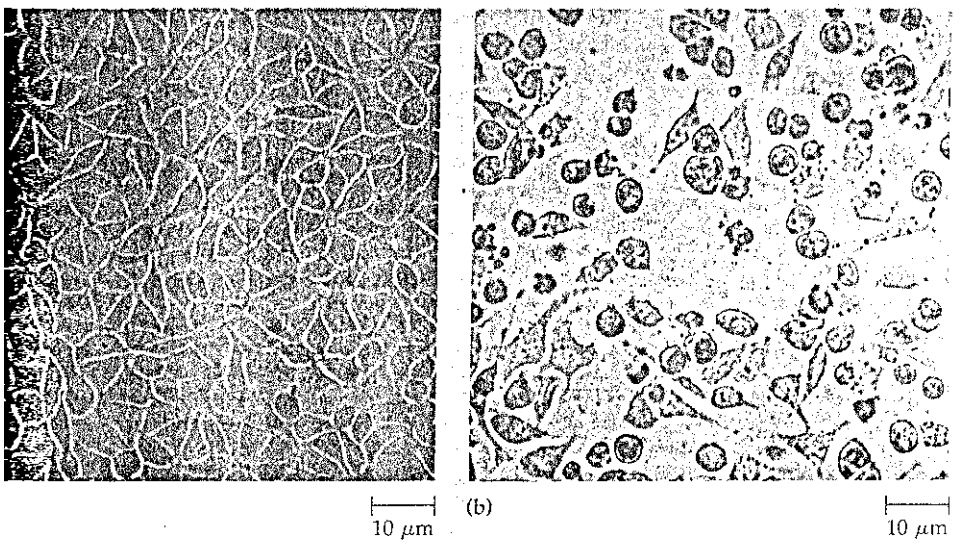

Fig.4.4: Cytopathic effects of viruses. (a) A monolayer of uninfected mouse L cells. (b) The same cells 24 hours after infection with VSV. Source: Tortora *et al,* 1992

CPE can be detected and counted in much same way as plagues caused by bacteriophages on a lawn of bacteria.

Primary cell lines: Derived from tissue slices, tend to die out after a few generations. Certain cell lines called **diploid cell lines**, developed from human embryos can be maintained for about 100 generations and are widely used for culturing viruses that require a human host. Cell lines developed from embryonic human cells are used to culture rabies virus for a rabies vaccine called **human diploid culture vaccine**.

Continuous cell lines: when viruses are routinely grown in a laboratory, a continuous cell lines are used. These are transformed cells that can be maintained through an indefinite number of generations and they are sometimes called "immortal" cell lines.

3.1.6 Storage of Virus Isolates

Short-term storage (minutes or hours) during experiments in the laboratory or glass house, virus are kept as isolates at or close to 0°C.

This is usually accomplished by plugging the tube containing the virus in an ice-bucket.

If the virus isolates are continually subcultured in the laboratory/green house host plants, problems may occur. For long term is bedeviled with several problems:

1. the virus may become contaminated by another virus, this can occur in the glasshouse by insect transmission or even by plants touching or rubbing together.
2. mutation or attenuation of the culture may occur, with the progressive selection of a typical strain during subculture
3. the culture may be lost through death of the host plant
4. the propagation host may occupy valuable glasshouse space over long periods of time.

3.1.7 Methods of Long-Term Storage of Virus Isolates

To maintain isolates in their original uncontaminated condition, the following methods are adoptable:

1 Dry the leaves rapidly over calcium chloride ($CaCl_2$) under a vacuum pressure. The dried materials may then be ground to a powder and stored (McKinney and Silber, 1968; Bos, 1969)

2 A more efficient method that is quite effective for some, but not all viruses is to freeze-dry (Lyophilisation) infected sap in the

presence of glucose and peptone. A suitable method is to add 0.7 %(w/v of D-glucose and peptone to filtered sap in a glass ampoule (Hollings and Stone, 1970). After Lyophilisation, the ampoule is covered and stored at a room temperature.

3 Lyophilised small samples of infected leaves are stored in an ampoule without grinding or the addition of other chemicals (Walkey, 1991).

3.2 Culture Media

3.2.1 Different Types of Media

A microbiological medium (media plural) is the food that is used for culturing bacteria, molds, and other micro-organisms. It exists in three consistencies: liquid, solid and semi-solid (Benson 1994).

a. **Liquid media**: include nutrients broth, citrate broth, glucose broth, litmus milk. These media are used for the preparation of large numbers of organisms, fermentation studies and various other tests.

b. **Solid media**: are made by adding a solidifying agent, such as agar, gelatin or silica gel to a liquid medium. A good solidifying agent is that which is not utilised by micro-organisms, does not inhibit bacterial growth and does not liquefy at room temperature. Agar and silica gel do not liquefy at room temperature and are utilised by very few organisms. Gelatin on the other hand is hydrolysed by quite a few organisms and liquefies at room temperature. Nutrient agar, blood agar and saboraud's agar are good examples of solid media that are used for growing colonies of bacteria and molds.

c. **Semi solid media**: fall in between liquid and solid media. Although they are similar to solid media in that they contain solidifying agents such as agar and gelatin, they are more jelly-like due to lower percentages of these solidifiers.

d. **Synthetic media**: These media are used in detecting motile or flagellated organisms. Media are prepared from chemical compounds that are highly purified and precisely defined. Such media are readily reproducible. These are known as synthetic media.

e. **Non synthetic media**: such as nutrient broth that contain ingredients of imprecise composition are called non-synthetic media.

We also have what is called special media. These are of two types:

a. **Selective media**: are media that allow only certain types of organisms to grow in or on them because of (i) the absence of certain critical nutrients that make it unfavourable for most but not all organisms. (ii) the presence of inhibitory substances that prevent certain types of organisms to grow on them. The inhibitory substances may be salt (sodium chloride), a toxic chemical (crystal violet), an antibiotic (streptomycin), or some other chemicals.
b. **Differential media**: are media that contain substances that cause some bacteria to take on a different appearance from other species, allowing one to differentiate one species from another.

3.2.2 Preparation of Sterilised Medium

As soon as the medium is composed as shown in 3.2.3, the pH of the medium is adjusted to the desired pH using hydrochloric acid (HCL) or sodium hydroxide (NaOH). If the pH is too high, add a drop or two of 0.1N HCL or 1.0N HCL depending on the volume of the medium you are dealing with. If the pH is too low, add one or two drops of 0.1N or 1.0N NaOH also depending on the volume being considered. Use a glass stirring rod to mix the solution as the drops are added.

The pH itself is determined by either Beckman's pH meter if available; if not the pH litmus paper can be adopted. These done known volumes of the medium are pipetted into test tubes. These test tubes are then capped with either cotton wool plug or aluminum foils. These tubes are then placed in a wire basket with a label tapped outside of the basket. The label should indicate the type of medium, the date and the name of the student.

The baskets containing the tubes are then placed in an autoclave chamber which sterilises best at 250˚F (121.6˚C). To achieve this temperature, the autoclave is then placed on heat to develop 15pounds per square inch (psi) of steam pressure. The medium for complete sterilisation should be allowed to remain in the autoclave chamber at this pressure for 10-15minutes.

After sterilisation, the medium can either be poured into sterile plates or slants of the medium can be made:

a. **Slants**: If you have a basket of tubes that are to be converted into slants, it is necessary to lay the tubes down in a near horizontal manner as soon as they are removed from the autoclave. Solidification occurs within 30-60minutes.

b. **Plates**: The sterilised mediums are poured into sterile petridishes e.g. 10.0mm in diameter with a sterile cover in a sterile inoculating chamber. The pouring is done when the medium has cooled down to 45-50˚C.

c. **Storage**: Tubes of broth, agar deeps, nutrient gelatin etc should be allowed to cool to room temperature after removal from the autoclave. Once they have cooled down, place them in a refrigerator or cold storage room.

3.2.3 Composition of some Culture Media for Fungal Isolation

(a)Soluble StarchYeast Agar
Soluble starch 10g
Yeast powder 2g
Agar 15g
Water to 1000ml.

(b) Malt Peptone Agar
Malt extract 30g
Peptone 5g
Agar 15g
Water to 1000ml.

(c) Corn-meal Agar
Ground corn 50g
Dextrose 2g
Agar 15g
Water to 1000ml.

(d) Potato-dextrose Agar
Potato (oxoid L 101) 4g
Dextrose 20g
Agar 15g
Water to 1000ml.

(e) Bean-pod Agar
Green string beans 20g
Agar 15g
Water to 1000ml.

(f) Plain Agar
Agar 20g
Water 1000ml.

(g)Yeast Extract Agar
Yeast extract 3g
Peptone 5g
Agar 15g
Water to 1000ml.

3.3 I solation of Bacteria

3.3.1 The Streak and Spread Plate Technique

It is essential to examine really young and fresh lesions in which hyphae or bacteria are active. Surface sterilisation is not practicable in this case unless the leaves are leathery such can be swabbed over with cotton-wool moistened with spirit. This cannot be done with thin leaves because by so doing the bacteria are likely to be killed especially those in between epidermal cells. Small and presumably younger and older spots in which a relatively large number of dead tissues are likely to harbour saprophytes.

A few typical spots should be cut out with sterilised scissors avoiding as much of healthy areas as possible, and placed in a drop of peptones water on a flamed sterile slide. The tissue is held firmly down with a sterile needle and cut nearly in half with a sharp sterile scalpel and protected with a cover slip. In a few minutes bacterial cells should diffuse out from the several edges of the discoloured tissues and form a distinct cloud easily detected under the medium powers of the microscope (x10, x40). The cover slip should then be removed and a little sterile water added by means of a sterile wire-loop or a sterile pipette. From this suspension, a film must be made on a perfectly clean slide, dried in air and stained by Grams method.

3.3.2 Preparation of Pure Cultures for Inoculations

Isolation plates are made from the watery suspensions from lesions obtained from above. This is done with the streaking method. This is a better method than dilution series as both time and materials are saved and the confusing appearance of buried colonies is also avoided. To procure separate colonies, the agar must be dry otherwise the growing colonies will tend to run into one another. The medium to be used is prepared the previous day because agar extrudes water as a thin film after the agar has set which contracts slowly into drops. The streaking is done with a small (2mm) loop of platinum or new nichrome wire dipped into the suspension. The Petri-plates are removed and placed in an inoculating chamber and the contents of the loop deposited on one small area near the edge of the plate by gently moving the loop to and fro several times over the same area of the agar. In this way, the bulk of the bacteria are removed from the loop. Without flaming the loop further streaks are made parallel to the first and 5mm apart sufficient in numbers to cover half of the agar surface. The plate is then inverted to prevent contamination from the air while the loop is flamed and, when cool is drawn at right angles to the streaks previously made except the first which must not be touched.

Three or four streaks are made this way at 5mm apart, so that only one quarter of the surface is left untouched. The loop is flamed again and drawn at right angles through the streaks just made. Three or four streaks 5mm apart and parallel with the first lot will cover the remaining quarter of the agar surface. The process is repeated once more, this time only over the last quarter of the plate and parallel to the second lot. Three replicates should be prepared in this manner and incubated at 25-27°C. After four days separate colonies will appear on the surface last streaked. The presence of bacteria is thus confirmed, the suspension can be used for inoculating isolation plates.

The streak-plate and the spread plate techniques can be performed expediently and with a minimal amount of equipment; these are routine procedures for isolation of bacteria in pure culture. One of the limitations, however, is that only a small amount of the specimen can be spread over the surface of the medium. The presence of bacteria is thus confirmed, the suspension can be used for inoculating plates.

3.3.3 Caution

1. The separation of the bacterial cells depends on the initial concentration of the suspension and on the degree of 'washing out' in the first streak. If the concentration has been low and the 'washing out' through, separate colonies may appear along the second and third streaks, while the rest of the surface remains sterile (not inoculated). However if a very concentrated suspension is used and washing out inadequate separation will not be effected even in the last quarter of the agar surface and the plate will be covered with two lots of parallel lines of confluent growth at right angles to each other.

(A) (B)

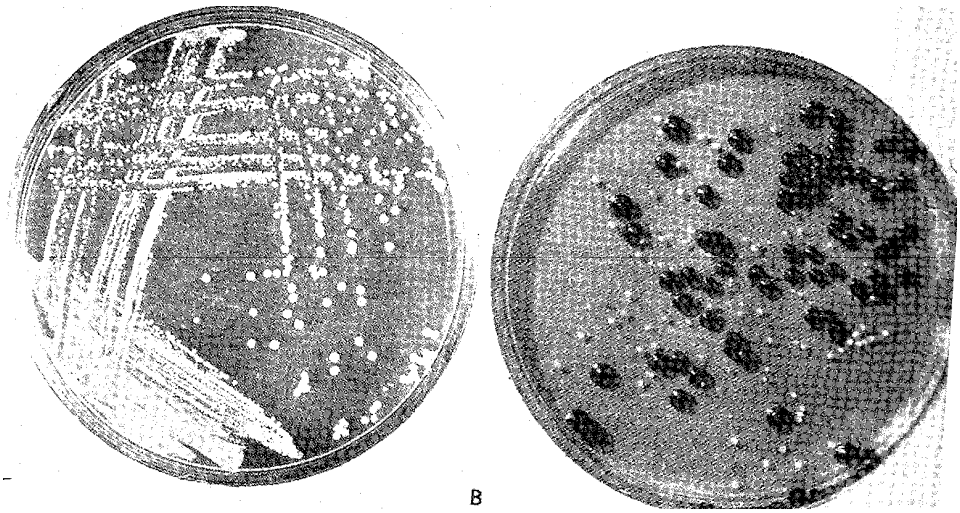

Fig.4.5: (A) streak-plate culture showing areas of isolated colonial growth. The lines of growth reflect how the plate was streaked (inoculated) and (B) Colonies of two different bacterial species on the same plate. The large colonies are *Serratia marcescens*, which has brick-red pigment, and the smaller light colonies are *Sarcina lutea*, which has a lemon-yellow pigment.

Source: Pelczar *et al.,* 1997

2. It sometimes happen that in streaking out very decomposed material or that which has been in contact with soil, the whole agar surface will be covered in a few hours with fast growing

saprophytic bacteria. *Proteus* which will either inhibit the growth of other bacteria or over grow the colonies that may have formed. It is therefore advisable not to suspend from badly decomposed tissues.

3. The most numerous colonies of similar appearance are not necessarily pathogenic, especially if they appear quickly and grow rapidly.
4. Those which came up more slowly are more likely to be the pathogens even if they appear in much smaller numbers. For example, in trying to isolate the leafy-gall organism *Corynebacterium fasciens,* the isolation plates are usually covered with fast growing saprophytes in 24 hrs and if not until 4 to 5 days later that a few yellow convex colonies of *C. fasciens* become visible.
5. When well separated colonies have appeared on the isolation plates, several subcultures can be made and incubated for 24-48 hrs by which time they will be turbid.
6. From both cultures agar slopes are inoculated and the resulting growth is used for inoculating suitable plants to find out which of the pathogenic bacteria are pathogenic.

3.3.4 Isolation of Bacteria using a Glass-Rod instead of Wire Loop

Unless the wire loop is very small (2mm) in diameter and very smooth i.e. not too rough from constant heating, bacteria are not always separated sufficiently on agar plates by streaking-out method of isolation.

The developing colonies are often too close together and tend to grow into one another. Better results can be obtained by substituting a fine glass rod for the ordinary wire loop. A 3mm glass rod heated to softness with Bunsen burner can be drawn out to give several 15cm lengths of a fine rod of about ½mm diameter. One end dipped into the suspension of bacteria will pick up a small drop which is rapidly streaked on to agar plates in parallel lines about 1cm apart. After suitable incubation, the individual colonies can be recognised under the low power (x10) of the microscope and transferred by capillary pipettes into tubes of broth (medium).

This isolation can be carried on any of the media listed below:

a) Yeastrel Agar or Yeastrel broth
b) Meat-infusion broth.
c) Glucose broth or Glucose Agar etc.

3.3.5 The Pour - Plate Technique

The principle of poured plate technique is a serial dilution of the specimens in tubes of liquid (cooled) agar medium. Dilution through more than one tube is necessary to obtain well isolated colonies, since the magnitude of the bacterial population contained in the original specimen is not known. The medium is maintained in a liquid state at a temperature of 45°C to permit a through distribution of inoculum within the medium and is poured into Petri-dishes, allowed to solidify and then incubated. Because some of the organisms are trapped beneath the surface of the medium when it solidifies, the pour-plate exhibits both the surface and subsurface colonies.

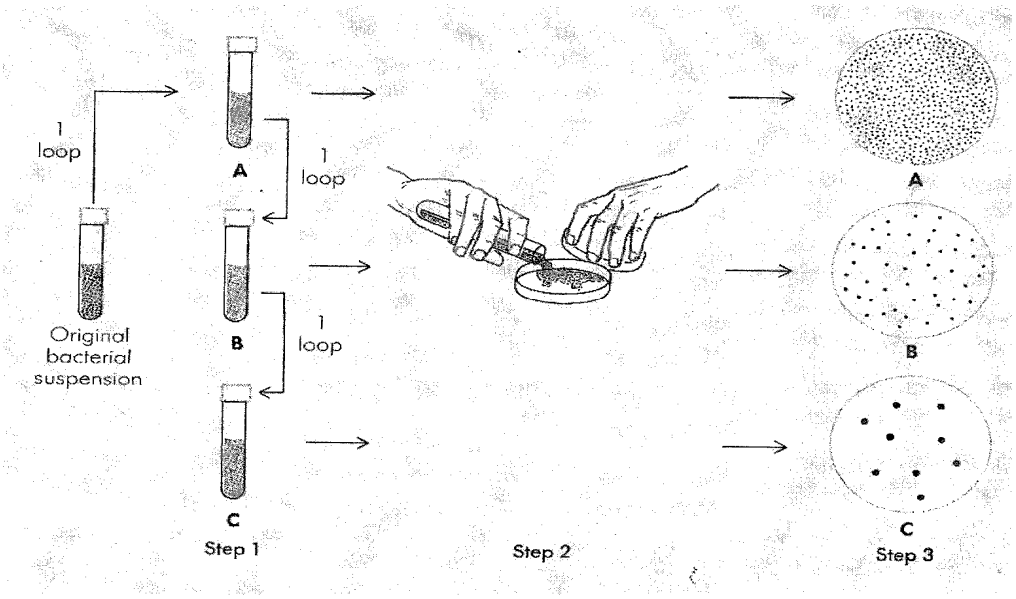

Fig.4.6: Pour- Plate Technique showing decreasing number of colonies resulting from the dilution

Source: Pelczar *et al,* 1977

If a quantitative procedure is used, it is possible to determine the number of bacteria (of a particular species) present in the specimen as well as isolate these in pure cultures.

The streak-plate(or spread plate) and pour plates techniques can be made more effective for isolating specific kinds of bacteria by using selective or differentiable culture media. It is also possible to treat the specimen before plating to eliminate unwanted bacteria.

3.3.6 Description of the Pour-Plate Technique

1) One loop full of original suspension of the inoculum is transferred to the first tube (liquid cooled agar medium). The first tube plus the content is rolled between the hands to effect through mixing of the inoculum. A similar transfer is made from the first

tube to the second tube and from the second tube to the third tube.

2) Contents of each of the tubes are poured into separate Petri-dishes.
3) After incubation, plates are examined for the one which contains isolated colonies.
4) From these plates, pure cultures of bacteria can be isolated by transferring a portion of a colony to a tube of sterile medium.

SELF-ASSESSMENT EXERCISE 1

Itemise the isolation of viruses.

3.3.7 Enrichment Culture Technique

To improve the chances of isolating some unusual physiological types of bacteria, the plating procedure may be preceded by growth in an enrichment culture. In principle, the technique environment (a medium of known composition and specific conditions of incubating) which will favour the growth of a particular type of bacterium being sought but will be unsuitable for the growth of other types. Enrichment cultures are used when the type of bacterium to be isolated is present in a small number and grows more slowly than many other species in the inoculum.

To isolate a soil bacterium capable of utilising α-conidendrine, constituent of wood source (cellulose). If nutrient agar is inoculated directly with the soil specimen, the chance of finding α- conidendrine utilising bacteria will be very limited. Thus the enrichment culture can be used and prepares liquid medium consisting of inorganic salt, an inorganic nitrogen source and α- conidendrine as the sole carbon source. Bacteria unable to utilise α- conidendrine will not grow. Incubate the culture for few days and then transfer a small amount from it into another flask of fresh medium of the same composition. Since the organisms are capable of utilising α- conidendrine, they will grow. α-conidendrine represents the substance from which the organism sources its energy and carbon for synthesis. A sample from the medium can then be streaked on the medium for the same composition solidified with agar. The α-conidendrine utilising bacteria will develop into colonies.

3.3.8 The Serial Dilution Technique

If the organism being sought in a mixed culture is present in a greater number than any other organism, it may be obtained in pure culture by series of dilutions in tubes of an appropriate medium. When greatly diluted the specimen contains only the species. It is advisable to confirm

by a plating procedure the purity of the culture isolated. This technique is adopted in making isolation of organisms from the soil.

3.3.9 The Single Cell Isolation Technique

A special equipment; the micromanipulator can be used in conjunction with a microscope to pick a single organism (cell, spore or conidium) from hanging drop preparation. The micromanipulator permits the operator to control the movement of a micropipette or a microprobe (a fine needle) in the hanging drop so that a single cell can be taken into the tube and transferred to a suitable medium for growth. This unique technique is reserved for use in highly specialised studies as it obviously requires a skilled operator.

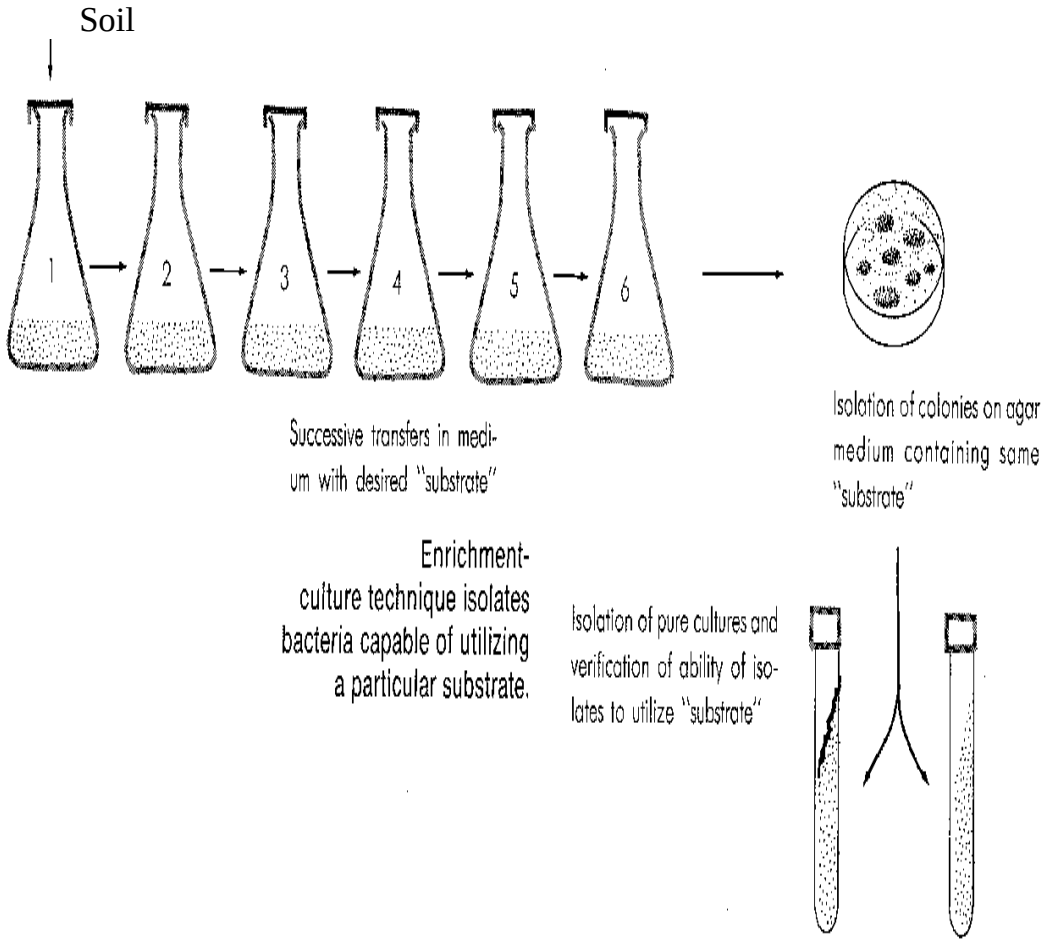

Fig.4.7: Enrichment Culture Techniques. Isolation of Pure Cultures and Verification of Isolates to Utilise "Substrate".

Source: Pelczar *et al.,* 1977

3.4 Isolation of Fungi by Single Conidium Technique

Fungi can be isolated by the same general methods used for bacteria. They all grow aerobically on the culture media at temperatures ranging from 20-30°C. Most of them grow more slowly than bacteria so that the media which support bacteria may be overgrown by bacterial contaminants in a mixed inoculum. Where fungi are to be isolated it is good to use a medium that favours the growth of fungi and not bacteria. There are two types of fungi:

a) Facultative fungi that can be isolated and grown in culture.
b) Obligate fungi that live only in the host but will not grow on any axenic medium.

3.4.1 Preparation of Inoculum

The fungus e.g. *Pyricularia oryzae* can be isolated from rice leaf lesions by the single-cell (condium) in this case isolation technique described by Shanta, 1954, adopted by Awoderu, 1969, 1972 and Pelczar *et al*, 1977, 1993. Wash infected leaves with sterile distilled water for 3 minutes and cut the lesions into pieces of 2-3cm square and surface sterilise with 1% mercuric chloride or sodium hypochlorite to disinfect the lesions from the superficial contaminants for 15seconds. Wash them in three changes of sterile distilled water and dried between sterile filter papers. Place them on moist sterile filter paper placed in 9-cm Petri dishes and incubate at 27°C under continuous daylight fluorescent tubes.

Examine the plates under 100x binocular microscope for conidia. When conidia are observed and the sporulating area located, a sharp flame-sterilised dissecting needle is dipped into soluble starch yeast agar, so that a layer of agar is formed around the needle. The needle is then used to touch the sporulating area. In this way a mass of conidia will be picked up each time and streaks of such conidia were made in plates of soluble starch-yeast agar. When fungal colonies develop, subcultures were made from the edges of these colonies and subculture on fresh culture medium of the same composition. Incubate the cultures at 25±2°C and examine daily under light microscope for conidial formation. When conidia are found, pure cultures are made re-isolating using the single-cell (monoconidial) culture technique (Pelczar *et al.,* 1977, 1993).

3.4.2 Preparation of Single-Cell (Monoconidial) Cultures

Monoconidial cultures can be prepared from these pure cultures in the way described from by Awoderu 1972 and Pelczar *et al,* 1977, 1993. The top of a hypodermic needle with a fine borne (0.5mm in diameter)

is fixed to top of a condenser lens of a microscope by means of melted wax in such a way that this bore could be seen in centrally through the eye piece when the microscope field is in focus.

A conidia suspension about 500 conidia per ml is prepared in sterile distilled water. Two milliliter of conidial suspension added to tubes containing 8ml of the molten agar previously maintained at 45°C in a water bath. The conidia are mixed with the agar by gently rocking the tube. The mixture is then poured into sterile plates and allowed to set. Six hours later after the conidia might have started germinating; the plates are inverted on the stage of the binocular light microscope and examined for isolated germinating conidia.

When germinating conidium is located by raising the condenser in such a way that the area containing the germinating conidium is cut with the needle. The condenser is then lowered and other germinating conidia located.

A small sterile needle is then used to transfer the cut discs into fresh culture medium (Soluble starch-yeast agar) on plates or slants in McCartney bottles or test tubes containing the same medium. Several single-cell (monoconidial) cultures can be prepared in this way.

Pure cultures of this single-cell isolation can be obtained by subculturing into fresh plates and incubating at 25±2°C.

Suspension of the monoconidial culture can be prepared for pathogenicity tests.

3.4.3 Isolation of the Saprolegniaceae

To isolate the members, go to the nearest pond and full a quart jar half full of water. Add some baits e.g. boiled maize grains, rice grains or wheat. In a few days, colonies of the fungi will be found around the baits. These are then transferred to sterile culture Petri-dishes each containing 25ml of half and half autoclaved tap water and sterile distilled water. If ponds are not nearby, collect some soil 1 to 2 inches below the surface of the soil and poured into a quart jar half full of autoclaved water. This will also give a good catch of the fungi using the same set of baits. Pure cultures are then obtained from these colonies.

SELF-ASSESSMENT EXERCISE 2

Differentiate between enrichment culture methods and spread plate technique in the isolation of micro-organisms.

4.0 CONCLUSION

There are different methods of isolation for micro-organisms because of the diversity of their nature, hosts and habitats.

5.0 SUMMARY

Methods involved in the isolation and preservation of viruses were discussed. The preservation of viruses can be short term or long term. Basically the preservation has to be done at $0^{\circ}C$ in an ice-bucket or in an ampoule containing the powder of the infected specimen.

The streak or spread plate technique, the pour-plate technique for special isolates and the serial dilution technique are usable for isolation of bacteria and fungi with little modifications where necessary. Both bacteria and fungi can be preserved in inoculated McCartney bottles or test tubes in the refrigerator at low temperatures of 0 - $10^{\circ}C$. The principles of sterilisation and disinfection are adopted and are pre-requisites to obtaining pure cultures of the organisms.

6.0 TUTOR-MARKED ASSIGNMENT

i. Distinguish between pure culture, axenic culture and mixed culture.
ii. Compare two named techniques for isolating micro-organisms.
iii. Why should pure cultures of micro-organisms be obtained?
iv. Outline a laboratory procedure for isolating a cellulose digesting bacterium.
v. How will you obtain a pure culture of a named micro-organism?
vi. Describe the single-cell technique for isolating a named organism.
vii. What precautions do you have to take during bacterial isolation?
viii. What general features should be observed in establishing the colonial characteristics of an organism?

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MODULE 2 STRUCTURE, REPRODUCTION AND IMPORTANCE OF MICRO-ORGANISMS

UNIT 1 STRUCTURE OF MICRO-ORGANISMS: THE FUNGI

CONTENTS

1.0 INTRODUCTION

In this unit, the structure of fungi will be discussed. The structure of a typical fungus is made up of the vegetative (nutritionally active) body and the reproductive unit. The structure is usually hidden and diffusely organised around and within the areas of their food sources. The yeasts are usually of single cells while the others have structures called hyphae (hypha singular); the hyphae form interwoven mats called the mycelium. This mycelium forms the feeding network of a fungus because it is embedded in the environment in which the fungus grows. Hyphae of fungi are either divided into cells by septa (septate hyphae) or are not divided (coenocytic hyphae). Their walls are mainly of chitin.

Parasitic fungi usually have some of their hyphae modified as haustoria. These hyphae when mature produce various types of spores released into the environment and dispersed by wind. The hyphae (mycelium) make up for the lack of mobility in fungi by swiftly extending the tips of hyphae into the immediate environment for food.

2.0 OBJECTIVES

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

- explain the basic structure of the fungi
- distinguish between the vegetative and reproductive structures of fungi
- discuss the diversity and complexity of the various structures of fungi.

3.0 MAIN CONTENT

3.1 Structure of Fungi

Asexual Reproductive Structures of the Mucoraceae

The Mucoraceae reproduces asexually by means of non-motile sporangiospores called *aplanospores* contained in a *sporangium.* They carry sporangia on simple *sporangiophore* as in Mucor (Figure 1.1)

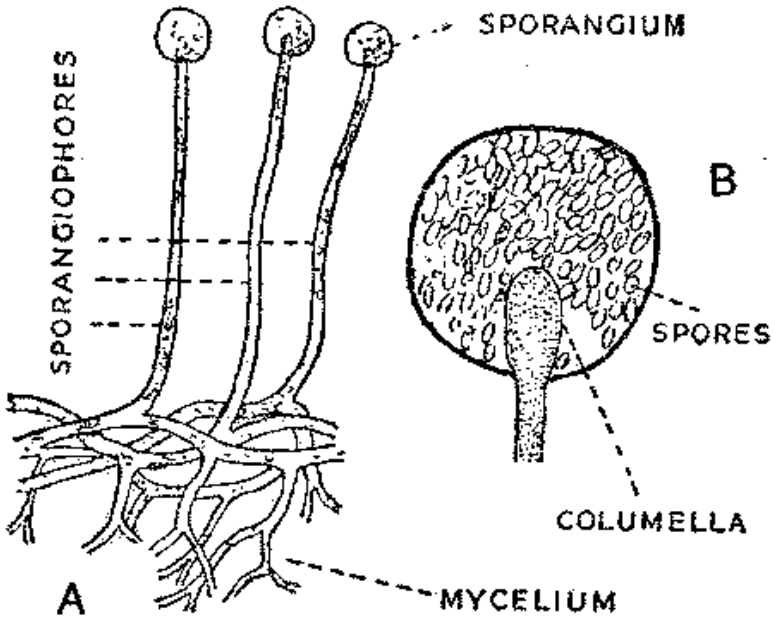

Fig.1.1: Mucor (A) Portion of a Mycelium Bearing Sporagiophores and Sporangia (B) Single Sporangium
Source*:* Lowson, 1962

This sporangium is formed at the tip of a sporangiophore as globose swelling in which central columella becomes separated from the outer region. The typical sporangium when developed under favourable conditions contains thousands of sporangiospores. *Rhizopus* which belongs to the same Mucoraceae family has a similar structure but differs in the sense that it lacks a columella and develops a hypophysics instead and root-like structure called the rhizoid (Figure 1.2).

3.2 Evolution of Conidia from the Sporangium

A typical sporangium is found in the family Mucoraceae- the largest and most primitive of the eleven families under the Order Mucorales. Such as a sporangiophore, as a globose swelling in which columella becomes separated from the outer region (Figures1.2 and 1.3). The typical sporangium developed under favourable conditions contains thousands of spores.

In contrast to the above, some Mucorales produce small sporangia with columella as in Mucor (Figure1.2) or without columella as in Rhizopus (Figure1.3) which contain few spores each and some species are monosporous. Such small sporangia are called Sporangiola (singular sporangiolum); (These are believed to have developed from sporangium with many spores) the end product being a sporangiolums with few or single spore. Sporangiola which contain only one spore are often regarded as conidia.

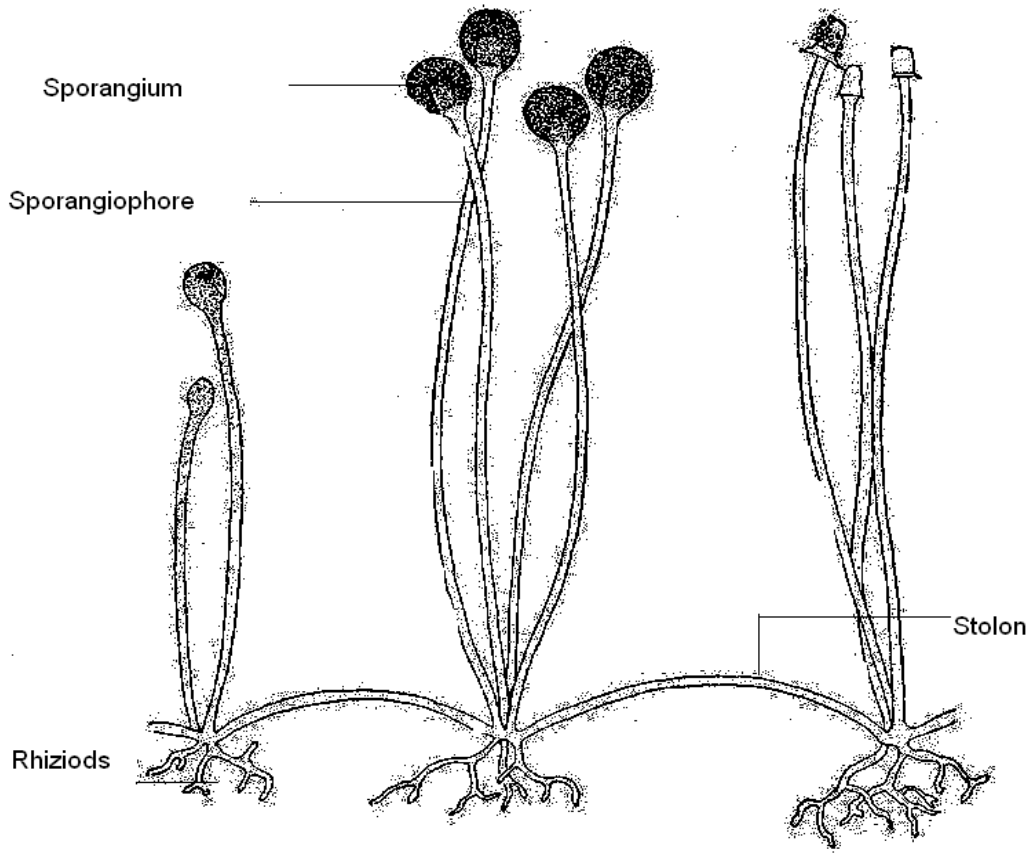

Fig.1 2: Rhizopus
Source*:* Smith, 1955

Thus depending on the conditions under which the fungus is growing *Blakeslea trispora* may produce sporangia with many spores (Figure 1.3A-D) produce sporangia with few Sporangiola are also applicable to structures in which the sporal and sporangial walls are fused and cannot be distinguished from each other as in the heads of sporangiola of *Choanephora cucurbitarum* (Figure1.3 E) and in *Cunninghamella echinulata (*Figure1.3 F). Either term sporangiola or conidia may be applicable to these structures, since it is probable that the conidia of these Fungi have arisen from sporangia by reduction of the number of spores from many to one and by the subsequent fusion of the wall of the sporangium with that of the single spore with it. Figure 1.3 shows structures to confirm that the conidium of the Mucorales has developed from the sporangium.

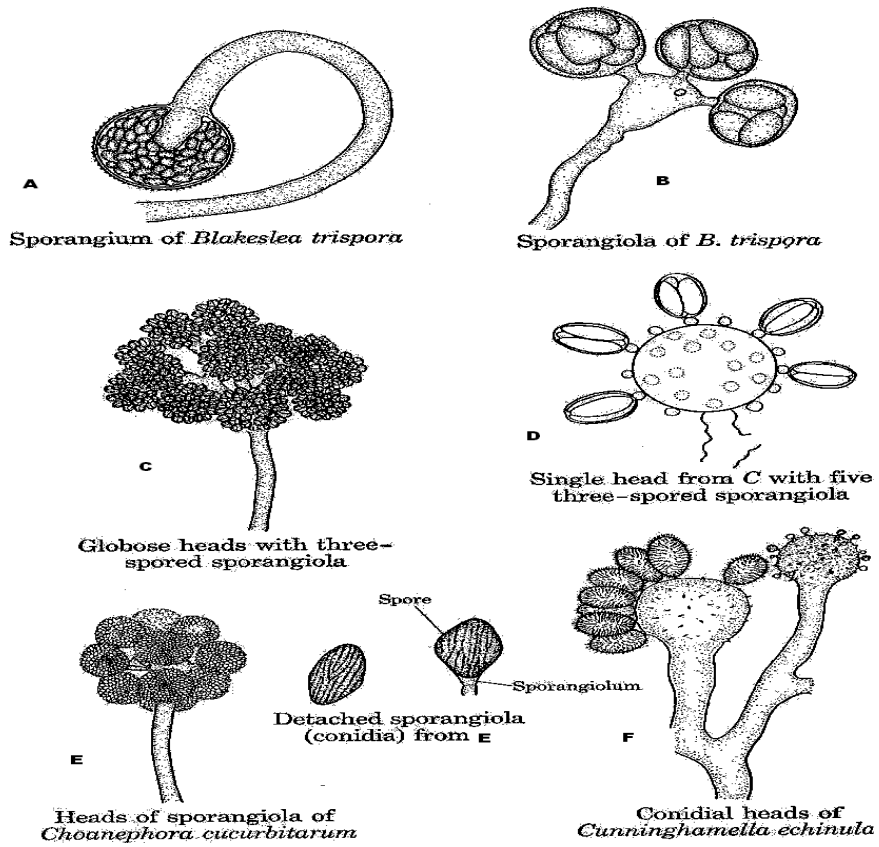

Fig.1.3: Series of Drawing Showing Probable Transition Stages in the Evolution of the Sporangium to a Conidium

Source: Alexopolous, 1962

3.3 The Merosporangia

The Merosporangia may be borne on the surface of an inflated sporangiophore and radiate out or they may be borne on the surface of an inflated sporocladia.

In *Syncephalastraceae*, the merosporangia contain uniseriate spores (Figure.1.4). The Piptocephalidaceae and Dimargaritaceae carry two spores. The kickxellaceae bear monosporous sporangiola on special cells produced on sporocladia.

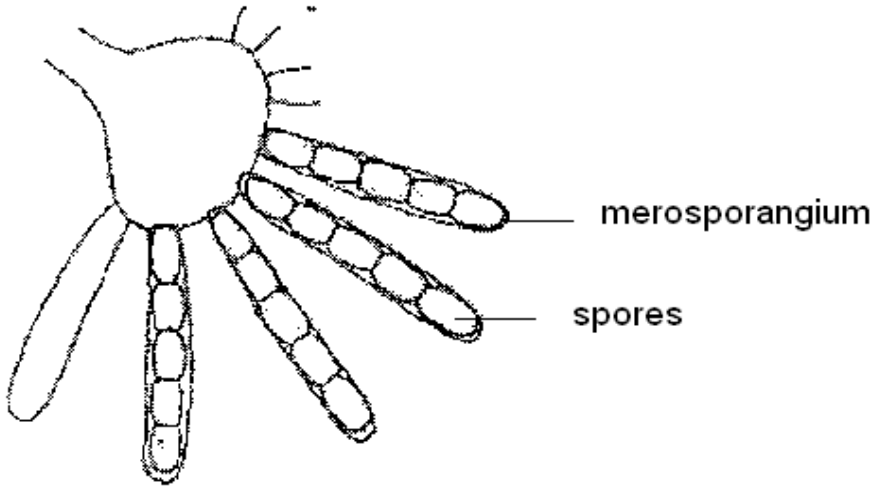

Fig.1.4: Merosporangium with Uniseriate Spores
Source: Deacon, 1984

3.4 Sporangial Apparatus of Pilobolus

The Pilobolaceae represented by *Pilobolus longipes* is a common inhabitant of cow intestine and cow dung. The entire sporangium is violently discharged off the sporangiophore. The sporangiophore of *Pilobolus* (Figure 1.5) consists of a swollen trophocyst, the sporangiophore proper, a swollen subsporangial vesicle and a sporangium whose wall is heavily cutinised.

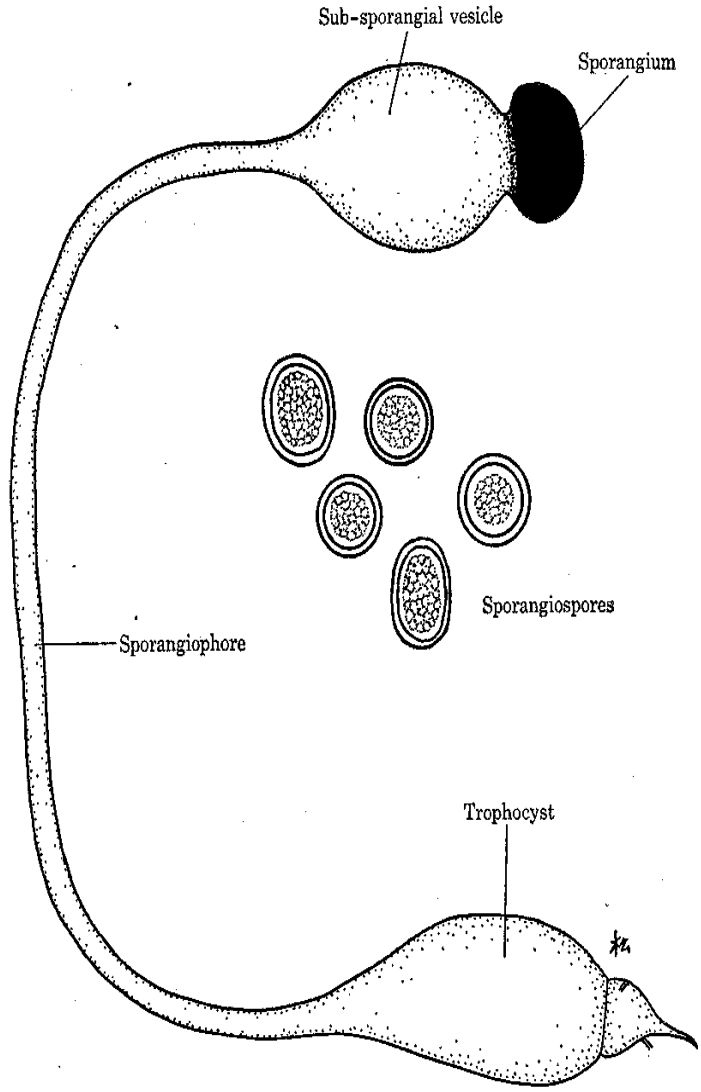

Fig. 1.5: ***Pilobolus Longpipes.*** **Sporangial Apparatus**
Source: Alexopolus, 1962

3.5 The Mastigomycota

A subdivision with the class Oomycetes is well represented by the Orders Saprolegniales and the Peronosporales, members of which are aquatic in nature. This characteristic is well illustrated in their asexual and sexual structures. They are coenocytic.

The asexual structure includes the zoosporangia containing biflagellated zoospores each bearing one tinsel flagellum directed forward and one whiplash flagellum directed backwards.

The zoospores are borne in sporangia of various types. Their sporangia are referred to as zoosporangia (Figure 1.6). In Saprolegnia, there could be the repetitive productions of sporangia within the old sporangium in a process called sporangial proliferation (Figure 1.6).

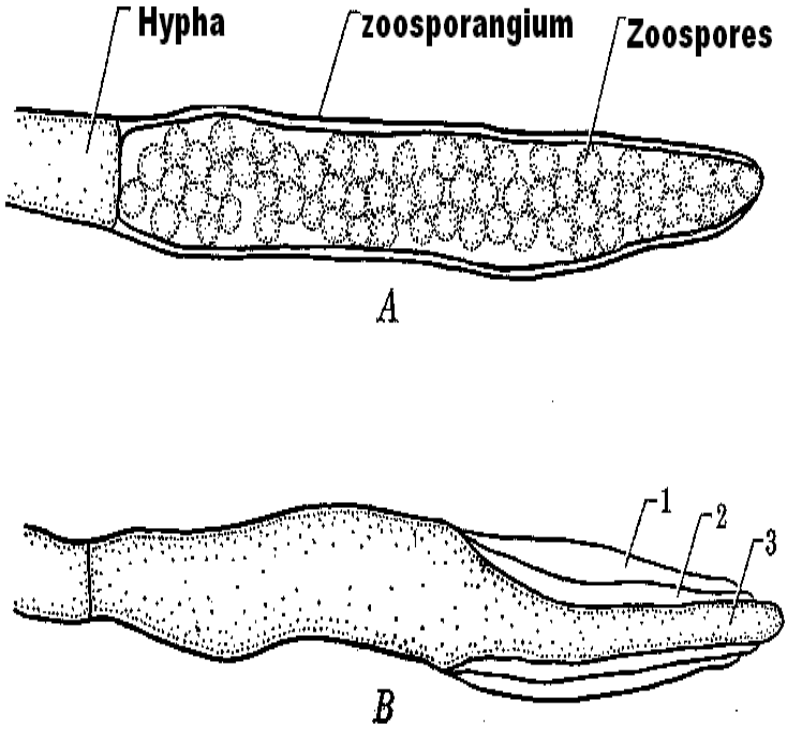

Fig.1.6: Zoosporangia of Saprolegnia. A. Mature zoosporangium of Saprolegnia sp. B Internal proliferation 1, 2 Empty zoosporangial cases. 3. Developing zoosporangium

Source: Alexopolous, 1962

The species that produce one type of spores are monomorphic and those that produce 2 types are dimorphic.

The genus *Pythiopsis* produce only one type of spores i.e. the primary zoospores. The genera *Saprolegnia, Leptolegnia and Isoachlya* produce both types of zoospores hence they are dimorphic.

In the genus Dictyuchus, no spores are produced.

3.6 The Peronosporales

The most advanced members of the Oomycetes. This Order includes aquatic, terrestrial and amphibious members culminating in highly specialised obligate parasites. They are destructive parasites of economic plants, frequently causing diseases with tremendous losses to

crops. The damping off fungi, the white rusts and the downy mildews belong to this Order.

They posses coenoeytic stout hyphae which branch freely. Many members produce haustoria by means of which the organisms obtain nourishment from the host cell.

The haustoria may be knob-like, elongated or branched within the host cells. The hyphae of the parasitic fungi are intercellular or intracellular, growing between cells or within cells.

The family Pythiaceae: aquatic, amphibious, and terrestrial fungi and disease causing agents causing serious diseases of economic crops. Coenoeytic hyphae with well developed haustoria (Figure 1.9). *Pythium debaryanium*, a member lives in the soil saprophytically on dead organic matter or parasitically on young seedlings of many seed plants (angiosperms). The hyphae are both intercellular and intracellular but no haustoria are produced. The sporangia are globose to avoid and are either terminal or intercalary on the somatic hyphae. Production of zoospores is preceded by the formation of a bulb-like vesicle at the tip of the long tube which emerges from the zoosporangium. The sporangial protoplast flows into the vesicle through the tube and differentiation of the zoospores takes place in the vesicle. The zoospores are kidney or bean seed shaped with 2 lateral flagella attached to the concave.

3.7 The Higher Fungi

The Ascomycota, the Basidiomycota and the Deuteromycota are sometimes called higher fungi as they all possess septate hyphae.

3.7.1 Ascomycota

The Ascomycetes are found in a variety of habitats. Many are small and inconspicuous and parasitic on plants and only seen from their effect on hosts. Others are saprophytic, live in soil or on decaying logs and leaf mold, produce large discernuable fruit bodies. Some are entirely *hypogeans* living under the ground. A number are *coprophilous* living only on the dung of certain animals.

Marine Ascomycetes may be saprophytic on various types of organic material submerged or floating in marine waters or may be parasitic on marine algae and higher plants. The asexual structure vary with the members but each of them produce conidia on conidiophores of different types ranging from long to short while some conidia are almost becoming sessile, some may be branched (Figure 1.7).

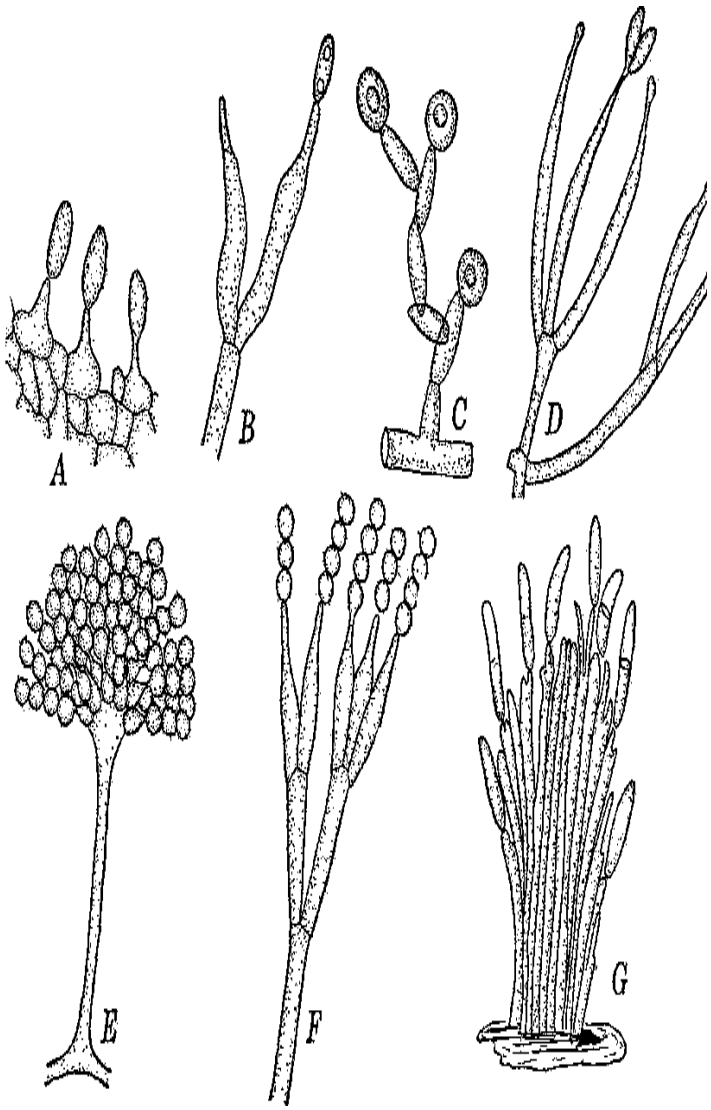

Fig. 1.7: **Various Types of Conidiophores Bearing Conidia *(A) Phyllosticta (B) Dendrophoma (C) Monopodium (D) Verticillium (E) Aspergillus (F) Penicillium (G) Isariopsis***

These conidiophores may be produced in fruit bodies like (A) the pycnidium, this is a hollow structure whose pseudoparenchymatous walls are lined with conidiophores (Figure. 1.8A) and (B) the acervulus is a mat of hyphae usually formed by parasitic fungi, below the epidermis or cuticle of the plant host and giving rise to short conidiophires closely packed together forming a bed like mass (Figure 1.8B). Conidiophores may also be closely packed to form a complex structure called Sporodochia and Synemata (Figure 1.8C and D).

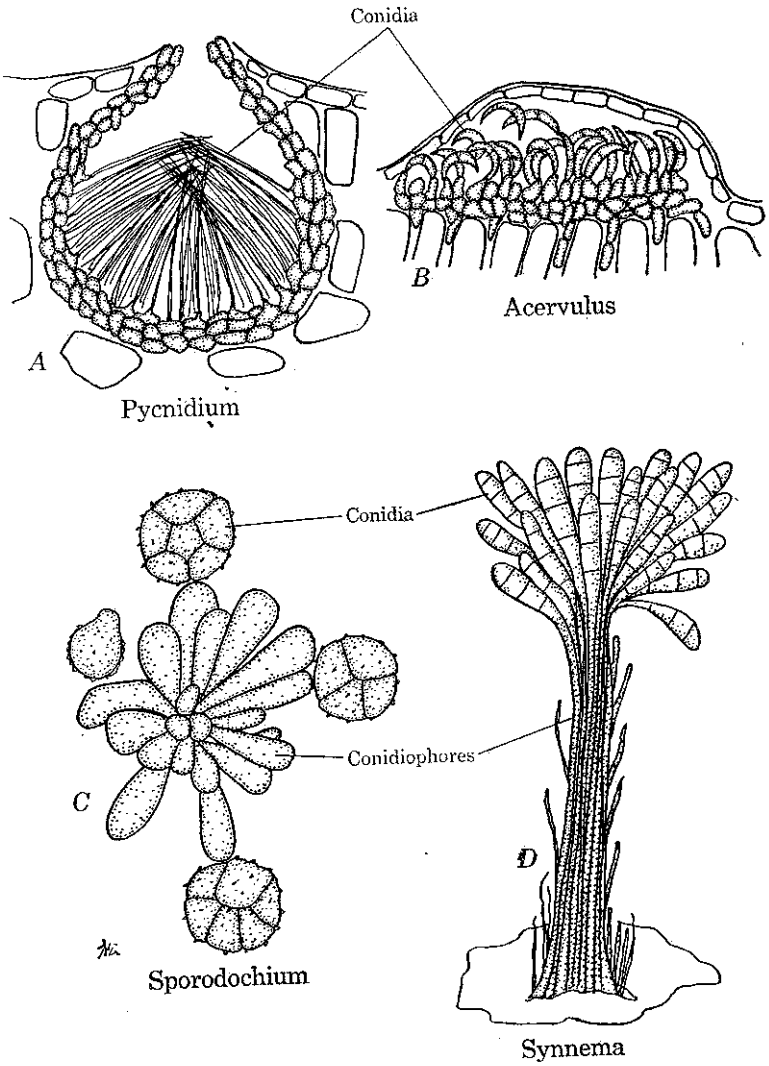

Fig.1.8: Four types of asexual fruit bodies. *(A) Septoria (B) Marssonin (C) Epicoccum (D)Arthrobotryum*
Source: Alexopolous, 1962

3.7.1.1 The Eurotiaceae

Two important genera are grouped in this family, namely the genus *Aspergillus* and the genus *Penicillium.* This is because the two molds produce the sexual spores known as ascospores.

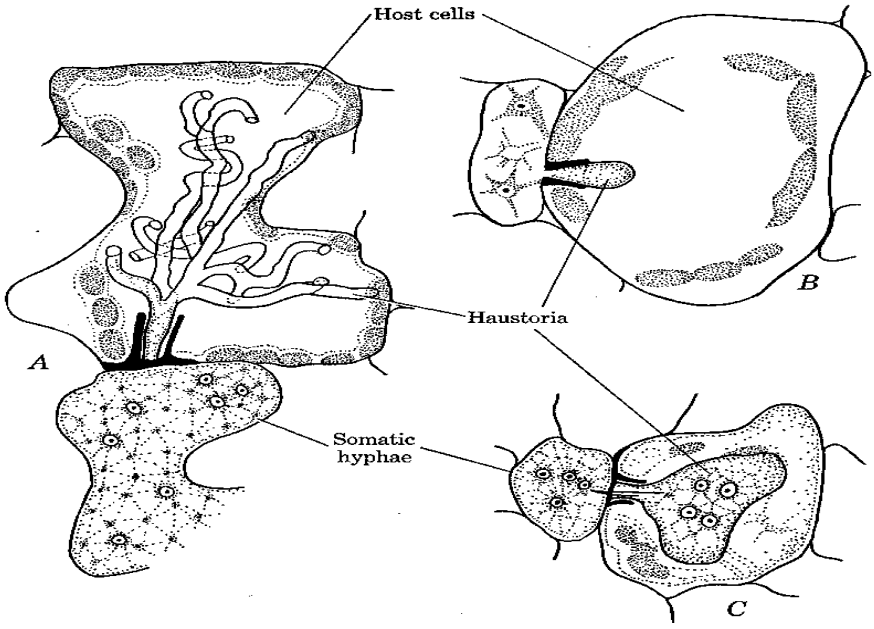

Fig.1.9: Haustoria of the Peronosporaceae
Source: Alexopolous, 1962

The *Aspergillus* species are the black mold (even though they can be other colours) which can be collected by exposing waste food to the air e.g. bread and or other food materials for at least a day, you find them growing. The soils also contain the spores of the *Aspergilli*. Examples of *Aspergillus* are *A.flavus* and *A.fumgatus.*

The *Aspergilli* are capable of utilising an enormous variety of substances for food because they produce several enzymes. They are found on exposed foodstuffs and cause decay. They are common contaminants of culture in the laboratory (bacteriological and mycological alike). The two common species are animal and human pathogens causing a number of diseases known as *Aspergillosis.*

3.7.1.2 The Asexual Reproductive Structures of Aspergillus

The mycelium of the *Aspergilli* resembles that of many fungi. The hyphae are well developed, branching profusely, septate and hyaline.

The mycelium produces conidiophores singly from somatic hyphae. The hyphal cell giving rose to the conidiophores is called foot cell. The conidiophores are long erect hyphae, each terminating in a bulbous head, the vesicle on which asexual conidia are formed. As the multinucleate vesicle develops, a large number of sterigmata are developed over the entire surface. One or two layers of sterigmata may be produced depending on the species. These are called primary and secondary sterigmata. When there are two layers, the conidia

automatically develop from the secondary sterigmata as the sterigmata reaches maturity; they begin to form conidia at their tips, one below the other in chains. The conidia are typically globose and unicellular with roughened walls. The conidia of *Aspergillus* are formed inside the tip of sterigmata which are tubes. A portion of the protoplasm with a nucleus at that of the sterigma is delineated by a septum. The protoplast rounds off, secretes a wall of its own within the tubular sterigma and develops into conidium. The conidial wall may fuse partially or completely with the wall of the sterigmata. A second protoplast below the first develops into a spore and pushes the first spore outward without disjunction, so that a chain of spores (conidia) is formed. This action continues until conidial chain forms a globose appearance on the vesicle. *Aspergillus* colour varies from black, brown, yellow or even green.

The colour depends on the species and on the medium on which the fungus is growing. Under a favourable condition, the conidia germinate by germ tube giving rise to a mesh of vegetative hyphae, from where new sets of sporangial heads are developed.

SELF-ASSESSMENT EXERCISE 1

Describe the asexual structure of a named member of the family Mucoraceae.

3.7.2 Deuteromycota

These are fungi reproducing through the production of asexual spores. Their sexual structures are not yet discovered. Most of their conidia represent the conidial stages of Asomycetes whose ascigerous stages are yet to be discovered or do not exist. Such fungi are described as “imperfect fungi” or “fungi imperfecti”. They are described as conidial stages of the Ascomycota or rarely Basidiomycota e.g. *Aspergillus* and *Penicillium* that belong to the class Plectomycetes of the *Ascomycota* with their fruit body as Cleisthothecia. They have septate hyphae with perforations on their septa for cytoplasmic streaming from one cell to the other.

Sporulation: Those that produce conidia freely on conidiophores produce more than those producing their sporodochia or synemata and acervuli. Temperature, nutrition, pH, light affect their rates of conidial production.

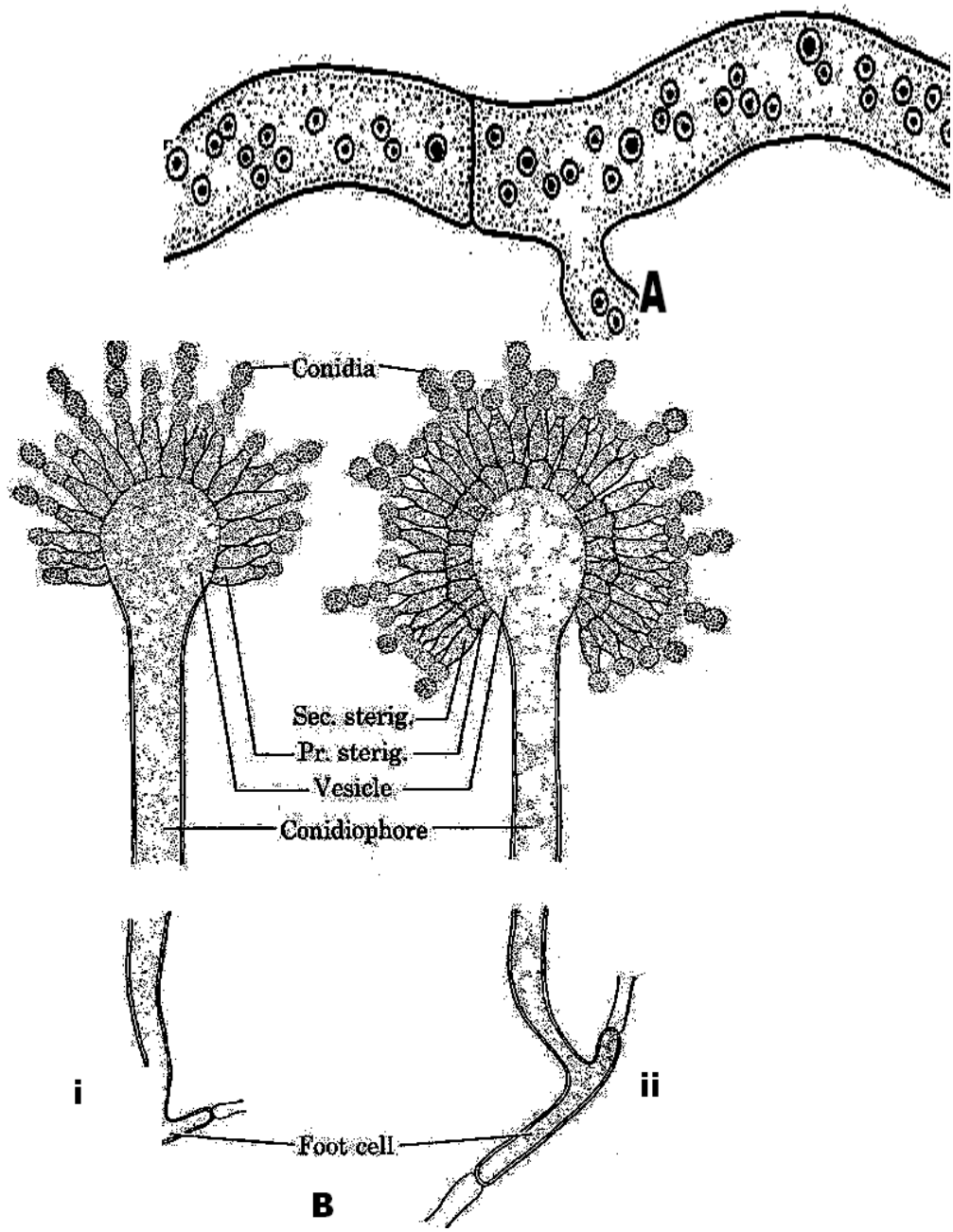

Fig.1.10: A and B. Asexual Reproductive Structures
Source: Alexopolous, 1962

3.7.2.1 The Hyphomycetes

The Hyphomycetes are those that produce their conidia on more or less loose cottony hyphae. Such hyphae bear sterigmata on which such spores are produced. Some conidiophores are inflated at the tip e.g. *Aspergillus.* Others are inflated at intervals forming knee-like structures on which conidia are grouped e.g. *Gonatobotrys*. While others have many branches arranged in whorls e.g. *Verticillium.* (Figure 1.7G).

A group of conidiophores often unite at the base to form synema (Figure 1.8D). The top of the synema is often branched, the conidia arising from the tips of the numerous branches.

3.7.2.2 The Pycnidium

In some fungi, conidia arise in globose or flask-shaped structures (pycnidia). The conidiophores are usually short e.g. *Phyllosticta*, are almost absent in others e.g. *Plenodomous.* They may be long in some cases e.g. *Dendrophoma* (Figure 1.8A).

However, whatever the length, they all arise from the internal wall of the Pycnidium. The pycnidia resemble the perithecium of the pyrenomycetes. The differences is in the content, perithecium contain asci and ascocarps while the other contains conidia. The variation in the structure of the pycnidia is used in separating the genera. (Figure 1.11).

The Acervulus: This is a flat open bed of generally short conidiophores growing side by side and arising from a stromatic mass of hyphae. Conidia are borne at the tip of the conidiophous. They are usually formed underneath the cuticle or epidermis of a host plant and eventually become erumpent.

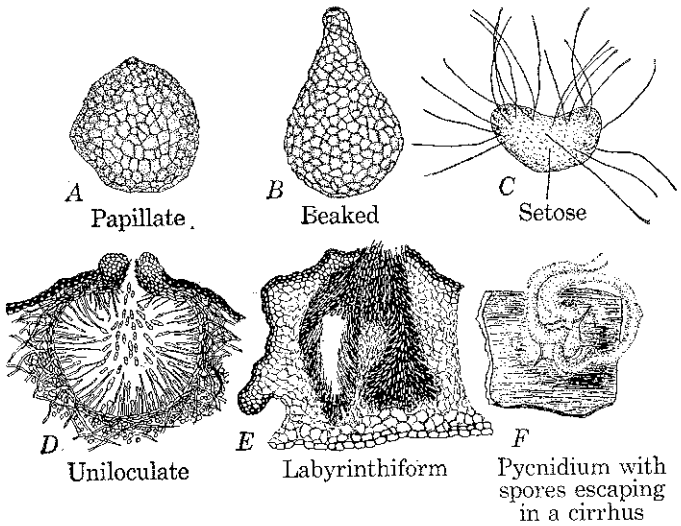

Fig.1.11: Various Types of Pycnidia. (A) *Zythia fragariae* (B) *Dendrophoma obscurans* (C) C*haetomella atra* (D) *Diploidia zeae* (E)*Fusicoccum viticolum*

Source: Alexopolous, 1962

In addition, some acervuli produce long, stiff pointed, dark structures that look like bristles, these are sterile structures called Setae.

Setae are present in some fungi e.g. *Colletotrichum* but absent in others e.g. *Gleosporium*, a distinguishing feature. (Figure 1.12).

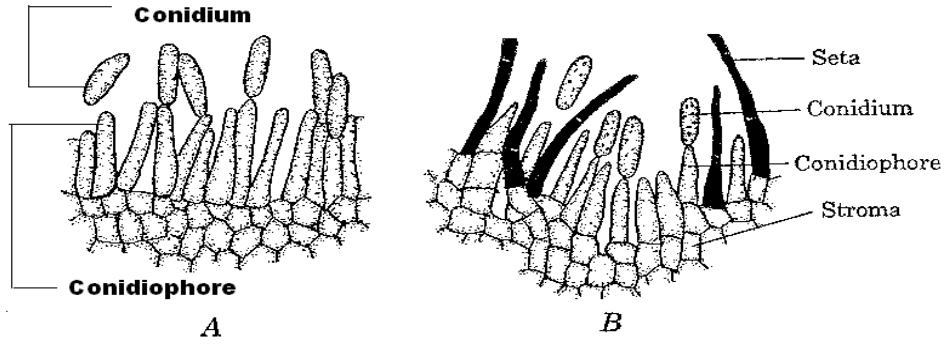

Fig.1.12: Acervuli (A)*Gloeosporium sp.* (B)*Colletotrichum*
Source: Alexopolous, 1962

The spores: Asexually produced spores are usually designated as conidia regardless of their method of origin. Other types of spores produced under the *Ascomycetes* are microconidia, chlamydospores, arthrospores and blastospores. Other spores include:

- Phialospores: are formed from special bottle shaped structure phialid.
- Porospores: are spores which are formed from pores of a conidiophore.
- Pycnidiospores: are spores formed in pycnidia.
- Dictyospores possess both vertical and horizontal septa.
- Scolecospores: are greatly elongated or worm like.
- Helicospores: are coiled spores.
- Blastomycetes: contains the yeast like members.
- Coelomycetes: are members that form pycnidia or acevuli.

3.7.2.3 Asexual Reproductive Structure of Penicillium

The morphology of the *Penicillium* differs considerable from that of *Aspergillus.* The mycelium produces simple, long and erect conidiophores which branch about two thirds of the way to the tip, in characteristics or asymmetrical, broom-like fashion. The conidiophores are commonly referred to as the penicillus (pl. penicillin; L penicillium = small brush, (Figure 1.7F).

The multiple branching of the conidiophores ends in a group of sterigmata which carry the long conidial chains (Figure 1.7F).

The conidia are globose to ovoid and under the microscope resemble glass beads. They are formed in the same manner as Aspergillus. The enormous quantity of greenish, bluish, or yellow conidia which are produced are chiefly responsible for the characteristic colony colour of various species of *Penicillium*.

Under favourable conditions, the conidia germinate by germ tubes which develop into vegetative hyphae from which a number of new generations of the asexual structures are developed.

3.7.3 Structure of the Basidiomycota

The basidiomycota consists of mushrooms, toadstools, puffballs and stink horns, shell fungi and bird nest. The Basidiomycota produce their basidia on highly organised bodies of various types. These fruiting bodies correspond to the ascocarps of the Ascomycetes and are called Basidiocarps.

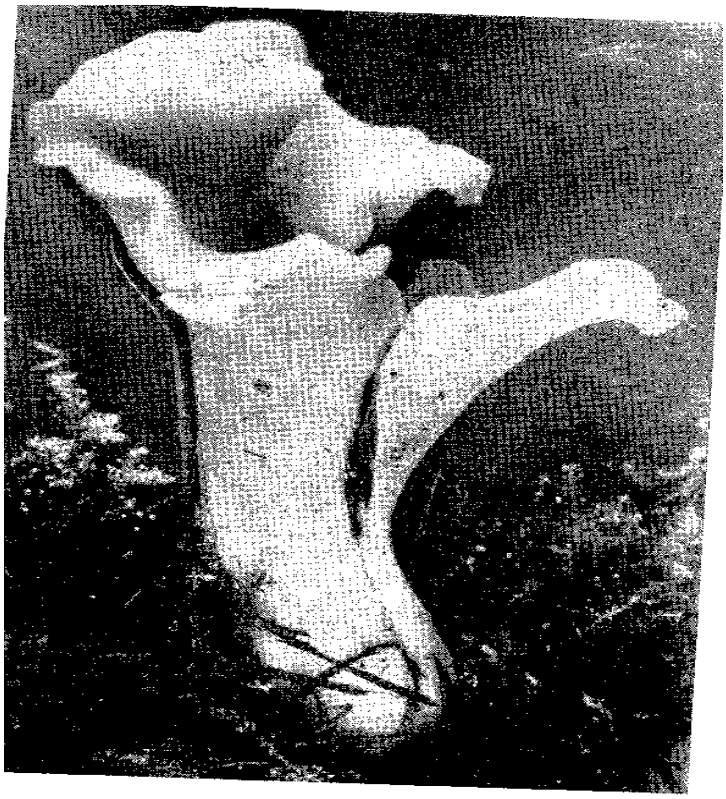

Fig.1.13: ***Phlogiotis helvelloides*****. ½ Funnel Shaped Basidiocarp**
Source: Alexopolous, 1962

Basidiocarps may be thin crust-like, gelatinous, cartilaginous, papery, fleshy, spongy, corky and woody. It is within this group that fruitbodies have reached the most advance stage amongst the fungi. However, the *rusts* and *smuts* belong to the orders Uredinales and Ustilaginales which do not produce basidiocarps.

3.7.3.1 The Primary Mycelium

Usually develops from the germination of basidiospores which may be multinucleate first, the nucleus or the nuclei at the basidiospore dividing many times as the germ tube emerges from the spore and begins to grow. Such multinucleate stage of the primary mycelium is short-lived, however because septa are soon formed which divide the mycelium into uninucleate cells. In some species, septum formation begins upon completion of the first division of the spore nuclei so that the primary mycelium is septate and uninucleate.

3.7.3.2 The Secondary Mycelium

Originates from the primary mycelium; its cells are typically binucleate. The binucleate condition begins when the protoplasts of the two uninucleate cells fuse without karyogamy taking place after plasmogamy. The binucleate cells thus formed produces a branch into which the nuclear pair migrates; the two nuclei divide conjugately and the sister nuclei septate into the two daughter cells.

3.7.3.3 The Tertiary Mycelium

Is represented by the organised specialised tissues which compose of sporophores of the higher basidiomycota. The cells of the tertiary mycelium are binucleate, the sporophores (structures which bear spores) actually originating when secondary mycelium forms complex tissues.
Thus the basidium, the dikaryotic mycelium, the formation of clamp connections, the dolipore septum mechanism is the special characteristics of the Basidiomycota.

The Basidiocarps: the higher members of the Basidiomycota produce basidia in highly organised fruiting bodies of various types. These fruiting structures, corresponding to the ascocarps of the highest Ascomycota are called Basidiocarps. Basidiocarps may be thin, and crust-like, gelatinous, cartilaginous, papery, fleshy, spongy, corky, woody, e.t.c.

Most Basidiomycota bear their spores on basidia but the Ustilaginales do not possess basidiocarps. Their conidia are exposed.

Fruiting bodies of basidiomycota are among the most familiar examples of fungi. Mushrooms, shelffungi, coral fungi, puffballs, earthstars, stinkhorns and birds net fungi are all examples of basidiocarps of fungi which bear them.

The basidiocarp of the family Tremellaceae is ½ funnel-shaped (Figure 1.13) as shown by *Phlogiotis helvelloides.* Members of the family Auriculaceae have ear shaped basidiocarps e.g. *Auricularia auricular* (Figure1.14).

Fig.1.14: Ear Shaped Basidiocarp
Source: Alexopolous, 1962

Members of the family Sporobomycetaceae do not develop basidiocarps. The rusts (Uredinales) produce their spores in Uredia and the spores produced are uredospores. The uredospores are attached on long stalks. The rust also produces aecia and aeciospores in the life cycle.

The Ustilaginales: the smuts produce no basidiocarp; the teleutospores are exposed and are produced in telia. The teleutospores may be sessile or stalked.

The Homobasidiomycetes: basidiocarps are produced in the order polyporales-family, polyporaceae produce shell-like or bracket like basidiocarps. In the order Agaricales, family Agaricaceae, distinct and most advanced basidiocarps are produced.

3.7.3.4 The Structure of Mushroom

The typical form of most Agaricales is the well known mushrooms. It begins as a tiny knot of hyphal cells; it eventually develops into a small globose or ovoid body commonly called the button stage. In some species, the margin of the young pileus or cap is connected to the stem (stipe) by a membrane, called inner cell. As the fruit body grows rapidly, the upper portion of the button expands into the cap or pileus and the inner veil tears and becomes severed from the margin of the pileus and remains attached to the stem (stipe) in the form of a ring or annulus as in Agaricus type (Figure 1.15).

In some others, mushrooms whose development may be different from above, as the inner veil tears, portions of it hang down from the cap like a thin, (cobwebby) the cortina curtain as in Amanita type.

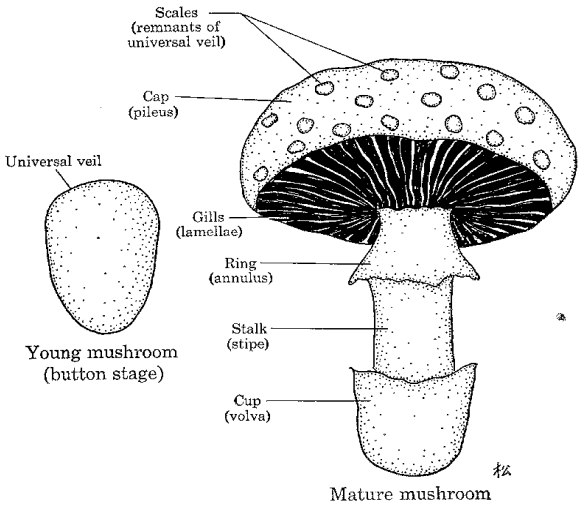

Fig.1.15: Basidiocarp of the Agaricaceae (Amanita Type)
Source: Alexopolous, 1962.

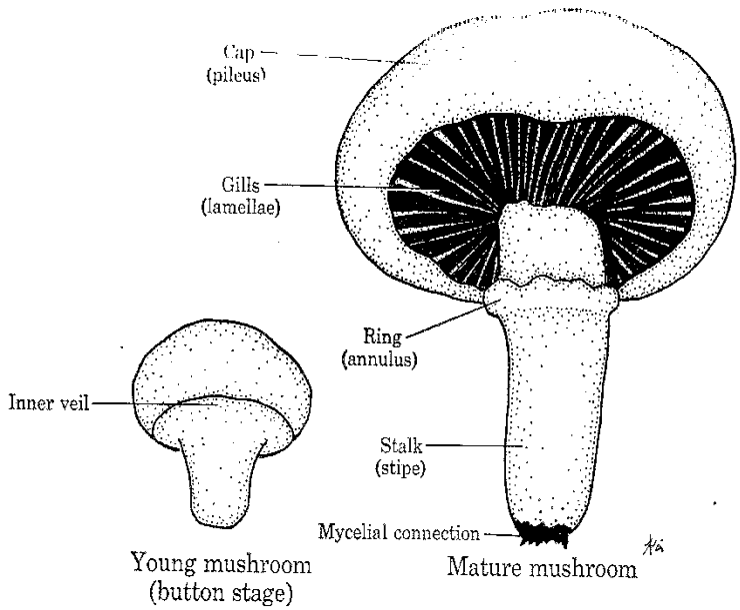

Fig.1.16: Basidiocarp of the Agaricaceae (Agaricus Type)
Source: Alexopolous, 1962

Fig.1.17: Armillaria mellea, Type (a Plant Pathogen)
Source: Guttman, 1999

SELF-ASSESSMENT EXERCISE 2

Describe the structures of *Aspergillus* and *Penicillium.* What are the basic differences and similarities?

4.0 CONCLUSION

As diverse as fungi are, they are organisms consisting of single cells and hyphae as the vegetative (parent) structure. Different types of the reproductive structures that produce spores have been discussed.

5.0 SUMMARY

The basic structures-vegetative and reproductive are related to the life cycles of the various fungi described. The vegetative structures can mature and give rise to the reproductive structure and vice-versa. The complex and diverse fungal structures make them unique in the world of microorganisms. The various spores produced by these various fungi help in their geographical distribution and spread in the environment.

6.0 TUTOR-MARKED ASSIGNMENT

i. Compare and contrast molds and yeasts.
ii. Draw and describe many spore types as possible. Where are they formed?
iii. Which of the following is (are) dimorphic?
   a. *Aspergillus*
   b. *Candida*
   c. *Penicillium*
   d. *Rhizopus.*
iv. Compare the vegetative and asexual structures of a named mold and yeast.
v. Compare the life cycle of *Rhizopus* and that of *Pythium.*
vi. Describe a typical sporangium of the zygomycetes. How does it differ from that of the oomycetes?
vii. Draw and label fully a fruit body of a named basidiomycete.

7.0 REFERENCES/FURTHER READING

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UNIT 2 REPRODUCTION IN MICRO-ORGANISMS: THE FUNGI

CONTENTS

1.0 INTRODUCTION

Reproduction is one of the life processes in living organisms. This process is fundamental to the existence of life otherwise it will be extinct. The process in which the race is maintained and keeps life going from generation to generation is called reproduction.

This process of reproduction also occurs in micro-organisms but with variation. Some methods of reproduction may be specific to certain micro-organisms and vary in some. This unit will deal with the different aspects of reproduction and the products of the various reproductive processes in the fungi.

2.0 OBJECTIVES

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

- explain the processes leading to various types of reproduction in fungi
- differentiate between asexual and sexual reproduction
- discuss the types of asexual reproduction
- discuss the types of sexual reproduction
- distinguish the various types of reproductive units (spores) produced.

3.0 MAIN CONTENT

3.1 Reproduction in the Zygomycetes

3.1.1 Asexual Reproduction in the Zygomycetes

As illustrated by *Mucor* or *Rhizopus* is by spore formation. The Mucoraceae is the largest and the most primitive in the order Mucorales with 11 families. A sporangium is formed at the tip of a sporangiophore as a globose swelling in which central columella becomes separated from the sporiferous region (Figure 2.1). Can you still remember Module 2 unit 1(Figure 1.1 and 1.2)? The typical sporangium develops under favourable condition with many thousands of spores. For asexual reproduction to begin, the spore are liberated by dissolution of the sporangial wall in *Mucor mucedo* and by explosion in the sporangium of *Rhizopus nigracans*, the top of the sporangium forms a cover over the lower part to allow the release of the sporangiospores. The sporangiospores are liberated under favourable conditions (optimum relative humidity and temperature), the spores germinate to give rise to a large mass of vegetative hyphae from where new sets of sporangiphores and sporangial will be developed. Other methods of asexual reproduction in the Mucoraceae include the formation of chlamydospores sometimes referred to as gemmae in the hyphae and the breaking up of the mycelium into yeast like bodies which reproduce by budding. Yeast-like cells are formed when the mycelium is growing in a liquid medium. These cells however are not yeasts.

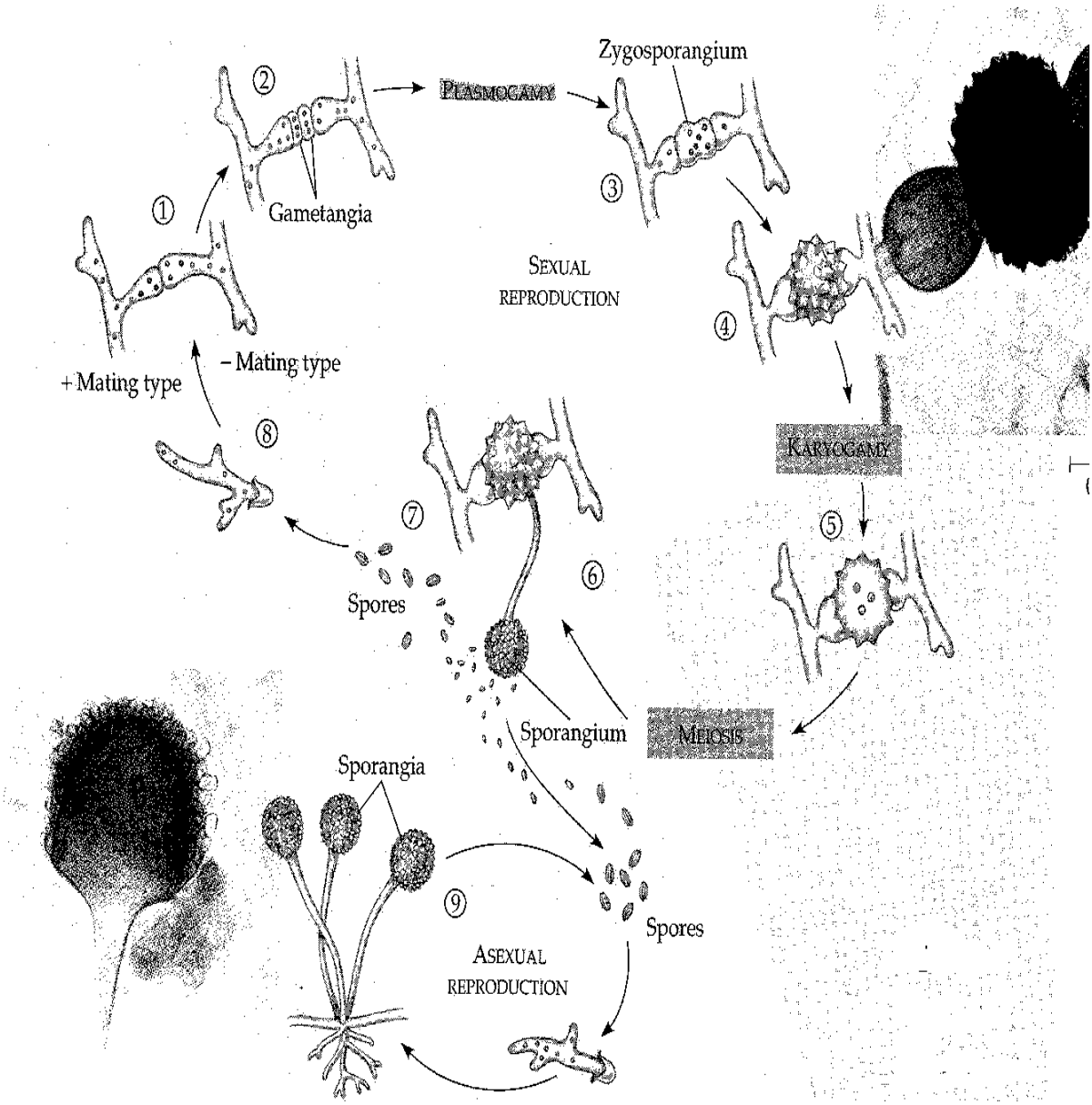

Fig.2.1: The Life Cycle of a Zygomycete (e.g.*Mucor*)
Source: Campbell, 1996.

3.1.2 Sexual Reproduction in the Zygomycetes

Occurs by copulation of two multinucleate gametangia which are similar in structure but which may differ in size. Thus this type of sexual reproduction is homothallic. The mating types are designated + and – (Figure 2.1). They form hyphal extension, each walled off around several haploid nuclei by a septum. The gametangia undergo plasmogamy (fusion of cytoplasmic contents). The zygosporangium develops rough, thick walled coating that can resist dry conditions and other inclement conditions. When conditions are favourable, karyogamy (fusion of nuclei) occurs giving rise to a diploid zygote known as

zygospore. This is followed by meiosis (reduction division). The zygosporangium then breaks dormancy germinating into a short sporangium that disperses the genetically divided haploid spores called sporangiospores (Figure 2.1). These spores germinate into new mycelia.

3.2 Reproduction in the Oomycetes

3.2.1 Asexual Reproduction

Occurs in a long, cylindrical, terminal zoosporangium typically produced by members of this family. The young sporangia are full of dense, granular protoplasm which gives them a brownish appearance by transmitted light under the microscope. Zoospores develop in the zoosporangium. Primary and secondary zoospores are commonly produced in the oomycetes.

Saprolegnia produce both primary and secondary zoospores. The primary zoospores are released from the zoosporangium and after a period of swarming they encyst. Secondarily zoospores are produces after two swarming periods occur. Saprolegnia is said to be diplanetic and the phenomenon is diplanetism. In Saprolegnia, the swarming periods are of considerable duration. In Achyla, the primary zoospores encyst just outside the mouth of the sporangium as soon as they are released. Eventually they germinate giving rise to a secondary zoospores. Achyla therefore exhibits a strong tendency to suppress the first swarming period. In the genus Dictyuchus, no primary zoospores are liberated. Instead they encyst within the sporangium and release a secondary zoospore which escapes from the sporangium, swarms for a time and encysts after a resting period. These cysts release another secondary zoospore which in turn warms and encysts. This process may be repeated several times, all swarming zoospores being secondary zoospores type. This phenomenon is called repeated zoospore emergence or polyplanetism.

In the genus Thraustotheca, the primary zoospores encyst within the sporangia and eventually liberate secondary zoospores which swarm but once (monoplanetic) and monoplanetism exhibited.

In Geolegnia which probably represents the culmination of this evolutionary series, both swarm periods have been suppressed and no zoospores are formed. Each of the aplanospores which escapes from the sporangium germinates by germ tube. Geolegnia is thus aplanetic.

Another method of asexual reproduction in the saprolegnuaceae is by means of chlamydospores sometimes called gemmae.

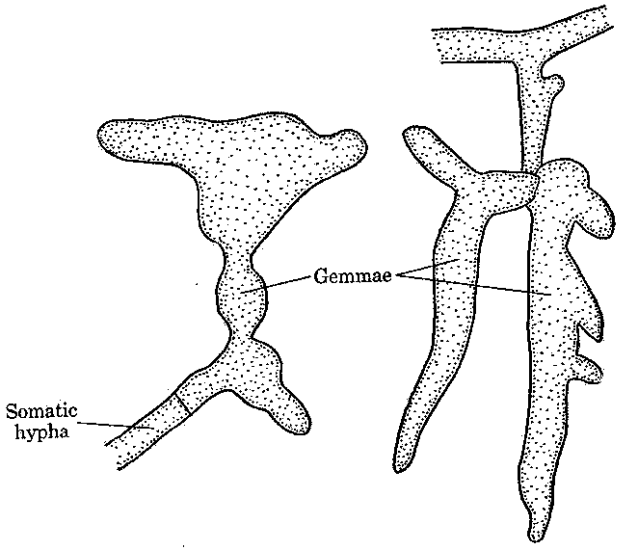

Fig.2.2: Gemmae of Saprolegnia
Source: Alexopolous, 1962.

These are generally borne terminally or in chains. In the later event, they become separated after mating. Gemmae germinate by means of germ tubes which either grow into hyphae or develop into short stalked sporangia typical of the species (Figure 2.2).

3.2.2 Sexual Reproduction in Oomycetes

Sexual reproduction is by means of gametangial contact. The passage of the male gamete into the female gametanigium is through a fertilisation tube (Figure 2.3). The sex organs being generally terminal but intercalary oognia could be formed. The oogonium is usually globose, its entire contents differentiating into one or more globular and uninucleate oospheres (egg cells). The elongated multinucleate antheridium originates either on the same hyphal branch (homothallic) or on which the oogonium is attached on a different thallus (heterothallic). One or more antheridia may become attached to the oogonium, pierce it, and branch out sending one branch to each oosphere. Within the oogonium, one nucleus of the antheridium now passes through each fertilisation tube. This is followed by plasmogamy and fuses with the egg nucleus (karyogamy).

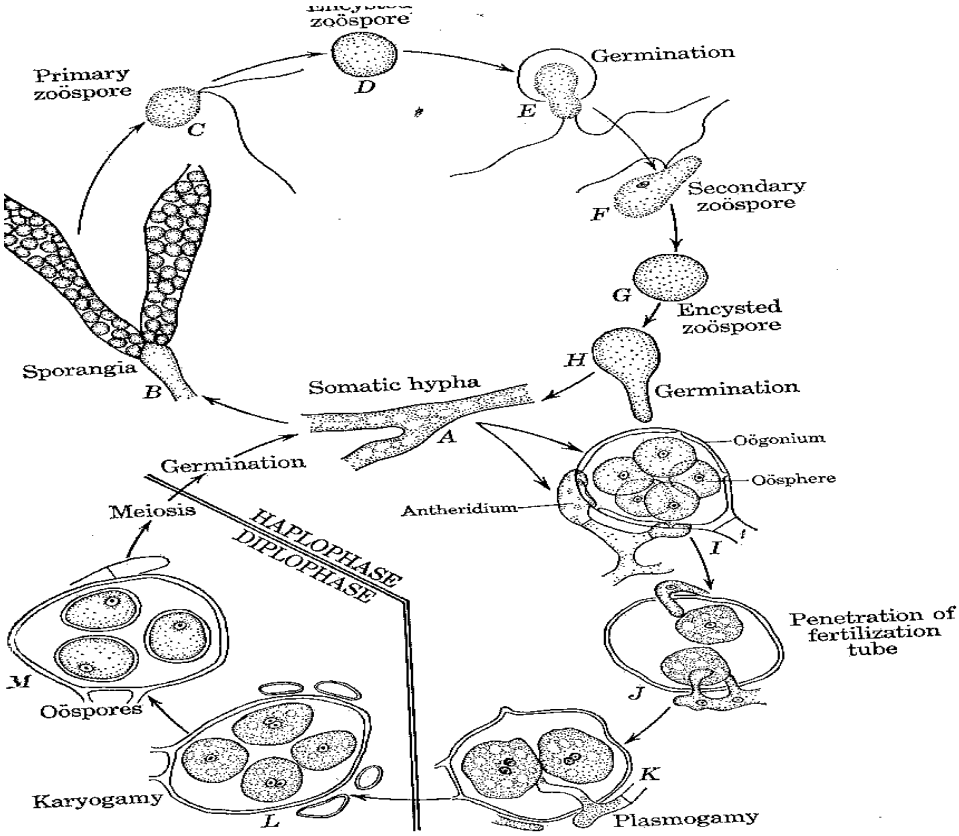

Fig.2.3: Life Cycle of Saprolegnia
Source: Alexopolous, 1962.

Fertilised oospheres develop thick walls and are converted to *oospore* (the zygote). After a period of rest, the *oospore* goes through meiotic division to give rise to haploid cells which now germinate by means of hyphal tubes which later develops vegetative hyphae from which new zoosporangia are formed followed by new sets of gametangia.(Figure 2.3).

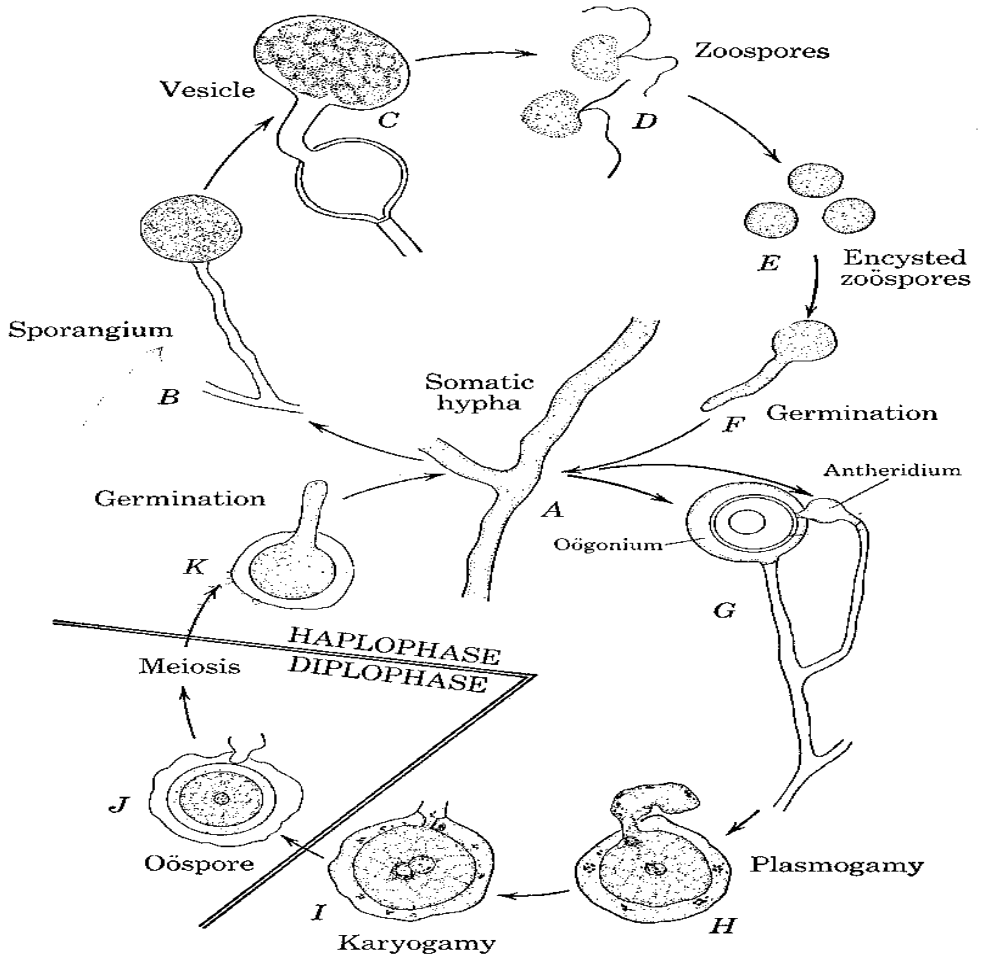

Fig.2.4: Life Cycle of *Pythium debaryanum*
Source: Alexopolous, 1962.

3.2.3 Reproduction in the Peronosporales

Represented by one of the families-Pythiaceae and the genus *Pythium debaryanum.* They possess coenocytic hyphae. They live saprophytically on dead organic matter or parasitically on young seedlings of economic crops. The hyphae are both intercellular and intracellular. They do not produce haustoria.

3.2.3.1 Asexual Reproduction in the Peronosporales

The asexual reproductive structure is a globose to oval sporangium and is terminal or intercalary on the somatic hyphae (Figure 2.4C). Either production of zoospores is preceded by the formation of a globose vesicle or bulbous structure at the tip of a long tube which emanates from the sporangium (Figure 2.4). The sporangial protoplast flows into the vesicle through the tube and differentiation of the zoospores takes place in the vesicle (Figure 2.4D). The narrow tube linking the

sporangium and the vesicle is very distinct, but the vesicular wall is thin. The sporangial protoplast moves rather rapidly through the tube into the vesicle and appears to remain there while the delimination of the zoospores is taking place. In 15-20 minutes, one can see a slight trembling as the crowded zoospores become restless and begin to move under the microscope. This motion becomes accelerated gradually but rapidly until the separate zoospores begin to move out of the vesicle. Suddenly the vesicle wall bursts like a soap bubble and the zoospores scatter to all directions dashing out in a rush (Figure 2.4D).

The zoospores are kidney shaped and have two lateral flagella attached to the concave side. After a period of swarming in the film of water in the soil, the zoospore come to rest, encyst and germinate by a germ tube (Figure 2.4E and F). *Pythium* is therefore monoplanetic i.e. one type of zoospores produced. They end up producing new set of hyphae from where the cycle is repeated.

3.2.3.2 Sexual Reproduction in *Pythium*

Sexual reproduction in *Pythium* is also heterothallic with male gametes (Antherielia) and female gametes (oognia) well developed in close proximity often on the same hypha with the antheridia just below the oogonium (Figure 2.4G). The oogonium is globose with multinucleate oosphere surrounded by a layer of periplasm. The antheridium is much smaller and somewhat elongated or club-shaped. Upon gametangial contact, a fertilisation tube develops and penetrates the original wall and the periplasm. In the meantime, nuclear division has taken place and all but one functionally nucleus in each have disintegrated. The male nucleus now passes through the tube into the oosphere approaches the female nucleus, followed by plasmogamy and karyogamy; the two nuclei unite to form a zygote (the oospore). The oosphere develops into a thick walled, smooth oospore which germinates after growing through meiotic division. Under favourable condition, the oospore germinates by germ tube which develops into mycelium.

SELF-ASSESSMENT EXERCISE 1

Compare the sexual reproduction in the Mucorales with that of the Saprolegniales.

3.3 Reproduction in the Ascomycota

3.3.1 Asexual Reproduction in the Ascomycota

Asexual reproduction in the Ascomycota occurs through fission, budding, fragmentation, arthrospores, chlamydospores or conidia depending on the species and the environmental condition.

a) Fission and budding are methods of propagation encountered in yeasts.

3.3.1.1 Budding in Yeast

This is to those yeasts which commonly reproduce by budding. In this process, the protoplasm of the cell covered by a thin membrane pushes out of the cell wall in the form of a bud and eventually forms daughter cells. The bud enlarges until it is separated from the mother cell by a constriction at the base. The daughter cell may in turn reproduce a bud while still attached to the parent cell (Figure 2.5A). A chain of cells may thus be formed. Such chains may branch if more than one bud is produced from any one yeast cell in the chain as it sometimes happens. During the process of budding the nucleus divides, one daughter nucleus passing into the bud, the other one remains in the mother cell e.g. *Saccharomyces cerevissae*.

3.3.1.2 Fission in Yeast

The yeast cell reproduces by transverse division. The parent cell elongates, the nucleus divides and a transverse wall (septum) is laid down somewhere near the middle, separating the mother cell into two uninucleate (Figure 2.6) daughter cells. The septum is formed by annular growth beginning at the wall and processing inwards (invagination). The new wall thickens inwards before the daughter cells break off.

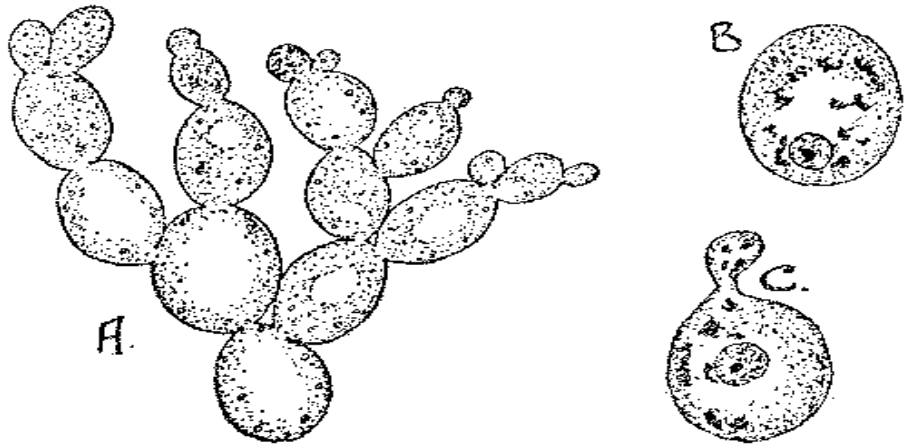

Fig.2.5: (A) Budding Yeast (B) Yeast Mother Cell; (C) Budding Yeast Cell
Source: Lowson, 1962.

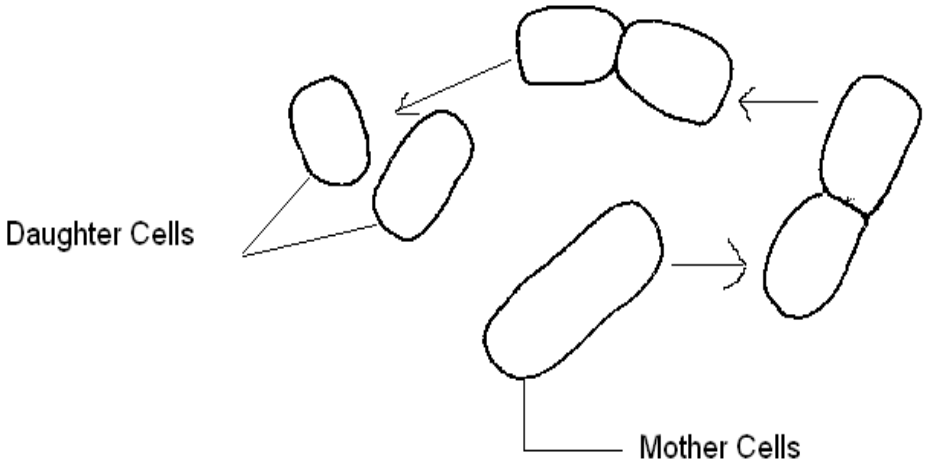

Fig.2.6: Fission in Yeast Cells
Source: Deacon, 1984.

3.3.1.3 Fragmentation (Arthrospores)

All living portions of the thallus are potentially capable of growth, fragmentation whether natural or artificial. Under favourable fragmentation condition, many new individuals as fragments may result. Each fragment is capable of germinating by germ tube to produce a new set of vegetative hyphae.

3.3.1.4 Conidia.

These are generally produced on conidiophores varying from extremely short to nearly absolute hyphae to those that are long and intricately branched.

3.3.1.5 Pycnidia and Acervuli

Conidiophores may be produced free from each other without any evident organisation arising from ordinary somatic hyphae or they may be organised into definite fruiting bodies. The most common of such fruiting bodies are the pycnidia and acervuli.

The *pycnidium* is a hollow, globose or flask-shaped structure whose pseudopanenchymatous walls are lined with short conidiophores.The acervulus is a mat of hyphae usually formed by parasitic fungi, below the epidermis or cuticle of the plant host and giving rise to short conidiophores closely packed together forming bed-like mass conidiophores which may also be cemented together to form complex structures such as s*porodochia* and s*ynemata*.

3.3.2 Sexual Reproduction in the Ascomycota (Plectomycetes)

In the Plectomycetes, the sex organs, antheridium and ascogonium are produced close to each other on somatic hyphae. Haploid mycelia of opposite mating types become intertwined. One acts as a female producing a structure called an *ascogonium* which receives many haploid nuclei from the antheridium of the male. The ascogonium has a pool of nuclei from both parents, but karyogamy does not occur at this time. The ascogonium gives rise to dikaryotic hyphae that are incorporated into an ascocarp, the cup of a cup fungus. A cup fungus of *Sarcoscypha coccineae* is illustrated in (Figure 2.7).

The tips of the ascocarps dikaryotic hyphae are partitioned into asci. Karyogamy occurs within these asci and the diploid nuclei divide by meiosis, yielding four haploid nuclei. Each of these haploid nuclei divides once by mitosis, and the ascus now contains eight nuclei. Cell walls develop around these nuclei to form ascospores (Figure 2.7) when mature, all ascospores in an ascus. A collapsing ascus breaks the neighbouring asci and causes them to fire their spores. The chain reaction releases a visible cloud of spores with an audible hiss. Germinating ascospores give rise to new haploid mycelia (Figure 2.7).

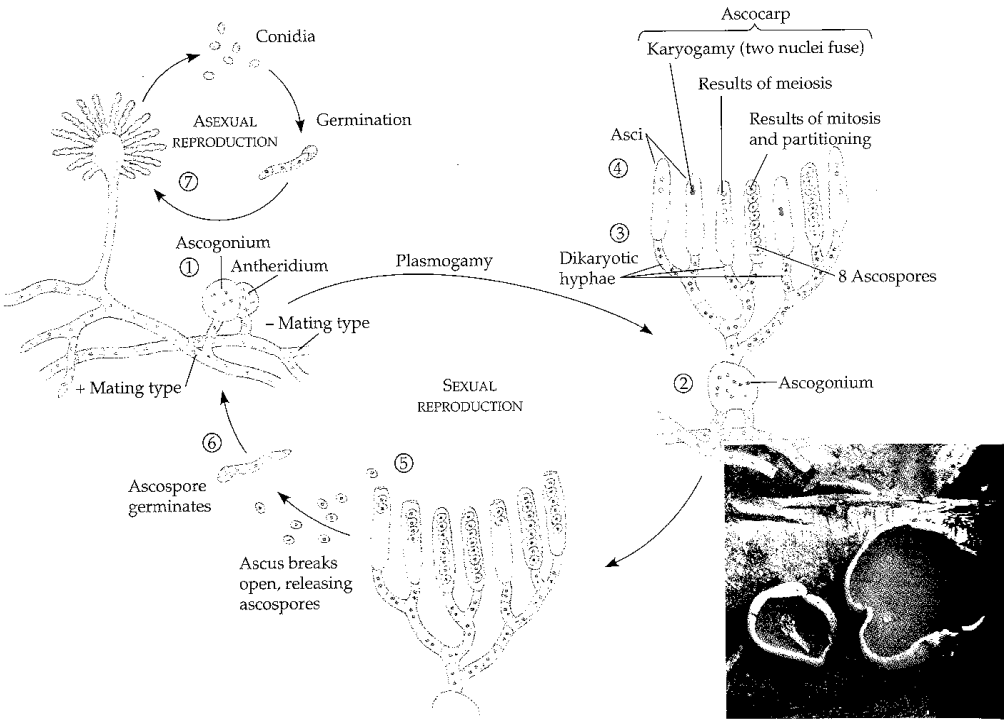

Fig.2.7: The Life Cycle of an Ascomycete e.g. *Sarcoscypha coccineae*

Source: Campbell, 1996,

3.4 Reproduction in the Basidiomycota

3.4.1 Asexual Reproduction in the Basidiomycota

Asexual reproduction in the Basidiomycota occurs by means of budding, by fragmentation of the mycelium and by the production of conidia, arthrospores and oidia.

(a) Conidia production is common in the smuts and the rusts where conidia are budded off both the basidiophores and the mycelium. The rusts also produce spores (uredospores) which are conidial in origin and function (Figure 2.8).

b) The hyphae of Basidiomycota often break into unicellular sections which without rounding or forming thick walls as *chlamydospores* do germinate into germ tubes which develop into mycelia. These mycelia fragment as the Arthrospores which are produced from the primary and secondary mycelium.

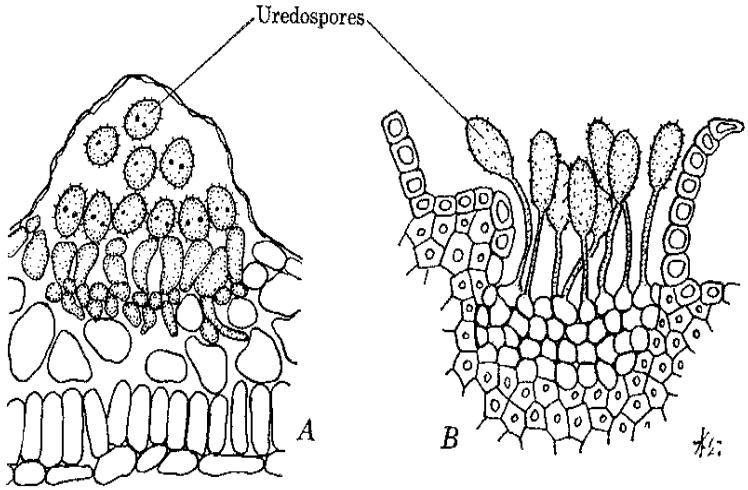

Fig.2.8: Two Types of Uredia. A. Uredospores Produced Successively one below the other; without stalks. B. Uredospores Produced Singly; Stalked.

Source: Alexopolous, 1962.

(c) Oidia are produced by special short hyphal branches, the oidophore (Figure 2.9)

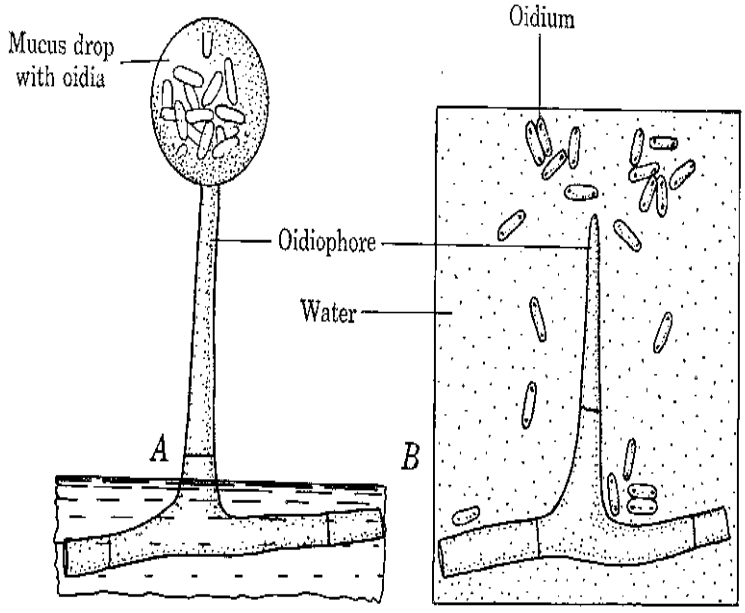

Fig.2.9: Oidiophore and oidia of *Coprinus lagopus*. (A) In the air, (B) After submerging in water

Source: Alexopolous, 1962.

The oidia may be uninucleate or binucleate depending on whether they are produced by primary or secondary mycelium. Such oidia perform two functions; they may either germinate or produce uninucleate primary mycelia or they may act as Spermatia uniting with somatic hypha.

3.4.2 Sexual Reproduction in the Basidiomycota

This is well illustrated in the life cycle of the Agaricus, a member of the family Agaricaceae and order Agaricales (Figure 2.10).

Haploid basidiospores germinate in a suitable environment and grow into short-lived haploid mycelia. Undifferentiated hyphae from two haploid mycelia of opposite mating types undergo plasmogamy (Figure 2.10), creating a dikaryotic mycelium that grows faster than and ultimately crowds out, the parent haploid mycelium. The dikaryons of basidiomycota are long lived, generally producing new crops of the basidiocarps (mushrooms), in this case each year. Karyogamy occurs in the terminal dikaryotic cells that line the surface of the gills. Each cell rapidly forms a diploid basidium which rapidly undergoes meiosis and yields four haploid nuclei. The *basidium* then grows four appendages (sterigma) and one haploid nucleus enters each appendage and develops into basidiospore.

When mature, the basidiospores are propelled slightly by electrostatic forces; into the spaces between the gills. After the spore drop below the cap, they are dispersed by wind and under a favourable condition new generations continue to be produced (Campbell, 1996). This life cycle is exhibited in Figure 2.10.

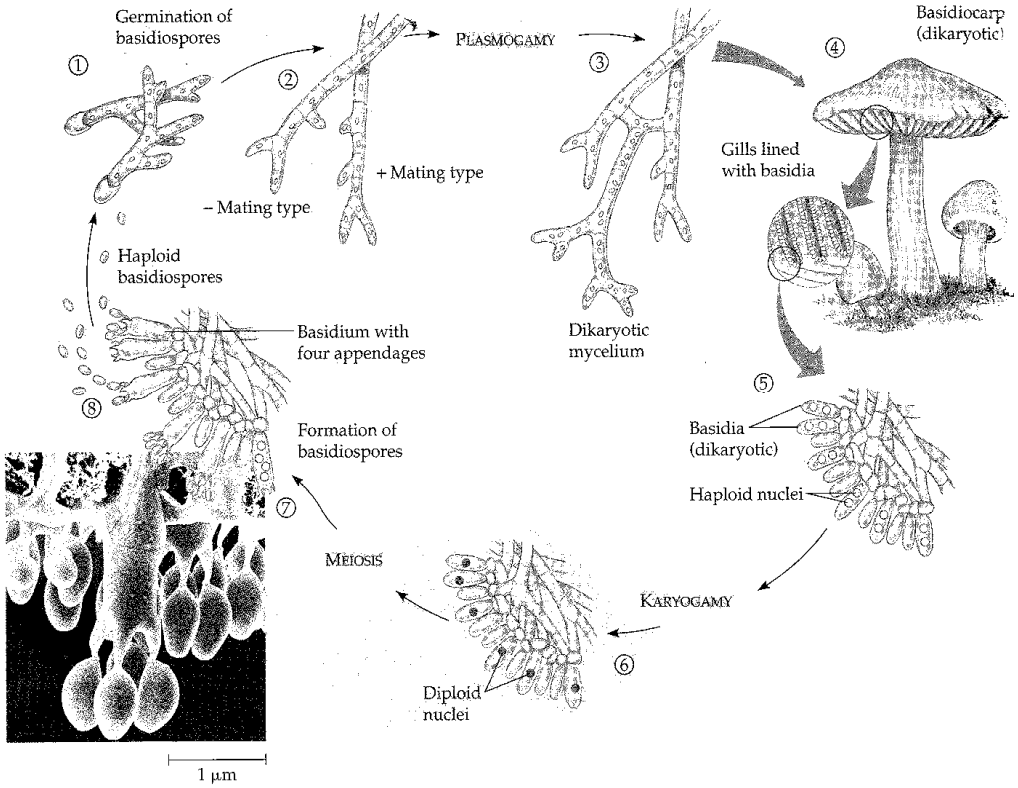

Fig.2.10: The Life Cycle of Basidiomycete
Source: Campbell, 1996.

SELF-ASSESSMENT EXERCISE 2

Describe the life cycle of a monokaryotic fungus and that of a dikaryotic fungus. What are the notable differences?

4.0 CONCLUSION

The various fungi described show diversities in both asexual and sexual reproductive processes where different types of spores as reproductive units are produced.

5.0 SUMMARY

In this unit, you have learnt the:

- Various reproductive methods in the fungi.
- Different types of reproductive organs in asexual and sexual reproduction of the fungi.
- Basic differences between asexual and sexual reproduction in the fungi.
- Formation, types and names of the different asexual and sexual spores.
- Sexual spores are peculiar to the classes to which the fungi belong.

6.0 TUTOR-MARKED ASSIGNMENT

i. Describe the morphology and reproductive processes in *Rhizopus.*
ii. Compare the life cycles of *Saprolegnia* and *Pythium.*
iii. List the differences in the sexual reproduction of *Mucor* and *Saprolegnia.*
iv. Name and describe three types of asexual fruitbodies.
v. Describe the typical structure of the Agaricus. How does this differ from the ascocarp?
vi. What are the similarities and differences between the life cycles of Ascomycetes and Basidiomycetes.

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UNIT 3 IMPORTANCE OF MICRO-ORGANISMS: THE FUNGI

CONTENTS

1.0 INTRODUCTION

The importance of fungi can be treated under two major heading i.e. merits (beneficial effects) and demerits (harmful effects). Fungi are useful to mankind in that they are used as a direct source of food e.g. in the production of soya sauce and Tempe. They are used in the industries in the fermentation processes. They are producers of antibiotics and produce enzymes. Mycorrhizal fungi are beneficial to plant partners (forest tress) for supplying nutrients to the trees. Some fungi are antagonistic and are utilised for controlling plant diseases and pests. They play an important role in increasing soil fertility especially in the recycling of essential nutrients. Fungi are rich sources of essential vitamins which are used as nutritional supplements and in medical

therapy. Some fungi have been used in genetics of certain compounds in chemical mixtures. This unit will discuss the economic importance of fungi.

2.0 OBJECTIVES

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

- discuss the beneficial effects of fungi to man, animals and plants
- list the effects of fungi to man, animals and plants
- list the effects on industries, food and Agriculture.

3.0 MAIN CONTENT

3.1 Importance of Fungi

3.1.1 Fungi as Food

a) Morels, mushrooms, truffles, puff balls and non woody-polypores have been used as food by man e.g.
   - *Lycoperdon gemmatum* (puff balls)
   - *Agaricus campestris* (mushrooms)
   - *Agaricus biosporus* (mushrooms)
   - *Volrariella volvacea* (straw mushroom)
   - *Polypores squamosum* (pore fungi)

b) Yeast containing protein, amino acids, fats, mineral salt and vitamins has been used in preparing yeast cakes. The yeast, S*accharromyces cerevisae* (the bakers yeast) is used in making bread. It is also used in the preparation of pizza and dumplings. Other fermenters are *Torulopsis, candida, Trichosporumn pullulan.*

c) *Penicillium roqueforts is* used for the production of cheese. *Aspergillus nidulans, A.sydowsi,*A. *fisheri, Penicillium piscarum and P.javanicus* are good source of fats.

3.1.2 Industrial Uses of Fungi

Fungal products produced in industries include alcoholic beverages, organic acids, enzymes and pigments.

3.1.2.1 Alcoholic Beverages

Saccharomyces cerevissae and *Rhizopus oryzeae* are utilised for the production of alcoholic beverages e.g. beer, wine, whisky, rum and gin. *Mucor javanicum and Penicillium patulum* are also used in alcoholic fermentation. Wines are produced from grapes or other fruits with *Sacccharomyces ellipsoideus.*

3.1.2.2 Production of Organic Acid by Fungi

Fungi are valuable in organic acids production.

Table 3.1: Production of Organic Acid by Fungi

ORGANIC ACIDS	PRODUCED BY
Citric acid	*Aspergillus niger*
Fumaric acid	*Rhizopus arrhizus,R. nigracans*
Gallic acid	*Penicillium glaucum, A,niger*
Gluconic acid	*Aspergillus niger*
Itaconic acid	*Aspergillus terreus*
Kojic acid	*Aspergillus flavus*
Lactic acid	*Rhizopus oryzae*
L-matic acid	*Schizophyllum commune*
Oxalic acid	*Aspergillus niger,Scherotinium sclerotiorum*
Trans-epoxysuccinic acid	*Aspergillus fumigates*

Source: Pandey and Trivedi, 2006.

3.1.2.3 Enzymes Production by Fungi

Various enzymes are produced on commercial scale by using different fungi e.g. *Aspergillusoryzae, A.niger, Trichodermaviride,* species *Mucor, Rhizopus and Penicillum* (Table 3.2).

Table 3.2: Enzymes Produced by Fungi

FUNGI	TYPES OF ENZYMES
Aspergillus spp.	Amylase, Pectinase, Protease and Glucoamylase
Aspergillus niger	Amylase, Amyloglucosoidase, Pectic enzyme and Glucose oxidase
Mucor spp	Rennat protease
Penicillium chrysogenum	Glucose oxidase
Rhizopus spp	Lipase, Pectic enzymes and glucosidase
Saccharomyces cerevisiae	Invertase
Trichoderma viride	Cellulase

Source: Kingsley *et al*. 2003; Pandey and Trivedi, 2006.

3.1.3 Pigments Produced by Fungi

Some fungi are adopted in the extraction and synthesis of some pigments on commercial basis for dying. Examples of various dye stuff from various fungi are shown in Table 3.3.

Table 3.3: Pigments Obtained from Fungi

PIGMENT	FUNGUS
Atromentin	*Paxillus atromentosus*
Beta-carotene	*Blakeslea trispora*
Catenarin	*Helminthosporium*
Citrinin	*Penicillium citrinum*
Neocercosporia	*Cerocospora kikuchii*
Phoenicin	*Penicillium pheonicum*
Spinulosin	*Penicillium spinolosum*
Emodic acid	*Penicillium spp.*

Source: Kingsley *et al*, 2003; Pandey and Trivedi, 2006.

3.1.4 Production of Antibiotic by Fungi

Several antibiotics have been produced from some fungi on commercial bases. These are listed below in Table 3.4. In 1991, Sir Alexander Flemming discovered the antibiotic Penicillin which was produced from *Penicillum notatun* and *P.chrysogenum* was found to produce penicillin better and in larger quantities. Since then a number of antibiotics produced by fungi have been reported (Table 3.4).

Table 3.4: Production of Antibiotics by Fungi

ANTIBIOTICS	SOURCE	BIOLOGICAL ACTIVITY
Penicillin	*Penicillium notatum*	Active against Gram positive bacteria
Griseofulvin	*Penicillium p.nigricans*	Antifungal
Cephalosporin	*Emericellopsis minimum*	Antifungal
Fumgillin	*Aspergillus fumigatus*	An amoebocite
Fumigatin	*A fumigates*	Antibacterial
Gliotoxin	*Gliocladium virens*	Antibacterial, antifungal
Patulin	*Aspergillus clavatus*	Antibacterial, antifungal
Sparassol	*Sparassis ramose*	Antifungal
Statolan	*Penicillium stolonniferum*	Antiviral
Ustilagic acid	*Ustilago maydis*	Antifungal, antibacterial
Wortmannin	*Talaromyces wortmannii*	Antifungal
Viridian	*Gliocladium virens*	Antifungal

Source: Pandey and Trivedi, 2006.

3.1.5 Vitamin Production by Fungi

The following examples are some of the vitamins produced by some fungi (Table 3.5).

Table 3.5: Vitamins Produced by Fungi

VITAMINS	PRODUCED BY
Vitamin B	Yeasts
Ergosterol (the precursor of Vitamin D2)	Yeast
Riboflavin	*Ashbya gossypii, candida spp, Eremothecium asbyi,*
Niacin	*Agaricus*
Vitamin A(Palmitate)	*Rhodotrula gracilis*
Vitamin C(ascorbic acid)	*Agaricus*
Cobalamins	*Agaricus*

Source: Pandey and Trivedi, 2006.

3.1.6 Uses of Fungi in Agriculture

a) In ecosystems, fungi are important decomposers of the plant and animal debris which lead to the maintenance of humus level and increase the soil fertility. They help in the recycling of nutrient elements in the soil especially as saprophytes and as symbionts (Lindhal *et al,* 2007; Barea *et al,* 2005). Can you still remember the biogeochemical cycles in Module 1 unit 2?

(b) Gibberellins are plant hormones produced by the fungus, *Gibberella fujikuroi.* It used to accelerate the growth of several horticulture crops.

c) Some members of the Order Enthomorphthorales, chytrids, a few yeasts, many Ascomycetes and Deuteromycetes are used in controlling plant pests. *Coelomomyces* and *Legenidium* are parasitic on the *anopheline* mosquitoes.
Generally, fungi can be parasitic on other parasitic fungi e.g. *Trichoderma viride* and *Pyricularia oryzae* may become suppressing the activities of such pests and diseases like insects, mites, weeds, nematodes and other pathogenic fungi on important crop plants.Entomopathgenic fungi can be used as biospecticides e.g *Beavveria brassiana,Metarhizium anisophiliae,Hirsutella spp, Paecilomyces spp* and *Verticillum lecanii.*

d) A dual (mutual) association of a root of higher plant and fungus that is not disease producing is referred to as mycorrhiza. Mycorrhiza enhances mineral supply to the forest trees (Pandey and Trivedi, 2006). They are also beneficial to the plant secreting hormones and antibodies that reduce the potential disease.

3.1.7 Fungi and Scientific Research

(a) Fungi are being used as research tools for the study of fundamental biological processes by cytologists, geneticists, biochemists and biophysicist. For example, *Saccharomyces cerevissae* is being used in genetics and cell biology. The yeast serves as a vector in gene transfer. The Neurospora (red-bread mold) is often used by geneticists and biochemists for the study of heredity

(b) The DNA can also be cloned in eukaryotic cell e.g. in genetic engineering.e.g. the yeast cells offer the advantage of having plasmids. Artificial chromosomes that combine yeast DNA and foreign DNA have been produced and cell division clones these chromosomes. Yeast is therefore being used as a vector in genetic engineering.

(c) By using recombinant DNA technology, fungi are being used to produce a number of natural proteins, vaccines, hormones, enzymes, antibiotics. Pigments, vitamins agricultural fungicides, plants growth regulators, organic acids and alcohols (Pandey and Trevedi, 2006)

3.1.8 Fungi as Test Organisms

The amounts and types of vitamins, aminoacids and elements are often determined by the use of fungi in bioassay studies.

For example, Scopulariosis is used for the detection of arsenic acid for the estimation of copper in the soil. (Pandey and Trevedi,2006). Fungi are used for vitamin-bioassays. The yeast, *Yarrowia lipolytica* is known to degrade palm oil mill effluent (Osival *et al*; 2002). The "white root" fungi can degrade insecticides, herbicides, heavy fuels and turn them into carbon dioxide, water and basic elements.

It should be noted that all the discussions under 3.1.1-3.1.8 represents the major beneficial importance of fungi.

SELF-ASSESSMENT EXERCISE 1

Discuss the contributions of fungi to medicine and agriculture.

3.2. Harmful Effects of Fungi

Micro fungi produce poisons or mycotoxins (aflatoxin) in food and feed products thus leading to mycotoxicoses in man and animals.

3.2.1 Fungi as Agents of Food Spoilage

Penicilium digitatum, Zygosaccharomyces, Debaromyces, Saccharomyces cause rotting or deterioration of fruits, seeds and vegetable. *Rhizopus nigracans could be* responsible for the deterioration in storage. The spoilage of sweet potatoes in storage. Some yeast species cause diseases of tomatoes, fruits, beans and cotton. *Penicillum expansum, Aspergillus glaucus, A.niger, A.clavaties A.repens. Mucor bracemosus, Neurospora sitophila, Odium lactis* and *Fusarium spp.* are responsible for meat spoilage in storage and transportation. Exposed bread is spoiled by *Mucor mucedo, Aspergillus glaucus, A.repens, A.flavus and Neurospora sitophila.* Milk, cream and other diary products are regularly spoiled by *A.repens, A.favus, A. fumigatus, Penicillium, Mucor and Oidium lactis* (Pandey and Trevedi, 2006). *Penicillium italicum and P. digitatum* cause blue and green mold on citrus fruits in storage. *P.expansum* causes decay of apples, pears, and grapes in storage. Fungal spoilage on food during storage leads to accumulation of a*flatoxins.*

3.2.2 Fungi and Spoilage of Industrial Products

Yeasts like S*accharomyces* and *Brettanomyces* in wine can cause spoilage and result in wine faults and subsequent spoilage of such wines.

3.2.3 Deterioration of Materials by Fungi

Many saprophytic fungi grow on a number of articles used by human e.g. paper, clothing materials, leather goods, photographic materials, optical instruments, tobacco, cigars and wood products, rubber when environmental conditions are favourable (Pandey and Trivedi, 2006).

a) Textiles: Wool is commonly destroyed by species of *Alternaria, Steinphyllum, Penicillum and Trichoderma.* Cotton is destroyed by species of *Starchybotrys*, *Chaetomium*, and *Steinphyllum*. Cotton industry faces serious damage from species of *Trichoderma, Fusarium, Aspergillus, Penicillium, Curvularia, Rhizopus and Yeasst.* Silk industry is affected by S*capularopsis, Stachybotripand Trichoderma.*

b) Paper: Many fungi e.g.C*haetomium, Mycotridium, Aspergillus, Torula, Cephalotheium, Alternaria Fusarium, Cladosporium, Cephalosporium* decompose cellulose and lignin and ruin papers. *Penicullium purpureum* spots printing papers, books and engravings.

c) Leather materials: Different leather goods, shoes, belts, bags are destroyed by *Asporgillus niger, Pencillum* and *Paecilomyces.*

d) Optical instruments: Fungi like *Aspergillus candida. A.nidulans, A.niger* and *Helminthosporium* grow on the surface of lenses in binoculars and camera lenses, thereby rendering them useless.

e) Rubber: Rubber products like rubber tiers, electrical insulators are spoiled and defaced by *Aspergillus candida, A.flavus, A.fumigatus, A.niger, A.terreus* and *Penicillum species* (Pandey and Trivedi, 2006)

f) Paints: Species of *Aspergillus, Penicillum, Cladosporium, Alternaria* are responsible for the mould spotting or discoloration of the painted surfaces under favourable environment conditions.

g) Fungi and wood decay: Most members of the polyporales and agaricales (Basidiomycetes) are responsible for wood decay e.g. *Polyporus tomentosus, P.schuweintizii, Fomes lignosus* and *Ganoderma* spp.

3.2.4 Fungi as Agents of Animal Diseases

Species of *Trichophyton* causes *dermatomycoses* and in animals these are other wise known as dermnatophytes. The ringworm disease of the horses is caused by *Microsporun canis.* Eating of sclerotia (ergot) by the grazing cattles results in abortion paralysis and gangrene of the hooves and tails. Mycotic abortion in cattle is caused by *Absidia sp* (Kingsley *et al*, 2003).

3.2.5 Fungi as Agents of Human Diseases

a) Mycoses: These are caused by some virulent fungi.
   i) Blastomycosis (*Blastomyces dermatitidis)*
   ii) Paracoccidiomycosis (Paracoccidiomyces brasiliensis).
   iii) *Histoplasmosis(Histoplasma capsulatum).*
   iv) *Coccidioidomycosis(Coccidiodies immitis).*

b) Systemic diseases: Some opportunistic fungi also cause diseases in compromising patients.
   i) Systemic candidiasis (C*andida albicans, C.parapsilopsis* and *C.tropicalis).*
   ii) Aspergillosis (Aspergillus especially *A.fumigatus).*
   iii) Cryptococcosis (*Crpytococcus informans).*
   iv) Fungal meningitis (*Cryptococcus neoformanus).*

c) Subcutaneous mycoses-(Dermatophytes): Three fungal genera are associated with these infections. *Trichophyton, Microsporum* and *Epidermophyton.* They infect hair, outermost layer of the skin and nails. They inflict the following infections:
   i. Tinea capitis (scalp ringworm)
   ii. Tinea pedis (athletes foot, foot-rot)
   iii. Tinea corporis (body infection).

3.2.6 Production of Mycotoxins by Fungi

Some fungi produce toxic materials that are deadly to man and animals on food products.

i) aflatoxin: (*Aspergillus flavus A.niger, A.parasiticus* on cereals and grains).
ii) z*eavalenone* and *sporofunsarin(Fusarium spp.)*
iii) ochratoxin: (*A.ochraceus*)
iv) fumigatin (*A. fumigates)*
v) ascladiol (*A. clavatus)*
vi) muscarine (*Amanita muscaria)*
vii) psotalens (*Sclerotinia sclerotium)*
viii) rubratoxin (*Penicillium rubrum)*
ix) screpenosis (*Fusarium nivale).*

3.2.7 Fungi as Plant Pathogens

Fungi inflict a number of diseases on cereals, legumes, cash crops and tree crops that can reduce their economic yield, reduce the quality of their product and consequently reduce the market value. Table 3.6 is an example of some pathogens of the rice plant and various types of diseases caused on the rice plant.

Table 3.6: Some Fungal Rice-Borne Diseases

DISEASES	CAUSAL ORGANISM
Blast	Pyricularia oryzae
Brown leaf spot	Drechslera oryzae
False smut	Ultiloginoidae virens
Udbatta disease	Ephelis oryzae
Narrow brown leaf spot	Cercospora oryzae
Foot rot	Fusarium moniliforme
Stock burn	Trichocoliella padwicki
Stem rot	Sclerotiurn oryzae
Kernel smut	Tilletia barclayana

Source: Awoderu, 1995.

SELF-ASSESSMENT EXERCISE 2

Fungi are enemies or friends of life, Discuss.

4.0 CONCLUSION

The beneficial and deleterious roles of fungi to man, animals and plants have been presented and discussed. They are very important in the existence of life.

5.0 SUMMARY

In this unit you have learnt that:

- Some fungi are used in the production of bread, alcoholic beverages, enzymes, organic acids and pigments.
- Some Fungi are edible.
- Many antibiotics can be produced from some fungi.
- Fungi form mycorrhiza.
- Fungi are agents of biological control.
- Fungi are employed in scientific research and genetic engineering.
- Many fungi are responsible for spoilage, deterioration of food and industrial products.
- Fungi are agents of plant, animal and human diseases.
- Some fungi are capable of producing deadly toxins in and on food materials.

6.0 TUTOR-MARKED ASSIGNMENT

i. Discuss the importance of fungi to Agriculture.
ii. Why is the mycorrhizal association of plants and fungi considered to be mutualistic?
iii. How important are fungi in medicine.
iv. Enumerate the diseases infecting man by fungi citing the diseases and their causing agents.
v. Describe the importance of molds e.g. Aspergillus, Penicillium and Rhizopus to life.
vi. Yeast is both beneficial and deleterious to life. Discuss.
vii. Fungi are enemies or friends, justify this statement.

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UNIT 4 STRUCTURE AND REPRODUCTION: THE BACTERIA

CONTENTS

1.0 INTRODUCTION

The bacteria belong to the prokaryotic group of organisms. Prokaryotes lack a true nucleus. These include the eubacteria (true bacteria) and archaeobacteria which look similar but they are not the same. These bacteria are differentiated by their morphological (shape, their chemical composition often detected by staining reactions), nutritional requirements, biochemical activities and source of energy (sunlight or chemicals). This unit will be dealing with the structure and reproduction of bacteria.

2.0 OBJECTIVES

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

- explain the typical structure of the bacterium
- describe the different morphological types of bacteria
- identify their extracellular structures

- list the major chemical constituents of the bacterial cell wall
- explain the various reproductions in the bacteria.

3.0 MAIN CONTENT

3.1 Structure and Exta-Cellular Structures in Bacteria

3.1.1 Structure of Bacteria

All bacteria are unicellular with variety of shapes and sizes but are always structurally small; hence they are regarded as micro-organisms. Their genetic material is not enclosed within a membrane. They lack other membrane bounded organelles. Their DNA is not associated with histone proteins (special chromosal proteins found in eukaryotes). The walls contain the complex polysaccharide peptidoglycan. They usually divide by binary fission. During this division, the DNA is copied and the cell splits into two cells. Binary fission involves fewer structures and processes. Bacteria can be seen under the light microscope but the details are revealed under the electron microscope.

The cell-wall of the bacterium contains polysaccharides but rarely cellulose, fatty materials, proteins and chitins may be present.

The outer wall is modified to form a mucilaginous capsula or structure which protects the bacterium against dessication. The capsules of many cells may run together to form a complete structure called zooglea in which many bacterial cells are cemented together by their gummy walls. The internal contents of the cell include the semi fluid cytoplasm and numerous small specks called ribosomes. Ribosomes are sites of protein synthesis. The single circular chromosome (chromatin or DNA) is found in the nucleoid region .i.e. no true nucleus. Motile bacteria may have long projections called flagella (singular-flagellum).Their genetic material is not enclosed within a membrane.

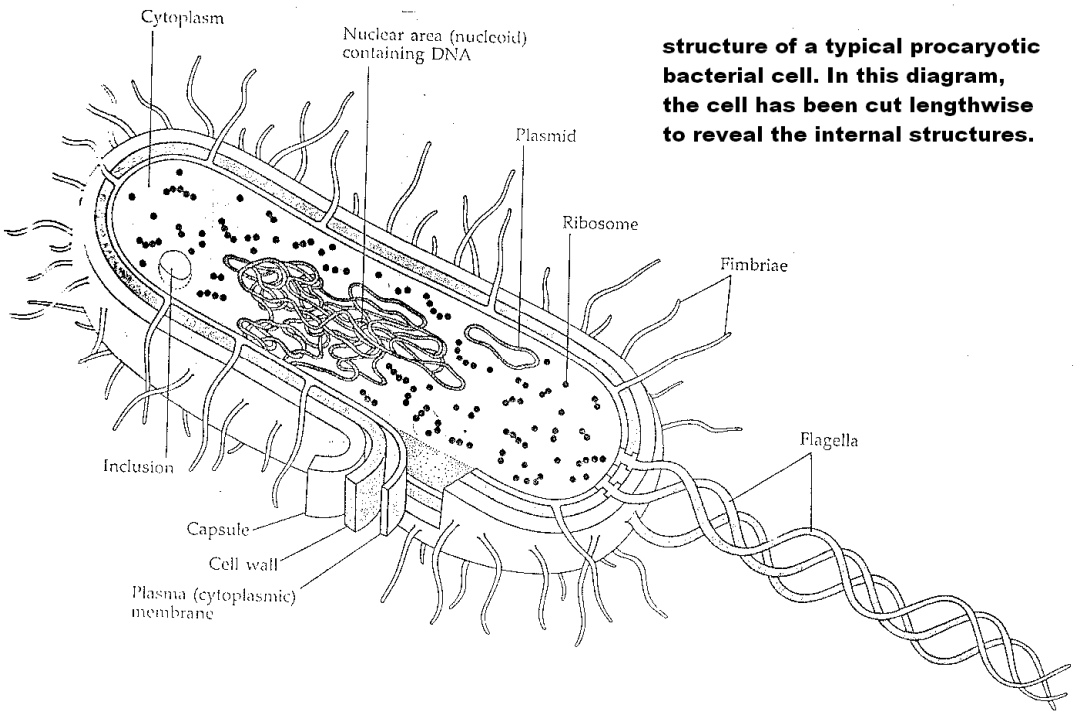

Fig.4.1: A Typical Bacterial Cell
Source: Tortora *et al;* 1992.

3.1.2 Types of Bacterial Cells

There are many sizes and shapes of bacteria. Most bacteria fall within a range of 0.20um in diameter and from 2-8um in length. They exhibit a few basic shapes. These are the spherical (coccus), rod-shaped (bacillus) and spiral.

i. The Coccus (cocci, plural)

The cocci are usually round but can be oval elongated or flattened on one side (Figure 4.2a). When cocci divide to reproduce, the two cells can remain attached to one another. Cocci that remain with one another after dividing are called diplococci (Figure 4.2a). Those that divide and remain attached in chain-like patterns are called streptococci (Figure 4.2a). Those that divide in two planes and remain in groups of four are called tetrads (Figure 4.2b).

Those that divide in three planes and remain attached in cube like groups of eight are called sarcinae (Figure.4.2c),while those that divide in multiple planes and form grape-like clusters or broad sheets are called staphylococci (Figure 4.2d).

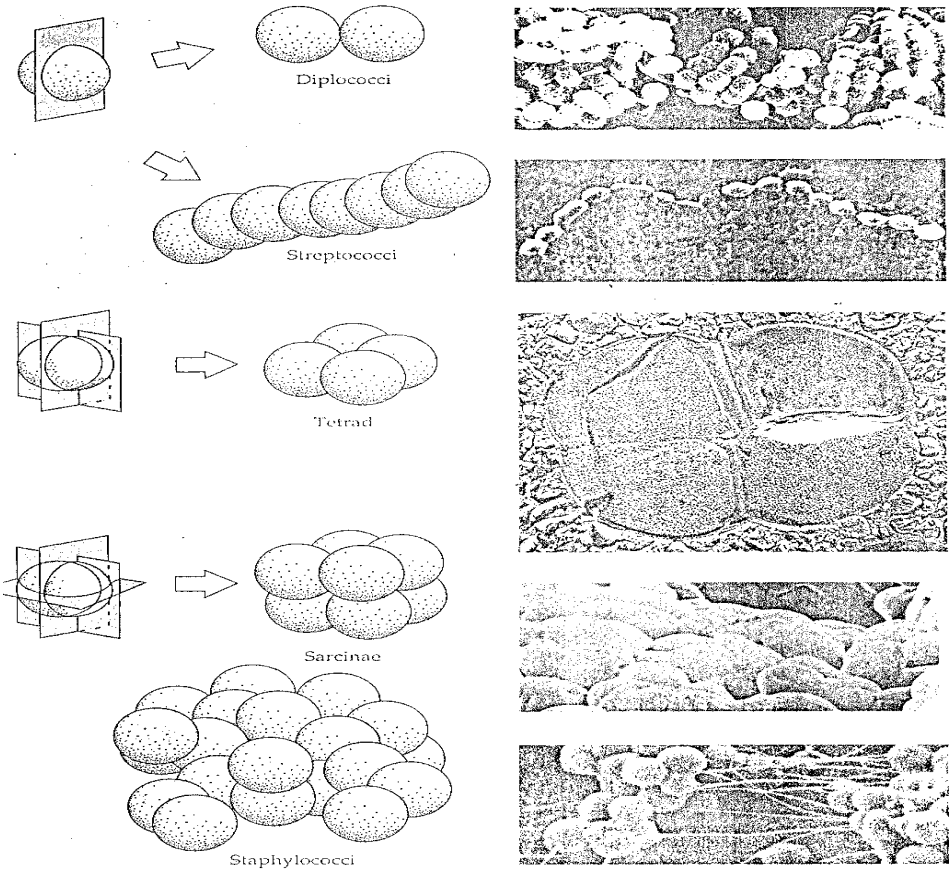

Fig. 4.2: Arrangements of cocci. The number of planes in which the cell divides determines the arrangement of cells. Shown are diagrams (left) and corresponding photos (right). (a) Division in one plane produces diplococci and streptococci. (b) Division in two planes produces tetrads (c) Division in three planes produces sarcinae, and (d) Division in multiple planes produces staphylococci.

Source: Tortora *et al;* 1992.

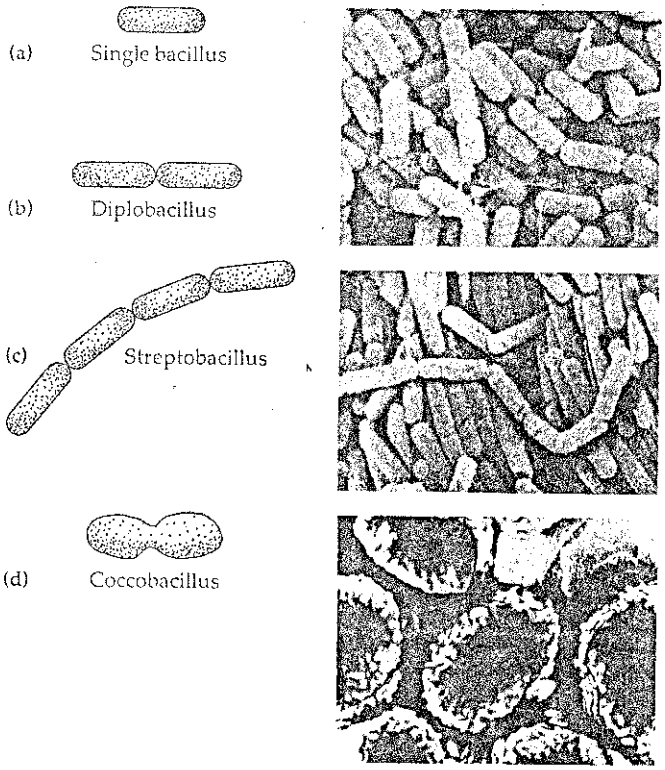

Fig.4.3: Shown in the diagram (left) and corresponding photos (right). a) Single bacilli. b) Diplobacilli. In the photos, a few joined pairs of bacilli serve as examples of diplobacilli. c) Streptobacilli d) Coccobacilli

Source: Tortora *et al;* 1992.

ii. The Bacillus (bacilli, plural)

The Single-bacillus is a single-rod-shaped cell (Figure 4.3a).The bacilli divide only across their short axis, so there are fewer groupings of bacilli than cocci. The Diplobacilli appear in pairs in a free division (Figure 4.3b).The Streptobacilli occur in chains after division (Figure 4.3c). The Coccobacilli have tapered ends like cigars; some others are oval and look so much like cocci (Figure 4.3d). The term bacillus refers to the shape of a bacterium and when italised become the name of bacterium (Bacillus).

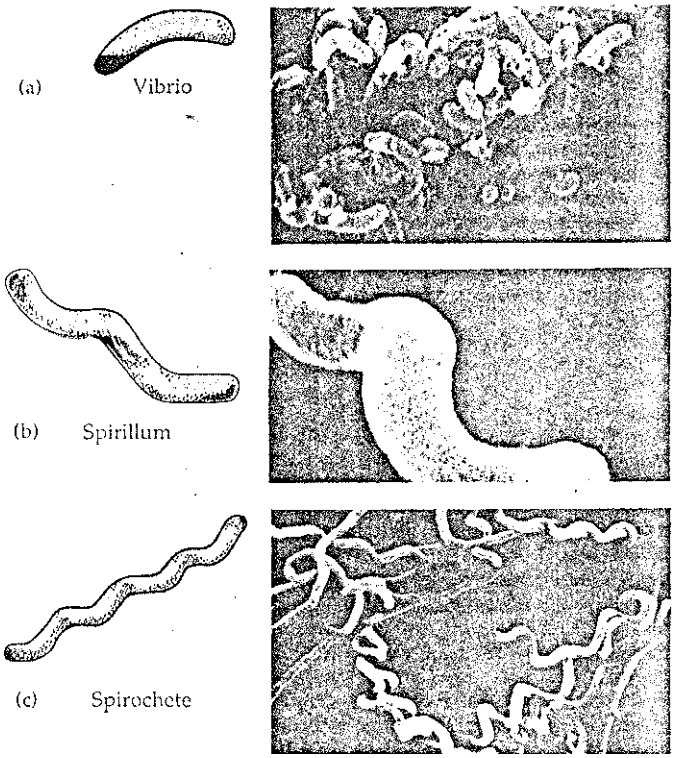

Fig.4.4: Spiral bacteria. Diagrams (left) and corresponding photos (right) of (a) Vibrio (b) Spirillum, and (c) Spirochete.

Source: Tortora *et al;* 1992.

iii. The Spirals

The vibrios are bacterial cells that are slightly curved and look like a comma, they are not twisted (Figure 4.4). The spirilla are bacteria with helical shape, like corkscrew and fairly rigid bodies, they are twisted (Figure 4.4). They have outside appendages called flagella. The spirochetes are helical and flexible spiral bacteria (Figure 4.4). The spirochetes have no flagella, move by means of an axial filament which resembles flagellum but is contained under an external flexible sheath.

iv. The Star-shaped and Square Cells

The genus Stella are star-shaped cells (Figure 4.5a). The square-shaped bacteria (flat-cells) are found in the halophilic archaeobacteria (Figure 4.5b).

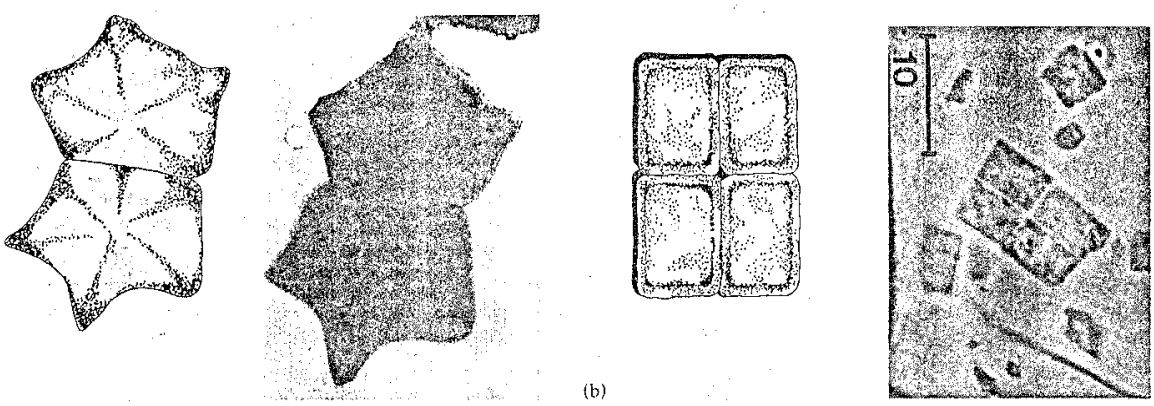

Fig.4.5: Star-shaped and square Cells (a) Stella (star-shaped). (b) Halophilic archaebacteria (square cells).

Source: Tortora *et al;* 1992.

3.1.3 The Extra-Cellular Structures in Bacteria

3.1.3.1 Glycocalyx

Is the substance that surrounds the cell. The bacterial glycocalyx is a viscous sticky gelatinous polymer that is external to the cell wall. It is composed of polysaccharides, polypeptide or both. These are made inside the cell and secreted to the cell surfaces. When the substance is organised and is firmly attached to the cell wall, the glycocalyx then becomes a capsule. It is the substance responsible for bacterial virulence in certain species. Capsules often protect pathogenic bacteria from phagocytosis by the cells of the host. Phagocytosis is a process by which certain white blood cells engulf and destroy microbes. *Streptococcus pneumoniae* causes pneumonia which is protected by a polysaccharide capsule. Uncapsulated S.pneumoniae cells are readily phagocytised and cannot cause pneumonia.

Another function of the capsule is that it allows the bacterium to attach to various surfaces in order to survive in various environments.

Through attachment, bacteria can grow on diverse surfaces such as rocks, in fast moving streams, plant roots in the soil, human teeth, and tissues and even on other bacteria.

Streptococcus mutans, cause of dental carriers, attaches itself to the teeth by glycocalyx. The capsule of *Klebsiella pneumoniae* prevents phycocytosis and allows bacterium to adhere to and colonise the respiratory tract. *S.mutans* may use its capsule as a source of nutrition by breaking it down and utilising the sugars when an energy store is low. A glycocalyx can protect a bacterium against dehydration.

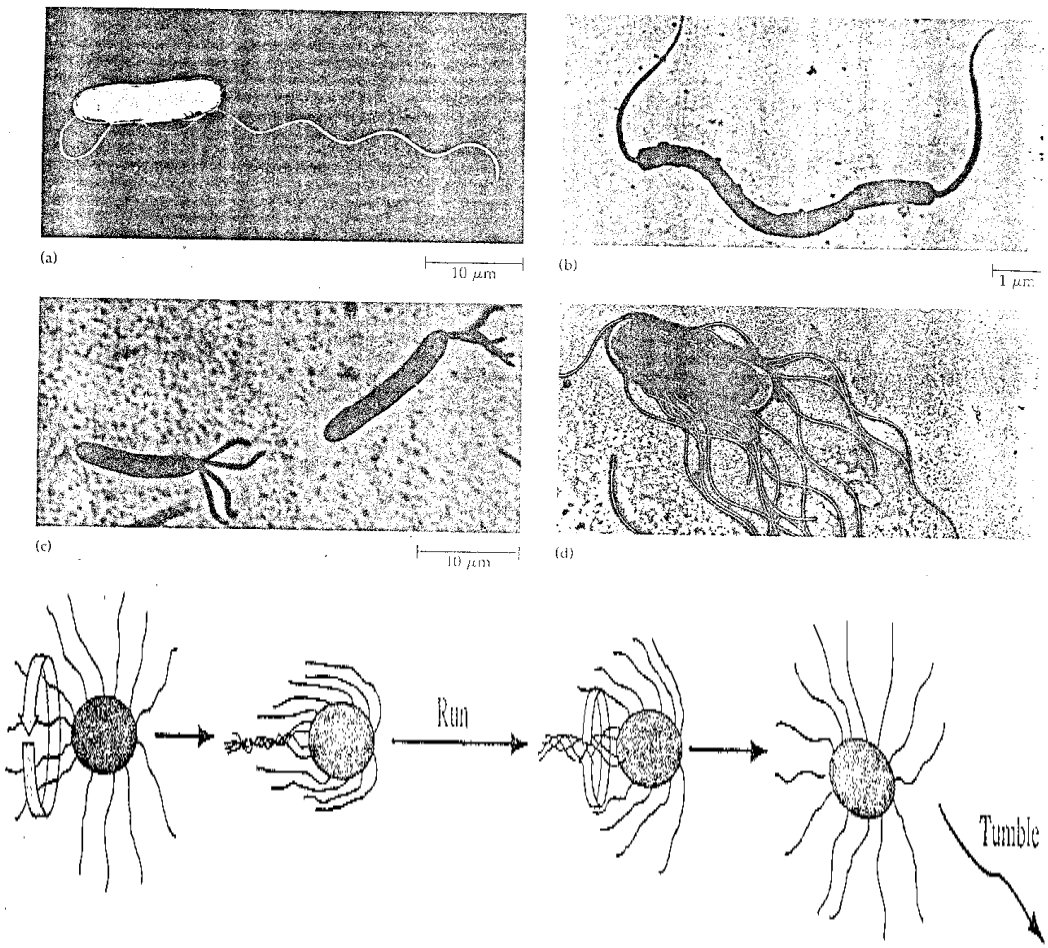

Fig.4.6: Flagella, Four Basic Types of Flagella Arrangements: a) Monotrichous (*Legionella pneumophila*) (b) amphitrichous c) Lophotrichous d) peritrichous (*Salmonella*). e) Types of bacterial motility, showing a "run" and a "tumble".

Source: Tortora *et al;* 1992.

3.1.3.2 The Flagella

These are long filamentous structures used for locomotion by bacteria (Figure 4.6). Bacterial cells have four arrangements of flagella:

a) Monotrichous: single polar flagellum only on one end (Figure 4.6a)
b) Amphitrichous: single flagellum at each end of the cell (Figure 4.6b)
c) Lophptrichous: two or more at one or both poles of the cell (Figure 4.6c).
d) Peritrichous: flagella distributed over the entire body of the bacterial cell (Figure 4.6d).

When a bacterium moves in one direction for a length of time, the movement is called “run” and “swim”. “Runs” are interrupted by periodic, abrupt, random changes in direction called “tumbles” then a run resumes again (Figure 4.6). Tumbles are caused by a reversal of flagella rotation. The proteus sp endowed with large numbers of flagella can “swarm” or show rapid wavelike growth across a solid culture medium.

The advantage of motility is that it enables a bacterium to move towards a favourable environment and away from an unfavourable environment. The movement of a bacterium towards or away from a particular stimulus is called taxis. Such stimuli include chemicals (chemotaxis) or light (phototaxis). Motile bacteria contain receptors in or just under the cell wall. These receptors pick up chemical stimuli e.g. oxygen, ribose and galactose, the information is passed to the flagella. If the chemotactic signal is positive, called attractant, the bacteria move towards the stimulus with many runs and few tumbles. If the chemotactic signal is negative, called repellent, the frequency tumbles increases as the bacterium moves away from the stimulus.

The Spirochetes have a unique structure and motility. One of the best known spirochete is *Treponema pallidum*, the causal agent of syphilis and *Borrelia burgdorferi*, the agent of Lyme disease. These two bacteria move by axial filaments, bundles of fibrils that arise at the ends of the cell beneath the outer sheath which are anchored at one end of the spirochete, have a structure similar to that of flagella. The rotation of the filaments produces an opposing movement of the outer sheath that propels the spirochetes by causing them to move like corkscrews (Figure 4.7).

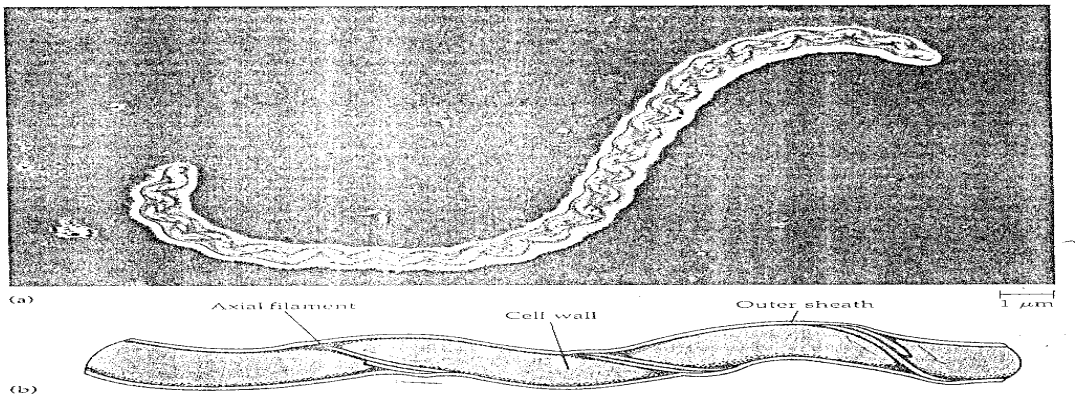

Fig.4.7: Axial Filaments a) Photomicrograph of the spirochete and Leptospira, showing an axial filament. b) Diagram of axial filaments wrapping around part of a spirochete.

Source: Tortora *et al;* 1992.

3.1.3.3 Fimbriae and Pili

Many Gram-negative bacteria contain hair like appendages (Figure 4.8) that are shorter, straight, thinner had more numerals than flagella and are used for attachment rather than for motility. These structures are made up of protein called pilin arranged helically around a central core and are divided into two types, fimbriae and pili, with very different functions.

Fimbriae can occur at the poles of the bacterial cell or they can be evenly distributed over the entire surface of the cell. They number from a few to hundreds per cell.

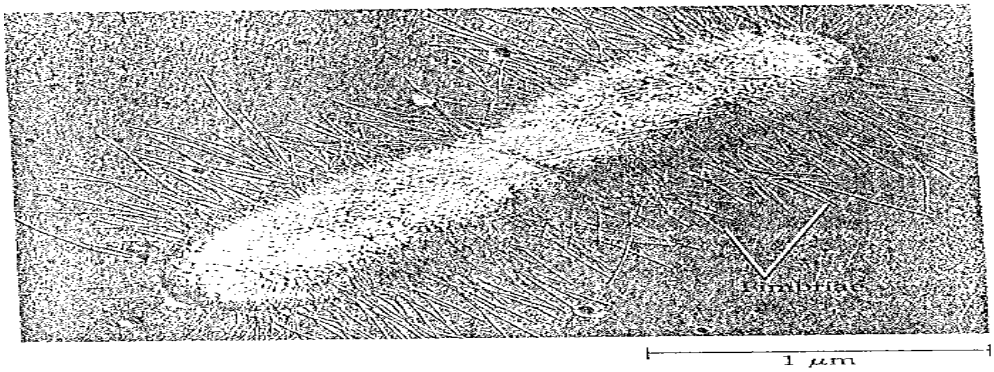

Fig.4.8: Fimbriae. The fimbriae seem to bristle from this cell of *E.coli* which is beginning to divide.
Source: Tortora *et al;* 1992.

Fimbriae also allow a cell to adhere to surfaces including the surface of other cells for example, fimbriae associated with the bacterium *Neisseria gonorrhoeae*, the agent of gonorrhea, help the microbe to colonise mucous membranes. Once established, the bacteria are capable of causing the disease. When fimbriae are absent, colonisation cannot occur and diseases are absent.

Pili are usually longer than fimbriae and number only one to two per cell. Pili function to join the bacterial cells prior to transfer of DNA from one cell to another. For this reason they are sometimes called sex pili.

3.1.4 The Cell Wall

The cell wall of bacteria cell is a complex, semi-rigid structure that is responsible for the characteristic shapes of the cell. The cell wall surrounds the underlying, fragile plasma membrane (cytoplasmic membrane) and protects it and the internal parts of the cell from adverse changes in the surrounding environment.

The major function of the cell wall is to prevent bacterial cells from rupturing, when the osmotic pressure inside the cell is greater than that outside the cell of a bacterium and serves as a point of anchorage for flagella. As the volume of bacterial cell increases, there is a corresponding extension of the plasma membrane and cell wall. Clinically, the cell wall is important because it contributes to the ability of some species to cause disease and is the site of action of some antibiotics. The cell wall is used to identify the major types of bacteria.

The bacterial cell wall is composed of a macromolecular network called peptidoglycan (murein) which is present either alone or in combination with other substances. Peptidoglycan is a mucopolysacccharide consisting of a repeating disaccharide attached to chains of four or five amino-acids. The component monosaccharides called N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) from murus, (meaning wall) are related to glucose. The structural formulars for NAG and NAN are shown in (Figure 4.9).

There are some prokayotes that have no cell walls but have very little wall materials. These include members of the genus Mycoplasma and related organisms. Mycoplasmas are the smaller known bacteria that cannot grow and reproduce outside the living cells of their host. They pass through most bacterial filters because they have no cell wall. Their plasma membranes are unique among bacteria in having lipids called Sterols which help to protect them from osmotic lysis.

Archebacteria may lack cell walls or may have unusual cell walls composed of polysaccharides and proteins but not peptidoglycan. These walls do contain a substance similar to glycan that contains N-acetyltalosaminuronic acide and instead of NAM but lacks the amino acid found in bacteria cell walls. The substance is called *pseudomurein.*

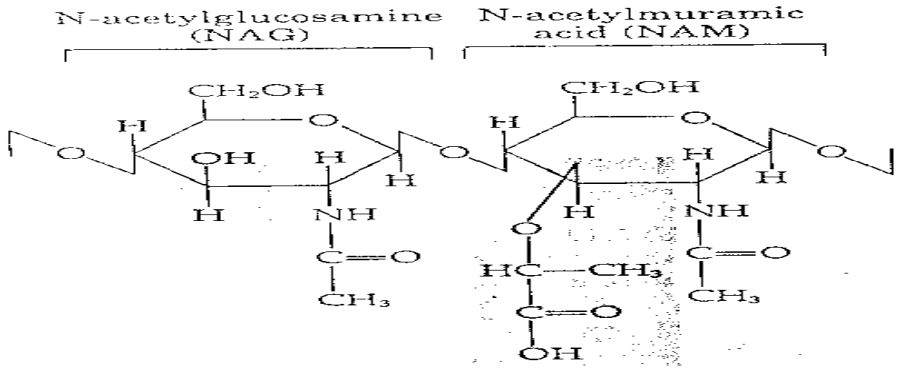

Fig.4.9: **N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) joined as in peptidoglycan. The areas showed the differences between the two molecules. The linkage between them is called a ß-1, 4 linkage.**

Source: Tortora *et al;* 1992.

Others are the forms named after Lister Institute where they were discovered. These are very tiny mutant bacteria with defective cell walls. Certain chemical and antibiotics e.g. penicillin induce many bacteria to produce L forms which tends to contain just enough cell wall material to prevent them from lysis in dilute solutions.

3.1.5 The Plasma Membrane

The plasma membrane (cytoplasmic membrane or inner membrane) is a thin structure lying inside the cell wall and enclosing the cytoplasm of the cell (Figure 4.10). The plasma membrane of prokaryotes consists of primarily of phospholipids (which are the most abundant chemicals in the membrane, and proteins. Prokaryotic plasma membranes are less rigid than eukaryotic membranes. The only exception to this is the acellular (no cell wall) prokaryote called Mycoplasma, which contains membrane sterols.

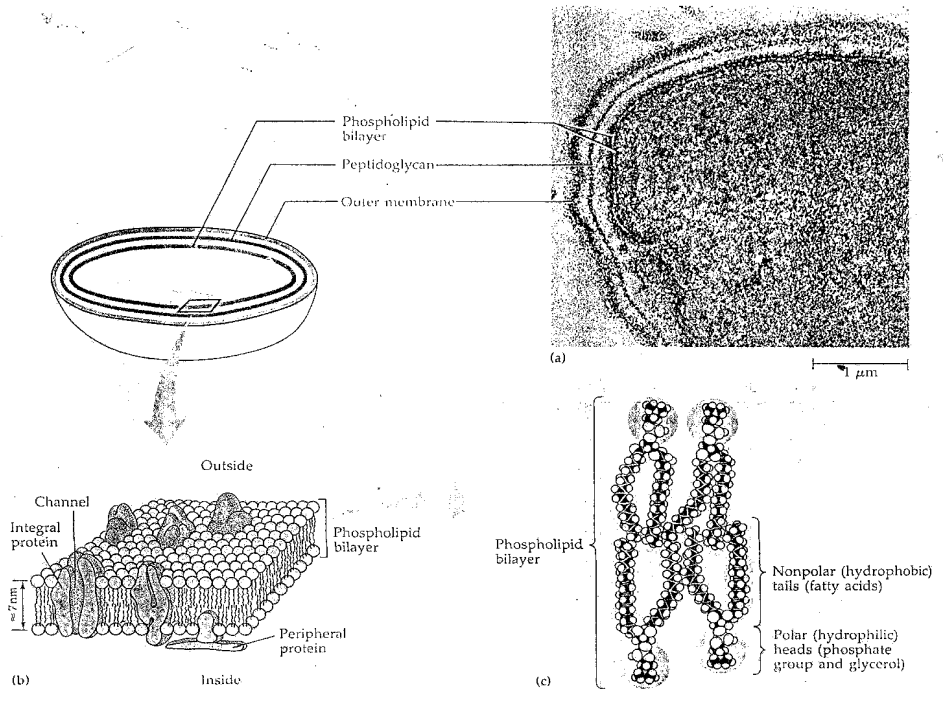

Fig.4.10: Plasma Membrane (a) Electron micrograph showing the phospholipids bilayer forming the plasma membrane of the bacterium *Bacillius brevis,* layers of the cell wall can be seen outside the plasma membrane. (b) Drawing of a membrane showing phospholid bilayer and proteins. The outermost membrane of Gram-negative bacterium is also a phospholid bilayer (c) Space-filling model of several molecules as they are arranged in the phospholipids bilayer.

Source: Tortora *et al;* 1992.

SELF-ASSESSMENT EXERCISE 1

Describe in detail the structure of a bacterial cell.

3.1.6 The Nuclear Area

The nuclear area (nucleoid) of a bacterial cell contains a single long circular molecule of double stranded DNA, the bilateral chromosomes.

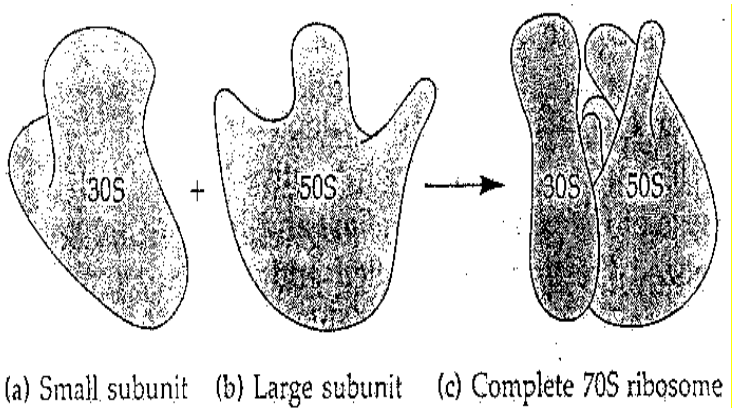

Fig.4.11: Prokaryotic Ribosome
Source: Tortora *et al;* 1992.

This is the cell genetic information, its DNA, carrying all the information required by the cell structures and functions. The bacterial chromosomes do not include the protein histones and are not surrounded by nuclear membranes. The nuclear area (nucleoid) can be spherical, elongated or dumbbell-shaped. In an active bacterial cell, 20% of it is occupied by DNA since such cells presynthesise nuclear materials for future cells. The chromosome is attached to the plasma membrane. Proteins in the chromosome are believed to be responsible for replication of the DNA and segregation of the new chromosomes to daughter cells in cell division. In addition to the cell chromosomes, these are small circular, double stranded DNA molecules called plasmids (Fig.4.1). These are extra chromosomal genetic elements which are not connected to the main bacterial chromosomes and they replicate independently of the chromosomal DNA.

Plasmids are an advantage to the cell; they carry 5 – 100 genes. Plasmids may carry genes for such activities as antibiotic resistance, tolerance to toxic metals, production of toxins and synthesis of enzymes. Plasmids can be transferred from one bacterium to another. Infact, plasmid DNA is used for genetic manipulation in biotechnology.

3.1.7 The Ribosomes

Ribosomes are cytoplasmic inclusions and all prokaryotes and eukaryotes possess ribosomes. They are the centers of protein synthesis. The cytoplasm of a prokaryotic cell contains thousands of these very small structures which give the cytoplasm a granular appearance (Figure 4.1).

Ribosome consists of two subunits, each subunit being composed of protein, and a type of RNA called Ribosome RNA (rRNA). Prokaryotic ribosomes are called 70S ribosome as against 80s of eukaryotic cells (Figure 4.11). The letter S refers to sedimentation coefficient, stands for Svedberg unit, a measure of sedimentation velocity during ultra high speed centrifugation. The higher the value of S, the faster the velocity. Therefore prokaryote ribosomes are smaller than those of the eukaryotes. The subunit of 70S ribosome consists of a small 30s subunit containing one molecule of rRNA and a larger 50s subunit containing two molecules of rRNA. Several antibiotics e.g. streptomycin, neomycin and tetracylin exert their antimicrobial effects by inhibiting protein synthesis on the ribosomes.

3.1.8 Other Cytoplasmic Inclusions

Within the cytoplasm of prokaryotic cells are several kinds of reserve deposits known as inclusions. Some inclusions are common to a wide variety of bacteria, where as others are limited to a small number of species and therefore serve as basis for identification. The following are examples:

(a) Metachromatic granules: These inclusions sometimes stain red with methyl blue and are collectively known as volutin granules. Volutin represents a reserve of inorganic phosphates (polyphosphate) that can be used in the synthesis of ATP. It is generally formed by cells that grow in phosphate rich environments. Metachromatic granules are found in algae, fungi, protozoans and bacteria. These granules are quite large and are characteristics of *Corynebacterium diptheriae*, the causal agent of diphtheria, thus they have diagnostic significance.

(b) Polysaccharide granules: They consist of glycogen and starch and their presence can be demonstrated when iodine is applied to the cells. In the presence of iodine, glycogen granules appear reddish brown and starch granules appear blue.

(c) Sulphur granules: The “Sulphur-bacteria” to which Thiobacillus belongs derive energy by oxidising sulphur and sulphur containing compounds. These bacteria may deposit sulphur granules in the cell where they serve as energy reserve.

(d) Carboxysomes: These are polyhedral and hexagonal inclusions that contain the enzymes ribosome 1-5-diphosphate carboxylase. Bacteria that use carbondioxide as their sole source of carbon require this enzyme for carbon dioxide fixation during photosynthesis. Among the bacteria containing carboxysomes are nitrifying bacteria, cyanobacteria and thiobacilli.

(e) Lipids inclusions: Lipid inclusion appears in various species of *Mycobacterium*, *Bacillus*, *Azotobacter, Spirillum.* Lipids inclusions are revealed by use of fat soluble dyes e.g. Sudan dyes.

(f) Gas vacuoles: These are hollow cavities found in many aquatic prokaryotes including cyanobacteria, anoxygenic photosynthetic bacteria and halobacteria. Each vacuole consists of rows of several individual gas vesicles which are hollow cylinders covered by protein. The function of the gas vacuole is to maintain buoyancy so that the cells can remain at the depths in the water appropriate for them to receive sufficient amounts of oxygen, light and nutrients.

3.1.9 The Endospores and their Formation

During inclement weather (e.g. lack of nutrients, absence of water), certain Gram-positive *Clostridium* and *Bacillus* form specialised resting cells called endospores. Endospores are highly durable, dehydrated cells with thick walls and additional layers. They are formed internal to the bacterial cell membrane. When released into the environment, they can survive extreme heat, lack of water, exposure to many toxic chemicals and radiation.

True endospores are usually found in Gram positive bacteria but one Gram negative species *Coriella burnelti* the cause of Q-fever, forms endospores. Endospores can be stained with endospore stains.

The process of endospores formation within a vegetative parent cell is known as sporulation or sporogenesis (Figure 4.12).

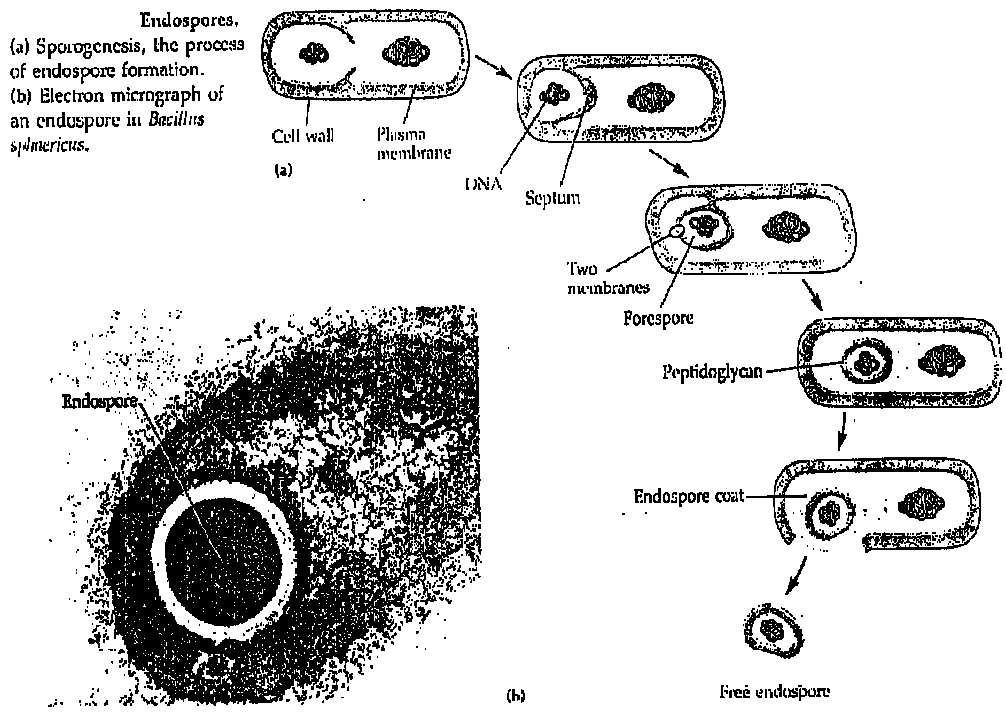

Fig.4.12: Endospores
Source: Tortora *et al;* 1992.

For sporogenesis to take place, a newly replicated bacterial chromosome and a small portion of cytoplasm are isolated by a growth of the plasma membrane called spore spectrum. The spore septum becomes a double-layered membrane that surrounds the chromosome and cytoplasm. This structures enclosed within the original cell is called a forespore. Thick layers of peptidoglycan are laid down between the two membrane layers. Then a thick spore coat of protein forms around the outside membrane. It is this coat that is responsible for the resistance of endospore to many harsh conditions. Depending on the species, the endospore might be located terminally (at one end), sub terminally (near the end or centrally inside the vegetative cell). When the endospore matures, the vegetative cell wall dissolves (lysis), killing the cell and the endospore is freed.

Most of the water present in the forespore cytoplasm is eliminated by the time sporogenesis is complete and the endospores do not carry out metabolic reactions. The highly dehydrated endospore core contains only DNA, small amounts of RNA, ribosomes, enzymes and a few important molecules. The latter include a large amount of an organic acid called dipicolinic acid (found in the cytoplasm), which is accompanied by a large amount of calcium ions. Endospores (a resting structure formed inside bacterial cell) can remain dormant for thousands of years. An endospore returns to its vegetative state by a process called germination. Germination is triggered by physical or chemical damage to the endospores coat. The endospore enzymes then break down the

extra layers surrounding the endospore, water enters, and metabolism resumes. Because one vegetative cell forms a single endospore, which after germination, remains one cell. Sporogenesis is therefore not a means of reproduction because there is no increase in the number of cells. They are a means of going through inclement weather. Endospores are resistant to processes that normally kill vegetative cells e.g. heating, freezing, dessication, use of chemicals and radiation whereas most vegetative cells are killed at a temperature of 70°C. Endospores can survive in boiling water for 19 hours. Endospores are therefore a problem in the food industry because they are likely to survive under processing and if conditions are favourable, some species produce toxins and disease e.g. *Clostridium botulinum* and *Bacillus anthracis.*

3.2 The Reproduction in Bacteria

(a) Bacterial growth refers to an increase in bacterial cell numbers and not an increase in size of the individual's cells. Bacteria normally reproduce by binary fission.

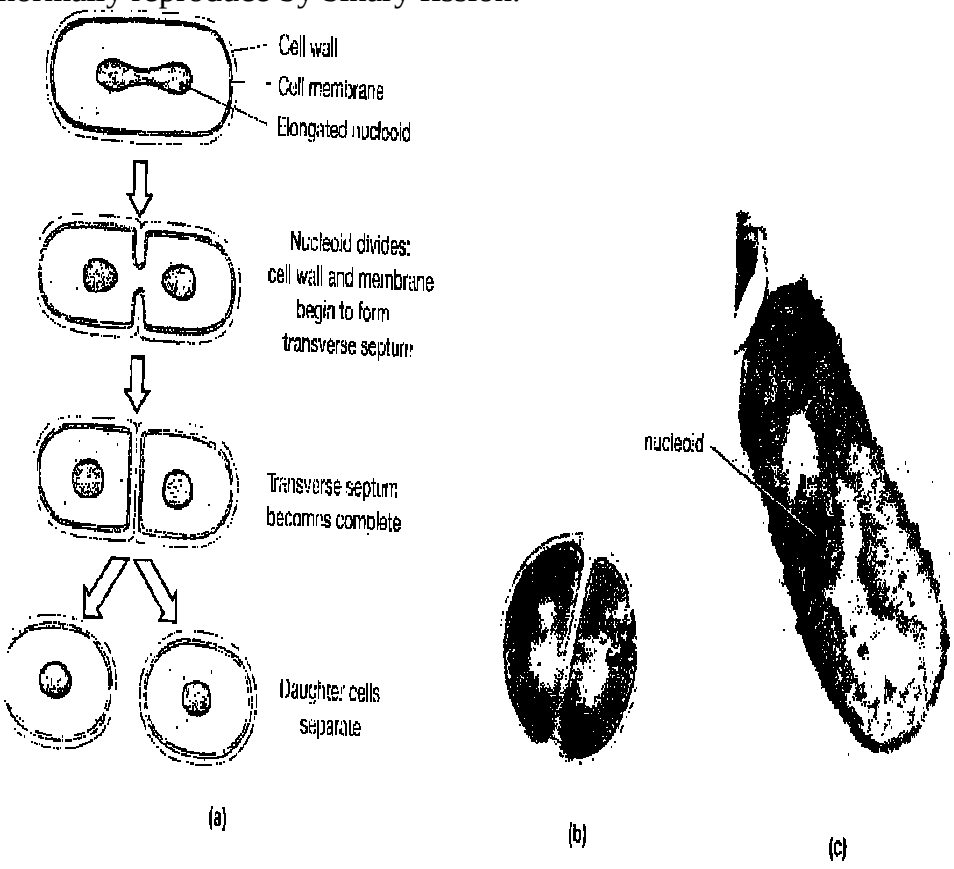

Fig.4.13: Binary Fission in Bacteria
Source: Jacquelyn, 2002.

The first step in division is the elongation of the chromosomal DNA. Eventually the growing cell walls meet and two morphologically and physiologically similar daughter cells are

formed, each of which is identical to the mother cell (Figure 4.13).

(b) A few bacterial species reproduce by budding, that is, they form initial outgrowth that enlarges until its size approaches that of the parent cell and then separates.

(c) Some filamentous bacteria, the actinomycetes reproduce by producing chains of spores carried externally at the tips of the filaments. A few filamentous species fragment to produce chlamydospores and arthrospores. These fragments initiate the growth of new cells.

(d) There could be exchange of genes between two DNA molecules of the bacterial chromosomes to form a hybrid gene. This is called genetic recombination.

(e) Genes can also be transformed from one bacterium to another in a process referred to as transformation.

(f) Another method of reproduction is by conjugation. Conjugation in bacteria is carried out by plasmids (Figure 4.1). The conjugating bacterial cells must be of opposite mating types. The donor is replicated during transfer of a single-stranded complementary strand. Note that conjugation requires that there must be direct contact unlike the transformation.

SELF-ASSESSMENT EXERCISE 2

Why are mycoplasmas resistant to antibiotics?

4.0 CONCLUSION

Most bacteria have cells while a few are acellular. Cellular bacteria are of diverse morphology and their cell wall is complex. There are structures outside the cell wall and reproduction in bacteria is discussed.

5.0 SUMMARY

In this unit, you have learnt the:

- basic structure of the bacterium
- different morphological types of bacteria
- extra-cellular components on the cell wall
- complex nature of the cell wall
- various types of cellular inclusions
- sporogenesis
- reproduction in bacteria.

6.0 TUTOR-MARKED ASSIGNMENT

i. Why is an endospore called a resting spore? What is the significance of the endospore?
ii. Of what use is each of the following to the cell?
    a) Metachromatic granules
    b) Polysaccharide granules
    c) Lipids inclusions
    d) Sulphur granules
    e) Carboxysomes
    f) Gas Vacuoles
iii. Draw the examples of bacteria listed below.
    a) spiral
    b) bacillus
    c) coccus
    d) spirochaete
    e) streptobacilli
    f) staphylococcus
iv. With relevant diagrams explain the meaning of
    a) Lophotrichous
    b) Monotrichous
    c) Peritrichous
v. Describe reproduction in the bacterial cell; illustrate your answer with diagrams.
vi. Write short notes on
    a) Bacterial ribosomes
    b) The nucleoid
vii. With appropriate diagram describe locomotion in the bacteria.

7.0 REFERENCES/FURTHER READING

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UNIT 5 IMPORTANCE OF MICRO-ORGANISMS: THE BACTERIA

CONTENTS

1.0 INTRODUCTION

Bacteria are generally known as enemies of man, plants and animals because of their role in spoilage of food, drinks and causing diseases like tuberculosis, tetanus, diphtheria and cholera. A large number of bacterial diseases are also inflicted on crops; they reduce the quality of crop products and their market value. Animals also suffer similar predicaments; the deleterious and beneficial effects of bacteria will be discussed in this unit.

2.0 OBJECTIVES

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

- explain the beneficial effects of bacteria in the environment, industries, medicine and agriculture
- discuss the harmful effects of bacteria on plants and human lives.

3.0 MAIN CONTENT

3.1 Importance of Bacteria

Bacteria are generally known to be important in the lives of man, plants, and animals. They bring about food spoilage and food poisoning to man. Bacteria are known to cause a number of diseases in plants, animals and man. Many bacteria however, are important because of their positive contributions to improve the life of mankind.

3.1.1 Beneficial Effects of Bacteria

(a) Bacteria as Decomposers

Saprophytic bacteria are decomposers, decomposing pure fraction of dead organic materials, plants debris and excreta. During the process, these compounds are reduced to carbon, oxygen, hydrogen, nitrogen, sulphur and phosphorous which are again made available to the living plants.

If it were not for fungi and bacteria as decomposers, these elements that are essential to life would have become buried in the dead plants and animals.

(b) In Sewage Disposal

Millions of aerobic and anaerobic bacteria (both pathogenic and non pathogenic) convert organic materials in the sewage to inorganic forms through mineralisation or stabilisation and reclaim water from domestic and industrial wastes. The methanogenous bacteria e.g. methane bacteria, *Methanococcus* and M*ethanocarcina* produce methane during the anaerobic process phase of the decomposition of the sewage. The methane is collected and used as a fuel.

(c) Bacteria in Dairy Products

Lactobacillus sp. in combination with yeasts and moulds are used in the preparation of fermented foods such as cheese, pickle, soy sauce, saucerkraunts, vinegar, wine and yoghurt.

(d) Bacteria in the Industry

i) Vinegar Production: Sugar solutions are commercially fermented by lactic acid bacteria e.g. *Mycodema aceti* into acetic acid (vinegar) and water.

ii) Manufacture of Acids: Lactic acid, citric acid, gluconic acid, and butyric acid are produced from glucose and other sugars (industrially) by the activity of species of *Lactobacillus, Bulgaricus, Delruickii and Clostiridium.* Lactic acid itself is used in the manufacture of food products, leather, and pharmaceuticals, paints and plastics.

iii) Manufacture of Acetone and Alcohol: Acetone, butyl alcohol and ethyl alcohol are commercially obtained by the distillation of molasses fermented by anaerobic bacterium C*lostridium acetoburylium.*

iv) Curing and Ripening of Tea and Tobacco: Bacteria are used for curing crude leaves of tea and tobacco using *Micrococcus candidians* to act on the hung leaves. This process adds flavour to tea and tobacco leaves, thus increasing their market value.

v) Fibre Retting: The fibre of jute, hemp, flax that held together in close association is separated by the reaction of bacteria including *Clostridium felsineum* and

C.pectirovorum. These plants are then soaked in water and swell. The bacteria hydrolyse the pectic substances of the middle lamellae of the cells. The fibres are thus separated and used in making ropes and sacks.

(e) Bacteria and Bioremediation

Bacteria have been used in waste processing and bioremediation. Bioremediation is the use of biologically mediated process to remove or degrade pollutants from specific environment (e.g. in oil spillage or pollution) they are capable of digesting or degrading the hydrocarbon in petroleum to clean up spill e.g.*Nocardia* and *Rhodococcus.* Bacteria are also used for the bioremediation of industrial toxic wastes. In the chemical industry, bacteria are most important in the production of pure chemicals for use as pharmaceutical or agrochemicals (Pandey and Trivedi, 2006).

(f) In Biological Control

Antagonistic bacteria e.g. *Bacillus thuringiensis* (BT) can be used in place of pesticides as biological control agents. Subspecies of this bacterium are used as lipidopteran-species insecticides under trade names as dipel and thuricide. Some antibiotics from this bacterium can be used to control plant diseases (Pandey and Trivedi, 2006).

(g) Bacteria in the Rumen of Cattle

The digestion of cellulose in the intestinal tracts of herbivores (cow and goat) is largely catalysed by enzymes secreted by bacteria e.g. *Ruminococcus albus, Bacteroides succinogenes.* No vertebrate produces cellulose digesting enzymes (the cellulases). The bacteria in their rumen (digestive tract) help them to break down the cellulose in the grass they eat when they graze.

(h) In Enzyme Production

Bacteria produce various types of enzymes that are used in food industry and in the production of chemicals. These include amylase *(Bacillus),* protease *(Bacillus subtilis),* penicillinase *(Bacillus subtilis, B. cereus),* streptodonnase amd streptorinase*(Streptococcus pygenes).*

(i) In Steroid Production

Steroids are hormones usually obtained from the body of animals and used for treatment of various diseases. Recently however, steroids have been obtained by using bacterial species of *Corynebacterium* and *Streptomyces.*

(j) In Vitamins Production

Several species of bacteria have been used to obtain vitamins e.g. C*lostridium butylinum* produces riboflavin (vitamin B2) *Propionibacterium shermaii* and *Pseudomonas denitrificans* are used to produce vitamin B12. Harmless *Escherichia coli* produces vitamin K12.

(k) Bacteria and Medicine

Because of their rapid growth rate and the relative ease with which they can be manipulated, bacteria are the work horses in molecular biology, genetics, biochemistry and biotechnology. Another important application of studies on recombination DNA technology established through *E.coli*, is the production of therapeutic proteins. These include insulin (to cure diabetes), growth hormones (for pituitary dwarfism), interferons (for curing cancer), erythropoilin (for anaemia interlewkine-2).

(l) In Gene-therapy

The goal of gene therapy is to correct genetic diseases caused by mutant genes that do not produce functional enzymes. To correct this, the normal gene is introduced into bacterial cells to restore proper enzymes production. This can be done in two ways. In one case (ex-vivo), cells are removed from the patient's body, the proper DNA is inserted and the cells are then returned into the patient's body or in other case (in-vivo) carrier molecules or vectors e.g. *E.coli*, and viruses are used to take the normal gene into cells in the patient's body.

(m) Bacteria as Vector in Biotechnology

The DNA engineered in the test tube must be returned to a living cell in order to function. Most genetic engineering procedures need carriers known as **cloning vectors** for moving recombitant DNA from test tubes back into cells. Bacterial plasmids is one of the two vectors used e.g. *Escherichia coli* and *Agrobacterium*. A plasmid is a double –stranded DNA molecule that can exist and replicate independently of the chromosome. Recombinant plasmids thus produced by splicing restrictions fragments from foreign DNA into plasmids isolated from bacteria can then be returned easily to bacteria. The bacterium replicates the recombitant plasmids as the original cell produces a colony (Campbell, 1999).

(n) In the Production of Antibiotics

Several antibiotics have been produced from bacteria which have proved effective against infections. These include:

i. actidione*(Streptomyces griseus*)
ii. aureomycin*(Streptomyces aurreofacines)*
iii. bactrin*(Bacillus subtilis)*
iv. chromycin*(Streptomyces venezula)*
v. kanamycin*(Streptomyces kanamyceticus*)
vi. neomycin*(Streptomyces fradiae*)
vii. rifanpin*(Streptomyces mediterranei*)
viii. viocin *streptomyces puniceus* produces
ix. erythrocin*(Actinobacteria and Streptomyces eruthreus*)
x. streptomycin(*Actinobacteria and Streptomycin griseus)*
xi. terramycin*(Actinobacteria and Streptomycin rimosus).*

(o) In Hydrogen Gas Production

Clostridium perfringens produce large quantities of hydrogen gas during fermentation of carbohydrates.

(p) Bacteria and Agriculture

Bacteria along with other soil micro-organisms play a dominant role in the recycling of mineral nutrients in the soil. This is achieved through the following:

i) Nitrogen Cycle: Through the activities of species of *Azotobacter, Clostridium, Rhizobium* (nitrogen fixing bacteria) converts nitrogen to ammonia (ammonification). This is acted upon by species of *Nitrobacter* and *Nitosomonas* which oxidise ammonia to nitrite and the *Nitrobacter* converts nitrite to nitrate which is made available to plants.

ii) Sulphur Cycle: The autotrophic bacteria oxidise sulphur to sulphate which is made available to the plants. These bacteria include *Thiobacillu thioxidans.* Heterotrophic bacteria also liberate sulphur in the form of hydrogen sulphide which can also be produced by reduction of sulphate by the species of Desulfovibrio and Desulfuromonas. Hydrogen sulphide can also be reduced to elemental sulphur by species of *Beggiatoa.*

SELF-ASSESSMENT EXERCISE 1

Describe the contribution of bacteria to industry.

3.1.2 Harmful Effects of Bacteria

Bacteria are also important in agriculture causing disease on plants, fruits and seeds, thereby reducing crop yield and quality. Certain bacteria are responsible for many human and animal disease conditions.

(i) In Reduction of Soil Fertility

Thiobacillus denitificans and *Micrococcus denitrificans* are capable of transforming nitrates to nitrogen which is released into the atmosphere. This process of denitrification can lead to loss of much needed nitrates in the soil.

(ii) Bacteria as Plant Pathogens

Table below shows some examples and names of diseases caused by some bacteria on their host plants.

Table 5.1: Some Plant Diseases that are Caused by Bacteria

DISEASE	CAUSAL ORGANISM	PLANTS
Citrus canker angular leaf spot	*Xanthomonas citri*	Citrus sp.
Blackarm of cotton	*Xanthomonas malvacearum*	Cotton
Bacterial wilt of maize	*Xanthomonas stewarti*	Maize
Bacterial tomatoe canker	*Corynebacterium micniganese*	Tomatoes
Black rot of cabbage	*Xanthomonas campestins*	Cabbage
Fire blight of apple	*Erwinia amylovora*	Apple
Leaf blight of rice	*Xanthomonas oryzae*	Rice
Redshine of sugar cane	*Xanthomonas rubric lineans*	Sugar cane
Nut disease of potatoes	*Pseudomonas solanacearum*	Potatoes
Yellow ear rot of wheat	*Corynebacterium tritici*	Wheat

Source: Pandey and Trivedi, 2006.

(iii) Some Bacterial Infections in Man

Many bacteria species which grow on food stuffs liberate exotoxins causing food poisoning in man. One of the bacteria responsible for this is *Staphylococcus* and *S.aureus* which are the most common source of food poisoning thereby releasing exotoxin known as enterotoxin while growing on food.

Other bacterial diseases in man are:

i. bacterial diarrhea(*Campylobacter fetus)*
ii. eye infection(*Bacillus circus)*
iii. pubonic plague *(Yersinia pestis)*
iv. meningococcal *meningitis* in both young and adults.
v. *(Neisseria meningittidis)*
vi. gonorrhoea*(Neisseria gonorrhoeae)*
vii. whooping cough*(Bordetella pertussis)*
viii. slain infections*(Staphylococcus* and *streptococcus).*

(iv) Pathogenic Bacteria in Animals and Man

Clostridium botulinum produces toxin in food and on wounds leading to botulism (Wells and Wilkins, 1996). *C.difficile* causes *pseudomembrane colitis; C.perfringes* synonymous with *C.welchii* causes food poisoning and gas gangrene and *enteroxemia* known as overeating disease or pulpy kidney disease in sheep and goats. *C.tetani* causes tetanus in man. *C.betalinum* bacteria from honey cause infant botulism in one year old and younger babies. The bacterium produces botulin toxin which

eventually paralyses the infants breathing muscles. *Escherichia coli* may cause serious food poisoning in the gut especially the virulent strains (Vogt and Dippoid, 2005).

*Enterotoxigenic E.coli (*ETEC) causes diarrhea without fever in humans, pigs and sheep. More than 200 million cases of this diarrhea and 380,000 deaths mostly children have been reported across developing countries. *Enteropathogenic E.coli (*EPEC) causes a syndrome identical to *Shigellosis* with profuse diarrhea and high fever.

*Enterohemorrhagic E.coli (*EHEC) causes bloody diarrhea without fever. EHEC can also cause heamolytic-uremic syndrome and sudden kidney failure.

Enteroaggregative E.coli (EAggEC) causes watery diarrhea without fever.

Salmonella enterica var chloeraesius causes hog cholera. *Salmonella enterica* var *thyphimurium* causes typhoid fever with watery stools in man and animals. *Vibrio cholera* causes cholera, *Mycobacterium tuberculosis* causes tuberculosis; *M.leprae* causes Hansens disease called leprosy in humans (Pandey and Trivedi, 2006).

SELF-ASSESSMENT EXERCISE 2

Enumerate, citing specific examples the ailments inflicted on man by bacteria.

4.0 CONCLUSION

Bacteria by their various activities can both be beneficial and harmful to the environment, plants, man and animals.

5.0 SUMMARY

In this unit, you have learnt the roles of bacteria in the:

- decomposition of dead organic matter in soils
- sewage dispersal, dairy products and industry
- bioremediation, biological control and rumen of herbivores
- steroids, vitamins, hydrogen gas, enzyme and antibiotic production
- fields of medicine, gene therapy, biotechnology and agriculture
- reduction of soil fertility
- establishment and production of infections in plants, animals
- and man.

6.0 TUTOR-MARKED ASSIGNMENT

i. Describe the importance of bacteria in medicine.
ii. How important are bacteria in agriculture?
iii. Enumerate the diseases inflicted on man by bacteria.
iv. What specific roles do bacteria play in the environment?
v. Bacteria are friends or foes. Discuss.
vi. Describe the contribution of bacteria in the industry.
vii. Describe the role of bacteria in sewage disposal.

7.0 REFERENCES/FURTHER READING

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UNIT 6 STRUCTURE AND REPRODUCTION: THE VIRUSES

CONTENTS

1.0 INTRODUCTION

Various researchers (Mayer in 1883, Ivanowsky in 1892, and Beijerinck in 1897) discovered viruses as filterable agents that are capable of causing diseases in plants, animals and man. Viruses were also found to replicate (reproduce) within a living host cell. This means that all viruses cannot live or survive outside their living hosts. Viruses have simple structures. A typical virus consists of a nucleic acid, ribose nucleic acid (RNA) or deoxyribose nucleic acid (DNA) and a protein coat, the capsid. Some viruses in addition to the single nucleic acid and capsid have additional structures. Some viruses infect bacteria and these viruses are called bacteriophage or simply phages. The identities (structures) of viruses can only be seen or revealed under the electron microscope and other techniques like the x-ray crystallography.

In this unit, you will be introduced to the various types of viruses, the nature and processes of their replication (reproduction).

2.0 OBJECTIVES

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

- describe the nature and structures of viruses
- explain the various types of viruses
- describe the various steps in the reproduction (replication) of viruses.

3.0 MAIN CONTENT

3.1 Structures and Reproduction of Viruses

3.1.1 Structure of Viruses

a. Viroid and Virion

A viroid is a plant pathogen composed of molecules of naked RNA only and several hundred of nucleotide. The virus takes the form of a particle called virion. A virion is a complete, fully developed viral particle composed of nucleic acid surrounded by a coat that protects it from the environment and serves as a vehicle of transmission from one host cell to another. A virion consists of a nucleic acid genome surrounded with a protein layer called capsid. The virion contains only one type of nucleic acid which could be either ribose nucleic acid (RNA) or deoxyribose nucleic acid (DNA). Virions have simple regular shapes and sizes. Virus= DNA or RNA+protein

A typical virus may contain single stranded RNA or DNA, double stranded RNA or DNA depending on the specific type of virus. A virus is called DNA or RNA virus depending on the type of genome (RNA or DNA) it carries. The protein structure that encloses the genome (capsid) are built from large number of proteins subunits called capsomeres. Some viruses have viral envelopes which are made from membranes cloaking their capsids. The envelopes are derived from host cell membrane plus phospholipids and proteins. Proteins and glycoproteins are covalently bonded to carbohydrates viral origin (e.g. *influenze virus).*

b **Viroids as Unusual Agents**

A few plant diseases e.g. potato spindle-tuber diseases are known to have been traced to viroids. A viroid is merely a circular, single-stranded RNA, a of molecule 250 to 370 nucleotides, much smaller than the smallest viral genome. After being transmitted from one plant to another mechanically or through pollens or ovules, viroids may multiply massively on the new host cells, mostly in the nucleoli. They do not act as mRNA to direct protein synthesis and it is not known how they cause diseases. In fact, the same viroid may have little effect on one host but produce a severe disease in another. Viroids show that nucleic acids, with their intrinsic property of replication, may reproduce in surprising ways, often at the expense of other biological systems (Campbell, 1996).

c **Virus Size**

The sizes of viruses were first estimated by filteration through membranes of known pore diameter. Viral sizes are determined

today by ultra configuration and by electron microscopy, which seems to produce the most accurate results. Viruses vary considerably in size. Although, most of them are quite a bit smaller than bacteria, some of the larger viruses (e.g. small viruses-poxyvirideae) are about the same size as some very small bacteria (e.g. mycoplasmas, rickettsias and *Chlamydia*). Viruses range from 20-300nm in diameter. The comparative sizes of several viruses and bacteria (Figure 6.1) as well as sizes of various viruses compared with a human red blood cell (Figure 6.2) are shown below.

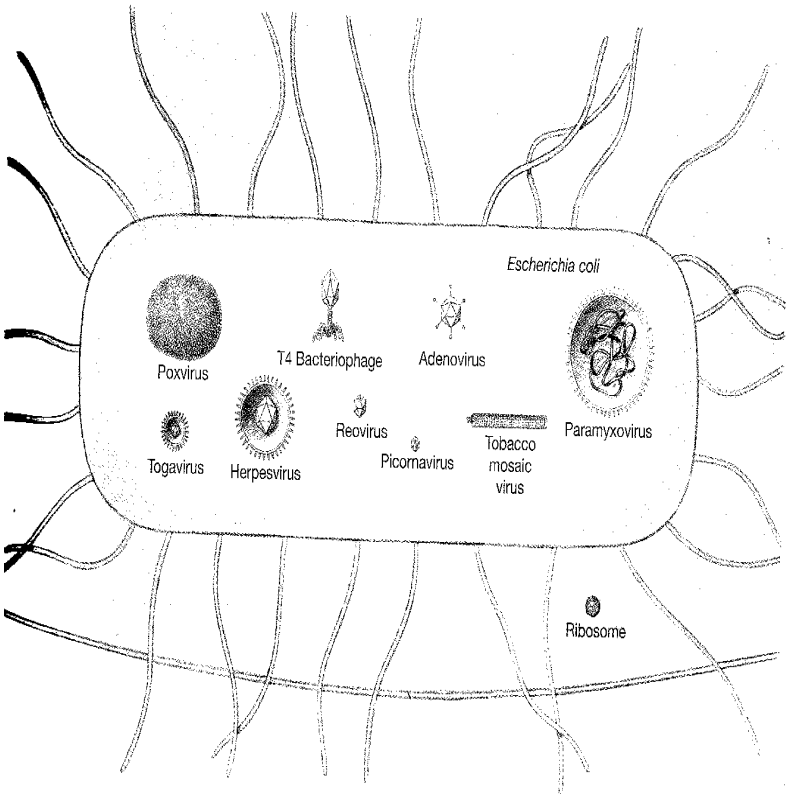


Fig.6.1: Variations in Shapes and Sizes of Viruses Compared with a Bacterial Cell, an Animal Cell and a Eukaryotic Ribosome

Source: Jacquelyn, 1996.

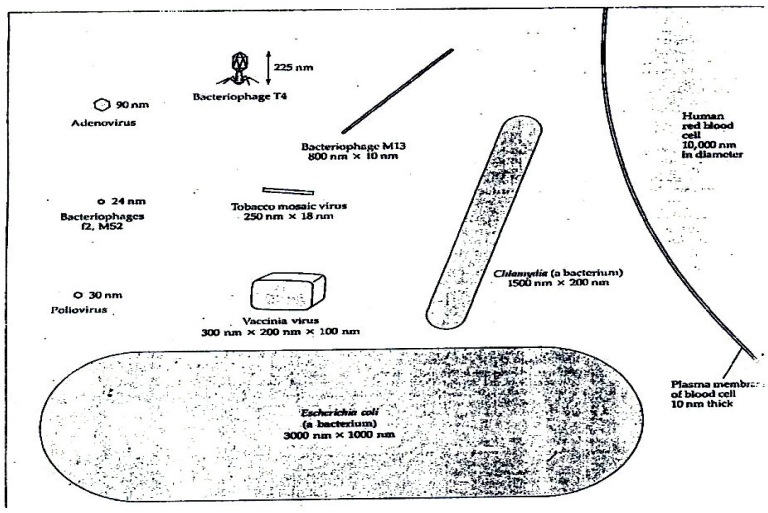


Fig.6.2: Virus Sizes. Sizes of several viruses compared with a human red blood cell, shown to the right of the microbes. Dimensions are given in nanometers (nm) and are either diameters or length by width.

Source: Tortora *et al*, 1992.

d **Viral Nucleic Acid**

The core of a virus contains either DNA or RNA, which is the genome material. The percentage of nucleic acid in relation to protein is about 1% for the influenza virus and about 50% for certain bacteriophages. The total amount of nucleic acid varies from a few thousand nucleotides (or pairs) to as many as 250 thousand nucleotides (*Escherichia coli's* chromosome consists of approximately 1million nucleotide pairs). In contrast to prokaryotic and eukaryotic cells, in which DNA is always the primary genetic material and RNA plays an auxiliary role, a virus can have either a DNA or RNA. Depending on the virus, the nucleic acid can be linear or circular. In some viruses, (e.g. the influenza virus) the nucleic acid is in several segments.

e **Capsid and Envelope**

The nucleic acid of a virus is surrounded by a protein coat called capsid (Figure 6.3a). The structure of the capsid is ultimately determined by the viral nucleic acid and accounts for most of the mass of a virus, especially of small ones. Each capsid is composed of protein subunits called capsomeres. In some viruses, the protein composing the capsomers is of single type. In other viruses several types of protein must be present. Individual capsomers are often visible in electron micrographs (Figure 6.3a) the arrangement of capsomers is characteristic of a virus.

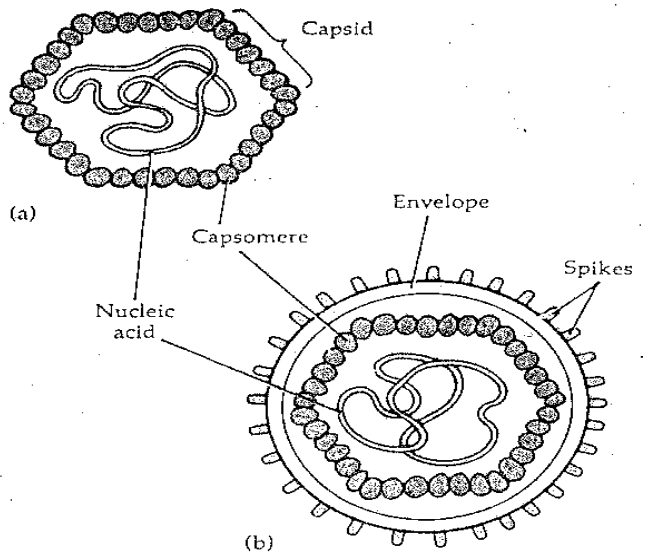


Fig.6.3: General Structure of Two Types of Viruses (a) Naked Virus (b) Enveloped Virus with Spikes
Source: Tortora *et al,* 1992.

In some viruses, the capsid is covered by an envelope (Figure 6.3b).
This usually consists of some combinations of lipids, proteins and carbohydrates. Some animal viruses are released from the host cell by an extrusion process that coats the virus with a layer of the host cells plasma membrane, that layer becomes the viral envelope. In many cases, the envelope is made up of proteins determined by viral nucleic acid and materials derived from the normal host cell components. Depending on the viral envelope it may be covered by spikes (Figure 6.3b) with the carbohydrates-protein complexes that project from the surface of the envelope (glycoprotein). Some viruses attach to the host cells by means of these spikes. Spikes are such reliable characteristics of some virus that they can be used as a means of identification. The ability of the influenza virus, to cling to the red blood cells and form bridges between them is due to its spikes. The resulting clump is called hemagglutination and is the basis of several useful laboratory tests (Tortora *et al,* 1992). Viruses whose capsids are not covered by an envelope are known as naked viruses or non enveloped viruses (Figure 6.3a). The capsid of naked virus protects the nucleic acid from nuclease enzymes in biological fluids and promotes the viruses' attachments to susceptible cells. When the host cells have been infected by a virus, the host immune system produces antibodies (proteins that react against the virus) to inactivate that virus and stop the infection. Some viruses can escape antibodies because of regions of genes that code for these viruses surface. Protein is susceptible to mutations. Mutant viruses alter their surface proteins so that

the antibodies are not able to react with them. Influenza virus frequently undergoes such changes on its spikes. This is why a certain individual can be infected with influenze several times. Although antibodies might have been produced to one influenza virus, the virus will mutate and be able to cause infection on the same host again.

f Virions

Virions have simple, regular shapes and sizes.The nucleic acid and capsid of a virion together forms a nucleocapsid and this can be of various shapes. Some nucleocapsids are enveloped- an envelope that is created from a membrane of the host cell but this does not give a virion the property of a cell. A protective capsid can be assembled around a nucleic acid to make either a helical or spherical structure. A helical structure is made by stacking identical subunits called capsomeres (Figure 6.3a) that enclose the nucleic acid in an internal groove. Some of them have such a nucleocapsid enclosed in an envelope. A spherical virion is made up of protein subunits that form a shell around a core of nucleic acid. In each case, the size of the virion reflects the size of nucleic acid. However all spherical capsids are icosahedral i.e. a solid with 20 identical triangular faces-pentagons or hexagons. Helical viruses (Figure 6.5) resemble long rods that maybe rigid, their capsid is a hollow cylinder with a helical structure. An example of helical virus that is a rigid rod is the tobacco mosaic. Another is bacteriophage M13. Many animal, plant and bacterial viruses are polyhedreal, that is they are many sided (Figure 6.4).

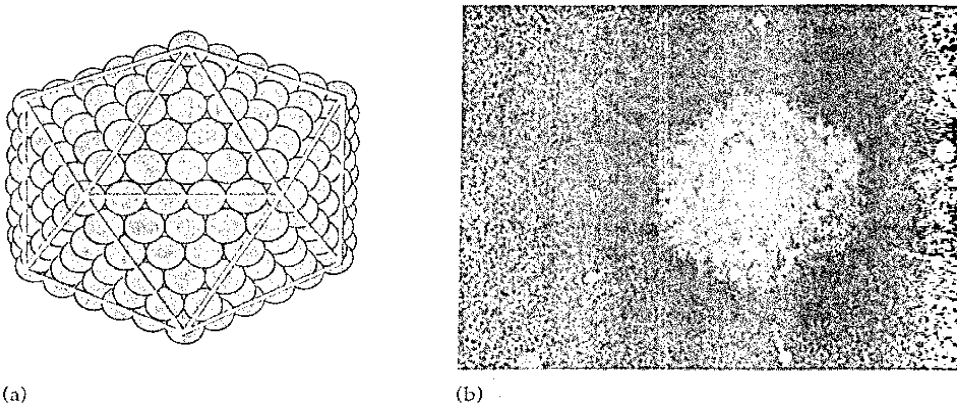


Fig.6.4: Morphology of a Naked Polyhedral Virus in the Shape of an Icosahedron. (a) Diagram of an icosahedron (b) Electron micrograph of an adenovirus. Individual capsomers in the protein coat are visible.

Source: Tortora *et al,* 1992.

The capsid of most polyhedral viruses is in the shape of an icohedron, a regular polyhedron with triangular faces and 12 corners (Figure 6.4). Example of polyhedral viruses is adenovirus and poliovirus.

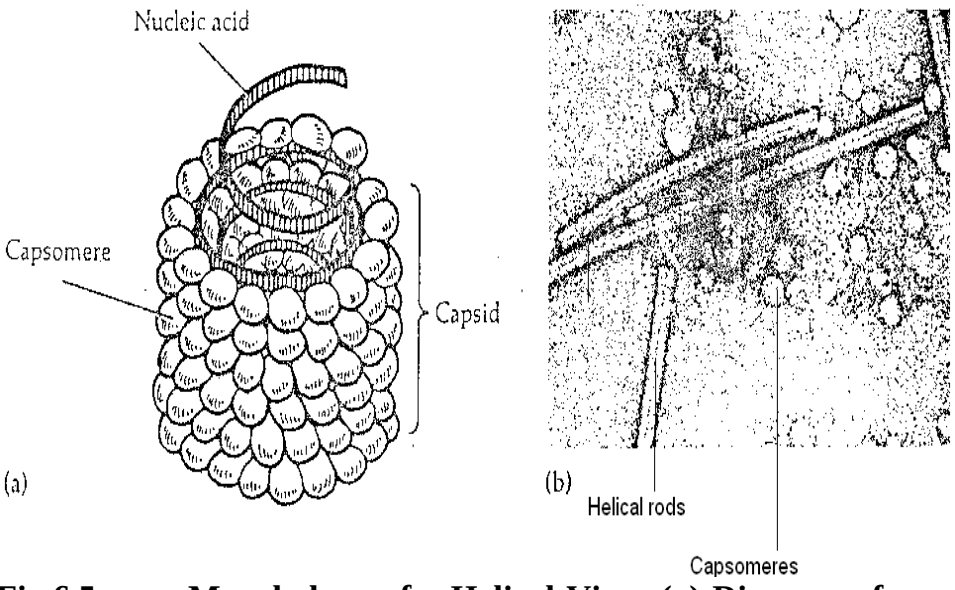


Fig.6.5: Morphology of a Helical Virus (a) Diagram of a portion of tobacco mosaic virus. Several rows of capsomers have been removed to reveal the nucleic acid. (b) Electron micrograph of tobacco mosaic virus showing helical rods.

Source: Tortora *et al,* 1992.

g. **Enveloped Viruses**

The capsid of some viruses is covered by an envelope. Enveloped viruses are roughly but highly pleomorphic (variable in shape) because the envelope is not rigid. When helical or polyhedral viruses are enclosed in an envelope, they are called enveloped helical or enveloped polyhedral viruses. Example of an enveloped helical virus is the *influenze virus* (Figure 6.6) and that of an enveloped polyhedral (icosahedral) virus is the herpers simplex virus (Figure 6.7). Some viruses, particallarly bacterial viruses, have very complicated structures and are called complex virus.e.g. poxviruses which do not contain clearly identifiable capsids but have several coats around the nucleic acid (Figure 6.8).

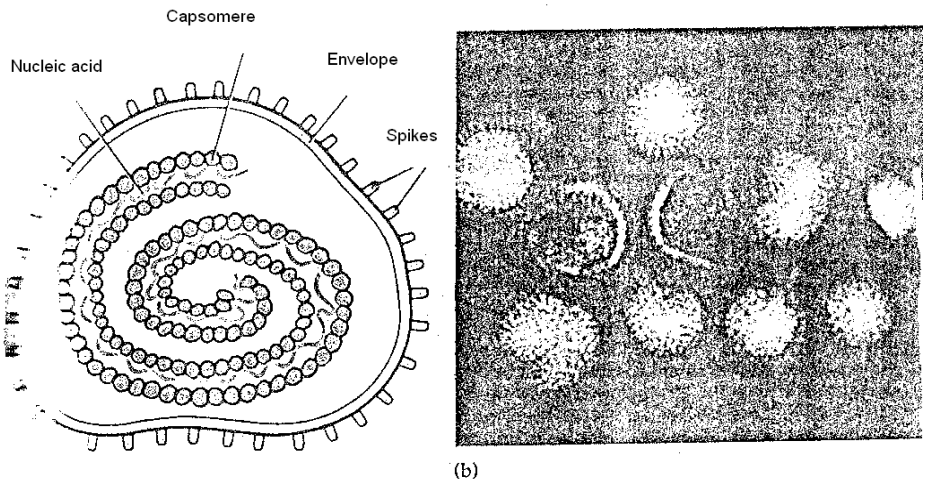


Fig. 6.6: Morphology of an Enveloped Helical Virus. (a) Diagram of an enveloped helical virus. (b) Electron micrograph of influenza viruses. *Source*: Tortora *et al;* 1992.

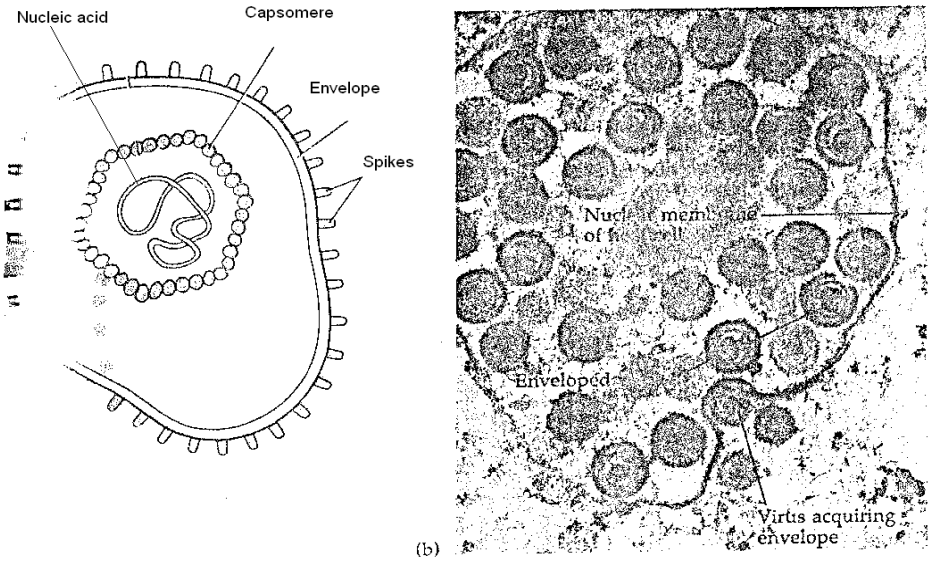


Fig. 6.7: Morphology of an Enveloped Polyhedral (icosahedral) Virus. (a) Diagram of an enveloped polyhedral virus. (b) Electron micrograph of a group of herpes simplex viruses. To the lower right, a virus particle is acquiring its envelope as it buds out through the nuclear envelope of a host cell.

Source: Tortora *et al*, 1992.

Certain bacteriophages have capsids to which additional structures are attached. The capsid (head) is polyhedral and the tail is helical (rod shaped). The head contains the nucleic acid (Figure 6.8a, b).

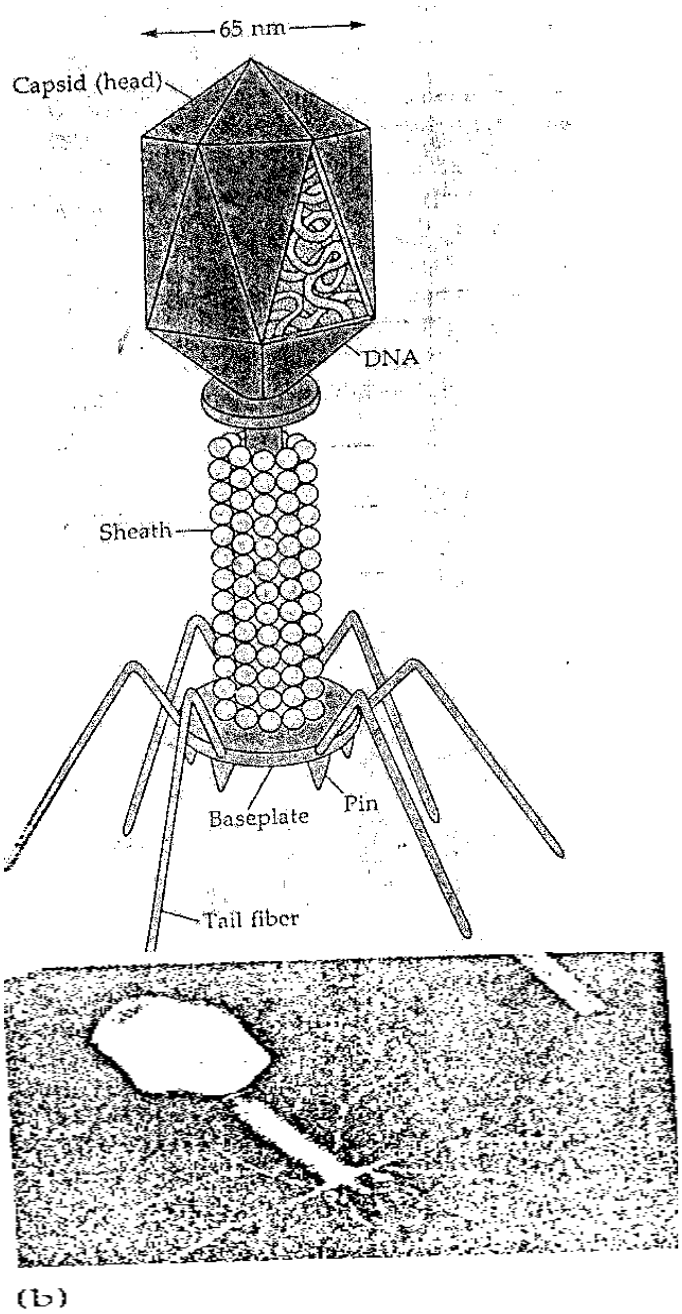


Fig. 6.8: Morphology of complex viruses (a) Diagram of a T-even bacteriophage (b) Electron micrograph of T4 bacteriophage

Source: Tortora *et al*, 1992.

h. Structure of a Retrovirus (e.g. HIV-AIDS)

The structure is spherical with projection emerging from the envelope (Figure 6.9) called the glycoprotein which enables the virus to bind to specific receptors on the surface of a host. The capsid encloses two identical genomes of RNA which are non-complementary strands. Below and above the arms of the genomes are the reverse transcriptase enzymes. Surrounding the

virus is an envelope developed from the membrane of the host cell.

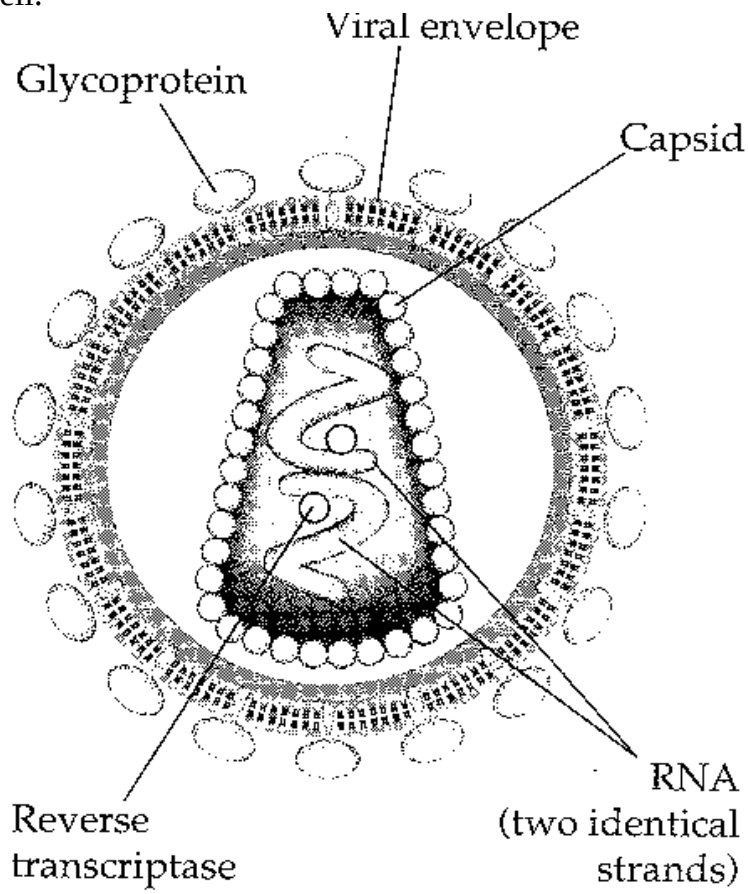


Fig.6.9: Transmission Electron Micrograph of a Retrovirus

Source: Campbell, 1996.

i. Structure of T-even Bacteriophage

Phages are viruses that infect bacteria. A T-even phage such as T4 has a complex capsid consisting of a polyhedral head and a tail apparatus. The DNA, the genome is stored in the head. The tail piece functions in the injection of this DNA into bacterium. The combination of this phage and the bacterial cell is the most complex among the viruses.

The even phages T2, T4 and T6 are very similar in structure. Their capsids have octahedral heads that enclose the genome (Figure 6.8a, b)

SELF-ASSESSMENT EXERCISE 1

Describe a typical structure of a virus and indicate the role of the component parts.

j. Host Range

The host range of a virus is the spectrum of host that the virus can infect. Viruses' mulitiply only in cells of particular species and these are divided into three classes: animal viruses, plant viruses and bacterial viruses (bacteriophages). Protists and fungi can also be hosts of viruses.

Within each class, each virus is able to infect cells of certain species only. The particular host of a virus is determined by the virus' requirements for its specific attachments to the host cell and the availability within the potential host of cellular factors for viral multiplication. For the virus to infect host cell, the outer surface of the virus must chemically interact with specific receptors sites on the surface of the cell.

3.1.2 Reproduction (Replication) of Viruses

The stages or phases that follow the replication of viruses in their host cells are usually described by using the bacteriophages. A bacteriophage (phage) is a bacterial virus. This example is used because the various stages involved in the replication process are relatively simple to understand.

Lytic or Virulent Bacteriophage Cycle

This is a reproductive cycle that ends in the death of the bacterium in which the virus multiplies. The term lytic refers to the last stage of infection during which the bacterial cell wall breaks open through the enzymic action of lysozyme, thereby releasing the new phages. The virus goes through the following stages in its replication cycles to produce more bacteriophages:

1. Adsorption: the attachment of viruses to host cells.
2. Penetration: the entry of virions or their genomes into the host cell.
3. Biosynthesis: The synthesis of new nucleic acid molecules, capsid proteins, and other viral components within host cells while using the metabolic machinery of the host cells.
4. Maturation: the assembly of newly synthesised viral components into complete phages.
5. Release: the ejection of new phages from host cells.

The phages designated T2, T4 and T6 (T stands for "type") are complex but well studied naked phages that have double stranded DNA as their genetic material. The most widely studied is the T4 phage, an obligate pathogen of the common enteric, *Escherichia coli*. T4 has a distinctly shaped capsid made of a head, collar and tail (Figure 6.8a). The DNA is packed in the polyhedral head, which is attached to a helical tail.

Infection and replication of new T4 phage occurs in the series of steps illustrated in Figure 6.10.

1. **Step1: ADSORPTION**: when T4 phages collide in the correct orientation with host cells, the phages will attach to or adsorb onto, the host cell surface by its tail fibres. Adsorption is a chemical attraction which requires specific protein recognition factors found in the phage tail fibres that binds to specific receptors sites on the host cell. The fibres bend and allow the pins to touch the cell surface. Although T4 attaches to the cell wall; other phages can adsorb to flagella or pili.
2. **Step2: PENETRATION**: the enzyme lysozyme which is present within the phage tail, weakens the bacterial cell wall, the tail sheath retracts or contracts so that the genome moves from the head into the host cell cytoplasm. The hollow tube in the tail is forced to penetrate and come into contact with the host cell membrane. The viral DNA then moves from the head through the tube into the bacterial cell. The phage introduces its DNA into the periplasmic space between the cell membrane and the cell wall. Either way the phage capsid remains outside bacterium.
3. **Step 3: BIOSYNTHESIS**: Viral genomes are too small to contain all the genetic information to replicate themselves. Therefore, they must use the biosynthetic machinery present in the host cells. Once the phage DNA enters the host cell, the phage's genes take control of the host cell metabolic machinery. Usually, the bacterial DNA is disrupted so that the nucleotides of hydrolysed nucleic acids can be used as building block for the new phage. Phage DNA is transcribed into mRNA and translated on host ribosomes, which direct the synthesis of capsid proteins and viral enzymes. Some of the enzymes are DNA polymerases that replicate the phage. DNA, thus the phage infection directs the host cell to make only viral proteins.
4. **Step 4: MATURATION**: The head of T4 phage is assembled in the host cell cytoplasm from newly synthesised capsid proteins. Then a viral DNA molecule is packed into each head. At the same time phage tails are assembled from newly formed base plates, sheath and collars. When the head is properly packed with DNA, each head is attached to a tail. Only after the heads and tails are attached that the tails fibres are added to form mature infective phages.
5. **Stage 5: RELEASE OR EJECTION**: The enzyme lysozyme which is coded by a phage gene breaks down the cell wall allowing viruses to escape. In the process, the bacterial cell wall is lysed. Thus phages such as T4 are called virulent or lytic phages because they destroy the bacterial cell wall they infect. The released phages can now infect susceptible *E. coli* bacteria,

starting the infection all over again. Such infections exhibited by virulent phages represents the lytic cycle of infection (Figure 6.10).

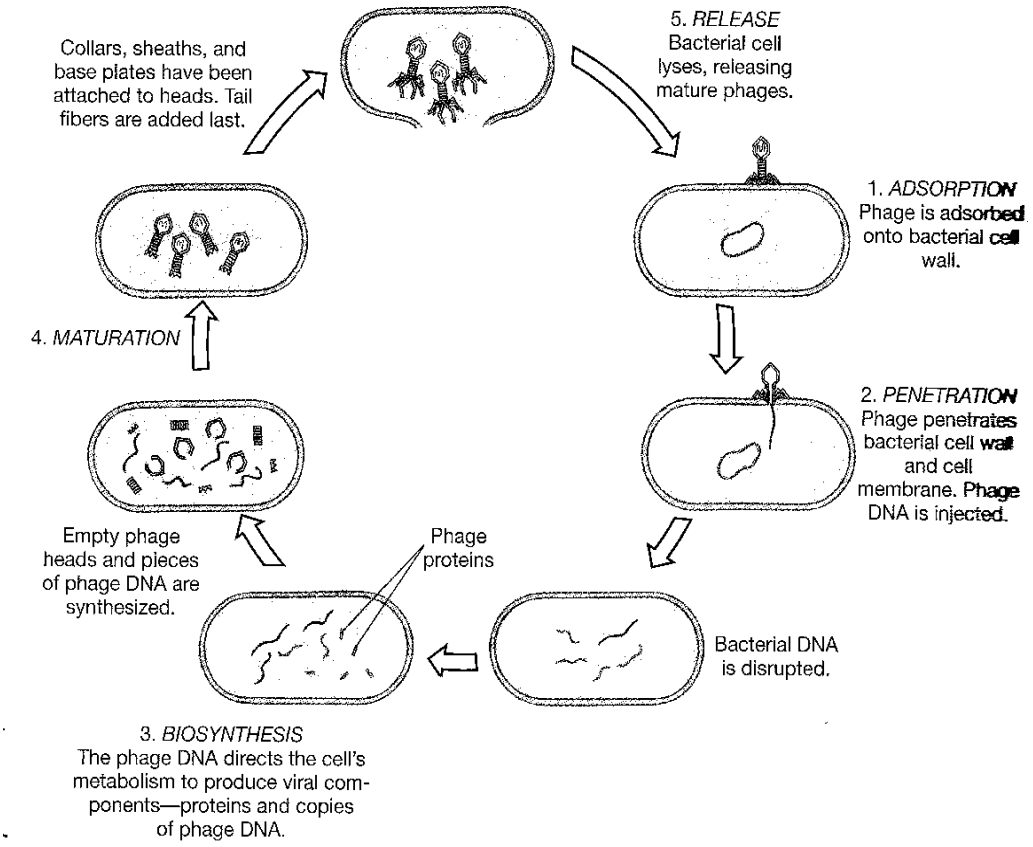


Fig.6.10: Replication of a Virulent Bacteriophage (T4)
Source: Jacquelyn, 1996.

The time from adsorption to release is called burst time; it varies from 20-40 minutes for different phages. The number of new released phages from each bacterial host represents the viral yield or burst size. In phage T4, 50-200 new phages may be released from one infected bacterium.

(b) **Phage Growth and the Estimation of Phage Numbers**

Like the bacterial growth, viral growth (biosynthesis and maturation) can be described by a growth curve which is based on observation of infected bacteria in the laboratory cultures (Figure 6.11).

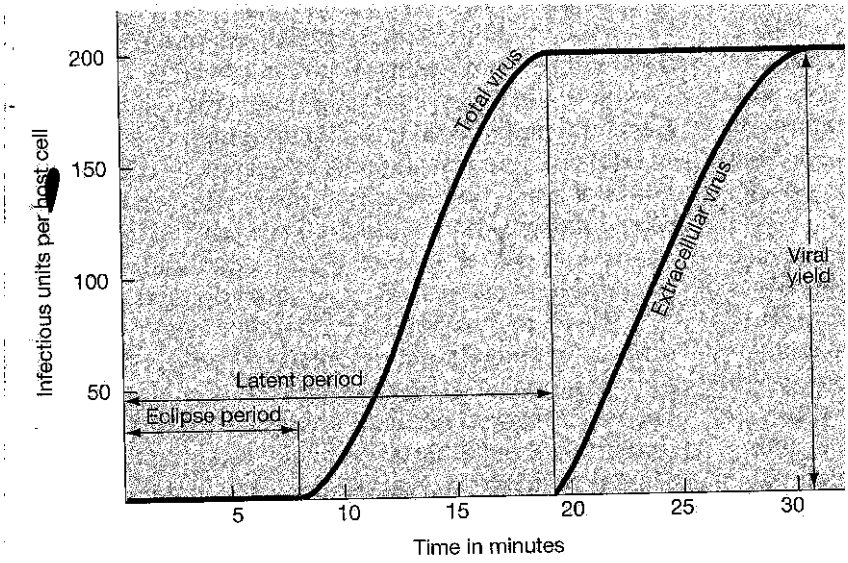


Fig.6.11: Growth Curve for a Bacteriophage. The eclipse period represents the time after penetration through the biosynthesis of mature phages. The latent period represents the time after penetration through the release of mature phages. The number of viruses per infected cells is the viral yield, or burst size.

Source: Jacquelyn, 1996.

The growth curve of a phage includes an eclipse period, which spans from penetration through biosynthesis. During the eclipse period, mature virions cannot be detected in host cells. The latent period spans from penetration through up to the point of phage release. In Figure 6.11, the latent period is longer than the eclipse period. The number of viruses per infected host cell rises after the eclipse period and eventually levels off. It is not possible to determine the number of viruses through counting usually or through the light microscope not even electron microscope. The only solution is The Plague Assay. To perform a plague assay, virologists start with a suspension of phages. Serial dilutions like those described for bacteria, are prepared. A sample of each dilution is inoculated onto a plate containing a susceptible bacterial lawn- a layer of bacteria ideally; one wants a dilution where only one phage will infect one bacterial cell. Consequently, new phages are produced from each infected bacterial cell, lysing the cell.

These phages then infect surrounding susceptible cells and lyse them. After incubation and several rounds of lysis, the bacteria lawn shows clear areas called plagues (Figure 6.12).

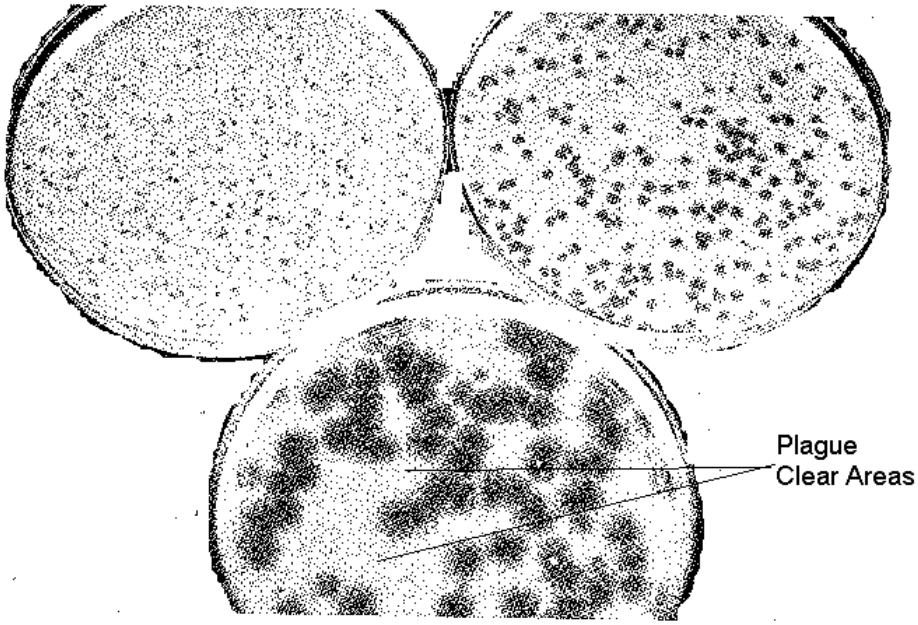


Fig.6.12: Result of a Plague Assay
Source: Jacquelyn, 1996.

The plague assay of the number of bacteriophages in sample is done by spreading the sample out over a ‘‘lawn’’ of solid bacterial growth. When the phages replicate and destroy the bacterial cells, they leave a clear spot called a plague, in the lawn. The number of plagues corresponds roughly to the number of phages that were initially present in the sample. Different types of phages produce plague of different sizes or shapes when replicating in the same bacterial species. In this case, it is *Esherichia coli*. The upper left-hand plate was inoculated with T2 phage, the upper right-hand plate, with T4 phage; and the lower plate, with lambda phage.

The plagues (the spots) represent areas where viruses have lysed host cells. In other parts of the bacterial lawn, uninfected bacteria multiply rapidly and produce a turbid growth layer. Therefore by counting the number of plagues, and multiplying that number by dilution factor, the number of phages in milliliters of suspension can be estimated. Sometimes, two phages can be deposited so close together that they produce a single plague. And not all plagues are infective. Thus counting the number of plagues in a plate will approximate, but may not be exactly equal to the number of infectious phages in the suspension. Therefore, such counts usually are reported as plague forming units (pfu) rather than the number of phages.

c LYSOGENY

An example of a temperate phage is lambda virus. There are weak bacteriophages (temperature phages) that do not destroy the host cells at the end of their multiplication, thus they exhibit Lysogeny, a stable long-term relationship between the phage and the host. Such participating bacteria are called lysogenic bacteria. The lambda phages attach bacterial cells and insert their linear DNA into the bacterial cells and then integrate into the circular bacterial chromosomes at a specific location.

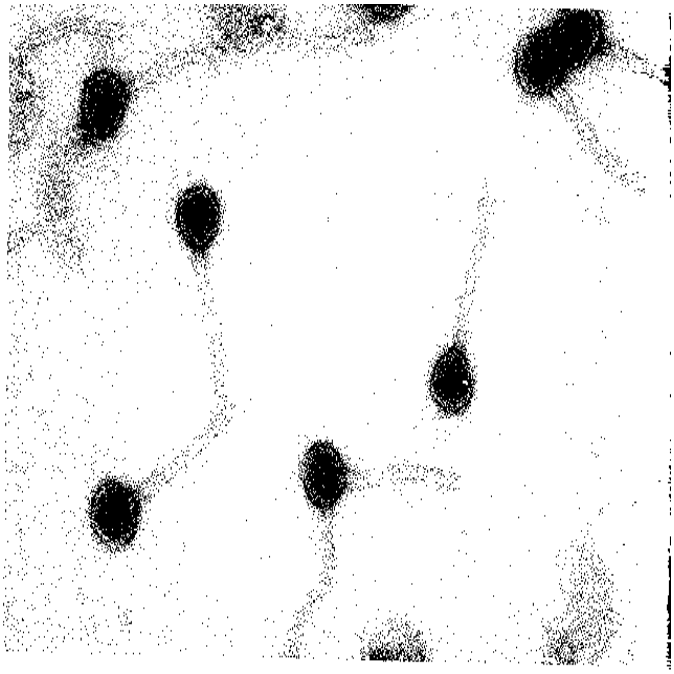


Fig.6.13: False Colour, Transmission Electron Micrograph (TEM) photo of the temperate phage lambda (84,000x), which infects the bacterium *Escherichia coli*.

Source: Jacquelyn, 1996.

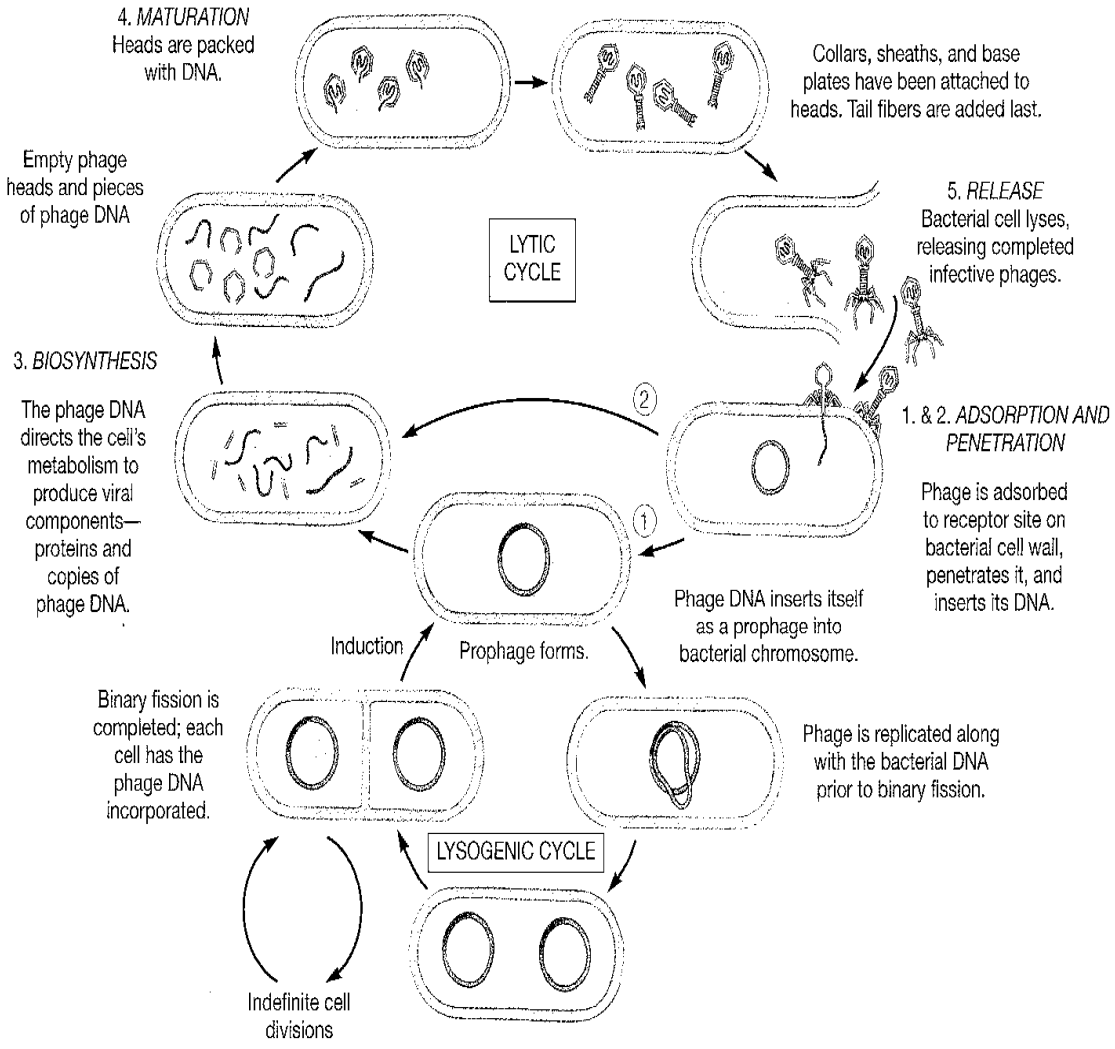
This viral DNA within the bacterial chromosome is called prophage.

The combination of a bacterium and a temperature phage is called a lysogen. Insertion of lambda phage into a bacterium alters the genetic characteristics of the bacterium. Two genes present in the prophage produce proteins that repress virus replication. The prophage also contains another gene that provides “immunity” to infection by another phage of the same type. This process is called lysogenic conversion which prevents the adsorption or biosynthesis of phages of the type whose DNA is already carried by the lysogen. The gene responsible for this

immunity does not protect the lysogen against a different type of temperate phage or by a virulent phage. Lysogenic conversion can be of medical significance because the toxic effect of some bacterial infections are caused by the prophages they contain.e.g. bacteria *Corynebacterium diptheriae* and *Clostridium botulinum* contain prophages that have a gene that codes for the production of a toxin. The conversion from non-toxin production to toxin production is largely responsible for the tissue damage that occurs in diphtheria and botulism respectively. A prophage is therefore the latest form of a temperate phage that remains within the lysogen, usually integrated into the host chromosome. Without the prophages the bacteria do not cause disease. Once established as a prophage, the virus can remain dormant for a long time. Each time a bacterium divides, the prophages is copied and is part of bacterial growth with a prophage represents a lysogenic cycle however, either spontaneously or in response to some outside stimulation, the prophage can become active and initiate a typical lytic cycle. This process is called induction, may be due to lack of nutrients for bacterial growth or the presence of chemicals toxic to the lysogen (Figure 6.14). The provirus seems to sense that living conditions are deteriorating and that it is time to find a new home. Through induction, the provirus removes itself from the bacterial chromosome. The phage DNA then codes for viral proteins to assemble new temperate phages in a manner similar to the lytic phages. Consequently, new temperate phage mature and are released through cell lysis (Figure 6.14).

d. Replication of a Retrovirus

The retroviruses present a complicated reproductive cycle. They are RNA viruses. Retro meaning “backward” refers to the reverse direction in which genetic information flows in the viruses. Retroviruses are equipped with a unique enzyme called reverse transcriptase which can transcribe DNA from RNA template, providing an RNA-DNA information flow. The newly formed DNA then integrates as a provirus into the chromosome of the nucleus of the animal cell. The host RNA polymerase transcribes the viral DNA into RNA molecules which can function both as mRNA for the synthesis of viral proteins and as new genomes for viral offsprings released from the cell. A retrovirus of particular importance is the HIV (Human Immunodeficiency Virus), the virus that causes AIDS (Acquired Immunodeficiency Syndrome). The glycoprotein of the envelope enables the virus to bind to the specific receptor on the surface of certain white blood cells.



① In the lysogenic cycle, temperate phages can exist harmlessly as a prophage within the host cell for long periods of time. Each time the bacterial chromosome is replicated, the prophage also is replicated; all daughter bacterial cells are "infected" with the prophage. Induction involves either a spontaneous or environmentally induced excision of the prophage from the bacterial chromosome. ② A typical lytic cycle, involving biosynthesis and maturation, occurs, and new temperate phages are released.

Fig. 6.14: Replication of a Temperate Bacteriophage
Source: Jacquelyn, 1996.

Although the RNA strands are identical, they are not complementary strands. The genome enters a host cell when the virus fuses with the plasma membrane and proteins of the capsids are enzymatically removed. Reverse transcriptase enzyme then catalyses the synthesis of DNA complementary to the RNA template provided by viral genome. The new DNA strand then serves as a template for the synthesis of a complementary DNA stand. The double stranded DNA is incorporated as a provirus into the host cell's genome (Figure 6.15).

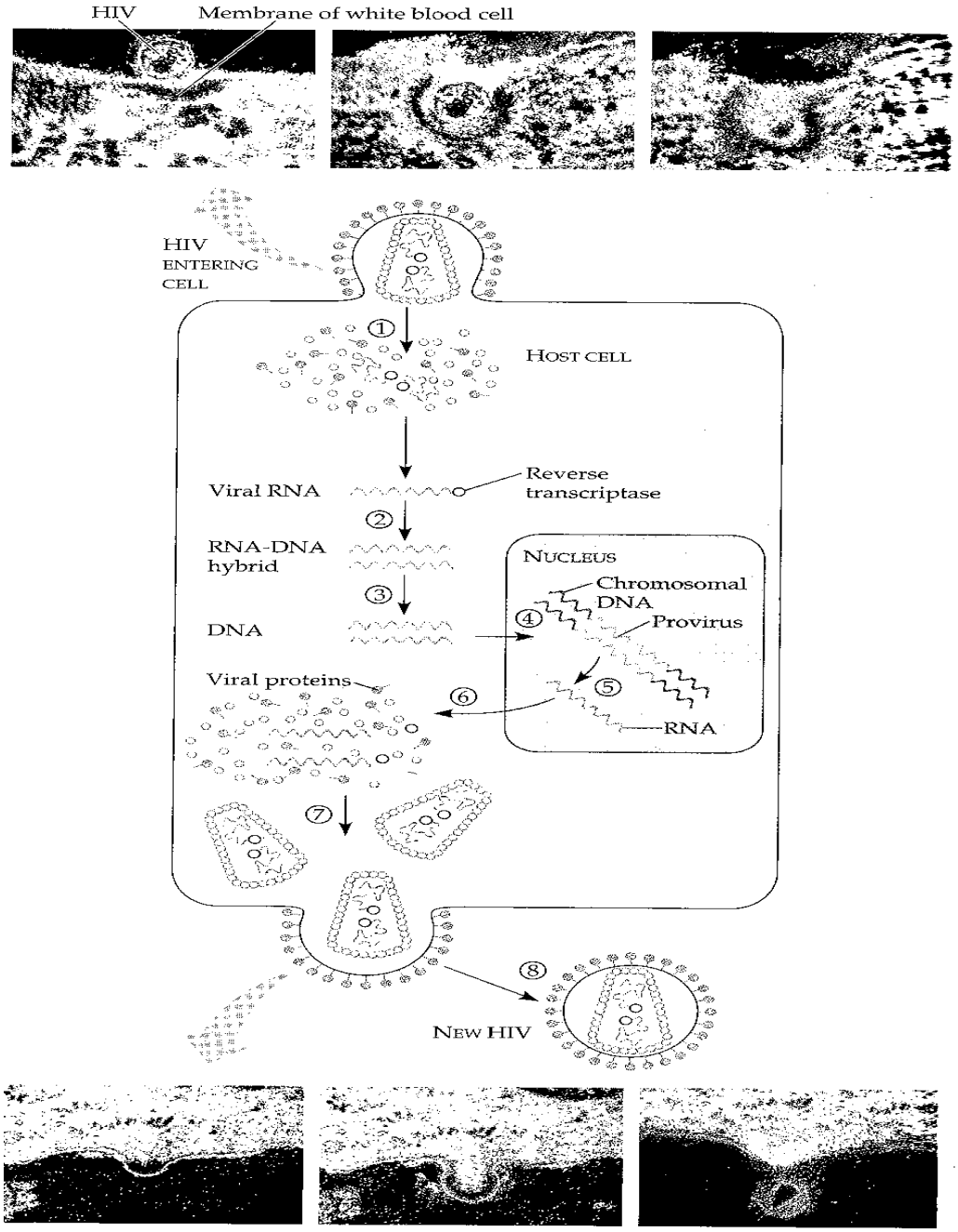


Fig.6.15: The Life Cycle of HIV, a Retrovirus. Transmission electron micrographs (artificially coloured) depict HIV entering (top) and leaving (bottom) a human white blood cell.

Source: Campbell, 1996.

SELF-ASSESSMENT EXERCISE 2

Differentiate between a lytic and lysogenic viral cycles.

4.0 CONCLUSION

The discovery, nature and structures of various viruses have been discussed. The replication processes in a bacteriophage, retrovirus and temperate virus have been highlighted.

5.0 SUMMARY

In this unit you have learnt the:

- nature of viruses as obligate pathogens
- structures of viruses
- multiplication (replication) of the viral particles as lytic, lysogenic and temperate viruses
- replication processes involved in the retrovirus.

6.0 TUTOR-MARKED ASSIGNMENT

i. What is a virion?
ii. Describe the typical structure of the virus.
iii. List the main characteristics of viruses.
iv. List the general steps in viral replication.
v. How do T even phages attach to and enter the host cells?
vi. What is lysogeny? How is it different from the lytic cycle?
vii. What are the differences in the replication process of retroviruses?

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UNIT 7 IMPORTANCE OF MICRO-ORGANISMS: THE VIRUSES

CONTENTS

1.0 INTRODUCTION

Viruses remain one of the most studied organisms by scientists yet they continue to cause daily diseases of plants, animals and man.

For instance the Acquired Immune Deficiency Syndrome (AIDS) and Ebola viral infections defy solutions. It has become increasingly difficult to treat viral infections using chemotherapeutic drugs because of the intracellular nature of viruses. Drugs that are targeted in killing or destroying viruses will kill the host cells. This unit will look at the impact and importance of viruses in the lives of man, plants and animals.

2.0 OBJECTIVES

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

- explain the importance of viruses to life
- discuss their impact to human life
- explain the importance of viruses on plants
- differentiate between their beneficial and harmful effects.

3.0 MAIN CONTENT

3.1 Importance of Viruses

3.1.1 Beneficial Effects

(a) Viruses as Cloning Vectors

Genetic engineering (e.g. recombinant DNA technology) procedure utilises carriers called **cloning vectors**. A virus is used as one of the cloning carriers (vectors). Certain types of bacteriophage (e.g.lambda) can also be used as cloning of bacterial cells. Fragments of foreign DNA can be spliced into a phage genome by using restriction enzyme and lipase enzyme. The recombinant phage DNA is then introduced into an *E.coli* cell through normal process of infection. Once inside the cell, the phage DNA replicates and produces a new phage into other bacterial cells by infection (Campbell, 1996). It is also possible to use viruses as vectors to genetically engineer animal cells e.g. the retroviruses which following infection integrates into chromosomes. This delivers recombinant DNA directly to a chromosome of the animal cell (Campbell, 1996).

(b) Viruses as Bioinsecticides

Viruses that are pathogenic on specific insects include nuclear polyhedrosis viruses (NPVs), granulosis viruses (GVs) and cytoplasmic polyhedrosis viruses (CPVs). Most of these viruses primarily affect butterflies and moths e.g. Lepidoptera. An important commercial viral pesticide is marketed under the trade name Elcar for control of the cotton bollworm *Helliothis zea* (Prescott *et al*, 2009).

One of the most exciting advances is the use of baculoviruses that have been genetically modified to produce a potent scorpion toxin active against insect larvae. After ingestion of the viruses by the larvae, these viruses are dissolved in the midgut of the larvae and are released. The ingested baculovirus is a genetically modified virus (i.e. recombinant virus) that produces this insect selective neurotoxin (the scorpion toxin). It also acts on the neurons of the larvae because it is more rapidly effective than the parent virus due to genetic modification. The larvae died and the leaf damage by these insects is markedly decreased.

SELF-ASSESSMENT EXERCISE 1

Enumerate the importance of viruses.

3.1.2 Harmful Effects

(a) Human Viral Infections and Diseases

Human beings and animals are also affected by viruses causing diseases of various types.

A number of viruses whether RNA-carrying viruses or DNA carrying viruses cause a number of human diseases, some of which can lead to mortality, if immediate medical care is not taken. Some of these are shown in Tables 7.1 and 7.2.

Table 7.1: Classification of Major Groups of RNA Viruses that cause Human Diseases

Family	Enveloped or Naked and Capsid symmetry	Typical size(nm)	Example(Genus or species)	Infection or Disease
(+) *Sense RNA Viruses*				
Picornaviridae (1 copy)	Naked, polyhedral	18–30	*Enterovirus* *Rhinovirus*	Polio Common cold
Togaviridae (1 copy)	Enveloped, polyhedral	40–90	*Hepatovirus* Rubella virus Equine encephalitis virus	Hepatitis A Rubella (German measles) Equine encephalitis
Flaviviridae (1 copy)	Enveloped, polyhedral	40–90	*Flavivirus*	Yellow fever
Retroviridae (2 copies)	Enveloped, spherical	100	HTLV-I HIV	Adult leukemia, tumors AIDS
(−) *Sense RNA Viruses*				
Paramyxoviridae (1 copy)	Enveloped, helical	150–200	*Morbillivirus*	Measles
Rhabdoviridae (1 copy)	Enveloped, helical	70–180	*Lyssavirus*	Rabies
Orthomyxoviridae (1 copy in 8 segments)	Enveloped, helical	100–200	*Influenzavirus*	Influenza A and B
Filoviridae (1 copy)	Enveloped, filamentous	80	*Filovirus*	Marburg, Ebola
Bunyaviridae (1 copy in 3 segments)	Enveloped, spherical	90–120	*Hantavirus*	Respiratory distress, hemorrhagic fevers
Double-Stranded RNA Viruses				
Reoviridae (1 copy in 10–12 segments)	Naked, polyhedral	70	*Rotavirus*	Respiratory and gastrointestinal infections

Source: Jacquelyn, 1996.

Table 7.2: Classification of Major Groups of DNA Viruses that cause Human Diseases

Family	Enveloped or Naked and Capsid Symmetry	Typical size(nm)	Example Genus or Species	Infection or Disease
Double-Stranded DNA Viruses				
Adenoviridae (linear DNA)	Naked, polyhedral	75	Human adenoviruses	Respiratory infections
Herpesviridae (linear DNA)	Enveloped, polyhedral	120–200	*Simplexvirus*	Oral and genital herpes
			Varicellovirus	Chickenpox, shingles
Poxviridae (linear DNA)	Enveloped, complex	230 × 270 shape	*Orthopoxvirus*	Smallpox, cowpox
Papovaviridae (circular DNA)	Naked, polyhedral	45–55	Human papilloma-viruses	Warts, cervical and penile cancers
Hepadnaviridae (circular DNA)	Enveloped, polyhedral	40–45	Hepatitis B virus	Hepatitis B
Single-Stranded DNA Viruses				
Parvoviridae (linear DNA)	Naked, polyhedral	22	B19	Fifth disease (erythema infectiosum) in childre

Source: Jacquelyn 1996

(b) Some Plant Viral Infections and Diseases

Most economic crop plants are infected by viruses. In most cases the virus will cause a reduction in yield and the quality of the infected crop will be reduced and hence reduced the market value.

Annual crops, vegetables and cereals that are usually grown from seeds have virus infections. Such crops may result in the complete loss of the crop in the season e.g. the rice yellow mottle virus, the Tungo virus and the cowpea mosaic virus are examples. Some of these are listed on Table 7.3.

Table 7.3: Examples of Yield Reduction caused by Various Plant Virus Diseases

Crop	*Virus*	*Yield reduction (%)*	*Country*	*Reference*
Beans (*Phaseolus vulgaris*)	Bean yellow mosaic	33	United States	Hampton (1975)
	Bean common mosaic	64		
Cabbage	Turnip mosaic	36	England	Walkey & Webb (1978)
Cassava	Cassava mosaic	24–75	Kenya	Seif (1982)
Lettuce	Lettuce mosaic	56	United States	Zink & Kimble (1960)
	Cucumber mosaic	8–50	England	Walkey & Ward (1983)
	Beet western yellows	7–58		
Pepper	Various	9–67	United States	Villalon (1981)
Potato	Potato leaf roll	65–92	United States	Harper *et al.* (1975)
Maize	Maize streak	25–60	Kenya	Guthrie (1978)
Wheat	Barley yellow dwarf	9–29	Australia	Smith & Sward (1982)
Apple	Various	39–51	Germany	Schmidt (1972)
Pear	Ring pattern	35	United States	Waterworth (1976)
Raspberry	Raspberry mosaic	50	United States	Converse (1963)
		11–14	Canada	Freeman & Stace-Smith (1970)
Strawberry	Various	14–38	Belgium	Aerts (1977)
Sweet cherry	Prunus ringspot and prune dwarf	70	England	Cameron (1977)
Tobacco	Tobacco etch	3–18	United States	Gooding (1970)
	Tobacco mosaic	5–16		

Source: Walkey, 1991.

SELF-ASSESSMENT EXERCISE 2

Enumerate the importance of viruses to man.

4.0 CONCLUSION

The harmful and beneficial impact of viruses to plants, animals and man have been presented and discussed.

5.0 SUMMARY

In this unit, you have learnt the:

- Beneficial importance of viruses
- Harmful effects of viruses.

6.0 TUTOR-MARKED ASSIGNMENT

i. Describe the use of viruses in biological control.
ii. Viruses and life; Friends or foes: Discuss
iii. Name two RNA viruses and two DNA viruses causing diseases on man.
iv. Name four viruses that are found on food crops.

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UNIT 8 STRUCTURE, REPRODUCTION AND IMPORTANCE OF MICRO-ORGANISM: THE ALGAE

CONTENTS

1.0 INTRODUCTION

What are algae? The term alga refers to relatively simple aquatic organisms that are photoautotrophs. This means that they carry out photosynthesis. Algae are studied because they are extremely important ecologically, accounting for about half the photosynthetic production of organic material on a global scale. They are usually found in a moist environment and also occur as fresh water and marine phytoplankton and intertidal weeds. A phytoplankton is a community of floating photosynthetic organisms in water. The algae except the blue-green algae (cyanobacteria) are eukaryotic organisms and are key producers (photoautotrophs) in moist aquatic ecosystems. This is due to the fact that they are the basis of aquatic food webs, supporting an enormous abundance and diversity of animals. Algae are of various colours such as green, yellow, orange, brown, red, blue and others. Apart from the presence of chlorophyll ‘’ a’’ in them, there are other accessory pigments that trap wavelengths to light. The other pigments tend to give them these various colour combinations. You will learn more about algae in this unit.

2.0 OBJECTIVES

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

- describe the characteristics of algae
- discuss the structures existing among the algae
- list the criteria used in classifying the algae

- discuss the economic importance of algae.

3.0 MAIN CONTENT

3.1 Classification of Algae

Algae are photoautotrophs, although some grow heterotrophically, when growing photosynthetically, they produce oxygen and use carbondioxide as the sole source of carbon. Every algal cell is photoautotrophic or can absorb nutrients directly. Many algae are motile or have motile stages during their life cycles. Algae are generally classified on the basis of the following:

- Nature and properties of pigment.
- Nature of reserve and storage products.
- Type and number of insertions (points of attachment and morphology of flagella).
- Chemical composition and physical features of the cell wall.
- Morphology and characteristics of cells and thalli.

All these characteristics are taken into account in assigning algae to divisions. The major groups of algae and their distinguishing characters are shown on Table 8.1. The Euglenoids, Chrysophytes and the Pyrophytes are unicellular. The other major groups are included in the genera that are multicellular. All algae possess chlorophyll as their primary photosynthetic pigment as well as carotenoid pigments (Table 8.1).

The Rhodophyta contain chlorophyll “a” and phycobillins, the other algae contain chlorophyll and either chlorophyll b or c. Members of the Euglenophyta and Pyrrophyta are animal-like, they do not have cell walls, other algae have cell walls composed of silica, cellulose, polysaccharides or organic acids. Algae are able to store energy reserves in the form of fats, oils and carbohydrates. Many algae are motile by means of flagella which vary in structure, number and point of attachment (Table 8.1).

Table 8.1: Characteristics of the Major Groups of Algae

Division	Habitat	Morphology	Pigments	Reserves	Cell-wall composition
Green algae (Chlorophyta)	Mostly fresh-water, some marine	Uni- to multi-cellular; some microscopic; two or more equal apical or subapical flagella	Chlorophyll *a* and *b*, carotenoids	Starch	Cellulose and pectin
Brown algae (Phaeophyta)	Almost all marine	Multicellular and macroscopic; two lateral flagella on zoospores	Chlorophyll *a* and *c*, carotenoids	Laminarin and fat	Cellulose with alginic acids
Red algae (Rhodophyta)	Mostly marine, some freshwater	Multicellular and macroscopic; no flagella	Chlorophyll *a*, *d* in some; carotenoids; phycobilins	Starch	Cellulose and pectin
Golden-brown algae, diatoms (Chrysophyta)	Mostly marine	Unicellular and microscopic; one or two apical equal or unequal flagella	Chlorophyll *a*, *c*; carotenoids	Chrysolaminarin, oils	Pectic compounds with siliceous material
Dinoflagellates (Pyrrophyta)	Marine and freshwater	Unicellular and microscopic; two lateral flagella	Chlorophyll *a*, *c*; carotenoids	Starch, oils	No cell wall
Euglenoids (Euglenophyta)	Freshwater	Unicellular and microscopic; one to three apical flagella	Chlorophyll *a*, *b*; carotenoids	Paramylon, oils	No cell wall

Source: Pelczar *et al*, 1993.

3.2 Characteristics and Morphological Forms of Algae

Single algal cells exist in a variety of forms, including spherical, curved and rod-like shapes. Aggregate of cells can form multicellular colonies or filaments that are either branched or unbranched. In some cases, these cells aggregates resemble more complex organisms (Figure 8.1), the individual cells of the aggregate act cooperatively to benefit the entire organism in the colony.

(a) ***Chlamydomonas***

This is one of the unicellular green algae. It is found chiefly in ponds and ditches. The motile cell is usually more or less spherical or ovoid in shape (Figure 8.1a). It has a cellulose wall which is in contact with the protoplasmic contents. The protoplasm in the anterior region gives off two **flagella** which pass through the cell wall. The protoplasm in this region also has

two vacuoles (i.e. space filled with liquid, which shows alternate expansion and contraction), situated at the base of the flagella, and an orange coloured **pigment spots or eye-spots** contains a single, large, more or less cup-shaped **chloroplast**, embedding a round body known as the pyrenoid (Figure 8.1).

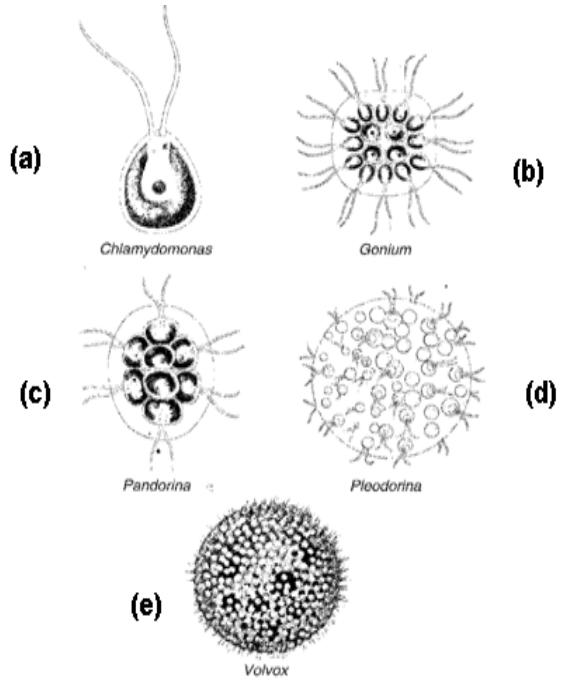

Fig.8.1: (a) *Chlamidomonas* and Some Colonial Algae
Source: Jacquelyn, 2002.

A single nucleus is present in the central region of the protoplasm enclosed within the cavity of the chloroplasts (Figures 8.1a and 8.2). The cells move through water by means of their flagella. The movement is autonomic but is often directed by external stimuli.e.g. light. The cells move towards bright diffuse light and away from light of great intensity. This sensitiveness of light is specially associated with the eye spot. The pyrenoid consists of proteins. It probably stores food materials. It is believed that it takes part in the photosynthetic action of the alga.
Chlamydomanas is capable of encystment to enable it pass through inclement weather. It produces a red pigment known as **haematochrome** .

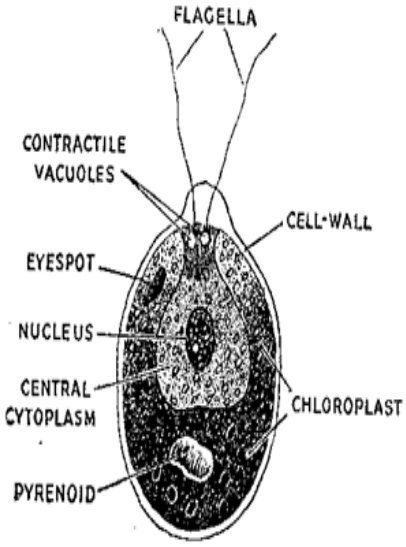

Fig. 8.2: Chlamidomonas
Source: Lowson, 1962.

(b) ***Spirogyra***

This is one of the freshwater green algae. It forms bright slimy masses in ponds and slow running streams. Its vegetative body is an unbranched filamentous thallus consisting of short cylindrical cells placed end to end and showing no distinction between base and apex. The filament increases in length by cell division and growth of the cells. All the cells have the same structure and are capable of division (Figure 8.3) and (Figure 8.4). *Spirogyra* is an example of mulitcellular alga which shows little or no division of labour. Infact each cell is self sufficient to the extent that each of them can be regarded as a complete organism and the whole filament as a colony of the individuals for each cell carries out all the vital functions necessary for its existence. The filament is in most species, invested by a delicate mucilaginous sheath formed by the cell walls. It is this that makes the mass of spirogyra filament slimy to touch.

Each cell is cylindrical in shape with transverse end walls (Figure 8.3a), and has the structure characteristics of parenchymatous cells. The wall consists of cellulose or a related substance. Inside the cell wall, there is a lining cytoplasm, from which delicate **protoplasmic strands** run across a central **vacuole** to the center of the cell (Figure 8.3b).

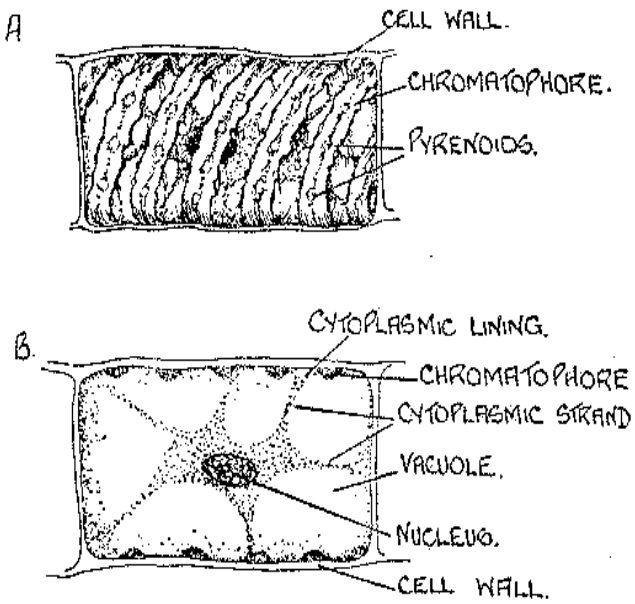

Fig.8.3: Spirogyra Cell. A. Surface View; B Optical Section

Source: Lowson, 1962.

The nucleus is usually embedded in the small central mass of cytoplasm. The most conspicuous structures in the cell are the green spirally coiled **chromatophores** (Chloroplasts). There can be one to seven of such per cell; the number varies depending on the species. They lie in the cytoplasm and each one contains a number of well marked p**yreonoid** (Figure 8.3a). The numerous species of *Spirogyra* are distinguished by the character of the transverse walls, the number of chloroplasts per cell, the number of spiral turns in one chloroplast, the characters of the cell containing the zygospores and of the zygospores themselves (Figure 8.4).

3.3 Reproduction in *Spirogyra* and *Chlamydomonas*

Algae reproduce either asexually or sexually. Most of them reproduce by fragmentation of cells from colonies or multicellular aggregates or by spore formation. The spores formed by algae during reproduction are either flagellated (zoospores) or non-motile (aplanospores). Zoospores are commonly produced by aquatic algae whereas aplanospores are usually found among terrestrial algae. Sexual reproduction in algae involve haploid sex cells (gametes) combining after fusion to form a zygote. Union of identical gametes (no sex differentiation) are said to be **isogamous** whereas unions involving distinct male and female gametes are termed **heterogamous**. When there is a difference in the sizes of the gametes -or gamete is small and motile and the other non-motile and large, the fusion is said to be **oogamous.** Certain terrestrial algae such as

the multicellular Ulva (sealettuce) exhibit a process called **alternation of generations.**

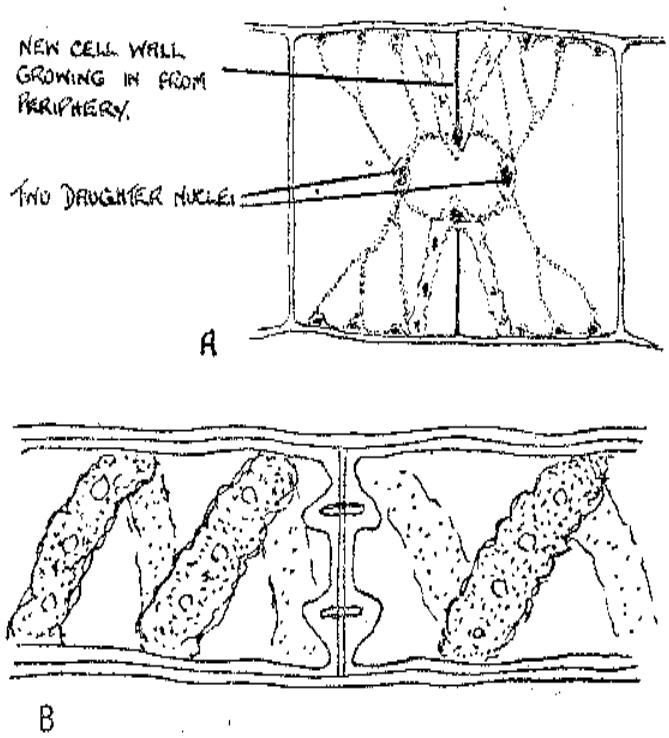

Fig.8.4: A *Spirogyra* Cell-Division
Source: Lowson, 1962.

(i) Asexual Reproduction in *Chlamydomonas*

Chlamydomonas is a unicellular chlorophyta (Figure 8.5). The mature of the organism is a single haploid cell. When it reproduces asexually, the cell withdraws its flagella and then divides twice by mitosis giving rise to four new cells (Figure 8.5). These daughter cells develop flagella and cell walls and then emerge as swimming zoospores from the wall of the parent cell which had encloses them. The zoospores grow into haploid mature cells, completing the asexual life cycle (Figure 8.5 and Figure 8.6).

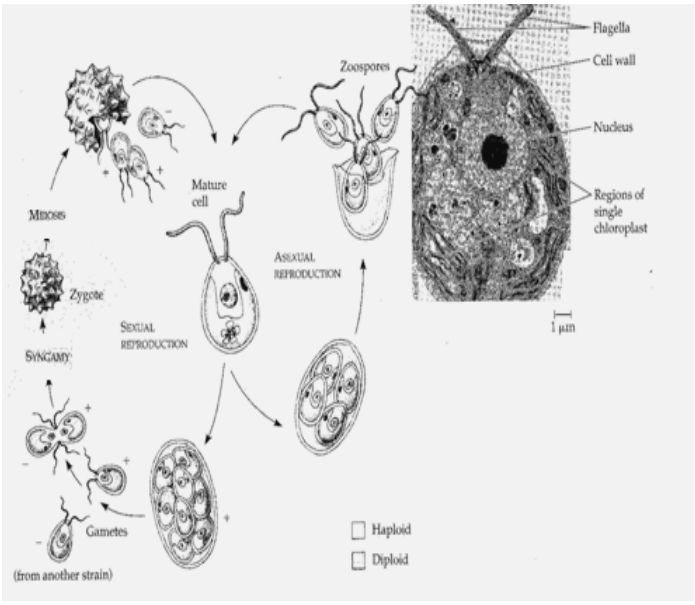

Fig. 8.5: The Life Cycle of *Chlamydomona*.
Source: Campbell, 1996.

(ii) Sexual Reproduction in *Chlamydomonas*

Sexual reproduction is triggered by a shortage of nutrients; drying of the pond or some other stress. Within the parent cell, mitosis produces many haploid gametes (Figure 8.5). After their release, gametes from opposite mating strains designated + and- strains pair up and cling together by the tips of their flagella. The gametes are morphologically indistinguishable, and their fusion is known as **isogamy**, which literally means a "marriage of equals". The gametes fuse slowly, forming a diploid zygote, which secretes a durable coat that protects the cell against harsh conditions (Figure 8.5). When the zygote breaks dormancy, meiosis produces four haploid individuals (two of each mating types) that emerge from the coat and grow into mature cells, complementing the sexual life cycle (Figure 8.6).

(iii) Asexual Reproduction in *Spirogyra*

There is no special method of asexual reproduction in *Spirogyra* but filaments may break into a number of fragments each one consisting of one or more cells, and these by ordinary cell division may form new filaments (vegetative reproduction). Fragmentation of this kind may occur naturally and results in a rapid multiplication of Spirogyra when conditions are favourable.

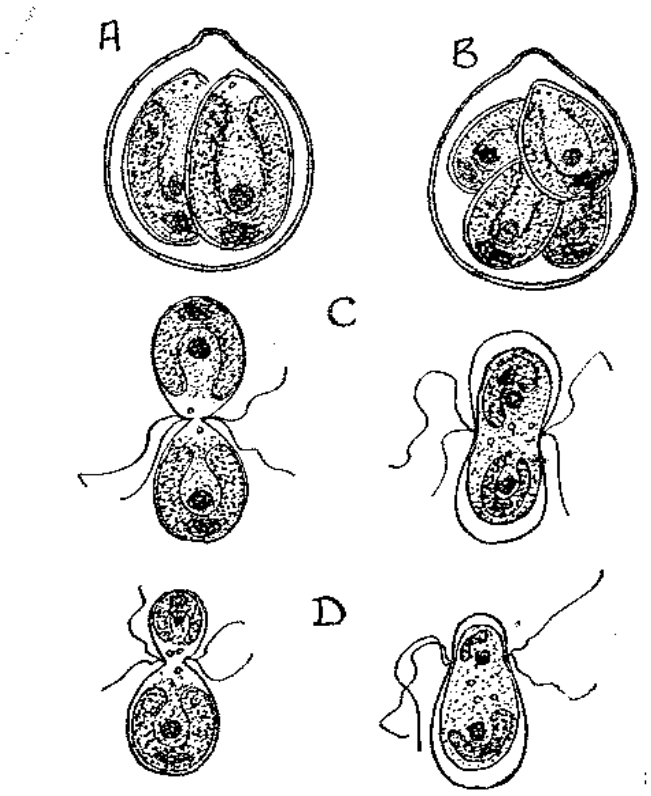

Fig. 8.6: ***Chlamydomonas.*** **(A, B) Asexual Reproduction. (C, D) Sexual Reproduction. (C) Isogamy, (D) Anisogamous.**
Source: Lowson, 1962.

(iv) Sexual Reproduction in *Spirogyra*

Sexual reproduction is **isogamous** and the process is termed **conjugation.** It usually occurs between two filaments, movements bring two filaments together and in close contact that they coalesce. Along the line of contact, slight protrusions arise from the cells of one of the two filaments and are followed by corresponding and opposite protrusions from the cells of the other filaments. As these protrusions elongate, the two filaments are gradually pushed apart and the conjugation tubes formed. When they have reached full growth their end walls disappear and the protoplasts are now in direct contact. In the early stages of the conjugation process, starch accumulates in the cells concerned and the protoplasts of the cells of one filament begin to show plasmolysis effects before those of the other side. For this reason and because of subsequent behaviour, the filaments are distinguished as male and female respectively. The male protoplasts as it contracts remains in contact with the female and passing over its contents into the female cell through the conjugation tubes. It is only at this stage that the female protoplast usually disintegrates at an early stage and the fusion of the nuclei into fat, which is often coloured red. A thick resistant

wall is secreted and the ripe zygospore is formed which may be spherical or ellipsoidal in shapes. The zygospores are liberated following the decay of the cell wall of the female filament and are capable of swimming a period of rest in substratum even though there may be a seasonal drying of the pond or stream. The method of conjugation described is known as ladder or **scalariform conjugation** (Figure 8.7). In some other species of spirogyra, conjugation may take place between cells of the same filament. This is known as **chain conjugation**.

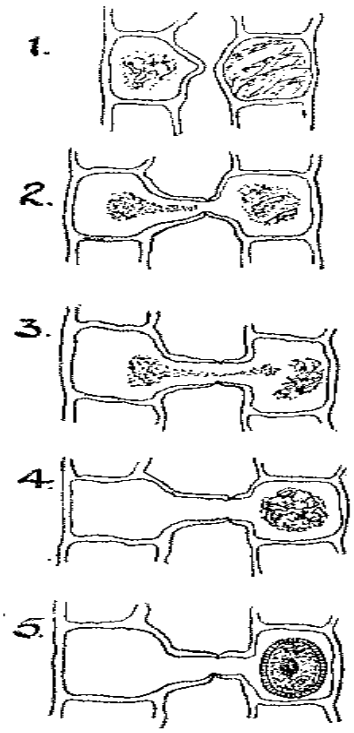

Fig. 8.7: Stages in Conjugation of *Spirogyra*
Source: Lowson, 1962.

(v) Germination of *Spirogyra* Zygospore

The result of conjugation is the formation of the zygospore. Meiosis may occur in the diploid nucleus after fusion or during the maturation of the zygospore or just before germination. There are two successive divisions associated with meiosis. One of the four resulting nuclei enlarges, the remaining three gradually abort. In preparation for germination, the stored fat is converted to starch and the chloroplasts become more readily discernible (Figure 8.8). A germ tube emerges at one end of the zygospore through the ruptured outer layers of the thick cell wall. This becomes divided into two cells by a transverse septum. The lower cell has little chlorophyll or may be almost colourless. The upper cell divides further to form the *Spirogyra* filament. *Spirogyra* is a member of the chlorophyceae known as conjugates.

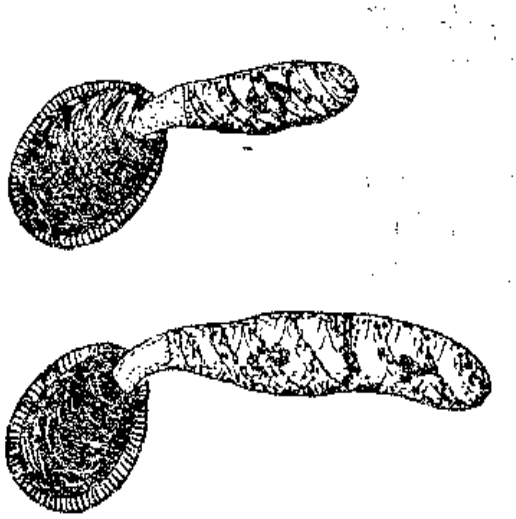

Fig. 8.8: ***Spirogyra.*** **Germination of Zygospore**
Source: Lowson, 1962.

SELF-ASSESSMENT EXERCISE 1

Describe with named examples the various morphological structures existing among the algae.

3.4 Importance of Algae

Algae are an important part of an aquatic environment food chain, because they fix carbondioxide into organic molecules that can be consumed by chemoheterotrophs, using the energy produced in photophosphorylation during the process of photosynthesis.

a. In Nature

Algae convert the carbondioxide in the aerosphere into carbohydrates in photosynthesis. Molecular oxygen (O_2) is a by product of their photosynthesis. The top few meters of any body of water contain planktonic algae. As three quarters of the biosphere is covered with water, it is estimated that 80% of the biosphere's photosynthesis is executed by planktonic (Tortora *et al*, 1992.).Seasonal changes in nutrients, light and temperature cause fluctuations in algal populations; the periodic increase in algal population is called **blooms.** Blooms of dinoflagellates are responsible for seasonal red tides. Blooms of a few species indicate that the water in which they grow is polluted because these algae thrive in high concentrations of organic materials that exist in sewage or industrial wastes. When algae die, the decomposition of the large numbers is associated with algae bloom. This depletes the level of dissolved oxygen in the water when diatoms and other planktonic organisms that grew several

years ago die and are buried by sediments, the organic molecule they contained did not decompose to be returned to the carbon cycle as CO_2. Heat and pressure resulting from the earths geologic movements altered the oil stored in the cells as well as the cell membrane. Oxygen and other elements are eliminated, leaving a residue of hydrocarbons in the form of petroleum and natural gas deposits.

Many unicellular algae are symbionts in animals. The giant clam **Tridacna** has evolved special organs that host dinoflagellates. As the clam sits in the shallow water, the algae proliferate in these organs when they are exposed to the sun. The algae release glycerol into the clam's blood stream, thus supplying the clam's carbohydrates requirement. Evidence suggests that the clam gets essential proteins by phagocyting old algae (Tortora *et al*, 1992).

b. Algae as Food

Algal culture is another avenue for increasing human food supplies. Algae such as *Scenedesmus* and *Chlorella* have been cultivated in Asia, Israel, Central America, several European countries and even the Western United States. Algae have been used as ingredients in ice-cream, (as well as non food consumer products such as diapers and cosmetics). The use of algae as human food shortens the food chain. If humans eat algae directly rather than eating fish that have been nourished on algae, the people will feed more on algae than on the fish.

Approximately 100,000kg of algae are required to produce 1kg of fish. Each acre of pond used to cultivate algae can produce 40 tons of dried algae-40 times the protein per acre from soyabeans and 160 times that from beef. Algal culture has proved to be economically visible only in urban areas where large quantities of treated sewage on which to grow the algal are available. In addition to the problem of getting people to accept algal products as food, growing algae in sewage creates a potential health hazard because the products may contain viral pathogens. e.g. *Cyanobacterium spirillum* (a blue-green alga), has been grown for centuries as food in alkaline lakes in Africa, Mexico and by the Incas in Peru. The *Cyanobacteria* are harvested, sun-dried, washed to remove sand and made into cakes for human consumption (Jacquelyn, 1996).

c. Algal Infections

Most algae manufacture their own food and are not parasitic but some strains of *Prototheca* have lost their chlorophyll and survive by parasitising other organisms found in water and moist soil. They enter the body through the skin wounds. By 1987, 45 cases of protothecosis had been reported, 2 from cleaning home aquariums. Protothecosis was first observed on the food of a rice farmer, and most subsequent cases have occurred in legs or

hands. In immunodeficient patients, the parasite can invade the digestive tract or peritoneal cavity. A few skin infections have responded to oral potassium iodide or intravenous amphotericin B and tetracycline therapy, but no satisfactory treatment has been found for others (Jacquelyn, 1996).

d. **As Soil Micro-organisms**

Algae are naturally present in the soil but living in the surface of the soil where they can carry on photosynthesis. In the desert or other barren soils, algae contribute significantly to the accumulation of organic matter in the soil.

e. **Algae in Aquarium**

The large numbers of algae usually develop in aquariums that make it look like a pea soup. This problem is solved by placing a few pennies in the tank. Enough copper to inhibit algal growth dissolves from pennies into the water. This will increase the visibility and the enjoyment of the fish.

f. Algae and Humans

Regardless of their niche, algae constitute a significant and vital part of the living world through photosynthesis; they provide a large portion of the oxygen and organic materials that is require by other life forms. Algae are important sources of protein and iodine when consumed, particularly for humans living in the Far East. Without eukaryotic algae, life would have a much more restricted existence (Lim, 1998).

g. Algae and Gel-forming Substances

Apart from coastal people, particularly in Asia where algae (sea weeds) are harvested for consumption as food, gel-forming substances in their cell walls e.g. algin in brown algae, agar and carageenan in red algae are extracted in commercial operations.

These substances are widely used in the manufacture of thickeners for such processed foods such as puddings and salad dressing, and also as lubricants in oil drilling. Agar is also used as the gel-forming base for microbiological culture media.

h. The Lichens

There had been various definitions for the lichens. A Lichen is an association of a fungus and an alga in which the two organisms are so intertwined to form a single thallus. The fungus component is called **mycobiont** and the algal component is **phycobiont.**

The lichen is again defined as an association between the fungus and the alga from which both partners benefit and the association is a perfect example of **symbiosis** (mutualistic association). The fungus derives food from the algal cells and in return protects the alga from adverse conditions particularly from drought (Figure 8.9) (Tortora *et al,* 1992).

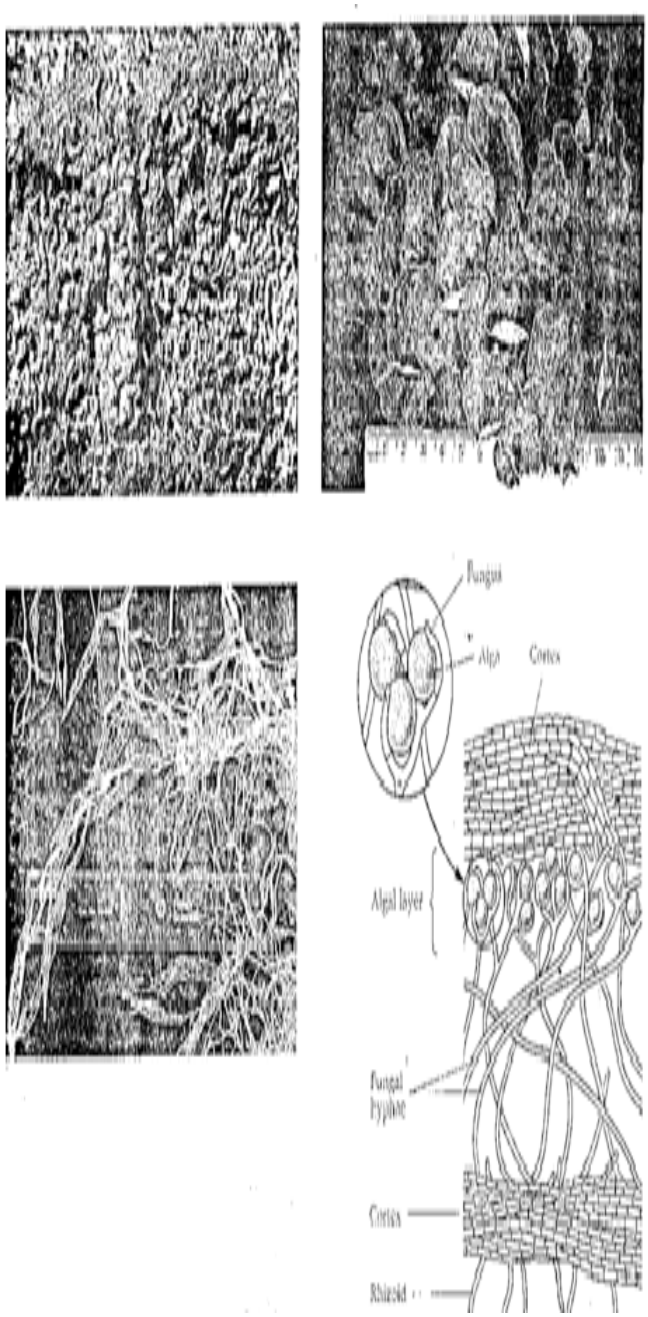

Fig. 8.9: Lichen
Source: Tortora *et al;* 1992.

Lichen grows in distinctive shape as if they are single species, but each is actually a specific association between a fungus and an alga. The two organisms form a minor ecosystem in themselves because the alga uses photosynthesis to make organic compounds that partially feed the fungus. The fungus on its own part provides water, minerals, protection and microenvironments suitable for the growth of the alga (Guttman, 1999). Mutualisms are common in nature but a remarkable number involve fungi. The best known is the lichen symbiosis, in which a fungus (the mycobiont) and an alga (the phycobiont) associate so intimately that they create a distinctive structure, a lichen thallus that

neither partner assumes by itself. The minority alga grows amidst the moist fungus body and photosynthesises, providing organic nutrients for both partners, while the fungus provides minerals and protection from drying and harsh light. The mycobionts and the phycobionts are species in their own right and some may grow independently but many may occur only in lichens. The lichen is sometimes considered a species of its own and named as such, but lichens are formally named for the mycobiont and classified on this basis. A single species of mycobionts could form lichens with several species of phycobionts (Guttman, 1999).

The lichen thallus is usually constructed in layers, including a dense protective layer and a distinct layer where the algae are scattered among the hyphae, and it grows in one of three general forms as in Figure 8.9 as **foliose, fruticose or crustose**. Lichens grow in living plants and on some animals e.g. on insects. A few species of lichens grow in fresh water, on ocean shores, and even submerged in the ocean. However; lichens have little or no ability to withstand drying, they dry out rapidly whenever their environment becomes dry, but rehydrates quickly when water returns. Within the thallus, some hyphae hold the algal cells in place while other hyphae (haustoria) penetrate the algae, though always leaving the algal plasma membrane intact. As the phycobiont photosynthesises, it exports over 90% of its carbohydrates product to the mycobiont. The mycobiont use some of this carbohydrate for growth but stores much of the mannitol, which provides insurance against its harsh living conditions. This storage is used during rehydration and as the dry lichen becomes rehydrated; its respiration rate rises to a high level for several hours before falling back to the normal rate of moist lichen (Guttman, 1999).

Lichens produce a variety of unusual compounds called lichen acid whose functions are not known; some are allomones, including antibiotic that inhibit the growth of bacteria that might destroy the thallus, and others inhibit the growth of mosses, other lichens and the seed plants. They may also discourage herbivores. Some may be light screens that protect the phycobiont, and others function in a mechanism in which the fungus regulates the rate of photosynthesis and the rate at which the alga exports carbohydrates (Guttman, 1999).

Lichens are very sensitive to air pollutants and like other sensitive organisms (e.g. canaries carried by miners) they may serve as monitor to air quality. Lichens have disappeared from badly polluted urban areas and have returned after the pollution has been reduced.

SELF-ASSESSMENT EXERCISE 2

Write an essay on lichens.

4.0 CONCLUSION

The structures, classification, characteristics and methods of reproduction of algae have been discussed. The nature of lichens and the importance of algae have also been treated.

5.0 SUMMARY

In this unit, you have learnt the:

- Nature of algae as photoautotrophs
- Diverse morphological structures of algae
- Different features used in the classification of algae
- Characteristics of the various algae
- Reproduction in *Chlamydomonas* and *Spirogyra*
- Importance of algae to life and nature
- Mutualistic association between an alga and a fungus.

6.0 TUTOR-MARKED ASSIGNMENT

i. What is the contribution of algae to life?
ii. What are the lichens? Discuss its contribution to the ecosystem
iii. Describe the life cycle of a chlorophyta
iv. Compare and contrast the structures of *Chlamydomonas* and *Spirogyra*
v. What are the roles of alga and the fungus in lichen?
vi. Briefly discuss the importance of algae
vii. Describe asexual reproduction in Chlamydomonas

7.0 REFERENCES/FURTHER READING

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MODULE 3 INTERACTIONS AND CONTROL OF MICRO-ORGANISMS

UNIT 1 ANTIGENS AND ANTIBODIES

CONTENTS

1.0 INTRODUCTION

The general practice today is to immunise people against future epidemics of some diseases. For instance, children are vaccinated against poliomyelitis at the early stages of their growth. The adults intending to travel outside the country are vaccinated against a number of diseases e.g. small pox, yellow fever, and cholera to mention a few. This process is called **immunisation**. What is injected is a **vaccine**. Also in the past, it is believed that once one had either an infection of small pox or chicken pox and got over it, the reoccurrence of the infection is ruled out. This indicated that the earlier infection must have some residual substances that will continue to protect the victim against a reoccurrence of the infection .i.e. an immune system has been developed following that infection. In this unit, the development of immunity which is the consequence of antigen - antibody reactions will be discussed.

2.0 OBJECTIVES

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

- define immunity, antigens, antibodies, serology and immunology
- explain the differences between innate and acquired immunity
- explain the differences between cell-mediated immunity and humoral immunity
- discuss the characteristics and the different types of antibodies
- explain primary and secondary responses to antigens.

3.0 MAIN CONTENT

3.1 Immunology and Serology

Immunology is the study of the process by which all living organisms, including man defend themselves against infection. Immunology is the science that deals with the immune system and attempts to understand the many phenomena that are responsible for both acquired and innate (natural) immunity. The term immunity means a high level of resistance to the infectious agents, foreign particles, toxins, living cells and cancer.

Serology encompasses the "in-vitro" study of antigen-antibody interactions, the science of immunity and a study of "in-vivo" development of antibody against specific foreign antigen. Serology is a branch of immunity that is concerned with in-vitro reactions involving one or more serum constituents e.g. antibodies and complements.

In the early days, it was observed that once a man contacted a disease and was cured, the disease never reoccurred. This prompted the Chinese in A.D. 1500 to develop a custom of inhaling crust of small pox lesions to prevent the development of small pox in later life (a very hazardous approach).

In 1798 an English physician Jenner published his work on vaccination describing a safe procedure. He found that people who had small pox never had it again during small pox epidemics in later life. To confirm this, he inoculated a boy with the pus from a lesion of small pox and subsequently re-inoculated the same boy several times with infections from a patient with small pox and observed that no disease was developed. The term vaccination was applied to the procedure and referred specifically to the injection of small pox **vaccine**. The term has now been applied to immunising procedure in which **vaccine** is used or injected. This is called immunisation.

Pasteur then reconfirmed Jenner's work by inoculating the causative agent of chicken cholera and found that it induced immunity against the disease.

Subsequently the term **antigen** was used to describe agent that confers immunity on the host by production of specific antibody. The modern science of serology was based on the above principle antigen-antibody reaction. Active immunity against disease is therefore achieved by the production of antibody in the host against an invading foreign antigen.

3.2 Antigens, Antibodies and Immunity

An antigen can be defined as a substance that is capable of stimulating the production of antibody specifically against it and that is able to react with that antibody.

An antibody can also be defined as a plasma protein, the **immunoglobulin** that is produced by the host in response to an antigen and directed specifically against that antigen. A special characteristic of the antibody is that once it is formed, it is capable of reacting with the antigen that stimulates its production. An antigen-antibody interaction brings about what is called immunity. There are two types of immunity: acquired and natural immunity.

a) **Acquired or Adaptive Immunity**: This is an immunity obtained in same manner other than by hereditary or the immunity achieved when antigens enter the body naturally or artificially.

Acquired immunity is due to the stimulation of antibody production and the production of memory cells keyed to the antigen. This can be achieved in two ways as active and passive immunity.

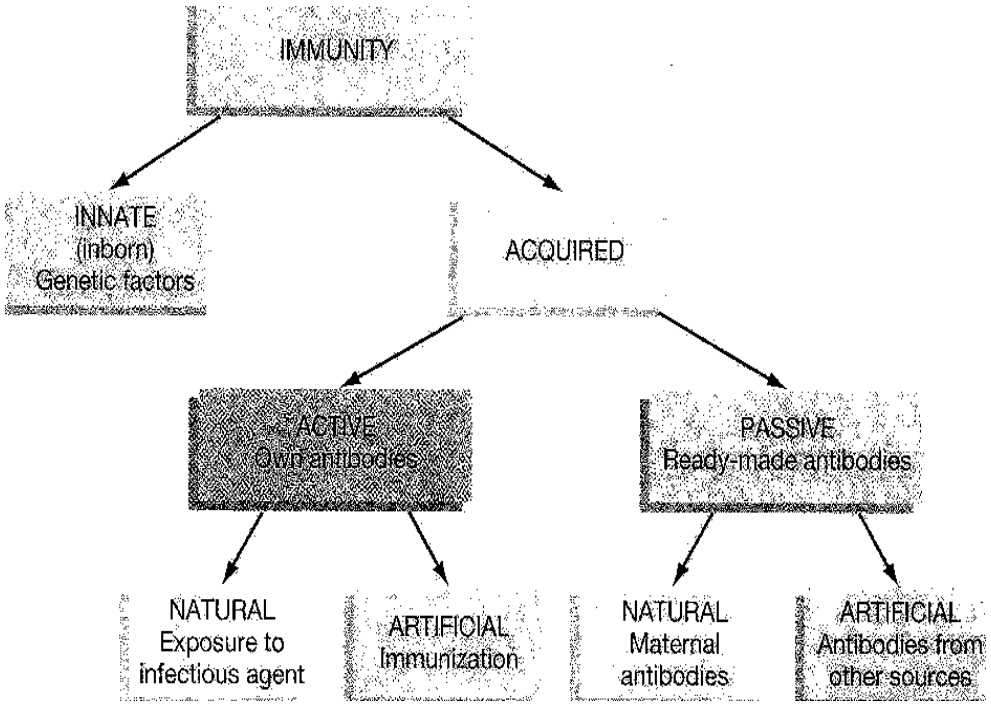


Fig.1.1: The Various Types of Immunity
Source: Jacquelyn, 2002.

Active immunity may be defined as the state of resistance developed by an individual following the effective contact with foreign micro-organisms or their products. Such contact may be achieved by

i) Clinical or sub clinical infection
ii) The injection of life or killed micro-organism or their antigen
iii) Absorption of bacterial products.

b) Natural Immunity: (Innate or non specific immunity)

i) Natural acquired active immunity: obtained when an individual is exposed to an infections agent, often having the disease and their own immune system responds in a protective way.

ii) Naturally acquired passive immunity: when antibodies made by another individual are given to a host e.g. mothers milk without intervention by man.

(c) Humoral Immunity

Humoral immunity develops from the formation of specific antibodies against antigens. This type of immunity protects a person from many types of micro-organisms that would normally cause diseases.

Lymphoid tissue and immune response: When the body is invaded by a foreign substance, it may induce humoral and cell-mediated immune responses. The body's lymphoid tissue plays an important role in this type of immune responses. Lymphoid tissue represents about 2% of the total body weight, mostly located in the lymph nodes and thymus.

3.2.1 Characteristics of Antigens

1. A molecule that can stimulate the production of antibodies.
2. The antibody it produces combines specifically with the antigen to elicit its production.
3. The antibodies produced are heterogenious with respect to immunoglobulin class, affinity for antigens and specificity.
4. Antigens of low molecular weight i.e. less than 5000 rarely stimulate the production of antibodies and with antigens they are known as *haptens.*
5. The larger or more complex the molecule, the more effective it will be as an antigen.
6. High molecular weight of molecules of 500,000 or greater with complex protein or polypeptide carbohydrates structure is the best antigens.
7. The antigen must posses a chemical structure that is different from that of the injected recipient.
8. The more diverse the chemical structure is, the more antigenic the molecule becomes.
9. The route of peritoneal administration of antigen is instrumental in the degree of antibodies produced.
10. Intravenous and intraperitoneal routes are effective.
11. Intradermal (into the skin) route offers stronger stimulus than the subcutaneous (beneath the skin) or intravascular (into the muscle) route.

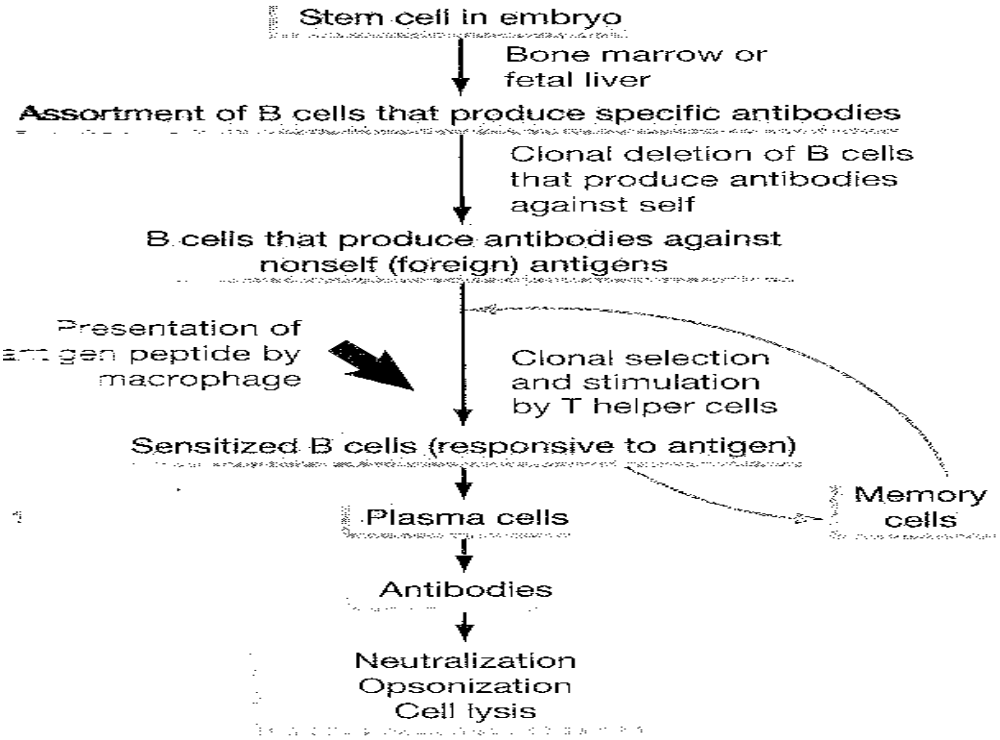


Fig.1.2: Summary of Humoral Immunity
Source: Jacquelyn, 2002.

The lymphoid tissue produces two types of cell populations (Figure 1.2) derived from bone marrow stem cells namely T-lymphocytes (T cells) and B-lymphocytes (B cells). T-lymphocytes are associated with cell mediated immunity while B-lymphocytes are associated with antibody production.

T (thymus-derived) lymphocytes mature in the thymus and are responsible for cell-mediated immunity and the regulation of immune responses. Thus the removal of the thymus in a newborn animal reduces its cell-mediated immune response.

B-lymphocytes mature in the bone marrow and are associated with antibody production. The term B (bursar of fabricus) lymphocytes came from the discovery, that in birds, these cells are produced in the bursar of fabricus, a small piece of lymphoid tissue attached to the posterior region of the intestine. Mammals do not have a bursar for B cell production instead; these cells are produced in the fetal liver and after birth, in the bone marrow (Figure 1.2).

3.2.2 Specific Features of Antigens

1) Antigens induce the production of antibodies.
2) Are able to react with that antibodies.
3) Are macromolecules with a high molecular weight less than 10,000 daltons.
4) Are usually foreign to the host.
5) Must have immunogenicity or the ability to elicit formation of antibodies.
6) Antigens may be bacteria, fungi, protozoa or other cells and viruses and molecules such as bacterial toxins, proteins or carbohydrates.
7) Antigen molecules are large; an antibody reacts with only a small part of this molecule called *epitope* or *antigenic determinant.*
8) Antigen determinants (*epitope)* are of proteins, carbohydrates of low molecular weight, or simple constituents of an antigen.
9) Slight changes in the chemical composition or physical configuration of determinant sites can alter the immunologic properties of antigens.
10) A single antigen may have one or more multiple determinant sites.
11) The term v*alence* refers to the number of determinant sites on an antigen.
12) Large antigenic molecules are multivalent and able to elicit formation of several different kinds of antibodies after introduction into a host.
13) Most determinant sites are located on the surface of antigens but some are hidden within them and exposed only if the antigenic molecule is hydrolysed.

3.2.3 Types and Properties of Antibodies

1. Antibodies are substances produced in response to antigenic stimulation that are capable of interacting with the antigen. They are glycoproteins.
2. Antibodies are specifically described as “immunoglobulin” because of the heterogeneity of the types of molecules that can function as antibodies. They are generally found in the body fluids and blood (serum).

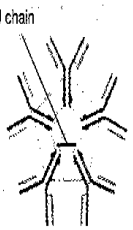
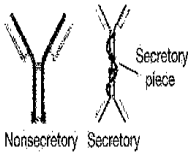
Five distinct structural types or classes of immunoglobulin have been identified in humans based on their physical, chemical and immunologic properties. They are:

i) Immunoglobulin G(1gG)
ii) Immunoglobulin M(1gM)

iii) Immunoglobulin A(1gA)
iv) Immunoglobulin D(1gD)
v) Immunoglobulin E(1gE)

Most antibodies are found within the gamma globulin.
The term 1g and gamma globulins are used to denote the immunoglobulin (antibody) with a specific antigen. It is also referred to as ***antiserum*** **(**antitoxin) because this serum has been obtained from an immunised host and contains the desired antibodies, it is called antiserum.

Table 1.1: Types and Characteristics of Antibodies

Properties of Antibodies					
	Class of Immunoglobulin				
Property	IgG	IgM	IgA	IgE	IgD
					
Number of units	1	5	1 or 2	1	1
Activation of complement	Yes	Yes strongly	Yes, by alternative pathway	No	No
Crosses placenta	Yes	No	No	No	No
Binds to phagocytes	Yes	No	No	No	No
Binds to lymphocytes	Yes	Yes	Yes	Yes	No
Binds to mast cells and basophils	No	No	No	Yes	No
Half-life (days) in serum	21	5–10	6	2	3
Percentage of total blood antibodies in serum	75–85	5–10	10	0.005	0.2
Location	Serum, extra-vascular, and across placenta	Serum and B cell membrane	Transport across epithelium	Serum and extra-vascular	B cell membrane

Source: Jacquelyn, 2002.

3.2.4 Classes of Immunoglobulins

The major classes of immunoglobulins are summarised in Table 1.2.

a. **1gG:** Immunoglobulin G (gamma globulin) is the predominant circulating immunoglobulin and constitutes 80% of all the antibodies found in the human body. It binds to micro-organisms

to enhance their phagocytosis and lysis, activate complements and is the major humoral line of defense in the body. 1gG is produced late in the immune response and can contribute to persistent immunity. It is the only immunoglobulin that is able to cross the placenta and it provides maternally acquired passive immunity in uterus and to neonate at birth.

b. **1gM** is generally the first antibody to be produced during an antibody response. It is able to bind bacteria surface antigens to activate complement. 1gM is the largest of the immunoglobulin. It has a molecular weight of 900,000 daltons and 10 antigens binding sites.

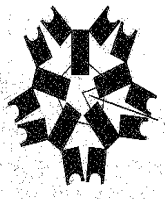
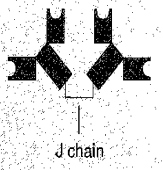
c. **1gA**: is found not only in serum 1gA but also in body secretions. Serum 1gA is the principal immunoglobulin in the mothers' milk (colostrums), saliva, tears and gastro-intestinal and respiratory secretions. It is often the first existing antibody to contact invading micro-organisms, in contrast to 1gM, which is the first antibody to be specifically produced in response to invaders. 1gA in colostrums provides temporary passive immunity to nursing new born infants. 1gA binds to surface antigens of micro-organisms and prevents the adherence of these organisms to the mucosal membrane of the respiratory, gastro intestinal and genitourinary tracts.

d. **1gD:** constitutes approximately 1% of the total serum immunoglobulin. Its role in immunity is unclear, although its presence on the surface of certain types of lymphocytes (B-lymphocytes) suggests that it may serve as a receptor for antigens and may regulate the synthesis of the other immunoglobulin.

e. **1gE:** is found in low concentration through out the body and is involved in Type 1 hyposensitive reactions, where 1gE molecules primarily attach to tissue mast cells and basophilic leucocytes. When 1gE on the surface of the cells binds antigen, the antibody-antigen complexes stimulate the cells to release vasoactive amines such as histamine and serotonin. These chemicals are responsible for the symptoms of hypersensitive reactions. Serum 1gE levels may increase several folds in allergic individuals.1gE level also increases in individual with intestinal parasitic infections, which suggest a role in immunity against parasitic diseases.

Table 1.2: Classes of Immunoglobulins

Classes of Immunoglobulins

Classes	Structure	Molecular Weight (Daltons)	% of Total Antibody	Serum Level (mg/ml)	Number of Antigen-Binding Sites	Heavy Chains	Light Chains	Major Characteristics
IgG		150,000	80	3	2	γ	κ or λ	Major circulating antibody
IgM		900,000 (pentameter)	10	1.5	10	μ	κ or λ	First antibody to be specifically produced during immune response
IgA		160,000 (monometer) 385,000 (dimer)	5–15	1.5–4	2 4	α	κ or λ	Often the first antibody to contact invading microorganisms; major secretory antibody; exists as monometer in serum and as dimer in secretions
IgE		190,000	0.002–0.05	0.0001–0.0003	2	ε	κ or λ	Involved in Type I hypersensitive reactions
IgD		185,000	1	0.03	2	δ	κ or λ	Present on surfaces of lymphocytes

Source: Lim, 1996.

SELF-ASSESSMENT EXERCISE 1

Give an account of the properties of antigens and antibodies.

3.3 Primary and Secondary Responses to Antigens

(i) **Primary Responses**: In humoral immunity, the primary responses to the antigen occur when the antigen is first recognised by host B-cells. After recognising the antigen, B cells divide to form plasma cells which begin to synthesise antibodies. In a few days, antibodies begin to appear in the blood plasma and they increase in concentration over a period of 1 to 10 weeks. The first antibodies are 1gM which can attack foreign substances directly. As 1gM production accelerates, eventually, its production also decreases. The concentration of both 1gM and IgG can become so low as to be undetectable in plasma samples. However, memory cells persist in lymphoid tissues. They do not participate in the initial response but they retain their ability to

recognise particular antigen. They can survive without dividing for many years (Figure 1.4).

(ii) **Secondary Responses**: When the antigen recognised by memory cells enters the blood, a secondary response occurs. The presence of memory cells in the blood makes the secondary response much faster than the primary response. Some memory cells divide rapidly producing plasma cells and others remain as memory cells. Plasma cells quickly synthesise and release large quantities of antibodies. In the secondary response, 1gM is produced before 1Gg. However, IgM is produced in smaller quantities than in the primary response. Thus the secondary response is characterised by a rapid increase in antibodies most of which are 1gG.(Figure 1.4).

3.4 Types of Antigen-Antibody Reactions

The antigen-antibody reactions are most useful in defending the body against bacterial infections, but they also neutralise toxins and viruses waiting to invade host cells. The defensive capability of humoral immunity depends on recognising antigens associated with the pathogen.

For bacteria to colonise surfaces or viruses to infect cells, these infective agents must first of all adhere to host cell surfaces. 1gA antibodies in tears, nasal secretions, saliva and other fluids react with antigens on the microbes. They coat bacteria and viruses and prevent them from adhering to the surfaces.

Microbes that escape 1gA invade tissues and encounter 1gE in lymph nodes and mucosal tissues. Gut associated lymphoid tissues releases large quantities of 1gE, which bind to **mast cells.** Mast cells are bone marrow- derived cells present in a variety of tissues that resemble peripheral blood-bone basophiles and contain an Fc Factor for IgE. These cells then release histamine and other substances that initiate and accelerate the inflammatory process, included in this process is the delivery of 1gG and complement to the injured tissue. Microbes that have reached lymphoid tissue without being recognised by B cells are acted on by macrophages and presented to B-cells. B-cells then bind the antigens and produce antibodies usually with the aid of helper T-cells.

Antibodies binding with antigens to the surface of microbes form antigen-antibody complexes. The formation of antigen-antibody complexes is an important component of the inactivation of infectious agents because it is the first step in removing such agents from the body. However, the means of inactivation varies according to the nature of the

antigen and the kind of antibody with which it reacts. Inactivation can be accomplished by such processes as **lysis** and **neutralisation**. These reactions occur naturally in the body and can be made to occur in the laboratory. Because bacterial cells are relatively large particles, the particles that result from antigen-antibody reactions also are large. Such reactions result in **agglutination** in the sticking together of microbes. 1gM produces **strong** agglutination and 1gG produces **weak** agglutination reactions with certain bacterial cells. Agglutination is the process where there is formation of an insoluble immune complex by the cross linking of cells or particles. The antibody responsible for this process is called an agglutinin.

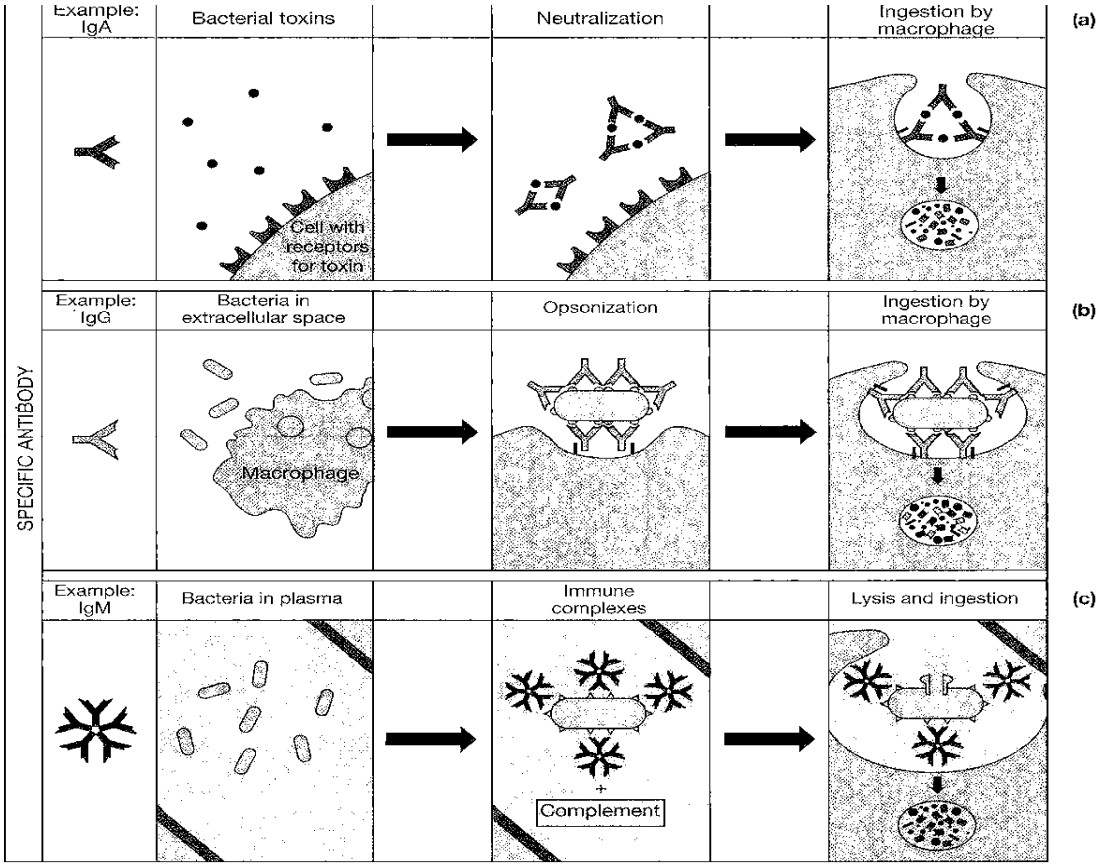


Fig.1.3: Antibodies produced by humoral immune responses eliminates in three ways (a) Neutralisation of pathogens by 1gA or 1gG. (b) Opsonisation of bacteria by 1gG. (c) Cell lysis initiated by 1gM or 1gG immune complexes allows for the formation of membrane attack complexes involving complement proteins.

Source: Jacquelyn, 2002.

(b) Opsonisation. Some antibodies act as **opsonins**. Some bacteria with capsules or surface proteins (M proteins) can prevent phagocytes from adhering to them. The complement system can counteract these defenses, making possible a more efficient

elimination of such bacteria. First, special antibodies called **opsonins** binds to and coat the surface of the infections agent. For example, *Clostidium* sp binds to these antibodies initiating the **cascade** (a fall). Phagocytic cells use two basic molecular mechanisms for the recognition of microbes: (i) opsonin dependent and (ii) opsonin independent recognition. Opsonisation is a process in which microbes or other particles are coated by serum components (antibodies, mannose binding proteins and/or complement C3b thereby preparing them(the antibodies) for recognition and ingestion by phagocytic cells. C3b is an example of a complement (recognition factor) from the serum. Others are C4, C5, C6 and C7.

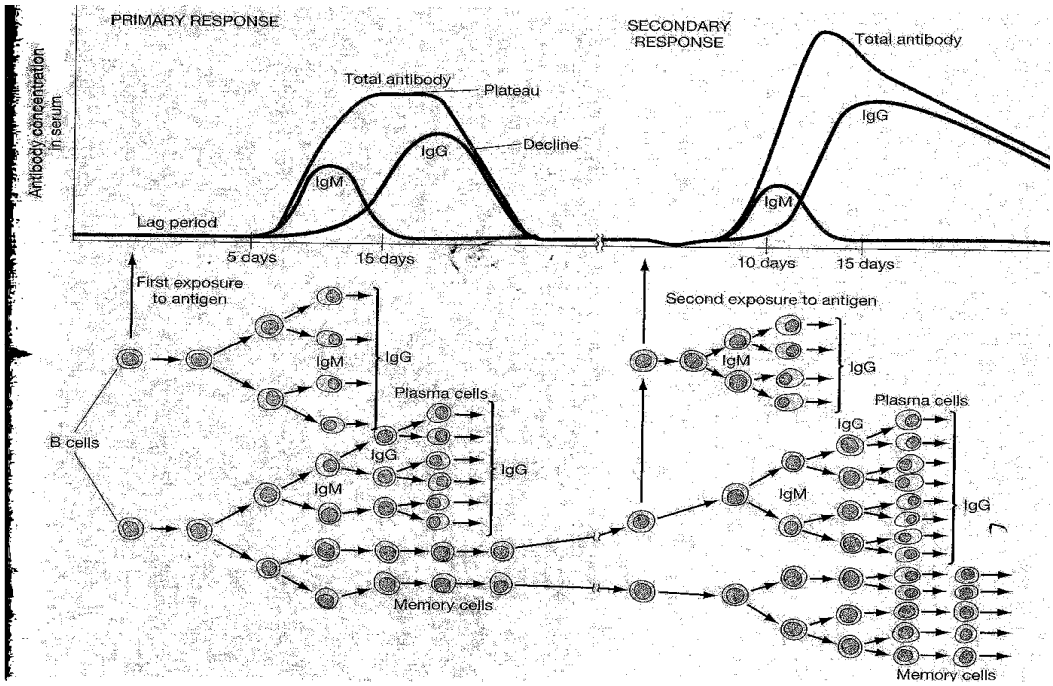


Fig.1.4: Primary and Secondary Responses to an Antigen
Source: Jacquelyn, 2002.

C3b then binds the surface of the microbe. Complement receptors on the plasma membrane of phagocytes recognises the C3b molecule, this recognition stimulates phagocytosis. This process instituted by **opsonins** is called **opsonisation** or **immune adherence** (Figure 1.6). Antibodies neutralise toxins and coat microbes so that they can be phagocytised.

Inflammation can also occur where the complement system is also potent in initiating and enhancing inflammation. The C3a, C4a and C5a enhance the acute inflammatory reactions by stimulating chemotaxis and thus phagocytosis occurs. Chemotaxis is a pattern of micro-organisms behaviour in which micro-organism moves towards chemical attractants (in this case chemical molecules of the C3a,C4a and C5a). These three complement proteins also adhere to the membranes of basophiles and

mast cells, causing them to release histamine and other substances that increase the permeability of blood vessels.

3.5 Membrane Attack Complexes

Another defense mechanism triggered by C3b is cell lysis. By a process called **immune cytolysis** complement proteins produce lesions in the cell membranes of micro-organisms and other types of cells. These lesions cause cellular contents to leak out. To cause immune **cytolysis,** C3b initiates the splitting of C5 into C5a and C5b. C5b then binds C6 and C7 forming a C5bC6C7 complex. This protein complex is hydrophobic (water fearing) (and form a barrier between the cell and its environments) and inserts this complex into the microbial membrane. By extending all the way, through the cell membrane, all these proteins form a **pore** and constitute the **membrane attack complex (MAC)**. The MAC is responsible for direct lysis of invading micro-organisms (Figure 1.5).

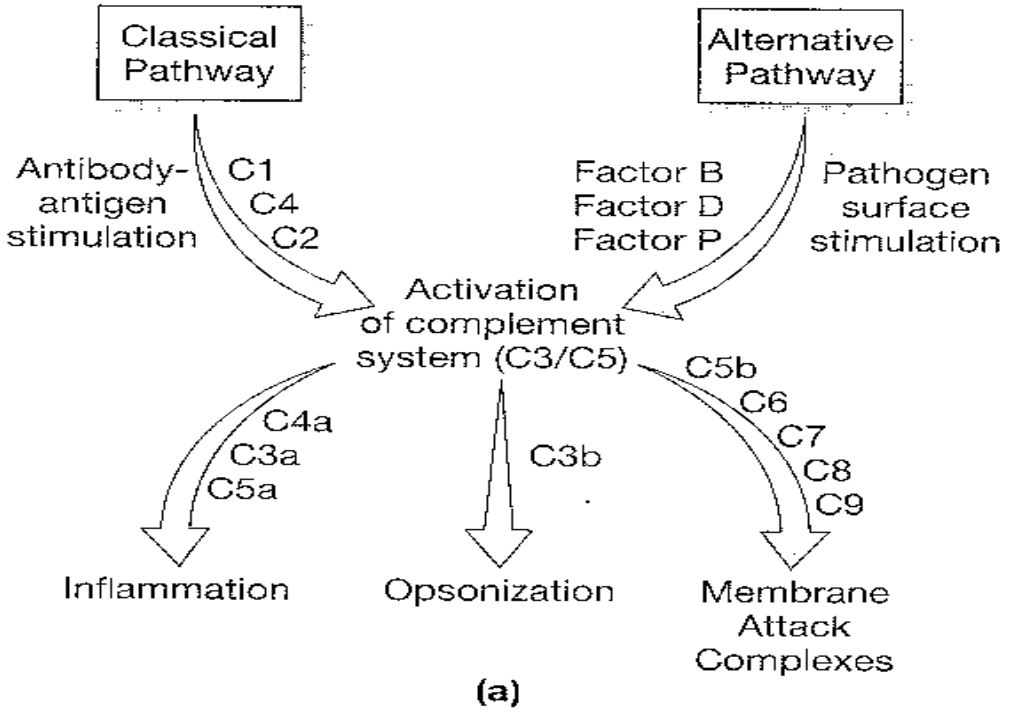


Fig.1.5: The Complement System: Classical and alternative pathways of the complement cascade.

Source: Jacquelyn 2002.

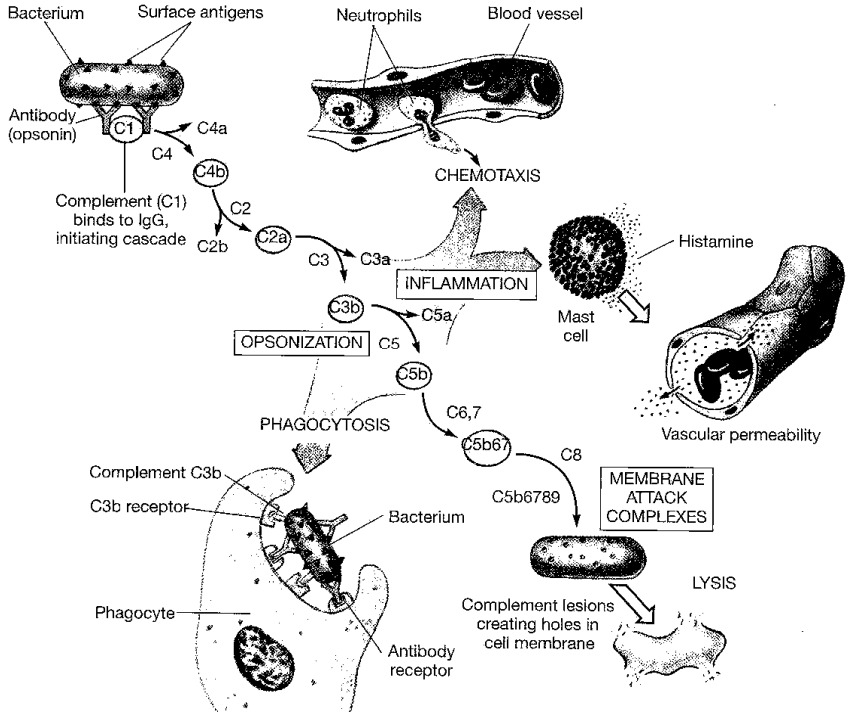


Fig.1.6: The Complement System
Source: Jacquelyn, 2002.

(a) Classical and alternative pathways of the complement cascade. Although the two pathways are initiated in different ways, they combine to activate the complement system. (b) Activation of the classical complement protein. In this cascade each complement protein activates the next one on the pathway. The action of C3b is critical opsonisation and along with C5b, for formation of membrane attack complexes. C4a, C3a and C5a also are important to inflammation and phagocyte chemotaxis. Host plasma-membrane contains proteins that protect against MAC lysis. These proteins prevent damage by preventing the binding of activated complement proteins to host cells. The MAC forms the basis of complement fixation. The advantage of the complement system to host defenses is that once it is activated, the reaction cascade occurs rapidly.

SELF-ASSESSMENT EXERCISE 2

Differentiate between primary and secondary responses to antigens in the human body.

4.0 CONCLUSION

The invasion of the human body by infective agents (antigens) stimulates the production of antibodies that bring about different types of antigen-antibody reactions. Immunity occurs in such an individual after these reactions.

5.0 SUMMARY

In this unit, you have learnt the:

- Process that is referred to as immunisation
- Types of immunity and how it is acquired
- Definitions of immunology, serology, antigens, antibodies and immunity
- Production of antibodies in the human body
- Features, types and characteristics of antigens and antibodies
- Primary and Secondary responses to antigens
- Types of antigen-antibody reactions
- Membrane attack complexes.

6.0 TUTOR-MARKED ASSIGNMENT

i. Differentiate between innate and acquired immunity.
ii. List the properties of an antigen.
iii. What are the properties of antibodies?
iv. Describe primary and secondary responses to antigens.
v. What is opsonisation? What is the significance of this in antigen-antibody reaction?
vi. What are the characteristics of antibodies?

7.0 REFERENCES/FURTHER READING

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UNIT 2 STERILISATION

CONTENTS

1.0 INTRODUCTION

It is of importance to state that it is necessary to control or eliminate microbial populations on inanimate objects e.g. eating utensils and surgical instruments. The process that makes it possible for these materials to be free of microbes and their products is called sterilisation. Sterilisation (Latin sterilis, unable to produce offspring or barren) is that process by which all living cells, viable spores, viruses, viroids and other injurious products are either destroyed or removed from an object or habitat (Prescott, *et al*, 2009). A sterile object is totally free of viable microbes, spores, and other infectious agents. How this condition can be obtained will be our discussion in this unit.

2.0 OBJECTIVES

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

- explain sterilisation and its importance
- discuss the various procedures to achieve sterilisation
- explain the factors that can affect sterilisation.

3.0 MAIN CONTENT

3.1 Definition and Uses of Sterilisation

Sterilisation: means the freeing of an article from all living organisms, including viruses, bacteria, fungi, and their propagules and their

products-toxins, enzymes etc. An article should not be described as being relatively sterile. It is either sterile or non-sterile.

Sterilisation is required for culture media, fluids, reagents, utensils, containers and equipment used in microbiology.

It is also required for medical and surgical instruments and materials used in procedures that involve penetration into the blood, tissues and other normal parts of the body e.g. in surgical operations, intravenous infusions, hypodermic injections and diagnostic aspirations (Duguid *et al*, 1987). The goal of sterilisation is two fold: (a) to destroy pathogens and prevent their transmission, and (b) to reduce or eliminate microbes responsible for the contamination of our water, food, environment and other substances.

3.2 Methods of Sterilisation

Four main methods are used for sterilisation. These are:

(a) **Heat:** The only method of sterilisation that is both reliable and widely applicable is by heating under carefully controlled environmental conditions at temperatures sufficiently higher that 100^{o}C to ensure the killing of even the most heat resistant micro-organisms and spores.

(b) **Filtrations**: Bacteria stopping filters are used in removing bacteria and all larger micro-organisms from liquids that are liable to be spoiled by heating e.g. blood serum and antibiotic solutions, and in which are residual contamination with filter passing viruses.

(c) **Irradiation**: With ultra-violet or ionising (e.g. gamma rays) radiation has special application including the sterilisation of disposable plastic equipment.

(d) **Chemical Disinfection:** This method is generally unreliable and unsuccessful in effecting sterilisation. Only a few of the more toxic and irritant disinfectants e.g. formaldehyde (formalin), glutaraldehyde and ethylene oxide, are capable of killing bacterial endospores. They are also effective only when used in an adequate concentration applied under carefully controlled conditions of temperatures, moisture e.t.c. This method is discussed in the next unit, (unit 3).

3.2.1 Factors and Sterilisation by Heat

Moist heat is much more effective than dry heat. Moist heat kills micro-organisms by coagulating and denaturing their enzymes and structural proteins, a process in which water participates. Spore sterilisation, i.e.

killing of the most resistant spores, requires exposure to moist heat at 121^{o}C for 10 to 30 minutes. Dry heat is believed to kill micro-organisms by causing destructive oxidation of essential cell constituents. Killing of the most resistant spores by dry heat requires a temperature of about 160^{o}C for 60 minutes. This high temperature causes slight charring of paper, cotton and other organic materials (Duguid *et al,* 1985). There are certain factors that affect the efficiency of heat to achieve the desired sterilisation.

The factors to be considered are the temperature and time of exposure, the number of vegetative micro-organisms and spores present, strains and spore-forming ability of the micro-organisms, and the nature of the material, containing the micro-organisms.

(i) **The Temperature and Time**: The temperature and time for killing is inversely related, shorter time suffice at higher temperatures. Thus to sterilise, the heating must be "hot enough and long enough". Published findings on the resistant spores show many discrepancies, but in practice the following may be taken as minimal sterilising exposures. For surgical and bacteriological sterilisation, most authorities consider that a 10 to 12 minutes exposure of organisms to moist heat at 121°C is sufficient. This ensures killing of all pathogenic sporing micro-organisms and all saprophytes except for some strict thermophiles that cannot grow at less than 40°C.

(ii) **Population and Spore Load of Micro-organisms:** The susceptibility and duration of survival to heat varies considerably among the individual cells, even in pure culture. The number of survivors diminishes exponentially with the duration of heating, and time for complete sterilisation increases in relation to the number initially present. In practice it is usual to minimise the number of contaminating bacteria by cleansing procedures before applying heat for the purpose of sterilisation. The species, strains and spore forming ability of the microbe greatly affect its susceptibility to heat. The amount of heat required to kill a given variety may be in terms of temperature and time of exposure, either as the thermal death point, i.e. the lowest temperature to give complete killing in aqueous suspension for 10 minutes, or as the thermal death point time, i.e. the shortest time for complete killing at stated temperature. The procedures are made under strictly standardised conditions, e.g. with sealed 9mm diameter hard glass tube containing 1to2 ml suspension of $5x10^{7}$ organisms per ml in a defined phosphate buffer solution at pH 7.0.This is because, thermal death point and time measurements depend on the killing variable tail of more resistant cells, amore reliable measurement is the decimal reduction time, or D value,

which is the time (in minutes) required to achieve a ten-fold reduction in viability of microbial population at a given temperature under standard conditions.

(iii) Susceptibility of Microbes

The vegetative forms of most bacteria, yeasts and fungi, and most animal viruses, are killed in 10 minutes by a temperature between 50°C (e.g. *Neisseria gonorrhoeae*) and 65°C (e.g. *Staphylococcus aureus*). Extreme suspecibility is shown by *Treponema pallidum* which is killed in 10minutes at about 43°C; *Coxiella burnetii* is a markedly resistant vegetative organism and extreme resistance is shown by *thermophilic saprophytic bacilli, e.g Bacillus stearothermophilus,* whose vegetative forms can grow at temperatures approaching 80°C. A few animal viruses are more resistant than majority; for example, that of poliomyelitis may require heating at 60°C for 30 minutes and that of serum hepatitis, when in serum, at 60°C for 15 to 30 minutes without affecting the virus. These viruses are killed by temperatures in the range 65°C to 80°C.

The spore forms of actinomycetes, yeasts and fungi are more resistant than parental vegetative forms, though not as highly resistant as bacterial spores. The more susceptible kinds are killed at 70°C in 5 minutes and the more resistant at 80°C to 90°C in 30 minutes. The resistance of bacterial spores varies considerably between different strains of the same species.

Dry heat at 100°C for 60 minutes is required to kill vegetative bacteria that would succumb to moist heat at 60°C in 30 minutes. Fungal spores are killed in hot air at 115°C within 60 minutes, and bacterial spores at temperatures in the range of 120°C to 160°C within 60 minutes.

The Nature of the Sterilising material

The sterilising material may affect the rate of killing. A high content of organic substances generally tends to protect spores and vegetative organisms against lethal action of heat. Proteins, gelatins, sugars, starch, nucleic acids, fats and oils all act in this way. The effect of fats and oils is greatest with moist heat since they prevent access of moisture to the microbes. The presence of an organic or inorganic disinfectant has opposite effect and promotes killing by heat. The pH is important; the heat resistant spores are greatest in neutral medium (pH7.0) and are diminished with increasing acidity or alkalinity. The effect of alkali has been used in disinfection of metal instruments by boiling at 100°C in water containing 2 percent of sodium carbonate; but this method is not as reliable as autoclaving. The conditions under which sporulating bacteria are grown influence the heat-resistance of the spores. Thus, the spores formed by soil bacteria and intestinal bacteria in artificial cultures

are sometimes less resistant than those formed in the organism's natural habitat.

3.2.1.1 Sterlisation by Dry Heat

(a) **Red Heat**: Inoculating wires, points of forceps and spatulas are sterilised by holding them in the flame of a Bunsen burner until they are seen to be red hot. This is part of the practice in microbiological laboratories.

(b) **Flaming**: Direct exposure for a few seconds in gas or spirit flame may be used for sterilising scalpels and needles. Needles, scalpels and basins are treated by immersing them in methylated spirit and burning off the spirit, but this method does not produce sufficiently high temperature for sterilisation.

(c) **Hot Air Oven**: This is the main means of sterilisation by dry heat. The oven is usually heated with electricity and has a thermostat that maintains the chamber air constantly at the chosen temperature and fan to assist circulation of air. Commonly, a temperature of 160°C is maintained for 1 hour (Duguid *et al,* 1985).

The hot-air oven is used for sterilising dry glassware, forceps, scalpels, scissors, throat swabs and syringes. It is also used for sterilising dry materials in sealed containers, and powders, fats, oil and greases which are impermeable to moisture.

The sterilising oven must not be overloaded and spaces must be left for circulation of air. It may be cold or warm when loaded, and is then heated up to the sterilising temperature in the course of 1 to 2 hours. The holding period of 1 hour at 160°C is timed as beginning when the thermometer first shows that the air in the oven has reached 160°C.

(d) **Infra-Red Radiation**: Another method of sterilisation by dry heat employs infra-red radiation. The infra-red rays are directed from radiation from electrically heated elements on to the objects to be sterilised, e.g. all-glass syringes, and temperatures of 180°C can be attained. Heating at or above 200°C by infra-red in *vacuum* has been employed as a means of sterilising surgical instruments. Cooling is hastened and oxidation prevented during the cooling period by admitting filtered nitrogen chamber.

SELF-ASSESSMENT EXERCISE 1

Describe in detail sterilisation by dry heat.

3.2.1.2 Sterilisation by Moist Heat

Killing by moist heat requires the micro-organisms to be in contact with hot water or steam. Moist heat may be employed: (1) at temperature below 100°C, 2) at a temperature above 100°C, i.e. in saturated steam under increased pressure in an autoclave.

(i) Moist Heat at Temperatures below 100°C
In the pasteurisation of milk, the temperature employed is either 63°C to 66°C (145°C to 150°C) for 30 minutes. These processes usually destroy all the non-spore forming pathogens such as *Mycobacterium tuberculosis*, *Brucella abortus* and various salmonellaceae that may be present in milk. *Coxiella burnetii*, the causative organisms of Q fever, is heat resistant and may survive pasteurisation. Unless large numbers of the organisms are present, the treatment usually reduces them to less than infective dose. Vaccines prepared from pure cultures of non sporing bacteria may be inactivated in a special water bath ('vaccine bath') at a comparatively low temperature; 1hour at 60°C is usually sufficient. Higher temperatures may diminish the immunising power of the vaccine (Duguid *et al*, 1987).
Eating utensils, clothing, bed –clothes and some items of nursing equipment may be disinfected by washing in water at 70°C to 80°C for several minutes.

(ii) Moist heat at a temperature of 100°C
Boiling at 100°C for 5 minutes to 10 minutes is sufficient to kill all non-sporing organisms. The method does not ensure sterility, but has been found satisfactory for certain purposes in bacteriology and medicine where sterility is not essential or better methods are unavailable.
When an instrument is removed from the boiling water, it should be allowed to dry e.g. scalpel blade or syringe needle, from becoming contaminated with skin bacteria carried from the fingers in the film of water on its surface.

(iii) Moist Heat Above 100°C (Use of the Autoclave)
Water boils when its vapour pressure equals the pressure of the surrounding atmosphere. This occurs at 100°C at normal atmospheric pressure (i.e.760mmHg.14.7lb. per square inch absolutely pressure or 0 1b/in2 'gauge pressure') thus when water is boiled within closed vessel at increased pressure, the temperature at which it boils, the steam if forms, will rise above 100°C.

This is the principle employed in the pressure cooker and the autoclave, which subject articles to moist heat at temperatures higher than 100°C.

Autoclaving is the method widely used for sterilisation of surgical supplies and bacteriological culture media. In the autoclave, all parts of the load to be sterilised must be permeated by steam. The steam should not only be saturated, i.e. at the point of condensing to liquid water, but also dry i.e. free from particles of liquid water once the whole of the load has been heated up to the temperature of the steam, there is a minimum holding time at that temperature necessary for sterilisation. The minimum holding times are 2 minutes at not less than 132°C (27Ib/in2 gauge pressure); 12 minutes at not less than 121°C (15Ib/in2 gauge pressure) and 30 minutes at not less than 115°C (101lb/in2 gauge pressure) (Duguid *et al*, 1987). They become 3, 18 and 45 minutes respectively.

All the air must be removed from autoclave chamber and the articles in the load, so that the latter are exposed to pure steam during the period of sterilisation. There are three reasons for this:

1) the admixture of air with steam results in a lower temperature being achieved at the chosen pressure;
2) the air hinders penetration of the steam into the interstices of porous materials, surgical dressings especially, and the narrow openings of containers, syringes, etc.; and
3) the air, being denser than the steam, tends to form a separate and cooler layer in the lower part of the autoclave, and so prevents adequate heating of the articles there. For examples, in an autoclave with no air discharge, a temperature of only 70°C was recorded at the bottom while that at the top was 115°C.

There is one exception to the necessity for complete air discharge from the load. Hermetically sealed bottles and ampoules containing aqueous solutions and culture media are satisfactorily sterilised in spite of the presence of some air in them. The contained water provides the conditions for moist-heat sterilisation, making unnecessary the entry of steam for this purpose, and contents are heated to the same temperature as the chamber steam, though to a higher pressure, by the conduction of heat through the container walls.

The properties of some physical antimicrobial agents are listed in Table 2.1.

Table 2.1: Properties of Physical Antimicrobial Agents

Properties of Physical Antimicrobial Agents		
Agent	Action	Use
Dry heat	Denatures proteins	Oven heat used to sterilize glassware and metal objects; open flame used to incinerate microorganisms.
Moist heat	Denatures proteins	Autoclaving sterilizes media, bandages, and many kinds of hospital and laboratory equipment not damaged by heat and moisture; pressure cooking sterilizes canned foods.
Pasteurization	Denatures proteins	Kills pathogens in milk, dairy products, and beer.
Refrigeration	Slows the rate of enzyme-controlled reactions	Used to keep fresh foods for a few days; does not kill most microorganisms.
Freezing	Greatly slows the rate of most enzyme-controlled reactions	Used to keep fresh foods for several months; does not kill microorganisms; used with glycerol to preserve microorganisms.
Drying	Inhibits enzymes	Used to preserve some fruits and vegetables; sometimes used with smoke to preserve sausages and fish.
Freeze-drying	Dehydration inhibits enzymes	Used to manufacture some instant coffees; used to preserve microorganisms for years.
Ultraviolet light	Denatures proteins and nucleic acids	Used to reduce the number of microorganisms in air in operating rooms, animal rooms, and where cultures are transferred.
Ionizing radiation	Denatures proteins and nucleic acids	Used to sterilize plastics and pharmaceutical products and to preserve foods.
Microwave radiation	Absorbs water molecules, then releases microwave energy to surroundings as heat	Cannot be used reliably to destroy microbes except in special media-sterilizing equipment.
Strong visible light	Oxidation of light-sensitive materials	Can be used with dyes to destroy bacteria and viruses; may help sanitize clothing.
Sonic and ultrasonic waves	Cause cavitation	Not a practical means of killing microorganisms but useful in fractionating and studying cell components.
Filtration membranes	Mechanically removes microbes	Used to sterilize media, pharmaceutical products, and vitamins; in manufacturing vaccines; and in sampling microbes in air and water.
Osmotic pressure	Removes water from microbes	Used to prevent spoilage of foods such as pickles and jellies.

Source: Jacquelyn, 2002.

3.2.2 Sterlisation by Radiation

There are four general types of radiation: (1) Ultraviolet light (2) Ionising radiation (3) Microwave radiation and (4) Strong visible light.

(a) Ultraviolet light (uv) consists of lights of wavelengths between 40-390nm but wavelengths in the 200nm range are most effective in killing micro-organisms by damaging their DNA and proteins. Ultraviolet light is absorbed by purine and pyrimidine bases of nucleic acid such absorption can permanently destroy these important molecules. Ultraviolet is especially effective in viruses. However, it kills fewer bacteria than one might expect because of DNA repair mechanism. Once the DNA is repaired new molecules of RNA and protein can be synthesised to replace the damaged molecules. Lying in soil and exposed to sunlight for decades, endospores are resistant to uv damage because of a small protein that binds their DNA. This changes the geometry of the DNA by untwisting it slightly, thereby making it resistant to the effects of uv irradiation (Jacquelyn, 2002).
Ultraviolet light is of limited use because it does not penetrate glass, cloth, covers or under laboratory benches. It penetrates air, effectively reducing the number of airborne micro-organisms and

killing them on surfaces in operating rooms and rooms that will contain caged animals.

(b) Ionising radiation X-rays which have wave length of 0.1 to 40nm and gamma which have shorter wavelengths are forms of ionising radiations, so named because they can dislodge electrons from atoms, creating ions. (longer wavelengths are forms of non-ionising radiation). These forms of radiation also kill micro-organisms and viruses. Many bacteria are killed by **millirads** of radiation; polyviruses are inactivated by absorbing 3.8millirads. A rad is a unit of radiation energy absorbed per unit gram of tissue. A millirad is one thousandth of a rad. Humans usually do not become ill from radiation unless they are subjected to doses greater than 50rad. Irradiation is used by hospitals to sterilise food for immune-compound patients. Ionising radiation damages DNA and produces peroxides which act as powerful oxidising agents in cells. This radiation can also kill or cause mutations in human cells if it reaches them. It is used to sterilise plastics in laboratory and medical equipment and pharmaceutical products. It can be used to prevent spoilage in seafoods by doses of 100-250kilorads in meats and poultry by doses of 50-100kilorads and in fruits by doses of 200 to 300 kilorads (one kilorad equals 1000 rads). Many consumers reject irradiated food for fear of receiving radiation, but such foods is quite safe free of both pathogens and radiation. In Europe, milk and other food materials are often irradiated to achieve sterility (Jacquelyn, 2002).

(c) Microwave radiation, in contrast with gamma, x-rays, and ultraviolet radiation falls at the long-wavelength end of the electromagnetic spectrum (Figure 2.1). It has wavelength of approximately 1nm to 1m, a range that includes television and police radar. Microwave oven frequencies are turned to match energy levels in water molecules. In the liquid state, water molecules quickly absorb the microwave energy and then release it to surrounding materials as heat. Thus materials that do not contain water, such as plates made of paper, china or plastic remain cool while the moist food becomes heated. For this reason, the human microwave cannot be used to sterilise items such as bandages and glasswares. Moreover, bacterial endospores, which contain almost no water, are not destroyed by microwaves. However specialised microwave oven has recently become available that can be used in sterilising media in just 10minutes.

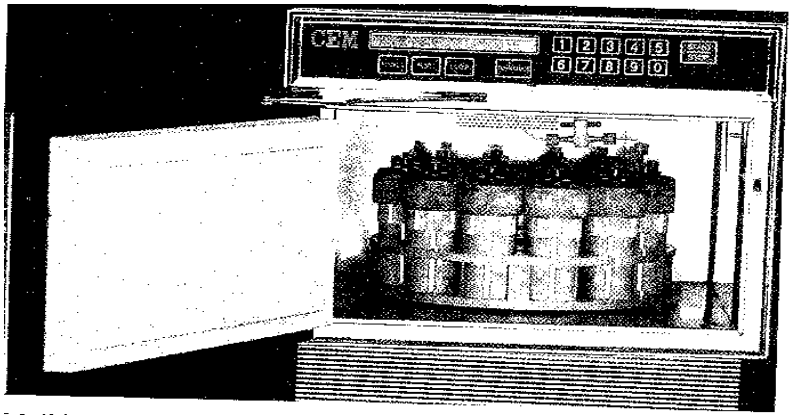

Fig.2.1: Microwave Sterilisation. The microclave system is specially designed for rapid sterilisation of microbiological media and solutions. Using microwave energy, it can sterilise 1.2 liters of media in 6.5minutes, or 100ml in 45seconds.

Source: Jacquelyn, 2002.

3.2.3 Sterilisation by Filtration

Filtration is the passage of a material through a filter or straining device. Sterilisation by filtration requires filter with exceedingly small pores. Filtration has been used since Pasteur' time to separate bacteria from media and to sterilise materials that would be destroyed by heat. Over the years filtration has been made of porcelain, asbestus, diatomaceous earth, and sintered glass (glass that has been heated without melting).

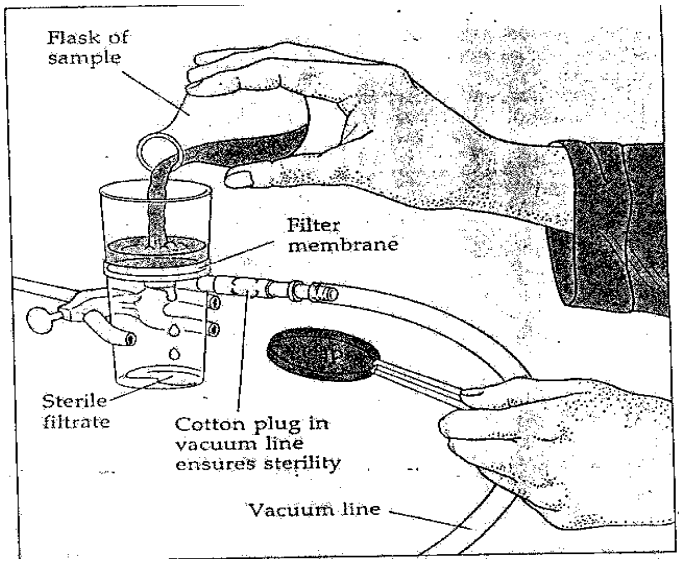

Fig.2 2: Sterilisation by Filteration

Source : Tortora *et al*, 1992.

Membrane filters (Figure 2.2). Consists of thin discs with pores that prevent passage of anything larger than its pore. It is made of nitrocellulose and has great advantage that it can be manufactured with specific pores sizes from 25um to less than 0.025um. Particles filtered by various pore sizes are summarised in Table 2.2. Membrane filters have certain advantages and disadvantages. Except for those with smallest pore sizes, membrane filters are relatively inexpensive; do not clog easily and can filter large volumes of fluid reasonably rapidly. They can be autoclaved or purchased already sterilised. A disadvantage of membrane filters is that many of them allow viruses and mycoplasmas to pass through. Other disadvantages are they absorb relatively large amounts of the filtrate and may introduce metallic ions into the filtrate. Membrane filters are used to trap bacteria from air and water samples can be transferred directly to agar plates, and the quantity of bacteria in the sample can be determined. Filtration is also used to remove micro-organisms and other small particles from public water supplies and in sewage treatment facilities. This technique however cannot sterilise, it merely reduces contamination (Table 2.2).

Table 2.2: Pore Sizes of Membrane Filters and Particles that Pass through them.

Pore Sizes of Membrane Filters and Particles That Pass Through Them	
Pore Size (in μm)	Particles That Pass Through Them
10	Erythrocytes, yeast cells, bacteria, viruses, molecules
5	Yeast cells, bacteria, viruses, molecules
3	Some yeast cells, bacteria, viruses, molecules
1.2	Most bacteria, viruses, molecules
0.45	A few bacteria, viruses, molecules
0.22	Viruses, molecules
0.10	Medium-sized to small viruses, molecules
0.05	Small viruses, molecules
0.025	Only the very smallest viruses, molecules
Ultrafilter	Small molecules

Source: Jacquelyn, 2002.

The various types of filter used in bacteriological work includes: (1) Earthen candles (Berkefeld, chamber land), (2) Asbestos and asbestus-paper disks (Seitz), (3) Sintered glass filters, and (4) Cellulose membrane filters.

SELF-ASSESSMENT EXERCISE 2

Enumerate the different methods of sterilisation by moist heat.

4.0 CONCLUSION

There is a big gap in the efficiency of sterilisation and that of disinfection. Disinfectants deals with the superficial contaminants while sterilisation brings about complete killing of the micro-organisms. Disinfectants will be discussed further in unit 3.

5.0 SUMMARY

Sterilisation and its uses have been discussed. Different methods by which sterilisation can be achieved were enumerated. Factors influencing the efficiency of sterilisation were also enumerated.

6.0 TUTOR-MARKED ASSIGNMENT

i. What is sterilisation? Cite a good example
ii. What factors control the use of sterilisation in combating micro-organisms?
iii. Describe the use of autoclave as a means of sterilisation.
iv. Discuss the procedures involved in sterilisation by heat.
v. What is filtration? How useful is this method in sterilisation?
vi. Discuss irradiation in relation to sterilisation.

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UNIT 3 DISINFECTION

CONTENTS

1.0 INTRODUCTION

Another way of controlling the population of microbes is by disinfection. Disinfection is described as the killing, inhibition or removal of micro-organisms that may cause disease. It should be noted that the primary goal of disinfection is to destroy potential pathogens, but disinfection also to a large extent reduces the microbial population. Disinfection when achieved is by the use of chemicals called disinfectants. Disinfectants are therefore agents, usually chemicals, used to carry out disinfection and are normally used only on inanimate objects. Please do not confuse disinfection with sanitisation. They are closely related. Sanitisation reduces microbial population to levels that are considered safe by public health standards. In this unit, you will learn more about the different types of chemicals used as disinfectants. For emphasis, sterilisation as a procedure kills all microbes while disinfection may or may not kill the microbes.

2.0 OBJECTIVES

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

- define disinfection
- list the procedures involved in disinfection
- explain the uses of disinfection
- discuss different methods of disinfection
- list common laboratory disinfectants.

3.0 MAIN CONTENT

3.1 Disinfectants as Antimicrobial Agents

Disinfection is used only in circumstances in which sterility is unnecessary or sterilisation procedures are impracticable, yet there is still some value in obtaining a particular or complete removal of non-sporing pathogens.

It is impracticable, for example to apply sterilising procedures to bedpans, baths, wash-basins, furniture, eating utensils, bed cloths and other formites that might spread infection in hospitals or even at homes. But because pathogens that might be present on these articles are capable of causing infections include those that form spores. It is useful to disinfect the articles by procedures lethal only to vegetative organisms.

It is also impracticable to apply sterilising procedures to the skin, but because the bacteria most commonly infecting bacterial wounds are non-spore forming. It is a valuable pre-operation procedure to treat the skin around the operative site with a disinfectant that will kill many of the vegetative bacteria on it and so reduce the chance of some of them being carried into the wound.

Antimicrobial agents as chemicals can either kill or prevent the growth of micro-organisms. Disinfectants fall into this group of such chemicals. Therefore a disinfectant can be referred to as a germicide (bactericide, fungicide, algicide or viricide).A germicide kills pathogens and many non -pathogens. However, other chemicals do not kill but they prevent growth. Such chemicals can be described as bacteriostatic or fungistatic. A disinfectant does not necessarily sterilise an object because viable spores and a few microbes may remain. Disinfection is therefore different from sterilisation. Disinfection however is necessary in many situations as you will encounter later in the unit.

3.2 Importance of Disinfectants

Chemical antimicrobial agents for use in the environment or on the skins are often wrongly called sterilising agents. They should be called disinfectants or antiseptics. These terms indicate the role of agents in killing or inhibiting many pathogenic micro-organisms without implying that they can be relied on to kill all micro-organisms and spores. The distinction between disinfectant and antiseptic is not clear-cut; it refers to the potency of the agent and many agents may be used as disinfectants in high concentration and as antiseptics in low ones.

3.3 Mode and Conditions of Action of Disinfectants

Remarkably little is known about the mechanism of action of many accepted antimicrobial chemicals. The protein-denaturing effects of acids and phenols, the oxidising action of the halogens, the lipids-solvent, and the detergent action of some organic solvents, and the detergent action of surface agents may account for some relatively non-specific antibacterial effects at the cytoplasmic membrane. Probably most disinfectants and antiseptics act by denaturing or altering proteins or lipids in the cytoplasmic membrane. Some antiseptics interfere with the energy yielding systems within the cell and some directly inhibit specific steps in biosynthetic pathways. It is known that mercuric salts combine with sulphydryl groups and that the antibacterial dyes combine with nucleic acid. This effect may be of practical use in the treatment of *herpes virus* infections. Heterocyclic dyes such as neutral red, proflavine and toluidine affect the guanine bases of nucleic acids. Thereafter, on exposure to fluorescent light, the dye absorbs energy and this may cause single-strand breaks by excision of guanine. The effect is referred to as 'photodynamic action.'

In general, the rate of inactivity of a susceptible bacterial population in the presence of an antimicrobial chemical is dependent on the relative concentrations of the two reactants, the bacteria and the chemical, but there are other variables that must be controlled if disinfection is to be ensured. The principal factors determining effective disinfection are the concentration of the disinfectants in use and the time during which it is effectively applied. The range of *dilution* over which a disinfectant is effective varies markedly with different chemicals and some disinfectants become quite inactive when diluted only for several times before use. Unless great care is taken in the preparations and replacement of 'in-use' dilutions, accidental over-dilution and thus failure of disinfectant may occur.

The velocity of the reaction also depends upon the number of organisms present, the species involved whether the organism is spore forming or not. In general, chemicals have an uncertain action on bacterial endospores. The microbicidal action of disinfectants is usually increased by heat within the limits of thermo stability of the substances and it is preferable to use disinfectants under warm rather than under cold conditions.

Some antimicrobial effects are very dependent on the correct pH, for their operation. Thus, the reaction of the suspending agent in any test of a disinfectant is very important. The 'hardness' of water may markedly interfere with the antimicrobial effect of surface-active agents with which it may be used. The presence of organic matter greatly influences

the efficacy of many disinfectants and the neutralising effect of proteins, e.g. in dirt, pus, blood, is well known.

Water is pre-treated by filtration or precipitation to remove gross organic matter such as algal debris before chlorine is introduced to kill the potentially pathogenic microbes.

3.4 Adequate Selection of Disinfectants

Successful disinfection depends on 1) the selection of disinfectant capable of performing the required task and 2) its careful use under appropriate conditions of concentration, duration of exposure to temperature, pH and absence of neutralising substances. Unfortunately, disinfectants are frequently chosen which are unsuited to their task or are used under conditions in which they have no chance of being effective.

There are three main purposes for which disinfectants are correctly used:

(a) Decontamination of Objects before Disposal or Re-use
Faeces, urine, pus, sputum and other potentially infective discharges, whether in a container, e.g. bed-pan, or on dressings or clothing may be disinfected before the container or soiled article is washed for re-use. Clinical instruments such as thermometers and spatula for body orifices may also be disinfected between the uses on different patients, although it is impracticable to sterilise them. The storage of cleaning mops, nail brushes and transfer forceps in disinfectants to prevent bacterial multiplication on them between different occasions of use is a similar application. In medical laboratories, contaminated slides, pipettes and other instruments are generally discarded into a jar of disinfectant; after several hours or days they are safely removed and washed. The best disinfectants for these purposes, especially when there is heavy soiling with organic matter, are the phenolic disinfectants of the black fluid, white fluid and Lysol types (but not the chlorinated phenols). Because they are cheap, have a wide spectrum of activity and are not very liable to become inactive in the presence of organic matter. These phenolic derivatives are the most commonly used general disinfectants.

(b) Reduction of Microbial Contamination of the Environment
In the hospitals, pathogenic *Staphylococci*, *Streptococci*, *Enterobacteria* and *Pseudomonas* may be present on the floors, walls and furniture of wards, surgical theatres and kitchens, and on bath, wash-hand basins and water-closets in the bathrooms used by patients. The risk of cross-infection of patients is

probably lessened if the amount of this environmental contamination is reduced by disinfectants. A disinfectant is unnecessary except for areas known to have been soiled with an infected body discharge; these areas should be wiped with a cheap phenolic disinfectant. Rooms vacated by patients with smallpox or tuberculosis may be wholly disinfected by filling with formaldehyde vapour for 24 hours, but such terminal disinfection is rarely undertaken for other kinds of infection. Baths and wash-hand basins may be source of cross-infection and should be regularly cleaned with a cleaning powder containing hypochlorite or with a mixture of hypochlorite and detergent. The seats, flushes and door handles in water-closets and working surfaces in kitchens should be wiped regularly with a hypochlorite solution. If cheap, toxic phenolic disinfectants were applied to these surfaces they would have to be removed before use by careful cleansing. Hypochlorite is preferred as a *surface disinfectant* for relatively clean objects because it leaves no objectionable residues.

For small, relatively clean areas, 70 percent isopropyl alcohol is a pleasant and moderately effective, residue-free disinfectant.

(c) Disinfection of the Skin, Hands and Operation Sites

Transient organisms that have been picked up by contact with outside sources and are lying on the surface of the skin are relatively easily removed by washing with soap and rinsing with a disinfectant. On the other hand, organisms such as *staph.aureus* that are resident or growing in the depths of skin are difficult or impossible to eliminate completely. Hands may be washed with liquid soap or detergent containing 3% hexachlorophene or rinsed with aqeous 0.5% chlorhexidine. The skin of operation sites may be washed repeatedly with hexachlorophene soap and painted preoperatively with 1% iodine or 5% chlorhexidine in 70 percent ethyl alcohol (70 percent industrial methylated spirit) or 70 percent isopropyl alcohol.

3.5 Properties and uses of Some Selected Disinfectants

The following disinfectants merit discussion. It gives comprehensive account of the clinical aspects.

(i) The Phenols

(a) The Phenol (carbolic acid) is powerfully microbicidal and the cheaper phenolic disinfectants derived from coal tar are widely used for decontamination of bathrooms, bed-pans and hospital floors. They are, however, too toxic and irritant to be applied to and left on objects that will come in contact with the skin. The preparations include Lysol

(liquor cresolis saponatus) and other cresol fluids active against a wide range of organisms and are not readily inactivated by presence of organic matter. They are good general disinfectants. Phenol itself is bactericidal at a concentration of one percent, but its activity is drastically reduced by dilution, and it is virtually inactive at 0.1 %. It is used at a concentration of 0.5% for preserving sera and vaccines. It is expensive and for general disinfection is replaced by cheaper preparations. **Phenol** and the coal tar derivatives are markedly toxic to man. Sudol is a less toxic substitute for Lysol; it contains xylenols and phenols, but must still be used with caution as a coarse disinfectant.

Jeyes fluid is a well known proprietary preparation. This group of compounds can be used to treat faeces or sputum to render them safe before disposal.

(b) The related cholorophenols and chloroxyphenols marketed as Hycolin and Dettol are less toxic and irritant but also are less active and are more readily inactivated by organic matter.

Hycolin is a green fluid containing a combination of synethtic phenols including 3:5-dimethyl 4-chlorphenol; 2-benzyl 4-chlorphenol; and sodium pentachlorphenate. It can be incorporated in a liquid soap, a hand cream or an antiseptic 1% aqeous solution. Dettol contain 4.8 % chloroxyphenol and is a very widely used fluid and a cream. These compounds are relatively inactive against *Pseudomonas* species; indeed Dettol can be incorporated in a selective, medium for the isolation of *Pseudomonas*.

(c) Hexachlorophane is an even blander agent and is incorporated in various antiseptic preparations for use on skin. It is effective against Gram-positive organisms but much less so against Gram-negative organisms and it is ineffective against *Pseudomonas* species. Combined as a 3% solution with a liquid detergent, it is marketed as **Phisohex**; it is also incorporated in soap as Gamophen, but is less active in powder form, it is available as Ster-Zac. These preparations have important applications in the control of pyogenic cocci in surgical and neonatal unit in hospital.

Their action is slow and repeated use is necessary before they exert a significant effect on the skin flora. Frequent whole-body application of a detergent emulsions containing hexachlorophane 3% to neonates or denuded surfaces, e.g. burns, may result in absorption of significant amounts into the blood. As hexachlorophane is potentially toxic, it should be used with care and whole body

application should be followed by rinsing, but its efficacy in the prophylaxis of staphylococcal infection in paediatric, maternity and surgical units is so generally accepted that its controlled use and its good reputation are likely to survive recent public anxiety.

(d) Chlorhexidine (**hibitane**) is recommended as a relatively non-toxic skin antiseptic for general use. It is most active against Gram-positive organisms and fairly effective against Gram-negative bacteria, it is inactivated by soap and organic matter. A 0.5 to 1.0% solution in 70% isopropyl alcohol are used for the treatment of wounds.

(ii) The Halogens

(a) Chlorine and Iodine are bactericidal and sporicidal. Chlorine has a special place in treatment of water supplies, and combinations of hypochlorite and detergents are useful for cleansing and disinfection in the food and dairy industries. Various hypochlorite preparations have a usefully wide spectrum of activity against viruses. Chloros, Euscol and Milton are aqueous solutions of hypochlorite and other salts; their antibacterial and antiviral efficacy is limited because chlorine releasing preparations are markedly inactivated by contact with other organic matter. Chloros, Eusol and Milton are aqeous solutions of hypochlorite yielding 100 000 parts per million of available chlorine. It is commonly used at 1 in 100 dilutions (yielding 1000 parts/ 10^6 chlorine) for general disinfection and disposal of contaminated glassware such as microscopes, slides and pipette, and at a 1 in 100 dilution (yielding 10 000 parts/10^6chlorine) for disinfection of equipment visibly contaminated with blood. The working dilution must be made up freshly each day in a carefully cleansed container (Duguid *et al,* 1987) and may be tested at intervals with a starch-iodine paper to confirm by demonstration of a dark-blue reaction that is still active. Milton is used for the cleansing and disinfection of babies' milk feeding bottle. Hypochlorite should not be applied to metal, which it corrodes, or to cloth, which it may damage.

(b) Iodine, like chlorine, is also inactivated by organic matter. Tincture of iodine (2.5% iodine and 2.5% potassium iodide in 90% ethanol and 2.5% iodine in 70% isopropyl alcohol) are powerful, rapid skin disinfectants and are valuable for preparation of the skin for surgery. A few individuals however are hypersensitive to iodine. Alcoholic solutions of iodine are too irritant for use on broken skin, and aqueous preparations are not as rapidly

effective. The iodophors, containing iodine complexes with an anionic detergent, are less irritant; an example is Betadine, a water soluble complex iodine and polyvinyl pyrrolid (povidone). Betadine solution contains 1% available iodine and can be used as a bactericidal disinfectant of superficial wounds. It is only slowly sporicidal but it is rapidly effective against vegetative organisms including fungi and *Trichomonas*. A compress of providone iodine applied to abraded skin 15 to 30 minutes markedly reduces the spore population; repeated applications of this type may, if need be, be used when operations are delayed for 2 or 3 days. It was reported that disinfection of intact skin at an operation site for 2 minutes with povidone-iodine containing 1 percent available iodine in 70% ethyl alcohol reduced the resident flora to a similar degree as that achieved with the alcoholic chlorhexidine (Duguid *et al*, 1987).

(iii) Metallic Salts

(a) Mercuric chloride is sometimes used as a disinfectant in a 1 in 1000 solution: mercuric salts are strongly bacteriostatic, but they are not effectively bactericidal. The use of these preparations as pre-operative skin taraldehyde disinfectants is no longer justified. 'Merthiolate', a proprietary name for sodium ethylmercurithiosalicylate, is used.

(b) Silver nitrate: It is an antibacterial agent for the eyes in newborn babies and is widely and successfully used as a prophylaxis in gonococcal ophthalmic. It has been largely replaced by more modern antiseptics such as chlorhexidine, but it still retains a place in treatment of extensive burns.

(iv) Formaldehyde and Glutaraldehyde

(a) Formaldehyde: is highly lethal to all kinds of microbes and spores, killing bacterial spores almost readily as the vegetative forms. It is applied as an aqueous solution or in gaseous form. It is cheap, and non-injurious to cloth, fabrics, wood, leather, rubber, paints and metals. In the gaseous form, it is used to disinfect rooms, furniture and wide variety of articles liable to damage by heat, e.g. wollen blankets and clothing shoes, respirators, hairbrushes, and gum-elastic cathers. Commercial 'formalin' is a 40% (w/v) solution of formaldehyde in water containing 10% methanol to inhibit polymerisation. Formalin is a preservative for dead animals and specimens including man (cadaver) in the laboratories and human anatomy rooms. A dilution containing 5% formaldehyde

in water is a powerful and rapid disinfectant when applied directly to a contaminated surface.

The formaldehyde gas is liberated by spraying or heating formalin, or by heating solid Para formaldehyde. The atmosphere must have a relative humidity over 60% and preferably 80 to 90%, and a temperature of at least 18°C. Moreover, the materials must be arranged to allow free access of gas to all infected surfaces, since its penetration into porous fabric is slow.

Small articles, such as instruments, shoes and hair-brushed, are disinfected by exposure for at least 3 hours to formaldehyde gas introduced into the air in cabinet by boiling formalin in an electric boiler. Blankets and the surfaces of mattresses are disinfected similarly in a large cabinet, where they are hung unfolded. Folded blankets and clothing can be disinfected if they are packed in chamber of a stream-jacketed autoclave and heated at 100°C for 3 hours in the presence of formalin vapour.

(b) Glutaraldehyde in 2% aqueous solution is even more effective. It is stable in acid solution but much more active in alkaline solution. Accordingly the commercially available preparation, Cidex is supplied together with a separate alkaline buffer containing a rust inhibitor which is added before use.

Cidex is bactericidal and sporicidal and effective against viruses. It is particularly useful for the sterilisation of items of equipment that cannot be subjected to sterilising temperatures, such as cystoscopes, anesthetic equipment, plastic materials and thermometers. Formaldehyde and glutaraldehyde are two few disinfectants that are sporicidal.

(v) Volatile Solvents

(a) Isopropyl alcohol is not subject to exercise duty and is cheaper than ethyl alcohol. Both alcohols are optimally bactericidal in aqueous solution at concentrations of 70 to 75%, and have very little bactericidal effect outside this range e.g. when ‘absolute’, i.e. undiluted with water, or when diluted too much. They are often used in skin disinfection before hypodermic injection, venepuncture, e.t.c; the skin should be dry before they are applied. Chlorhexidine (0.5%) or iodine (1 to 2%) or laurolinum acetate (5%) in 70% alcohol are used for the same purpose and such mixtures are superior to alcohol alone.

(b) Acetone and ether are only weakly antibacterial and are not effective alternatives to alcohol as skin disinfectants; they should not be used.

(c) Chloroform has a limited use as a bacterial agent in bacteriology. It is rapidly bactericidal to vegetative bacteria and readily removes itself from a treated medium or culture by evaporation.

(vi) Soaps and Detergents

(a) Ordinary soaps are anionic detergents and have a degree of antibacterial activity; they contribute greatly to hygiene by aiding the mechanical removal of organisms during washing. There is evidence that soaps of saturated fatty acids are mildly effective against some pathogenic intestinal bacteria and that soaps of long-chain unsaturated fatty acids are active against some of the respiratory pathogens. Surface-active agents have general purposes wetting and cleansing properties as well as some disinfectant activity. The synthetic anionic detergents such as sodium alkyl sulphates inhibit Gram-positive bacteria to some extent, but they are much less effective against Gram-negative bacteria.

(b) Cationic detergents: The quaternary ammonium compounds are most useful. They combine antibacterial properties with detergent activity and being relatively non-toxic and bland, they are popular cleansing agents in the treatment of accidental wounds. They are essentially bacteriostatic and more active against Gram-positive than Gram negative bacteria. They are inactivated by organic matter. *Pseudomonas pyocyanea* is notoriously resistant to them and this organism has been cultured from the corks of bottles of their solutions.

(c) The Quaternary compounds: Commercially available preparations include Cetavlon (cetrimide), Roccal and Zephiran (benzalkonium chloride) and Laurodin (laurolinium acetate). There is an interest in Laurodin as an effective skin antiseptic. Picloxydine is biguanide with marked antibacterial activity. A 1% solution in combination with octylphernoxypolyethoxy ethanol (11%) and benzalkonium chloride (12%) is marketed as Resiguard, which, at dilution of 1 in 60, is active against both Gram-positive and Gram-negative organisms. It is relatively non-toxic and does not irritate the eyes or lungs. It may be used with a fogging or spraying machine for the disinfection of walls and other surfaces.

(d) Tego compounds are bacteriostatic, ampholytic surface-active derivatives of dodecyldi (amino-ethyl) glycine. At a concentration of 1% in water, they are said to be effective against a wide range of Gram-positive and Gram negative organisms and some viruses, but the antimicrobial effect

of Tego compounds is markedly reduced by a variety of substances including organic matter and even hard water. They cannot be recommended for general use. They have been recommended in the past for the use in animal houses, partly on the basis of their food detergent properties and their relative non-toxicity, but there are many detergents in the market that are less expensive.

SELF-ASSESSMENT EXERCISE 1

Describe and discuss decontamination of the skin.

(vii) The Oxidising Agents

Hydrogen peroxide and Potassium permanganate have been used as antiseptics in the past. Oxidising agents disrupt the structure of membranes and proteins. Hydrogen peroxide (H_20_2) which forms highly reactive peroxide is used to clean puncture wounds. When hydrogen peroxide breaks down into oxygen and water, the oxygen kills obligate anaerobes present in the wounds. Hydrogen peroxide is quickly inactivated by enzymes from injured tissues. It is also very effective in disinfecting contact lenses, but all traces of it must be removed before use, or it may cause eye irritation. A recently developed method of disinfection that uses vapourised hydrogen peroxide can now be used for small rooms on areas such as glove boxes and transfer hoods (Figure 3.1).

Another oxidising agent that is used is Potassium permanganate. It is also used to disinfect instruments and in low concentration to clean skin. Boric acid has an interesting antibacterial activity; however it can produce toxic reactions and it is now replaced by effective agents. It is used as a bacteriostat in preserving samples of urine prior to examination of viable counts in the laboratory.

Sodium azide is sometimes used as a preservative in biological preparations including experimental antisera. It inhibits esterification. It can be used at low concentrations (0.08%). It is very toxic to man and animals.

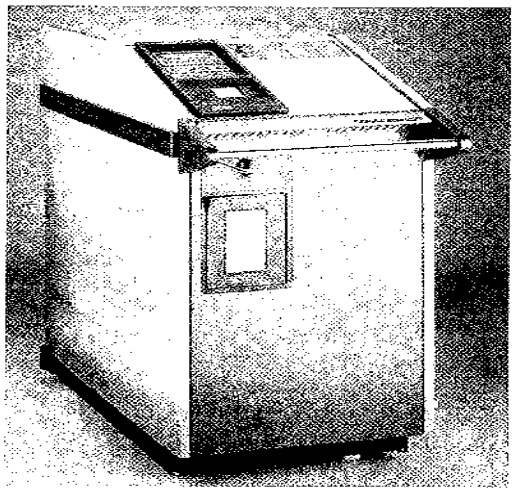

Fig.3.1: Hydrogen Peroxide Disinfection
Source: Jacquelyn, 2002.

(viii) Ethylene Oxide

This gaseous disinfectant is also highly lethal to all kinds of microbes and spores, but is capable of much more rapid diffusion into dry, porous materials. It is particularly valuable for sterilising articles liable to damage by heat, e.g. plastic and rubber articles, blankets, pharmaceutical products and complex apparatus such as heart-lung machines (Duguid *et al*, 1987).

It must be used only in a special chamber or apparatus since it is toxic and forms an explosive mixture when more than 3% is present in the air.

A non-explosive mixture of 10% ethylene oxide in carbon dioxide or a halogenated hydrocarbon may be employed for sterilisation. The sterilisation time depends among other factors, on the temperature of the reaction and the relative humidity, which should be between 20 and 40 %. Gaseous ethylene oxide has extra-ordinary penetrating power used at a concentration of 500ml/litre at 50°C for 4 hours. It sterilises rubber goods, mattresses, plastics and other materials destroyed by higher temperatures.

Also, ethylene oxide has been used to sterilise space probes that might otherwise carry earth microbes to other planets. Special equipment used during oxide sterilisation is shown in Figure 3.2.

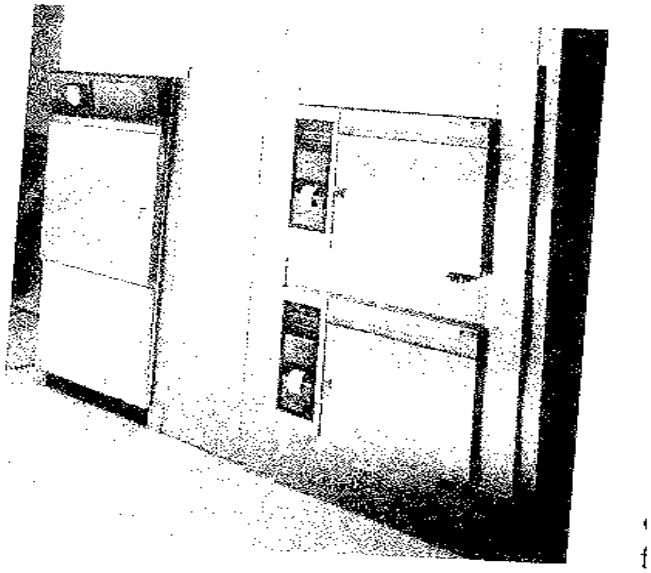

Fig.3.2: Ethylene Oxide Sterilisation
Source: Jacquelyn,2002.

An ampoule of endospores should be processed with ethylene oxide sterilisation to check the effectiveness of sterilisation. All articles sterilised with ethylene oxide must be well ventilated for 8-12 hours with sterile air to remove all traces of this toxic gas, which can cause burns if it reaches living tissues and is also highly explosive. After exposure to ethylene oxide, articles such as catheters, intravenous lines, in-line valves and rubber tubing must be thoroughly flushed with sterile air. Both the toxicity and flammability of ethylene oxide can be reduced by using it in gas that contains 90% carbon dioxide. It is exceedingly important that workers be protected from ethylene oxide vapours, which are toxic to skin, eyes, and mucuous membranes and may also cause cancer (Jacquelyn, 2002).

SELF-ASSESSMENT EXERCISE 2

Describe the properties and mode of action of some disinfectants.

4.0 CONCLUSION

Disinfection is not as efficient as sterilisation in destroying the microbial contaminants. Disinfection deals with the superficial contaminants while sterilisation totally kills all the associated contaminants.

5.0 SUMMARY

Different methods of using disinfectants have been listed. These include disinfection by chemicals, differentiating between mild and strong disinfectants. Disinfection refers to the reduction in number of pathogens or micro-organisms on objects or materials so that the organism can no longer pose a disease threat. Certain disinfectants can also destroy microbes.

6.0 TUTOR-MARKED ASSIGNMENT

i. Why are soaps used in cleaning things?
ii. What are the draw backs of using ethylene oxide as a disinfectant?
iii. Explain the antimicrobial actions of hydrogen peroxide.
iv. Describe the actions of ethylene oxide gas in microbial control. Is this sterilisation or disinfection?
v. List the factors to consider before selecting a disinfectant.
vi. Discuss the use of formaldehyde and glutaraldehyde in microbial disinfection.
vii. Describe the mode of action and use of three of the following types of disinfectant.
    a. Phenols
    b. Chlorine or iodine
    c. Ethylene oxide
    d. Oxidising agents.

7.0 REFERENCES/FURTHER READING

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