Course Title: Agricultural Microbiology

Credit: 2(1+1)

Course offered during: B.Sc. (Agri) I Year, II Semester

Title of the topic: Agricultural Microbiology

Name of the college: College of Agriculture, Tikamgarh

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Lecture Notes

On

Agricultural Microbiology

Contents

Syllabus of Agricultural Microbiology 2(1+1) as per the recommendations of the ICAR Fifth Deans 'Committee

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- Bacterial genetics: Genetic recombination- transformation, conjugation and transduction, plasmids, transposon.
- Role of microbes in soil fertility and crop production: Carbon, Nitrogen, Phosphorus and Sulphur cycles. Biological nitrogen fixation- symbiotic, associative and asymbiotic. Azolla, blue green algae and mycorrhiza. Rhizosphere and phyllosphere.
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- Staining and microscopic examination of microbes.

Chapter 1

INTRODUCTION TO MICROBIOLOGY

Chapter Objectives

- **This chapter introduces the subject of microbiology with specific focus on its significance in agriculture science.**
- **It highlights the major benchmarks achieved in the field of microbiology, which have led to wider emergence of the subject.**
- **It cites major theories advocating microbial development and evolution.**
- **Towards the end, the chapter presents applied area of microbiology with specific reference to agriculture, medical, industrial and research sectors.**

1.1 An introduction

Microbiology is the science of small living organisms and deals with the wide occurrence of such organisms in soil, water and air. Being too small to be seen with unaided eyes, these organisms are termed as microorganisms. Microorganisms refer to express viruses, bacteria, fungi, protozoa and some algae.

Microorganisms are recognized as integral and functionally important components of diverse habitats, ranging from soil collectives to the human surroundings. Virtually microorganisms are omnipresent and are imposed on all aspects of life; therefore the science of microbiology has assumed a central position of great significance in biological science as whole. Conventionally, in elementary cycle of the nature, microorganisms used to be described only as decomposer whereas green plants as producers and animals as consumers. However, with the advances in our biological knowledge base, it has been realized that plants, animals and microorganisms are interdependent in many sense.

Among microorganisms, viruses are the smallest while algae are considered as largest microorganisms. Despite categorised under microorganisms, there are few microbes such as some fungi and algae that have visible sizes. The relative sizes of different microorganisms are shown in Table 1.1. Viruses are not considered as independent living cell because of their incapability of existence outside the living bodies. They are just genetic material surrounded by protein coat. Bacteria, fungi, protozoa and algae are fairly simple organisms as most of them are single celled with no complex cellular organization even multicellular microorganisms do not have diverse range of cell types.

The study of microorganisms is called as microbiology. The study of microorganisms is quite significant for their possible role in natural processes such as decomposition of organic matter, fixation of atmospheric nitrogen in soil, release of nutrients from soil and water to plants and animals. Study of microorganisms also help us to get insight into the elementary mechanism of cell`s function. In the last few decades, microbiology has emerged as an applied science for their industrial applications on one hand while on the other for their pathogenic properties too. Biotransformation potentialities of the microorganisms and their enormous utilization in molecular and biotechnological research have also fetched attention on wider scale.

Table 1.1: Types of microorganisms, their sizes and class

Microbes	Approximate range of sizes	Cellular class
Viruses	0.01 to 0.25 μm	Acellular
Bacteria	0.1 to 10 μm	Prokaryotic
Fungi	2 μm to 1m	Eukaryotic
Algae	1 μm to few meters	Eukaryotic
Protozoa	2 μm to 1000 μm	Eukaryotic

1.2 Prokaryotic and Eukaryotic microorganisms

Two different classes of cell types are found within the microorganisms; prokaryotes and eukaryotes. Bacteria exclusively come under prokaryotes while fungi, protozoa and algae are eukaryotic microorganisms. Prokaryotes lack a distinct membrane surrounding their chromosome and do not have different organelles like mitochondria, chloroplast, endoplasmic reticulum and Golgi apparatus to perform special functions. In contrast, nucleus of the eukaryotes is enclosed by two concentric membranes and cytoplasm contains several membrane bound structures to perform specialized functions like energy generation and electron transport in and across the cells. A comparison of the main features of these two classes of the cell is shown in Table 1.2.

Table 1.2: The major differences between prokaryotic and eukaryotic microorganisms

Points of differences	Prokaryotes	Eukaryotes
Cellular organization	• Cell walls made up of peptidoglycans	• Cell walls made up of polysaccharides either cellulose (algae) or chitin (fungi)
	• Ribosomes-70S	• Ribosomes-80S (mitochondrial and chloroplast ribosomes are 70S)
	• Flagella consist of single protein called flagellin	• Flagella have more complex structure with 9+2 microtubular arrangement
	• Energy metabolisms associated with the cytoplasmic membranes	• Mitochondria present for the energy metabolism (except in some anaerobic microbes)
	• Photosynthesis associated with the membrane system and vesicles in the cytoplasm	• Chloroplast present in algal and plant cell, in addition, internal membranes such as endoplasmic reticulam and golgi bodies are associated with protein synthesis and targeting.
Genetic materials and replication	• No nuclear membrane present, DNA free in the cytoplasm	• True Membrane bound nucleus is present with DNA in it.
	• DNA associated with histone like proteins	• DNA is complexed with histone proteins
	• May contain extra-	• Plasmids rarely found

chromosomal DNA called plasmids	
• Cell division by binary fission	• Cell division by mitosis
• Transfer of genetic information occurs by conjugation, transduction and transformation	• Exchange of genetic information occurs during sexual reproduction. Meiosis takes place for the production of haploid cells (gametes)

1.3 Microbiology– in context to agriculture

Microbiology in general, has very diverse utility in agriculture, horticulture, animal sciences, fisheries and forestry and hence studied as a different branch termed as agriculture microbiology. A big number of harmful microorganisms called pathogens are responsible for majority of plant diseases**.** Large numbers of these microbial pathogens are routinely found in the soil, air and water and can infect the plant through the roots and leaves. Getting inside into the causes, mode of dissemination, prevalence and control of diseases requires basic understanding of microbiology under sub-discipline called plant pathology or phytopathology**.**

1.3.1 Plant Pathology

Plant pathology is basically the study of microorganisms that cause disease in plants. It also involves the understanding of interaction of environmental factors and host with infecting microorganisms. The microorganisms which cause diseases in the plants are called as plant pathogens. Table 1.3 presents major plant diseases caused by microorganisms.

Table1.3 Major Plant diseases caused by microorganisms

Diseases	Pathogen
Bacterial Diseases	
Soybean Blight	*Pseudomonas glycinea*
Halo Blight of Beans	*P. phaseolicola*
Leaf spot of Tobacco	*P. angulate*
Muko of Banana	*P. solanacearum*
Olive knot disease	*P. savastanoi*
Blight of Beans	*Xanthomonas phaseoli*
Blight of Rice	*X. oryzae*
Black rot of crucifers, citrus canker	*X. campestris*
Leaf spot of fruits	*X. prini*
Wilt of cucurbits	*Erwinia tracheiphila*

Wilt of corn	*E. stewartii*
Soft rot of fruit, Black leg of potato	*E. carotovora*
Wilt of alfalfa	*Corynebacterium insidiosum*
Wilt of tomato	*C. michiganeae*
Leafy gall of ornamentals	*C. facians*
Scab of potato	*Streptomyces scabies*
Crown gall of various plants	*Agrobacterium tumefaciens*
Hairy root of various plants	*Agrobacterium rhizogenes*
Aster Yellows	*Mycoplasma spp.*
Peach X disease	*Mycoplasma spp.*
Bermuda grass witches	*Spiroplasma spp*
Broom corn stunt	*Spiroplasma spp.*

Viral Diseases	
Rosette of Groundnut	Groundnut Mosaic Virus
Tobacco Mosaic	Tobacco Mosaic Virus
Tobacco Leaf Curl	Tobacco Leaf Curl Virus
Potato Mild Mosaic	Potato Virus-x
Potato Rugose Mosaic	Potato Virus-x and Potato Virus-y
Potato Leaf Roll	Potato Leaf Roll
Potato Crinkle	Potato Virus-x and Potato Virus-y
Tomato Bunchy Top	Tomato Bunchy Top
Little leaf of Brinjal	Brinjal Little leaf Virus
Papaya Mosaic	Papaya Mosaic Virus
Stunt of Cardamon	Cardamon Stunt Virus
Ratoon Stunt of Sugarcane	Ratoon Stunt Virus
Bunchy Top of Banana	Banana Bunchy Top Virus
Little Leaf of Cotton	Cotton Little Leaf Virus
Quick Decline (Tristeza) of Citrus	Citrus Tristeza Virus

Fungal Diseases	
Leaf spots and blight of various plants	*Alternaria spp.*
Anthracnose of crops	*Colletotrichum spp.*
Root rot of many plants	*Fusarium spp.*
Blight of cereals	*Helminthosporium spp.*
Smut of corn, wheat and others	*Ustilago spp.*
Rust of cereals	*Puccinia spp.*
Heart rot of trees	*Fomes spp.*
Powdery mildew of grasses	*Erysiphe spp.*
Soft rot of fruits	*Rhizopus spp.*
White rust of crucifers	*Albugo spp.*
Root disease of cereals	*Polymyxa spp.*

Nematodal disease	
Root-knot disease	*Meloidogyne spp*
Soybean cyst	*Heterodera glycines*
Pine wilt	*Bursaphelenchus xylophilus*
Root-knot disease	*Meloidogyne* spp

1.3.2 Antagonism

In contrasting to plant pathogens, certain native microorganisms present in the soil feed upon (or antagonistic to) these pathogens and can prevent the infection of crop plants. This particular behaviour of microorganisms is called antagonism. Literally, antagonism is an interaction between two organisms where one organism benefits at the cost of harm to another organism. Example includes predation or a predator eating prey or parasitism. In case of plant pathogens, antagonism usually involves competition between two microorganisms for food, nutrients and production of inhibitory compounds such as antimicrobial metabolites, secondary metabolites, antibiotics and extracellular enzymes.

1.3.3 Bio-pesticides

Some soil microorganisms produce compounds that stimulate the natural defence mechanisms of the plant and improve its resistance to pathogens. Commercially, these microorganisms are categorised under collective name of biopesticides. The term biopesticides are defined as the compounds derived from some living organisms and used to manage insect-pests by means of specific biological lethal effects. As compared to broader synthetic chemical pesticides, biopesticides are natural and have biological origin. It generally refers to products containing biocontrol agents or substances (including their genes or metabolites) derived from natural materials (such as bacteria or other microbes, animals and plants) used for controlling pests. The biopesticides would be dealt in detail in chapter 5.

1.3.4 Organic farming

The practices of traditional or chemical intensive agriculture are now being revaluated and are coming under increased scrutiny of our awareness regarding health and environmental issues. Modern agriculture seeks to introduce agricultural practices that are health savvy and maintain the long-term ecological balance of the soil ecosystem. In this context, use of microbial inoculants in agriculture (as biofertilizers, phytostimulators and biopesticides) under the name of organic farming represents an attractive environment friendly alternative of mineral fertilizers and chemical pesticides.

1.3.5 Genetic engineering

Recent advances in molecular biology have enabled the transfer of genes from one organism to another. In this direction, the specific genes from particular bacteria that can kill

certain insects but do not cause harm to humans have been successfully transferred to plants. The transferred genes get expressed in the form of protein in the host plant. This protein is toxic to the insects, so that when the insect feeds on the plant, the insect dies. Similarly many qualitative and quantitative improvements in plants have become possible today by means of bacterial gene transfer. The relatively easy manipulation of bacterial gene led to the enormous progress of biochemical and genetic research. These gene transformation aspects are now very elaborative science and studied under the subject Genetic engineering.

1.3.6 Food and Fermentation technology

Fermentation technology is although an old science but now emerging as an adequate technology to aid up food industries. Some of the promising examples where the microorganisms have been extensively used to aid up food industries are- Beer and wine production by yeast, bread making, processing of milk to dairy products by lactic acid bacteria and the production of vinegar by acetic acid bacteria. The food and fermentation technology would be dealt in detail in chapter 2.

1.3.7 Soil Microbiology

The soil represents a favourable environment for a diverse range of microorganisms including bacteria, fungi, algae, viruses and protozoa and therefore these microorganisms are abundantly and sometime quite densely found in soil. On an approximation, generally per gram of soil encompasses almost one to ten million of microorganisms. Among all microorganisms, bacteria and fungi are the most prevalent. All these microorganisms interact with one another, the environment and with the soil to create constantly altering conditions. The interactions between these multiple factors are responsible for the variation of soil types of a particular place and constitute distinct branch of agriculture microbiology called Soil Microbiology. The activity of all these microorganisms is vital for the soil and is accountable for soil quality, texture, structure and other properties as well. The topic has been comprehensively described in chapter 4.

1.3.8 Dairy Microbiology

Milk and milk processing industry is an excellent example where bacteria, yeasts, moulds and viruses are very important in determining the quality of final product. Dairy Microbiology deals with microorganisms associated with milk and milk products. The science comprises of the study of the control and destruction of undesirable microorganisms leading to spoilage of the milk and milk products on one hand while on the other, dairy microbiology also deals with intentional and directional introduction of beneficial microorganisms.

1.3.9 Environmental microbiology

Environmental microbiology is all about the study of microbial communities in the environment. It specifically deals with the composition and physiology of microbial

communities living in the soil, water and air. It also covers the microorganisms inhabiting on animals and plants.

1.3.10 Industrial Microbiology

Industrial microbiology is an applied area and gaining lot of attention in recent years. Traditionally, microorganisms used to be exploited for synthesis of many important chemicals such as acetone, butanol and acetic acid etc. However, more recently with the advent of genetic engineering tools, cloning of pharmaceutically important polypeptide genes e.g. insulin into microbes are carried out, which may then be produced on large scale.

1.4 History of Microbiology

From the ancient times, a lot of significant resources have been used to study microbiology; however, all those initial explorations were focussed primarily on fighting infectious diseases and their causes. With the advent of advanced tools and microscopic techniques, the field of microbiology is developing day-by-day with the inclusion of practical application and aspects of human welfare. It is also interesting to mention here that relatively recent advances of microscopic tools have made certain old concepts and claims somewhat inaccurate. Many individuals have made significant contributions to the development of microbiology (Table 1.4). Some benchmark points of microbiology is furnished as follow-

- Historians dedicated for microbiological chronology are not unanimously of the view that who made the first observations of microorganisms.
- Early civilizations (e.g., Crete, India and Scotland) showed signs of using toilets and sewers dating back as far as 2800 BC.
- The first record of human using microbes comes from ancient tablets from mid east. Over 8000 years ago, Babylonians were using yeast to make beer and acetic acid bacteria to make vinegar over 6000 years ago.
- About 5000 years ago, Persia (Now Iran) region recorded the wine making. The Romans used to consider God for that was specific for microorganisms. The roman God of mold and mildew was *Robigus* and *Robigo* which means crop rust. (Rust is one of the plant disease caused by fungus). God Robigus was very much feared because of crop lost.
- About 2000 years ago, Romans proposed that diseases were caused by tiny animals. But, fundamentalist religions had a strong hold over the progress.
- The real microbiology history starts from 1600s, when people began to make crude lenses and microscopes.
- Robert Hooke was the first person to report seeing microorganism under a microscope. He saw cells in a piece of cork in 1665, but his lenses were apparently too poor to “see” bacteria.

- In 1673, Anton van Leeuwenhoek, a dutch businessman reported to design a microscope with a 270X amplification power, which allowed him to see microorganisms for the first time.
- Anton Van Leeuwenhoek wrote a series of letters to the Royal society of London on microorganisms between 1673 and 1723 (until his death). He regarded these microorganisms as animalcules. Later these animalcules have been identified through his drawings as bacteria and protozoa.
- In those years around 1665, the theory of spontaneous generation or abiogenesis was put forward and strongly debated. According to the theory, living organisms spontaneously arose from lifeless matter such as beef broth. This theory was supported by John Needham.
- In 1668, Francesco Reddi, an Italian physicist conducted experiments to disprove spontaneous generation. Lazzaro Spallanzani (1767) an Italian naturalist and Rudolf Virchow (1768) a German scientist also challenged spontaneous generation theory with the concept of non-spontaneous generation or biogenesis theory.
- In 1798, Edward Jenner developed the technique of vaccination. He vaccinated with scrapings collected from cowpox blisters. He inoculated a healthy volunteer with cowpox material by scratching the person's arm with a pox contaminated needle. The scratch turned into a raised bump. The volunteer became mildly sick within a few days and recovered, but never contracted either cowpox or small pox.
- Louis Pasteur is credited for several discoveries in the field of microbiology. In 1857, he demonstrated that souring of milk is due to the action of microorganisms.
- In 1861, Louis Pasteur completely disproved spontaneous generation theory by conducting several experiments. For discarding abiogenesis theory, he poured beef broth into a long necked flask. In the second step, he heated the long neck of flask and bent it into an S-shaped curve. He found that microorganisms did not appear in the cool solution even after a long period.
- In 1864, Louis Pasteur enunciated the concept of pasteurization (that heating at moderate temperature could kill the microorganisms in milk).
- In 1880, Louis Pasteur discovered the principle and working of vaccination. Pasteur in his experiments noted that the bacterium responsible for fowl cholera or chicken cholera lost its virulence after the bacterium was grown in the laboratory for long periods. He noted that the strains with decreased virulence were capable of inducing immunity against subsequent infections by their virulent counterparts.
- The experiments of Louis Pasteur also encouraged the belief that microorganisms were in the air and could cause disease. Pasteur postulated the germ theory of disease**,** which states that microorganisms are the causes of infectious disease.
- Pasteur's attempts to prove the germ theory were unsuccessful. However, the German scientist Robert Koch in the year 1877 provided the proof by cultivating anthrax bacteria apart from any other type of organism (also known as Koch's hypothesis).

1.4.1 Koch`s postulates

The procedures used by Robert Koch to demonstrate the germ theory is popularly known as Koch's postulates**.** Basically, these postulates were based on the few hypotheses. Robert Koch hypothesized that *anthrax bacillus*, a gram positive bacterium was the source of the antrax disease. He could successfully validate his hypothesis by infecting mice with the strains of *anthrax bacillus* isolated from the spleens of animals that had died from the disease. He had demonstrated that the infected mice had identical symptoms. Koch also grew the bacilli for several generations and showed that those bacilli could cause anthrax in later generations also. On this way, Koch could enunciate the postulates. The points or steps of Koch's postulates are used to relate a specific microorganism to a specific disease. They are as follow:

(1) A particular microorganism is observed in a sick animal (*step a of Fig 1.1*)
(2)This microorganism can be cultivated in the lab as culture (*step b of Fig 1.1*).
(3)The growing culture of microorganisms, if injected into a healthy animal, produces the same disease (*step c and d of Fig 1.1*).
(4)The organisms are observed in the sick animal and can be re-isolated in the lab as pure culture (*step e and f of Fig 1.1*).

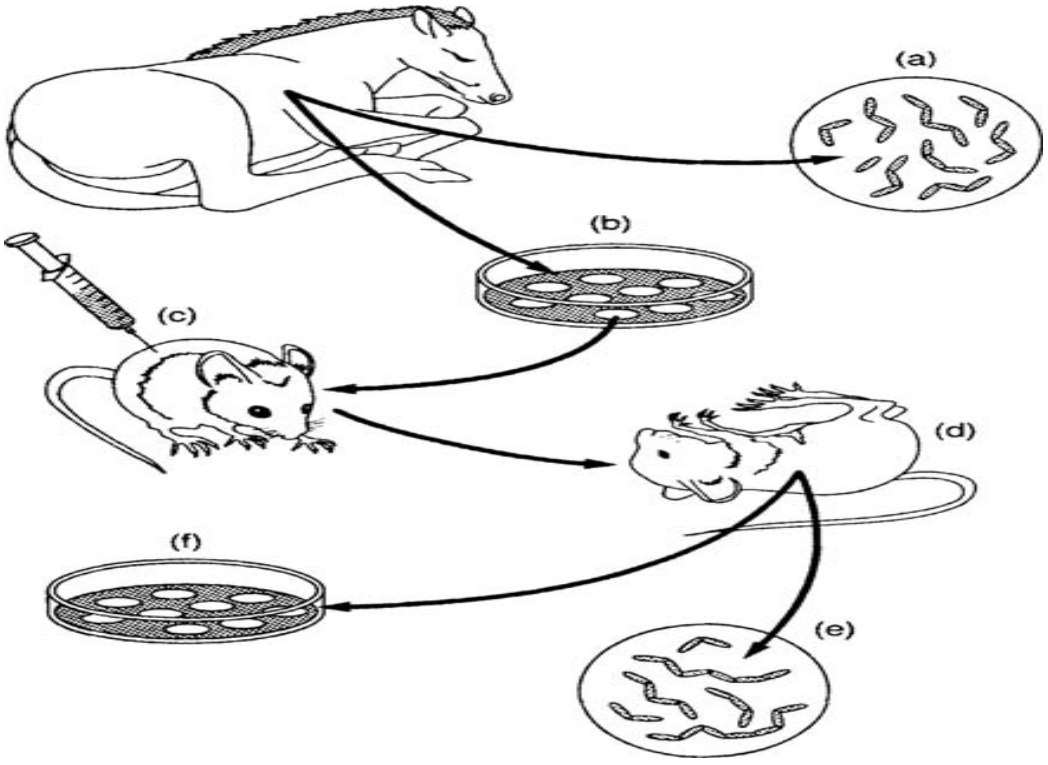

Figure 1.1: Schematic representation of steps of Koch`s postulates

Although, the Koch's postulates are still relevant in the present days practice of diagnosis of a specific disease, they are certain limitations too. Even Robert Koch had noticed the same. For example, Microorganisms were the cause of the major diseases such as cholera and leprosy, but but fail to comply all four postulates. Besides, Koch also found that the pathogen of cholera namely *Vibrio cholerae* could be isolated from both healthy as well as sick people and thus invalidating Koch's postulate

Table 1.4. A chronology of important developments in the field of microbiology

Year	Scientist	Contribution/Discovery
1590	Z. Janssen and H. Janssen	Assembled the first compound microscope
1665	Robert Hook	First description of cell (dead cells), published *Micrographia*
1677	A. V. Leeuwenhoek	Observed sperm of mammal`s including man
1745	John Needham	Proposed spontaneous generation or abiogenesis theory
1769	L. Spallanzani	Disproved spontaneous generation theory of microorganisms
1857	Louis Pasteur	Demonstrated that microorganisms are responsible for fermentation
1859	Charles Darwin	Published the book " Origin of Species"
1864	Louis Pasteur	Discovered pasteurization
1867	J. Lister	Discovered aseptic surgery
1877	Robert Koch	Proved that anthrax is caused by bacterium
1880	Louis Pasteur	Developed vaccines and treatment of rabies
1882	Robert Koch	Discovered *Mycobacterium tuberculosis* as causal organism of tuberculosis
1883	Robert Koch	Discovered *Vibrio cholerae* as causal organism of Cholera
1884	Christian Gram	Developed gram staining technique
1884	Escherich	Discovered *E. coli*
1885	A. Nicolaier	Discovered *Clostridium tetani* as causal organism of tetanus
1890	E. Von Bering and S. Kitasato	Discovered bacterial toxins and developed anti toxins
1890	P. Ehrlich	Proposed theory of immunity
1892	D. Ivanovski	First demonstration of virus
1894	A. Yersin	Discovered the cause of plague (*Yersinia pestis*)
1896	E. Van Ermengen	Caused of botulism food poisoning
1898	K. Shiga	Discovered the cause of dysentery (*Shigella dysenteriae*)
1898	E. Nocard and E.R. Roux	Discovered the cause of pleuroneumonia in cattle caused by mycoplasma (*Mycoids*)
1901	Martinus Beijernick	Developed enrichment culture method
1905	F.R. Schaudin and P.E. Hoffman	Discovered Syphilis
1906	J. Bordet and O. Gengou	Discovered the cause of whooping cough (*Bordettela*

		pertussis)
1907	E.F. Smith	Discovered the cause of Crown gall disease in plants (*Agrobacterium tumefaciens*)
1909	H.T. Riketts	Discovered the cause of rocky mountain spotted fever (*Rickettsia*)
1929	Alexander Fleming	Discovered Penicillin
1935	G. Domagk	Discovered chemotherapeutic value of sulphonamides (sulpha drugs)
1940	C.N. Acharya	Pronounced better utilization of gel waste for the production of biogas and compost
1944	O. Avery, C. Macleod and M. McCarty	Working with *Pneumococcus* bacteria proved that DNA, not protein, is the hereditary material in most living organisms
1952	Selman Waksman	Isolated streptomycin (this was the first effective drug available to treat infections with gram negative bacteria and tuberculosis)
1952	N.D. Zinder and J. Lederberg	Described transduction in *Salmonella*
1952	A.D. Hershey and M. Chase	DNA is the genetic material in *E. coli* phage T_2
1953	J.D. Watson and F.H.C. Crick	Proposed the double helix model of DNA
1953	C.C. Lindergren	Gene conversion in *Saccharomyces.*
1955	S. Benzer	Presented fine structure of the r^{II} locus of T_4 phage of E. coli; coined the term 'cistron', 'recon' and 'muton'
1955	Volkin and Astrachan	Reported existence of mRNA (studied T_2 infected *E. coil*)
1955	H. Frankel Conrat and R.C. Williams	Demonstrated that RNA is genetic material in TMV
1955	F. Jacob and E.L. Wollman	Discovered that bacterial conjugation involved transfer of a piece of DNA from donor to recipient cells.
1958	F. Jacob and E.L. Wollman	Discovered that *E. coli* linkage group is circular
1958	M. Meselson and F.W. Stahl	Demonstrated semi-conservative replication of DNA in *E. coli.*
1959	K. McQuillen, R.B. Roberts and R.J. Britten	Discovered that *E. coli* ribosomes are sites of protein synthesis.
1961	F. Jacob and J. Monod	Proposed the operon concept of gene regulation; proposed the existence and function of mRNA
1961	S.B. Weiss and T. Nakamoto	Isolated RNA polymerase
1963	J. Monod and S. Brenner	Discovered the replication model in *E. coli*
1963	J. Cairns	Presented auto-radiographic picture of replicating chromosome of *E. coli*; circular with Y-shaped replication

		forks
1970	R.J. Swaby	Developed biosuper by mixing rock phosphate, sulphur and thiobacilli
1977	W. Gilbert and F. Sanger	Developed a method to sequence DNA
1983	Karry Mullis	Invented Polymerase Chain Reaction
1993	Michael Smith	Discovered site-directed mutagenesis
1996		Genome of *Saccharomyces cerevisiae* was sequenced
1996	Bishop and Varmus	discovered oncogenes
1996	Peter C. Doherty and Rolf M. Zinkernagel	Discovered how the immune system knows which cells are virus-infected
1997	Stanley B. Prusiner	Discovered and characterized prions as a new biological infectious agent containing only protein and no nucleic acid
1998		DNA sequence for *Mycobacterium* tuberculosis was decoded
2000		Genetic blueprint of the cholera bacterium was developed
2001	Leland Hartwell Paul Nurse Tim Hunt	Identified key molecular steps in the cell cycle using yeast as a model organism
2005	Barry Marshall and Robin Warren	For the identification of Helicobacter pylori and its role in gastritis and peptic ulcer disease
2008	Harald zur Hausen	for his discovery that human papilloma viruses can cause cervical cancer, and Françoise Barré-Sinoussi and Luc Montagnier, for their discovery of HIV
2011	Bruce A. Beutler Jules A. Hoffmann Ralph M. Steinman	Discoveries concerning the activation of innate immunity Discovered the role of dendritic cell in adaptive immunity
2012	Sir John B. Gurdon and Shinya Yamanaka	Discovered that mature cells can be reprogrammed to become pluripotent
2016	Yoshinori Ohsumi	Discovered mechanisms for autophagy in microorganisms
2018	James P. Allison and Tasuku Honjo	Discovery of cancer therapy by inhibition of negative immune regulation
2019		Discovery of a novel corona virus "2019-nCoV". First ever appeared in Wuhan city, in China December 2019. Accounted for thousands of deaths around the world.

While the technologies today is far more complex than it was in the 1900s or before, much of the progress made in microbiological fields had taken place in those era only. However, with the advent of advanced molecular techniques, the chronology of microbiology is getting updated day-by-day. Those described here simply represent the benchmarks of microbiology and will surely help the students to have an insight.

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Chapter 2

BACTERIA: STRUCTURE, CLASSIFICATION, NUTRITION AND GROWTH

Chapter Objectives

- **This chapter describes the size, basic classification, and morphological diversity of bacteria.**
- **It highlights the bacterial structure and the function of the different bacterial components.**
- **It covers the aspects on bacterial cell growth, different phases of growth curve and the factors affecting the growth of bacteria with appropriate examples.**
- **It describes all major modes of bacterial nutrition with appropriate examples.**

2.1 Introduction

Bacteria are the biological cells that constitute a large domain of prokaryotic microorganisms. Typically very small in size or merely a few micrometres in length, bacteria have a number of shapes, ranging from spheres to rods and spirals. Bacteria are thought to have been the first organisms to appear on earth, about 4 billion years ago and have adapted to almost all available ecological habitats.

Some bacteria are pathogenic that means responsible for diseases in humans, animals, or plants; however most of bacteria are not dangerous. Few bacteria are good example of beneficial ecological agents whose metabolic activities support the life of other higher organisms while some bacteria are symbionts of plants and invertebrates, where they facilitate host by carrying out important functions such as cellulose degradation and nitrogen fixation.

Soil bacteria use decaying materials as food. These bacteria maintain the soil fertility by breaking down the complex organic matter, excess inorganic nutrients such as N, P, and S and release them into the soil. Some bacteria are commercially used in the preparation of different foods, chemicals, and antibiotics.

As a group, bacteria display exceedingly diverse metabolic capabilities and can use almost any organic compound and some inorganic compounds, as a food source. Owing to this, they are used in a number of industrial and medicinal processes also. A growing interest in the bacterial shapes, structures and metabolisms is shedding new light on the roles, bacteria play in agriculture, live stocks and human health.

2.2 Bacterial Size

Bacterial cells are about one-tenth the size of eukaryotic cells and are typically 0.5–5.0 micron in length. "Micron" or micrometre (denoted as μm) is the unit of measurement that is used to measure the bacterial size. One micron equals to one thousandth of a millimetre or 10^{-6} of a meter. It is worth mentioning that the limit of resolution with the unaided eye is merely about 200 microns while most commonly the bacteria are 0.2 – 1.5 μm in diameter and 3 – 5 μm in length. Thus, bacteria are smaller and can only be visualized with the aid of light microscope. The power of a light microscope is limited by the wavelength of visible light, which is about 0.5 μm.

2.3 Bacterial Morphology

Bacterial morphology is very diverse. The bacterial shapes directly affect biological functions, including mode of nutrition, motility, dispersion, stress resistance and interactions with other organisms. Although, bacterial shape is genetically determined, but physical or environmental forces (may be internal and/or external) exerted on cells are increasingly recognized as responsible players in deciding bacterial shapes.

Based on their morphology bacteria are grouped as

(i) *Cocci* : These are are spherical or oval cells (the word cocci is taken from kokkos meaning berry). The cocci bacterial cells occur either singly or in pairs, as tetrad, in chains and in bunch.

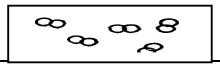
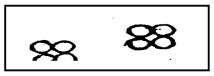
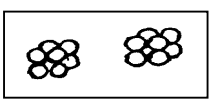
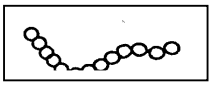
(ii) *Bacilli* : The cylinder shaped bacteria vary in length, diameter and may have square, round or pointed ends (the word bacilli is taken from bacilus meaning rod).

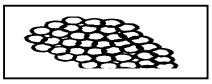
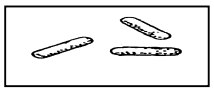
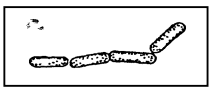
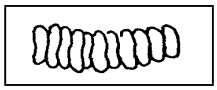
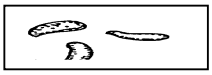
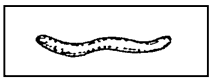
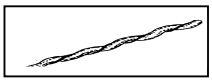
(iii) *Spirilli (curved)* : The curved forms are found as slender, spiral spirochaetes and bent rods.

In addition to above defined shapes, some other structural diversity also exists. The *Actinomycetes* or *Streptomycetes* are the bacteria which form branched filamentous hyphae having resemblance with fungal hyphae. *Actinomycetes* are so called because of a fancied resemblance to the radiating rays of the sun when seen in tissue lesions (from actis meaning ray and mykes meaning fungus).

Mycoplasma is another example of structural variant. Mycoplasmas are the bacteria which are cell wall deficient and hence do not possess a stable morphology. They grow as round or oval bodies as interlacing filaments. The different shapes and arrangement of bacterial cells are described in Table 2.1. In addition to these mentioned shapes, there are some other morphological characters in bacteria such as endospore formation, presence of capsule, presence and arrangement of flagella etc. which are useful in their identification.

Table 2.1. Different Shapes and Arrangements of Bacterial Cells

Shape and arrangement	**Term used**	**Genus**	**Appearance**
Spherical (Coccus)			
Single	Monococcus	*Micorcoccus* sp.	
Pairs	Diplococcus	*Neisseria gonorrhoeae*	
Quadruple	Tetrad	*Pediococcus* spp.	
Cuboidal packets	Sarcinae	*Sarcina ventriculii*	
Chains	Streptococcus	*Streptococcus* spp.	

Bunches	Staphylococcus	*Staphylococcus* spp.	
Cylindrical (Bacillus)			
Single	Bacillus	*Bacillus* spp.	
Chains	Streptobacillus	*Lactobacillus* spp.	
	Pallisade	*Corynebacterium Diptheriae*	
Curved (spirillum)			
Curved rod	Vibrio	*Vibrio cholerae*	
Helically curved	Spiral	*Spirillum*	
Corkscrew	Spirochaete	*Treponema pallidum*	
Star formation	Rosette	*Caulobacter* spp.	
Pleomorphic	Variable in shape	Mycoplasma, *Corynebacteria*	Variable shapes

2.4 Bacterial structure

Bacteria, being unicellular prokaryote, have simpler internal structure. Unlike eukaryotes, it lacks all membrane bound cell organelles such as nucleus and nucleolus, mitochondria, lysosome, golgi, endoplasmic reticulum, chloroplast, peroxisome, glyoxysome, and true vacuole. The outer layer of the bacterial cell consists of two components, the outer rigid cell wall and inner plasma membrane. The cell envelope surrounds the cytoplasm and other inclusions such as ribosomes and mesosomes, granules, vacuoles and the nuclear body. Thus, the bacterial structure can best be studied by bifurcating them as the structures outside the cell wall and those inside the cell wall. These structures are listed in table as follow:

Table 2.2 Components of bacterial cell wall

A. Structure/components outside cell wall	B. Structure/components inside cell wall
a. Capsule	a. Cytoplasmic membrane
b. Flagella	b. Cytoplasm
c. Pili	c. Ribosome
d. Slime	d. Mesosome
	e. Cytoplasmic Inclusions
	f. Nucleoid
	g. Spore

2.4.1 **Cell wall**

The cytoplasm of all the bacteria is enclosed within cell membrane, external to which a very rigid cell wall is present that gives shape to the bacterial cell. Cell wall constitutes a significant portion of the dry weight of the bacterial cell and is very essential for bacterial growth and division. The major functions of the bacterial cell wall are as follow:

(i) Protection from osmotic lysis: the cell wall prevents the cell from expanding and eventually bursting due to water uptake (the pressure inside the cell = 300 lbs/in^2)

(ii) Virulence factor: cell wall can be responsible for causing virulence in host organisms.

(iv) Defence against host immune response

(v) Protection from some toxic substances

2.4.1.1 **Chemical composition of bacterial cell walls**

Chemically the cell wall is composed of peptidoglycan or murein which is made up of sugar and amino acids. The structure of peptidoglycan consists of long polymers of two sugar derivatives N acetyl glucosamine (NAG) and N acetyl muramic acid (NAM) with side chains of four alternating D-and L-amino acids attached to the NAM (fig 2.1). The peptidoglycan and peptide chains are cross linked to provide rigidity to the cell wall.

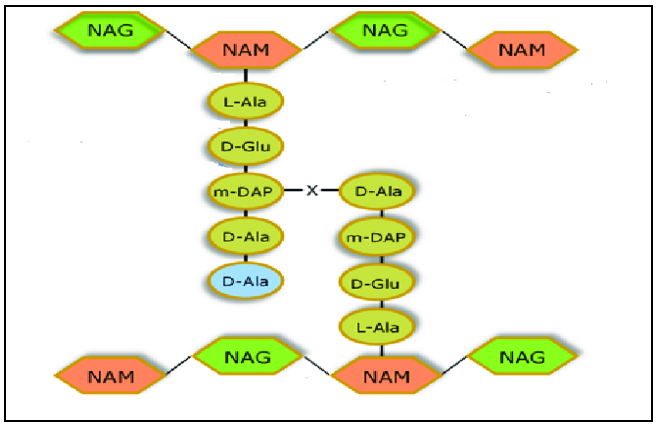
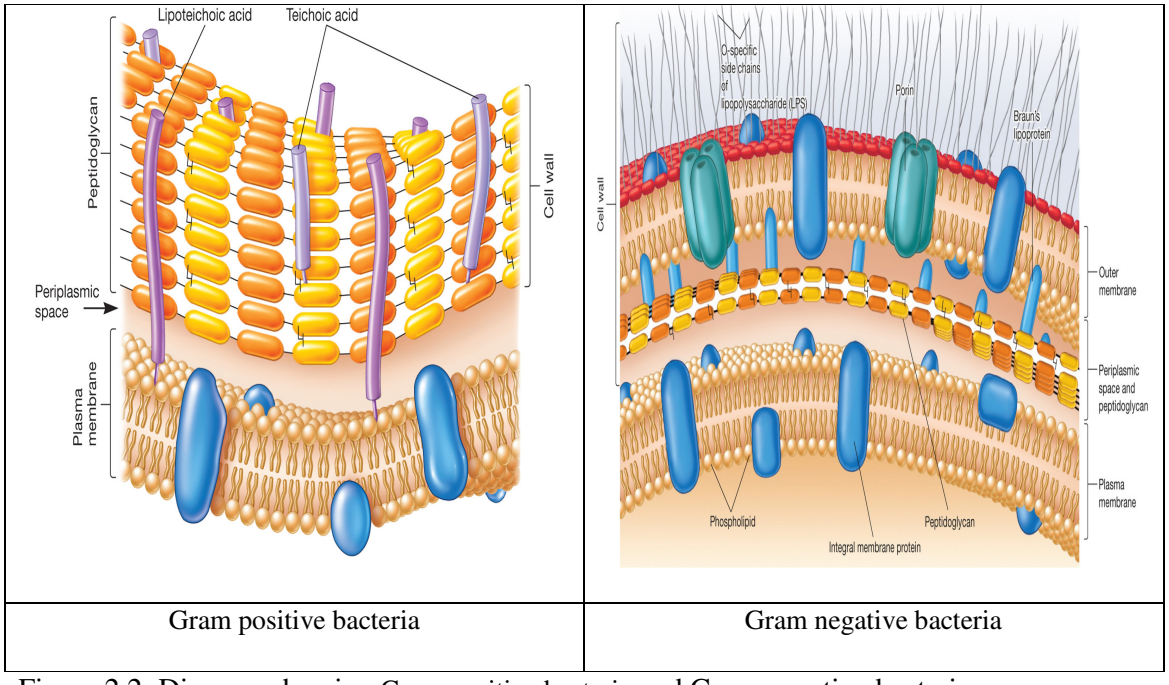


Figure 2.1: The cross linked peptidoglycan and peptide chains in the bacterial cell wall

A polyalcohol called Teichoic acid is embedded in it and responsible for linking peptidoglycan to cytoplasmic membrane and thus provides rigidity to the peptidoglycan. On the basis of cell wall composition, bacteria are classified into two major group ie. Gram Positive and Gram negative. Actually, Bacteria are termed gram negative or gram positive based on a staining called Gram staining and that primarily depends on the type of cell wall that bacteria have.

Gram positive bacteria have cell walls made up of peptidoglycan. The cell wall of the gram-positive bacteria also contains teichoic acid**,** which is made up of alcohol (glycerol or ribitol) and phosphate. These bacteria would retain the gram stain and observed as violet colored after the application of iodine (as mordant) and alcohol (Ethanol as decolorizer). The bacteria whose cell walls are made up of an outer membrane in addition to the inner peptidoglycan layer are called as gram-negative (figure 2.2). The outer membrane is made up of Lipopolysaccharides, lipoproteins, and phospholipids. These bacteria would be decolorised by alcohol owing to the lipidous outer membrane and lose the stain crystal violet, so they have to be counterstained by secondary stain safranin to appear pink in color. The major differences between gram-positive and gram-negative bacteria are presented in table 2.3.



Gram positive bacteria	Gram negative bacteria

Figure 2.2: Diagram showing Gram positive bacteria and Gram negative bacteria

Table 2.3: Major differences between Gram-positive and Gram-negative bacteria

Bacterial characters	**Gram Positive**	**Gram Negative**
Cell Wall	Their cell wall is smooth and single layered	They have a wavy and double layered cell-wall
Cell Wall thickness	The thickness of the cell wall is 20 to 80 nanometres	The thickness of the cell wall is 8 to 10 nanometres
Peptidoglycan Layer	It is a thick layer	It is a thin layer
Teichoic acids	Presence of teichoic acids	Absence of teichoic acids
Outer membrane	The outer membrane is absent	The outer membrane is present
Porins	Absent	Occurs in Outer Membrane
Morphology	Cocci or spore-forming rods	Non-spore forming rods.
Flagella Structure	2 rings in basal body	4 rings in basal body
Lipid content	Very low	20 to 30%
Lipopolysaccharide	Absent	Present
Toxin Produced	Exotoxins	Endotoxins or Exotoxins
Resistance to Antibiotics	More susceptible	More resistant
Examples	*Staphylococcus, Streptococcus* etc.	*Escherichia, Salmonella*, etc
Gram staining characteristics	These bacteria retain the crystal violet color even after they are washed with acetone or alcohol and appear as purple colored when examined under the microscope after gram staining.	These bacteria do not retain the stain color even after they are washed with acetone or alcohol and appear as pink colored when examined under the microscope after gram staining.

2.4.1.2 **Gram staining**

Gram staining method is one of the important and most explicit procedures in Microbiology. It was developed by Danish physician Hans Christian Gram in 1884. Gram staining is widely used as the primary basis of bacterial identification and frequently used for taxonomic division. Classic Gram staining techniques usually involve three following steps/processes as follow:

(a) **Staining with crystal violet (a water soluble dye)**: crystal violet stains are applied onto the bacterial cell. Due to the presence of the peptidoglycan layer on the cell walls of gram positive bacteria, these bacteria will retain the crystal violet stain.

(b) **De-colorization (using ethanol/acetone)**: The ethyl alcohol or acetone is used to decolorize the bacterial sample. Actually ethanol dehydrates the peptidoglycan layer and thus tightens and condenses it more tightly. In this way, the tight layer of peptidoglycan inhibits the crystal violet to penetrate across it, and hence the crystal violet stain is trapped in the cell wall of gram positive bacteria.

(c) **Counterstaining (using red dye Safranin)**: while on the other hand, as the gram negative cells cannot retain the crystal violet iodine complex so when safranin is applied on the outer membrane of gram negative bacteria the color is lost. Actually, the safranin is relatively a lighter stain as compared to crystal violet therefore it does interrupt the purple coloration in the gram positive cells.

2.4.2 **Structures outside cell wall**

(a) Capsule- The capsules are the outmost structures of bacterial cells. These are the gelatinous secretion of some bacteria which provides cell with additional protection helps them in preventing phagocytosis of bacteria. Phagocytosis is a type of endocytosis in which any cells uses their plasma membrane to swallow up a large external particle. These capsules are secreted by the cell into the external environment and are highly impermeable. However, the capsules are considered to be a major virulence factor of bacteria. That means almost all the bacterial pathogens including *Streptococcus pneumoniae, Klebsiella pneumonia, Neisseria meningitidis, Haemophilus influenza* and *Escherichia coli* etc. have polysaccharide capsules on their surface.

(b) Flagella- These are long (about 20 nm) hair or whip like helical filaments extending from cytoplasmic membrane to exterior of the cell. These flagella help bacteria to move towards nutrients and other stimuli. The long filament of flagella comprises of many subunits of a single protein called flagellin. This protein is synthesized within the cell and extends through the centre of flagella. Flagellin is highly antigenic and have key role in cell motility. The position of the flagella varies with the bacterial species. Functionally and structurally, it is divided into three parts, the filament, hook and the basal body. Filament is connected to the hook at cell surface, the hook and basal body are bordered in the cell envelope. The arrangement of flagella are described as follow (figure 2.2)

(i) Monotrichous – single flagella on one side
(ii) Lophotrichous – tuft of flagella on one side
(iii) Amphitrichous – single or tuft on both sides
(iv) Peritrichous – surrounded by lateral flagella along the periphery

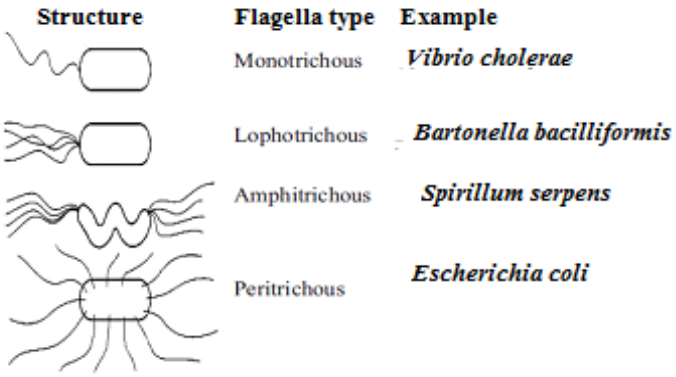


Figure 2.2: Arrangement of flagella in bacterial cell

(c) **Pili / Fimbriae** - It is hair-like proteinaceous appendage used for adherence to a host (in case of a pathogen), or for transferring DNA when bacteria conjugate. As compared to flagella, fimbriae is thinner, smaller and more in number (as many as 1,000 fimbriae in one bacterial cell). Also, they do not have role in cell motility. Bacteria use fimbriae to adhere to other bacteria or animal cells. The fimbriae is comprised of a protein subunit called pilin.
(d) **Slime (extracellular polysaccharide)** - This is an extracellular material, loosely associated with some bacterial species. Slime facilitates colonization of smooth, prosthetic surfaces such as intravascular catheters.

2.4.3 **Structures inside cell wall**

(a) Cytoplasmic membrane- It is present just below the cell wall and present in both Gram positive as well as Gram negative bacteria. It is a thin but semi-permeable layer that encloses the cytoplasmic contents of the bacterial cell and is made up of a phospholipid bilayer and proteins. Being hydrophobic in nature, it acts as a barrier and prevents the outflow of the cytoplasmic constituents which is hydrophilic.

(b) Cytoplasm- Similar to the eukaryotes, bacterial cytoplasm is also a colloidal system consisting of a variety of organic and inorganic constituents such as 80% Water and 20% Salts, Proteins. They are rich in ribosomes, DNA and fluid. Apart from chromosomal DNA, the extra choromosomal DNA is characteristically closed and circular. These extra chromosomal DNA is called Plasmids. They are highly coiled and complexed with polyamines and other support proteins.

(c) Ribosomes- Ribosomes are the platform of protein synthesis whereby they receive the genetic commands and translate these in the form of specific proteins. Ribosomes are composed of ribosomal RNA and protein. The bacterial ribosomes are slightly smaller than the ribosomes of eukaryotic cells and composed of two subunits namely 50S and 30S as opposed to 60S and 40S in eukaryotes. These two subunits combine together to form complete 70S ribosomes during protein synthesis (also called translation process). Here "S" denotes a Svedberg unit which is basically a non-metric unit for the sedimentation rate or

sedimentation coefficient and is considered as a measure of time defined as 10^{-13} seconds. The sedimentation coefficient refers to the rate at which a molecule or particle precipitate at the bottom of a test tube under the centrifugal force of an ultra-high speed centrifuge.

(d) Mesosomes- They are vesicular structure produced by localized and inward folding of plasma membrane into the cytoplasm. Mesosomes are rich in respiratory enzymes and other enzymes responsible for DNA replication and cell division.

(e) Nucleoid- The nucleus is not distinct in prokaryotes and hence called nucleoid. It doesn't have a uniform shape and size as there is no nuclear membrane around it. The nucleoid is principally composed of several copies of DNA which exist in the form of closed, continuous and coiled thread. In addition, nucleoids also have some RNA and proteins.

(f) Spore- Some bacteria form highly resistant resting stage called spores, which helps them to sustain in adverse environmental conditions. They are neither a reproductive form nor a storage granule. These spores enable bacteria to be resistant against the adverse environmental conditions and bactericidal agents as well as. There exists three layers in the spore namely core, cortex and spore coat.

2.5 **Growth and multiplication of bacteria**

Bacteria multiply by binary fission where the cell divides to form two daughter cells. Nuclear division takes place before cell division and therefore, in a growing population, many cells having two nuclear bodies are commonly found.

Bacterial growth may be considered as two levels, first is the increase in size of individual cells and second is the increase in number of bacterial cells. Growth in numbers of bacterial cells can be studied by bacterial counts as against that of total and viable counts. The total count indicates the number of cells either living or dead and the viable count represents the number of living cells that are capable of multiplication.

2.5.1 Bacterial Growth Curve

When bacterial cells are grown *in vitro* or cultured in a suitable culture media followed by incubation, their growth follows a particular pattern. If bacterial counts are carried out at regular intervals after inoculation and plotted with respect to time, a growth curve is observed. The curve shows the following phase-

(i) Lag phase

Immediately following the bacterial inoculation, there is no appreciable increase in number, though there may be an increase in the size of the cells. This early period is actually the time required by the bacteria for adaptation to the new environment. This lag phase varies with the species, nature and composition of culture medium and temperature.

(ii) Log or exponential growth phase

The lag phase is followed by log or exponential phase when the bacterial cell starts dividing and their numbers amplify exponentially with time.

(iii) Stationary phase

After the exponential growth phase, cell division is stopped due to exhaustion of nutrients and accumulation of toxic products in the media. The viable cell counts remain stationary as there remains equilibrium between the dying cells and the newly formed cells.

(iv) Decline Phase

This is the last phase when the bacterial population declines due to cell death.

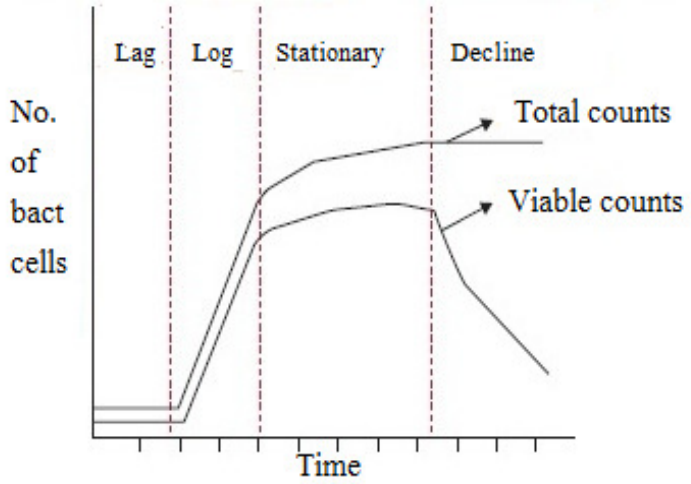


Figure 2.3: Bacterial Growth Curve

The different phases of bacterial growth curve are directly related to the morphological and physiological adaptations of the cells. The maximum cell size is attained towards the end of the lag phase. In the second or log or exponential growth phase, cells are smaller in size and stained uniformly. In the third or stationary phase, bacterial cells are commonly gram variable and show uneven staining owing to the presence of intracellular storage granules. Generally spore formation takes place at this stage. Besides, many bacteria produce secondary metabolites such as exotoxins and antibiotics. Cell deaths are the common feature in the phase of decline.

2.5.2 **Factors affecting the growth of bacteria**

The generation and multiplication time of the bacteria is affected by several factors such as nutrition, temperature, oxygen, carbon dioxide, light, pH, moisture and salt concentration.

(a) Nutrition- Water, proteins, polysaccharides, lipids, nucleic acid and mucopeptides are the principal constituents of the bacterial cells. In addition, for adequate growth and multiplication, bacteria require a source of energy, carbon, nitrogen and some inorganic salts.

Bacteria can be classified on the basis of their nutritional requirements as well as based on their energy requirement and also on the basis of their ability to synthesise essential metabolites (explained later in this chapter).

(b)Temperature- The optimum temperature requirement for bacterial growth varies with the species. Bacteria which grow best at temperatures of 25-40°C are called mesophilic bacteria. Psychrophilic bacteria are those that growth is best favoured at temperatures below 20°C. Another group of non pathogenic bacteria called thermophiles that grow adequately at higher temperatures that are at 55-80°C.The lowest temperature that completely checks the bacterial growth or kills a bacterium under standard conditions in a given time is known as thermal death point.

(c) Oxygen-Bacteria are divided into aerobes and anaerobes, based on their dependence on the influence of oxygen on growth and viability. Bacteria that necessitate oxygen for their growth are called aerobic. Aerobic bacteria may be obligate or facultative aerobes. The bacteria such as *cholera* and *vibrio,* which will grow strictly in the presence of oxygen, are called obligate aerobes while facultative anaerobes are the bacteria which are normally aerobic but can grow even in the absence of oxygen. The bacteria, such as *clostridia* grow in the absence of oxygen are classified under anaerobic bacteria. They are also the obligate anaerobes and Microaerophilic anaerobes. The obligate anaerobes do not survive on exposure to oxygen, whereas, microaerophilic bacteria grow adequately in the presence of low oxygen tension.

(d) Carbon Dioxide- Small amounts of carbon dioxide are also required by all bacteria for their growth. The atmospheric carbon dioxide fulfils this demand. However, some bacteria like *Brucella abortus* need much higher levels of carbon dioxide for its growth.

(e) Light- Bacteria are generally negatively phototropic, they die if exposed to light and on the contrary, grow well in the dark. Bacteria are sensitive to ultraviolet light and other radiations too.

(f) pH- The requirement for pH also varies with the bacterial species**.** Each bacterial species has a specific pH range, above or below which it cannot grow and survive. Similarly, every species has an optimum pH at which it grows well. Most of pathogenic bacteria grow best at either neutral or slightly alkaline pH (7.2 – 7.6).

(g) Moisture-Water is a vital constituent of bacterial protoplasm. Lack of moisture or drying is lethal to the bacterial cells; however, the effect of moisture or drying varies in different species.

(h) Salt concentration of the culture media- Salt concentration of the culture media is also an important consideration for bacterial growth. However, bacteria are more tolerant to osmotic variation as compared to other cells because of good mechanical strength of their cell

wall. Plasmolysis (or shrinkage of cell) may occur on abrupt exposure of bacterial cells to a hypertonic solution because of osmotic withdrawal of water from the protoplasm.

2.6 Nutritional diversity in bacteria

Bacteria require energy and nutrients to build complex biomolecules such as proteins, lipids and polysaccharides to synthesize structural membranes and drive metabolic processes. The bacteria can be grouped according to the requirement of carbon and the energy source. Some bacteria require dead organic molecules to produce energy, while other bacteria generate their own energy from diverse inorganic sources. On the basis of nutritional requirements, bacteria are classified as follow-

(a) **Autotrophs and Heterotrophs-** like plants and algae, there are some bacteria that make their own food by converting light energy and chemical energy. These bacteria are called autotrophs. For example *Cyanobacteria*, purple bacteria, green sulfur bacteria. These bacteria get their energy from the oxidation or breakdown of several organic or inorganic food substances available in their surroundings.

(ii) On the other hand, some bacteria acquire energy by consuming organic molecules. Like animals and fungi, these bacteria are called as heterotrophs.

(iii) **Autotrophic bacteria that eat inorganic compounds**- Some autotrophic bacteria find their nutrition from inorganic compounds. For such bacteria, carbon dioxide is generally the sole source of cellular carbon. These autotrophs also use hydrogen sulphide (H_2S), ammonia (NH_3) or hydrogen gas to convert carbon into sugars required for energy. Nitrifying bacteria, which oxidize and convert ammonia to nitrites and nitrates are specifically categorized as **chemoautotrophic nutrition**.

(iii) **Heterotrophic bacteria that consume organic compounds**- Heterotrophic bacteria require organic molecules such as sugars, fats and amino acids for example Saprophytic bacteria**.** They manage their nutrition from dead and decaying organic matters. These bacteria will break down complex compounds with the aid of enzymes to release energy. Saprophytic bacteria are decomposers and play an important role in ecosystem by releasing simpler products which plants and animals can use.

(iv) **Bacteria that use light as food**- Phototrophic bacteria are autotrophs that absorb light energy, to create cellular energy through photosynthesis. There are two types of phototrophs. Anaerobic phototrophs are the bacteria that do not produce oxygen as a byproduct, while aerobic phototrophs are those which produce oxygen. For example, Cyanobacteria. Both autotrophs and heterotrophs can be phototrophs. Heterotrophic phototrophs use organic compound, besides producing organic molecules through photosynthesis.

(v) **Bacteria that eat chemicals-** These bacteria find chemical energy from their surroundings and convert it into adenosine triphosphate (ATP) for cellular use. These bacteria are considered as chemotrophs. These bacteria also acquire energy from oxidation-reduction reactions of inorganic compounds such as ammonia (NH_3, hydrogen sulphide (H_2S). For instance, sulfur bacteria are chemoautotrophs which produce energy by oxidizing hydrogen sulfide into sulphur and water.

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Chapter 3

BACTERIAL GENETICS

Chapter Objectives

- **This chapter describes the gene expression along with various elements controlling the same in bacteria.**
- **It focuses on mechanisms of genetic recombination in bacteria.**
- **It takes comparative account of different mode of genetic recombination *i.e.* transformation, conjugation and transduction.**
- **It refers involvement of bacteria in desirable genetic manipulation of our food.**

3.1 Introduction

Genetics is the study of heredity and variations. Actually, genetic variation is the key to the survival of a species, allowing a species to adapt to changes in their environment by natural selection. That's true for bacteria as well. Like other living organisms, bacteria also multiply and pass on their characteristics from generation to generation. There are also the possibilities of variations in a small proportion in the inherited characters of their progeny.

Bacteria reproduce by binary fission. As a result of binary fission, bacteria produce clones or genetically identical copies of the parent bacterium. Since the offspring bacteria are genetically identical to the parent, binary fission doesn't provide an opportunity for genetic recombination or genetic diversity (aside from the occasional random mutation) in bacteria. In fact, the three other mechanisms responsible for transfer of genetic materials take place in bacteria. These are termed as transformation, conjugation and transduction.

3.2 Genetic recombination in bacteria

Bacteria can transfer genes from one strain to another by three different mechanisms: transformation, conjugation and transduction, these events clearly show the universality of sexuality in the living world. The genetic recombination in bacteria has a very vital significance. Some of them are as follow:

- **Transfer of antibiotic resistance genes**: The remarkable spread of resistance to multiple antibiotics may have been aided by the transfer of resistance genes within populations and among species.
- **As a tool to study advances of molecular biology and biotechnology**: Many bacteria have enzymes that enable them to destroy foreign DNA that gets into their cells. It seems unlikely that these would be needed if that did not occur in nature. In addition, the prime enzymes of bacterial reproduction namely **restriction endonucleases** have provided the tools of molecular biology. Most of the advances in biotechnology industry directly depend on the use of these enzymes.
- **Study of Evolution**: The recent completion of the sequence of the entire genome of a variety of bacteria (and archaea) suggests that in the past the genes have moved from one species to another by horizontal gene transfer.

3.3 **Transfer of genetic material**

Whenever two fragments of DNA come into contact with each other, exchange between the sections of each DNA takes place. This stage is called as crossing over during which, the DNA breaks and is attached on the other DNA strand leading to the transfer of genes and possibly the formation of new genes. This phenomenon is called as genetic recombination. Hence genetic recombination is the transfer of DNA from one organism to another.

The donor DNA that got transferred may be integrated into the nucleoid of recipient bacteria by a range of mechanisms. In homologous recombination, the DNA sequences having nearly the same nucleotide sequences (termed as homologous) get exchanged by means of breakage followed by reunion of paired DNA segments. Genetic information can be transferred from organism to organism through vertical transfer (from a parent to offspring) or through horizontal transfer methods such as transformation, conjugation and transduction. Generally, bacterial genes are transferred to members of the same species but sometimes, transfer of genes to other species can also happen.

3.3.1 Transformation

Transformation involves the uptake of free or naked DNA released by donor bacteria by a recipient one. Transformation was the first example of genetic exchange in bacteria and has been first ever demonstrated in an experiment conducted by Griffith in 1928 (in box).

It is basically a natural mode of gene transformation and has a large impact on lateral gene flow in nature therefore remains a matter of speculation.

Box-3.1: Griffith`s experiment to demonstrate genetic transformation in bacteria

Fredrick Griffith (1928) found that there were two different strains of bacteria ***Streptococcus pneumoniae*** (also known as the *pneumococcus*).

a) An 'S' or smooth coat strain of it, usually surrounded by a gummy capsule made of a polysaccharide. The presence of a capsule around the cells in bacteria gives the colonies a glistening, smooth (**S**) appearance. This strain is lethal to mice.

b) An 'R' or rough strain, surface of its colonies is wrinkled and rough (**"R"**). With no capsule, the bacteria also do not possess virulence i.e. the strain is non lethal to mice.

c) Griffith observed that on injecting mice with small number of avirulent 'R'cells together with heat killed 'S' cells, mice died. thus it was concluded that, there was some material in the heat killed 'S' strain that was responsible for transforming the 'R' strain into lethal form

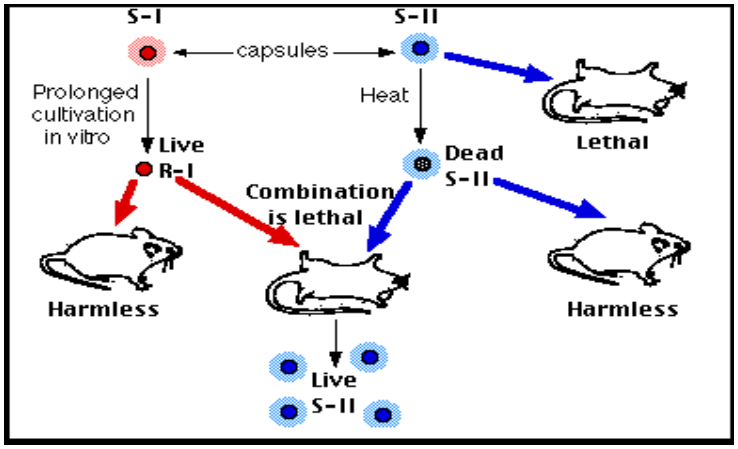

Figure 3.1: Bacterial transformation

Later in 1944, Oswald Avery, C.M. Macleod and M. Maccarty conclusively and explicitly demonstrated that the only material that was responsible for transformation was DNA.

3.3.2 Conjugation

In conjugation**,** DNA is transferred through a tube between two bacteria cells. This tube is termed as conjugation tube. In 1946, Joshua Lederberg and Tatum discovered that some bacteria can transfer genetic information to other bacteria through a tube known as conjugal tube. Conjugation involves the transfer of DNA in the form of a plasmid from donor bacterium to recipient bacterium. Plasmid transfer in Gram-negative bacteria takes place only either between strains of the same species or closely related species. Some plasmids are designated as F factor (also called "F" plasmid, fertility factor or sex factor) as these plasmids carry genes that mediate their own transfer from host to the recipient. An important character of the F factor is that it can replicate independently in the cell. These genes are responsible for encoding the production of the sex pilus and also the enzymes necessary for conjugation. The bacterial cells with F plasmids act as donors and represented as F+ (male). On the other hand, those cells lacking this F plasmid act as recipient and are represented as F- (female). The process of conjugation is completed in following stages (fig.3.1).

(i) An F+ donor cell contains its chromosomal DNA and an F plasmid. It has a rod like pilus. It has been earlier mentioned that a recipient F- cell has only a chromosome but no F plasmid.

(ii) The donor cell uses its pilus to attach to the recipient cell, and the two cells are pulled together.

(iii) A conjugal tube forms between the cytoplasms of the two cells, and a single strand of the F plasmid is fed through.

(iv) Both of the cells now have an F plasmid and are F+. Finally, the former recipient cell now becomes a new donor and has ability to form a pilus.

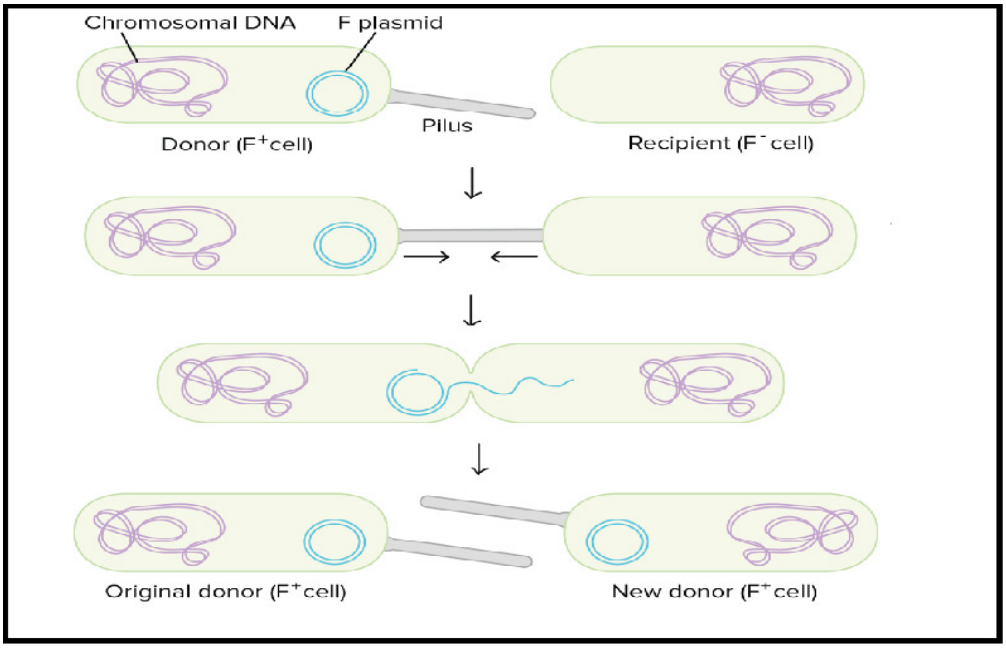

Figure 3.2: Bacterial conjugation

3.3.3 Transduction

In transduction**,** a bacterium transfers its DNA (or a portion of it) to another bacterium (that is not its progeny) through a virus. Actually, the viruses (called as bacteriophages) that infect bacteria move short pieces of chromosomal DNA from one bacterium to another. It means, physical contact does not occur between the DNA donating bacteria and the DNA receiving bacteria (as happen in conjugation).

3.3.3.1 **Types of Transduction**

The gene transfer through transduction is very common among bacteria. There are two types of transduction take place in bacteria, they are: generalized and specialized transduction.

(a) Generalized transduction (Lytic Cycle)

Generalized transduction or Lytic Cycle of bacteria takes place when a virus accidently gets inside into the bacterial cell and transfers its genetic material. Viruses (or bacteriophages) cannot replicate their own DNA; rather they infect bacteria and use their machinery (enzymes and template etc) to replicate their own DNA. When the replication is completed, parts of the virus are recombined and released to infect other cells. The lytic cycle or generalized transduction involve following stages:

1. An infecting virus (bacteriophage) adsorbs to the cell surface of a bacterium.
2. The bacteriophage genome gets inside into the cell of bacterium. The phage DNA uses the bacterium's machinery (DNA template and enzymes) to synthesize bacteriophage components and enzymes as well.
3. Once reassembled, the bacteriophages kills its host by bursting the cell (hence *lysis*) and are released to infect other cells.
4. The bacteriophage carrying the DNA of the donor bacterium again adsorbs to another recipient bacterium.
5. The DNA from donor bacterium thus gets exchanged by recombination of the recipient's DNA and confers new property to the bacterium.

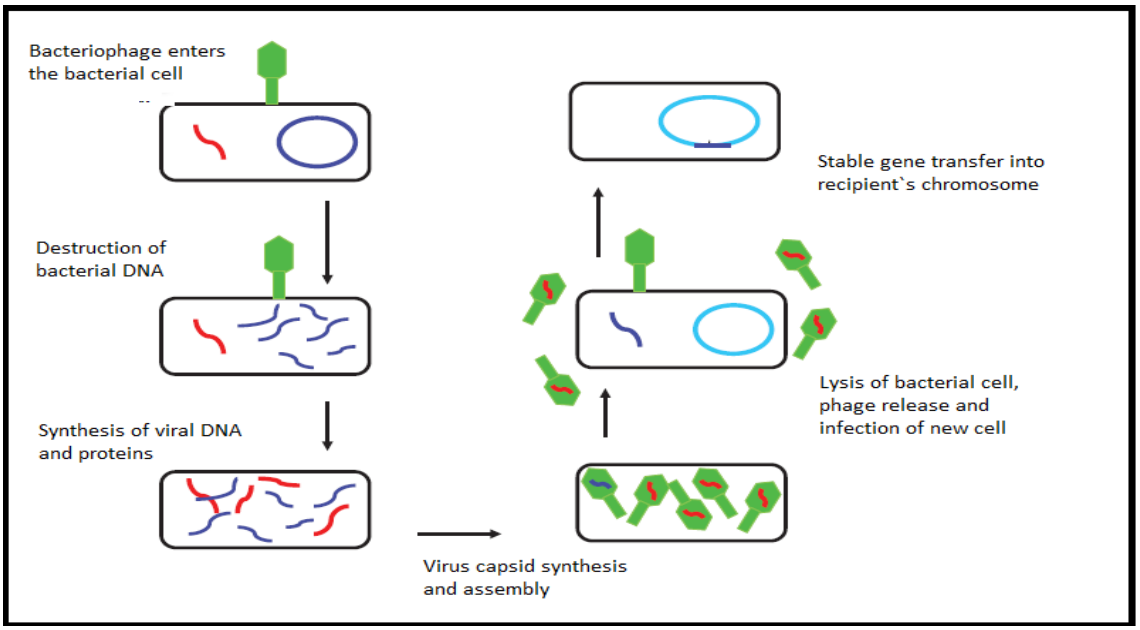

Fig. 3.3 Generalized transduction (Lytic Cycle)

(b) Specialized transduction (Lysogenic Cycle)

In case of specialized transduction or lysogenic cycle, the phage DNA gets incorporated into the bacterium chromosome. This is called a prophage and it behaves as if it were a part of bacterial chromosome. Once incorporated, the genes of the phage DNA also get expressed in the bacterium. Sometimes, the prophage gets detached from the host chromosome during multiplication of lysogenic host bacteria, and in this course, it may carry along fragments of bacterial chromosome with itself. The detached prophage then starts a new lytic cycle. This prophage may have a piece of chromosomal DNA of bacteria. When such phage infects another bacterium, it may incorporate the gene that was picked up from the preceding host into the new host genome. the examples of specialized transduction include λ phage in *Escherichia coli*. The mechanism of eukaryotic gene transfer by transposable elements is quite similar to prokaryotic transduction.

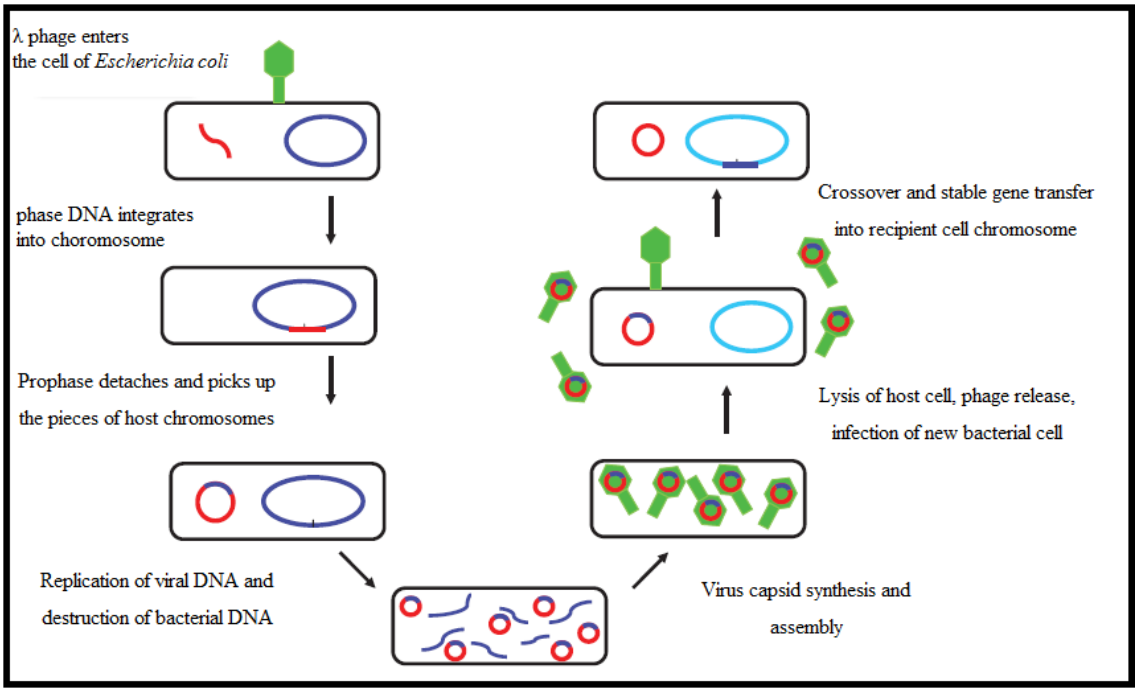

Figure 3.4: Specialized transduction ((Lysogenic Cycle)

3.4 Transposable elements (Transposons)

Transposon or transposable element is a DNA sequence that can "jump" to different locations within a genome. Although these DNA sequences change their position, however, they are always present as integrated sequences within a genome. Transposons are considered to be very important in bacterial genetics as they lead to mutation by way of duplication of the same genetic material. A mutation occurs when there is any genetic change in DNA or chromosomes. The transposons in bacteria can also move from chromosomal DNA to plasmid DNA and vice versa. In this way, these transposons also lead to addition of genes (another type of mutation).

The transposons were first ever discovered by Barbara McClintock in 1940. While working on maize genetics, McClintock noticed that different kernels of corn displayed diverse colors in the same cob (fig.3.5). Her studies revealed that each corn kernel looked differently in colour because it was genetically dissimilar. McClintock explained that the different colour could only appear if the genes were not static, but moving or changing their places from one chromosome to another (jumping).

Fig.3.5: Different kernels of corn displayed diverse colours in the same cob

In bacteria, transposable elements sometimes carry genes for antibiotic resistance and diseases. If one of these transposable elements that carries antibiotic resistance genes or disease causing genes jumps from the chromosome to a plasmid, then these genes can be easily passed to other bacteria by way of transformation or conjugation.

3.5 Plasmids

The extra-chromosomal DNA found inside a bacterium cell is called Plasmid. The plasmids are not considered as essential for the survival of the bacteria; however they give some extra advantages to the bacteria such as resistance to the cell against antibiotics. The salient features of a typical plasmid are as follow:

(i) **Number and size:** A bacterium can have no plasmids at all or have many plasmids (20-30) or multiple copies of a plasmid. Usually they are closed and circular molecules; however they occur as linear molecule in *Borrelia burgdorferi*. Their size may vary from 1 Kb to 400 Kb.

(ii) **Multiplication:** Plasmids multiply independently of the chromosome and are inherited regularly by the daughter cells.

3.5.1 Significance of plasmids

1. **Confer resistance to several antibiotics**: Gram-negative bacteria have plasmids that give resistance to several antibiotics such as kanamycin, neomycin, tetracycline, streptomycin, chloramphenicol, penicillins and sulfonamides.
2. Plasmid genes code for production of toxins: Some bacteria also produce toxins through plasmid genes (table3.1)

Table: 3.1 Some toxins produced by bacterial plasmids

Bacteria	**Toxins**
Escherichia coli	Enterotoxins
Vibrio cholerae	Choleragen
Staphylococcus aureus	Exfoliative toxin
Clostridium tetani	neurotoxin

3. Plasmids carry genes for resistance/sensitivity to heavy metals such as Hg, Ag, Cd, Pb etc.
4. Plasmids carry virulence genes. Eg, *vir* genes of *Agrobacterium tumefaciens.*
5. Plasmid genes code for DNA repair enzymes (DNA damaged by UV light are repaired by these enzymes).
6. Plasmid is responsible for colonization factors in bacteria. Colonization in bacteria is necessary for their attachment.

3.5.2 Applications of plasmids

(i) As vectors: plasmids are used in cloning short segments of DNA in genetic engineering.
(ii) To replicate proteins: Plasmids can be used to have proteins such as insulin that codes for insulin, in large amounts.
(iii) In gene therapy: Plasmids are popularly used for transferring genes into human cells as part of gene therapy.

3.6 Episomes

The term episome was enunciated by François Jacob and Elie Wollman in 1958. Previously, it was considered synonymous with plasmids. Now, episome is specifically defined as a genetic element inside some bacterial cells, particularly the DNA sequence of some bacteriophages, that can either replicate independently of the host or in association with a the chromosome with which it gets integrated. In other words, the "F factors plasmids" that can code for self transfer to other bacteria are called episomes. The bacterial cells with episomes are called Hfr cells i.e. high frequency of recombination.

3.7 Genetic engineering

Recent advances in genetic technology and better understanding of molecular biology have allowed greater molecular level understanding of physio-chemic system of several model organisms including microorganisms, animals and plants. Modification of pathways and products of these physio-chemic systems holds great potential for improvement of the organisms for human welfare.

The technology dealing with selective, deliberate transfer of beneficial gene(s) from one organism to another to create new improved crops, animals or materials is termed as genetic engineering. Genetic engineering with the use recombinant DNA techniques, cell

hybridization or protoplast fusion can now take away genes from cells of one organism and introduce them into the cells of other organisms to do particular functions.

Gene transfer between organisms has two major applications: firstly, to produce large amounts of biologically useful proteins including many enzymes of industrial importance and drugs for various diseases. Secondly, the gene transfer method is widely used to generate organisms with altered but desired characteristics. Successful gene transfers have been achieved in several plants, animals and microorganisms.

3.8 Genetically modified Organisms

Advances in cell biology and molecular biology have enabled the scientists to generate organisms with modified genes. Any organism whose genetic material has been transformed or altered using genetic engineering tools is said to be genetically modified organism (GMO). There are many successful attempts where genes have been transferred within the same species, across the species and even across kingdoms. The organisms where the new genes have been introduced are called as transgenic organisms. With the help of genetic engineering tools, the new genes can be introduced or the existing genes can be deleted, altered or knocked out.

So far, a wide array of genetically modified organisms has been developed by researchers for countless purposes: to make copies of genes or proteins, to determine gene function, to create models for human disease and to study gene expressions. One major purpose of GMO has been to generate food crops that are modified in a way to fight the biotic and abiotic challenges. For example, currently the genetically modified crops such as soybean, corn, alfalfa, cotton available in the market have bacterial genes introduced into their genomes that encode for pest or herbicide resistance. Soybean is the most common genetically modified edible crop worldwide. Soybean has been genetically modified to have a gene that makes it resistant to the herbicide Roundup.

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Chapter 4

SOIL MICROBIOLOGY

Chapter Objectives

- **This chapter describes soil microbial diversity.**
- **It focuses on mechanisms of microbial transformation of Carbon, Nitrogen, Phosphorus and Sulphur.**
- **It focuses on mechanisms of biological Nitrogen fixation.**
- **It takes an account of different microphlora of Rhizosphere and Phyllosphere.**
- **It refers involvement of microorganisms in composting.**

4.1 Introduction

Traditionally, the soil microbiology has been the study of microorganisms and their processes in soil. In fact, soil is an active habitat for enormous variety of life-forms including microorganisms. The interaction of enormous microorganisms with each other and also with their environments is covered under the branch of science called soil microbiology.

Soil gives a mechanical support to plants from which they extract nutrients. Besides providing shelters to a variety of animals, it provides habitats colonised by several kinds of microorganisms. All these microorganisms interact with one another and with the soil and thus alter the soil conditions continuously. Indeed, the live and continuous interactions among multiple soil components including microorganisms are responsible for the variation of soil types. The activity of living organisms in soil helps to control its quality, depth, structure, texture and other properties. The climate, slope, local environmental conditions also contribute to the nature of soil in different locations. Consequently, the same fundamental soil structure in different locations may be found to support very different biological communities including microorganisms.

4.2 Microbial groups in soil

The soil represents a favourable habitat for microorganisms and is inhabited by a wide range of microorganisms, including bacteria, actinomycetes, fungi, cyanobacteria, algae, viruses and protozoa. Microorganisms are found in large numbers in soil - usually between one and ten million microorganisms are present per gram of soil - with bacteria and fungi being the most prevalent.

(I) Bacteria:

i. Bacteria constitute the most dominant group of microorganisms in soil and probably equal one half of the microbial biomass in soil.

ii. They are present in all types of soil but their population decreases with increase in the depth of soil.

iii. Bacteria thrive in soil as cocci (spheres, 0.5 μ), bacilli (rods, 0.5 to 3.0 μ), or spirilli (spirals). The bacilli are very common in soil, whereas spirilli are rarely found in natural environments.

iv. The most commonly occurring soil bacteria belong to the genera *Pseudomonas, Arthrobacter, Clostridium, Achromobacter, Bacillus, Micrococcus, Flavobacterium, Corynebacterium, Sarcina*, and *Mycobacterium. Escherichia* is encountered seldom in soils except as a contaminant from sewage, whereas *Aerobacter* is frequently encountered and is probably a normal inhabitant of certain soils.

v. Soil bacteria are primarily classified into autotrophic and heterotrophic categories. Several of the reactions involved in nitrogen transformations in soil depend on the chemoautotrophic Nitrobacter and Nitrosomonas and hence chemoautotrophy of bacteria in soil is intimately related to crop production.

(II) Actinomycetes

(i) Actinomycetes have characteristics common to bacteria and fungi and yet possess sufficient distinctive features to delimit them into a distinct category.

(ii) Taxonomically, actinomycetes are clubbed with bacteria in the same class of Schizomycetes but confined to the order Actinomycetales.

(iii) They bear certain similarities to Fungi Imperfecti in the branching of the aerial mycelium which profusely sporulate and in the formation of distinct clumps or pellets in liquid cultures.

(iv) The number of actinomycetes increases in the presence of decomposing organic matter.

(v) The most conducive range of pH is between 6.5 and 8.0. Waterlogging of soil is unfavourable for the growth of actinomycetes whereas desertic soils of arid and semi-arid zones sustain sizeable population, probably due to the resistance of spores to desiccation.

(III) Fungi

(i) Fungi dominate all soils and possess filamentous mycelium composed of individual hyphae. The hyphae may exist as uni-, bi- or multinucleate and either as non-septate (without cross walls) or septate.

(ii) Like bacteria and actinomycetes, the quality and quantity of organic matter present in soil have a direct effect on fungal numbers in soil since most fungi are heterotrophic in nutrition. However, fungi are dominant in acid soils but also present in neutral or alkaline soils and some can tolerate pH beyond 9.0.

(iii) Broadly speaking, the soil fungi are classified into Phycomycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti. Many fungi which are commonly isolated from soil are categorized under the class Fungi Imperfecti as they produce abundant asexual spores and lack sexual cycle.

(iv) The genera of fungi which are most commonly encountered in soils include *Aspergillus, Alternaria, Botrytis, Cephalosporium, Cladosporium, Monilla*, *Penicillium, Trichoderma, Trichothecium, Verticillium*, and *Fusarium* (Fungi Imperfecti).

(v) Degradation of organic matter and help in soil aggregation is one of the main functions of fungi in soil. Besides this property, certain species of Alternaria, Aspergillus, Cladosporium and Helminthosporium produce substances similar to humic substances in soil and hence may be important in the maintenance of soil organic matter.

(IV) Cyanobacteria

(i) Several cyanobacteria such as Anabaena, Nostoc, Wollea, Lyngbya, Calothrix, Chroococcus, Gloeocapsa, Plectonema, Microcystis, etc. are generally found in soil and are reported to have the capability of fixing atmospheric nitrogen from the air.

(ii) Many soil cyanobacteria can resist long spells of drought. Upon contact to moisture, these cyanobacteria grow and develop slowly in comparison to bacteria and green algae but become dominant over them quickly. For example *Nostoc muscorum* and *Nodularia harveyana* appeared from soil that had been dried for 79 years.

(V) Algae:

(i) Algae differ from other soil microorganisms by having photosynthetic pigments.

(ii) The maximum depth to which microalgae have been recorded in soil is 2 m. Also, because of their requirement for light, microalgae develop most abundantly when the soil is not heavily shaded by vegetation or surface litter.

(iii) Green microalgae (*Chlorophyceae*) are the group most commonly present in soil. Yellow green algae (*Xanthophyceae*) are less common while the red algae (*Rhodophyceae*) are rarely found except in abnormal saline soils.

(iv) Besides their photosynthetic activities, soil microalgae add certain compounds to the soil, either through death or by secretion from the cells. Eg. Members of *Chlorophyceae* release polysaccharides and Chlorella produce growth substances and antibiotics which may affect the soil flora.

(VI) Viruses

(i) Viruses are the smallest inhabitants of the soil and they generally attack the cells of bacteria (bacteriophages) and *actinomycetes* (cyanophages).

(ii) The viruses possess a head-like and a tail-like structure. The tail attaches itself to the surface of the host and gains entry into host's protoplasm. Lysis sets in when the virus multiplies resulting in the liberation of many more progeny phages to reinfect new bacterial cells.

(VII) Protozoa

(i) Protozoa that occur in soil are unicellular and generally they lack chlorophyll barring few exceptions.

(ii) They are characterized by a cyst-stage in their life cycle which can help them to withstand adverse soil conditions.

(iii) Important genera of protozoans that occur in soil are Cercomonas, Entosiphon, Allantion, Heteromita, Monas. Spiromonas, Spongomonas and Tetramitus etc..

(iv) The protozoans prefer certain species of bacteria for their nutrition. For example, some protozoas thrive in soil by ingesting bacteria belonging to the genera *Aerobacter*, *Agrobacterium*, *Bacillus, Escherichia, Micrococcus* and *Pseudomonas* into their protoplasm.

(v) Use of organic manures in soil increases the number of soil protozoans which is again a reflection on the corresponding increase in the bacterial flora due to the application of organic matter.

4.3 Role of microbes in soil fertility and crop production

The major roles of soil microbes in increasing and maintaining the soil fertility and thereby promoting the crop production are as follow:

(i) *Physical support*: Microorganisms contribute to soil formation through nutrient cycling and organic matter production. Microbial products play very important roles for to soil aggregation and improved soil structure.

(ii) *Raw materials*: Soil microbes also produce antimicrobial agents and enzymes used for physiological as well as reproductive growth of the plants.

(iii) *Growth medium for plants*: Soil microbes mobilize nutrients from insoluble minerals to support plant growth.

(iv) *Buffering water flows*: Soil macropores are formed by plant roots, earthworms and other soil biota, which may depend on soil microbes as food or for nutrients.

(v) *Nutrient cycling*: The activities of soil bacteria, archaea and fungi drive nutrient cycling in soils and are involved in weathering minerals.

(vi) *Recycling of wastes and detoxification*: Microbial processes like mineralization and immobilization are responsible for recycling of wastes and detoxification of certain toxic elements in the soil.

(vii) *Biological control of pests, weeds and pathogens*: Beneficial species include bacteria, archaea, and fungi that support plant growth through increasing nutrient availability and by outcompeting invading pathogens.

(viii) *Carbon storage and regulation of greenhouse gas emissions*: By mineralizing soil carbon and nutrients, microbes are major determinants of the carbon storage capacity of soils. For example, denitrifying bacteria and fungi regulate nitrous oxide (N_2O) while methanogenic bacteria regulate methane (CH_4) emissions from the soils.

4.4 Biogeochemical cycling of nutrients

Soil microorganisms have a profound effect on the transformations involved in a large number of biogeochemical cycles of nutrients including carbon (C), nitrogen (N), phosphorus (P) and sulphur (S) and other various micronutrients and macronutrients.

4.4.1 The Carbon cycle

Carbon exists in the atmosphere mainly as carbon dioxide gas. The gaseous carbon dioxide gets dissolved in the atmospheric water to produce bicarbonate. Plants, bacteria, and algae convert these carbon dioxide or bicarbonate into organic molecules by the process of photosynthesis. These organic molecules enter into food chains and passed through several higher organisms. These higher organisms' converts the organic carbon back into carbon dioxide gas through cellular respiration.

Long term storage of organic carbon takes place when living organisms is either buried underground or sinks to the bottom of the ocean and forms sedimentary rock. Volcanic

activity sometimes or more commonly burning of fossil fuels brings this stored carbon back into the carbon cycle, although the formation of fossil fuels takes place at very slow pace on geologic timescale.

(i) The biological carbon cycle

Carbon dioxide is taken by all the autotrophs including terrestrial and aquatic organisms and through this; carbon enters into the food chains and food webs. Photosynthetic organisms inhale carbon dioxide from the air or intake bicarbonate ions from the water and convert them into organic compounds such as glucose, starch or glycogen. These organic molecules are the feed of Heterotrophs such as humans or other higher organisms. Upon intake of organic molecules by the heterotrophs, the organic carbon is entered into the food chains and food webs. When the organisms die, the decomposition of the dead materials begin. Decomposers break down dead organisms and waste products and release organic compounds and carbon dioxide as well. In this way, carbon cycle occurs through this biological pathway. As per estimation, an estimated 1,000 to 100,000 million metric tons of overall carbon move through the biological cycle each year.

(ii) The geological carbon cycle

The geological carbon cycle is relatively much longer than the biological carbon cycle and usually takes millions of years to complete the geological pathway for carbon. Carbon in different forms such as ocean sediment, soil, rocks, fossil fuels remain stored in the atmosphere for long periods of time. The atmospheric level of carbon dioxide is influenced by the reservoir of carbon in the oceans. The formation of bicarbonate ($H_2CO_{3)}$ by the reaction of carbon dioxide from the atmosphere and water is the key phenomenon of geological carbon cycle (reaction below).

$$CO_2+H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3 + H^+ \rightleftharpoons CO_3^{2-}+2H^+$$

The carbonate combines with calcium (Ca^{2+}) ions to form calcium carbonate. The calcium carbonate is the major component of the shells of marine organisms. Upon death of the marine organisms, carbonic shell may sink and become part of the sediment on the ocean floor. Over the passage of several years, the sediment transforms into limestone, which is the largest carbon reservoir on Earth.

The terrestrial carbon is stored in soil as organic or inorganic carbon. The organic form of carbon such as fossil fuels is formed as a result of decomposition of living organisms whereas inorganic carbon comes from weathering of rock and minerals. When fossil fuels (oil, coal, and natural gas) are burnt, carbon is released into the atmosphere as carbon dioxide. The inorganic carbon may enter the atmospheres by volcanic eruptions. In the ocean floor, carbon-containing sediments are taken deep within the Earth in a process called subduction, in which one tectonic plate moves under another. Carbon

dioxide is formed in the process which can be released into the atmosphere by volcanic eruptions. The schematic representation of the carbon cycle is depicted in figure 4.1

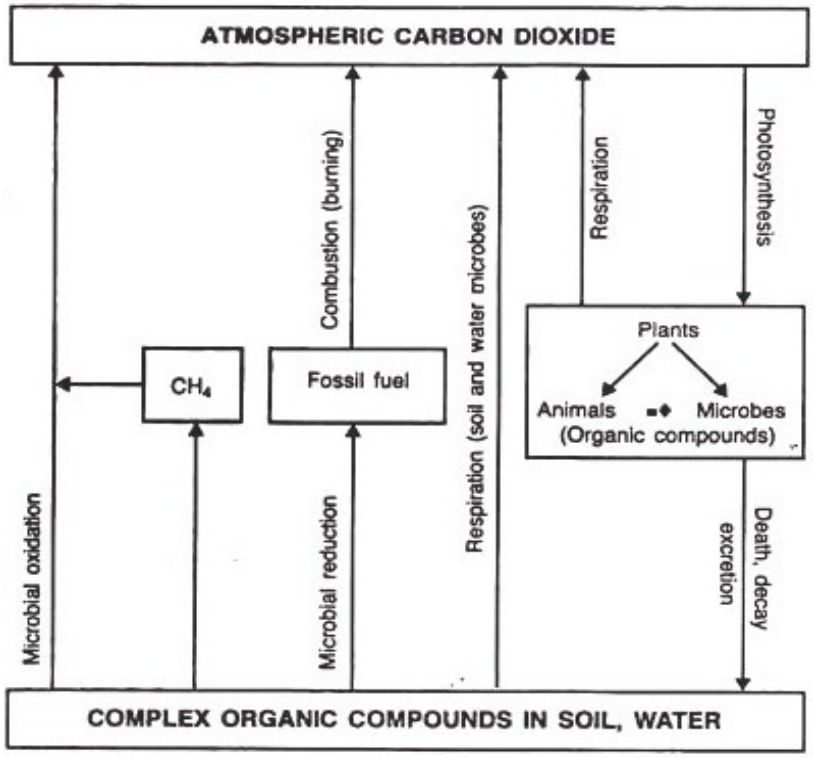

Figure 4.1 Schematic representation of the carbon cycle

4.4.2 The Phosphorus cycle

In this cycle, phosphorus moves through the lithosphere, hydrosphere, and biosphere. It is a slow process, which involves some key steps, as shown in the figure 4.2 below and described as follows:

(i) **Weathering**: In nature, phosphorus is found mostly in the form of phosphate ions (PO_4^{3-}). Phosphate compounds are found in sedimentary rocks, and as the rocks weather down over long time periods, the phosphorus they contain slowly leaches into surface water and soils.

(ii) **Absorption by Plants and Animals**: Phosphate compounds in the soil can be taken up by plants and, from there, transferred to animals that eat the plants. Phosphates may be taken up from the dead animals or dead plants or their wastes by detritivores or returned to the soil. The surface run-offs also carry phosphorus-containing compounds to rivers, lakes, and oceans, where they are taken up by aquatic organisms.

(iii) **Return to the Environment via Decomposition**: When plants and animals die, decomposition results in the return of phosphorus back to the environment via the

water or soil. This environmental phosphorus is then used by plants and animals and step 2 of the cycle is repeated.

(iv) **Uplift**: New phosphorus-rich sedimentary layers are formed when phosphorus-containing compounds from the bodies or wastes of marine organisms sink to the floor of the ocean. Over long periods of time, this sedimentary rock may be shifted from the ocean to the land surface by a process of geological progression called uplift. Although, uplift process is extremely slow like the average phosphate ion has an oceanic residence time (that is the time in the ocean) is approximately of 20,000 to 100,000 years.

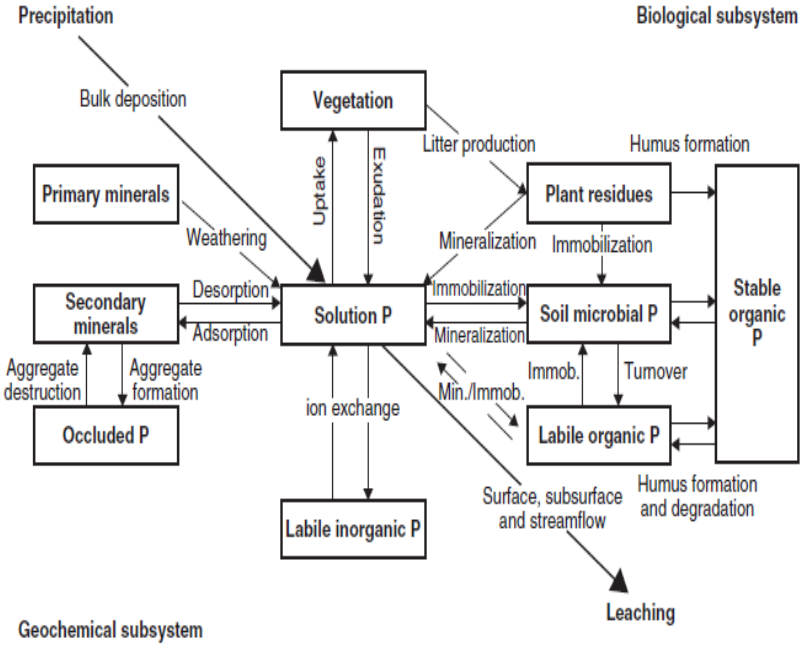

Figure 4.2: Schematic representation of the phosphorus cycle

4.4.2.1 Microbial transformations of phosphorus

(i) **Mineralization:** Organically bound P is not directly available to organisms because it cannot be absorbed into cells in this form. For cellular uptake of phosphorus, it must be released from the organic molecule through a process of mineralization. The specific enzyme called phosphatase has key roles in the final conversion of organically bound phosphorus to inorganic phosphate. These enzymes are produced by microbial population including bacteria such as *Bacillus megaterium*, *B. subtilis*, *Serratia* spp., *Proteus* spp., and *Streptomyces* spp. and fungi such as *Penicillium* spp., *Aspergillus* spp. and *Rhizopus* spp. After mineralization, phosphorus is absorbed up by plants, immobilized by the different microorganisms, absorbed to mineral surfaces and/or precipitated in inorganic complexes.

(ii) Immobilization: Soil microorganisms are responsible for fixation or immobilization of phosphorus. Microorganisms do this either by the formation of

inorganic precipitates or by synthesis of organic cell constituents or intracellular polyphosphate granules.

(iii) Oxidation and Reduction: A number of soil bacteria and fungi have the potential of oxidizing reduced phosphorus compounds (e.g., phosphate, hypophosphite) either aerobically or anaerobically.

(iv) **Solubilization:** The low solubility of P in soils makes it one of the major nutrients limiting plant growth. Frequent applications of soluble forms of P are needed because only a fraction is used by plants while the rest rapidly forms insoluble complexes. Production of traditional phosphorus fertilizer used to be based on chemical processing of insoluble mineral phosphate ore, which is quite expensive and also environmentally unsafe. In those areas where commercially produced phosphorus fertilizer is too expensive, the microbial solubilization of phosphate rock can be a viable alternative. Phosphate-solubilizing microorganisms such as *Pseudomonas*, *Bacillus*, *Micrococcus*, *Aspergillus*, and *Fusarium*, etc convert the insoluble rock phosphates into soluble forms by the processes of acidification, chelation, and exchange reactions.

4.4.3 The Nitrogen cycle

Nitrogen Cycle refers to conversion of nitrogen into many forms through biogeochemical process. In this process nitrogen from the atmosphere passes to the soil, taken by the organism and finally reaches back into the atmosphere. Nitrogen Cycle integrates several processes such as nitrogen fixation, nitrification, denitrification, decay and putrefaction.

The nitrogen gas occurs in organic as well as inorganic forms. Organic nitrogen is the key element found in almost all the living organisms. Several organic compounds consists of nitrogen get passed from primary producer to the consumers through the food chains. Inorganic forms of nitrogen are abundantly found in the atmosphere. This nitrogen is made available to plants through the process called symbiosis involving symbiotic bacteria. These specific bacteria are capable of transforming the inert nitrogen into usable different forms such as nitrites and nitrates.

4.4.3.1 Steps of Nitrogen Cycle

The process of Nitrogen Cycle takes place in several steps which are as follows:

A). Nitrogen fixation

It is the very primary step of the nitrogen cycle. Atmospheric nitrogen (N_2) which is mainly available in an inert form gets transformed into the ammonia (NH_3). The inert form of nitrogen gas is available as deposition into soils and gets precipitated into surface water. Later, two nitrogen atoms this inert nitrogen gets separated and combines with hydrogen to form ammonia (NH_4+).

Symbiotic bacteria such as *Azotobacter* and *Rhizobium* also have a major role in the entire process of Nitrogen fixation. These bacteria possess nitrogenase enzymes which catalyze the reaction of gaseous nitrogen with hydrogen to form ammonia. Nitrogen fixation can occur either naturally by the process called atmospheric nitrogen fixation or through man-made processes called industrial nitrogen fixation. The atmospheric nitrogen fixation simply involves lightening while industrial fixation involves manufacturing of ammonia and nitrogen-rich fertilizers under high temperature and pressure condition.

Types of Nitrogen Fixation

1. **Atmospheric fixation:** It is the natural phenomenon where the energy of lightning breaks the atmospheric inert nitrogen into nitrogen oxides.
2. **Industrial nitrogen fixation:** It is industrial process of nitrogen fixation by the use of ammonia. Under high temperature and pressure, ammonia is produced by the direct combination of nitrogen and hydrogen and later, also converted into various fertilizers such as urea.
3. **Biological nitrogen fixation:** The unavailable forms of nitrogen get fixed in the soil by these certain microorganisms. Bacteria such as *Rhizobium* and blue-green algae convert the unusable form of nitrogen into other usable compounds that are more readily usable.

B). Nitrification

In this process, the ammonia gets converted into nitrate in two steps by the presence of bacteria in the soil. Firstly nitrites are formed by the oxidation of Ammonia with the help of bacterium species namely *Nitrosomonas*. Later, the produced nitrite gets converted into nitrates by the bacterium *Nitrobacter*. This conversion is very important for plants as ammonia gas is toxic for the organisms including plants.

The two steps reaction (reduction and oxidation respectively) involved in the process of nitrification is as follows:

$$2\,NH_4^+ + 3O_2 \xrightarrow[\text{(Obligate autotrophic bacteria)}]{\textit{Nitrosomonas}} 2\,NO_2^- + 2H_2O + 4H^+$$

Reduction

$$2NO_2 + O_2 \xrightarrow[\text{oxidation by } \textit{Nitrobacter}]{\text{Through}} NO_3^-$$

Oxidation

Figure 4.3: Nitrification process

C) Assimilation

Primary producers or plants intake the nitrogen compounds from the soil with the help of their roots, which are available in the form of ammonia, nitrite ions, nitrate ions or ammonium ions. In case of nitrate absorption, the nitrate is first reduced to nitrite ions and then ammonium ions. These ammonium ions get incorporated into amino acids, proteins, nucleic acids (DNA), and chlorophyll.

D) Ammonification

When plants or animals die, the nitrogen present in the organic matter is released back into the soil. The microbial decomposers such as bacteria or fungi present in the soil convert the organic compound back into ammonium. Ammonia gas is produced in this process of decomposition, which can be used further for other biological processes. The reaction proceeds as follow:

$$\text{Amino acids} + 1/2O_2 \xrightarrow{\textit{Soil enzymes}} CO_2 + H_2O + NH_3$$

E) Denitrification

Denitrification is the conversion of nitrates back into the largely inert nitrogen gas (N_2). It is a reduction process that completes the nitrogen cycle. The process is anaerobic and performed by bacterial species such as *Pseudomonas* and *Clostridium,* which reduces nitrate to gain oxygen and gives out free nitrogen gas as a by-product. These bacteria reside in the deep aquatic soil where conditions are mostly anaerobic. However, these anaerobic bacteria can also thrive in aerobic conditions.

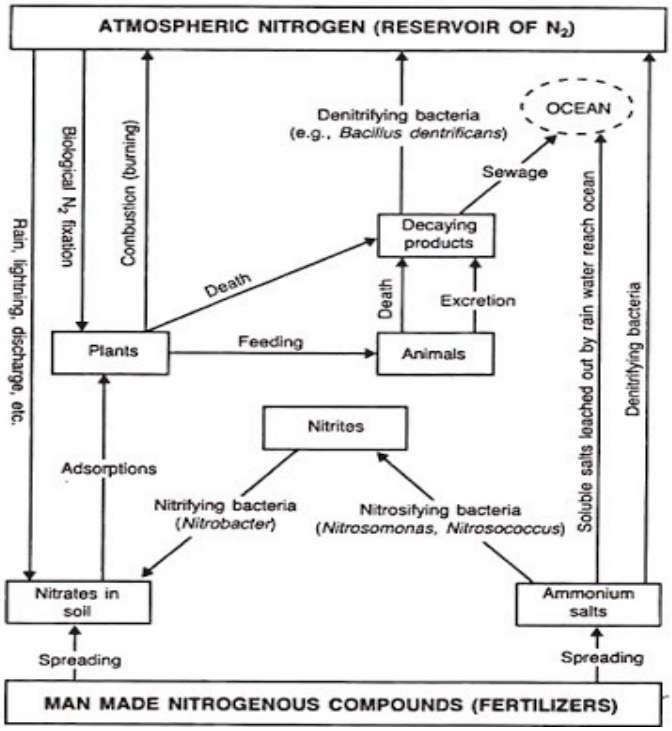

Figure 4.4: Schematic representation of the Nitrogen cycle

4.4.3.2 Importance of Nitrogen Cycle

Nitrogen is very vital element as it is an indispensable constituent of several biomolecules essential for life. Amino acids, proteins, DNA, and chlorophyll are the examples having nitrogen as an integral component. Despite, nitrogen is very abundantly available in the atmosphere dinitrogen gas (N_2), the same is not accessible to most organisms as such, making nitrogen often limiting factor for plants and other organisms. The prime importances of the nitrogen cycle are as follows:

i. Helps plants to synthesise chlorophyll from the nitrogen compounds.
ii. Helps in converting inert nitrogen gas into a usable form for the plants through the biochemical process.
iii. In the process of ammonification, the bacteria help in decomposing the animal and plant matter, which indirectly helps to clean up the environment.
iv. Nitrates and nitrites are released into the soil which helps in enriching the soil with necessary nutrients required for cultivation.
v. Nitrogen is an integral component of the cell, and it forms many crucial compounds and important biomolecules.

4.4.4 The Sulphur cycle

The sulphur is one of the macronutrients required by plants and is obtained by them from the soil and from the atmosphere. Biologically, it is present in amino acids and proteins and responsible for a distinctive odour. It is also a component of a large number of enzyme systems. Several groups of prokaryotes utilize and release sulphur.

The sulphur cycle typically involves significant interactions among different components of atmosphere that is the pedosphere, the hydrosphere and the biosphere. Globally, the major storehouses for sulphur cycle are pyrite and gypsum (an evaporite of seawater) in the lithosphere and in seawater respectively. Only a trace amount of sulphur is found in living organisms, however, under the swampy regions and marine muds, where organic matter gets accumulated under prolonged anaerobic conditions, substantial amounts of sulphur is present.

Increasing amounts of atmospheric sulphur compounds are the direct result of human activities and are principal components of air pollution in industrial areas. Mostly these sulphur containing pollutants are short-lived in the air and washed out with the rain popularly known as acid rain.

A lot of similarities are there between sulphur and nitrogen cycles. Here also, the short-term movements of sulphur elements from the atmosphere take place through the metabolism of bacteria. The gaseous sulphur moves in a closed cycle from the air to the soil and back. There are several sub-cycles, which are as follow:

1.) A prolonged cycle of weathering, erosion, deposition of rocks,

2.) An atmospheric cycle, in which bacteria decompose the dead organic matter and release sulphur to the atmosphere. This sulphur is washed back to the soil by precipitation. The process is called acid rain,

3.) A marine cycle where sulphur is released to the atmosphere from sea evaporation temporarily and again falls back into the sea,

4.) A soil–plant ecosystem cycle where organic sulphur found in manure or other fertilizer is used to sustain soil microbes and plants. A variety of soil organisms disintegrate sulphur-containing proteins into their constituent amino acids. These sulphurs of the amino acids are converted to hydrogen sulfide (H_2S) gas by another series of soil microorganisms. Under aerobic condition, hydrogen sulfide is converted to sulphur and then to sulfate by sulphur bacteria. Eventually the sulfate again transforms into hydrogen sulfide. Hydrogen sulfide gas quickly oxidizes to gases that dissolve in water to form sulphurous and sulphuric acids. These compounds are largely responsible for the "acid rain" and accounting for death of a number of sensitive aquatic organisms. The acid rain has also a role in damaging marble monuments and stone buildings.

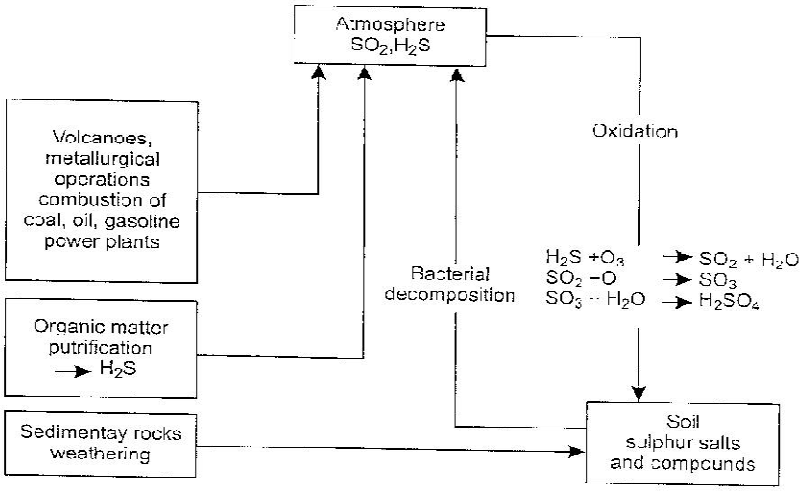

Figure 4.5: Schematic representation of the Sulphur cycle

4.5. Microflora in Rhizosphere and Phyllosphere

4.5.1 Rhizosphere microflora

The soil regions around the plant roots are characteristically different from the other soils or the soils which are not directly in contact with plant roots. This soil region is called rhizosphere and is defined as the soil region around the plant roots extending up to a few millimeters off the root surface, in which the types, counts, and activities of microorganisms differ from that of the bulk soil. Although, a number of microorganisms are found in the rhizosphere include bacteria, fungi, algae, viruses, nematodes, protozoa, and arthropods, but

bacteria are the dominant and have direct prominent roles in plant growth. Rhizosphere plays a very important role through microorganisms in regulating the decomposition of soil organic matter and nutrient cycling. The integration of root structure and microbial processes has roles to play in the nutrients absorption and water uptake. The interaction between roots and microorganisms decide growth conditions for both the plant and the microorganisms in the rhizosphere. The plant roots uptake several mineral nutrients and water for plant growth, and in turn release a wide range of organic compounds and enzymes in the soil. As a result, an intense microbial activity is aggravated in this rhizosphere. A generalized picture showing different types of association between plant roots and beneficial soil bacteria present in the rhizosphere is depicted in fig. 4.6.

4.5.2 Importance of Rhizosphere

Although the rhizosphere represents an important soil habitat and many microorganisms are beneficial for plant growth and development, there are also some plant pathogenic microorganisms such as *Agrobacterium* bacteria that colonize the soil around plant roots and cause diseases. Besides, some facultative human pathogenic bacteria like *Staphylococcus Burkholderia*, *Stenotrophomonas Enterobacter* and *Pseudomonas* are also found in the rhizosphere and can be carried on in plant tissues. Knowledge of rhizosphere is vital for adaptive or curative measures from these pathogenic microorganisms. As far as the beneficial effects are concerned, the rhizosphere represents a congenial soil habitat where introduction of beneficial microorganisms as inoculants such as biofertilizers, phytostimulators, and biopesticides can result in significant improvements in crop yield and/or crop quality.

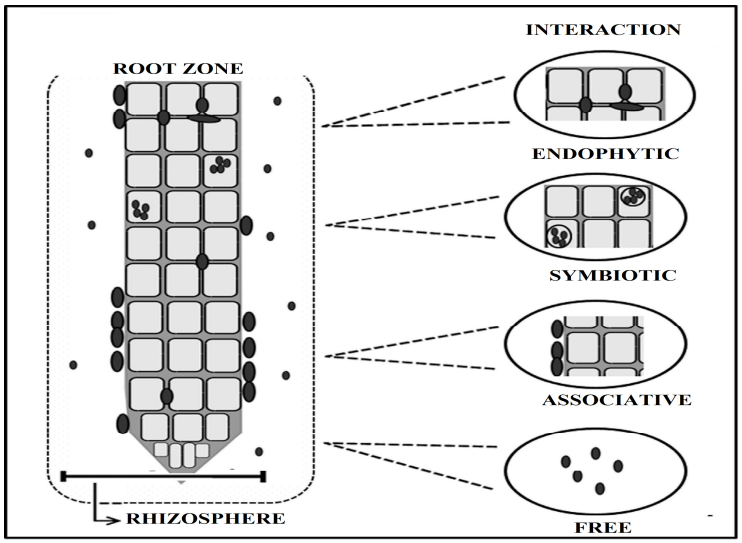

Figure 4.6: Overview of different types of association between plant roots and beneficial soil bacteria present in the rhizosphere

4.5.3 Phyllosphere microflora

The above-ground parts of plants are also generally colonized by a variety of microorganisms as leaf and stem surfaces facilitate a favourable environment to allow growth of microbial communities. This above-ground habitat of microorganisms is termed as the phyllosphere, and the inhabiting microorganisms are called epiphytes. These epiphytes are known for their enormous potentiality in promoting the plant growth and productivity. The epiphytes of phyllosphere are very wide and cover many different genera of bacteria, filamentous fungi, yeasts, algae, and, less frequently, protozoa and nematodes.

4.5.4 Importance of Phyllosphere

Phyllosphere plays key role in affecting the plant-microbe interactions on plant leaf surfaces not only for beneficial plant growth but also for disease suppression. Bacteria, actinomycetes and fungi are the microbial groups that directly control the growth and development plants as well as regulate plant pathogens. The growth of epiphytes is directly influenced by some external and internal factors such as nutrient availability, moisture, temperature, topography, leaf architecture, presence of enzymes and growth inhibitors etc.

Epiphytes are also involved in biogeochemical cycles such as carbon and nitrogen cycles. In carbon cycle, epiphytes intercept the carbon compounds released from plants or removed by sucking arthropods, while in the nitrogen cycles, epiphytes are involved in nitrification of ammonium pollutants and nitrogen fixation.

Many phyllosphere microbial inhabitants are very important to plant health, as there exists a better understanding of the interactions of microbes with plants and among themselves. An understanding of Phyllosphere microflora interaction may contribute also to have an insight of the ecology of human-pathogenic bacteria on plant surfaces and provide clues for the development of control measures.

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Chapter 5:

MICROORGANISMS IN AGRICULTURE

Chapter Objectives

- **This chapter describes the implications of different classes of microorganisms in agriculture.**
- **It defines biofertilizers, and describes their classes and importance with suitable examples.**
- **It defines biopesticides and highlights different classes with suitable examples. It also highlights the advantages of using biopesticides over chemical pesticides.**
- **It covers the aspects on silage production, procedure and advantage of its application over hay or fodder.**
- **It describes biofuel and primarily focuses on biofuels made from microbes which have potential to replace our present day fuels, either alone, by blending, or by chemical conversion.**
- **The chapter covers the aspects on generation of enormous amount of agro-waste, their ill effects on environments and management of these wastes through biodegradation.**

5.1 Introduction

Modern agricultural practices largely depend on massive and blunt use of mineral fertilizers to achieve higher production. It also involves non-judicious applications of chemical pesticides and weedicides to protect crops against pathogens and pests including weeds. These practices are now being globally criticized and are put under increased scrutiny as we are being more aware of potential health and environmental consequences of excessive mineral fertilizer and chemical pesticide.

It is widely felt that massive applications of mineral fertilizers (more specifically nitrogenous fertilizers) can cause groundwater contamination by nitrates leaching. Under certain soil conditions, denitrification of applied nitrogen fertilizer can give rise to gaseous nitrogenous compounds that volatilize from soil into the atmosphere. Some of them (e.g. nitrous oxide) are thought to contribute to the greenhouse effect and/or the alteration of the ozone layer. Similarly, use of chemical pesticides has raised concern about their possible presence and/or that of their residues in the food chain and in the environment.

5.2 Microorganisms in agriculture

Microorganisms have a vital role in supporting all plants and thus animals although soil organisms comprise <1% of the total mass of a soil. The increased awareness and concerns about the possible health impacts and environmental hazards of using mineral fertilizers and chemical pesticides non-judiciously have fetched wider interest in alternative strategies to ensure competitive production and protection of crops. The approach involves new agricultural practices that are environment friendly and maintain ecological balance of the soil ecosystem on sustainable basis. In the new non-chemical approach of farming, microorganisms play a dynamic role. Soil is the base of diverse array of biological processes such as biological nitrogen fixation and denitrification, decomposition of plant residues, mineralization and immobilization and biogeochemical cycling etc. All these processes are directly or indirectly regulated by microorganisms. Under the current scenario of excessive use of chemical fertilizers and pesticides, a considerable proportion of the beneficial soil microorganisms have been lost. To achieve better yield and sustainability in agriculture, there is a serious demand to enrich the soil with beneficial microorganisms. Followings are the few major application of soil beneficial microorganisms.

(i) **Microbes break down the complex organic matter:** Microorganisms play an important role in the decomposition of organic matter present in the soil. Microorganisms are specific to the types of organic matter to be decomposed.

(ii) **Microbes help in recycling of nutrients:** Soil inhabiting microorganisms have vital roles in transforming the nutrients to different forms. For example, sulphate-reducing bacteria are actively involved in decomposing and oxidizing various organic matters.

(iii) **Microbes help in maintaining the soil moisture:** As stated earlier, microorganisms have considerable role in the decomposition of complex organic matter. The complex organic matters of dead plant and animal are firstly broken down into smaller fragments called humus. In fact, humus contains many nutrients (most prominently carbon and nitrogen) that improve the soil health, structure and texture. Also, humus helps to retain moisture in the soil and improves soil's cation exchange capacity. Humus, being negatively charged molecules bind to positively charged cations of plant nutrients.

(iv) **Microbes create soil structure:** Some specific soil microorganisms secrete biomolecules like polysaccharides, gums and glycoproteins, which stick soil minerals together and thus, form a good soil structure. Fungal hyphae and plant roots are also known for binding soil aggregates together.

(v) **Microbes fix nitrogen:** Agriculture depends heavily on the ability of certain bacteria to convert atmospheric nitrogen (N_2 gas) to ammonia (NH_3). Some of these bacteria live freely in the soil, while others grow in association with plant roots. The classic example is *Rhizobium bacteria,* which grows in the roots of legumes to make the nitrogen available for the growth of plants.

(vi) **Microbes promote plant growth**: Some soil microbes produce a variety of substances that promote plant growth, including auxins, gibberellins and antibiotics.

(vii) **Microbes control pests and diseases:** The best known example of the use of soil microorganisms in pest control is the commercial production of the soil bacterium *Bacillus thuringiensis* (Bt) to control polyphagous pests namely *helicoverpa* of crops. There are reports where some strains of Bt were successfully used to mamage beetles and flies as well. Several strains of the fungal genus *Trichoderma* have been developed as biocontrol agents against fungal diseases of plants, mainly root diseases. Various other genera of fungi are also used for the control of insect pests.

On account of above mentioned applications of microorganisms in agriculture, the, microbial inoculants on large scale is used in different forms, the major among them are biofertilizers, phytostimulators and biopesticides.

5.3 Biofertilizers

The use of beneficial microorganisms as biofertilizers has become a popular alternative in agriculture. Technically, biofertilizers refer to the preparations containing living microbial inoculants of bacteria, algae, fungi either alone or in combination that help crop growth by augmenting the availability of nutrients. These biofertilizers are applied through seed or soil application in order to make the nutrients available to the plants through their interactions in the rhizosphere. Biofertilizers are applied also to hasten certain microbial processes in the soil so as to augment the availability of nutrients for the plants in easily absorbable form. Besides, application of plant growth promoting *rhizobacteria* (PGPRs),

cyanobacteria, *endo* and *ectomycorrhizal* fungi make the plant tolerance to abiotic and biotic stress.

5.3.1 Classes of Biofertilizers

Biofertilizers can be grouped in different ways based on their nature and function.

(i) N_2-fixing bacteria

Although free or inert nitrogen (N_2) is quite abundant (approximately 80 percent) in atmosphere but it is not available for plant uptake. There exist some bacteria which are capable of N_2 fixation from this atmospheric nitrogen. These bacteria form different associations with plants such as many free living bacteria N_2 fixing bacteria grow in the soil. Some bacteria form symbiotic relationship with plants. In addition, there are some bacteria that live in close association with the plant root zone without having any intimate endophytic symbioses. Different classes of N_2 fixing bacteria as biofertilizers are shown in table 5.1

Table 5.1: Different classes of N_2-fixing biofertilizers

Type/Nature of Habitat		**Examples**
Free living	Aerobic	*Azotobacter, Beijerinckia, Anabaena*
	Anaerobic	*Clostridium*
	Facultative anaerobic	*Klebsiella*
Symbiotic		*Rhizobium, Frankia, Anabaena azollae*
Associative symbiotic		*Azospirillum*
Endophytic		*Gluconacetobacter, Burkholdria*

(a) Free-living N_2 fixing bacteria

Many free-living bacteria such as *Azotobacter, Beijerinckia,* and *Clostridium* fix atmospheric N_2 in some plants like alfalfa *(Medicago sativa)* cucumber and barley plants. These free living N_2 fixing bacteria may have three variants namely aerobic, anaerobic and Facultative anaerobic (table 5.1).

(b) Symbiotic N_2 fixing bacteria

The most widely known symbiotic N_2 fixing bacteria are *Rhizobium* (family *Rhizobiaceae*). These bacteria harbour in the root nodules of legumes. Besides, *Frankia* is another important genus that is capable of N_2-fixing through infecting and nodulating trees or the plants of woody families. The N_2 fixing potential of these genera largely depends on host plant species and bacterial strains. Therefore, for the selection of biofertilizers, microbial strain and host compatibility must be taken into considerations. For application purpose, these microbial inoculum can be made and applied in several ways such as powder, liquid or in granular formulations.

(c) Associative symbiotic N_2 fixing bacteria

There are some bacteria which in place of nodulating remain associated with the host plants. Examples include *Azospirillum, Acetobacter diazotrophicus* and *Herbaspirillum* spp. which colonize a number of annual and perennial crops such as sorghum, sugarcane and maize. Apart from nitrogen fixation, these associative bacteria are also known to increase the growth of a range of crops including sunflower, cotton, pepper, tomato and eggplant etc. Therefore, these bacteria are better known as growth-promoting N_2-fixing biofertilizers.

(d) Endophytic N_2 fixing bacteria

These are the bacteria that get inside into the host plant tissues (for example pineapple) and colonize the interior of the host without giving any harm to the host. Having the place inside the host tissues, these endophytic bacteria can make the fixed nitrogen available directly to the plants. Examples of endophytic bacteria include *Azoarcus* spp., *Gluconacetobacter* and *Burkholdria* etc.

(e) Cyanobacteria

These are blue-green bacteria also called cyanobacteria, generally found in water and on land. They also help fix atmospheric nitrogen. Examples are *Oscillatoria, Nostoc, Anabaena* etc. Cyanobacteria is considered as potential biofertilizer for reclamation of extreme, arid environments. These are known to increase soil nitrogen content very efficiently. In the cultivation of rice, cyanobacterial N_2 fixation (mainly through *Nostoc* and *Anabaena*) is essential. The symbiotic association between the aquatic fern Azolla and *Anabaena* is very important for rice fields. In this association, *Anabaena* receives nutrients like carbon and nitrogen from the plants in exchange for fixed nitrogen. This in turn adds organic matter to the soil enhancing the fertility of rice fields.

(ii) Phosphorus solubilizing microorganisms (PSM)

Like atmospheric inert nitrogen the, phosphorus present in the soil is also not accessible by the plants as such. There are some bacteria that solubilise phosphorus and transform it into vailable form for plants to uptake. The most widely known phosphorus-solubilizing bacteria (PSB) belong to the genera *Bacillus* and *Pseudomonas* while the common phosphorus-solubilizing fungi belong to genera *Penicillium* and *Aspergillus* (table 5.2). Application *of* PSB has been a viable option to supplement phosphorus from the relatively cheaper rock phosphate instead of superphosphate.

Table 5.2: Examples of Phosphorus solubilizing microorganisms **(PSM)**

Microbes	**Examples**
Bacteria	*Bacillus megaterium* var. phosphaticum, *B. subtilis*, *B. circulans, Pseudomonas striata*
Actinobacteria	*Actinobispora yunnanensis, Actinomodura citrea, Microtetrospora astidiosa*
Fungi	*Penicillium bilaii*, *Aspergillus awamori*
Mycorrhiza	Genera *Glomus, Funneliformis, Rhizophagus*

Endophytes	Bacteria	*Achromobacter, Enterobacter cloacae, Pantoea agglomerans, Acinetobacter,*
	Fungi	*Piriformospora indica*

(iii) Phosphorus mobilizers

There are some fungi that increase the uptake of soluble phosphates. The most prominent examples include Arbuscular mycorrhizal fungi (AMF), Ectomycorrhizal fungi, Ericoid mycorrhiza and Orchid mycorrhiza.

(iv) **Zinc and Silicate solubilizers**

For efficient zinc supplementation, applications of zinc solubilizing bacteria have been widely practiced. Zinc solubilizing bacteria is an economical option to convert complex inorganic zinc to available forms for the plants. The major examples include *Pseudomonas fragi*, *Pantoea dispersa* and *Pantoea agglomerans* etc.

Silicate solubilizing bacteria (SSB) has also an important role in solubilizing insoluble forms of silicates. In addition, these bacteria also solubilize potassium and phosphates. Therefore biofertilizers enriched with SSB are known to have good impact on increasing soil fertility and plant productivity. For example, the photosynthetic efficiency of *B. juncea* is improved with the application of SSB -enriched biofertilizers (*Bacillus sp*.).

(v) **Plant growth promoting rhizobacteria (PGPR)**

A variety of bacteria can promote plant growth and can improve plant health. Collectively these bacteria are called plant-growth-promoting promoting rhizobacteria (PGPR). Such soil bacteria have been found to produce some biomolecules such as vitamins and plant hormones that improve plant health and thus contribute to higher crop yield. These bacteria are termed as 'phytostimulators'. Examples include *Rhizobacteria*, *Pseudomonas* species and many more. These bacteria promote plant growth mainly through Phosphorus solubilization, nutrient uptake enhancement, or production of plant growth hormones and other essential biomolecules. A rhizobacterium belonging to the genus *Achromobacter* is reported to increase root hair number and length in oilseed rape *(Brassica napus).* Similarly, another rhizobacterium *Achromobacter* enhanced nitrate and potassium uptake to increase the crop yield.

(vi) **Fungi as biofertilizers**

The fungi play very important role in absorption of the phosphorus salts from the soil to be used by plants as nutrients. Fungi have characteristic filaments that increase the surface area for absorption of other mineral nutrients from the soil too.

(a) Mycorrhizal Fungi

Mycorrhiza are nowadays being used as an important biofertilizers. Basically mycorrhizal association is the mutualistic symbioses between fungi and plants. The fungi belongings to genus *Glomus* forms mycorrhizal association with the roots of the plants. This association

between plant roots and colonizing mycorrhizal fungi is a functional symbiosis where the mycorrhizal fungi are obligately or facultatively dependent on host for metabolites or nutrients and energy. The fungal mycelium extending from the root surfaces to the soil absorbs nutrients from the soil. Hence, the primary purpose of mycorrhizal association is the transfer of nutrients from fungus to the plants. In most of the cases, transfers of metabolites from the plants to fungi also take place. For example, a crop plant is able to use insoluble sources of phosphorus in presence of mycorrhizal fungi but unable to access phosphorus in the absence of mycorrhizal inoculation.

(b) **Arbuscular Mycorrhizal (AM) fungi**

Arbuscular mycorrhizal (AM) fungi are the oldest and most common symbiosis with land plants including vegetable crops, fruit crops and ornamental plants. AM fungi prominently facilitate nutrient uptake by forming vesicles, arbuscules, and hyphae in the plant roots. They also produce spores and form hyphae in the rhizosphere so as to enhance the access of roots to a large soil surface area leading to better plant growth and development. Although, AM fungi mainly facilitate uptake of nutrients specially phosphorus by the extra-radical mycorrhizal hyphae, but also protect plants against pests and diseases.

(c) **Ectomycorrhizal (**EcM) **fungi**

Ectomycorrhizal association are mutualistic association between higher fungi and Conifers and woody plants/trees and forest trees (gymnosperms or angiosperms). It is rarely found association and merely 10% of terrestrial plant species are ectomycorrhizal on Earth. This association is formed mainly on the fine root tips of the plant spread unevenly in the rhizosphere. The ectomycorrhizal associations consist of soil mycelium network, mycorrhizal roots (particularly root tips) and storage or reproductive structure. The ectomycorrhizal root is typically characterised by the presence of Hartig net. Examples of ectomycorrhizal fungi include *Basidiomycetes, Ascomycetes* and few *Zygomycetes*.

(d) Endomycorrhizal Fungi

Endomycorrhizal association is based on the exchange of nutrients from inside of the root cells and the hyphae extended outside the root. In this association, the fungal hyphae grow inside the roots andt penetrate the root cell walls. Endomycorrhizal association is formed mostly in green leafy plants and most commercially produced plants. Examples include vegetables crops, grasses, fruit trees, shrubs and flower plants. Examples of endomycorrhizal fungi include *Ascomycota.*

5.3.2 Importance of Biofertilizers

Biofertilizers are widely utilized for improving crop yields and reducing costs for inorganic fertilizers. In addition, biofertilizers are known for a number of encouraging contributions in agriculture; the major ones are as follow:

(i) They supplement fertilizer supplies for meeting the nutrient needs of crops in economically and environmentally viable way.

(ii) Biofertilizers can supplement almost 20 to 200 kg N/ha (through fixation) under optimum conditions and solubilise and/or mobilize 30 to 50 kg P_2O_5/ha.
(iii) They also promote plant growth by enhancing photosynthetic or metabolic efficiencies.
(iv) Some growth promoting substances and vitamins are also supplemented through biofertilizers that help to maintain soil fertility.
(v) They protect the plants from the incidence of pests-pathogens and control diseases.
(vi) They can increase the crop yield by an extent of 10 to 50%. N_2 fixer biofertilizers can reduce exhaustion of soil nutrients substantially.
(vii) Biofertilizers improve soil physical properties like structure, texture, tilth and overall soil health.

5.4 Biopesticides

The pesticides synthesized from natural origin in called biopesticides. It generally refers to the specific pesticides derived either from natural materials such as animals, plants, bacteria, or certain minerals. Examples include fungi such as *Beauveria* sp., bacteria such as *Bacillus* sp., neem extracts and pheromones derived from some plants. Similarly some other materials like Canola oil, tea tree oil, cayenne pepper, neem cake, extract from lemon grass and baking soda have pesticidal properties and therefore are considered as biopesticides. In other words, biopesticide is a formulation for controlling pests and is made from naturally occurring substances in non toxic and eco-friendly manner.

Biopesticides include several types of natural pest management intervention such as through predatory, parasitic, or chemical relationships. However, biopesticides are generally less toxic or not lethal to the user and non-target organisms thereby making them an ideal and sustainable tool for disease management.

5.4.1 Types of Biopesticides

Biopesticides are broadly classified into five major classes:

(i) **Biochemical pesticides**: These are naturally occurring substances or simply the plant extracts that control insect-pests by non-toxic mechanisms. In contrast, the conventional pesticides are the synthetic toxins that directly kill or inactivate the pest. Biochemical pesticides include substances that generally interfere with mating such as insect sex pheromones and other various scented plant extracts or trap that attract insect pests.
(ii) **Microbial pesticides:** These are the microorganisms (e.g., a bacterium, fungus, virus or protozoan) that act as the active ingredient in the formulations meant for killing insect-pests. Microbial pesticides can manage many different kinds of pests; however, each separate active ingredient is relatively specific for its target pest[s]. For example, there are fungi like *Colletotrichum*, and *Sclerotinia* that control certain weeds and other fungi such as *Basidiomycota*, and *Ascomycota* are known to infect and kill insects.

Nowadays, the most widely and popularly used microbial pesticides are subspecies and strains of bacteria *Bacillus thuringiensis* (Bt). Each strain of *Bacillus thuringiensis* produces a different toxic protein to kill one or a few related species of insect larvae. While some Bt proteins kill moth larvae found on plants, the other Bt proteins are specific for killing larvae of flies and mosquitoes. The target insect species are determined on the basis of production of the particular toxic protein that can bind and cut to a larval gut receptor, thereby killing them.

(iii) **Plant-Incorporated-Protectants (PIPs):** These are pesticidal gene products that plants produce from the foreign genetic material that has been added to the plant. The plant synthesizes the pesticidal protein that kills the pests when they feeds on the plant. For example, the genes from *Bacillus thuringiensis* bacterium coding for toxic proteins have been introduced in a number of crops through genetic engineering to manage the attacking larvae of *Helicoverpa.*

(iv) Botanical pesticides: These are the plant materials that have traditionally been practiced for pest management, although very few botanicals are currently used for the purpose. Pyrethrum and neem are well proven to manage insect pests and are being commercially exploited also. In addition, a number of plant products are known for their use as pest repellents, anti-feedants, and toxicants. For example a number of plants based pestiticides such as nicotinoids, natural pyrethrins, rotenoids, neem products etc are commercially used. These botanicals may be exploited as crude preparation of the plant parts such as leaves stem and bark etc crushed to produce powder or dilute form. The mode of action of botanical pesticides is similar to that of the chemical pesticides. For example, "Azadirachtin", the extract from neem tree (*Azadirachta indica*) kill the invading pests by disrupting the reproductive and digestive system of pests.

(v) Biotic agents (parasitoids and predators):

(a) **Predators**: The predators of insects include beetles, bugs, flies, spiders, wasps, and predatory mites. These predators are found all over the plants body including underground roots and consume their enemy prey over the course of their development. Predators are generally free living and are usually as big as or bigger than their prey. Some predators have specific prey while some predators can kill or consume any pests.

(b) **Parasitoids**: Presently, parasitoids are the most commonly used biological control measures around the world. The term parasitoid refers certain insects that have free-living adult stages but lay eggs inside a host body. The eggs go on to parasitize the host and eventually kill. Actually, the parasitoids larvae kill their hosts to complete their life cycle within the host from egg to adult. The larva that hatches from each egg of the parasitoid feeds on the host's tissues and body fluids, consuming it slowly till death of the host. The parasitoid usually needs only one

host to feed on and reach adulthood. The major examples of parasitoids are *Bathyplectes, trichogramma, encarsia* and *muscidifurax* etc.

5.4.2 Advantages of biopesticides

(i) Biopesticides are generally less toxic than conventional chemical pesticides.
(ii) Biopesticides are target specific and generally affect only the target pest or closely related organisms, whereas the conventinal broad spectrum chemical pesticides that may affect diverse organisms such as birds, insects and mammals.
(iii) Biopesticides often are required and effective in trace amount and often decompose quickly. This results in lower exposures to the users and lessens the pollution problems caused by conventional pesticides.
(iv) As an effective component of Integrated Pest Management (IPM) programs, biopesticides can greatly reduce the uses of conventional pesticides with crop yields remain high.

5.5 Silage production

Silage is high-moisture stored fodder produced by controlled fermentation which can be either fed to cattle, sheep and other such ruminants or used as a biofuel feedstock for anaerobic digesters. The process of fermenting and storing silage is called ensilage or silaging. The silage is usually made from crops of graminaeae family such as maize, sorghum, barley or other cereals. Many weeds such as spurrey (*Spergula arvensis*) also have ensilaging potential. Special names are given to silage depending on depending on types of raw materials, for example oatlage for oats and haylage for alfalfa.

Basically, hay and silage are the main methods of conserving forage. On one hand, hay is preserved by drying, whereas silage involves natural fermentation, which produces lactic and other acids, which preserve the forage.

The crops most often used for ensilage are the ordinary grasses, oats, maize, sorghum, pearl millet, napier, and alfalfa etc. These crops are characteristically rich in soluble carbohydrates and have 50% to 60% moisture content. The silage quality largely depends on the means of storage, the degree of compression, and the amount of water that would be lost during storage.

5.5.1 Silage production: Procedure

(i) Construct a silage storage structure (called silo) of dimension on cubic meter space. This structure can store 500-600 kg of green fodder.
(ii) Harvest the crop at 30-35 % dry matter (DM) stage.
(iii) Slice the fodder into small pieces of 2-3 cm size and fill the chopped fodder into the silo.
(iv) Press the chopped fodder layer by layer in the silo. Each layer should be of 30-45 cm.
(v) Filling and pressing should be completed as fast as possible.

(vi) Use additive (such as molasses) during filling of fodder in the silo, if required. Molasses allow the bacteria to produce the organic acids immediately. The more is the molasses added, the quicker the acidification and preservation take place. In addition, some additives such as common salt, formic acid, urea or lime are also used to facilitate good fermentation process.

(vii) After filling and pressing, seal the silo with thick polythene sheet.

(viii) Put weight through mud layer sand bags/tyres on the sheet to prevent air flow beneath the sheet.

(ix) Open the silo for feeding, minimum after 45 days, as per need. Initially, silage can be fed @ 5 kg/animal.

5.5.2 Advantages of silage making

(i) Silage furnishes high quality forage in any season throughout the year and that too at a low expense.

(ii) There is an acute shortage of green fodder or any suitable feed during the summers, silage can meet this deficiency during that part of the year.

(iii) Grass silage preserves 85 percent or more of the feed value of the crop, where as hay making will preserve significantly less percentage of nutrients.

(iv) It is the most economical and exploitable form in which the whole stalk of maize or sorghum can be processed to a good quality feed and stored for longer time. Otherwise considerable part of this crop is wasted during the course of feeding in dry seasons.

(v) During the monsoon months, it is very difficult to convert dry grasses into hay. The adequately stored and preserved silage can avoid this problem.

(vi) Weed species may also produce good quality silage. The ensiling process also manages all weeds that are present in the field. The weeds are harvested before seed formation and hence dissemination of their seeds is checked.

(vii) Silage is a quite palatable feed and moderately laxative in nature.

(viii) Silage is a very good protein and vitamins especially carotene source as compared to the dried forage.

(ix) Wastage of the plant is substantially less as the whole plant is being utilized for ensiling.

(x) The silage requires reasonably less space for storage than dry fodder of the same quantity.

(xi) Silage preparation helps to manage weeds in a field to a significant level, which are often spread through hay or fodder.

5.6 Biofuel

Biofuel includes solid biomass, liquid fuels and various biogases derived from plant or microorganisms or animal waste. In the scene of environmental pollutions from gas emissions from the fossil fuels, a panic of energy insecurity in the future and oil price hike, biofuels are fetching the interests of public as well as scientific communities.

Biofuel is considered as a cost-effective and environment friendly alternative to fossil fuels such as petroleum, coal and others.

Commercially, biofuel production primarily aims at producing energy products such as bioethanol (particularly ethanol, but also propanols and butanols, propane and butane diols etc.), biodiesel and biogas from natural or biological sources. Generally, two main classes of biofuels are there, primary and secondary biofuels. Primary biofuels are those which are used as they are or unprocessed. Examples include firewood, forest materials, crop residues or animal wastes. Secondary Biofuel is a type of fuel that is derived from biomass conversion. The secondary biofuels are further classified into first, second, and third-generation biofuels depending on the on the feedstock used.

The processes of biofuel conversion from the natural sources primarily involve the use of acidification to produce glucose from woods and subsequent fermentation by microorganisms under anaerobic conditions. Currently, the potential of various fungi and bacteria to degrade cellulose and other plant polymers has been fully explored. Also the powers of anaerobic microorganisms to ferment sugars to alcohols and ketones have been thoroughly and successfully demonstrated.

5.6.1 Different sources of biofuel

There are 4 sources biofuel production which are being globally exploited.

(a) Algae

Algae are the most commonly and recently used feedstock for biofuel production and present a positive hope for algae-derived fuel for the future. Algae offers several advantages over earlier plant or crops based feedstock such as sugar cane and corn (considered as first generation of biofuel production) and vegetable or animal waste streams (considered as second generation of biofuel production, while algal biofuel is termed as third-generation biofuel). The major advantages as follow:

(a) Algae feedstock can yield up to 300 times more oil per acre than plant or crops based feedstock as the algal feedstock has high concentrations of lipids, fatty acids, oil-containing molecules to create high efficiency biofuels.
(b) The algal biofuels do not emit CO2 back into the atmosphere.
(c) Among all other biofuels, algae feedstocks have been demonstrated to be used as a new form of green jet fuels.

(b) Carbohydrate (sugars) rich biomaterial

Fermentation of starches derived from carbohydrate (sugars) rich crops such as corn, sugarcane, barley, wheat, beets, and other similar food crops offer promising biofuel. The biofuels from these agricultural products have been used in an existing gasoline engine and auto industry, however the costs involved in production, harvesting and processing do not result in a sustainable gain for long term.

(c) Oils rich biomaterial

It is considered as first generation biofuel and is derived from existing food crops like corn, canola, rapeseed and sunflower. In addition, jatropha, a non-food crop has also been exploited for extracting oil from its seeds. The oils from these food crops are extracted after these have been used for other food purposes. The oil mainly consists of triglycerides-esters of glycerol with three fatty acids that make it as biofuel. The oils from canola and corn have been used for the creation of biodiesel fuel for home heating and automobiles.

(d) Agriculture wastes (organic and inorganic sources)

Agricultural wastes are the most commonly used traditional source of biofuels in India. These are mainly the residues left after production and processing of crop products. Agricultural wastes are used as biofuel either simply by burning or with very little processing. The use of agricultural wastes as biofuel fetches some controversy too as they are rich with inorganic nutrients and can better be used as compost in agriculture.

5.6.2 Approaches of Biofuel production

In general, two main approaches are currently used in biofuel production aiming at alcohol production: (i) Direct fermentation and (ii) Indirect fermentation.

(i) **Direct fermentation**: It depends on the conversion of various plant materials to biofuels, mainly ethanol. In principle, two processes are involved here:
(a) The degradation of starting plant materials (e.g. molasses from sugar cane, starch from corn kernels etc.) into fermentable sugars through the use of a glucoamylase enzyme to cleave starches and dextrins -1,4-glucosidic linkages, which releases sugar (glucose and maltose) for fermentation, and
(b) The conversion of these sugars to alcohol by yeasts or genetically engineered bacterial strains.

(ii) **Indirect fermentation:** It is less commonly used, and depends on pyrolysis (burning) of the starting plant materials, followed by the conversion of the produced gas (for example, Syngas, a mixture consisting mainly of carbon monoxide, hydrogen, and carbon dioxide) to ethanol using acetogenic bacteria.

5.6.3 Biofuels utilization

In principle, there are primarily three strategies to utilise energy from biofuels in different types of engines. The first one is either the engines need to be adapted to the existing fuels or secondly, the biofuel has to be composited to exhibit the similar features of traditional fossil fuels. The third strategy is using biofuels blended with traditional fuels. This often results in an advantageous modification of the fuel's properties, such as an increased octane rating without the use of environmentally harmful additives. Alcohol-based biofuels like methanol, ethanol and N-butanol can not only extend the supply of gasoline and diesel, or even replace them, but are also proved to be good additives to existing fossil fuels as oxigenisers, liquefiers or anti-knocking agents. However, there are limitations with energy-

rich microbial fermentations to be used as fuels as they may cause corrosion or swelling with certain materials or may have other undesirable characteristics.

5.6.4 Metabolic procedures of biofuel production

During the anaerobic metabolism of microorganisms, a balanced redox reaction takes place. In this reaction, CO_2 is removed from carbohydrates which have a Carbon: Hydrogen: Oxygen ratio of 1:2:1. One molecule of Glucose ($C_6H_{12}O_6$), is converted to two molecules of CO_2 and two molecules of ethanol (C_2H_5OH).

$$C_6H_{12}O_6 \xrightarrow[@\ 25–37°C]{redox} 2CO_2+2C_2H_5OH$$

Ethanol, with its high (Carbon and Hydrogen) to Oxygen ratio, holds most of the original energy content in combustion. As a microbial cell can liberate much less energy (2–3 ATP) from this anaerobic reaction as compared to oxidative respiration (26–38 ATP), it requires to consume about ten times the amount of substrate to gain the same amount of energy, compared to oxidative respiration.

5.6.5 Biological systems for Biogas production

Biogas formation from plant fibres is generally a three stage process involving a different set of anaerobic and facultatively anaerobic microorganisms in each stage:

(i). Hydrolysis of polysaccharides (starch, cellulose, hemicelluloses etc.), proteins and fats into oligosaccharides and sugars, fatty acids and glycerol. The hydrolysis process is followed by acidogenesis. Acidogenesis is the fermentation of these products into mainly acetic, propionic and butyric acid, carbon dioxide and hydrogen, alcohols and other minor compounds.

(ii). Acetogenesis: The production of acetic acid and carbon dioxide by the bacteria. It involves long generation time and therefore seems to be the limiting process step.

(iii). Methanogenesis: the methanogenic bacteria including slow growing archaea have specific coenzymes and pathways that use hydrogen to reduce carbon dioxide or in some cases acetate to produce methane. The main metabolic reaction here is the reduction (addition of Hydrogen) of carbon dioxide (CO_2) to methane (CH_4). The CO_2 and H_2 are produced as a result in these fermentation reactions. The methane and residual CO_2 are released as biogas which is also called marsh gas.

The bacterial community engaged in these three stages may be similar to those in cows rumen (such as *Ruminococcus species* and *Fibrobacter succinogenes* and *Butyrivibrio fibrisolvens*) or wastewater treatment plants (such as *Caldilinea*,

Dechloromonas, *Rhodospirilaceae, Nitrospira*, *Sphingobacteriales*, *Thiobacillus*, *Sinobacteraceae* and *Comamonadaceae*).

5.6.6 Biodiesel

Biodiesel refers to a monoalkyl ester of fatty acids from vegetable oil. Biodiesel is produced by enzymatic transesterification with petrochemically derived methanol, also called alcoholysis (e.g. rape seed oil methyl ester.). Instead of using vegetable oil, nowadays, microalgae are also being grown in bioreactors for the production of suitable biodiesel. Biodiesels from microalgae promise to cover a good market share in the future because of their high oil productivity and very low area requirement.

As a first step, alcohols from microbial fermentations such as ethanol, propanol and butanol can be used in place of methanol. Even a mixture of alcohols characteristic of acetone–butanol fermentation can be used for the process of enzymatic trans-esterification. The glycerol produced during trans-esterification can be fermented to 1,3-propanediol and possibly to other products by metabolically engineered bacteria or to highly inflammable methane in biogas plants. The methane it can be added in low concentrations as co-substrate in other fuels also. As stated earlier, microorganisms involved in biodiesel degradation is of great concern as unavoidable water content during bacterial oxidation may lead to corrosion problems.

Although the technology for conversion of plant oil to biodiesel is in nascent stage, it is envisaged that inclusion of biologically fermented ethanol and butanol in the current day fuels will be a feasible and economical option. In India and abroad, most diesel cars are now designed and licensed to use a biodiesel–diesel blend of up to 5% (v/v), however, such engines require more frequent oil changes.

5.7 Agro-wastes

With the intensification of agricultural production, higher quantities of livestock waste, agricultural crop residues and agro-industrial by-products also resulted. In the coming days too, it is likely to have considerable increase in agricultural wastes globally.

Agricultural wastes or otherwise called agro-waste are defined as the residues from the growing and processing of raw agricultural products such as fruits, vegetables, meat, poultry, dairy products, and crops. Roughly, agricultural wastes can be categorized into four classes (i) crop residues (ii) agro-industry wastes (iii) livestock wastes, and (iv) food grains, vegetable and fruit wastes.

The crop residues primarily involve straws of rice, wheat, corn, oats and barley crops. The agro-industry wastes consist of sugarcane bagasse, rice bran, De-oiled seed cakes, rice husk, apple and amla pomacea, orange, banana and other fruits peel etc,. Besides, hazardous and toxic agricultural waste (pesticides, insecticides and herbicides, etc) also aid to the agro wastes. Livestock wastes encompass cattle manure, swine manure and animal fat and carcasses. Food grains, vegetable and fruit wastes are primarily comprised of food processing

waste (for example, only 20% of maize is canned and 80% is waste), crop waste (corn stalks, sugarcane bagasse, drops and culls from fruits and vegetables).

5.7.1 Generation of agro-wastes

Agricultural intensification is normally accompanied by lot of wastes from the non-judicious application of various intensive farming methods and the blind use of chemicals in cultivation at the same time, adversely affect the environments. The nature of agro-waste is largely dependent on the type of agricultural activities carried out.

(i) Wastes from Cultivation Activities

Farmers, in general, use a massive amount of pesticides and insecticides in order to manage insect-pests and the spread of epidemic diseases. After use, most of the bottles and packages holding these pesticides are thrown bluntly into fields or ponds nearby. According to an estimate, about 1.8% of the chemicals remain as it is in their packaging. These wastes have the potential to cause unpredictable environmental consequences such as food poisoning, unsafe food hygiene and contaminated farmland due to their potentially lasting and toxic chemicals. Similarly, fertilizers play an important role in maintaining the productivity and quality of plants. Inorganic fertilizer is inexpensive and characterized by high productivity. Among the excess fertilizers applied to the field, some portion is retained in the soil, few portions goes into ponds, lakes and/or rivers through either surface runoff or irrigation resulting in the pollution of surface water. A portion of these fertilizers also enters the ground water while some portion evaporates or becomes de-nitrated, causing air pollution too.

(ii) Wastes from Livestock Production

Livestock waste implies the solid waste such as manure and organic materials from the animal excreta, wastewater such as urine, wastes from the slaughterhouses and wastewater from the bathing of animals, air pollutants such as H_2S and CH_4. In livestock waste, the major proportion is of water (about 75–95% of total volume), while the rest includes solid organic matter, inorganic matter, and many species of microorganisms and parasite eggs.

(iii) Waste from Aquaculture

One of the major wastes generated in aquaculture is metabolic waste of aquatic organisms which could be dissolved or suspended. Feeding rates of aquatic organisms are dependent on the ambient temperature. Increase in temperature results in increased feeding which gives rise to increased generated waste.

5.7.2 Utilization of agro-wastes

Agricultural waste management system must either use the residues rapidly, or store the residues under conditions that do not cause spoilage or render the residues unsuitable for processing to the desired end product. There are a number of applications to which these wastes can be managed. They include:

(i) Fertilizer Application

The utilization of animal manures for fertilizer has a definite impact on input energy requirements at the farm level. For example, poultry manure contains high phosphorus which has positive effect on the growth and productivity of crops. Adding manure to soil increases its fertility because it increases the nutrient retention capacity (or cation exchange capacity), improves the physical condition, the water-holding capacity and the soil structure stability.

However, fertilizer use of manures from large confinement is associated with high energy costs for transport, distribution, storage facility requirements, odour problems and possibility of groundwater contamination.

(ii) Anaerobic Digestion

Methane gas can be produced from agricultural wastes particularly from manures. The anaerobic digestion of agricultural waste to form methane-rich gas is being widely practiced. However, there are also some disadvantages of the methane generation process. The high capital costs and the explosive properties of the methane gas are some of the major bottlenecks in its wider adoption.

(iii) Adsorbents in the Elimination of Heavy Metals

Industrialization and urbanization led excessive release of heavy metals into the environment pose a big problem worldwide. In recent years, agricultural wastes have proven to be a low cost alternative for the treatment of effluents containing heavy metals through the adsorption process. The low cost agricultural waste such as sugarcane bagasse, rice husk, sawdust, coconut husk, oil palm shell, neem bark etc., for the elimination of heavy metals from wastewater have been investigated by various researchers.

(iv) Pyrolysis

Pyrolysis is a systems of heating agricultural waste at a a temperature of 400-600°C in the absence of oxygen in order to vaporize a portion of the material and getting a char behind. The system is used for the preparation of chemicals from agricultural waste as well as for energy recovery. Pyrolysis of agricultural waste yields oil, char and low heating value gas.

(v) Animal feed

Crop residues have high fiber content and are low in protein, starch and fat. These problems of scarcity of fodder and pastures may be circumvented by utilizing residues to feed domesticated animals.

5.7.3 Agricultural Waste Management

The traditional approach to agricultural waste management has been discharge to the environment with or without treatment. There is need to consider wastes as potential

resources rather than undesirable and unwanted, to avoid contamination of air, water, and land resources, and to avoid transmission of hazardous materials.

Therefore, the system of agricultural waste management must be adopted as a "planned system in which all necessary components has to be installed and managed to control and use byproducts of agricultural production in a manner that sustains or enhances the quality of air, water, soil, plant, and animal resources".

5.7.3.1 The '3R'Approach to Agricultural Waste Management

The concept of minimizing waste is getting popularity as the wastes can be restructured to produce same or modified economic products. The most logical approaches involve reducing quantity of wastes, reusing the waste products with simple treatments and recycling the wastes by using it as resources. This is usually referred to as '3R'. Thus, The principle of reducing waste, reusing and recycling resources and products (3Rs) aims at achieving efficient minimization of waste generation by:

(i) Choosing to use items with care to reduce the amount of waste generated.
(ii) Repeated use of items or parts of items which still have usable aspects.
(iii) The use of waste itself as resources.

The wastes when managed properly through the application of the knowledge of agricultural waste management systems such as the "3Rs" can be transformed into beneficial materials for human and agricultural usage. It is very important that proper waste collection, storage, treatment, transfer, and utilization is a panacea to a healthy environment. Proper waste utilization will certainly assist in developing our agricultural sector and provide viable biofuel resources for the future.

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