Uttar Pradesh Rajarshi Tandon Open University

M.Sc.

Environmental Science

PGEVS-103 (N)

Environmental Microbiology

COURSE INTRODUCTION

In this course learn will known about the microbial diversity and how they will useful agriculture and environmental. The course mainly focuses on the studying microorganisms in their natural environments, including soil, water, air, and various ecosystems. These microorganisms can include bacteria, archaea, fungi, viruses, and other microscopic life forms. Environmental microbiologists investigate the roles microorganisms play in nutrient cycling, biodegradation, and their impact on environmental health. They also study microbial diversity, adaptation to extreme conditions, and their applications in fields like bioremediation and wastewater treatment. Understanding the interactions between microorganisms and their environments is crucial for managing and preserving ecosystems, as well as for addressing environmental challenges such as pollution and climate change. The course is organized into following blocks:

Block 1 covers the microbial diversity and microbial techniques

Block 2 deals the soil water and air microbiology

Block 3 describes in agriculture microbiology

Block 4 this block covers the microbes in organic matter and microbial diseases

PGEVS-103N

Rajarshi Tandon Open University, Prayagraj

Environmental Microbiology

Block- I

Microbial Diversity and Microbial Techniques

UNIT -1

Microbial Diversity

UNIT-2

Cultural Media and Nutrition

UNIT-3

Microbial Techniques

PGEVS-103N

Rajarshi Tandon Open University, Prayagraj

Environmental Microbiology

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Introduction

This is the first block on microbial diversity and microbial techniques. It consists of following three units such as:

Unit-1: This unit covers the microbial Diversity among microorganism. Modern approaches to bacterial taxonomy, polyphasic classification, general characteristics of primary domains and of taxonomic groups belonging to bacteria, archaea and Eukarya.

Unit-2: This unit's culture media and nutrition. Construction of culture media, types of microbial culture media, principles of microbial nutrition, microbial nutritional types and modes of nutrition in bacteria

Unit-3: This unit covers the microbial techniques. The theory and practice of sterilization, pure culture techniques, enrichment culture techniques, isolation and culture of aerobic and anaerobic bacteria, preservation and maintenance of microbial cultures are cover in this unit.

Unit-1: Microbial diversity and systematic

1.1. Introduction

The microorganism is an organism which is only visible under microscope i.e. microscopic in nature and cannot seen by naked eyes, and commonly known as microbes. Microbial taxonomy is the classification, nomenclature and identification of microbes (algae, protozoa, slime moulds, fungi, bacteria, archaea and viruses). The naming of organisms by genus and species is governed by an international code. Bacteria can be separated into two major divisions by their reaction to Gram's stain, and exhibit a range of shapes and sizes from spherical (cocci) through rod shaped (bacilli) to filaments and spiral shapes. In clinical practice, bacteria are classified by macroscopic and microscopic morphology, their requirement for oxygen, and activity in phenotypic and biochemical tests. Various diagnostic test systems are used to detect specific bacteria in clinical systems, including specific gene probes, reaction with antibodies in ELISA formats, immunofluorescence and, increasingly, PCR-based technology. Different bacterial species, often exhibit different population structures, highly diverse (panmictic) or

relatively uniform (clonal) depending mainly on the frequency of gene recombination (from external sources).

Objectives:

- To study of modern approach for bacterial taxonomy
- To known the bacterial nomenclature
- To discuss the microbial diversity by using molecular methods

1.2. Modern approaches to bacterial taxonomy

The term taxonomy is the science that studies the relationships between organisms. It comprises classification, nomenclature, and identification of organism. The role of taxonomy is very useful in understanding of nature of organism. However, the bacterial taxonomy is called polyphasic, because it is based on studies of several molecular techniques, such as each one retrieving the information at different cellular levels (proteins, fatty acids, DNA). The obtained results are combined and analyzed to reach “consensus taxonomy" of a microorganism. Within time duration, lots of techniques were involved for the identification of microorganism such as polymerase chain reaction (PCR), becomes most useful techniques which create great changes to identification of microbes. If we see, nodulating bacteria, it has been repeatedly modified over the last 20 years, is identified with current microbial techniques. A "natural" taxonomy would be based on evolutionary relatedness. Thus, organisms in same "genus" ,a collection of "species", would have similar properties in a fundamental sense A natural taxonomy of microbes, has long been possible. The larger organisms have many easily distinguished features , for e.g., body-plans and developmental processes, that can be used to describe hierarchies of relatedness.

As we know that taxonomy comprises three components such as

Classification or orderly arrangement of units.

Identification, is known by characterization and by their special features

Nomenclature ,is known by their name or their class or units

Classification refers to the arrangements of bacteria into groups or taxa (sing, taxon) on the basis of their mutual similarity or evolutionary relationships. The bacterial classification was

firstly done by Mueller in 1786 and Ehrenberg in 1838, when very little was known about bacteria. In 1866, the Haeckel classified it as unicellular organism as Protista, while Cohn (1872-75) gives its classification on morphological basis. However, the bacterial classification represent special problems, thus Linnaeus (1735) divide all living beings into two kingdoms, plant and animal. The bacteria has been place in the plant kingdom and designated as *schizomycetes* .The kingdom are divided successively into division, class, order, family, tribe, genus and species. For example the taxonomic position of Escherichia coli is

Division: Protebacteria

Class: Gammaproteobacteria

Order: Enterobacterales

Family: Enterobacteriaceae

Gunus: *Escherichia*

Species: *E. coli.*

Species is the standard taxonomical unit of living organisms with high form of life, a species unit constitutes a stage of evolution, with a characteristic morphology. But, due to absence of fossil remains in bacteria, the evolutionary status of species cannot be established. However, the morphological deference is insufficient for the definition of bacterial species. In spite of these difficulties, the concept of species provides a convenient unit in bacterial taxonomy. As species is a genetic concept, that gives genetic information by comparison of the nucleotide base ratios, which are constant for any one species but may be different for other species.

The bacteria are indentified by many characters as the scientific study of organisms with the ultimate object of characterizing and arranging them in an orderly fashion. Identification represents the practical side of taxonomy, which is the process of determining that a particular isolate belongs to a recognized taxon. Therefore, systematic encompasses disciplines such as morphology, ecology, epidemiology, biochemistry, molecular biology, and physiology of bacteria are needed.

Nomenclature is the discipline concerned with the assignment of names to taxonomic groups as per published rules. The need for applying generally accepted names for bacterial species is self evident. The two kinds of names are given to bacteria. The first is causal or common and second is scientific or international name. The scientific name consists of usually two world, the first being the name of the genus and the second the specific epithet such as *Bacillus subtitles.* The generic name is generally Latin noun. The specific epithet is an adjective or noun and indicates some properties of the species. The generic name always be with a capital letter and the specific epithet with a smaller latter such as *Pseudomonas aeruginosa.* The genus *Xenorhabdus* contains a number of species including *Xenorhabdus nematophilus, Xenorhabdus beddingii, Xenorhabdus bovienii,* and *Xenorhabdus poinarii etc.*

1.3.Polyphasic classification:

The polyphasic classification is based up on the phenotype and genotype character of bacteria. Apart from them, some others identical approach such as genotypic, chemotaxonomic, is also included in the polyphasic classification. However, it is complicated in prokaryotic, phylogenetically old, phototrophic cyanobacteria, which contain very simple unicellular forms up to multicellular types with a differentiated and diversified thallus. Various genotypes are adaptable to various specialized ecosystems. It became possible with the aid of newer molecular techniques, such as the sequencing of ribosomal RNA (rRNA), DNA, and proteins. These techniques have made phylogenetic analysis of prokaryotes practicable. The complete 16S rRNA (is the RNA components of the 3OS submit of prokaryotic ribosome's) gene sequencing and its comparative analysis by phylogenetic trees, DNA-DNA hybridization studies with related organisms, analyses of molecular markers and signature pattern(s), biochemical assays, physiological and morphological tests. Collectively, these genotypic, chemotaxonomic and phenotypic methods for determining taxonomic position of microbes constitute what is known as the 'polyphasic approach' for bacterial systematic.

The rRNA sequence is used to construct phylogenic tree by applying distance-matrix method. The evolutionary distance (ED) is determined by recording the differences in the sequences of two or more organism (s) by software computer analysis. The contraction of

phylogenetic tree after ED measurement is mentioned in Fig.1.1. The difference in ED of two organisms is directly proportional to the total length of branches separating them.

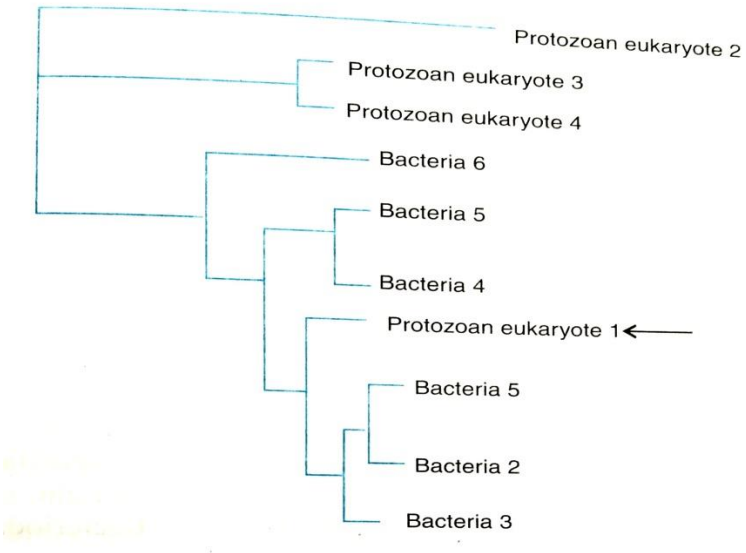


Fig. 1.1: Phylogenetic tree of bacteria

The polyphasic phylogenetic approach is basically most popular for the classification of bacteria and other microbes. This approach is highly useful when rapid development in molecular biological techniques are occurs. However, several DNA-based methods are available that provide information for delineating bacteria into different genera and species, and have the potential to resolve differences among the strains of a species. In the future, polyphasic taxonomy will have to cope with (i) enormous amounts of data, (ii) large numbers of strains, and (iii) data fusion (data aggregation), which will demand efficient and centralized data storage. Therefore, newly isolated strains must be classified on the basis of the polyphasic approach. Thus, current techniques enable microbiologists to decipher the natural phylogenetic relationships between microbes.

1.4.General characteristics of primary domains

The classification of living organism has been carried by E. Haekel (1866) in three groups as plant, animal and Protista. In which Protista is considered as primitive organism. After long time ,Sedillot has used word microbe. Later on, the microbes are classified is into different classes, based on the five kingdom, eight kingdom and three domen classification system. In 1970, Carl

Woese has noted that bacteria are distant from animals, therefore, they established a new concept of domains over the kingdom and he proposed three domains, Bactria, Archaea and Eukarya, is based on ribosomal RNA (rRNA) sequences which is widely accepted by scientists, The three domains are

1. Bacteria
2. Archaea
3. Eukarya

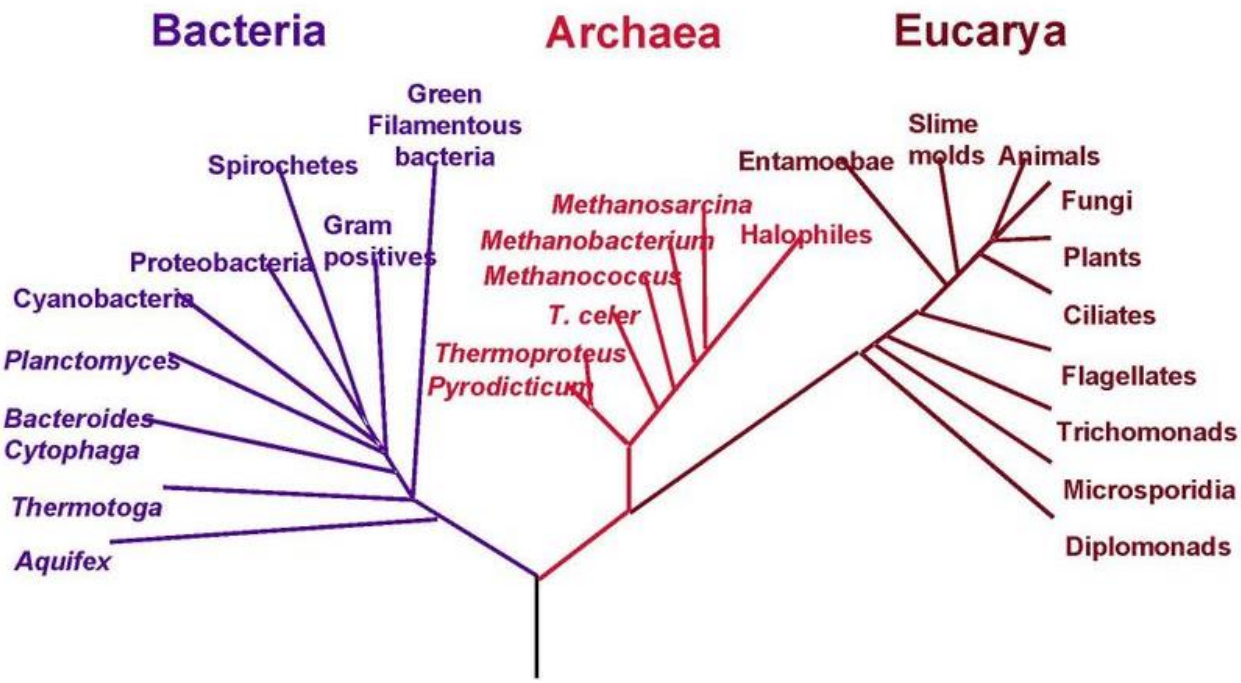


Fig.1.1: Demonstration of phylogenetic tree of life.

The Bacteria (Eubacteria)

Eubacteria are prokaryotic microorganisms consisting of a single cell lacking a true nucleus and containing DNA as a single circular chromosome. Eubacteria, or "true" bacteria, are single-celled prokaryotic microorganisms that have a range of characteristics and are found in various environmental conditions throughout all parts of the world. All types of bacteria fall under this title, except for archaebacteria. Bacteria are sensitive to traditional antibacterial antibiotics but are resistant to most antibiotics that affect Eukarya. It also contains rRNA that is unique to the Bacterial cell. The examples of bactreria are mycoplasmas, cyanobacteria, Gram-positive bacteria, and Gram-negative bacteria. The structures found in eubacterial cells are either external

or internal to the cell wall. Structures external to the cell wall may be flagella, fimbriae, axial filaments, glycocalyx, or pili. Each of these structures has its distinctive function where some eubacteria have flagella to facilitate their movement. Glycocalyx surrounds some eubacterial cells. It is a viscous polymer composed of polypeptides or polysaccharides and functions to protect the bacteria. Structures internal to the cell wall include cell membrane, cytoplasm, DNA, plasmid, and ribosomes. The example of Eubacteria is E. *coli*, *Lactobacilli,* and *Azospirillum.*

The Eubacteria has following characteristics such as

- **i.** The bacteria are unicellular microorganisms of a prokaryotic cell.
- **ii.** They contain DNA as a circular chromosome.
- **iii.** Its cell wall is composed of peptidoglycan.
- **iv.** They greatly differ in terms of morphology and physiology.
- **v.** Their cells lack true nucleus and membrane bound cell organelles which are present in eukaryotic cells.
- **vi.** Their DNA is not inside a nucleus and present in cytoplasm

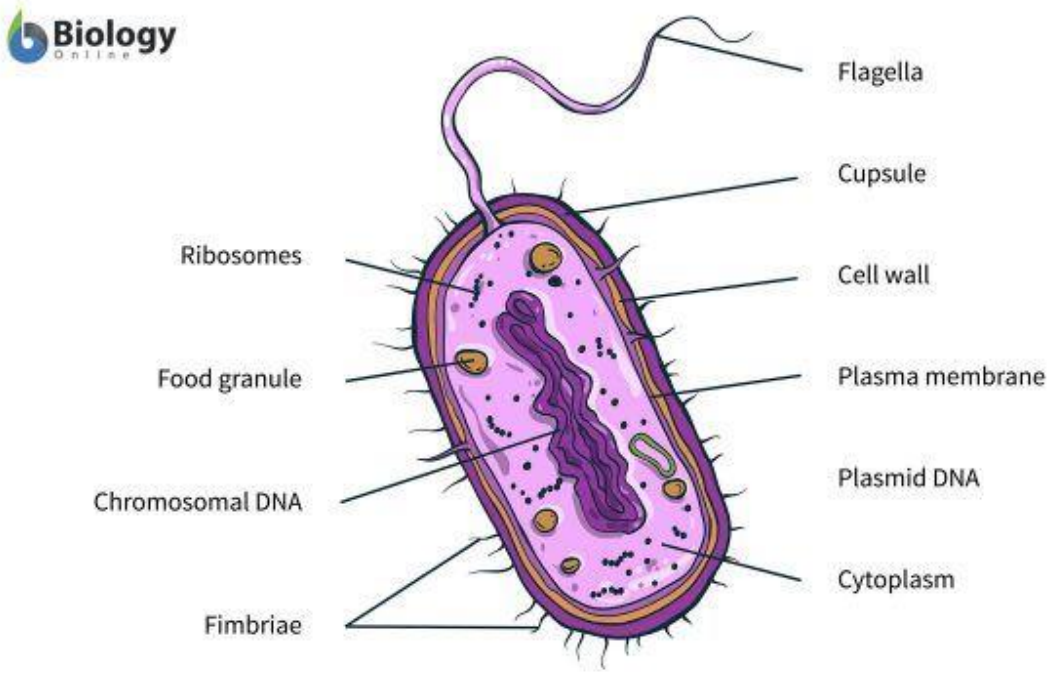


Fig.1.3: Structure of Eubacteria

The Archaea (archaebacteria)

The Archaea are primitive, single-celled microorganisms that are prokaryotes i.e.with out true nucleus.It is also known as early nucleus or primitive nucleus. Archaebacteria are a group of microorganisms considered to be an ancient form of life that evolved separately from the bacteria and blue-green algae, and they are sometimes classified as a kingdom. The archaea are prokaryotic cells which have membrane composed of branched chains hydrocarbon and does not contain peptidoglycan. Archaea are not sensitive to some antibiotics that affect the Bacteria, but are sensitive to some antibiotics that affect the Eukarya. Archaea also contain rRNA. Archaea often live in extreme environmental condition and include methanogens, extreme halophiles, and hyperthermophiles. One type of archaebacteria is crenarchaeota, which can live in extreme temperatures or acidity. Another type of archaebacteria is euryarchaeota, which include ones who produce methane or live in water with high salt content.

The common characteristics of Archaebacteria known up to date are:

i. The presence of characteristic tRNAs and ribosomal RNAs.
ii. The absence of peptidoglycan cell walls, with in many cases, replacement by a largely proteinaceous coat;
iii. The occurrence of ether linked lipids built from phytanyl chains and
iv. in all cases known so far, their occurrence only in unusual habitats.

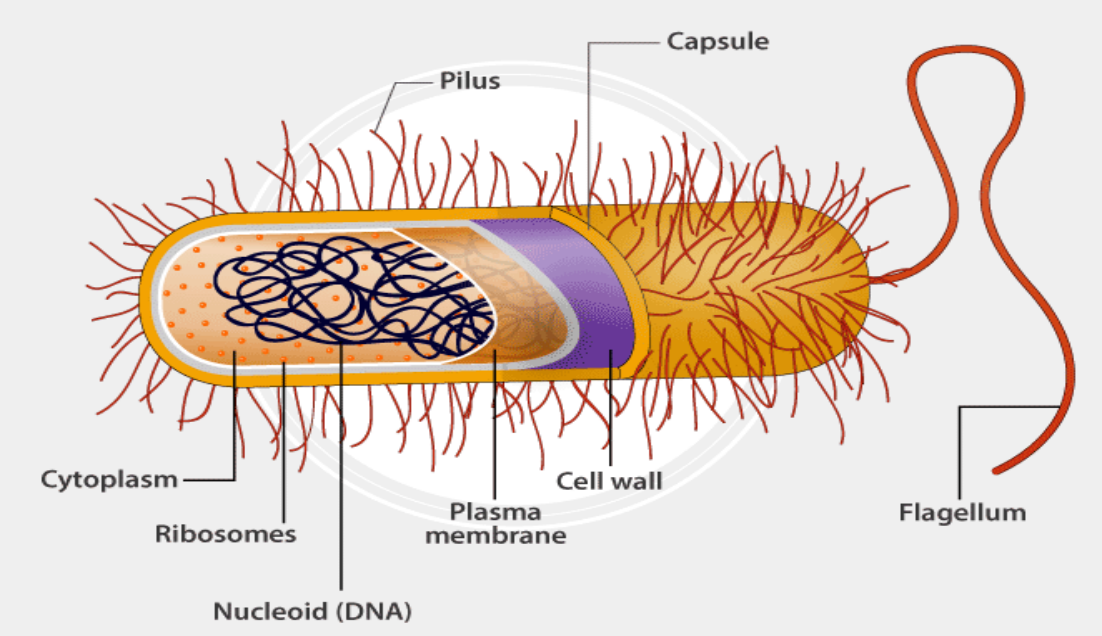


Fig.1.2: Morphology of archaebacteria

The *Eukarya* (eukaryotes)

Eukaryote, any cell or organism that possesses a clearly defined true nucleolus. In eukaryotes, the cell's genetic material or DNA, is contained within an organelle called the nucleus, where it is organized in long molecules called chromosomes. Eukaryotic cells also contain other membrane bound cell organelles, including mitochondria, which generate energy. Eukarya are resistant to traditional antibacterial antibiotics but are sensitive to most antibiotics that affect eukaryotic cells. Eukarya contain rRNA that is unique to the Eukarya as indicated by the presence molecular regions distinctly different from the rRNA of Archaea and Bacteria. There is a wide range of eukaryotic organisms, including all animals, plants, fungi, and protists, as well as most algae. Eukaryotes may be either single-celled or multicellular organisms. The cells of eukaryotes divide by mitosis (in vegetative cells) and meiosis (in reproductive cells). While, mitosis gives rise to two *daughter cells*, similar to parent cell and meiosis gives rise to four daughter cells. The cells from meiosis will be haploid after two consecutive divisions. The single cell is an entire organism , capable of performing all the fundamental functions (e.g. ingestion, respiration, excretion, osmoregulation, homeostasis, etc.) in unicellular organism .However,these are performed by different systems in a multicellular organism.

The *Eukarya* are subdivided into the following four kingdoms:

- **Protista Kingdom:** Examples includes slime molds, euglenoids, algae, and protozoans.
- **Fungi Kingdom:** Examples include sac fungi, club fungi, yeasts, and molds.
- **Plantae Kingdom:** Examples include mosses, ferns, conifers, and flowering plants
- **Animalia Kingdom:** Examples include sponges, worms, insects, and vertebrates.

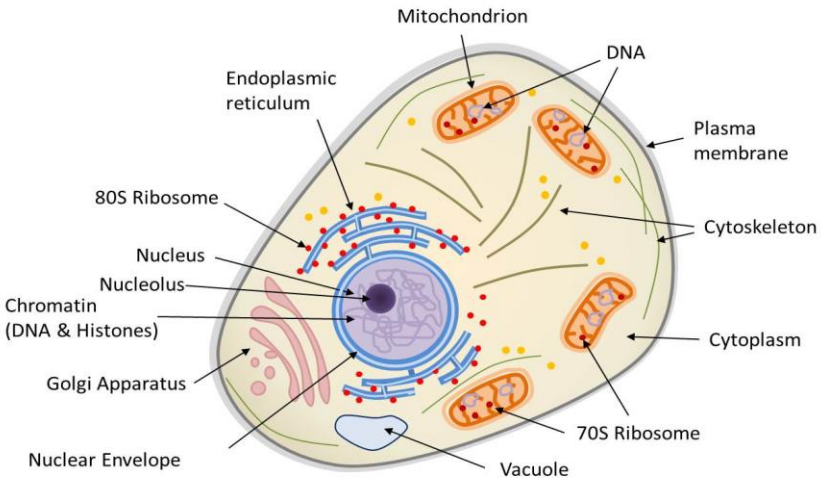


Fig.1.4: Cellular structure of eukaryotes

In modern sense, bacteria, cynobacteria, Actinomycetes are distributed in the modern domain bacteria. The methanogens, extremely themophilic organisms, extremely halophilic orgmsim etc are the domain Archaea, and molds, yeast, basidiomycetes, algae and protozoa etc in the domain of Eukarya.

1.5. Primary domains and taxonomic groups

Taxonomy refers to the arrangement of biological organism on the basis of their mutual similarities into units called taxa (singular taxon). The taxonomic unit, taxon, may have different levels depending on the extent of similarities among the organisms included in it. Each level or rank has a different designation and these ranks form a hierarchical arrangement. The taxonomy, described as completely as possible the basic taxonomic units and also to devise an appropriate way of arranging and cataloguing these units. These individual of organisms that follow the degree of phenotype similarity are assemblage. Every assemblage of individuals shows some degree of internal phenotypic diversity because of genetic variation.

A natural classification should have good predictive value (information content). In contrast, a special or artificial classification yields particular information to the specialized user. If we accept this distinction, it is clear that the phenetic classification would allow the most general predictive properties, whereas the phyletic system would offer information that is primarily of use to evolution, i.e., it is a special classification.

The basic taxonomic unit of biological organisms is a species. In case of bacteria, a species is defined as a collection of strains which resemble each other in many characteristics and differ significantly from other collections of strain. A colony ideally is formed from a single cell or spore growing on an agar medium. So, a bacterial species, according to the above definition, comprises a number of strains which are closely similar, differing in one or a few characteristics.

A taxonomic character is any attribute of a member of a taxon by which it differs or may differ from a member of a different taxon. A characteristic by which members of two taxa agree but differ from members of a third taxon is a taxonomic character.

1.5.1. Classical Taxonomy:

The characterization of any organism in done on the bases of stable character. This depends on the nature of taxa. Such as bacteria has been classified on the basis of similarity of their phenotypic characteristics like morphological features, response to Gram stain, cultural characteristics, physiological and biochemical properties, pathogenicity, antibiotic sensitivity, serological relationships etc. Taxonomically important morphological, cultural and physiological- biochemical characteristics are shown in Table 3.1, 3.2 and 3.3 respectively.

Table 3.1 : Morphological characteristics of taxonomic value	
Characteristics	**Variations**
Cell morphology	
Unicellular	Cocci, bacilli, vibrios, spirilli, spirochaetes, prosthecate, stalked, sheathed.
Multicellular	Mycelial, filamentous.
Cell arrangement	Single, pairs, chains, bunches, packets.
Staining property	
Gram staining	Gram-positive, Gram-negative.
Acid fast staining	Acid-fast, non-acid fast.
Flagellation	Monotrichous, lophotrichous, amphitrichous, peritrichous endoflagellate or non-flagellate.
Motility	Non-motile, flagellar locomotion, gliding movement, motility due to endoflagella.
Glycocalyx	Capsule present or absent, slime layer.
Spores	Non-sporing, endospore, exospore, conidia, myxospores.
Sporangium	Shape, location of spore.
Cell inclusions	Poly β-hydroxybutyrate, volutin, polysaccharides, sulfur droplets, parasporal protein crystals.
Ultra-structural features	Surface structures of cells, — flagella, pili, fimbriae, texture of slime layer.

Source: Taxonomy: Definition, Objectives and Characteristics (biologydiscussion.com)

Table 3.2 : Cultural characteristics used in bacterial classification	
Characters	**Variations**
Oxygen relationship	Aerobic, microaerophilic, facultatively anaerobic, obligately anaerobic.
Temperature relationship	Psychrophilic, mesophilic, facultatively thermophilic, obligately thermophilic, hyperthermophilic
pH relationship	Optimum pH in medium range, acidophilic, alkalophilic.
Culture medium composition and ability to grow	Inorganic salts medium with and without an organic carbon source; medium without combined nitrogen; medium containing complex organic compounds, like beef-extract, yeast-extract, etc.
Colony morphology on agar media	Colony discrete or spreading; surface texture, colour, elevation; margin; colony reverse (underside); soluble and insoluble pigments, colour of the pigment.

Source: Taxonomy: Definition, Objectives and Characteristics (biologydiscussion.com)

Table: 3.3: physiological and biochemical characteristics of bacteria

	Escherichia coli DH5α Inactive	*Enterobacter aerogenes*	*Citrobacter freundii*	*Proteus mirabilis*	*Serratia marcescens*
Risk Group	2	2	2	2	2
Cell length (µm)	2-6	1.2-3.0	1-5	2-4	5-30
Gram Stain	-	-	-	-	-
Cell shape & arrangement	Straight bacillus/rods: single or pairs	Rod shaped w/ round ends	Long rod-shaped	Rod-shaped	Short and rod-shaped
Colonial characteristics (pigment colour)	Smooth convex grey moist or rough flat dry dull wrinkled	Smooth, irregularly round, yellow pigmentation	Smooth, convex, uniform, transparent, shiny	Colourless	Round, elevation-convex and slightly umbonate, margin-entire, yellowish orange, opaque
Preferred Temperature Range (°C)	21-37	20-37	30-37	37	36
Oxygen requirement (FTM)	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe	Facultative anaerobe
Catalase	+	+	+	+	+
***Glucose fermentation (24 hr KIA)**	A	Both A and G	Both A and G	A	A
Lactose fermentation (24 hr KIA)	-	+	+	-	-
H_2S (KIA or SIM)	-	+	+	+	-
Methyl Red Test	+	-	+	+	-
Voges-Proskauer	-	+	-	-	+
Citrate Test	-	+	+	+	+
Indole Test	+	-	+	-	-
Gelatin hydrolysis	-	-	-	-	+
Motility (SIM)	V	Motile	V	Motile	Motile

1.5.2. Molecular Taxonomy:

The modern characterization techniques reveal the molecular structure of organism in the twentieth century, those brigs drastic changes in taxonomic arrangement of microbial world. This approach reveals the similarity or dissimilarity of macromolecules such as protein, carbohydrate and nucleic acids that could be used as an indicator of evolution of living organisms was first suggested by Zuckerkandl and Pauling in 1965. This new approach has given rise to the molecular taxonomy. Although, initially amino acid sequencing of proteins was used as a parameter for determination of phylogenetic relations, nucleic acids soon replaced proteins. Among the characteristics of nucleic acids, DNA base composition, DNA homology, DNA sequencing, r-RNA sequence analysis etc. have been used for solving taxonomic problems. The

first characteristic that was applied in solving taxonomic problems was the base composition of DNA. A unique feature of DNA is that the ratio of (G + C): (A + T) is more or less constant for a biological species. In bacteria this value varies from about 25% to 80%.

The Nucleic acid hybridization has also been used for solving of taxonomical problem. The most similar genomes of two organisms can be obtained by DNA-DNA hybridization that gives heteroduplex between two single stranded DNA molecules, derived from two organisms depends on the degree of complementarity of the two single strands. Various methods have been developed for quantitative determination of heteroduplex formation. It should be noted that for determining relationships among distantly related organisms, DNA-DNA hybridization, cannot give any positive information, because DNAs of such organisms do not possess enough base-pair complementarity to allow heteroduplex formation. Only information that may be obtained is that the organisms concerned are not related to each other. From taxonomic point of view, such information is not of much value.

Ribosomal RNA homology, which discover in 1965, reveal in organism the DNA segments transcribing ribosomal RNA (r-cistrons or r-DNA) have changed more slowly in course of evolution than the rest of the genome. This provided an instrument for comparing the phylogenetic relationships between distantly related organisms through determination of base sequences of r-RNA or r-DNA. Ribosomal RNA of most of the major taxonomic group has been found to possess one or more unique sequences, which are known as their oligonucleotide signature. One of the major impacts of r-RNA studies on taxonomy is the recognition of three major domains- the Archaea, the Eucarya including all eukaryotes, and the Bacteria. It has been claimed by Woese, Kandler and Wheelis (1990) that the three major evolutionary lines diverged from a common ancestral form.

1.5.3. Taxonomic groups

Taxonomy is the branch of biology that classifies all living organism. It was developed by the Swedish botanist Carolus Linnaeus, who lived during the 18th Century, and his system of classification,known as binomial nomenclature system, is still used today. Taxonomic ranks are always capitalized, except for species. There are eight general taxonomic groupings, starting with the most general and ending at the most specific. These groupings are:

Domain: A domain is the highest (most general) rank of organisms. The three domains of life form are Bacteria, Archaea, and Eukaryota.

Kingdom: kingdom is the second highest taxonomic rank. In the past, the different kingdoms were Animalia, Plantae, Fungi, Protista, Archaea, and Bacteria

Phylum (or Division for plants): Phylum (plural: phyla) is the next rank after kingdom

Class: The class is a group of related orders. For example order Primata, comprising monkeys, gorillas and gibbons are placed in Class Mammalia along with order Carnivora which includes animals like tiger, cat, and dog all having a common feature that is hair on skin and milk glands.

Order: Order is more specific than class. There are between 19-26 orders of Mammalia, depending on how organisms are classified

Family: Family is, in turn, more specific. Some families in the order Carnivora

Genus: Genus (plural: genera) is even more specific than family. It is the first part of an organism's scientific name using binomial nomenclature; the second part is the species name. Genus and species are the only taxonomic ranks that are italicized

Species: it is the most specific taxonomic rank some time divide in to subspecies. The species name is always italicized, but never capitalized. It is the only taxonomic rank that is not capitalized. It consists of individuals which have fundamental similarities and can be distinguished from other closely related species due to distinct morphological characters.

Importance of taxonomy

- Permits the organization to organize huge amounts of information about organism
- Allows predictions and hypotheses to be made upon this information
- Places organisms in useful groups with precise names that permit effective communication between investigators
- Essential for the identification of organisms

Numerical Taxonomy

- The branch of taxonomy that uses mathematical methods to evaluate observable differences and similarities between taxonomic groups.
- It aims to create a taxonomy using numeric algorithms like cluster rather than using subjective evaluation of their properties.
- The concept was first developed by Robert R. Sokal & Peter H. A. Sneath in 1963 but first it was approached by Adanson and hence it is called as Adansonian Taxonomy.
- All characteristics are given equal weight (either 0 , 1 or +, -) and a computer based analysis is carried out to group the bacteria according to shared properties.

The numeric taxonomy is used for data collections, data coding, calculation of similarity and dissimilarity matrix based, on computer analysis. It does also utilize formation of dendrogram.

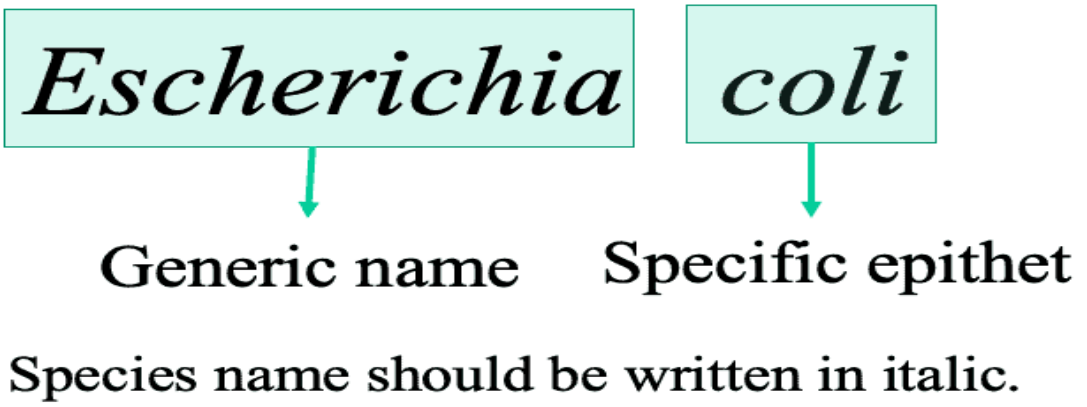
1.6. Nomenclature

The bacteria are microscopic organism so that its classification is quite difficult, but to make easy of bacterial study, it necessary to classify it properly. Previously, it is categories by using old staining techniques, such as Gram's staining for differentiation of bacteria on the basis of cell wall structures, their morphology, and biochemical characters. Nowadays, the use of advamce modern characterization techniques, such as restriction fragment length polymorphism (RELP), DNA-DNA hybridization and 16S rRNA sequencing make the bacterial studies easier. These techniques create interest in bacterial taxonomy in modern time.

Bacteria are scientifically recognized, using a binomial nomenclature system using two words that refer to the genus and the species. The names assigned to microorganisms are in Latin. The first letter of the genus name is always capitalized. The name should be internationally accepted.

However, first nomenclature was based on the disease caused. But letter on this system of nomenclature was discarded due to reason that some bacteria cause several different diseases. For example *Escherichia* coli, caused diarrhea, hemorrhagic colitis, dysentery like illness similar to *Shigella* infection. After Carl Linnaeus, who gave classification system, which was already accepted to assign both plants and animals. According to this, order of bacteria divided into

family and in each family, there are number of genera, each genera comprise with one or more species. This nomenclature is based on genus and species system called binary system of nomenclature, where genus name should be write always in capitalized, often to just the first letter is in capitalized letter and species name is written with a lower initial letter. For example



* Species name should be written in italic.

Other, sometime the common genus name uses as abbreviate, in which the first few letters are written for example Staphylococcus aureus abbreviated as Staph aureus, Staphylococcus *pyrogenes* abbreviated as Staph *pyrogenes* etc. Currently the bacterial nomenclature is regulated by a committee of systematic nomenclature. List of approved names are published in Internal Journal of Systematic and Evolutionary Microbiology.

Generally the name are organized into a following hierarchical system such as

Phylum: a group into which animals, plants, etc. are divided

Class: The type of a class is one of the orders. The class is named after the type genus of the type order of the class.

Order: The type of an order is one of the genera. The order name is named after the type genus of the order. E.g. The order Pseudomonadales is named after the type genus Pseudomonas.

Family: In general, the family name is named after the type genus of the family, e.g. The family Pseudomonadaceae is named after the type genus Pseudomonas.

Genus: A genus is a taxonomic rank used in the biological classification of living and fossil organisms

Species: A group of organism that are all the same and that can breed together.

Subspecies: Subspecies are created only when it is necessary.

For example:

Rank	Suffix	Example
Order	-ales	*Pseudomonadales*
Suborder	-ineae	*Pseudomonadineae*
Family	-aceae	*Pseudomonadaceae*
Genus		*Pseudomonas*
Species		*Pseudomonas aerugiosa*

1.7. Summary

The term "*taxonomy*" was developed from two Greek words, "*taxis*," meaning arrangement, and "*nomia*," meaning distribution or method. The scientific definition of taxonomy is that it involves in the classification of organisms ,both alive and extinct. Also, it includes the naming and arranging of organisms in higher groups. Taxonomy involves seven different types of processes: description, naming, recognition, comparison, and classification of taxa, genetic variation, identifying specimens, and defining taxa in the ecosystem. Nomenclature is the discipline concerned with the assignment of names to taxonomic groups as per published rules. The need for applying generally accepted names for bacterial species is self evident. Nucleic acids sequence comparisons and structural and biochemical comparisons consistently categorize all living organisms into 3 primary domains: Bacteria, Archaea, and Eukarya (also called Eukaryotes; these terms can be used interchangeably). The bergey's manual of systematic bacteriology has four volumes that contain the internally recognized names and descriptions of bacterial species. Denaturing gradient gel electrophoresis (DGGE) is a commonly used molecular technique for rapid fingerprint analysis of microbial community composition, diversity, and dynamics. The method is rapid and affordable, allowing multiple samples to be processed simultaneously.

1.8. Terminal questions

Q.1: What do you understand by modern approach of taxonomy? Discuss briefly.

Answer:----------

Q.2: What do you mean by nomenclature, discuss the role of primary domain in nomenclature?

Answer:----------

Q.3: What do you understand by Polyphasic classification?

Answer:----------

Q.4:What are microorganisms? Discribe in short.

Answer:----------

1.9. Further suggested readings

1. Bergey's Manual of Systematic Bacteriology- David Hendricks Bergey,1923
2. Manures, Fertilizers And Pesticides- Amiitava Rakshit, Priyankar Raha And Nirmal DeFertilizers A Text Book- Ranjan Kumar Basak
3. R.C. Dubey and D.K. Maaheshwari, A Textbook of Microbiology, S. Chand Publication-2013.
4. Ian L. Pepper, Charles P. Gerba, Terry J. Gentry, A Microbiology, Academic Press-2015.
5. K Vijaya Ramesh , Environmental Microbiology, MJP Publication-2019.
6. P.K. Mahapathra, A Textbook of Environmental Microbiology, I K International Publishing House Pvt. Ltd-213.
7. Barbara Kołwzan et al., Introduction To Environmental Microbiology, academic teachers from Wroclaw University of Technology, Poland

Unit-2: Culture Media and Nutrition

2.1. Introduction

The diversity refers the variability among the individuals of microbes. It is found in all kinds of microbes. The main key of microbial diversity on the earth is due to evolution. The new species of microbes evolved through the interaction of their genomes within the environment. Metabolic diversity is used as a physiological or ecological concept that referring to the metabolic repertoire available to any group of organisms. However, microbes are present on the earth about 3.5 to 4 billion years ago and involve in creating to new environment. They replicating quickly, exchanging genetic material with each other and with other organisms, bacteria and archaea have become ubiquitous. Due to its manipulation in the environments in which they live, they develop new ecosystems. The microbial diversity play important role in the recycling of essential nutrients on Earth. Microorganisms vary with regard to the sources of energy which they use for assembling macromolecules and other cellular components from smaller molecules. Phototrophs obtain their energy from light; chemotropism use chemicals as energy sources. Many organisms use organic compounds as sources of energy, these are known as the chemoorganotrophs. In contrast, the chemolithotrophs use inorganic chemicals as energy sources. Microbial diversity describes the number of different species of microbes present and

their distribution. The different kinds of microbes are distinguished by their differing characteristics**.**

Objectives:

- to study different types of culture media
- to discuss different types of microbial nutrition
- also discuss the mode of nutrition in bacteria

2.2. Culture Media Preparation

Culture medium has nutrient or combination of nutrients, and it is used for the growth and multiplication of microorganisms. The aim of culture medium is to provide a balanced mixture of the required nutrients that will permit good growth of the microorganism. Microbiological culture media (sign. Medium), however, consists of various nutrient substances like inorganic and organic compounds. The media may be grouped into (1) those media that requires living cells or tissues and (2) those media that do not require living cells or tissues. This media is again divided into following two categories:

(a) Non synthetic or complex media

(b) Synthetic media or defined media

(a) Non synthetic or complex media: In this medium, exact chemical composition of each of the constituents is not known with certainly. Potato-Dextrose Agar, Soil Extract Agar, Oatmeal Agar, Malt Extract Agar and Waksman's medium are some of the most widely used non-synthetic media. The undefined chemical composition medium is used to grow either *Escherichia coli or Leuconostoc mesenteroides:* The composition of the medium is given below:

Glucose ……. 15g

Yeast extract…..……. 5g

Peptone……..……. 5g

KH_2PO_4 ……. 2g

Distilled water..……. 1,000 ml

pH ……...……. 7

Non-synthetic media often has digests of casein (milk protein), soybeans, beef extract, yeast cells or any of a member of highly nutritious but chemically undefined substances. Such digests are available commercially in powdered form. They can be weighed and dissolved in distilled water to prepare a medium.

(b) Synthetic media or defined media: In this medium only pure chemical are used in definite concentrations. Due to known chemical composition, these media are useful for nutritional and metabolic studies. Czapek's Dox medium (GM-9) and Richard's solution (GM-27) are the most widely used synthetic media. A defined medium used for *Escherichia coli* is a follows:

K_2HPO_4 ……. 7g

KH_2PO_4 ……. 2g

$(NH_4)_2SO_4$ ……..…. 1g

$MgSO_4$……..0.1 g

$CaCl_2$…….0.02 g

Glucose ……. 4-10g

Trace elements (Fe, Co, Mn, Zn, Cu, Ni, Mo)…….2-10 μm each

Distilled water..……. 1,000 ml

pH ……..……. 7

On the basis of their physical state ,media may be divided into following categories :

(a) Liquid media

(b) Semisolid media

(c) Liquefiable solid media

(d) Solid media

(a) **Liquid media.** These media are used in liquid form e.g. Nutrient broth, Skimmed milk, Peptone solution, etc.

(b) **Semisolid media.** These media contain a smaller amount (0.5% or less) of agar which imparts a "custard consistency", e.g. Cystine trypticase medium.

(c) **Liquefiable solid media.** It is also called 'solid reversible to liquid medium'. These media are prepared by adding suitable amount of gelatin or agar to the liquid medium. This medium remains solid when cooled and became liquid when warmed or vice-versa, e.g. Nutrient agar medium, Nutrient-gelatin medium, Potato dextrose agar medium.

(d) **Solid media.** It always remains solid, e.g. Potato slices (used for special cultivation of bacteria), Coagulated blood serum, Coagulated egg and trypticase-soy-agar medium.

Bacteriological Media:

The following media are used for the growth of bacterial microorganism:

(a) Cultivation Media

(b) Storage media

(c) Enrichment media

(d) Differential media

(e) Assay media

(f) Maintenance media

(a) **Cultivation Media:** This medium is used for the general cultivation of bacteria,e.g. Nutrient broth, Nutrient agar, etc.

(b) **Storage media:** In storage media, bacteria are stored in "stock culture" condition for longer periods to provide a source of viable culture, e.g. Yeast extract mannitol agar medium.

(c) **Enrichment media.** The medium in which nutritional environment is adjusted in such a way so that the growth of a certain type of bacteria in isolation and identification of pure cultures from an initially mixed population of bacteria, e.g. Addition of extracts of plant or animal tissues to nutrient broth or nutrient agar media provides additional nutrients which favors the growth of fastidious heterotrophic bacteria.

(d) **Differential media:** This medium is used to determine differential reactions which allow presumptive identification of bacterial species. Blood agar medium is a good differential medium. If a mixture of bacteria is inoculated on a blood agar medium, some of the bacteria may hemolyze the red blood cells, while others do not show hemolytic reactions. Thus a clear zone of hemolyzed red blood cells around certain colonies of bacteria is seen.

(e) **Assay media:** This media is of prescribed composition and have profound influence on the bacterial cells with respect to formation of enzymes, toxins, antibiotics and other products. Such media are also called media for special purposes, for example, Pyridoxine deficient growth medium for *streptococcus faecalis* which yields cells containing large amounts of tyrosine decarboxylase apoenzyme.

(f) **Maintenance media:** These media are different from growth media and are required to maintain the viability and physiological characteristics of bacteria.

Some Microbiological Media:

I. Media for bacteria :

(a) **Nutrient Broth (**or **Beef-extract Peptone Broth).** This liquid medium is widely used for cultivation of aerobic bacteria and as a basal medium for a variety of physiological tests.

The composition of the medium is given below:

Beef-extract …….. 3g

Peptone…… ……..... 5g

Agar…….. …….. 15g

Distilled water..…….. 1,000 ml

The water containing beef extract and peptone is heated to 60^0C to promote solubilizaton of the ingredients. It is now cooled and the pH is adjusted (6.8-7.2), and is dispensed in tubes or other containers, and is autoclaved at 15 lb pressure (at 121^0C temperature) for 15 minutes.

(b) **Nutrient Agar or Beef extract Agar:** This medium is used for the growth of many heterophic bacteria.

The composition of the medium is given below:

Beef-extract …….. 3g

Peptone…… ……..... 5g

Agar…….. …….. 15g

Distilled water..…….. 1,000 ml

The above ingredients are added in water and heated to 60^0C to promote solubilization of the ingredients. It is now cooled and the pH is adjusted to 6.8-7.2. The medium is dispensed in tubes or other containers and autoclaved at 15 lb pressure at 121^0C temperature for 15 minutes.

(c) **Trypticase Soya Agar:** This is solid medium for a general purpose. It favors better growth of more bacteria than does nutrient agar medium.

The composition of the medium is given below:

Trypticase (peptone)……15g

Phytone (peptone)…… ……... 05g

Sodium chloride……05g

Agar…….. …….. 15g

Distilled water..…….. 1,000 ml

pH……7.0±

(d) **Sodium Caseinate Agar:** This medium is often used for the enumeration of bacteria is soil.

The composition of the medium is given below:

Sodium Casenate……01g

Glucose ……..……. 01g

$MgSO_4$…….0.2 g

K_2HPO_4 ……. 0.2g

FeSO4…….trace

Agar…….. …….. 15g

Distilled water..…….. 1,000 ml

pH……7.0±

II. General purpose media for cyanobacteria (blue green algae): This medium is generally used for culture of cyanobacteria. If 0.20g of potassium nitrate is added, the medium supports the growth of many non-nitrogen fixing cyanobacteria.

The composition of the medium is given below:

Magnesium Sulphate ($MgSO_4$, $7H_2O$)…… 0.025g

Calcium Chloride…….…… 0.05g

Sodium chloride…… 0.20g

Dipotassium hydrogen phosphate……..…… 0.35g

A_5 trace elements stock solution…… 1.0ml

Glass-distilled water……..…… 1,000ml

III. General purpose media for fungi

(a) Potato- Dextrose Agar Medium. This medium is widely used for the culture of both yeasts and molds.

The composition of the medium is given below:

Potato (peeled and sliced)…..…….. 15g

Dextrose…...…….. 1,000 ml

Agar…….. …….. 15g

Distilled water..…….. 1,000 ml

pH……5.6±

(b) Malt-Agar medium. This medium is used for the culture of yeasts and molds.

The composition of the medium is given below:

Malt extract ……30g

Agar…….. …….. 15g

Distilled water..…….. 1,000 ml

pH……5.5±

(c) Czapek-Dox Agar medium. This medium has been used for culture of saprophytic fungi.

The composition of the medium is given below:

Sucrose ……30g

Sodium nitrate……..…...3g

Dipotassium phosphate……..1g

Magnesium Sulphate……..….0.5g

Potassium chloride……..0.5g

Ferrous sulphate…….0.01g

Agar…….. …….. 15g

Distilled water..…….. 1,000 ml

pH……7.3±

3.3.Types of Microbial Culture media

Bacteria identified by the bacterium population rather than the single bacterial cell. It is very difficult to recognize their morphology alone. If we study, the human or animal cell or any other natural media, the mixed population of bacteria are seen there. So for the study of bacterial cell and their morphology, it should be used to appropriate procedure for separately growth (isolated) on culture media and obtained pure culture for study.

Numerous culture media has been in practice for development of different bacteria. These media has different composition of nutrients as per requirement of desire commonsial.

First, the original media used by Louis Paster were liquid such as urine or meat broth. However, liquid has many disadvantage because, the liquid media support mixed bacterial population growth. Which create difficulty to isolate different types of bacteria form mixed population. Thus it does not support isolated study of bacterial cell. But in some cases, the liquid media is very useful, such as during identification of bacteria growth form blood or water, when large volume have to be tested.

Bacteria in liquid media get diffuse, procedure discrete visible growth on solid media, if inoculated in suitable dilution, bacteria forms colonies which are made up of cells originating from single bacterium cell. While , solid media produce distinct colony of morphology along with other characteristics features.

Agar which is obtained from some types of seaweeds generally universally used to prepare solid media. Agar has long chain polypeptide as chief constituents and also contain some inorganic and some small quantity of protein like substance. Agar is hydrolyzed at high temperature, high pressure and high acidic pH . Agar get melt at 98 °C and usually set at 42 °C is employed for solid media.

Peptone is also one of the universal ingredients of common solid media. Peptone constituents are mainly proteases, polypeptides, amino acid and partial amount of digested protein. Apart from this, variety of inorganic salt, including phosphate, potassium and magnesium are also found. As per requirement, the media have been classified in several ways

1. Solid media, liquid media, semisolid media
2. Simple media, complex media, synthetic and define media, specific media.
3. Aerobic media and anaerobic media

Solid media

It is also known as basal media, which support the growth of most of non-fastidious bacteria. It consists of protein, water, meat extract, sodium chloride and nutrient agar (NA). Simple media are generally used for primary isolation of microorganism. The concentration of nutrition agar play significance role in basal media as solidifying agent. If concentration of agar is reduced to 0.2-05 % than semi solid or sloppy agar is obtained, while concentration of agar increases upto 6 % than solid medium is obtaind, it presents spreading or swarming by organism such as proteus microbils a gram negative bacterium, causes urinary tract infection (UTI).

Enriched media

In this media, substances like blood, serum, egg yolk etc added to basal media. Enriched media are used to grow nutritionally extracting bacteria, Example of enriched media are blood agar, chocolate agar, woffer's serum slope etc. Chocolate agar is known as heated blood agar or lysed blood agar.

Selective media

This media prevent the growth of unwanted commnensal or containing bacteria and help to recover pathogen form a mixture of bacteria. In this media, the inhabitation substance is added

in solid media, which enables a greater number of required bacterium to form colonies than another bacteria. For example deoxycolate citrate medium for dysentery bacteria. Any agar media can be made selective by the addition of certain inhibitory substance that doesn't affect the pathogen of interest.

Indicator media

Certain media recognized such a way, those different bacteria can be recognized on the basis of their colony, color for that some substance are used in media to change the color, when bacteria grown in them. Such media is called differential media or indicator media. For example, in incorporation of sulphite in Wilson and Blair medium, *Salmonella typhi* reduce sulphite to sulphide in the presence of glucose and the colonies of S. thyphi have a black metallic seen.

Synthetic or defined media

Those media which are prepared only by pure known chemical is known as synthetic media. The synthetic media used for special study such as metabolic requirements, there is yeast ,plant or animal tissue present. Example- Dubos culture medium with tween 80.

Sugar media

The sugar media contain following sugar

Monosaccharide's

Disaccharides

Polysaccharides

Trisachhrides

Alcohol

Glycosides

Non-carbohydrates

Usually sugar media consists of 1% of the sugar in peptone water along with a appropriate indicator.

Transport Media

The transport media use for transport of newly commnensal, such as media prevent drying of a specimen, mention the pathogen to commnensal ratio, also inhibit the growth of unwanted bacteria, example, Stuarts medium – a non-nutrient soft agar gel contain a reduce agent to prevent oxidating and charcoal to neutralize certain bacteria including for gonococci.

Anaerobic media

Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation reduction potential and extract nutrients. Media for anaerobic bacteria may have to be supplemented with nutrient like hermin and vitamin K, such media may also have to be reduced by physical or chemical means. Boiling of the medium causes to expel any dissolve oxygen. Adding 1% glucose, 0.1% thioglycollate, 0.1 ascorbic acid, 0.005% cystic or red hot iron filing can render a medium reduced. Examples- thioglycollate broth and cooked meat broth.

2.4. Principles of Microbial Nutrition

The microorganisms are most versatile and diversified in their nutritional requirements. Some microorganisms require few inorganic substances, while others requires complex organic compounds for nutrition. All organisms need some common nutrients like carbon, nitrogen and water. Water is especially important to microorganisms, because they absorb nutrients only when it is in dissolved form. Carbon is needed to synthesize the organic molecules from which organisms are built up. Hydrogen and oxygen are also important elements as they are found in many organic molecules. Electrons are also required by organisms due to two reasons. (1) The movement of electrons occurs through electron transport chain during oxidation- reduction reaction which provides energy to microorganism for physiological work. (2) Electrons are also required to reduce molecules during biosynthesis.

When microorganisms are in nature, they take their nutritional requirement from the environment. But when they are cultivated in the laboratory, microbiologist use culture media, which provide the proper essential chemical elements to them. The main elements for cell growth include Carbon, nitrogen, hydrogen, oxygen, sulphur and phosphorus.

Mineral Nutrients:

The microbial nutrients are classified as **macro** (major) **nutrients,** and **micro** (minor) **nutrients** or **trace elements** on the basis of their amount required.

1. Marco or Major Nutrients

The microbial cells contain 80-90% water of their total weight, therefore, the water is always the major essential nutrient in quantitative terms. In addition to **oxygen** and **hydrogen,** the other macro (major) elements are **carbon, nitrogen, phosphorus, sulphur, potassium, magnesium, sodium, and calcium** .

Carbon is the main constituent of all cell materials and represents about 50% of cell's dry weight, CO_2 is the most oxidized form of carbon and the photosynthetic microorganisms reduce CO_2 to organic cell constituents. The non-photosynthetic microorganisms obtain their carbon requirement mainly from organic nutrients which contain reduced carbon compounds. The sulphur and nitrogen requirements of most organisms can be met with amino acids. A few microorganisms are capable of reducing elemental nitrogen to ammonia by biological nitrogen fixation.

Most of the microorganisms need **molecular oxygen** for respiration. Because,the oxygen serves as terminal electron acceptor, and such organism are called **'Obligate aerobes'**.As opposed to this, there are a few organisms which do not use molecular oxygen as terminal electron acceptor and called **'obligate anaerobes'**. Aerobes which can grow in the absence of oxygen are called **'facultative anaerobes'** whereas, anaerobes which can grow in the presence of oxygen are referred to as **'facultative aerobes'**.

(2) Micro or Minor Mineral Nutrients or Trace elements:

Besides macro elements, the microorganisms also use iron, cobalt, copper, manganese, molybdenum, nickel, selenium, tungsten, vanadium and zinc in very small amount. These elements are known as **minor** (micro) **nutrients** or trace elements. Besides the mineral nutrients, the microorganisms also need some organic compounds like **amino acids, purines, pyrimidines,** and **vitamins**. The nucleic acids are the constituents of proteins and purines and pyrimidines are constituents of nucleic acids. Vitamins, however are the most commonly needed growth factor and forms parts of the prosthetic groups of certain enzymes.

Nutritional forms of Microorganisms:

On the basis of nutritional sources of carbon, the microorganisms have following groups:

1. **Chemoutotrophs** those organisms that use inorganic chemical substances as sources of energy and carbon dioxide as the main source of carbon.

2. **Chemoheterotrophs** those that use organic chemical substances as sources of energy and organic compounds as the main source of carbon.

3. **Photoautotrophs** those that use sun light as a source of energy and carbon dioxide as the main source of carbon

4. **Photoheterotrophs** those that use light as a source of energy and organic compounds as the main source of carbon

It should be remembered that some species of microorganisms cannot be categorized exclusively into one of the four above groups. For example, certain phototrophic bacteria can also grow as chemotrophs. In the absence of oxygen (anaerobic conditions), *Rhodospirillum rubrum* depends on light as its energy source of lives, as a photoheterotroph. However in the presence of oxygen (aerobic conditions), it can grow in the dark, as a chemoheteroph. The microorganisms also require sources of electrons for growth and can be categorized as **lithotrophs** and **organotrophs,** on the basis of their electron sources. **Lithotrophs** (i.e. "rock-eater") are those that use reduced inorganic substances as their electron source, whereas, **organotrophs** extract electrons from organic compounds.

The **lithotrophs** are divided into two groups:

(i) **Photolithotrophs** use light as source of energy and includes photosynthetic microalgae, cyanobacteria and photosynthetic bacteria

(ii) **Chemolithotrophs:** which use inorganic chemical as source of energy and consists of entirely bacteria such as sulphur bacteria, iron bacteria, nitrifying bacteria, hydrogen and methane bacteria.

The **organotrophs** are also divided into two groups:

(i) **Photo-organotorphs:** These bacteria are intermediate between photoautophs and chemoautotrophs. They can utilize light energy like phototrops and synthesize their food from organic raw material, absorbed from the environment like chemoautotrophs, e.g. purple non sulphur bacteria.

(ii) **Chemoorganoheterotrophs:** They cannot synthesis their own food and take it directly from external environment using chemical energy source. It includes protozoa, fungi and majority of bacteria.

2.5. Microbial nutrition

Nutrients are materials that are acquired from the environment, and are used for growth and metabolism of microorganism. There are two categories of essential nutrients: macro-nutrients and micro-nutrients. Macro-nutrients usually help to maintain the cell structure and metabolism. Micro-nutrients help in enzyme function and maintain protein structure. The microorganism requires ten elements in large quantity, because, they used to construct macromolecules like carbohydrate, proteins, lipid and nucleic acids. However, all microbes have a need for three things: carbon, energy, and electrons. There are specific terms associated with the source of each of these items, to help define organisms. All organisms are carbon-based with macromolecules proteins, carbohydrates, lipids, nucleic acid having a fundamental core of carbon. On one hand, organisms can use reduced, preformed organic substances as a carbon source. These are the **heterotrophs** or "other eaters." Alternatively, they can rely on carbon dioxide (CO_2) as a carbon source, reducing or "fixing" this inorganic form of carbon into an organic molecule. These are the **autotrophs** or "self feeders." Before going to more discussion about microbial nutrition, let's know about nutrients.

Nutrients are materials that are obtained from the environment, and are used for growth and metabolism of microorganism. Microorganisms (or microbes) vary significantly in terms of the source, chemical form, and amount of essential elements they need. Some examples of these essential nutrients are carbon, oxygen, hydrogen, phosphorus, and sulfur etc. There are two categories of essential nutrients: macro-nutrients and micro-nutrients. Macro-nutrients usually help to maintain the cell structure and metabolism. Micro-nutrients help in enzyme function and to maintain protein structure. The microbial cell is made up of several elements such as carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, and magnesium. These are also known as macro elements or macronutrients, because these elements are required in high amounts by the microbes. On the other hand, several other elements are also required by the microbes in a very small amount which are known as microelements or micronutrients or traces

elements. These nutrients include iron, manganese, zinc, cobalt, molybdenum, nickel, and copper. These are not essential elements for the growth of the microbes but these are involved in biological functions of bacterial cell in several ways.

Cultivation of microorgmsim requires the appropriate culture media. Media are nutrient preparations used for the growth of microbes in the laboratory. Many microbes as well as cells of both, plants and animals can be grown in *in vitro* in prepared media. In order to grow, all organisms need a variety of chemical elements as nutrients. These elements are necessary for both, the synthesis and the normal functions of cellular components. Microbes need carbon, hydrogen, oxygen and a source of electrons.

Carbon

Carbon is needed to synthesize the organic molecules. It is the basic structural component of compounds. All microorganisms require carbon in same form. However, carbon makes the backbone of the three major classes of organic nutrients such as carbohydrate, protein and lipids. Because, these compound provide energy for growth and development. Those microbes that use organic compounds as their major carbon source are called heterotrophs. While, organism that use carbon dioxide as their major or even sole source of carbon are called autrophs.

Nitrogen

Nitrogen is the essential part of amino acids and is basic unit of proteins, means protein is synthesized by the use of nitrogen. However, all organisms require nitrogen is same form. While bacteria are versatile in utilization of nitrogen. Some bacteria use atmospheric nitrogen by the process of biological nitrogen fixation. Nitrogen is the part of various enzymes and synthesized different nitrogenous compounds such as nitrates, nitrites or ammonium salts.

Phosphorus

Phosphorus is essential for the synthesis of nucleic acids and adenosine triphosphate (ATP). ATP is the main energy compound form of energy storage and transfer. Almost all microorganisms used inorganic phosphate as their phosphorus source or incorporate it directly. However, some microbes such as Escherichia coli used both organic and inorganic phosphorous.

Hydrogen, oxygen and sulfur

Apart from carbon, phosphorus and nitrogen, some other elements such as hydrogen, oxygen, sulfur etc. are also essential for growth of microorganism. The hydrogen and oxygen are the part of many compounds. Sulfur is needed for the biosynthesis of the some amino acids. The four sulfur containing amino acids are cysteine, methionine homocysteins and taurine, but only two are incorporated in proteins. Most of organism used sulfate as a sources of sulfur after reducing it. While other microbes require an organic sulfur such as the amino acid cysteine.

Many other essential elements are require in smaller amount such as sodium (Na^+), iron ($Fe^{+)}$, Zinc (Zn^{2+}), cupper ($Cu^{2+)}$, Manganese (Mn^{2+}), Molybdenum (Mo^{2+}) and cobalt (Co^{2+}) etc. Na+ is requiring for transport of sugar. Iron is requiring for enzymes such as cytochromes, catalase, and succinic dehydrogenase, molybdenum is require for nitrogenase etc.

2.6. Types of microbial nutrition

Microbes can be grouped nutritionally on the basis of how they satisfy their requirements for carbon, energy and electrons or hydrogen. The microbes that used chemical compound for their energy sources are called chemotrophs. Those depend primarily on radiant energy of sun light are called phototrophs. Microorganisms may be grouped on the basis of their energy sources. Two sources of energy are available to microorganisms. Microbes that oxidize chemical compounds (either organic or inorganic) for their energy source are called *chemotrophs*, and those that use light as their energy source are called phototrophs. A combination of these terms with those employed in describing carbon utilization results in the following nutritional types:

1. ***Chemoautotrophs*:** microbes that oxidize inorganic chemical substances as sources of energy and carbon dioxide as the main source of carbon.
2. ***Chemoheterotrophs***: microbes that use organic chemical substances as sources of energy and organic compounds as the main source of carbon. Chemoheterotrophs contributes to biogeochemical cycles such as carbon and nitrogen cycle. In which nutrients are converted into different form of organic compounds.
3. ***Photoautotrophs:*** microbes that use light as a source of energy and carbon dioxide as the main source of carbon. It use light energy to drive the process of photosynthesis which generate the ATP and chemically link together CO_2 molecules to form glucose.
4. ***Photoheterotrophs:*** microbes that use light as a source of energy and organic compounds as the main source of carbon.

Microorganisms also have only two sources of hydrogen atoms or electrons. Those that use reduced inorganic substances as their electron source are called *lithotrophs.* Those microbes that obtain electrons or hydrogen atoms (each hydrogen atom has one electron) from organic compounds are called *organotrophs.* A combination of the above terms, describes four nutritional types of microorganisms:

1. Photolithotrophic autotrophy
2. Photo-organotrophic heterotrophy
3. Chemolithotrophic autotrophy
4. Chemo-organotrophic heterotrophy.

In addition, some species of microbes are versatile in their nutritional need. They cannot be characterized exclusively into one of above four groups such as some phototrophic bacteria (*Rhodospirillum rubrum*) can also grow as chemotrophs. In the absence of oxygen, they depend on light as its energy sources. However, in presence of oxygen, it can grow in dark as a chemoheterotrophs. The characteristics of these types with representative microoganisms as well as other organisms are shown in Table 2.1.

Table 2.1: Nutritional types of microorganism

Nutritional Type	Energy source	Electron or hydrogen source	Carbon source	Examples of organisms
Photolithotrophic autotrophy	Light	Inorganic compounds, water	Carbon dioxide	Purple and green sulphur bacteria; algae; plants; cyanobacteria
Photo-organotrophic heterotrophy	Light	Organic compounds	Organic compounds	Purple and green non-sulphur bacteria
Chemolithotrophic autotrophy	Inorganic compounds	Inorganic compounds	Carbon dioxide	Nitrifying, hydrogen, iron, and sulphur bacteria
Chemo-organotrophic heterotrophy	Organic compounds	Organic compounds	Organic compounds	Most bacteria, fungi, protozoa, and animals

2.7. Mode of nutrition in bacteria

Autotrophic and heterotrophic are the main mode of nutrition in bacteria. The autotrophic bacteria make their own food from outside sources of energy. They are further divided into chemo and photo autotrophs. While, heterotrophs do not make their own food and they depend

on readymade organic food from outside for their survival. Saprotrophic, symbiotic and parasitic are their sub types.

Photoautotrophic bacteria: They have photosynthetic pigments which are bacteriochlorophyll and bacteriophaeophytin. These pigments occur in thylakoids. They are anaerobic type with no oxygen involvement in this type of process. As no oxygen is required, these bacteria can survive in areas where there is low oxygen cocentration. In this process, instead of water, hydrogen is used as a source of reducing power. The source of hydrogen is organic and inorganic compounds. This hydrogen is picked by NAD ions. In this type of nutrition photosynthetic pigments synthesize ATP.

Chemoautotrophic Bacteria: They make their food from inorganic compounds and make their energy from chemical oxidation reactions involved in external medium. This energy is used in carbon assimilation. Nitrifying, sulphur oxidizing and iron bacteria comes under this category. Energy released in this process is trapped inside ATP structure.

Nitrifying bacteria: They make energy by oxidation reaction involving ammonia and nitrate. Sulphur oxidizing bacteria oxidize sulphur compound, hydrogen bi sulphide to sulphur. This oxidation process releases energy. An iron bacterium liberates energy by involving ferrous and ferric compounds. Some chemosynthetic bacteria depend upon liberation of carbon dioxide and water for energy.

Saprophytic bacteria: These are free living organisms which depend upon organic remains for their food. This bacteria leads to fermentation (anaerobic break down of carbohydrates), putrefaction (anaerobic break down of proteins) and decay (anaerobic break down or decomposition of organic compounds). Their presence is beneficial as well as harmful for human interest. They serve to clean the environment. Fungi also play an important role in it. They dispose of the organic remains and are referred to as Natures Scavengers. They dispose sewage and cure tea, coffee and tobacco. They cause food poisoning, disintegration of food along with destruction of common household stuffs.

Symbiotic bacteria: They live in cordial environment which is suitable to both the organisms. They check the growth of putrefying bacteria and produce vitamin B and K. They live in human intestine, like E. coli.

Parasitic bacteria: These bacteria live with other living beings. They depend on them for their survival and derive their food from them. They may or may not be disease causing. Disease causing is referred as pathogenic parasitic bacteria, which may cause disease by attacking host cells or by releasing toxins. Toxins may be further sub divided into exotoxins and endotoxins.On the other hand, non pathogenic bacteria do not cause any disease in host.

2.8. Summary

Culturing bacteria is the initial step in studying its morphology and its identification. Bacteria have to be grown (cultured) for them to be subsequent clinical diagnosis. The food material or substances required for growing microorganisms in vitro (outside the body) is called culture medium. Aseptic technique is a procedure that is performed under sterile conditions, a method that prevents the introduction of unwanted organisms or contaminants into an environment. Broth is one method of bacterial culture, is liquid culture, in which the desired bacteria are suspended in a liquid nutrient medium, such as Luria Broth, in an upright flask. This allows a scientist to grow up large amounts of bacteria for a variety of downstream applications. Sterilization is the process in which media are used for the growth of bacteria or other microbes should be sterilized by heating in an autoclave at 15lb pressure and 121 ℃ temperature for about 15 min. However, the sterilization refers to the killing or elimination of all microorganisms, including highly resistant bacterial spores.On the basis of nutrition, bacteria may be autotrophic or heterotrophic. Heterotrophic bacteria pass their life as parasite, saprophyte or symbiont.

Bacteria requires various types of essential mineral nutrient elements for their growth and development.On the basis of their amount of requirement,these mineral elements are classified into two types- Macro nutrient elements(required in larger amount) and Micro nutrient elements(required in very smaller amount).Macro elements have mainly structural function,where,micro elements have catalytic function and participate in enzyme structure.

2.9. Terminal questions

Q.1: What do you understand about the culture medium ? Discuss the role of agar in preparation of culture medium.

Answer: ----------

Q.2: What is the microbial nutrition? Discuss it.

Answer: ----------

Q.3: Discuss the role of microbial nutrient in microbial growth.

Answer: ----------

Q.4: Discuss the mode of microbial nutrition.

Answer: ----------

Q.5: Discuss the different types of microbial culture media.

Answer: ----------

Q.6: What is cultural tetchiness? Discuss it briefly.

Answer: ----------

2.10. Further suggested readings

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Unit-3: Microbial techniques and Growth

Contents

3.1. Introduction

The word microbiology is made up of Micro=small, Bio=Living and logy = to study. They are seen only under microscope and not seen by naked eyes. Thus microbiology means study of small living organisms i.e. microbes or microorganisms. The microbes are algae, fungi, bacteria and virus. Microbiologist uses various methods for isolation, observation and identification of microbes in laboratory. The various methods include sterilization, preparation of

pure culture, knowledge of microbial nutrition, inoculation and incubation of microbes. In the inoculation, microorganism is introduced into a sterilized culture medium with the help of inoculation needle under aseptic conditions and incubated under suitable environmental conditions for proper growth. The pure cultures are preserved and maintained for long time using various microbial techniques. These methods constitute a core of technique common to all in microbiology labs.

Objectives:-

After studying of this unit, students will be able to know:

- Various methods of sterilization used in microbiology.
- Isolation and culture of aerobic and anaerobic bacteria
- Methods of preservation and maintenance of pure culture.

3.2. Theory and Practice of Sterilization

Sterilization is a process by means of which all forms of microbial life is destroyed.

Methods of sterilization:

The various methods of sterilization fall under the following categories:

1. Physical methods of sterilization
2. Chemical methods of sterilization
3. Gaseous methods of sterilization.

1. **Physical methods of sterilization:-** These methods include dry heating, moist heating, radiations, filtration and electricity.

a) **Dry Heating:** This method is used for sterilization of glassware and small objects like inoculating loops and needles. The glasswares, e.g. test tubes and petri dishes are first dried, wrapped in brown paper and then exposed to hot air in an over (either electric or gas) to a temperature of 160^0c for two hours. At this temperature, destruction of all living cells and viable spores take place, and thus complete sterilization of glassware take place. Temperature is usually not controlled in an oven. Therefore precaution is taken to put off the switch as soon as the required temperature obtained.

Inoculation loops and needles are used for inoculation. Which was originally made up of twisted metal wire (such as platinum or tungsten) but disposable molded plastic version is now commonly used. Metal innoculation loops and needles are first dipped in absolute alcohol and then burn it into the flame of sprit lamp or burner till red-heating to a temperature high enough to destroy any organism present upon the surface.However, plastic versions were first dipped in absolute alcohal to distroy any organism present upon surface.

b) Moist Heating: In sterilization, moist heating is more effective and efficient than dry heating method because heat conduction is less rapid and process takes much longer time. The commonly used methods of moist heat sterilization are:

i. Streaming Steam: It is done by an apparatus known as Arnold steam sterilizer which allows live steam to the material to be sterilized. This live steam has a temperature of 100^0C. A single exposure for 90 minutes can satisfactorily sterilize the materials.

ii. Tyndallization: The process involves heating of material at 100^0C for 30 minutes at atmospheric pressure followed by cooling and incubating on three successive days. Thus, this process has three incubation periods, which allow heat resistant spores surviving in the previous boiling period to germinate to form the heat- sensitive growing stage, which can be killed by next boiling step. This process is time consuming and non nutrient solution can not be sterilized by this method.

iii. Steam under Pressure: Autoclave is a laboratory apparatus made up of tough double walled chamber designed for sterilization of media as well as apparatus including glassware. The autoclave works under the same principle as the pressure cooker where water boils at increased atmospheric pressure at temperature more than 100^0C. For sterilization of liquid media, a pressure of 15lb/in^2 at temperature 121^0C is given for 10-15 minutes. This high temperature is sufficient to kill vegetative cells and spores of most of bacteria. In autoclave, the condensation of steam generates extra heat and allows the steam to penetrate rapidly into porous materials.

c) Filtration: The filtration is used for the sterilization of biological fluids such as solutions of antibiotics, vitamins, tissue extracts etc. The seize filter, chamber land Pasteur fitters, Berkefed filter and Membrane filter are suitable for filtration. The first three filters are bacteriological filter, i.e., they retain only bacteria while, the membrane filters retain all forms of microorganisms including bacteria.

d) Radiation: Some of the radiations such as ultraviolet light (U.V. light), x-rays and gamma rays are used in sterilizing heat sensitive microorganisms. U.V. light has poor penetration into the material, therefore, it has limited sterilization power. It is used in irradiation of air in operating rooms and T.B. laboratories. Gamma radiations are ionizing radiation and have greater energy than U.V. light. It is mainly used in the sterilization of disposable plastic syringes, gloves, specimen's containers and Petri dishes.

e) Electricity: High and low frequency electric current are used for sterilization. In this, electric current passed through liquid containing micro-organisms. It kills a considerable portion of the microbial flora of the liquid. Since complete sterilization is not achieved, therefore, this process is applicable in pasteurization of milk and fruit juice as well as in the disinfection of water.

2. Chemical Methods of Sterilization

Non-volatile chemicals are used in laboratory for sterilization of glassware, desk, hand gloves etc. These chemicals basically kill the potentiality of microorganisms present on such articles and also reduces microorganism from the laboratory atmosphere. There are wide varieties of disinfectants; some of them are as follows:

- Chlorine and its compounds work as general disinfectant and sanitizer.
- Iodine and Iodophores work as antiseptic.
- Ethyl and Isopropyl alcohols work as skin antiseptic.
- Heavy metals like mercuric chloride and organomercurials are used for sterilization of surface of the bench tops and other inanimate objects in laboratory and operation theatre.
- Detergents like soap are used as disinfectants for utensils and glassware. It is also used as antiseptic for skin.
- Phenolic compounds like Lysol, cresol is effective against wide range of microorganisms.
- Silver nitrate is used for disinfecting the surface of test material.

3. Gaseous Methods of Sterilization.

This method is used only in special cases when sterilization by any other method is not possible, because, *the gas may be explosive and toxic to man*, e.g. ethylene oxide. The vapors of ethylene oxide are used under pressure by special equipment. Its vapors are highly toxic to viruses, bacteria, fungal cells, heat resistant spores and endospore.

Ethylene oxide is widely used in hospitals for disinfecting chemical respirators, heart-lung machines, ophthalmoscope etc.

3.3. Pure Culture Technique

In nature, microorganisms occur in air, soil and water as mixed population. For the study of specific microorganism and their role in the environment, the isolation of the same in pure culture is required. Pure culture involves isolation of individual microorganisms from a mixed population and their maintenance. Isolation of individual microorganisms from natural habitat and their growth under laboratory conditions require laboratory manipulation. When inoculum taken from natural habitat and is grown in culture medium, a large number of crowded diverse colonies develop there. These colonies lose their individuality, therefore, colonies are picked up and grown separately for detailed individual study. Several methods are in use for obtaining pure cultures. Some of common methods of them are streak plate method, pour plate method, spread plate method and serial dilution method.

3.3.1. Streak Plate Method:

This method is used to isolate bacteria. A small amount of mixed culture is taken on the tip of inoculating needle and is streaked across the surface of the agar medium. In this method, the inoculum sufficiently thin out in successive streaks and microorganisms are also separated from each other. Now these plates are incubated and allow to the growth, and colonies of microorganisms ultimately formed. By streaking, a dilution gradient is established and each colony is the progeny of single microbial cell, which represents a clone of pure culture (Fig. 3.1).

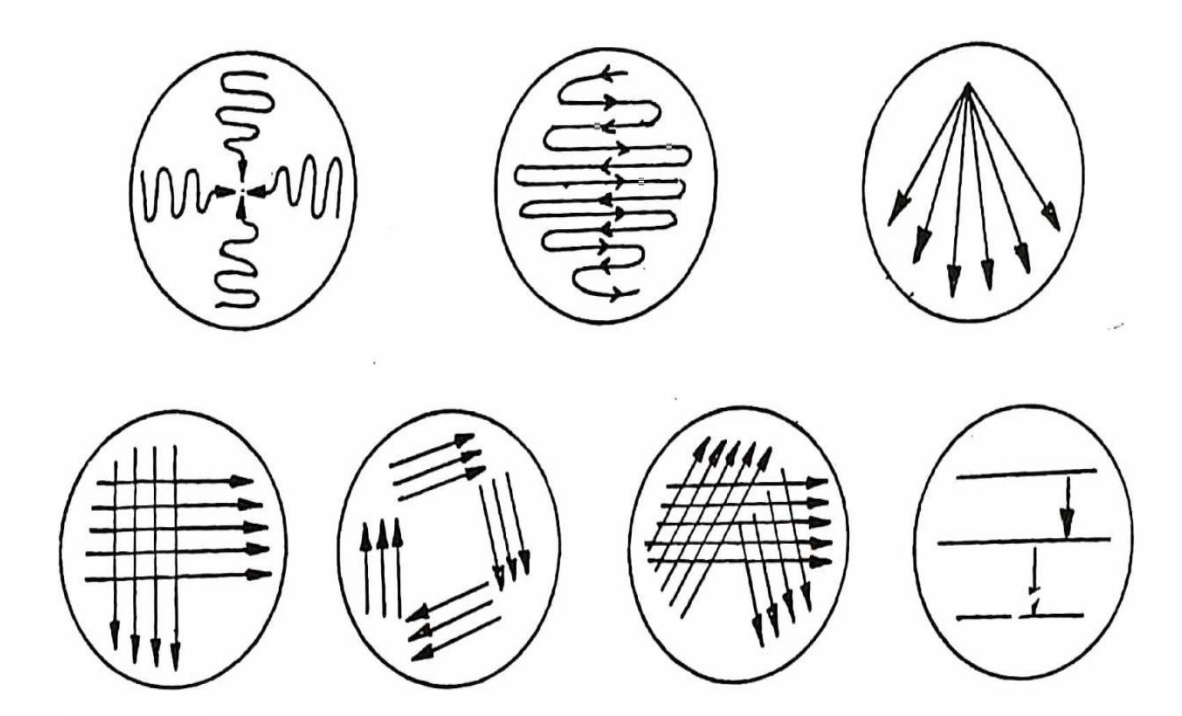
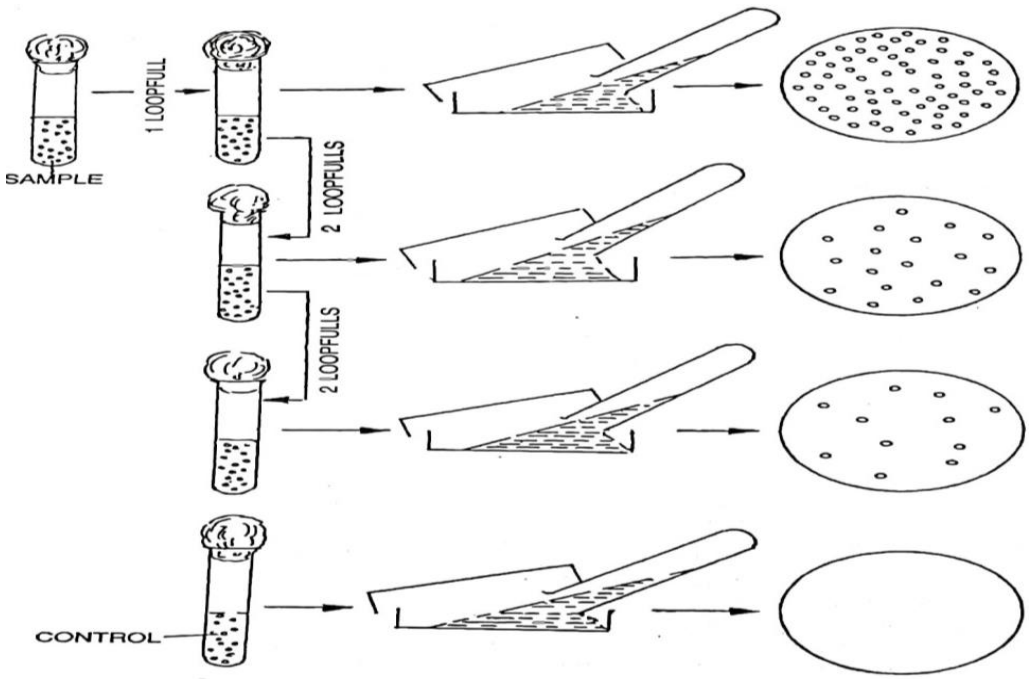


Fig.3.1: Different methods of streaking

3.3.2. Pour Plate Method:

In this method, diluted samples of inoculums mixed with melted agar medium, is plated in petri dishes. The main principle of this method is to dilute the inoculums in successive tubes having liquid medium.

The mixed culture of bacteria is diluted in tubes containing melted agar medium and mixed well. The temperature of liquid medium is maintained between 42-45^{0}C, because, agar solidifies below 42^{0}C. Now the contents of each tube are poured in to pertri dishes and allow to solidify. The inoculated pertri dishes are incubated for growth of bacterial colonies. The bacterial colonies develop both within the agar medium and on the agar medium. The isolated colonies are now picked up with the help of inoculating needle and streaked into another peridish to insure purity of the culture (Fig.3.2). The pour plate method has some disadvantages like (1) The picking up of surface colonies needs digging them out of agar medium and (2) The isolated microorganism must be able to with stand at 42-45^{0}C temperature of liquid medium. Due to which this technique is unsuitable for the isolation of psychrophilic microorganisms.



Source Microbiology by R.P. Singh

Fig. 3.2: Pour plate methods a. Media/dilution; b. pouring the plate; and c, colony development after incubation.

3.3.3. Spread Plate Method:

The mixed culture of microorganism is diluted in a series of tubes having sterile distilled water. Now a drop of such diluted liquid from each tube is placed on the centre of sterilized petri dish having agar medium. The drop is spread evenly over the surface with the help of sterilized bend glass rod. Now, these petri dishes are incubated for the growth of isolated microbial colonies. The isolated colonies are picked up and transferred into fresh culture medium to ensure their purity (Fig. 2.3).

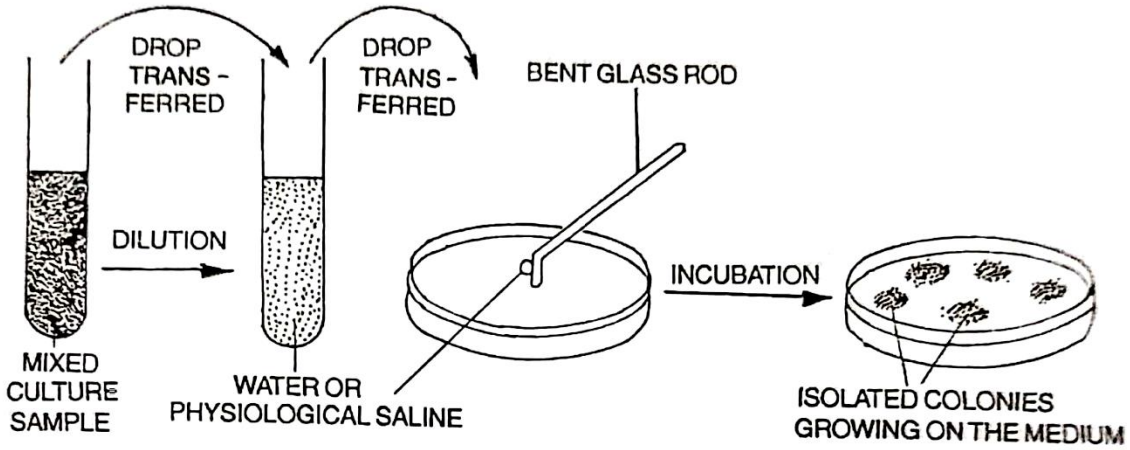


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Fig. 3.3: Speared plate method

3.3.4. Serial Dilution Method:

This method is used to isolate pure cultures of those microorganisms which grow only in liquid medium. As its name indicates, the inoculums are subjected to serial dilution in a sterile liquid medium as given below:

Step-1 Suppose, we have a culture containing 10ml of liquid medium, containing 1,000 microorganisms.

Step 2: Take out 1ml of the above medium which contains about 100 microorganisms and mix it in another tube having 9 ml medium. Now, the tube will have 10ml medium and 100 microorganisms.

Step 3: Take out 1ml medium from the tube of step 2 in another fresith tube having 9 ml medium and mixed it. Now this tube will have 10 ml medium with 10 microorganisms.

Step 4: Take out again 1 ml medium from the tube of step 3 which contains 1 microorganism and mix it in another tube having 9ml medium. Now the tube will have 10 ml medium with one microorganism. The tube is incubated for the growth of microorganism. The medium of this tube contains millions of microorganism but, since they all are originated from a single microorganism, it represents the pure culture of that particular microorganism.

3.4. Enrichment Culture Techniques

Enrichment culture technique is a very valuable technique used to enhance the population density of a particular group of microorganism with the total microbial population of a sample. The basic principle involved is that of selection. Often, it is desired to isolate bacteria that are relatively in very low numbers. This creates a problem in isolation. Normally, bacteria are isolated using the streak plate technique. Each cell multiplies in the culture during incubation to form visible colony of the same strain. But, if the desired organism is in minority in the sample, i.e., 0.1% of the total, then, one would have at least a thousand isolated colonies on a plate to have a chance of seeing just one of the desired bacterium. This is physically impossible. The medium of known composition and specific conditions of incubation favors the growth of desired microorganisms, but, it is not suitable for the growth of other types of microorganisms. Therefore, this enrichment culture technique provides a specially designed cultural environment by (1) Modifying physical conditions of the culture medium, (2) Modifying the nutrient content of the culture medium.

3.4.1. Modifying Physical Conditions of the Culture Medium:

In this technique, the physical condition of the culture medium is modified to provide growth conditions favourable for the organism of interest and unfavourable for other competing organisms.For e.g., to isolate a thermophilic bacterium which grow at high temperature, i.e. 55^0C, the inoculated sample is incubated at high temperature. The organisms that cannot tolerate high temperature (psychrophiles) will die or fail to grow, while, thermophiles will grow normally and they increase in number.

3.4.2. Modifying the Nutrient Content of the Culture Medium

Enrichment can also be achieved by modifying the nutrient content of the culture medium.For e.g., in case of soil bacteria (present in soil), which convert the free inorganic atmospheric nitrogen into fixed organic nitrogen form, ammonia.This process is known as biological nitrogen fixation. Such organisms can be isolated by incubating a soil sample in the culture medium having all the ingredients necessary for the growth except nitrogen. The nitrogen fixing bacteria synthesize their own nitrogenous nutrients for growth, and increases in number in the enriched culture medium, where, others cannot.

3.5. Isolation and Culture of Aerobic and Anaerobic Bacteria

Isolation of bacteria: The isolation of aerobic and anaerobic bacteria is done by using following methods:

- Surface plating method is employed in clinical bacteriology and it enables the isolation of distinct colonies which may be picked out, if necessary for further purification and study.
- Enrichment are selective and indicator media. It is widely used for the isolation of pathogens from specimens such as feces, with varied flora.
- Pure cultures may be obtained by pretreatment of specimens with appropriate bactericidal substances which destroy the unwanted bacteria. This method is the standard practice for the isolation of tubercle bacilli from sputum and other clinical specimens, by treatment with alkali, acid or other substances to which most commensals are susceptible but tubercle bacilli are resistant.
- Obligate aerobes and anaerobes can be separated by cultivation under aerobic or anaerobic conditions. Veillon tubes were in use formerly, but are now obsolete. This consists of a glass tube open at both ends. One ends is closed with a rubber stopper, and molten glucose agar in which the inoculums is evenly dispersed is poured into the tube and allowed to set in vertical position. The top of the tube is closed with the cotton plug. On incubation, the bacteria in the inoculums differentiate depending on their oxygen requirement. The obligate aerobes grow at the top and the anaerobes at the bottom, while the facultative bacteria grow throughout the column. The entire medium can be extruded on to a plate and the different colonies fished out.
- Separation of bacteria with different temperature optima can be effected by incubation at different temperatures. Only thermophilic bacteria grow at 60^0C. A mixture containing *Neisseria meningitides(gram- negative bacteria, causing cerebrospinal meningitis)* and *N. catarrhalis (now known as Moraxella catarrhalis, causing otitis media and sinusitis in childhood)* can be purified by incubation at 22^0C when only the latter grows.
- By heating a mixture containing vegetative and spore forming bacteria, at 80 ^{0}C, the former can be eliminated. This method is useful for the isolation of tetanus bacilli from dust and similar sources.
- Separation of motile from no motile bacteria can be effected using *Craigie's tube*. This consists of a tube of semisolid agar, with a narrow tube open at both ends, placed in the

centre of the medium in such a way that it projects above the level of the medium. The mixture is inoculated into the central tube. On incubation, the motile bacteria alone traverse the agar and appear at the top of the medium outside the central tube. A U-tube also serves the same purpose, inoculation being performed in one limb and the subculture is taken in other limb. This method can also be used to obtain phase variants in Salmonella species.

- Pathogenic bacteria may be isolated from mixtures by inoculation into appropriate animal host. *Anthrax bacilli* can be distinguished from other aerobic speculating bacilli by inoculation into mice or guinea pigs. *Anthrax bacilli* produce a fatal septicemia and may be cultured pure from the heart blood.
- Bacteria of differing sizes may be separated by the use of selective filters. Filters are widely used for separating viruses from bacteria.

Numerous culture media has been in practice for growth and development of different bacteria. These media has different composition of nutrients as per requirement of desire commensals.

First, the original media used by Louis Paster were liquid such as urine or meat broth. However, liquid has many disadvantages, because, the liquid media support mixed bacterial population growth which create difficulty to isolate different cell. But in some cause, the liquid media is very useful such as during identification of bacterial growth form the blood or water when large volume have to be tested.

Bacteria in liquid media get diffuse, procedure discrete visible growth on solid media if inoculated in suitable dilution, bacteria form colonies which makes colonel of cells originating from single bacterial cell. While in solid media produce distinct colony morphology along with other characteristics features.

As per requirement, the media have been classified as:

1. Solid Media, liquid media, semisolid media.
2. Simple media, complex media, synthetic media.
3. Aerobic media and anaerobic media

Lots of bacterial media used for their culture is already described in this unit but for the culture of anaerobic bacteria media description is required.

Anaerobic media

Anaerobic bacteria needed special media for growth, because need low oxygen content in system, reduce oxidation- reduction potential and extract nutrients. Media for anaerobic may have to be supplemented with nutrient like hermit and vitamin K. Such media may also have to reduce by physical or chemical means. Boiling the media causes to expel any dissolve oxygen. Adding 1% glucose, 0.1% thioglycollate, 0.1 ascorbic acid, 0.005% cystic or red hot iron filing can render a medium reduced.

Culture for Anaerobic bacteria:

Anaerobic bacteria differ in their requirement and sensitivity of oxygen. Some, such as *Clostridium historlyticum*, are aerotolerant and may produce some growth on the surface of aerobic plates, while, others such as *Cl. tetani* are strict anaerobes and form surface growth only if the oxygen tension is less than 2 mmHg. A number of methods have been described for achieving anaerobiosis, by exclusion of oxygen, displacement of oxygen, chemical or biological methods and reduction of oxygen.

- **Exclusion of oxygen:** Cultivation in vacuum was attempted by incubating cultures in a vacuum desiccators, but this method is unsatisfactory as some oxygen is always left behind. Fluid cultures may boil over and the media may get detached from the plates in the vacuum produced. This method is not in use now.
- **Displacement of oxygen:** Displacement of oxygen with gases such as hydrogen, nitrogen, helium or carbon dioxide is sometimes employed, but this method rarely produces complete anaerobiosis. A popular, but ineffective method is the candle jar. Here inoculated plates are placed inside a large airtight container and a lighted candle kept in it before the lid is sealed. The burning candle is expected to use up all the oxygen inside before it is extinguished, but some oxygen is always left behind. The candle jar provides a concentration of carbon dioxide which stimulates the growth of most of bacteria.
- **Chemical or biological method:** This method, first introduced by Buchner in 1888, has been employed with different modifications, for providing anaerobiosis. Pyrogallic acid

added to a solution of sodium hydroxide in a large test tube placed inside an airtight jar provides anaerobiosis but a small amount of carbon monoxide remains, which is formed during the reaction, may be inhibitory to some bacteria.

Instead of alkaline pyrogallol, anaerobiosis has also been produced within jars with a mixture of chromium and sulphuric acid (Rosenthal method) or with yellow phosphorous. The most reliable and widely used anaerobic method is the *Mcintosh- Fildes' anaerobic* jar. It consists of a stout glass or metal jar with a metal lid which can be clamped air tight with a screw. Inoculated culture plates are placed inside the jar and the lid clamped tight. This method ensures complete anaerobiosis.

3.6. Preservation and Maintenance of microbial cultures

Maintenance of viability and purity of microorganisms during preservation is necessary. Normally, this is done by periodic transfer of microorganism into a fresh culture under aseptic conditions. It is difficult to maintain a large number of pure cultures successfully for a long time and there is a risk of contamination of cultures also. Therefore, some modern methods like refrigeration, paraffin method, cryo preservation and lyphilizaton are used for maintenance and preservation of microbial cultures. These are as follows:

(i) **Refrigeration:** Pure cultures can be stored at 0-4^{0}C in refrigerators or cold rooms. At this low temperature, the metabolic activities of microorganisms are slowed down and they can be preserved for short duration, i.e. 2-3 weeks for bacteria and 3-4 months for fungi.

(ii) **Paraffin method:** By this method cultures can be preserved for several years. The sterile liquid paraffin is poured over the slant of culture and stored upright at room temperature. The layer of paraffin ensures anaerobic conditions and also prevents dehydration of medium.

(iii) **Cryo-preservation:** The cultures of microorganisms are rapidly frozen in liquid nitrogen at -196^{0}C in presence of glycerol. This method has long storage time for pure culture.

(iv) **Lyophilization:** The culture is rapidly frozen at -70^{0}C and then dehydrated by vacuum. In this method, the microorganisms go into dormant state and retain their viability for years.

3.7. Microbial Growth

The growth is defined as an orderly increase in cellular components. Microbes grow in a variety of physical and chemical environments very quickly, provided with the right growth conditions ,for e. g.,food, correct temperature, etc . Depending on the situation, this could be a good thing or bad thing for humans. It is important to have knowledge of their growth, so we can predict or control their growth under particular condition. Bacteria are mostly unicellular organisms that multiplying rapidly under favorable growth conditions, makes colonies of millions or even billions of organisms within a space as small as a drop of water. Bacteria are widely studied microorganisms of great economic, medical and societal interest. Much of our understanding of bacterial life cycles seems from monitoring its growth and development. Growth of bacterial cultures is defined as an increase in the number of bacteria in a population rather than in the size of individual cells. The growth of a bacterial population occurs in a geometric or exponential manner. Bacterial growth is proliferation of bacterium into two daughter cells, the process called binary fission. Providing no event occurs, the resulting daughter cells are genetically identical to the original cell. Both daughter cells fromed by the division do not necessarily survive. However, if the number of surviving exceeds unity on an average, the bacterial population undergoes exponential growth. Bacterial population growth studies require cultivation of viable cells in a fresh sterile broth culture medium and incubation in a closed culture vessel with a single batch of medium under optimum temperature, pH, and gaseous conditions. Under these conditions, the cells will reproduce rapidly and the dynamics of the microbial growth can be charted by means of a population growth curve, which is constructed by plotting the increase in cell numbers versus time of incubation, and can be used to delineate the stage of the growth cycle.

Bacterial growth occurs by the division of one bacterium into two daughter cells in a process called binary fission. But some time one bacterium is able to divide a once in every 12 to 15 minutes. However, some require more time and a few, very long bacteria, may require more than 24 hours per cell division. The growth of microorganisms reproducing by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time. The resulting curve has four distinct phases such as lag phase, exponential or log phase, stationary phase, and death phase.

The growth of microorganism can be measured by

1. Increase in size but this is a poor criterion of growth.
2. Increase in the number of bacterial cell by counting the number of living cells.
3. Measurement of some component of cell structures such as protein or DNA as an indicator of microbial increase (growth) or decline (death)

3.7.1. Bacterial Growth Factors

Some microbes can synthesize certain organic molecules that they need from scratch, as long as they are provided with carbon source and inorganic salts. Other microbes require that certain organic compounds exist within their environment. These organic molecules essential for growth are called **growth factors** and fall in three categories: 1) amino acids (building blocks of protein), 2) purines and pyrimidines (building blocks of nucleic acid), and 3) vitamins (enzyme cofactors).

3.7.1.1. Physical Requirements for Growth

- **pH**: pH is the most important parameters of microbial growth. The most prefer pH of growth is between 6-8. But bacteria such as *Helicobacter pylori* which inhabit the stomach with a pH approaching pH 1. This organism makes ammonia to neutralize the stomach acid around it. Many fungi prefer low pH, pH < or = 5.
- **Temperature**: temperature is another parameter for growth regulator. There are three types of microbes exists in nature.These are
- **Psychrophiles** have a low temperature optimum. Such as Listeria monocytogenes grows best at low temperatures and cultures can be enriched by incubation at refrigerator temperature.
- **Mesophiles** have an optimum growth temperature around human body temperature.
- **Thermophiles** have a hot optimum growth temperature.

  Cold temperatures are often used to slow microbial growth and thus preserve foods. Freezing temperatures do not kill microbes but preserve them in "suspended animation." Freeze-drying or **lyophilization** is often used to preserve microbial cultures.

Osmotic Pressure: it is most often adjusted by altering the salt or sugar concentration and is often used to preserve food. Some organisms, the **halophiles**, can tolerate and grow at high concentrations of salt or sugar. The Staphylococci are an important group of medical microbes which fall into this category.

3.7.1.2. Nutritional requirement for Growth:

Water is main component require for microbial growth. Apart from this, some other nutritional elements are require such as carbon, hydrogen, oxygen, nitrogen, phosphorous, sulfur and other micronutrients etc. Organic compounds such as glucose and other sugars, amino acids, sometimes complex preformed organic compounds used in microbial growth.

3.8. Microbial Growth measurement

A numbers of methods are available for the measurement of microbial growth. Its depends on the objectives and usefulness for measurement.

Optical density (OD600) is most common method for microbial growth measurement where optical density at 600 nm is used. Thus method is based on absorbance detection mode. In this method detect the portion of light passes through a sample. The particles in solution scatter light and the more particles (microorganisms) can be found in a solution, the more light is scattered by them. Therefore, a replicating population of bacteria or yeast increases light scattering and measured absorbance values. This method based on a light-scattering, where the OD600 value can be directly related to the number of microorganisms in very low-density suspensions. The number of organisms in such a culture can only be calculated after a calibration of OD600 values to a count of organisms.

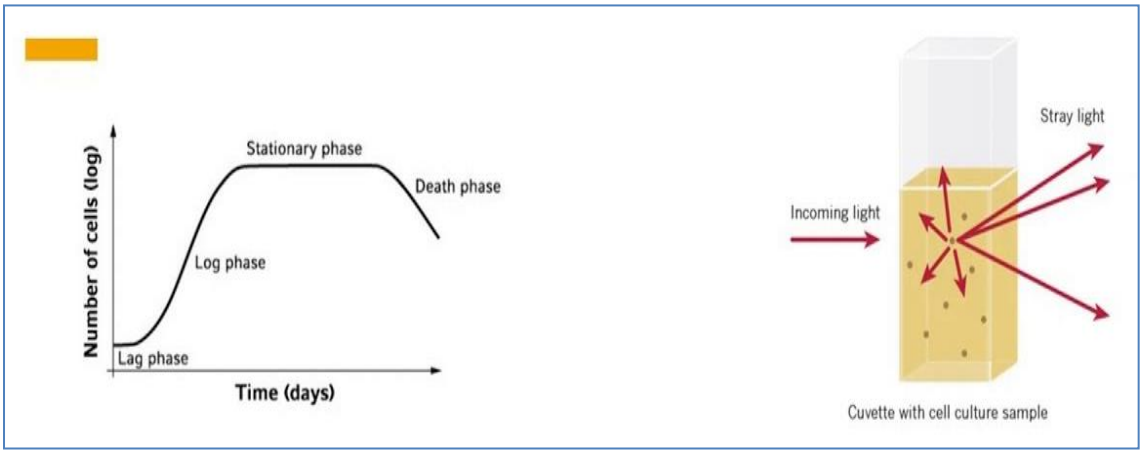

Fig.3.4: Microbial growth curve and OD measurement

In addition of OD_{600}, the bacterial growth can be measured either, i) by colony counting or cell counting, ii) by weighing the cell i.e. cell mass measurement, iii) by cell activity measurement.

i. By colony counting or cell counting

Direct microscopic counts are rapid but limited for their inability to distinguish between living cell and dead cell. The bacterial cell can be accurately count by using Petroff Housser counting chamber. This chamber includes a glass slide, a cover slip which is kept 1/50 mm above the slide. Where, bacteria suspension is present in each ruled square of the slide.

ii. By weighing the cell, i.e. cell mass measurement:

The viable cell rises to one colony so that colony count on agar plate is carried out for microbial population. For this measured amount of the sample of bacterial suspension is mixed in agar medium where each organism grows, reproduce, and form a visible mass in the form of colony. This colony can be seen with the help of magnifying lens. This method is called pure plate method. In this method, 0.1 ml sample containing bacteria is spread over the surface of an agar plate using a sterile glass spreader. In this method, the bacterial suspension is incubated until the colonies appear and colony is counted. To obtain the appropriate colony number, the sample must be diluted. Thus serial dilution for the sample are usefully adopted and this techniques in useful for the microbial count from cell sample.

iii. By cell activity measurement,

The cell mass is directly proportional to cell number that can be obtained by centrifugation of a known volume of culture and weighting the pellet obtained. Cell mass and number also counted by using optical density method. This method is based on turbidity of sample, means more turbid sample contain more bacterial count. The turbidity can be measure by photometer device that detect the amount of unscattered light recorded in photometer.

3.9. Mathematical expression of microbial growth

The growth of microorganisms involves the conversion of nutrients in the environment into biomass, mostly proteins and other macromolecules. Mathematical expression of microbial

growth is a powerful tool for gaining and understanding response of different factor for the growth and development of microbes. These measurements have usually been carried out during steady-state exponential or balanced growth for determination of growth rate and generation time.

3.9.1.Growth Rate and Generation Time

Bacterial cell generate by binary fission of mother cell, resulting the number of viable bacterial cells produced. In this process, the number of bacterial cell gets at specific time intervals because each binary fission takes in a specific duration of time. Generation times for bacteria vary from about 12 minutes to 24 hours or more. The generation time for E. coli in the laboratory is 15-20 minutes under optimal conditions. But in the intestinal tract, the coliform's generation time is estimated to be 12-24 hours. Symbionts such as Rhizobium and pathogenic bacteria such as *Mycobacterium tuberculosis* and *Treponema pallidum* have especially long generation times. Although, it has been noted in nature, many bacteria have generation times of several hours. Generation times for a few bacteria are shown in Table 3.1.

Table 3.3: generation time of some common bacteria

Bacterium	**Medium**	**Generation Time (minutes)**
Escherichia coli	Glucose-salts	17
Bacillus megaterium	Sucrose-salts	25
Streptococcus lactis	Milk	26
Streptococcus lactis	Lactose broth	48
Staphylococcus aureus	Heart infusion broth	27-30
Lactobacillus acidophilus	Milk	66-87
Rhizobium japonicum	Mannitol-salts-yeast extract	344-461
Mycobacterium tuberculosis	Synthetic	792-932
Treponema pallidum	Rabbit testes	1980

When growing exponentially by binary fission then increase in cell number is a function of the exponent (2^1, 2^2, 2^3, 2^4 --------2^n). Means if we start with one cell, when it divides, there are 2 cells in the first generation, 4 cells in the second generation, 8 cells in the third generation, and so on. The generation time is the time interval required for the cells (or population) to divide.

$$\mathrm{G\ (generation\ time)} = \frac{\mathrm{t\ (time, in\ minutes\ or\ hours)}}{\mathrm{n(number\ of\ generations)}}$$

Where,

t = time interval in hours or minutes

B = number of bacteria at the beginning of a time interval

b = number of bacteria at the end of the time interval

n = number of generations (number of times the cell population doubles during the time interval)

b = B x 2n (This equation is an expression of growth by binary fission)

Solve for n:

$$\log b = \log B + n\log 2$$

$$n = \frac{\log b - \log B}{\log_2}$$

$$n = \frac{\log b - \log B}{0.301}$$

$$n = \frac{3.3 \log}{B}$$

$$G = \frac{t}{n}$$

Solve for G

$$G = \frac{t}{\frac{3.3 \log b}{B}}$$

For example: if the bacterial cell increases from 10,000 cells to 10,000,000 cells in four hours than generation time is calculated by apply formula

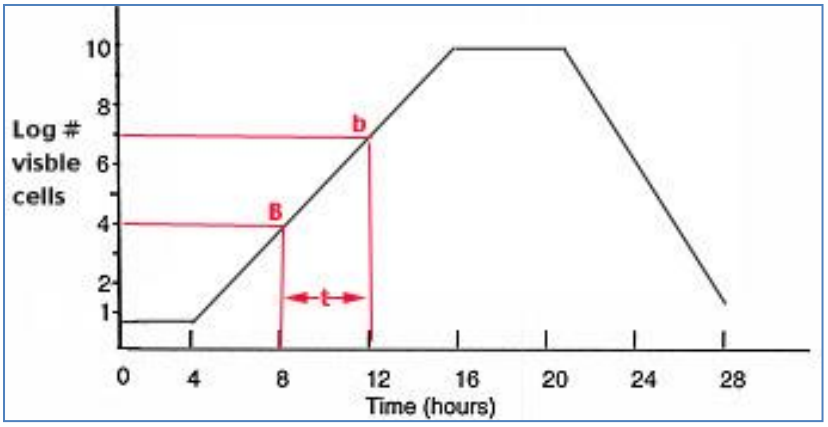

Fig: 3.5: Graph number of visible cell vs time

$$G = \frac{t}{\frac{3.3 \log b}{B}}$$

$$G = \frac{240 \text{ minutes}}{\frac{3.3 \log 107}{104}}$$

$$G = \frac{240 \text{ minutes}}{3.3 \text{ X } 3}$$

$$G = 24 \text{ minutes}$$

Another mathematical method of bacterial growth is used in practice that occurs in balance growth. The rate of increase in bacteria at any particular time is proportional to the Cell number of mass or bacteria present at that time (Fig. 3.3). The constant of proportionality is an index of the rate of growth and is called the exponential growth rate constant (K). It is defined as number of doublings in unit time, and is usually expressed as the number of doubling in an hour.

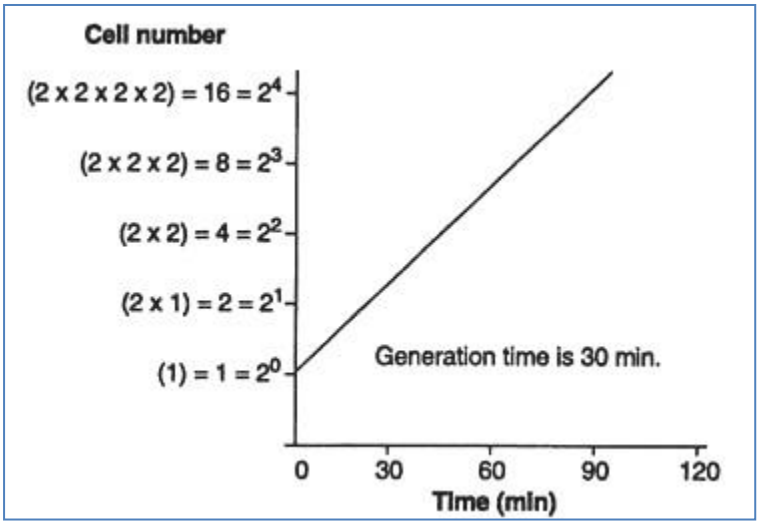

Fig. 3.6: Relationship of generation time and growth

$$B_n = B_o \, x \, 2K^t$$

B_n = Population at time t.

B_0 = Population at time zero.

Taking the logarithms

$$\log B_n = \log B_0 + Kt \log 2$$

Solving the equation for K

$$K = \log B_n - \frac{log B_0}{t \log 2}$$

The exponential growth rate constant is therefore reciprocal to generation time, i.e.

$$G = \frac{1}{K}$$

For example, generation time of E. coli is 20 minutes, i.e. 1/3 hour.

$$1/3 = \frac{1}{K}$$

K = 3 doublings per hour.

3.10. Bacterial Growth Curve

A conceptual plot of microbial cell concentration vs time for the batch system is called a growth curve. A batch culture is that in which growth of microbes occurs in a limited volume of liquid medium. During growth in liquid medium of unicellular microorganisms, the increase in cell number is logarithmic (exponential) for the same time. Thus, when microorganism grows in a suitable liquid medium (batch culture or closed system), it follow the four general patters of microbial growth. If bacterial counts are carried out at intervals after inoculation and plotted in relation to time, a growth curve is obtained (**Fig.3.4)**. The typical growth curve is divided into the following phase:

1. Lag phase
2. Log phase or exponential phase
3. Stationary phase
4. Death or decline phase

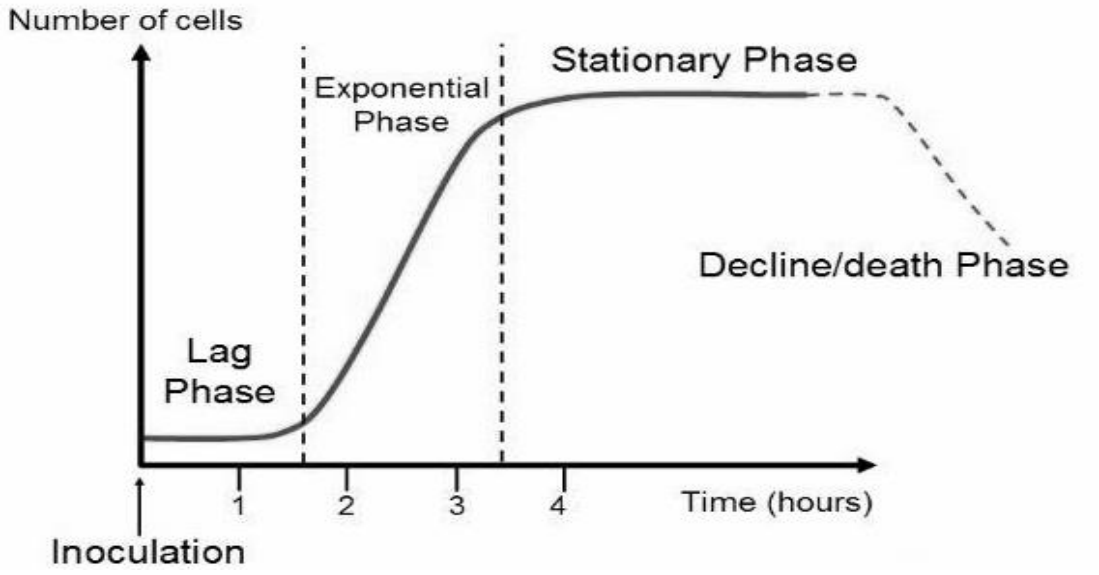

Fig.3.7: Bacterial Growth Curve-'S'shape

1. **Lag phase:** In this phase, bacteria adopt themselves to growth condition and also this phase follow stationary condition, in which population of bacteria remain constant for long period of time. During inoculation the bacteria, they do not immediately reproduce, and the population size remains constant and this phase is called constant phase. During this period,

called the lag phase, the cells are metabolically active and increase only in cell size. The lag phase is characterized by

- No cell division
- Cells are metabolically active and large in size
- Increase in size of bacteria
- Synthesis of RNA, enzyme, and co enzymes for physiological activity

This lag phase bacteria shows lot of variations according to condition. For example, if the culture microorganisms are taken form old culture, the duration will be longer. But if the culture is fresh, duration is short. Likewise, if the culture media is different from the previous culture then duration is long because bacteria takes some more time to adapt to the fresh media.

2. **Lag or exponential phase:** Log phase also called exponential or logarithmic phase. This is period characterize for doubling. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. During exponential phase, microorganisms start dividing at a constant rate and number of microorganism going to be double with time. However, bacteria have smallest size and generally the short time require for this phase. For the log phase, the rate of exponential growth varies between bacterial genera and is also influenced by culture growth condition.
3. **Stationary phase:** A stationary phase is attained at a bacterial population level of around 109 cells per ml. The stationary phase is often due to a growth-limiting factor such as the depletion of an essential nutrient. During stationary phase, the population size of bacteria remains constant, however, in this phase, some cells continue to divide and others begin to die. The rate of bacterial cell growth is equal to rate of bacterial cell death. The stationary phase has following characteristics, such as

- There is no net increase in the number of bacterial cell.
- Rapid cell division stops due to nutrient exhaustion and accumulation of toxic products.
- The viable count remains stationary as equilibrium exists between the dying cells and the newly formed cells.

- Production of antibiotics such as Penicillin, streptomycin etc and enzymes by certain bacteria occur during this phase.
- In endospore forming bacteria, speculation occurs as the bacteria enter stationary phase.

4. **Phase of decline**: This phase occurs when the population decreased due to cell death. The death phase caused due to lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious condition. Since it is a closed system, there is no way to add nutrients or remove the waste products. Eventually, this leads to unfavorable conditions and a decrease in the number of living cells in the population.

3.11. Growth yields

The yield of microbes determine in stationary phase. Yield (X) of a culture is the difference between the initial biomass (X_0) and the maximum biomass at the end of the growth phase (X_{max}):

$$X = X_{max} - X_D$$

The yield can be related to the amount of substrate used. This is the yield coefficient (Y), which is the ratio of the biomass formed (measured in g) to the mass of substrate (e.g. glucose) consumed (S, also in g):

$$Y = \frac{X}{S}$$

Thus the yield coefficient, commonly referred to as the substrate-to-biomass yield, is used to convert between cell growth rate dX/dt and substrate utilization rate dS/dt. Because of the morphological difference between unicellular bacteria or yeasts and apically-elongating, non-fragmenting, hyphal fungi, culture absorbance is a less reliable way of assessing the biomass of filamentous fungi than of bacteria or yeasts. Nevertheless, it is non-destructive and useful method for most growth experiments with fungi.

3.11.1.Microbial Growth Kinetics

In growth curve, the lag phase dX/dt and dS/dt are essentially zero. Using these corresponding observations of dS/dt and dX/dt, we can calculate yield coefficient, Y_{XS} and the specific growth rate μ as:

$$\text{Yeild cofficeint } (Y_{xs}) = \frac{\text{mass of new cells}}{\text{mass of substrate consumed}}$$

$$\mu = \frac{dX}{X_o dt} = \frac{\text{mass of cells produced}}{\text{original mass of cells.time}}, \left[\frac{1}{\text{time}}\right]$$

The yield coefficient, commonly referred to as the substrate-to-biomass yield, is used to convert between cell growth rate, dX/dt and substrate utilization rate, dS/dt.

3.12. Synchronous growth and Continuous culture

Synchronous culture refers to the growth process of the microbial population, where individual cells show **synchrony** with the other cells in the same culture medium by growing at the **same growth phase** for the given generation time. A synchronous or synchronized culture is a microbiological culture or a cell culture which contains the cells that are all in the same growth stage. In this process, the closely resemble and amplifies behavior of any single cell is considered. Where, these cells are physiologically identical and are in the stage of division cycle. The Synchronous culture also known as the synchronous growth, because here all the cells of the culture remain at the same stage of growth and phase. In some stage of microbial growth, synchromous population can be generated by manipulating their physical environments or physical composition of the medium. The cells of the synchronously growing culture divide at a time, their growth curve forms a Zig Zag pattern. To obtain synchronous population, the cell may be inoculated into a medium at a sub optimal temperature. After some time, they will metabolize slowly but will not divided. When temperature is raised to optimum, the cell will undergo the synchronized division. The dry mass of the cell, optical density, total proteins, or RNA contents per cell increase at a constant rate. The chemical growth inhibitors can be used to stop cell growth. When the growth is completely stopped for all cells, then remove the inhibitor from the culture and the cells will begin to grow synchronously. For example, Nocodazole is used in biological research for synchronization.

Recently, to obtain synchronous culture, the exponentially grown culture is centrifuge in order to separate cells, based on their density, which is directly related to the age. The microbial cell also shows the synchronization growth in random culture medium. The synchronous growth depends on the following parameters such as

1. The mitotic index
2. Generation time for doubling the microbial population

Let us see the growth pattern of cells by plotting a graph between doubling time vs a logarithmic number of cells, and time vs corresponding mitotic indices for a synchronous and random growth, respectively.

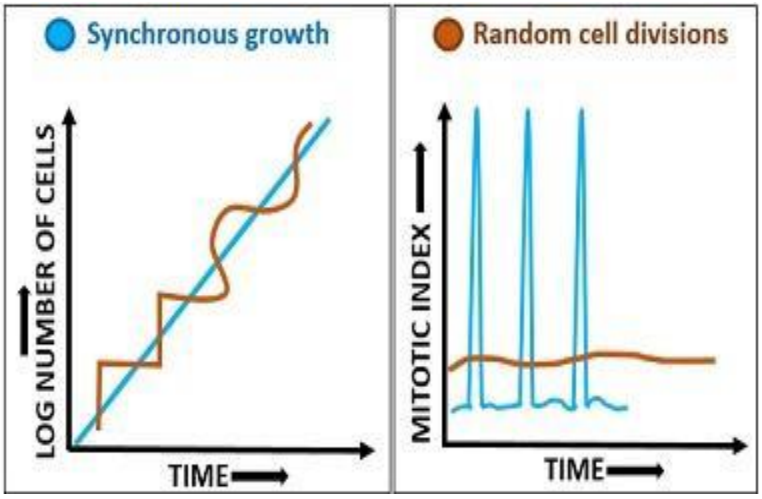

Fig.3.8: Synchronous growth of bacteria

Synchronous growth can only be maintained for a few rounds of growth and division. Ultimately, the inherent randomness of bacterial population growth again dominates. Synchronous culture helps in the separation of the smallest cells from an exponentially growing culture. In the laboratory, it is used to study the cell cycle, genetics and metabolic activity.

3.13. Summary

Sterilization is the first step of microbial methodology which includes physical, chemical and gaseous methods. Physical methods include dry heating, moist heating, radiations, filtration

and electricity. Chemical methods includes various non volatile chemicals such as chlorine, iodine, detergent, phenolic compounds etc. for sterilization of glass-wares, desk, gloves. In gaseous method, vapors of ethylene oxide are used for sterilization. The microorganisms are isolated in pure culture using streak plate method, pour plate method, spread plate method and serial dilution method. The microorganisms requires various nutrients like Carbon, nitrogen, phosphorus, sulphur , potassium, magnesium, sodium and calcium in larger amount and known as macro nutrients, and iron, cobalt, copper, manganese, molybdenum etc in smaller amount and known as micro nutrients. On the basis of nutrition source of carbon, microorganism may be chemoautotrophs, chemoheterotrophs, photoautotrophs and photoheterotrophs. There are lithotrophs and organotrophs also. Microorganism are isolated and grown under aseptic condition, Refrigeration, paraffin method, cryo preservation and lyophilization are used for maintenaning and preservating viability and purity of microorganisms in cultures. Microorganisms exist everywhere and can manipulate in all the environments in which they live. Diversity means “variation”, “differentiation”, or “diversification”, in contrast to “uniformity”. Nutrients mean all chemicals or substance that use for energy and cell growth is called nutrients. There are some essential nutrient used by microbes such macronutrients, required in large quantities, such as oxygen, hydrogen, carbon, nitrogen, phosphorus, and sulphur. They are main constituents for building of organic molecules. These organic molecules play principal roles for cell growth, energy needs and cell structure etc.

The microbial growth refers to the growth of a population (or an increase in the number of cells),and not to an increase in the size of the individual cell. Cell division leads to the growth of cells in the population. A microbial lab culture, typically passes through 4 distinct, sequential phases of growth that form the standard bacterial growth curve. These are Lag Phase - In the lag phase, the number of cells doesn't increase, Log Phase - cell numbers increase exponentially, Stationary Phase - The number of cells doesn't increase, but changes in cells occur and dearth Phase - In this phase, cells begin to die out.

3.14. Terminal Questions

Q.1: Describe chemical methods of sterilization.

Answer:- ----------

Q.2: What are pure culture techniques?

Answer:- ----------

Q.3: Describe different media used for culture of bacteria in general.

Answer:- ----------

Q.4: Describe methods used for isolation of aerobic and anaerobic bacteria.

Answer:- ----------

Q.5: What do you learn from microbial growth?

Answer:- ----------

Q.6: Discuss the role of nutrients in microbial growth.

Answer:- ----------

Q.7: Discuss about mathematical expression for microbial growth and culture.

Answer:- ----------

3.15. Further suggested readings

1. Bacterial Growth and Form by **Koch** and Arthur.
2. Prescott' microbiology, eighth edition by By Joanne Willey and Kathleen Sandman and Dorothy Wood.
3. A textbook of Microbiology, R.C. Dubey and D.K. Maheshwari,, S Chand & Company P Ltd, New Delhi
4. Text book of microbiology by Ananthanarayan and paniker's, Seventh edition, Orient longman private limited.
5. Microbiology: An Introduction, 13th Edition by Gerard J. Tortora, Berdell R. Funke and Christine L. Case.

Rajarshi Tandon Open University, Prayagraj

Environmental Microbiology

Block- II

Soil Water and Air Microbiology

UNIT -4

Soil Microbiology

UNIT-5

Water Microbiology

UNIT-6

Microbiology of Air

PGEVS-103N

Rajarshi Tandon Open University, Prayagraj

Environmental Microbiology

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Introduction

This is the second block on soil water and air microbiology. It consists of following three units such as:

Unit-4: This unit covers the soil as habitat for microbiology, soil microbes-algae, bacteria, actinomycetes, bacteriophage, protozoa, nematodes and fungi. The microbial balance in nature and rhizosphere and rhizoplane microorganism also covers in this unit.

Unit-5: This unit covers the water as habitat for microbiology, fresh and marine water microbiology. The microbial analysis of water-coliforms test, purification of water by microbes, microorganism associated with organic matter decomposition in water is also discussed.

Unit-6: This unit covers the aeromicrobiology. In this unit the aeromicroflora of pharmacy, aeromicroflora of hospital and other houses discussed. The phylloplane microflora, phylloplane pathogens and characteristic of phylloplane microflora also discussed here.

Unit-4: Soil Microbiology

Contents

4.1.Introduction

Soil is a complex ecosystem in a state of dynamic equilibrium bounded by physio-chemical parameters. The relative stability of this system depends upon the relative stability of its biological composition and regulating parameters. Long association between organisms in the same environment brings about a kind of balance among them, which is commonly known as equilibrium between organisms and their environment. The zone (region) of soil surrounding the plant root, where the nutrients released from the root, increase the microbial population and its

activities is termed the rhizosphere. Soil is a unique habitat which harbouras (house) of a variety of micro flora and faunna ,and gives mechanical and nutritional support to higher plants. Soil quality can be defined as the capacity of a soil to function within the boundaries of ecosystem, to sustain biological productivity, maintain environmental quality and promote plant growth and animal health. The plant root surface, usually including the adhering soil particles, is called rhizoplane. Functionally, the rhizosphere can be defined as the region extending a few millimeters from the surface of each root, where the microbial population of the soil is influenced by the chemical activities of the plant. Microorganisms growing under the influence of roots are often qualitatively and quantitatively different from those inhabiting remote or away from this influence in the soil environment. Therefore, the rhizosphere is a unique subterranean habitat for microorganisms.

Microbes of all major taxonomic groups, bacteria, fungi, algae, protists, and viruses are found in soil, acting as decomposers to breakdown the organic matter into smaller nutrients that are used by plants and by the microbes themselves. Algae are pioneer colonizers of barren volcanic areas, desert soils and rock surfaces. Four major groups of algae are found in surface soil. The green algae are most common in acidic soils. Members of chrysophycophyta (yellow-green algae) as *Navicula* and *Botrydiosis* are also found. Red algae as *Porphyridium* may also be present.

Blue-green algae (BGA) or cyanobacteria are free living nitrogen fixing, photosynthetic algae that are found in wet and marshy conditions. Nitrogen fixation takes place in specialized cells called the heterocysts which depend on vegetative cells for energy requirement and fix free molicular atmospheric nitrogen due to presence of enzyme nitroginase. While, the fixed nitrogen is utilized by the vegetative cells for growth and development. One of the important role of blue-green algae as biofertilizers in agriculture microbiology is due to its nitrogen fixing activity

4.2.Objectives

After going through the course of this unit, student will be able to:

- Understand the habitat of microorganisms found in soil.
- Know about the types of soil microorganism
- To know about the fungal-microbial balance in soil.
- Understand about the rhizosphere

- To know about the microorganism associated with rhizoplane.

4.3.Soil structure

Soil can be defined as loose surface material forming the upper part of the earth crust. It consisting of inorganic mineral particles, gases, liquids, organisms and organic matter, formed by gradual weathering of rock, and gives plant both, mechanical and nutritional support.

Soil formation is a complex process require very long duration of time.They are produced from rocks,the parent material, through the processes of weathering and natural erosion. Different soils may share common parent material but develop quite unique properties depending on their environment. A three dimensional body of soil is called a pedon. The minimum size of pedon that can be considered as soil is 1 to 10m^2 x 1.5 m deep. The pedon contains the solum (commonly referred as soil) which lies on the parent material, the mineral material from which the soil originates. The pedon can be divided into horizons. Soil categorized into three layers called soil horizons, the **top soil** (rich in humus, minerals, soil microbes and plant roots), **subsoil** (composed of fine particles and minerals) and **parent material** (the weathered rock composed of inorganic materials).

Table.4.1: Mechanical composition of soil and relative size of size particles

Soil type		Particle Size (mm)
Clay		<0.002
Silt		0.002-0.075
Sand	Fine	0.075-0.42
	Medium	0.42-2.0
	Coarse	2.0-4.75
Gravel		4.75-75

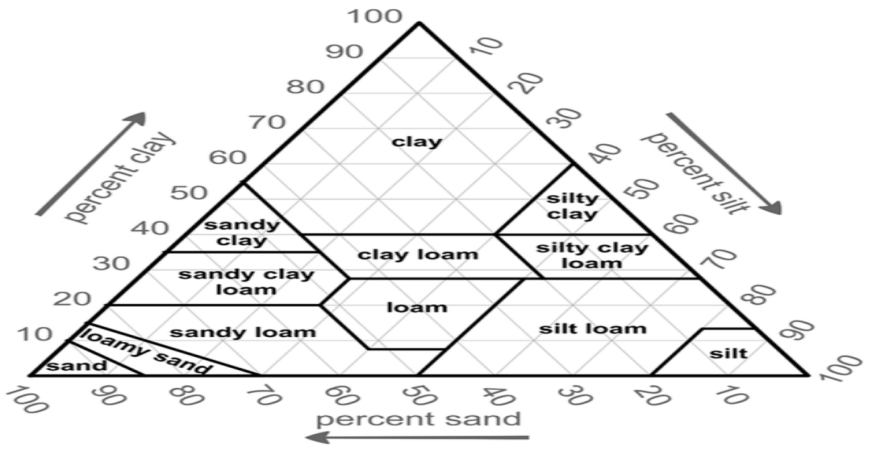


Fig.4.1: Soil texture depends on the percentage of clay, slit and sand particles in the soil

Soil is made up of particles composed of both, inorganic and organic substances. The inorganic components include rock, minerals, water and gases and the organic components include humus (non-living organic matter) and living organisms. The composition of the particles along with associated moisture contents are used to classify soils as clay, sand, silt or loam. Each of these soil types varies as to organic content, moisture content , pH, oxygen, mineral content and carbon dioxide concentration.

Top soil contains the greatest number of microorganisms and the zone of maximum microbial activity, because, it is well-supplied with oxygen and nutrients. Lower layer of soil contains less oxygen and nutrients contain with fewer microorganisms. The microbial activity decreases with the increase of depth of soil. Rock decomposition releases various sized particles ranging from rocks, pebbles and sand grains to microscopic morsels that lie in a loose aggregate. The water content of soil is directly related to its oxygen content, since both, oxygen and water compete for the pockets or spaces present in the soil.

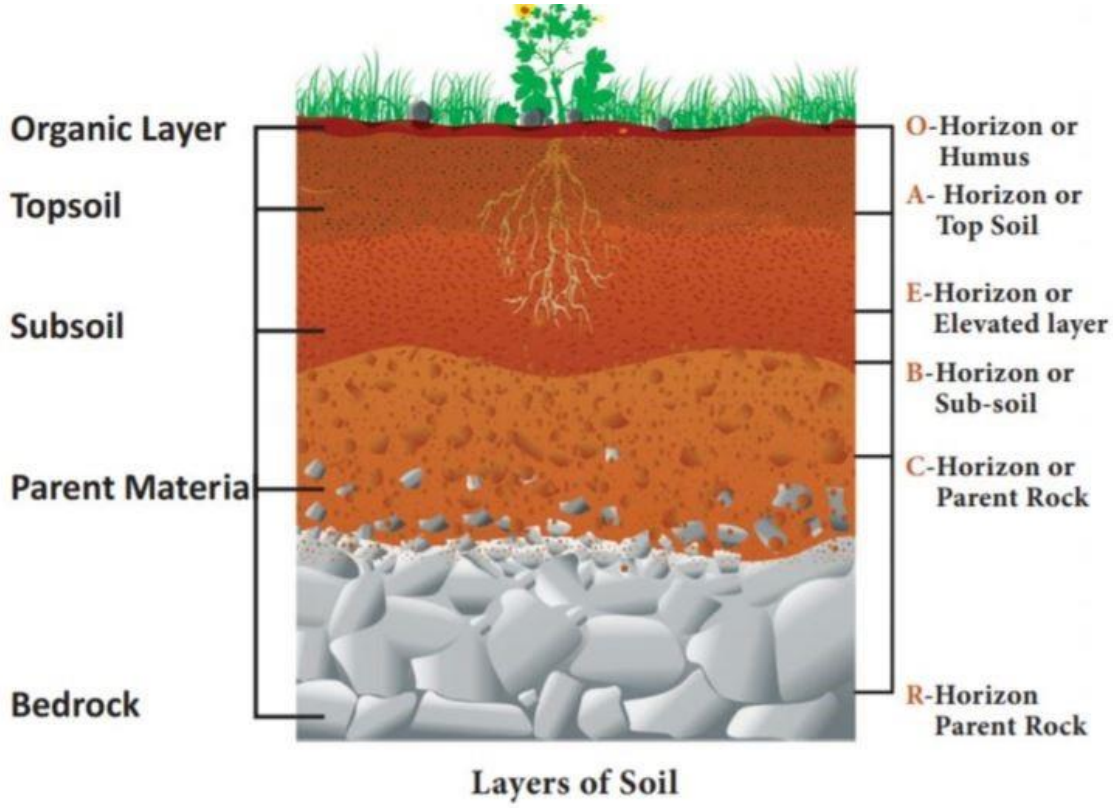


Fig.4.2: Soil profile

Humus is the amorphous, microbially altered, nonliving, relatively stable portion of soil organic matter, forms from condensation of phenolic and amino compounds derived from organic matter breakdown and condensation of aminoquinone intermediates. The soil humus is resistant to degradation, hence is nonliving in nature. Soil also has carbohydrates, especially polysaccharides, which play an important role in the formation and stabilization of soil aggregates. The humus content varies with climate, temperature, moisture, mineral content and microbial action.

4.3.1.Quality of Soil:

Soil quality can be defined as the capacity of a soil to function within the boundaries of ecosystem to sustain biological productivity, maintain environmental quality, and promote plant growth and animal health. Soil quality encompasses its capacity for crop productivity, food safety, and health of animals and humans. Soil quality can be improve or deteriorate, depending up on the various influencing factors. The factors that cannot be affected are geology,

topography, climate and time. The factors which can be influenced are humus content, phosphorus status, degree of saturation etc.

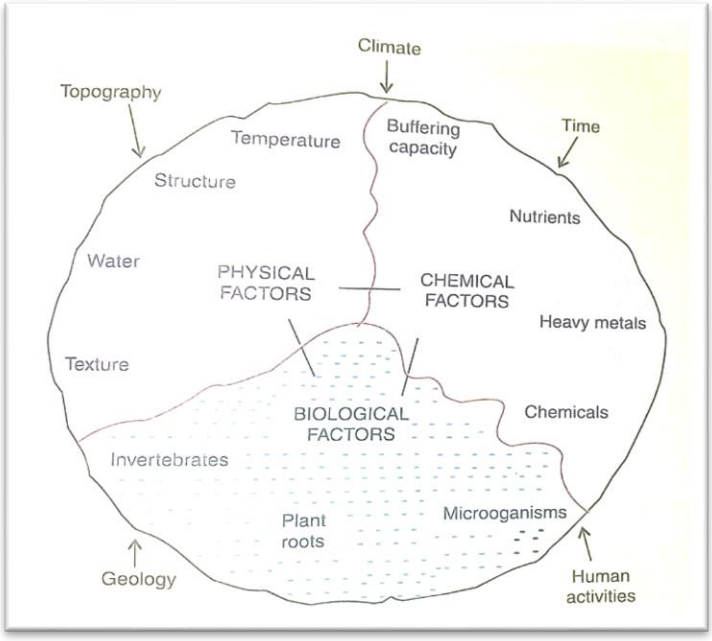


Fig.4.3: The complex structure of soil, created by the influence of geology, topography, climate, time and human quality

4.3.2.Physico-chemical properties of soil

It includes soil texture, water, air, inorganic chemicals and organic matter. The biological factors of soil are soil flora and fauna. Mechanical composition of soil is determined on the basis of size of soil particles,i.e. sand, silt and clay particles. The ratio of soil mineral particles governs the porosity (pore size of soil), soil water, air, temperature, pH, inorganic and organic matters and microorganisms.

The sources of inorganic material in soil are the parent rocks which get changed by the physical and chemical processes of weathering. Soil consists of different sized mineral particles, which ratio determines the characteristic of soil. On the basis of size, soil particles are divided into:

- **Sand particles** (about 50µm diameter) which are the fragment of rock materials.
- **Silt particles** (2-50µm diameter) which contain primary minerals (quartz)

- **Clay particles** (less than 2 µm diameter) composed of secondary minerals (such as kaolinite, montmorillonite, elite etc.)

Clay particles are negatively charged particles which are of the important components of soil environment influencing the physic-chemical and biological properties of soil (Gray and Williams, 1971). Chemical nature of soil minerals divided into **silicates** and **non-silicates**. The non silicate group includes oxides, hydrides, sulphates, chlorides, carbonates and phosphates.

The silicates are very complex in structure, but vary widely in its stability and resistance to decomposition. The most influential soil particles, as far as microbial activity is concerned, are the colloidal size clays and humic materials. It plays significant role in determining the availability of nutrients, and in the interaction of extracellular enzymes and antibiotic substances produced by the microorganisms (Burns, 1983).

The surface layer of soil consists of a relatively unchanged mass of plant and animal remains called litter. After microbial decomposition, organic matter is converted into unidentified amorphous material which is known as humus.

4.4.Soil as habitat for microorganisms

Soil is a unique habitat which harbouras (house) of variety of micro flora and fauna, and gives mechanical and nutritional support to higher plants.

Soil microbiology is defined as the branch of soil science concerned with soil inhabiting microorganisms, their functions and activities. The soil having a diversity of useful microorganisms of industry, agriculture and geochemical cycles. It is also the habitat of number of fungi and bacteria that are pathogens \for plants, animals and humans. Study of soil microbiology is essential to understand agriculture and environmental science.

Microbes of all major taxonomic groups such as bacteria, fungi, algae, protozoa, actinomycetes, bacteriophage, nematodes and viruses are found in soil.They acting as decomposers which breakdown the organic matter into smaller inorganic nutrients that are used by plants and by the microbes themselves. Some of the common microorganisms found in soil are:

Soil algae: Both prokaryotes and eukaryotes algae luxuriantly grow where adequate amount of moisture and light are present. They play a variety of roles in soil. The

photoautotrophic are predominantly present in areas where sunlight can penetrate the surface of the soil. They could be found at a depth, as some green algae and diatoms, can grow hetero as well as photo autotrophically.

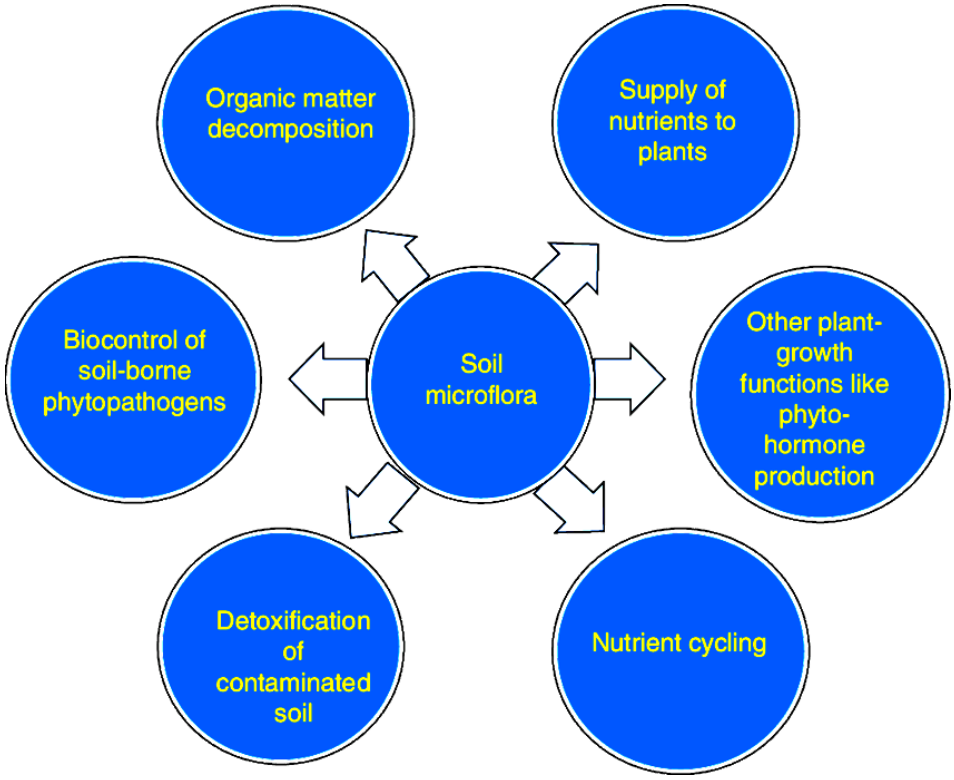


Fig.4.4: Microbial flora of soil environment showing possible interactions between microbes with energy substrate, physical factors and themselves

The algal populations are highest in the surface of 10 cm of soil, where their populations range from 5,000 to 10,000/g of soil. A visible algal bloom developing in surface of water may contain millions of algal cells. Algae are pioneer colonizers of barren volcanic areas, desert soils and rock surfaces. Four major groups of algae are found in surface soil.

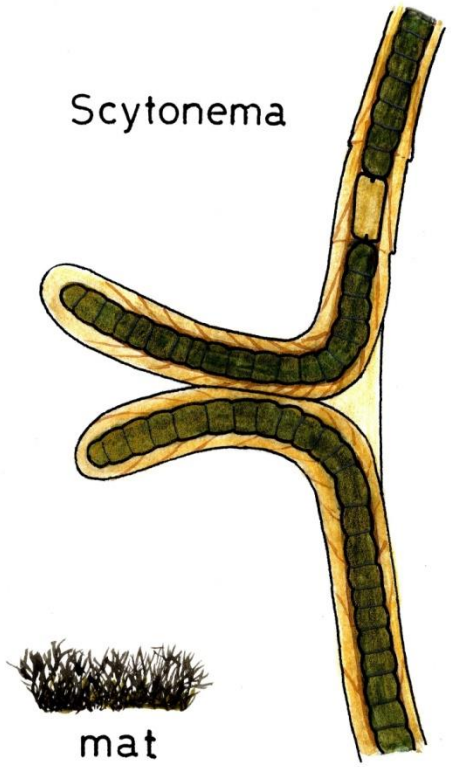
The green algae are most common in acidic soils. Members of chrysophycophyta (yellow-green algae) as *Navicula* and *Botrydiosis* are also found. Red algae as *Porphyridium* may also be present.

Blue-green algae (BGA) or cyanobacteria are free living nitrogen fixing photosynthetic algae, that are found in wet and marshy conditions. Nitrogen fixation takes place in specialized cells called the heterocysts which depend on vegetative cells for energy requirement to fix

nitrogen while the fixed nitrogen is utilized by the vegetative cells for growth and development. One of the important role of blue-green algae as biofertlizer in agriculture microbiology is due to this biological nitrogen fixing activity.

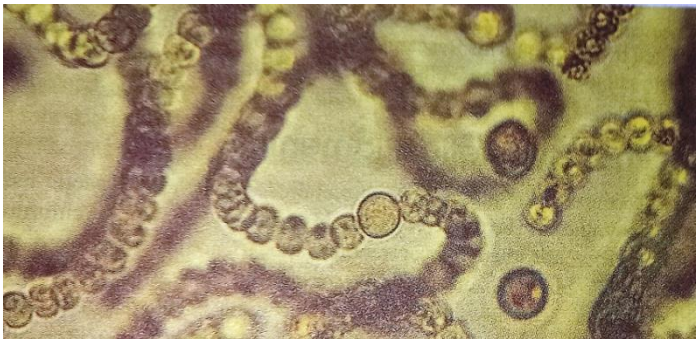
BGA are very common in the rice fields. They can also be used in reclamation of sodic soil, i.e. alkaline soil, sewage treatment etc.

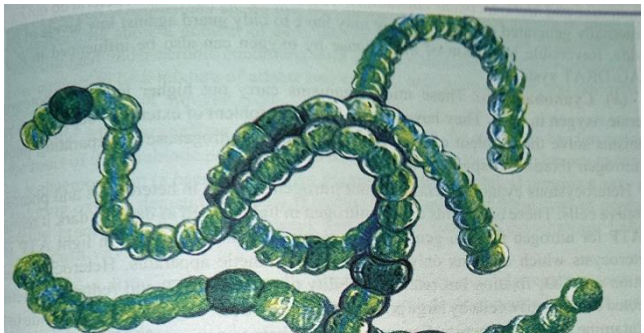
The prominent genera of BGA are *Anabaena*, *Calothrix*, *Oscillatoria*, *Aulosira*, *Nostoc*, *Scytonema*, *Tolypothrix* etc.



A **B**

Fig. 4.5: A. *Scytonema* B. *Aulosira*





A **B**

Fig. 4.6: A. *Nostoc* **B. *Anabaena***

Bacteria: bacteria are the smallest unicellular prokaryotes (0.5-1 x 1.0-2.0 µm), the most abundant group and usually more numerous than others, the number of which varies between 10^8 and 10^9 cells per gram soil. Bacteria are divided into two groups:

i. **Soil indigenous (true resident) or autochthonous**

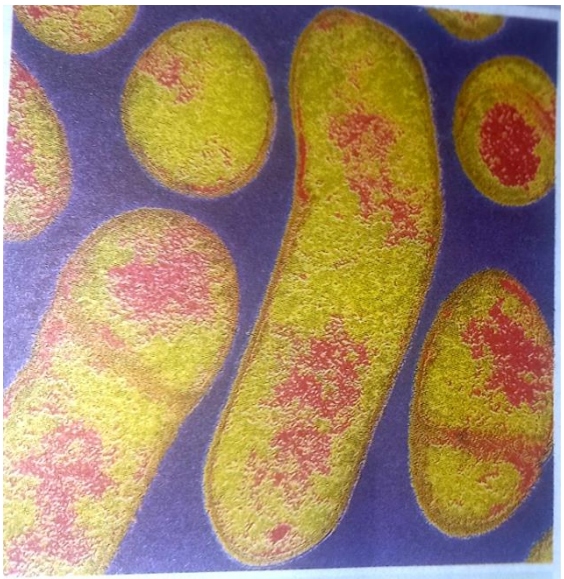
ii. **Soil invader or allochthonous**

The number and types of bacteria are influenced by soil types and their microenvironment, organic matter, cultivation practices etc. They are found in high number in cultivated than virgin land, maximum in rhizospehere and less in non-rhizosphere soil, possibly due to aeration and nutrient availability (Rovira, 1965, Alexander, 1977).

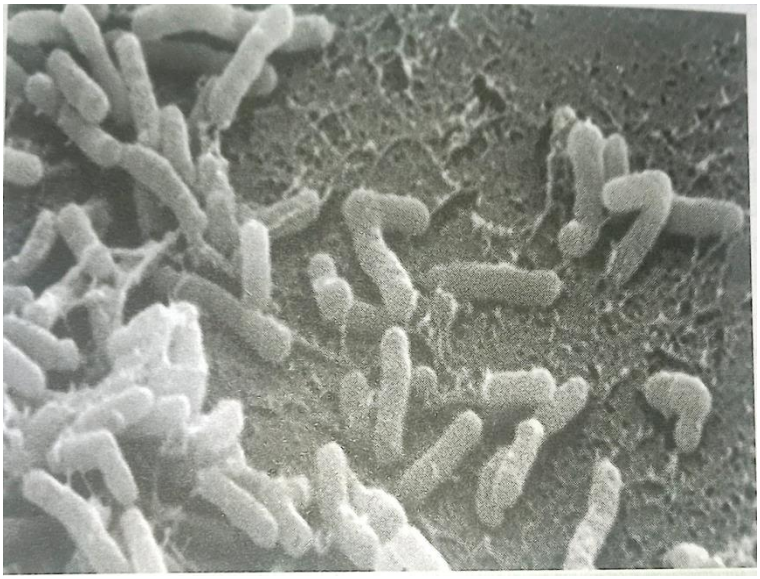
The inner region of soil aggregates contained higher level of Gram-negative bacteria, while the outer region contained higher level of Gram-positive, may be due to polymer formation, motility, surface changes and life cycle of bacteria involved.

Table 4.2: Bacteria commonly found in soil

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



A

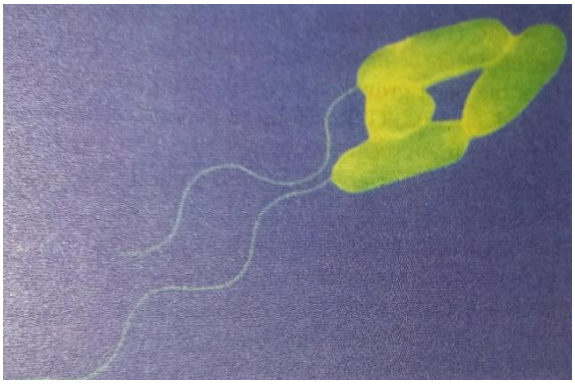


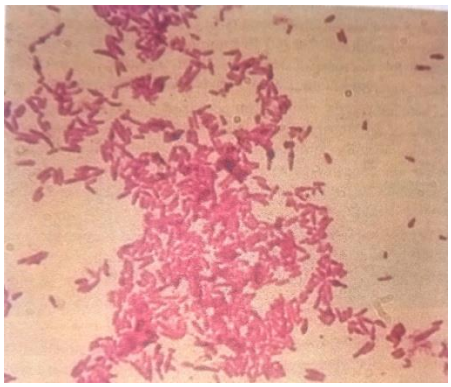
B

Fig.4.7: A. *Clostridium botulinum* B. *Agrobacterium* spp

Bacteria do not occur freely in the soil solution but are closely attached to soil particles or embedded in organic matter. They play a major role in organic matter decomposition, bio-transformation, biogas production; nitrogen fixation etc. Population of aerobic bacteria is much higher than the anaerobic population in soil.

Autotrophs and heterotrophs, mesophiles, thermophiles, psychrophiles, cellulose digesters, nitrogen fixing, nitrifying and denitrifying bacteria are found in the soil. These have

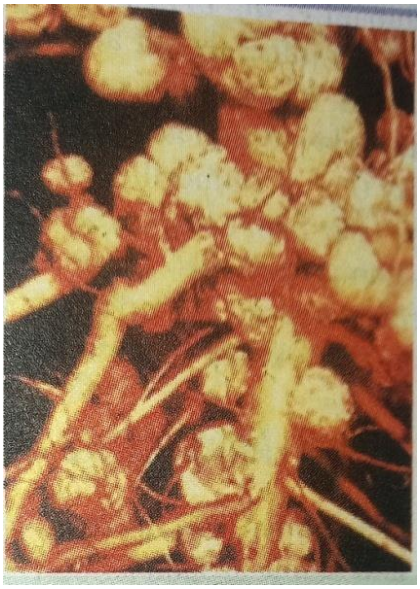




the ability to decompose complex substances such as cellulose, pectin, protein, butyic acid and urea. Some of soil bacteria are *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Alcaligens*, *Clostridium*, *Corynebacterium*, *Erwinia*, *Nitrosomonas*, *Nitrobacter*, *Pseudomonas*, *Rhizobium*, *Thiobacillus* etc.

A **B**

Fig. 4.8: A. *Pseudomonas solanacenum* B. *Corynebacteri*



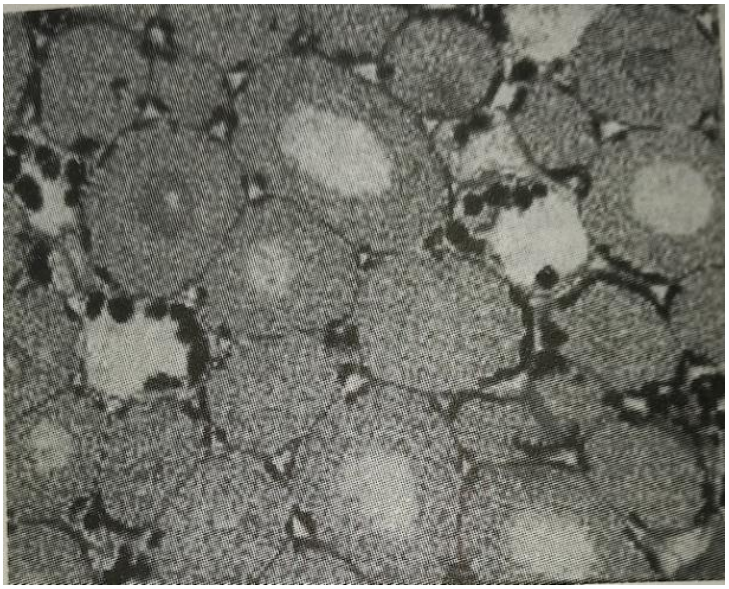


Fig. 4.9: A. Root nodules of leguminous plant have *Rhizobium* B. *Rhizobium*

Actinomycetes:

They share the characters of both, bacteria and fungi, and commonly known as "ray-fungi" because of their close affinity with fungi. They are Gram-postive and release antibiotic substances. Population of actinomycetes in soil remains greater in grass land and Pasteur soil than in the cultivated land. In temperate zones, the number of actinimycetes ranges from 10^5 and 10^8 cells per gram soil. The most limiting factor is the pH, which governs their abundance in soil. Neutral or alkaline pH (6.0 to 8.0) is favored its luxuriant growth (Garrett,1981). The important members of actinomycetes are *Actinomycetes, Actinoplanes, Micromonospora, Microbispora, Nocardia Streptomyces* and *Thermoactinomycetes* etc.

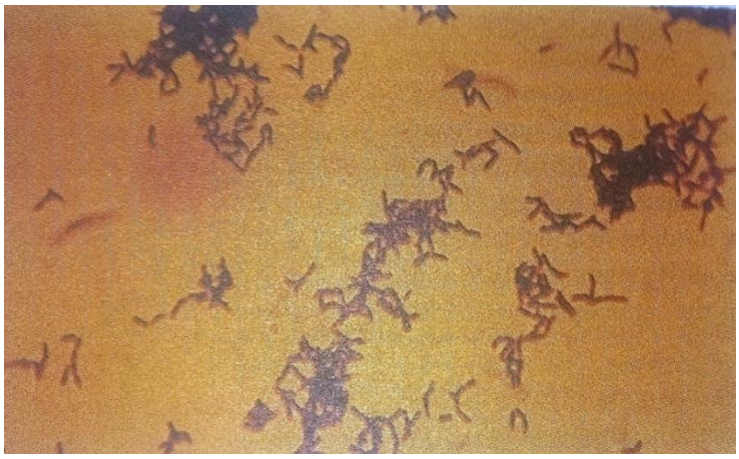


Fig.4.10: Actinomycetes

Bateriophage: Bateriophage as well as plant and animal viruses also found in the soil. Viruses persist in soil as dormant particles that retain parasitic ability. Soil viruses infect mostly bacteria but a few infect fungi and plants. Plant viruses (mostly having RNA genome) rarely survive in soil, while some insect viruses remain infective for years.

Protozoa: in moist soil, most of the members of micro fauna remain in encysted form. The role of soil protozoa is predatory as these eat upon bacteria and thereby regulate their population. The number of protozoa can be correlated with plant root growth and indirectly with status of soil nutrients (Griffin, 1972; Garrett, 1981). Mostly, amoebas and flagellated protozoa are found in many soils. Protozoa require a water film for locomotion and feeding, their activity is limited to the water filed pore space in soil. They can withstand in drying of the soil and other adverse conditions by forming resistant cysts. They have a significant role in the regulation and modification of the size, and composition of the microbial community and in the acceleration of the turnover of microbial mass, soil organic matter and nutrients. Protozoa feed on bacteria and may help in control of bacterial population.

Nematodes: most nematodes are microscopic (less than 50 µm wide by 2000 µm long) and transparent, and are the most numerous animal group found in soil. Free living nematodes are mostly found in the upper portions of the soil profile. They are active in water films, although, some persist through forming resistant cysts. At neutral pH, their population is higher. Some nematodes species parasitize upon living plant roots, while, others feed on the microbial community found near roots as compared to the rest of soil. Nematodes derive nutrients for their growth and reproduction from the cell contents and cytoplasm of protozoa, bacteria, fungi etc.

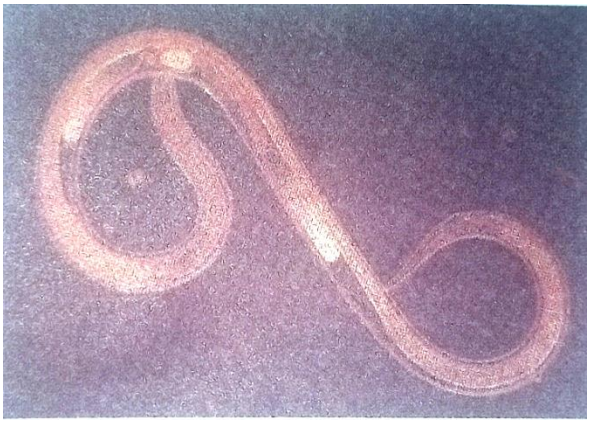


Fig. 4.11: Nematode

Fungi: they are the largest fraction of the microbial biomass in most well-aerated soils. The population ranges from 2x104 to 1x106 fungal propagules per gram of soil. Fungi derived nutrients for their growth from organic matters, living animals (protozoa, arthropods, nematodes etc.) and living plants, establishing different types of relationships. More than half of the fungal biomass is of basidiomycetous fungi alone. Soil fungi are dominated by the members of

Deuteromycetes (anamorphic fungi), Zygomycetes (Mucorales) and Ascomycetes, few belonging to the Chydriomycetes and Oomycetes.

Garrett (1950) classified soil fungi on the basis of substrate-specialization and duration of parasitism.

a. **Root inhabiting soil fungi:** they are characterized by an expanding parasitic phase on the living host with a little declining saprophytic phase.
b. **Soil inhabiting soil fungi:** they are characterized by the ability to survive indefinitely in soil as saprophyte.

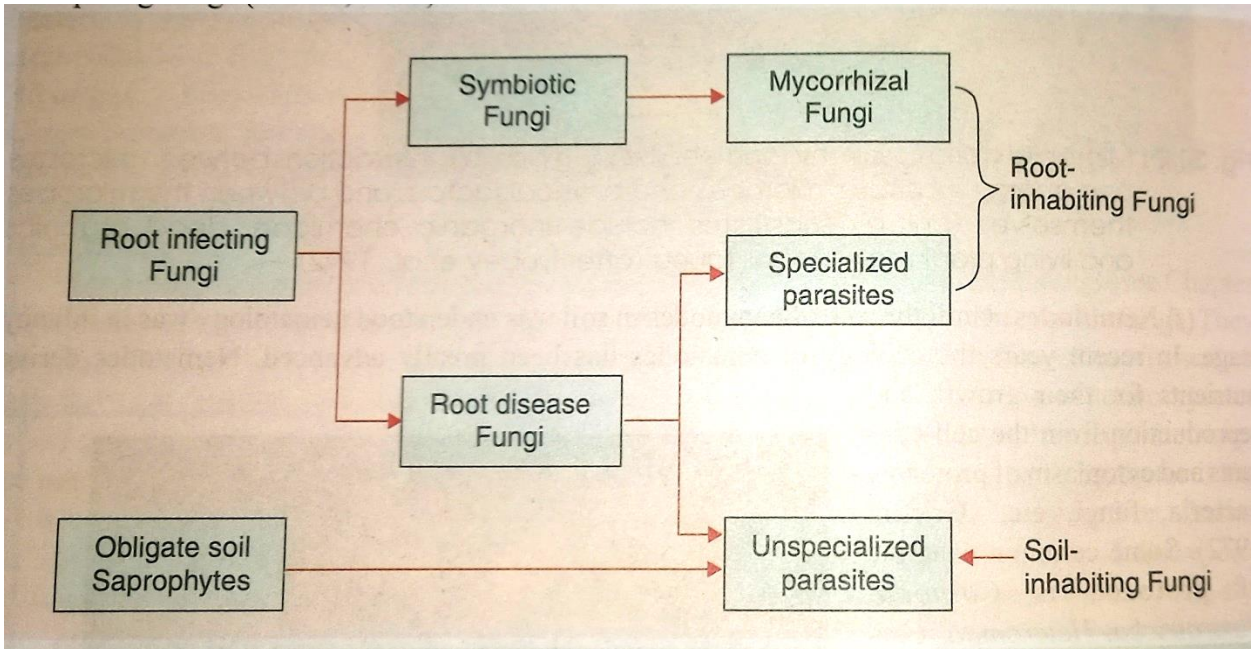
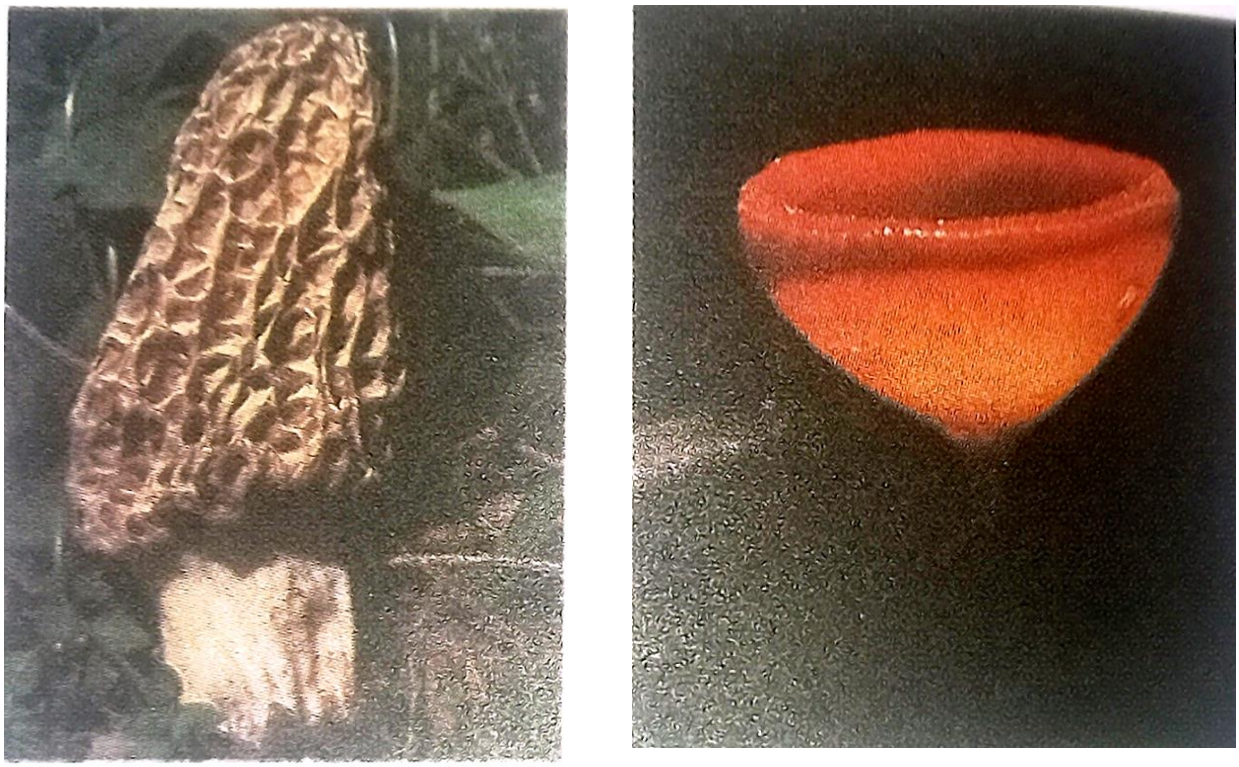


Table.4.3: Classification of soil fungi (Garrett, 1950)

Success in competitive colonization of any particular soil fungus, depend directly on its competitive saprophytic ability (CSA), inoculums potential at the surface of the substrate and inversely as the aggregate inoculums potential of the competing fungi.

Some of the soil saprophytes are *Alternaria, Aspergillus, Cladosporium, Dematium, Gliocladium, Helminthosporium, Humicola, Metarrhizium* etc.

Fungi associated with plant diseases are *Armillaria, Fusarium, Helminthosporium,Ophi0bolus, Phytophthora, Plasmodiophora, Pythium, Rhizoctonia, Sclerotoum, Thielaviopsis, Verticillium* etc.



A Morchella esculenta **B Auricularia auriculata**

Fig. 4.12: Member of Ascomycetes and Basidiomycetes

4.5.Microorganisms balance

Soil is a complex ecosystem in a state of dynamic equilibrium, bounded by physio-chemical parameters. The relative stability of this system depends upon the relative stability of its biological composition and regulating parameters (Kruetzer,1965). Long association between organisms in the same environment brings about a kind of balance among them which is commonly known as equilibrium between of organisms and their environment. The equilibrium is possible only when during coexistence action and interaction between different microorganisms if varied potential go on. Thus at a given time, population of one species increases while that of the others, perhaps decreases due to microbial interaction in an ecological

niche. In natural soil, the greater number of interacting factors results in the more stationary microbial balance (Wilhelm, 1965). In rhizosphere, the balance would be less stable due to continuous release of energy sources for microorganisms.

Baker and Cook (1974) pointed out that the presence of microorganisms at a given place and time is determined by (a) its having or being introduced there (b) the existence of physio-chemical environment favourable to its development (c) the presence of associated organisms (symbionts, hosts) favourable to its development or organisms (host or parasite) required for its survival and (d) the inhibition or absence of organism (disease organisms, pests, antagonists) so detrimental to it as to cause its extinction. An organism will increase until the limitations imposed by the biotic and abiotic environment just counter balance the rate of increase. Thus limitations check the chaos and epidemics of microorganisms.

4.6. Microorganisms of rhizospher and rhizoplane

The zone (region) of soil surrounding the plant root where the nutrients released from the root increase the microbial population and its activities is termed the rhizosphere. The term rhizosphere was coined by the German scientist Lorenz Hiltner (1904).

The plant root surface usually including the adhering soil particles, is called rhizoplane. Functionally, the rhizosphere can be defined as the region extending a few millimeters from the surface of each root, where the microbial population of the soil is influenced by the chemical activities of the plant. Microorganisms growing under the influence of roots are often qualitatively and quantitatively different from those inhabiting remote or away from this influence in the soil environment. Therefore, the rhizosphere is a unique subterranean habitat for microorganisms.

The rhizosphere microflora of one plant differ from the rhizosphere microflora of other plant. The rhizosphere region can be divided into two zones: the inner rhizosphere which is in a close vicinity of root surface, and the outer rhizosphere embracing the immediate adjacent soil. Clark (1949) has suggested to use the term rhizoplane for root surface itself.

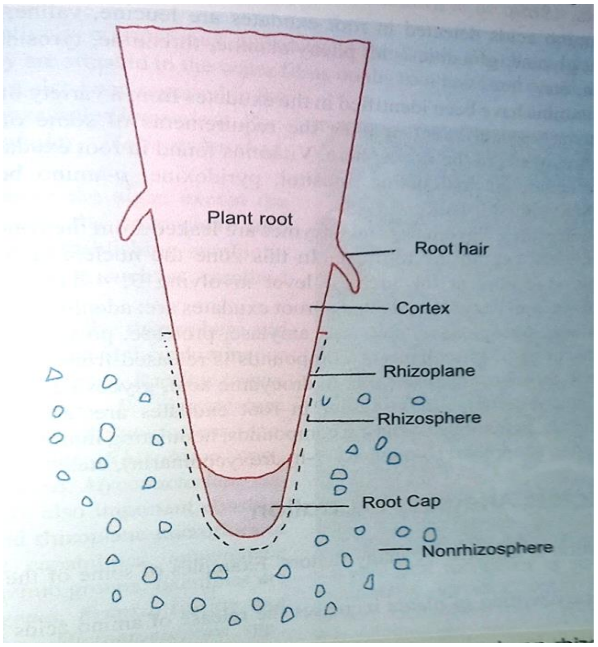


Fig.4.13: A typcal plant root showing rhizosphere, rhizoplane and non-rhizosphere regions

Soil	Average number of fungi ($\times 10^4$/g soil) Growth Stages				
	SDL	PRF	FLR	PFL	SNT
Non-rhizosphere	5.037	5.064	7.060	6.622	5.187
Rhizosphere	8.460	12.770	20.019	17.406	15.076
R:S Ratio	1.69	2.521	2.835	2.629	2.906

SDL: seedling; PRF: preflowering; FLR: flowering; PFL: post-flowering; SNT: senescent; R:S: rhizosphere and non rhizosphere ratio

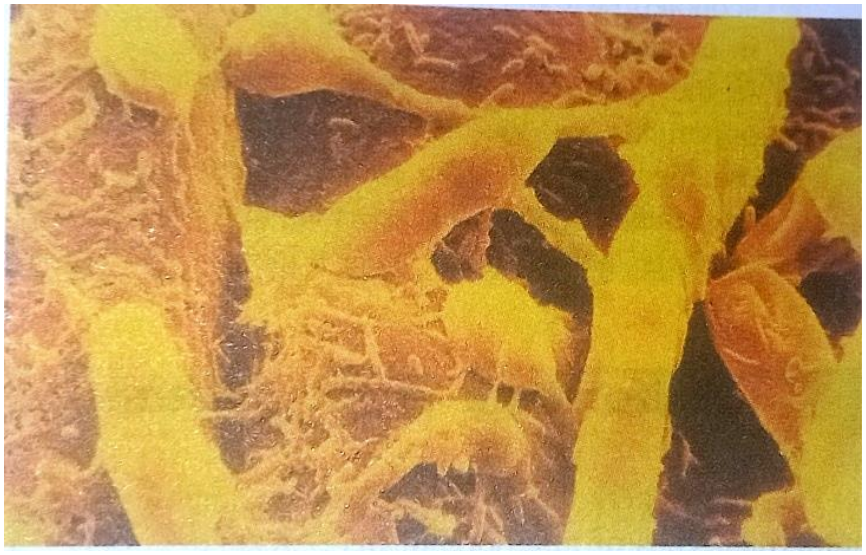


Fig. 4.14: A SEM micrograph showing bacteria and fungi colonizing the rhizosphere

Rhizoplane microorganism:

The rhizosphere region is a highly favourable habitat for the proliferation and metabolisms of numerous types of microorganisms.

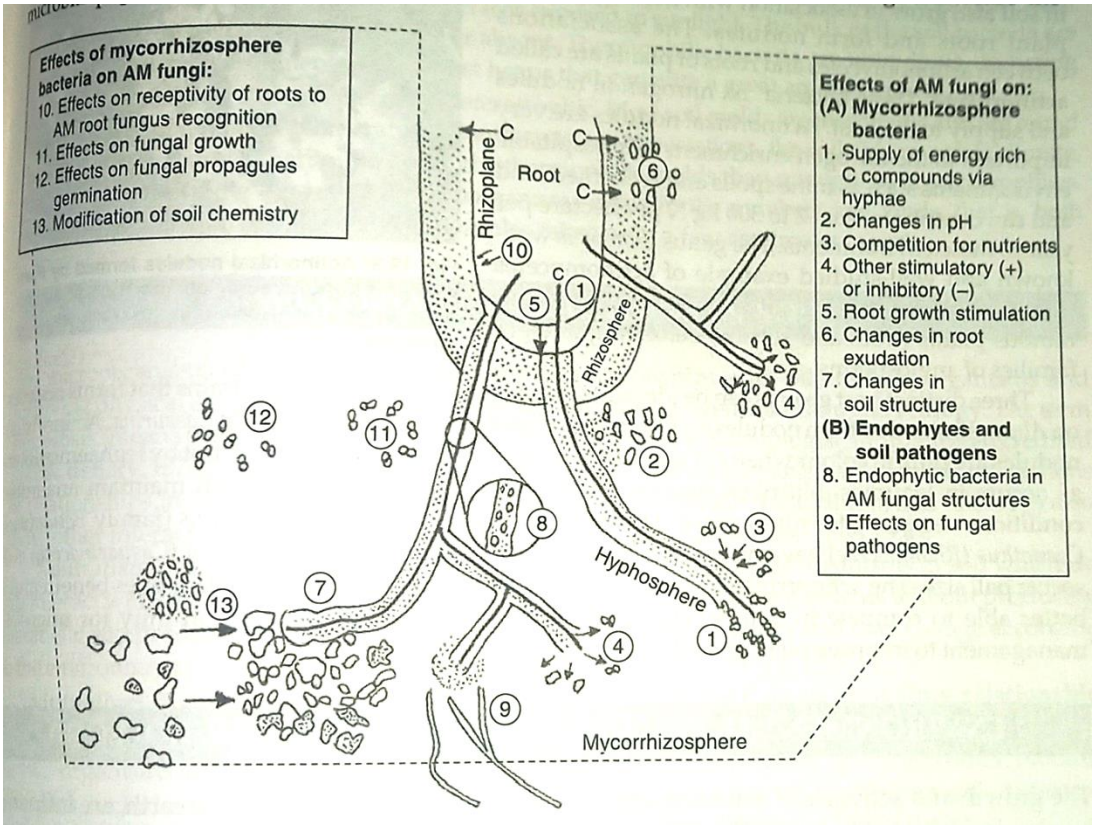


Fig.4.15: Mycorhizosphere: possible interactions among different components of the mycorhizosphere

The microbial community of this rhizophore zone can be examined by means of cultural, microscopic and manometric techniques.

The dominant fungi of rhizosphere *are Aspergillus flavus, A. fumigatus, A. luchuensis, A. niger, A. terreus, Cladosporium cladosporioides, Curvularia lunata, and Fusarium oxysporum, F.solani, Macrophomia phaseolina, Neocosmospora vasinfecta* and *Rhizoctonia solani*.

The small flagellate, large ciliates and amoeboidal forms of protozoa are also found. They are situated in the water films on the root hairs and on the epidermal tissue of root. Cysts of nematodes have also been reported in the rhizosphere region, for example, *Heterodera*, *Pectus, Tylenchus, Acrobeles, Helicotylenchus, Meloidogyne* etc.

Blue-green algae present in the rhizosphere and establishing symbiotic associations in certain plants such as coralloid root of *Cycas*.

Dominant bacteria in rhizosphere are *Agrobacterium radiobacter, A. tumifacians, Arthrobacter, Pseudomonas, Bacillus brevis, B. circulans, B. polymaxa, B. megaterium, Azotobacter, Flavobacterium, Rhizobium spp., Cellulomonas, Micrococcus, Mycobacterium* etc.

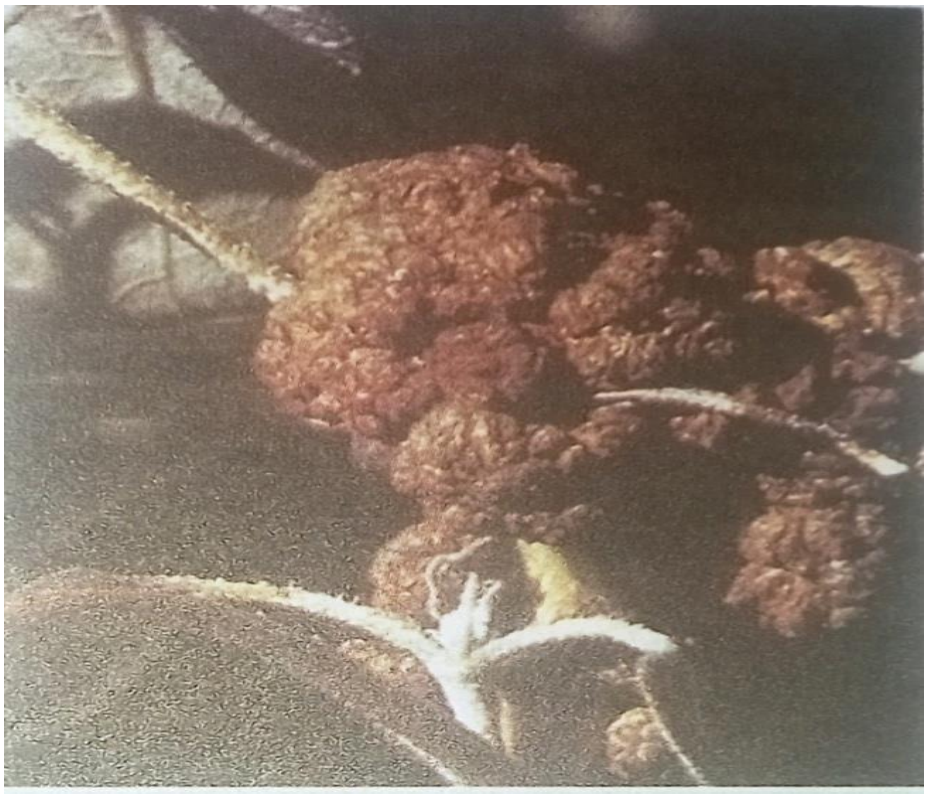


Fig.4.16: *Agrobacterium tumefaciens* colonises the rhizospere

Actinomycetes are also important constituents of rhizoshere and rhizoplane microflora of different biosynthetic capabilities, antagonistic, potentiality and taxonomic groups. Some are

Actinomycetes chromogenes, Frankia, Actinoplanes, Micromonospora, Microbispora, Nocardia spp., Streptomyces antibioticus, S. scabies, S. griseus etc.

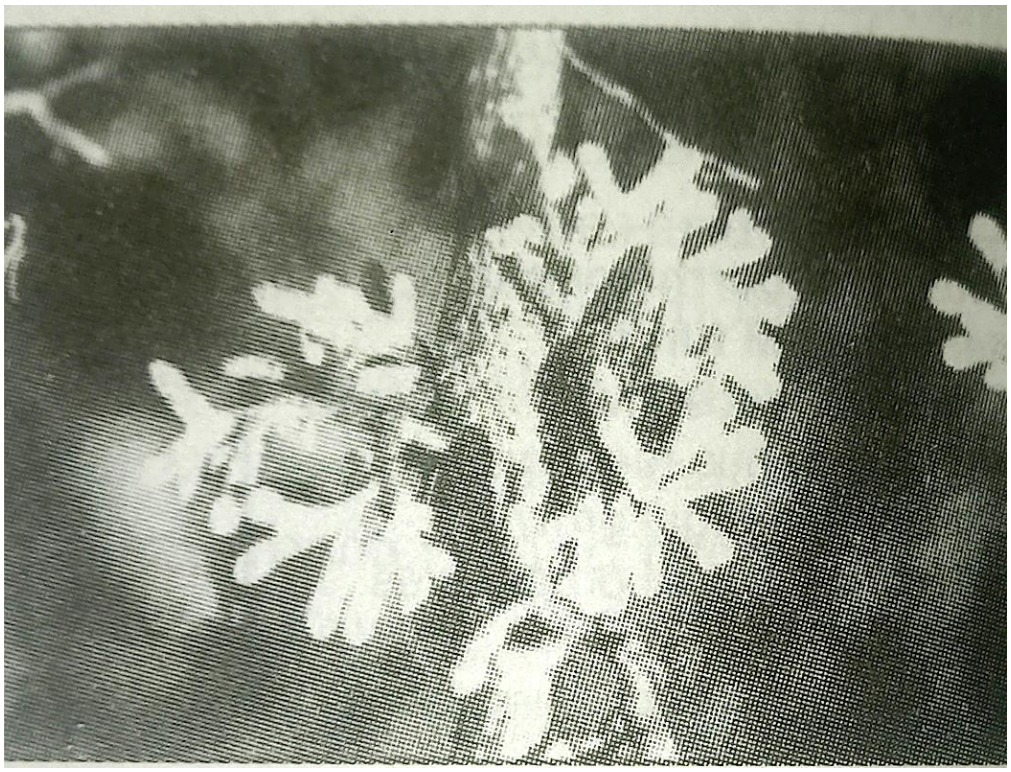


Fig.4.17: Actinorhizal nodules formed by *Frankia* on the root of Ceanothus

4.7.Summary

Soil microbiology is defined as the branch of soil science concerned with soil inhabiting microorganisms, their functions and activities. The soil having a diversity of useful microorganisms to industry, agriculture and geochemical cycles. It is also the habitat of number of fungi and bacteria that are pathogens for plants, animals and humans. Study of soil microbiology is essential to understand agriculture and environmental science. Soil formation is a complex function of climate, topography, parent material, time and biota. Different soils may share common parent material but develop quite unique properties depending on their environment. A three dimensional body of soil is called a pedon. The minimum size of pedon

that can be considered as soil is 1 to $10m^2$ x 1.5 m deep. The pedon can be divided into horizons. Soil categorized into three layers called soil horizons, the **top soil** (rich in humus, minerals, soil microbes and and plant roots), **subsoil** (composed of fine particles and minerals) and **parent material** (the weathered rock composed of inorganic materials). Top soil contains the greatest number of microorganisms and the zone of maximum microbial activity, because, it is well-supplied with oxygen and nutrients. Lower layer of soil have less of oxygen and nutrients contain, and fewer organisms. Humus is the amorphous, microbially altered, nonliving, relatively stable portion of soil organic matter, forms from condensation of phenolic and amino compounds, derived from organic matter breakdown and condensation of aminoquinone intermediates. Physico-chemical properties of soil include soil texture, water, air, inorganic chemicals and organic matter. The biological factors of soil are soil flora and fauna. Mechanical composition of soil is determined on the basis of size of soil particles, i.e., sand, silt and clay particles. The ratio of soil particle governs the porosity (pore size of soil), soil water, air, temperature, pH, inorganic and organic matters and microorganisms. On the basis of size of soil particles, soil particles are divided into: **Sand particles** (about 50µm diameter) which are the fragment of rock materials, **Silt particles** (2-50µm diameter) which contain primary minerals (quartz), **Clay particles** (less than 2 µm diameter) composed of secondary minerals (such as kaolinite, montmorillonite, elite etc.). Chemical nature of soil minerals divided into **silicates** and **non-silicates**. The non silicate group includes oxides, hydrides, sulphates, chlorides, carbonates and phosphates. The silicates are very complex in structure but vary widely in its stability and resistance to decomposition. The most influential soil particles, as far as microbial activity is concerned, are the colloidal size clays and humic materials.

The prominent genera of blue green algae are *Anabaena*, *Calothrix*, *Oscillatoria*, *Aulosira*, *Nostoc*, *Scytonema*, *Tolypothrix* etc.They are able to fix atmospheric nitrogen.

Bacteria do not occur freely in the soil solution but are closely attached to soil particles or embedded in the organic matter. They play a major role in organic matter decomposition, bio-transformation, biogas production, biological nitrogen fixation etc. Population of aerobic bacteria is much higher than the anaerobic population in soil. Autotrophs and heterotrophs, mesophiles, thermopiles, psychrophiles, cellulose digesters, nitrogen fixing, nitrifying and denitrifying bacteria are found in the soil. These have the ability to decompose complex substances such as cellulose, pectin, protein, butyric acid and urea. Some of soil bacteria are *Agrobacterium*,

Arthrobacter, Bacillus, Alcaligens, Clostridium, Corynebacterium, Erwinia, Nitrosomonas, Nitrobacter, Pseudomonas, Rhizobium, Thiobacillus etc.

Actinomycetes share the characters of both, bacteria and fungi and commonly known as "ray-fungi", because of their close affinity with the fungi. They are Gram-postive and releases antibiotic substances. The important members of actinomycetes are *Actinomycetes, Actinoplanes, Micromonospora, Microbispora, Nocardia Streptomyces* and *Thermoactinomycetes* etc.

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Fungi may derived nutrients for their growth from dead organic matters as saprophyte or living animals (protozoa, arthropods, nematodes etc.) and living plants as parasite by establishing different types of relationships. More than half of the fungal biomass is of basidiomycetous fungi alone. Soil fungi are dominated by the members of Deuteromycetes (anamorphic fungi), Zygomycetes (Mucorales) and Ascomycetes, few belonging to the Chydriomycetes and Oomycetes.

The dominant fungi of rhizosph*ere were Aspergillus flavus, A. fumigatus, A. luchuensis, A. niger, A. terreus, Cladosporium cladosporioides, Curvularia lunata, and Fusarium oxysporum, F.solani, Macrophomia phaseolina, Neocosmospora vasinfecta* and *Rhizoctonia solani*. The small flagellate, large ciliates and amoeboidal forms of protozoa are found.. Cysts of nematodes have also been reported in the rhizosphere region, for example, *Heterodera, Pectus, Tylenchus, Acrobeles, Helicotylenchus, Meloidogyne* etc. Dominant bacteria in rhizosphere are *Agrobacterium radiobacter, A. tumifacians, Arthrobacter, Pseudomonas, Bacillus brevis, B. circulans, B. polymaxa, B. megaterium, Azotobacter, Flavobacterium, Rhizobium spp., Cellulomonas, Micrococcus, Mycobacterium* etc. Actinomycetes are also important constituents

of rhizoshere and rhizoplane microflora of different biosynthetic capabilities, antagonistic potentiality and taxonomic groups. Some are *Actinomycetes chromogenes, Frankia, Actinoplanes, Micromonospora, Microbispora, Nocardia spp., Streptomyces antibioticus, S. scabies, S. griseus* etc

4.8.Terminal Questions:

Q.1: Soil is a habitat for microorganisms. Discuss critically.

Answer:----------

Q.2: Briefly discuss about rhizoshere and rhizoplane regions.

Answer:----------

Q.3: Write an essay on microbial community in soil.

Answer:----------

Q.4: Write short notes on the following:

a. Microbial balance

b. Soil profile

Q.5: **Answer**:----------

Q.6: Briefly describes about microorganisms of Rhizosphere.

Answer:----------

Q.7: Write about the physio-chemical properties of soil.

Answer:----------

4.9.Further suggested readings

1. R.C. Dubey and D.K. Maaheshwari, A Textbook of Microbiology, S. Chand Publication-

2013.

2. Ian L. Pepper, Charles P. Gerba, Terry J. Gentry, A Microbiology, Academic Press-2015.
3. K Vijaya Ramesh , Environmental Microbiology, MJP Publication-2019.
4. P.K. Mahapathra, A Textbook of Environmental Microbiology, I K International Publishing House Pvt. Ltd-213.
5. Barbara Kołwzan et al., Introduction To Environmental Microbiology, academic teachers from Wroclaw University of Technology, Poland

Unit-5: Water Microbiology

Contents

5.1. Introduction

Water is one of the naturally occurring essential requirements of all forms of life. The quality of water is decided by its physical, chemical, biological and microbiological composition. The quantity and quality of microorganisms present in water, effect purity of water, diseases caused by them, and effective means of their removal is known as water microbiology. Major area (about three-fourth) of earth surface is covered by water of oceans and to some extent by lakes, rivers, streams, pond etc. Water is constantly in continues circulation known as hydrological cycle or water cycle.

Water is also present in the form of ice in South and North poles of the earth, on the top of high mountains etc. Water receives microorganisms from air, soil, sewage, organic wastes, dead plants, animals etc. The study of diversity of microorganisms-viruses, bacteria, algae, protozoa and microscopic fungi which inhabit and perform activities essential to their life in fresh, estuarine (partiallly enclosed coastal body of brackish water with one or more rivers

flowing into it,and with a free connection to the open sea) and marine waters, including springs, lakes, rivers, bays and seas comes under water microbiology.

A major aim of water microbiology is to control pathogen survival and transfer, and creation of efficient sewage treatment systems. Water microorganisms occupy a central position in the food chain, by providing rich nourishment for the next trophic level, and they are instrumental in recycling of elements. Due to high rate of population growth and increasing industrialization, sources of available water for various purposes such as drinking, recreation, agriculture and aquaculture have been adulterated. Water is the most important common source of various infectious disesases. Waterborne diseases are cholera (*Vibrio cholera*), typhoid fever (*Salmonella typhi*), dysentery (*Entamoeba histolytica*), legionellosis (*Legionella pneumophila*) and giardiasis (*Giardia lamblia*). Hepatitis A and Norwalk viruses are the most significant waterborne viral pathogens.

Objectives

After going through the course of this unit, student will be able to:

- Understand the habitat of microorganisms in water
- Know about the types of microbes in fresh and marine water.
- To know about the microbial analysis of water-coliforms test
- Understand the purification of water by microbes
- To know about the microorganism associated with organic matter decomposition in water

5.2. Water as habitat for microorganisms

Natural water is divided into four types: atmospheric water, surface water, stored water and ground water.

a) **Atmospheric water**: snow and rain fall down on the earth's surface carries dust particles and associated microorganisms in the form of cells or dormant propagules. Quantity and quality of microorganisms varies with the locality. After heavy snow or rain fall, the atmosphere is free from suspended dust particles and microorganisms, that is why, rain water collected after the first shower is free from microbes.

b) **Surface water**: water occurring on the surface of ground in the form of lakes, rivers, streams, wells and ocean water etc. is known as surface water. It is contaminated by microorganisms, soil, domestic and industrial wastes, human and animal wastes in the form of urine and excreta, which are unfit for human consumption. Water without microorganism and chemical substances which is good for health and suitable for drinking is known as potable water. While, contaminated water is known as unpotable water which is unfit for drinking.

c) **Stored water**: stagnant land water present in ponds, lakes etc is known as stored water. Number of microorganisms in this water is affected by several factors.These are –

- **Temperature:** Increasing temperature is harmful to microorganism but some pathogenic microorganisms, e.g., *E. coli*, multiplies well at 37°C. Raw water stored at 22°C shows large number of bacteria.
- **Light Rays:** Prolonged exposure of direct sunlight kills the spores of microorganisms and vegetative cells. Diffused light causes no effect.
- **Food supply:** Increased food supply in water increase the number of microorganisms. Several waste materials present in water act as food bases for microorganisms. In water,there are dissolved gases, such as CO_2, H_2, etc., which have harmful effect on water microflora. These factors also changes the pH of water which later on effect the microbial community.
- **Sedimentation:** Microorganisms have specific gravity, slightly more than distilled water, therefore, they slowly settle down at the bottom of water bodies. Bacterial cells get attached to suspended particles that cause sedimentation.
- Interaction of Other Microorganism: Predatory protozoa need bacteria for their food. Sufficient amount of oxygen increase the population of bacteria which are engulfed by protozoa. Absence of dissolved oxygen and bacteria reduce the population of protozoa.

d) **Ground water:** it originates from deep wells and subterranean springs. Due to filtering action of soil, deep sand and rock, it is free from microorganisms.

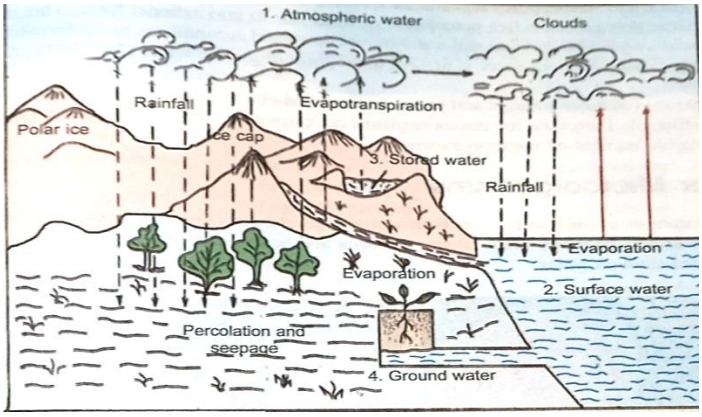


Fig.5.1: Hydrological cycle (based on Ambasht, 1984)

5.3. Water microbiology

Water provides an environment for a wide variety of microorganisms to survive and function. Defining characteristic of water for microbial growth, in comparison to air, is that oxygen which diffuses at a slow rate (average 1 / 10,000). Oxygen once dissolved in water, it is used by microbes at a faster rate, that is why anaerobic zones are created. A water body (fresh or marine) represents a complex ecosystem.

Sun light energy is converted into chemical energy by primary producers algae, cyanobacteria (in aerobic conditions) or bacteria (in anaerobic conditions) through photosynthesis. Carbondioxide utilized by primary producer as their carbon source.

A large number of microorganisms, such as fungi, bacteria, algae, protozoa, nematodes and several animal viruses are found in water.

Both, saprophytic as well as parasitic pathogenic fungi are found in water, e.g., *Achlya americana*, *A. androcomposita*, *A. debaryana* (Khulbe & Sati,1983), *Allomyces neo-moniliformis* (Bhargava, 1945), *A. laevis* (Khulbe & Sati,1983), *Blastocladia simplex* (Mishra & Dwivedi, 1987), *Chytridium breviceps, Cladochytrium setigerum*, *Dictyuchus pisci* (Khulbe & Sati,1983), *Pythium undulatum*, *P. echinulatum, Saprolegnia* spp. (Khulbe & Sati,1983), *Olpidiopsis luxurians* (Khulbe & Sati,1983), *Rhizophydium* sp. (Mishra & Dwivedi, 1987), *Sapromyces indicus.*

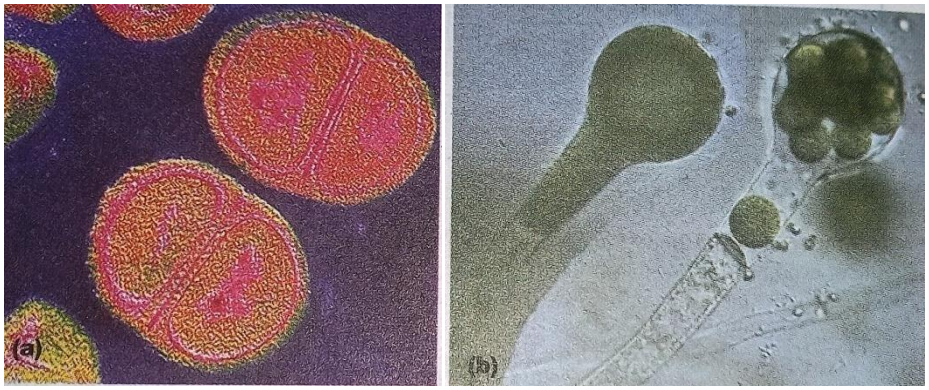


Fig. 5.2: a) The Domain Bacteria, b) *Saprolegnia*- an aquatic fungus

Several bacteria found in water belonging to different groups, such as coliform group (*E. coli*, *Aerobacte*r, etc.), fluorescent bacteria (e.g. *Pseudomonas*, *Alginomonas* etc.), proteus group, non gas forming, non-chromogenic and non-spore forming rods, spore forming bacteria (*Bacillus)*, pigmented and non-pigmented cocci (*Micrococcus*). Many water borne protozoa like *Giardia lamblia* (causes Diarrhea), *Cryptosporidium* (causes Scute enterocoliis), *Acanthamoeba* (causes Corneal ulcers) and *Entamoeba histolytica* (causes Amoebiasis) are found in water.

Table 5.1: Water-borne pathogenic microbes

Disease and Transmission Route	Microbial Agent	Sources of Agent in Water Supply	General Symptoms
Legionellosis (two distinct forms: Legionnaires' disease and Pontiac fever)	Caused by bacteria belonging to genus *Legionella* (90% of cases caused by *Legionella pneumophila*)	*Legionella* is very common organism that reproduces to high numbers in warm water, but only causes severe disease when aerosolized.	Pontiac fever produces milder symptoms resembling acute influenza without pneumonia. Legionnaires' disease has severe symptoms such as fever, chills, pneumonia (with cough that sometimes produces sputum), ataxia, anorexia, muscle aches, malaise, and occasionally diarrhea and vomiting.
Leptospirosis	Caused by bacterium of genus *Leptospira*	Water contaminated by the animal urine carrying the bacteria	Begins with flu-like symptoms, then resolves. The second phase then occurs involving meningitis, liver damage (causes jaundice), and kidney failure.
Otitis Externa (Swimmer's ear)	Caused by a number of bacterial and fungal species	Swimming in water contaminated by the responsible pathogens	Ear canal swells, causing pain and tenderness to the touch.
Salmonellosis	Caused by many bacteria of genus *Salmonella*	Drinking water contaminated with the bacteria. More common as a food-borne illness.	Symptoms include diarrhea, fever, vomiting, and abdominal cramps.
Typhoid fever	*Salmonella typhi*	Ingestion of water contaminated with feces of an infected person	Characterized by sustained fever up to 40°C (104°F), profuse sweating; diarrhea may occur. Symptoms progress to delirium, and the spleen and liver enlarge if untreated. In this case it can last up to four weeks and cause death. Some people with typhoid fever develop a rash called "rose spots", small red spots on the abdomen and chest.
Vibrio illness	*Vibrio vulnificus*, *Vibrio alginolyticus*, and *Vibrio parahaemolyticus*	Can enter wounds from contaminated water. Also acquired by drinking contaminated water or eating undercooked oysters.	Symptoms include abdominal tenderness, agitation, bloody stools, chills, confusion, difficulty paying attention (attention deficit), delirium, fluctuating mood, hallucination, nose-bleeds, severe fatigue, slow, sluggish, lethargic feeling, weakness.

5.3.1. Fresh water microbes

Fresh water environments are characterized by low salinity, and larger variability in temperature, pH, oxygen concentration and different groups of microorganisms. Fresh water lakes and ponds have a characteristic functional stratification. The aquatic ecosystems are classified as the photic and profundal zones, based on the light penetration. The littoral zone or shoreline is an area of shallow water near the shore where light penetrates up to the bottom. The

limnetic zone is above water area, away from the shore, where light genrally does not penetrate all the way to the bottom.

Microorganisms include algae, cyanobacteria, pseudomonads and *Caulobacter*. Profundal zone found between limnetic zone and lake sediment, dominated by purple sulphur and green sulphur bacteria. The soil at the bottom of the water column comprises the benthic zone which is composed of organic debris and mud. *Desulfovibrio* and methane bacteria found in benthic zone. Oxygen rich area contains aerobic bacteria (*Bacillus*) while anaerobic bacteria (*Clostridium*) found in oxygen deficient areas. Fresh water includes ground water and surface water (e.g., lakes, ponds, rivers and springs).

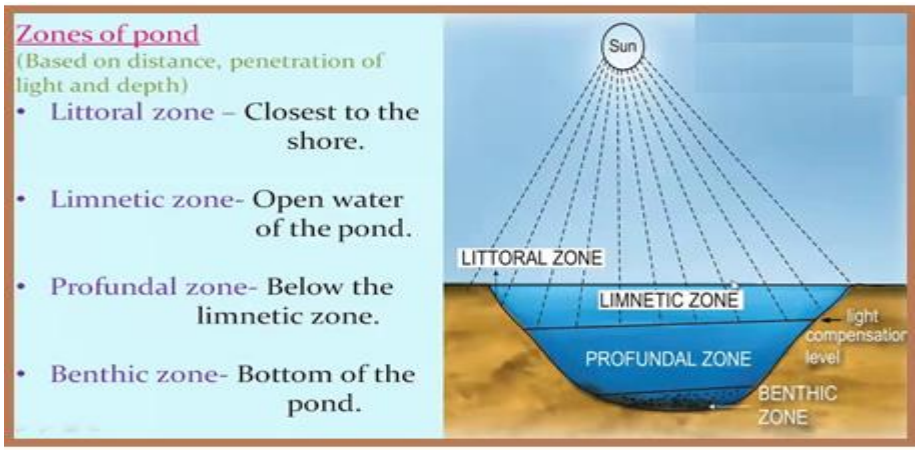


Fig.5.3: Stratification in a fresh water lake

Two important bacteria, *Bacillus* and *Clostridium* involved in recycling of organic detritus. Aquatic fungi are the important parts of the freshwater ecosystems. These include the chytrids and Ingoldian fungi. Chytrids characterized by motile uniflagellate zoospores with a single whiplash flagellum. Ingoldian fungi characterized by the unique tetraradiate conidia belongs to class Hyphomycetes (or Deuteromycetes) for eg. *Alatospora*, *Clavatospora*, *Tetrachaetum* and *Lemonniera*.

The nutrient poor lakes are known as oligotrophic lakes, whereas the nutrient rich lakes are called eutrophic lakes. The eutrophic lakes support luxuriant growth of bacteria and algae. Dal Lake (Srinagar, Jammu & Kashmir) and Naina Lake (Nainital) are the two important fully eutrophicated lakes of India. Some of the fast growing algae, at optimum condition, bloom well

showing their maximum population known as water blooming and microorganisms associated with it called water blooms.

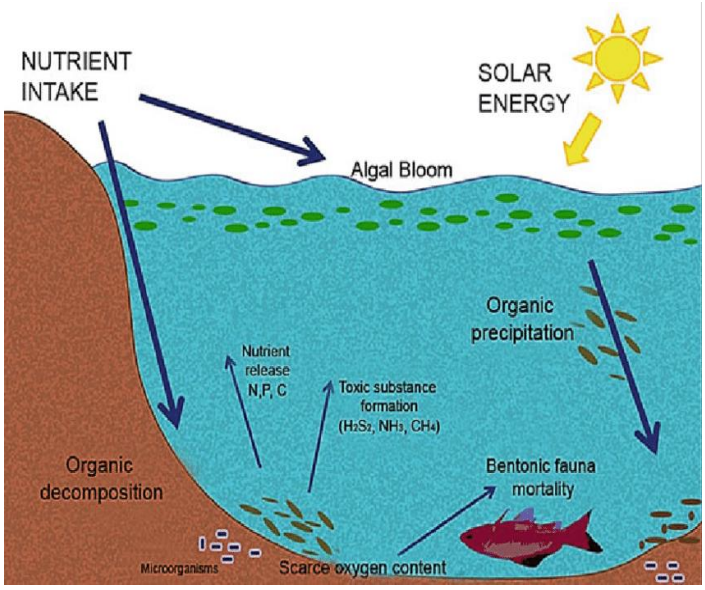


Fig. 5.4: A Eutrophic lake

Nostoc, *Anabaena*, *Microcystis*, *Oedogonium*, *Oscillatoria*, *Spirulina*, diatoms and Protozoa etc. are the microorganisms growing in lakes.

Estuaries are complex systems which receive inputs from various sources. Microbial flora of the estuary is matter to extensive fluctuations in temperature, salinity, turbidity and nutrient load over a wide gradient of space and time. Ascomycota, Chytridiomycota and Deuteromycota divisions of fungi occur in estuary. *Hyphomicrobium*, *Caulobacter* and *Gallionella* bacteria are found in nutritionally poor estuaries.

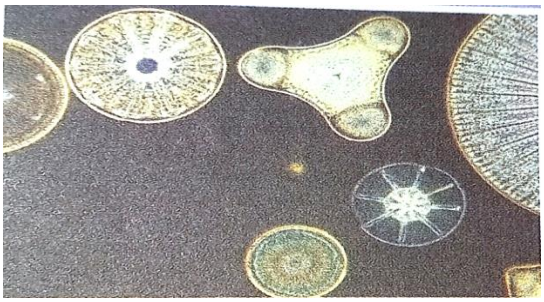


Fig. 5.5: Diatoms growing in a lake

Estuaries have domestic pollution rich with organic nutrients comprising bacteria such as coliforms, fecal *Streptococci*, *Bacillus*, *Clostridium*, *Sphaerotilus*, *Beggiatoa*, *Thiothrix*, *Thiobacillus* etc.

5.3.2. Marine water microbes

Marine environments are characterized by high salinity (3.5% salts), smaller variability in temperature, pH (8.0) and oxygen concentration. The marine environment covers about 70% of the earth's surface and contains 97 % of earth's water. Algae, fungi and bacteria are found in marine environments. The phytoplankton such as Cyanobacteria, diatoms, dinoflagelltes, chlamydomonads and a variety of other protists eukaryotic algae are the primary producers.

A common planktonic alga is *Synecococcus*, *Picocyanobacteria*.

In lower strata heterotrophic bacteria are present, which oxidize organic materials to carbon dioxide, which in turn produce oxygen through photosynthesis. Produced oxygen can be utilized in oxidative respiration. The bacteria also produce vitamins (B_{12}), essential for the growth of many algae.

The bacteria growing in marine environment can be categorized in three groups:

a) Barotolerant (growing between 0 and 400 atm)
b) Moderate barophiles (growing optimally at 400 atm)
c) Extreme barophiles (growing only at higher pressure 6000 to 11,000 meter depth)

Some bacteria living in gut of deep sea invertebrates (eg. Amphipods and Holothurians). These basophilic bacteria play a significant role in nutrient cycling. Recently, Archaebacteria has been discovered from the marine system.

An unusual marine microbe, *Thiomargarita namibiensis* (means the sulphur pearl of Namibia), the world's largest known bacterium, resembles a string of pearls, is found in the coast of Namibia in West Africa.

5.4. Microbial analysis of water- coli forms test

Contaminated, polluted and potable are three categories of water. When water contains a chemical or biological poison and infectious agent are called polluted water. Potable water is free from pathogens, dissolved toxins and disagreeable turbidity, colour, odour and taste.It is fit

for human consumption and is the basis of good health. Water pollution can be physical due to particulate matter (sand or soil), chemical due to inorganic and organic wastes, and biological which develop by microorganisms that comes from human wastes, food processing, meat packing plants and medical facilities.

Human pathogens in water come from contamination of the water with human feces that contain bacteria, viruses and protozoa, chemicals include lead, nitrates, arsenic and radium. Main source of water pollution is municipal and industrial wastes in landfills, residential septic tank systems, active and abandoned oil and gas wells, coals and mineral mines, underground storage tanks at gas stations, and use of large quantities of fertilizers to increase the crop production, and pesticides to control pests and plant diseases by farmers.

Biodegradation of organic wastes, suspended in water, by aerobic decomposers provided that the water contains sufficient oxygen for microorganisms to decompose these substances. The oxygen required for biodegradation is termed as biochemical oxygen demand (BOD). When BOD is high, the water can be depleted of oxygen rapidly, resulting in the decrease in number of aerobic decomposers, and an increase in the population of anaerobes, and slowing the decomposition process.

Purification procedures of water for drinking purposes are determined, depending on the type of impurities present in the water. Monitoring, identification by indicator, and removal of disease causing microorganisms are main part of water purification. Water is tested for faecal contamination, indicating presence of microorganisms (bacteria and viruses). Presence or absence of Escherichia coli indicates that the water is contaminated with faecal material or not. Diatoms represent water quality and pollution tolerance for various environmental parameters, such as pH, nutrients, temperature and salt concentration. Coliform bacteriophages and reoviruses are good indicators of faecal pollution, but their detection is tough. Coliforms are defined as facultative aerobic, gram negative, nonspore forming, rod shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C. Coliforms are the members of the family Enterobacteriaceae which includes *E. coli*, *Enterobacter aerogenes*, *Salmonella* and *Klebsiella pneumonia*.

Faecal coliforms are tested for their presence in water by Klein and Casida, 1967. This test done by standard multiple tube fermentation technique. This methods involve three routine standard test:

i. **Presumptive Test:** A series of fermentation tubes containing lactose broth or lauryl tryptose broth of known concentration, are inoculated with test water. These tubes are incubated at 35°C for 24 to 48 hours. Five fermentation tubes containing single or double broth are inoculated with 10 ml water, 5 tubes with 1 ml water and 5 tubes with 0.1 ml water. After 24 hours of incubation, the tubes indicate that coliforms are absent or present.

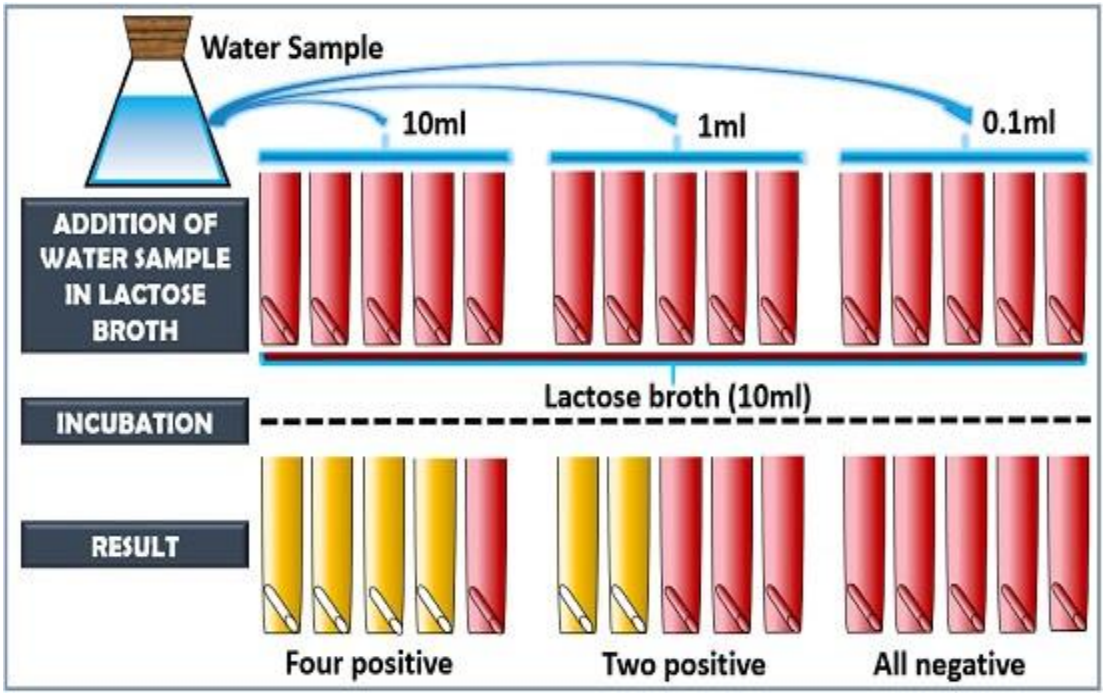


Fig. 5.6: The multiple tube fermentation tests

40 to 390 million per ml coliforms were required to produce visible gas in fermentation broth (Chambers, 1950). However, in most cases 75 million coliforms per ml required to produce thegas. Positive test of gas production indicate the coliforms may be present.

ii. **Confirmed Test:**

Positive test of gas production does not mean coliforms are present. The other organisms also give false positive presumptive test due to their capability to lactose fermenting into acid

and gas production. For example, if yeasts, species of Clostridium and some other microorganisms are present, gas is also produced. Therefore, a confirmed test is performed for the presence of coliforms.

All fermentation tubes showing gas within 48 hours at 35°C are used for confirmed test. It is of two types:

a. The positive presumptive fermentation tube is generally shaken. A drop of its culture is transferred to brilliant green lactose bile broth fermentation tube. These tubes are incubated for 48 hours at 35°C. The appearance of gas within this period indicates for positive confirmed test. The dye (brilliant green) inhibits the Gram-positive bacteria and synergistic reactions of Gram-positive and Gram-negative bacteria for a common food base.

b. The second confirmed test is done by eosine methylene blue (EMB) agar or endoagar method.

In eosine methylene blue agar method, a definite amount of two stains (eosine and methylene blue) is added to a melted lactose agar. The medium is poured into Petri dishes. Over the surface of EMB agar medium, a loopful culture from each positive fermentation tube is streaked. Petri dishes are incubated for 24 hours at 35°C keeping them in inverted position. There develop three types of colonies:

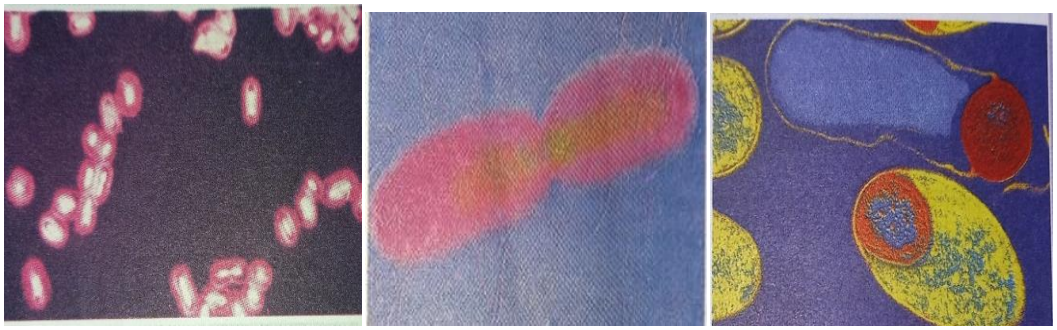
- Typical colonies (nucleated, with or without metallic sheen).
- Atypical colonies (opaque, non nucleated mucoid after 24 hours of incubation, pink).
- Negative colonies (all other types).

The development of typical colonies shows that the confirmed test is positive.

The endoagar medium is prepared by adding basic fuchsin (previously decolouised with sodium sulfite) to a melted lactose agar base. Medium is poured into Petri dishes. A loopful culture from each fermentation tube is streaked over the surface of medium. Petri dishes are incubated at 35°C for 24 hours. Different types of colonies develop. After lactose fermentation, acetaldehyde is produced which is trapped into endoagar.

Acetaldehyde reacts with sulfite to form an additional compound. This results in release of the basic fuchsin from the combination into the agar. Consequently, agar turns into a deep red

colour. The metallic gold like sheen appearing on the surface of typical colonies is due to the precipitation of liberated stain. The restored stain appears purple in colour.



E. coli *Clostridium* **bacteria** *Klebiella pneumonia*

Fig. 5.7

iii. **Completed Test:** it is performed to ascertain about the presence of coliforms in test water. The purpose of the completed test is to determine whether the colonies growing on EMB or endoagar are again capable of fermenting lactose, and forming acid and gas, and the organisms transferred to agar slants show the morphological appearance of coliform group. Each colony that gives positive confirmed test is transferred to lactose fermentation tube and to nutrient agar slants. These tubes are incubated at 35°C for 48 hours.

Production of gas in fermentation tubes and demonstration of Gram-negative, non spore forming rods on the agar slants, constitute a positive completed test for coliforms. The absence of gas and the rod production confirms for negative test of coliforms.

The Most Probable Number (MPN) of Coliforms: Hoskins (1934) computed the MPN to evaluate coli-aerogenes test by fermentation tube method.

The Membrane filter technique: A new method for enumeration of coliform organisms in water described by Goetz and Tsuneishi (1951) named as milipore filter technique. Commonly known as membrane filter technique. The filtering apparatus consists of a glass or stainless steel funnel and a flask. The funnel of stainless steel is clamped to a base containing a molecular filter. The stem of base is inserted into a filter flask through a rubber stopper. A sterile membrane filter (0.45 µm) disk is placed in the sterilized holding apparatus (A). A volume of water is passed through filter disk. Bacteria present in water sample are retained on filter disk. The sides of funnel and membrane are rinsed with sterile distilled water. Therefore, the membrane filter disc

is aseptically removed by a sterile forcep, and placed on absorbent disk, saturated with culture medium and contained in Petri dish (C). The medium passes through the pores of membrane and nourishes the bacteria present on it (D). After proper incubation at 35°C for 24 hours, each bacterium multipies to form a visible colony on membrane (D). The colonies are easily counted. This method has both advantages and disadvantages:

Advantages:

- It permits the small numbers of bacteria from large quantities of water. Therefore, it increases the accuracy and reliability of counting bacterial colonies.
- It does not allow spreading the combination of any number of bacteria from a few to 5,000 at a time. There is no need of making dilutions of water.
- It permits the separation of bacteria from their nutrients at any time.
- It allows the direct counting of microorganisms instead of counting most probable number.
- It is time saving method, permitting faster differentiation of bacteria and giving a permanent record if filter disks are preserved.
- This method is very useful in emergency.

Disadvantages:

- In turbid waters, containing algal growth and other materials, the pores of membrane filter are blocked. Therefore, filter prevents the testing of sufficient sample and fails to give accuracy of coliforms.
- High populations of non coliforms and other bacteria cause overgrowth. Therefore, cannot be counted accurately.
- Metals and phenols can be absorbed to membrane filter and therefore, inhibit growth of bacteria.

Table 5.2: Most Probable Number (MPN) of colform organisms present in 100 ml water.

Sample number	Quantity of water (ml)			MPN per 100 ml
	10	1	0.1	
	No. of samples of each quantity tested			
	3	3	3	
	Number of tubes giving positive reactions (acid and gas)			
1	2	3	1	36
2	3	2	2	210
3	3	3	2	1100
4	2	0	3	16
5	3	3	2	1100
6	3	3	0	240
7	2	3	3	53
8	3	3	3	1100
9	3	3	3	1100
10	3	1	0	43

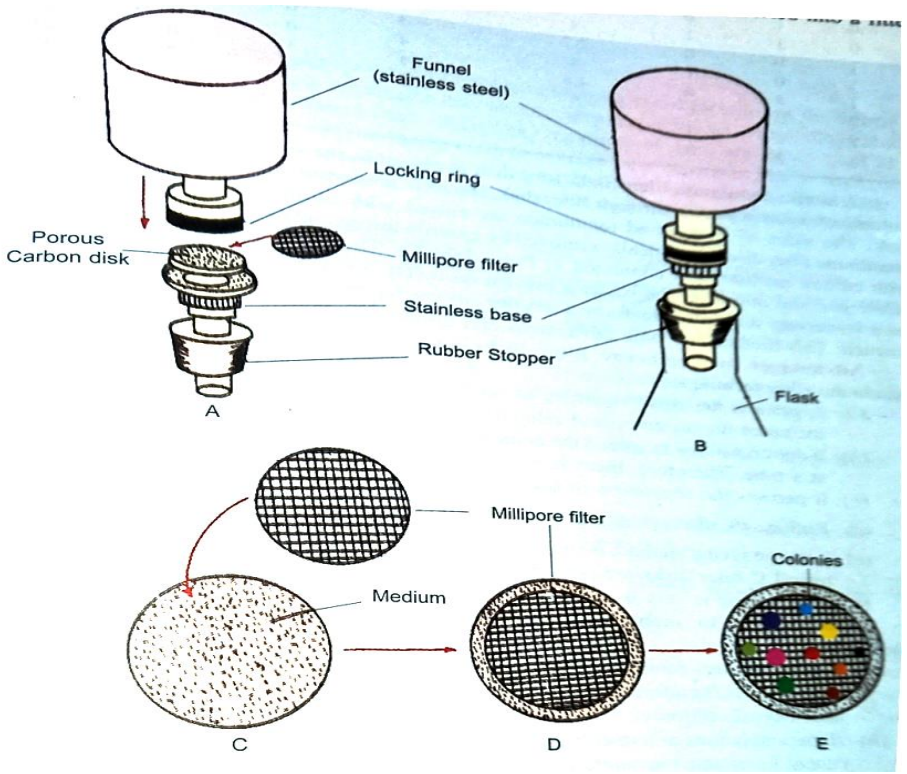


Fig.5.8: A Millipore filter system: A. unassembled view B. assembled view C. plating of membrane filter containing filtered water sample on appropriate medium D. Incubation for 24 hours E. colonies of typical coliforms growing over membrane filter.

5.5. Purification of drinking water

Water purification is essential before its consumption so that disease cycle of pathogenic microorganisms can be broken. Water purification is done with the prospect of making it satisfactory in appearance, taste, odour and free from pathogens. There are three chief methods which are used for the purification of drinking water in municipal supplies i.e., sedimentation, filtration and disinfection (Kabler, 1962).

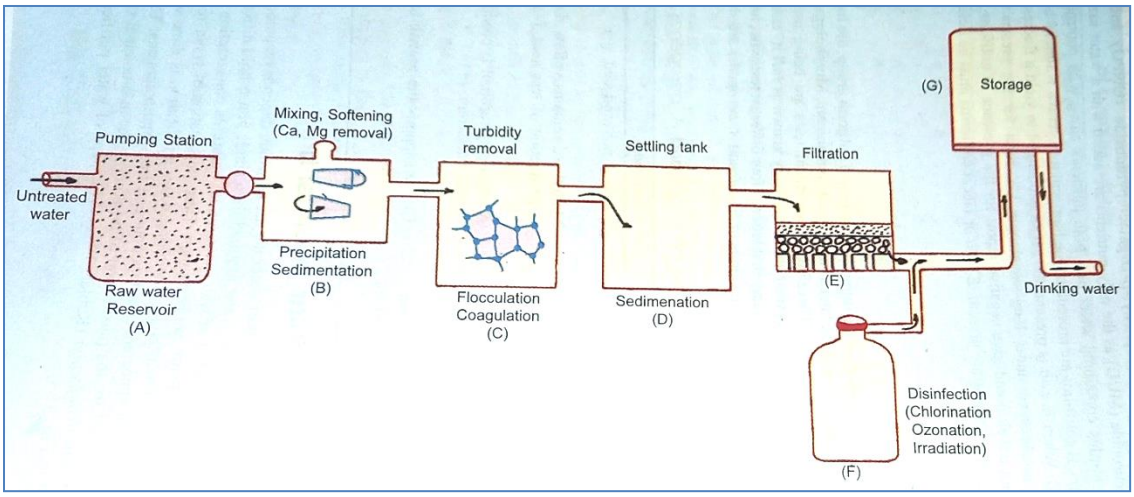


Fig.5.9: Steps of water purification of municipal water supplies.

a. Sedimentation:

This method use when water consists of large sized organic materials such as leaves, and gravels which have run off from the soil. Depending on their size, weight and conditions of the stored water, suspended particles were settled down. Sedimentation is done in large reservoirs or in restricted area of settling tank. The rate of sedimentation is enhanced by adding alum, iron, salts, colloid silicates which act as coagulants. The suspended materials and microorganisms are entrapped by coagulants and settle down rapidly.

This procedure is called coagulation or flocculation. The microorganisms remain viable for some time. Thus, sedimentation provides partial reduction of microorganisms in water due to their settling down on bottom but does not sterilize the polluted water.

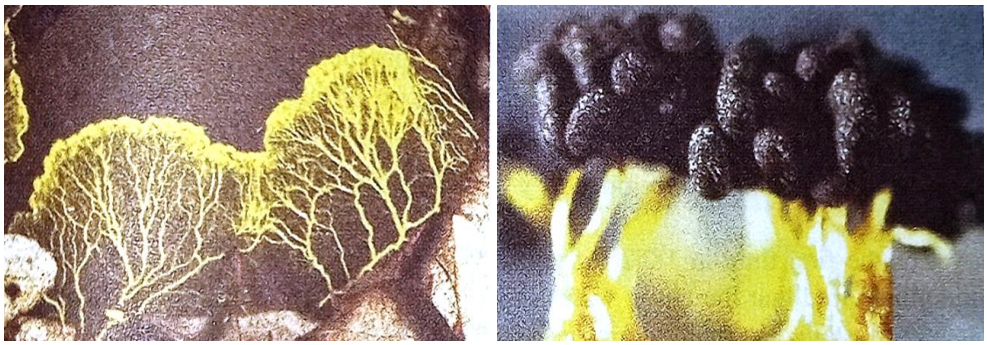


Fig. 5.10: *Physarum* (a slime mould) is reduced as it settles down on bottom

b. Filtration:

It is the second step of purification of water. This is the effective means of removing microorganisms and the other suspended material from the water. After sedimentation, the water is further purified by passing in to filtration unit. **There are two types of sand filters which are used in water purification:**

i. **Slow Sand Filter:**

The rate of filtration of water is slow; hence the plant requires a considerable area. This plant consists of a concrete floor containing drainage tiles (for collection of filtered water). The tile is covered with first coarse sand and finally 1 to 2 feet of sand at the top of plant.

Water passes slowly through the filter and collected by tile drain pipes at the bottom, which later on is pumped into a reservoir. Filters are blocked, if water is turbid. Therefore, turbid water should be clarified first by sedimentation, thereafter, passed through slow sand filters. The capacity of slow sand filter plant is to filter about 5 million of water per acre per day.

Water purification is done not only by physical action but also by physiological mechanisms, supported by microorganisms. In the surface of layers of fine sand, a colloidal material, consisting of bacteria, algae and protozoa, is attached. This mucilaginous material makes the pores more effective by closing the pores between the sand grains. Sand grains have positive charges and bacterial cell walls have negative charge.

Therefore, bacteria are adsorbed on the surface of sand. Protozoa ingest bacteria. Due to intense microbial interactions, chemical concentration of water is reduced.

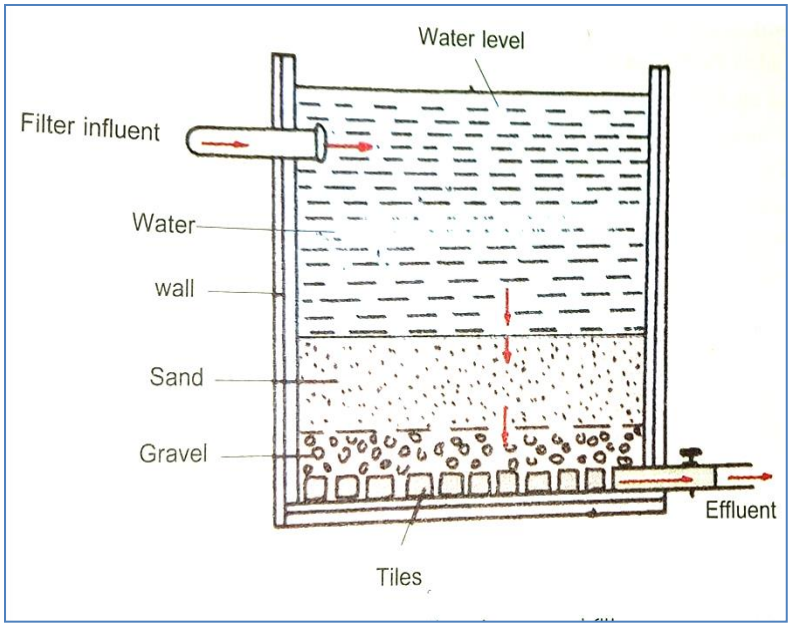


Fig. 5.11: Slow Sand Filter

When filtration efficiency of the plant is reduced, due to deposition of thick mucilaginous material, the plant is taken out for cleaning. Through this plant, the pathogenic microorganisms such as *Giardia* and its cysts, which are not removed by any other methods, can be filtered from water.

ii. **Rapid Sand Filter:**

This water filter plant consists of layers of sand, gravel and rock. Before filtration, water is treated with alum or ferrous sulphate in a settling tank where precipitates settle down. Then, water is allowed to pass through rapid sand filter plant. This plant depends on physical trapping of fine particles and floes or coagulants. The pores of the plants are soon blocked. It is cleaned by forcing cleaned water backward.

About 99% bacteria are removed by this plant. But unfortunately the use of coagulants, rapid filtration and chemical disinfection often does not remove *Giardia lamblia* cysts, *Cryptospordium* oocysts, *Cyclospora* and viruses.

Rapid sand filter plant operates about 50 times faster than slow sand filter plant, and can delivers about 150 to 200 million gallons of water per acre per day. It requires less land area, less cost and less maintenance.

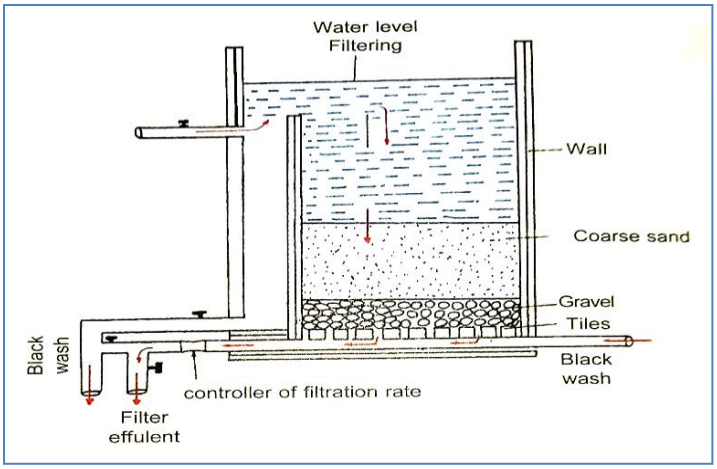


Fig. 5.12: Rapid Sand Filter

c. Disinfection:

Disinfection is the final step of water purification. Solutions of sodium hypochlorite are treated in small towns but in recent years, chlorination of public water supply has become popular. Some of the bacteria pass through filter even after filtration which must be killed before consumption of water. Therefore, disinfection of public water supply needs to be done. Chlorination involves the release of chlorine gas in water which gets readily mixed up with water. The amount of chlorine required depends on organic matter and number of microorganisms present in water, and duration of time to act upon. High concentration of chlorine quickly acts upon microorganisms and vice-versa. Therefore, the amount of chlorine required for disinfection is called chlorine demand. Chlorinated water contains about 0.1 to 0.2 ppm of residual chlorine which reaches to this concentration after 20 minutes of its addition.

Prolongs chlorine action in water containing high amount of organic matter, chloramines are formed. Change in odour and taste of water is due to the formation of chlorophenols. In the presence of high organic matter, chlorine reacts with it and produces halomethanes which are a group of carcinogenic compounds. The mechanism of action of chlorine on microorganisms is obvious. After reacting with water, chlorine is converted into hypochlorous acid which in turn quickly releases nascent oxygen.

The nascent oxygen soon oxidizes the cellular components of microorganisms as well as organic matter. However, chlorine fails to kill the microbial spores. The other gas which behaves

like chlorine is the ozone. The simplest method to make water free from microbes and for consumption is boiling for 10-15 minutes.

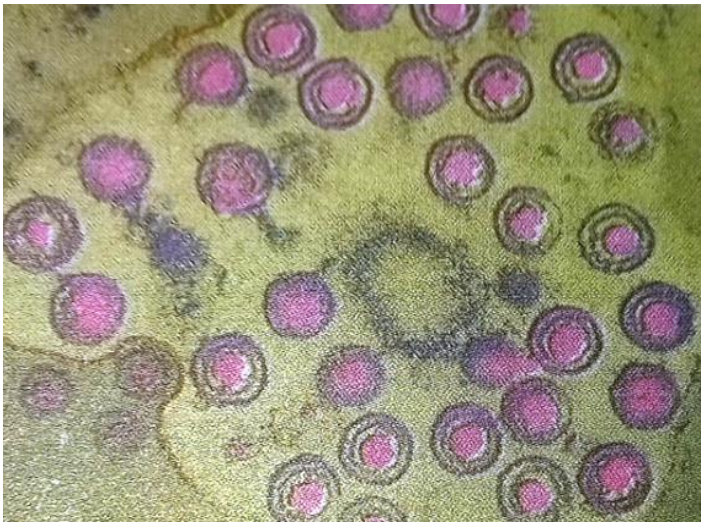


Fig. 5.13: Viruses are not removed by the use of coagulants and rapid filtration

5.6. Microorganism associated with organic matter decomposition in water

Acceleration of biodegradation of specific compounds by inoculating bacterial cell is called bioaugmentation (use of blends of microorganisms). Some bacteria contain specific plasmid which encodes enzymes for degradation of orgaic compounds. A variety of plasmids have been reported from *Alcaligenes*, *Acinetobacter*, *Arthrobacter*, *Beijerinkia*, *Klebsiella*, *Flavobacterium* and *Pseudomonas*. Several genetically engineered strains have been developed exploiting *Pseudomonas*.

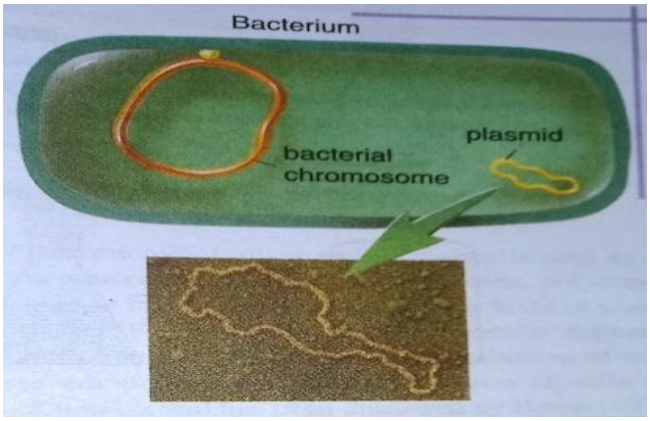


Fig.5.14: Bacterial plasmids encode enzymes for biodegradation of specific compounds

Microorganisms capable of degrading herbicides and other chemicals in industrial water are isolated from waste water, compost, sludge etc. Some of the strains may be irradiated to enhance their ability and mutants are selected. They are tested in laboratory before their use in the environment for their biodegradation ability. Selected strains are used in large fermentor to get mas culture. They are preserved through lyophilization, drying and freezing. Commercial bioaugmentation products are single culture of consortia of microorganisms with certain degradative properties of their desirable characters.

Phenol, ethylene, glycol and formaldehyde are hazardous wastes preset in water treated with the help of microorganisms. *Candida tropicalis* cells are used for removal of high concentration of phenol in fresh water. Ability of a bioreactor to dichlorinate 3-chlorobenzoate was increased after addition of anaerobic bacterium,Disulfomonile tiedjei to a methanogenic up flow anaerobic granular sludge blanket.

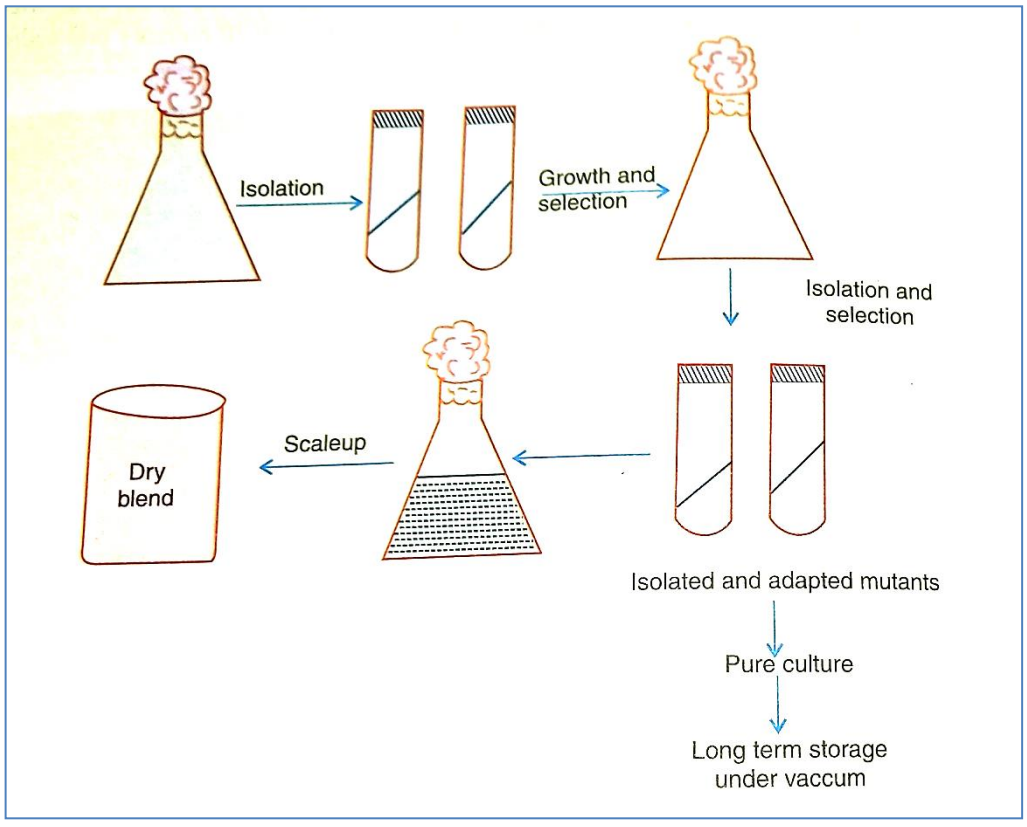


Fig. 5.15: Isolation and Purification of microbial blends use for pollution control

Anoxygenic phototropic bacteria have also been considered for the degradation of toxic compounds in wastes (Bitton, 1999). Application of biaugmentation includes:

a) The increased BOD removal in wastewater treatment plants
b) Reduction of sludge volume by about 30% after addition of selected microorganisms.
c) Use of mixed cultures in sludge digestion
d) Biotreatment of hydrocarbon waste
e) Biotreatment of hazardous wastes

There are several microorganisms growing in marine water, fresh water and waste water. They are removed by use of metals by the following mechanisms: adsorption, complexaton, precipitation and volatilization.

Desulfovibrio and *Desulfotomaculum* transform SO_4 to H_2S which promotes extracellular precipitation of insoluble metal suphides. *Klebsiella aerogenes* detoxifies cadmium to cadmium sulphate which precipitate on cell surface.

Bacillus licheniformis and *Zooglea ramigera* have been isolated from activated sludge. They produce extracellular polymer. The complex which accumulate metals, such as iron, copper, cadmium, nickel or uranium. The accumulated metals are released from biomass upon treatment with HCl. Fungal mycelia (*Aspergillus* and *Penicillium*) also remove metals from waste water. Aspergillus oryzae can remove Cadmium efficiently from solution.

Genetically engineered microorganisms (GEMs) are use to detect the pathogen and increase biodegradation of xenobiotics in waste water purification. Nucleic acid probes and PCR are the major tools of recombinant DNA technology to detect pathogens in effluents of waste water. *E. coli*, *Shigella flexneri*, *Salmonella*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Yersinia*, *Hepatitis* A virus, HIV and *Giardia* are some pathogens detected by PCR.

Genetically engineered microorganisms are used in several areas of waste water treatment, such as in biomass production, biodegradation of recalcitrant, removal of toxic metals, in fermentation (methane and organic acid production), in enhancement of enzyme activity, and increased resistance to toxic inhibitors.

Recombinant DNA technology is involved in two steps: Searching out microorganisms of desired function and transfer of character of desired function to the other microbes relevant to environment.

Table 5.3: Microorganisms involved in metal removal from industrial waste water

Class of Microorganisms	Heavy Metal Removed
1. Bacteria	
Bacillus cereus strain XMCr-6	Cr (VI)
Kocuria flava	Cu
Bacillus cereus	Cr (VI)
Sporosarcina ginsengisoli	As (III)
Pseudomonas veronii	Cd, Zn, Cu
Pseudomonas putida	Cr (VI)
Enterobacter cloacae B2-DHA	Cr (VI)
Bacillus subtilis	Cr (VI)
2. Fungi	
Aspergillus versicolor	Ni, Cu
Aspergillus fumigatus	Pb
Gloeophyllum sepiarium	Cr (VI)
Rhizopus oryzae (MPRO)	Cr (VI)
3. Yeast	
Sacharomyces cerevisiae	Pb, Cd
4. Algae	
pirogyra spp. and *Cladophora* spp.	Pb (II), Cu (II)
pirogyra spp. and *Spirullina* spp.	Cr Cu, Fe, Mn, Zn
tylon, Oedogonium and *Rhizoclonium* spp.	As

Many plasmids containing *Pseudomonas* strains are used to degrade several components of crude oils. These technologies increase the level of several enzymes and improve enzyme stability and catalytic efficiency, increase their substrate range, such as tryptophan synthetase, α-amylase, DNA ligase, benzylpenicillin acylase.

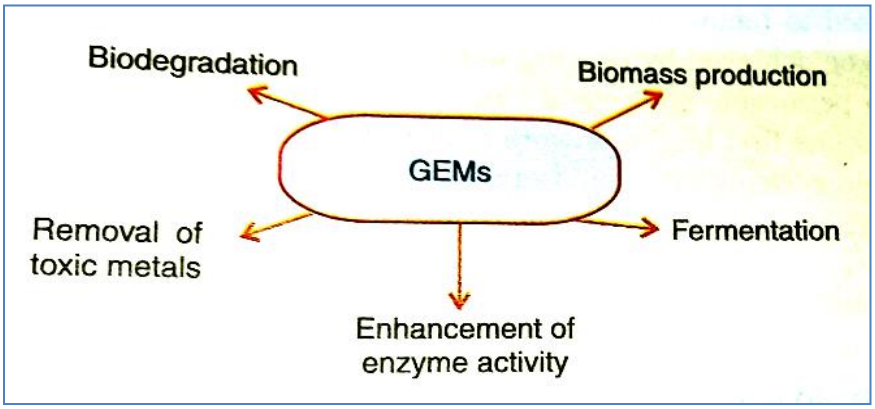


Fig. 5.16: Application of Genetically engineered microorganisms (GEMs) in different Areas.

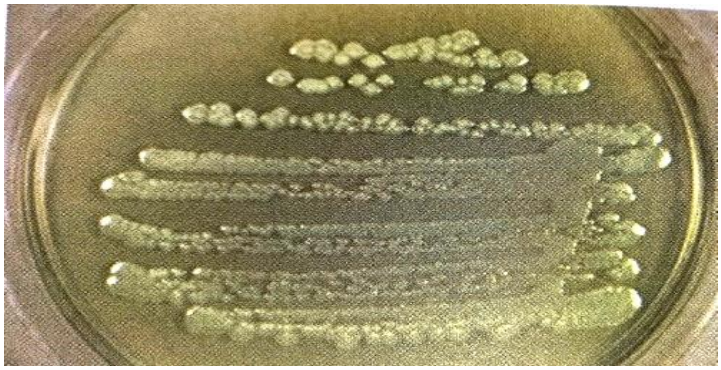


Fig. 5.17: Genetically engineered *Pseudomonas* strains

5.7. Summary

Water is one of the naturally occurring essential requirements of all forms of life. The quality of water is decided by its physical, chemical, biological and microbiological composition. The quantity and quality of microorganisms present in water, affects the purity of water, diseases caused by them and effective means of their removal, is known as water microbiology. A major aim of water microbiology is to control pathogen survival and transfer, and creation of efficient sewage treatment systems. Natural water is divided into four regions: atmospheric water, surface water, stored water and ground water. **Atmospheric water-** snow and rain fall down on the earth's surface carries dust particles and associated microorganisms in the form of cells or dormant propagules. **Surface water-** occurring on the surface of ground, in the form of lakes, rivers, streams, lakes, wells and ocean etc. It is contaminated by microorganisms, by soil, by domestic and industrial wastes, by human and animal wastes in the form of urine and excreta, which are unfit for human consumption. **Stored water-** stagnant land water present in ponds, lakes etc.,have reduced microorganism number. However, this number is affected by several factors, such as temperature, food supply, light rays and sedimentation. **Ground water – it** originates from deep wells and subterranean springs. Due to filtering action of soil, deep sand and rock, it is free from microorganisms.

Water provides an environment for a wide variety of microorganisms to survive and function. A body of water (fresh or marine) represents a complex ecosystem. Sun light energy converted into chemical energy by primary producers algae, cyanobacteria (in aerobic conditions) or bacteria (in anaerobic conditions) through photosynthesis. Carbondioxide utilized

by primary producer as their carbon source. Both, saprophytic and parasitic pathogenic fungi are found in water, e.g., *Achlya americana*, *A. androcomposita*, *A. debaryana*.. Several bacteria found in water belongs to different groups, such as, coliform group (*E. coli*, *Aerobact*er, etc.), fluorescent bacteria (e.g. *Pseudomonas*, *Alginomonas* etc.), proteus group, non gas forming, non-chromogenic and non-spore forming rods, spore forming bacteria Bacillus, pigmented and non-pigmented cocci (*Micrococcus*). Fresh water environments are characterized by low salinity and larger variability in temperature, pH, oxygen concentration and different groups of microorganisms. Fresh water lakes and ponds have a characteristic functional stratification. Microorganisms include algae, cyanobacteria, pseudomonads and *Caulobacter*. Profundal zone found between limnetic zone and lake sediment, dominated by purple sulphur and green sulphur bacteria. Two important bacteria *Bacillus* and *Clostridium* involved in recycling of organic detritus. Marine environments are characterized by high salinity (3.5% salts), smaller variability in temperature, pH (8.0) and oxygen concentration. A common planktonic alga is *Synecococcus, Picocyanobacteria.* Faecal coliforms are tested for their presence in water by Klein and Casida, 1967. This test done by standard multiple tube fermentation technique. These methods involve three routine standard tests:

Presumptive Test: A series of fermentation tubes containing lactose broth or lauryl tryptose broth of known concentration, are inoculated with test water**. Confirmed Test:** Positive test of gas production does not mean coliforms are present. **Completed Test:** it is performed to ascertain about the presence of coliforms in test water.

The purpose of the completed test is to determine whether the colonies growing on EMB or endoagar are again capable of fermenting lactose, and forming acid and gas, and the organisms transferred to agar slants show the morphological appearance of coliform group. Hoskins (1934) computed the MPN to evaluate coli-aerogenes test by fermentation tube method. Microorganisms capable of degrading herbicides and other chemicals in industrial water are isolated from waste water, compost, sludge etc. Some of the strains may be irradiated to enhance their ability and mutants are selected. Phenol, ethylene, glycol and formaldehyde are hazardous wastes present in water are treated with the help of microorganisms. *Candida tropicalis* cells are used for removal of high concentration of phenol in fresh water. Ability of a bioreactor to dichlorinate 3-chlorobenzoate was increased after addition of anaerobic bacterium, Disulfomonile tiedjei to a methanogenic up flow anaerobic granular sludge blanket.

5.8. Terminal Questions

Q.1: Give a detailed account on different types of water found in environment.

Answer: ----------

Q.2: Write an essay on microorganisms of water.

Answer: ----------

Q.3: Write in brief about chief methods of purification of drinking water.

Answer: ----------

Q.4: Discuss about the completed tests for coliforms.

Answer: ----------

Q.5: Write short notes on the following:

a. Microorganisms of organic matter decomposition in water
b. Sedimentation
c. Membrane filter technique
d. BOD.
e. MPN
f. COD

Q.6: Briefly explains Marine microbiology.

Answer: ----------

5.9. Further Readings

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Unit-6: Microbiology of Air

Contents

6.1. Introduction

Air is not a good medium for microorganism, but it is a carrier of particulate matter, dust and water vapour droplets, which is usually place of various microorganisms. Air does not contain the necessary amount of moisture and nutrients needed for growth and metabolism of microorganisms. Therefore,they do not grow and reproduce in air, and also due to high light intensities and extreme temperature variations. Air normally contains plenty of microorganisms which enter into it from soil and dry decomposed matter exposed to the action of wind. Healthcare units, industrial operations and agricultural practices also spread microorganism in the air. A variety of microorganisms such as bacteria, fungi, actinomycetes, algae, spores of pteridophytes, pollen grains, micro-insects and viruses are present in the air.

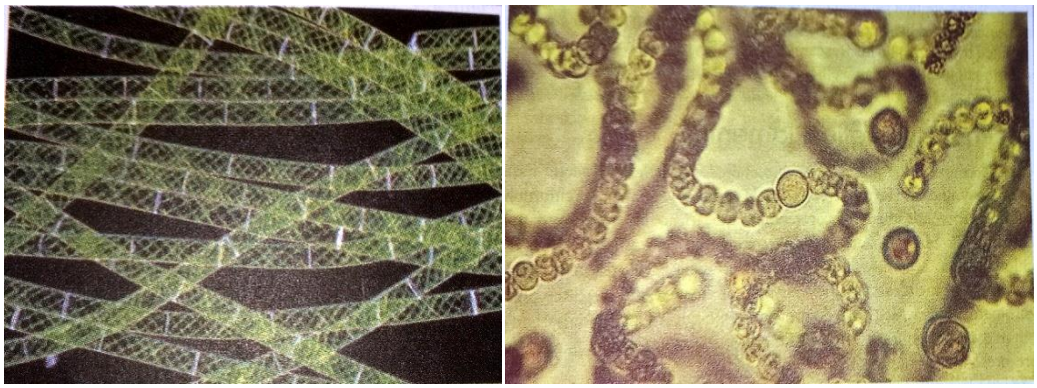


Fig.6.1: a. Spirogyra and b. Nostoc

The most common algae found in air are the species of *Chlorella*, *Chlorococcum*, *Chlamydomonas*, *Aulosira*, *Nostoc* and *Phormidium*. Diatoms, *Protococcus*, *Spirogyra*, *Oscillatoria* etc. The most common lichens were the species of *Cladonia*, *Heteroderma*, *Parmelia*, *Usnea* etc.

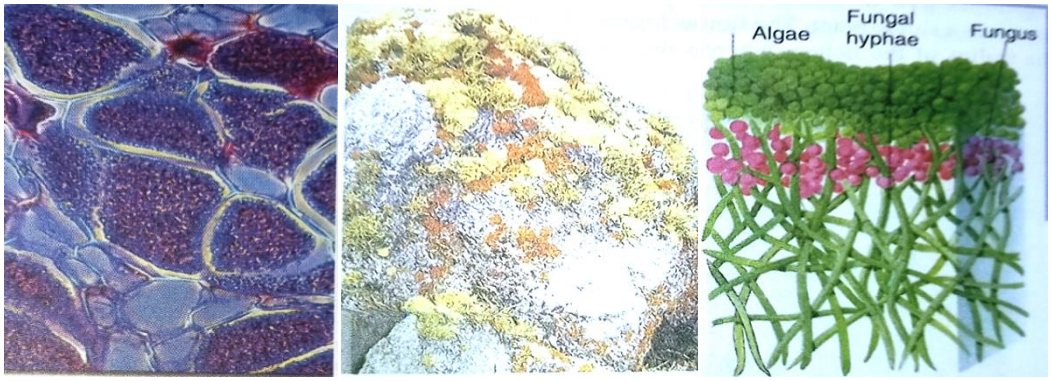


Fig.6.2: a. Lichen and b. T.S. of a Lichen thallus

In rainy season beauty of the world fame Taj Mahal fades due to the growth of algae on it. Hydrogen sulphide, sulphur dioxide, carbon monoxide, chlorine, hydrogen fluoride, ozone etc. are the important gases that affect the microorganisms growth. Airborne biological materials are known as airspora or bioaerosols. Exposures to bioaerosol, pollution cause contagious infectious diseases, such as whooping cough (*Bordetella pertussis*), tuberculosis (*Mycobacterium tuberculosis*), diphtheria (*Corynebacterium diptheriae*), leprosy (*Mycobacterium leprae*), allergies and cancer.

Objectives:

After going through the course of this unit, students will be able to:

- Understand the aeromicrology
- Know about the types of aerofungi.
- Understand the types of aeromicroflora of pharmacy, hospital and houses
- To know about the microflora of Phylloplane and their characteristic
- Understand the Phylloplane pathogens

6.2. Define Aeromicrobiology

The study of microorganisms present in the air, their influence on other living organisms and methods of their removal is known as Aeromicrobiology.

F.C. Meier in 1930, coined the term aerobiology. Since then, aerobiology has been defined by many workers as "the study of the aerosolization", "aerial transmission, and deposition of biological materials". Others have defined it more specifically as the study of diseases that may be transmitted via the respiratory route (Dimmick and Akers, 1969), "the study of airborne organisms and their effect on human health and the environment". In wider aspect aeromicrobiology is the study of all forms of microbial life present in air. According to environmental view, it is the study of various aspects of intramural (indoor) and extramural (outdoor) aerobilogy in relation with the airborne transmission of environmentally relevant microorganisms, including viruses, bacteria, fungi, yeast and protozoans. Several microbes like *Pseudomanas fluorescence* and *Mycobacterium* species can degrade simple polyaromatic hydrocarbons such as naphthalene and more toxic benzopyrene found in coal tar and cigarette smoke.

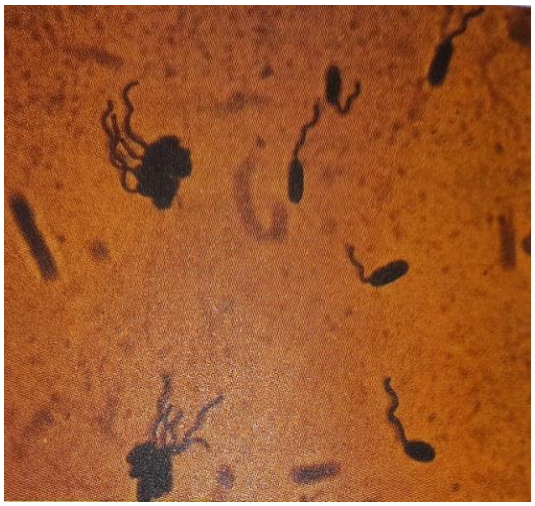


Fig. 6.3: Pseudomanas fluorescence

6.3. Aerofungi

Fungal aerosols are present in much higher concentration. Moulds and yeasts are commonly found in the air due to resistant to light. The fungi release their spores in dry state into the air. Spores and hyphal fragments are two forms of fungi present in the air. Spores are ideally adapted to air transport. They are inspite of being subjected to gravity, remain suspended in the atmosphere and are dispersed through air flow. Hyphal fragments constitute population of air borne microflora and asexual conidiophores. These infect leaves of plants and dispersed by mechanical action of air current. The air microflora mainly consists of spores of *Alternaria*, *Cercospora*, *Helminthosporium*, *Puccinia*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Homodendrum*, *Zygosporium*, *Aureobasidium*, *Pithomyces*, *Mucor* , *Rhizopus*, *Streptomyces*, *Bacillus*, *Clostridium*, *Stemphylium*, *Curvularia*, *Acremonium*, *Fusarium* and *Nigrospora*. Brown and black rust of wheat are disseminated from the hills of South India to Central India by "hop jump" in which a storm depression formed in Bay of Bengal / Arabian Sea reaches Central India (K.C. Mehta, 1940-52; Joshi et al., 1972; Nagarajan and Singh, 1973). Nagarajan and Singh, 1973, found that the satellite television cloud photography as a possible tool for forecasting the spread of several plant pathogens such as *Alternaria*, *Cercospora*, *Helminthosporium*, *Puccinia* etc. Aeromicrobial investigations on several crops, such as rice, wheat, jwar, bajra, cotton, banana, citrus, sugarcane and vegetables have been carried out by many scientists. Studies on microbial components of air over crop fields are useful in understanding the plant pathogens and in establishing the forecasting system for disease control. Navneet (1995-96) studied aeromycoflora over potato field at Kurukshetra for two years and rcorded 25 fungal species. The dominant fungi were *Alternaria alternate*, *Aspergillus flavus*, *A. niger*, *Cladosporium herbarum*, *Epicoccum nigrum*, *Penicillium citrinum*, *P. cyclopium* and *Trichothecium roseum*. Rain has the propound effect on aerofungi.

Fig. 6.4*: streptomyces and Aspergillus*

Verma et.al. (1998) recorded 62 aerospora over rice field by using Tilak sampler. The most dominant fungal taxa was *Aspergillus* (75%), followed by *Penicillium* (10%), *Alternaria* (3%) and *Cladosporium* (2%). Commonly aspergilli forms a major component of aerospora including pathogenic species, *A. Niger*. Many species of *Alternaria* (*A. brassicae*, *A. dauc*i, *A. porri*, *A. solani*) cause disease on several crop plants.

Some saprophytic fungi secreting aflatoxins as secondary metabolites which are injurious for human health. The species of fungi secreting aflatoxins are *Aspergillus flavus, A. parasiticus* and others. Environmental factors are responsible for occurrence of aerofungi in different regions. Choudhary (1991) surveyed the climatic conditions on incidence and severity of aflatoxin contamination of field maize crop during 1986-1990 which were cultivated as Kharif crop in Bihar. He noticed the temperature during July-August and prevalence of relative humidity for prolonged time are the major determinants of aflatoxin contamination.

6.4. Aeromicroflora of pharmacy

Escherichia coli, *Salmonella typhoid's*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* detected in pharmaceuticals raw materials of natural or biological origin such as starch, gums, gelatin etc. *E. coli* is a bacterium commonly encountered in the gastrointestinal tract of mammals and detectable in their faeces. Some strain of *E. coli* exist as harmless commensals in the intestine, while, some are pathogenic and produce enterotoxins, which are responsible for diarrhoeal disease. Exclusion of *E. coli* from pharmaceuticals materials is essential to avoid the risk of infection. *Staphylococcus aureus* inhibits in the human skin and nose without any apparent ill effect. Some strain exhibit a marked pathogenic potential and cause serious infections. They may originate on the skin but progress to other site.

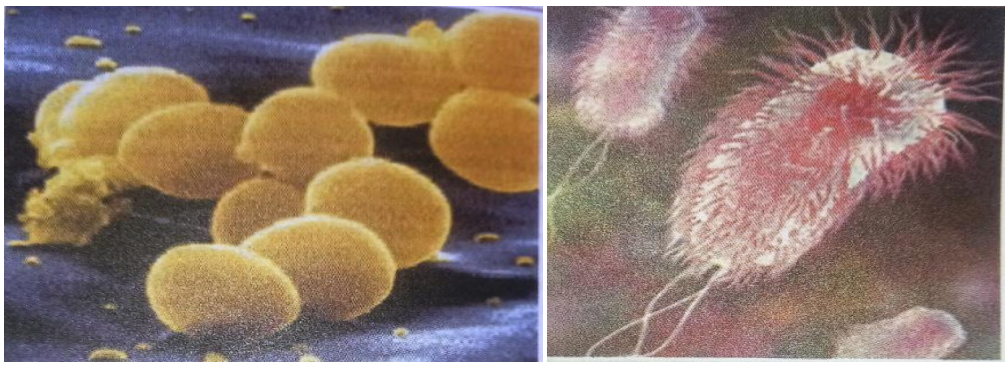


Fig. 6.5: *E. coli and Staphylococcus aureus*

Salmonella is also an inhabitant of mammalian intestine. Its pathogenic potential is substantially greater than that of *E. coli*. Some strains can initiate infections from the ingestion of very small number of cells.

Pseudomonas aeruginosa is a potential pathogen capable of causing infection at most vulnerable sites in healthy persons such as eyes. It may cause infection in many regions of the body with underlying disease or impaired immunity. This organism would be a potential problem as contaminant of pharmaceutical material due to resistant to preservatives. This bacterium is a fast growing with low nutritional requirements. Water is one of natural habitats of this bacterium. Stored water is more likely to be contaminated than freshly purified water.

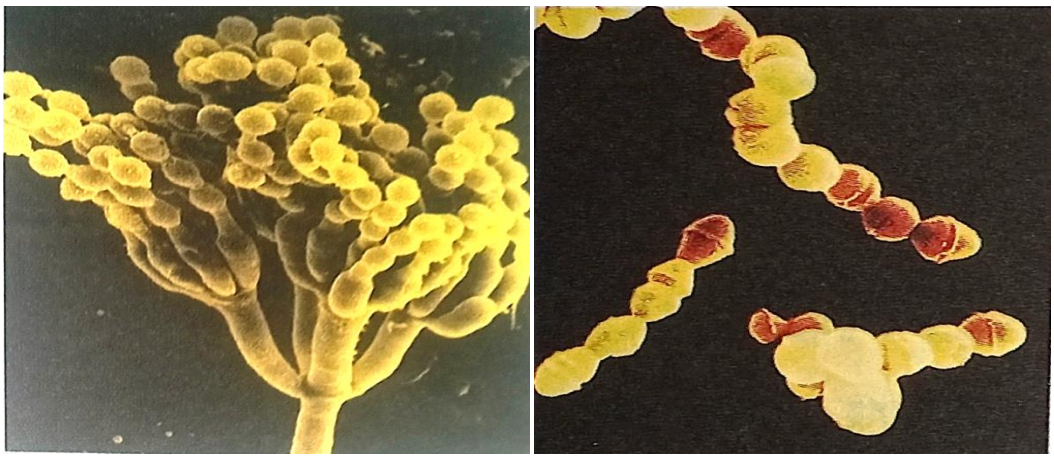


Fig.6.6: *Penicillium sp and Pseudomonas* sp.

Cladosporium cladosporioides, Alternaria sp., Penicillium cyclopium, Epicoccum nigrum etc. are reprted by Sharma and Navneet (1996) from the fermerntation unit of Gurukul Kangri, Pharmacy, Haridwar.

The environmental factors, such as relative humidity and temperature affected their occurrence. Microbial quality of non sterile pharmaceuticals products can be controlled by the adopting two types of standard:

i. Limit of the total numbers of viable organisms in a given weight or volume of liquid known as total viable count (TVC). This determined by using membrane filtration techniques, plate count method, multiple-tube or serial dilution method.

ii. Total exclusion of specific pathogens.

6.5. Aeromicroflora of hospital and other houses

Aspergillus flavus, *Aspergillus fumigates*, *Mycobacterium tuberculosis*, *Candida albicans*, *Staphylococcus aureus*, influenza virus etc. (Tilak, 1982) are hospital transmitted pathogens. The species of *Aspergillus* causes lung infection in hospital environment. *Candida albicans* caused candidiasis and contaminates the hospital wards through direct contact with fingers. Coughing and sneezing cause the spread of microorganism and viruses. Squames from the skin of persons in the operation theatre transmit pathogenic microorganisms.

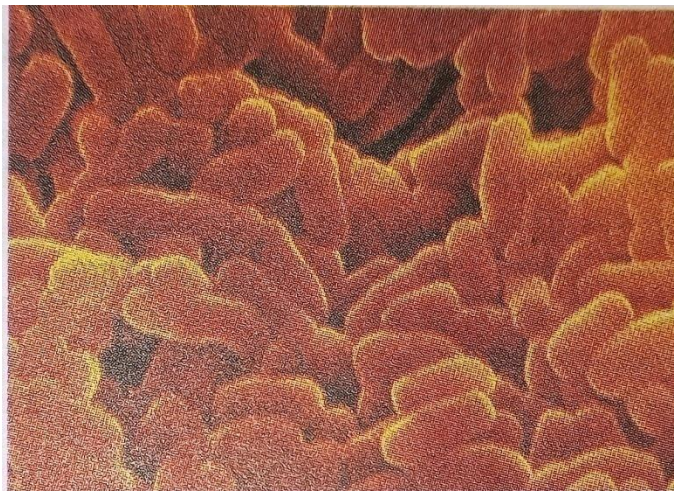


Fig.6.7: ***Mycobacterium tuberculosis***

Table.6.1: Some important human diseases transmitted by airborne particles

Viral diseases	**Bacterial diseases**
Chickenpox (Varicella)	Whooping Cough (Bordetella pertussis)
Flu (Influenza)	Meningitis (Neisseria Meningitidis)
Measles (Rubeola)	Corynebacterium dephtheriae
German measles (Rubella)	Pneumonia (Mycoplasma pneumonia, Streptococus pneumonia)
Mumps (Mumps)	Tuberculosis (Mycobacterium tuberculosis)
Smallpox (Variola)	

Several valuable materials are stored in houses which are deteriorated by aeromicroflora, such as printed and hand written books. The major constituent of paper is cellulose. Cellulose degrading microorganisms colonies and degrade the papers. The common degrading fungi are

the species of *Alternaria, Aspergillus, Curvularia, Bispora, Cladosporium,Cladosporium, Fusarium, Helminthosporium, Nigrospora, Rhizopus, Stemphilum, Trichoderma* etc.

Allergy is caused by certain biological and abiological agents presents in the atmosphere. The allergy causing agets present in the air are called aeroallergens and the allergy caused by them is called aeroallergy. Some of the prominent aeroallergens are house dusts, pollen grains, cosmetics, microbial spores or cells. In India, due to great diversity in vegetation and climatic conditions, the aeroallergens are also of different types in different region and places of country. Allergic pollen grains mainly belonging to the family Poaceae, Chenopodiaceae, Amaranthaceae and Asteraceae. Pollen grains get disseminated and dispersed through wind, water and insects, commonly known as vectors.

In the houses, air conditioners and coolers (humidifiers) are used, generally colonized by fungi *Aspergillus*, *Geotrichium*, *Penicillium*, *Phialophora*, yeast, and bacteria etc. House dust is a mixture of hair, moulds, bacteria, decomposed parts of cloths or furniture, small mites, insects etc. The most common fungal spores are the species of *Penicillium*, *Aspergillus*, *Cladosporium*, *Helminthosporium*, *Phoma*, *Fusarium*, *Cephalosporium*, *Curvularia, Nigrospora* and *Epicoccum* etc. Mites are the important components of house dust and strong allergens for humans. *Dermatophagoides pteronyssinus* is known as house dust mite. *D. pteronyssinus* is found in large number on mattresses, pillows, blankets etc., where human scales are also in abundant. The other species are *D. farinae, D. domesticus, D. destructer* etc.

Table.6.2: Common Aeroallergens

Aeroallergens	Examples
Alage	*Aulosira, Chlamydomonas, Lyngbya, Nostoc, Phormidium, Gloeotrichia, diatoms, Oscillatoria, Chlorella, Plectonema*
Fungi	*Alternaria, Aspergillus, Candida, Chaetomium, Curvularia, Fusarium, Monilia, Penicillium, Phoma, Trichodera*
Lichens	*Cladonia, Heterodermia, Parmellia, Usnea*
Pollen grains	*Ageratum conyzoides, Amaranthus sp. Argemone Mexicana, Azardirachta indica, Carica papaya, Cassia fistula, Cocos.*
Others	*Dust, mites, viruses, bacteria, protozoa, moss spores, fern spores, speed spiders etc.*

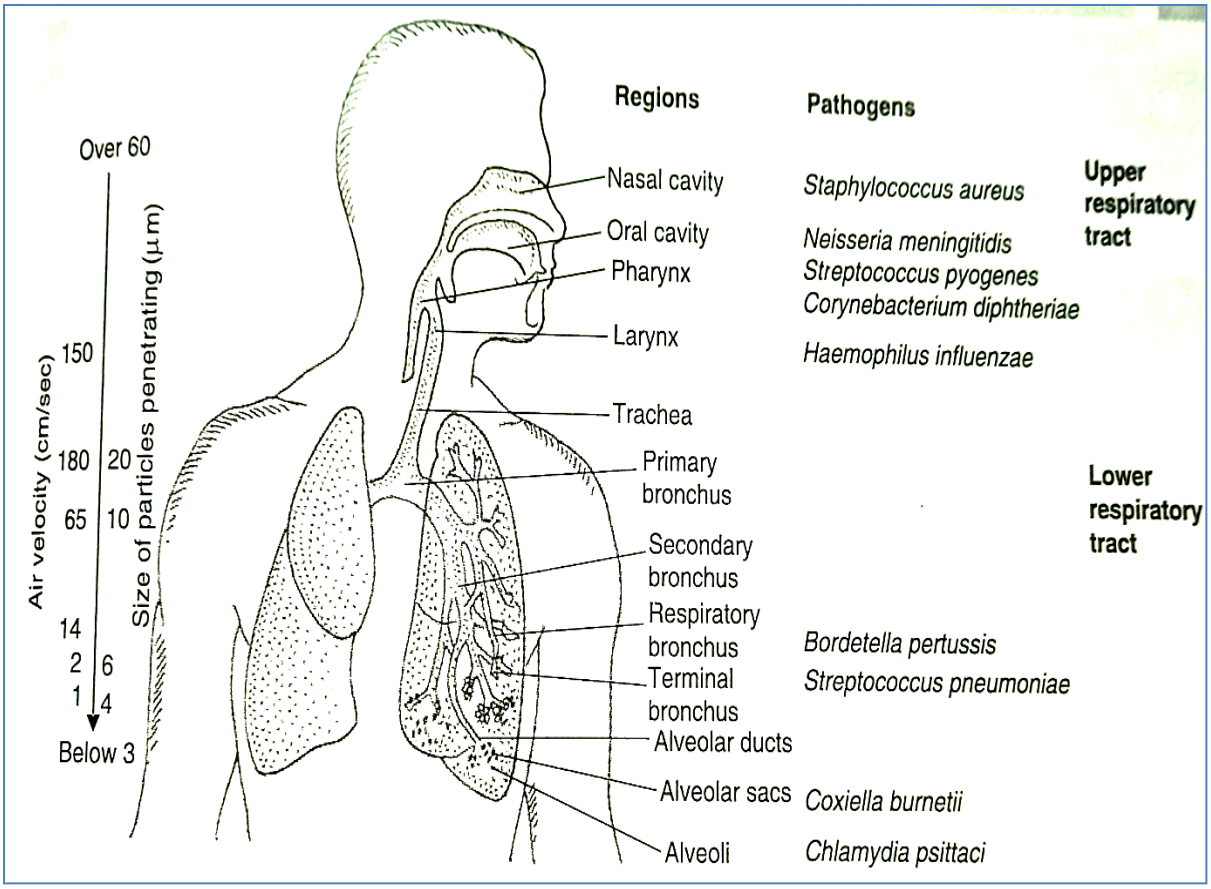


Fig. 6.8: Respiratory infections transmitted through airborne bacterial pathogens

Table.6.3: Common sources of Aeroallergens

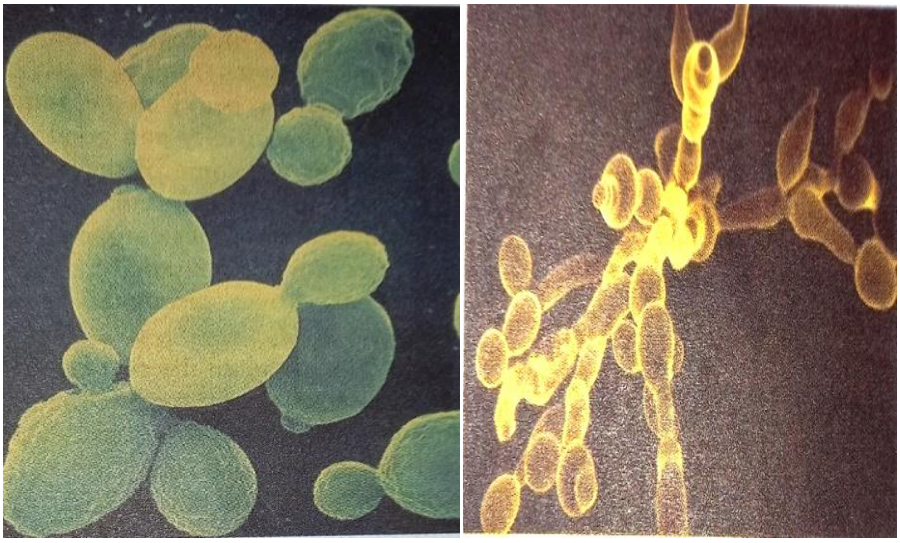
Aeroallergens	Sources
Pollen grains	Wind-pollination plants e.g. grasses, weeds, trees.
Moulds	Saprophytic fungi multiply by dead organic substrate in the presence of optimum moisture and temperature
Dander's	Feature of chickens, ducks, hair of cat, dogs, sheep, cattle etc
House dust	A composite of all dust contain specific components related to mites, algae, etc
Insecticides	Insecticides contain pyrethrum as common ingredients
Paint and Varnishes	Linseed oil, organic solvents act as irritant

6.6. Phylloplane microflora

Britain and Ruinen (1956), introduced the term phyllosphere to describe the leaf surface as the habitat of microorganisms. Kerling (1958) use the term phylloplane for leaf surface. Phylloplane is a natural habitat on leaf surface which supports heterogeneous population, comprising both, pathogens and non pathogens parasite. The leaf surface acts as the landing

stage for the microbial propagules. These are deposited by impaction, boundary layer exchange, sedimentation under the influence of gravity and in rain as splash droplets (Pugh, 1984).

The phylloplane microorganisms including yeasts, filamentous fungi, bacteria, blue green algae and ferns spore. Microflora of phylloplane is of great significance, as some of them have antagonistic action against fungal pathogens, degrade plant surface wax and cuticle, decompose plant material, and activate plants to produce phytoalexins, act as a source of allergic airborne spores and influence growth behavior and root exudation of plants.



Some common phylloplane microflora are yeasts (*Candida albicans*, *Sacchromyces cerevisiae*, *Torulopsis colliculosa*, *Cryptococcus diffluens*), bacteria (*Pseudomonas trifoli*, *Beijerinckia* sp. etc.) and fungi (*Aspergillus niger*, *A. terreus*, *Cephalosporium roseo-griseum*, *Curvularia lunata*, *Epicoccum purpurascens*, *Phoma glomerata*, *Myrothecium roridum*, *Fusarium oxysporum*, *Trichoderma harzianum*, *T. viride*, *Penicillium oxalicum*, *Alternaria alternate*, *Drechslera australiensis* etc.

6.7. Phylloplane pathogens

Fungal pathogens which produce spores or conidia on the surface are disseminated by the wind, such as downy mildews, powdery mildews, rusts, smuts, leaf spot causing pathogens and sooty molds. Some plant pathogenic bacteria are also carried to short distances by wind.

The pathogen must produce numerous spores which are successfully liberated, dispersed and deposited in a viable condition on susceptible plants under conditions conducive to infection.

Many fungal pathogens are well adapted to wind dissemination as they produce and liberate small and very light spores into the air in countless numbers, that are carried to long distances. Propagative spores are produced in many different ways in sporangia, in ascocarps and upon basidium.

Table.6.4: Fungal diseases of plants transmitted by airborne spores

Fungal agent	Sources	Disease
Aspergillus clavatus	Moldy malt	Malt worker's lung
Aureobasidium pullulans	Steam	Sauna-taker's lung
Alternaria spp.	Wood	Wood worker's lung
Botrytis cincerea	Moldy fruits	Winegrowers' lung
Cryptostroma corticale	Wood	Maple bark stripper's lung
Farnai rectivirgula	Straw	Potato riddler's lung
Serpula (Merulius) lacrymans	Moldy building	Dry rot lung
Penicillium spp.	Cork	Suberosis, woodman's disease
Penicillium casei	Cheese	Cheese worker's lung
Mucor stolonifer	Moldy paprika	Paprika worker's lung
Trichosporon cutaneum	House dust	Japan summer pneumonitis

Table.6.5: Phylloplane pathogens

Name of the disease	Pathogen
Late blight of potato	*Phytophthora infestans*
Early blight of potato	*Alternaria solani*
White rust of crucifers	*Albugo candida*
Ergot of rye	*Claviceps purpurea*
Brown spot of paddy	*Helminthosporium oryzae*
Leaf spot or tikka disease of groundnut	*Cercospora arachidicola* *Cercospora personata*
Powdery mildew of wheat	*Erysiphe graminis tritici*
Peach leaf curl	*Taphrina deformans*
Leaf spot of turmeric and zinger	*Taphrina maculans*
Rust of linseed	*Melampsora lini*
Downy mildew of crucifers	*Peronospora parasitica*

Downy mildew of pea, beans	*Peronospora viciae*
Downy mildew of grape-vine	*Plasmopora viticola*
Rust disease of wheat	*Puccinia graminis tritici*
Rice blast disease	*Pyricularia oryzae*
Rust of pea	*Uromyces fabae*
Leaf spot of crucifers	*Alternaria brassicae*

6.8. Characteristic of Phylloplane microflora

Phylloplane microflora has certain characteristic features so that it can survive within environment.

6.8.1.Morphological characteristics of phylloplane microflora: These include the development of pigment in their mycelia and formatting spores, pycnidia, apothecia, cleistothecia for proection against strong light and desiccation.

The dark pigments act as light screen and often referred to as melanin.

6.8.2.Physiological characteristics of phylloplane microflora:

a) **Nutrition:** phylloplane microfungi have ability to decompose cellulose by producing cellulases. In many fungi, such as *Alternaria alternate, Aureobasidium pullulans, Botryis cineria, Cladosporium herbarum*, pectinases, cutinases, proteases have been estimated.

b) **Relative humidity:** phylloplane fungi get advantages at low levels of relative humidity. Faster growth of germ tube and mycelium of some fungi occurs at 100% humidity. During rain and dew formation, high relative humidity is found on leaf surface.

c) **Radiations:** High intensity of UV radiation becomes lethal to microorganisms. High intensity of light inhibits mycelia growth, while normal light intensity not harmful to pylloplane fungi. The UV spectrum plays a significant role. Melanin pigment found in some fungi is resistant to UV exposure as compared to hyaline mycelia. *Aureobasidium* and

Sporobolomyces containing hyaline spores are killed by exposure of UV for five minutes, while dark spores of *Alternaria* and *Epicoccum* survive even after exposure of UV for 35 minutes.

d) **Temperature:** Phylloplane fungi are mesophilic growing at temperature between 20-25° C. Some fungi, such as *Alternaria alternate, Aureobasidium pullulans, Cladosporium herbarum* and *Botryis cineria* can grow below this temperature.

Rai and Singh (1980) investigated the antagonistic activities of some phylloplane fungi (of mustard and barley) against *Alternaria brassicae* and *Drechslera graminea*. The antagonistic fungi are *Aureobasidium pullulans, Epicoccum purpurescens, Cladosporium cladospor*ioides and *A. alternat*e. The most significant effect was observed when the spores of leaf surface fungi or their metabolites were sprayed on leaves prior to inoculation of the pathogens.

6.9. Summary

The study of microorganisms present in the air, their influence on other living organisms and methods of their removal is known as aeromicrobiology. Air does not contain the necessary amount of moisture and nutrients needed for growth and metabolism of microorganism. They do not grow and reproduce in air due to high light intensities and extreme temperature variations. Airborne biological materials are known as airspora or bioaerosols. A variety of microorganisms such as bacteria, fungi, actinomycetes, algae, spores of pteridophytes, pollen grains, micro-insects and viruses are present in the air. The most common algae found in air are the species of *Chlorella*, *Chlorococcum*, *Chlamydomonas*, *Aulosira*, *Nostoc* and *Phormidium.*

Hydrogen sulphide, sulphur dioxide, carbon monoxide, chlorine, hydrogen fluoride, ozone etc. are the important gases that affect the growth of microorganisms. Fungal aerosols are present in much higher concentration. Moulds and yeasts are commonly found in the air due to resistant to light. The air microflora mainly consists of spores of *Alternaria*, *Cercospora*, *Helminthosporium*, *Puccinia*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Homodendrum*, *Zygosporium*, *Aureobasidium*, *Pithomyces*, *Mucor*, *Rhizopus*, *Streptomyces*, *Bacillus*, *Clostridium*, *Stemphylium*, *Curvularia*, *Acremonium*, *Fusarium* and *Nigrospora*. Aeromicroflora of pharmacy includes *Cladosporium cladosporioides, Alternaria sp*., *Penicillium cyclopium*, *Epicoccum nigrum* etc. Hospitally transmitted pathogens are *Aspergillus flavus, Aspergillus fumigates, Mycobacterium tuberculosis*, *Candida albicans, Staphylococcus aureus,* influenza

virus etc. Allergy is caused by certain biological and abiological agents presents in the atmosphere. In the house, air conditioners and coolers (humidifiers) are used, generally colonized by fungi *Aspergillus*, *Geotrichium*, *Penicillium*, *Phialophora* yeast and bacteria etc.

Phylloplane is a natural habitat on leaf surface which supports heterogenous population comprising both, pathogens and non pathogens parasite. The leaf surface acts as the landing stage for the microbial propagules. The phylloplane microorganisms including yeasts, filamentous fungi, bacteria, blue green algae and ferns spores. Microflora of phylloplane is of great significance as some of them have antagonistic action against fungal pathogens, degrade plant surface wax and cuticle, decompose plant material, and activate plants to produce phytoalexins, act as a source of allergic airborne spores and influence growth behavior and root exudation of plants.

Fungal pathogens which produce spores or conidia on the surface are disseminated by the wind, such as downy mildews, powdery mildews, rusts, smuts, leaf spot causing pathogens and sooty molds. Some plant pathogenic bacteria are also carried to short distances by wind. Phylloplane microflora develops pigment in their mycelia, spores, pycnidia, apothecia, cleistothecia for proection against strong light and desiccation. Phylloplane microfungi have ability to decompose cellulose by producing enzyme cellulases. They are mesophilic growing at temperature between 20-25° C. High intensity of UV radiation becomes lethal to microorganisms.

6.10. Terminal questions

Q.1: What is aeromicrobiology? Write in brief aeromicroflora of house and hospitals.

Answer:----------

Q.2: What are aeroallergens? Briefly describe its bio-chemical components.

Answer:----------

Q.3: Write the characteristics of phylloplane microfungi.

Answer:----------

Q.4: Describe some airborne microbial diseases and their causative agents.

Answer:----------

Q.5: What is phylosphere? Briefly describe phyllosphere microflora.

Answer:----------

Q.6: Write short notes on the following:

a. Aerofungi and aflatoxins

b. Indoor microbiology

c. Phylloplane pathogens

d. Biodegradation

Answer:----------

6.11. Further Suggested Readings

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PGEVS-103N

Rajarshi Tandon Open University, Prayagraj

Environmental Microbiology

Block- III

Agriculture Microbiology

UNIT -7

Microbial Biofertilizers

UNIT-8

Microbial pesticides

UNIT-9

Microbiology degradation

PGEVS-103N

Rajarshi Tandon Open University, Prayagraj

Environmental Microbiology

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Block-4 *PGEVS-103N*

Introduction

This is the third block on agriculture microbiology. It consists of following three units such as:

Unit-7: This unit covers microbial inoculants, inoculants carrier, and production of microbial fertilizers, algae biofertilizers, Cyanobacterial biofertilizers, mycorrhizal biofertilizers, and phosphorous adding biofertilizers.

Unit-8: This unit covers the microbial insecticides, microbial herbicides, pseudomonas as bacterial insecticides, bacillus species as bacterial insecticides, virus insecticides

Unit-9: This unit covers the microbial degradation. The sewage degrading microorganism, microbial degradation of petroleum, microbial degradation of xenobiotics compound, microorganism in abatement of heavy metal pollution, and heavy metal tolerance in microbes cover in this unit.

Unit 7: Microbial Biofertilizers

Contents

7.11. Further suggested readings

7.1. Introduction

Continuous cropping, i.e, growing one crop after another, resulting in decrease in soil fertility due to decrease in nutrient content of soil. Soil fertility refers to the ability of soil to sustain agricultural plant growth and results in sustained and consistent yield of high quality and quantity. Soil fertility is complex process. Soil productivity mainly depends on soil fertility to great extent. However, a fertile soil is not necessarily a productive soil. Soil erosion (loss of top layer of soil by water and air) contributes to a loss of inherent soil fertility levels.

Farmers normally use chemical fertilizers, such as urea, ammonium nitrate and ammonium phosphate etc. (inorganic or artificial chemical fertilizers) to increase fertility and crop production of soil which contain three basic nutrients- nitrogen, phosphorus and potassium, some chemical fertilizers also contain micro nutrients such as zinc. However, excessive use of these chemical fertilizers decreases the fertility of soil, crop production, harden the soil, polluted air and water (cause eutrophication), releases greenhouse gases, kill soil microorganisms, lead to acidification in soil and chemical burn to the crop. So, the avoidance of chemical fertilizers and the use of natural fertilizers, such as biofertilizers and organic fertilizers (vermicompost, compost and green manure),and biopesticides and bioherbicides can be a sustainable approach to crop productivity.

Organic fertilizers contain plant or animal based material and have low level of nutrients but provide nutrients for longer duration of time than inorganic chemical fertilizers. Organic fertilizers may also contain beneficial microorganisms similarly to biofertilizers but their number is not specific in it, while biofertilizers contain specific number of microbes. Uses of organic fertilizers improving soil structure, texture and

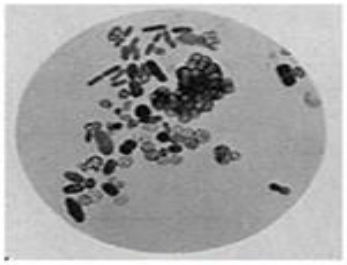
aeration, increasing the soil water retention ability, stimulating healthy root development, eco-friendly in nature and beneficial to microorganisms of soil making them the best fertilizer.

Microbial inoculants are soil amendments that can be added to your soil to improve the overall quality of the soil as well as enhance the growth of strong and healthy plants. It is a mixture of microorganisms that work with the soil and soil life to improve its fertility and health quality. These microbes are not only helpful in increasing the growth of plants, but also stimulative and beneficial to other organisms and insects that aid in plant growth overall development. This is much more organic and natural approach than adding unnatural additives in soil to increase fertility of soil and growth of plants. Use of synthetic fertilizers in high amount may be harmful to the soil and atmospheric area around them. Hower, on other hand, microbial inoculants are composed of active strain of microorganisms which directly or indirectly stimulate microbial activity, and hence improve mobility of nutrients from soil. Microbial inoculants are much safer and more natural approach to encouraging plant and soil health. They may be phyto-stimulants, bio-fertilizers or microbial biocontrol agents. They are low-cost, renewable sources of plant nutrients. These are the strains of beneficial soil microorganisms, which are cultured and packed in suitable carrier in laboratory, and are used as biofertilizer in increasing soil fertility and crop production in sustainable agricultural development.

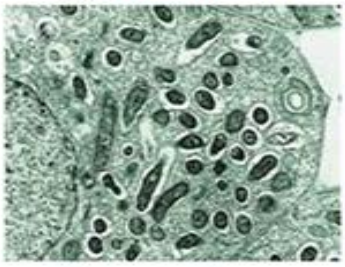
“Green Revolution”practices are also working for the increase in productivity of agriculture and reduce the risk of inorganic chemical based fertilizers on human health as well as the envinoment.Green technology discusses several aspects of the use of cyanobacteria to improve the crop productivity and soil fertility.

(a) *Nitrobacter*

(b) *Anabaena*

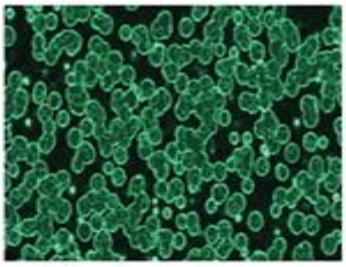


(c) *Azotobacter*

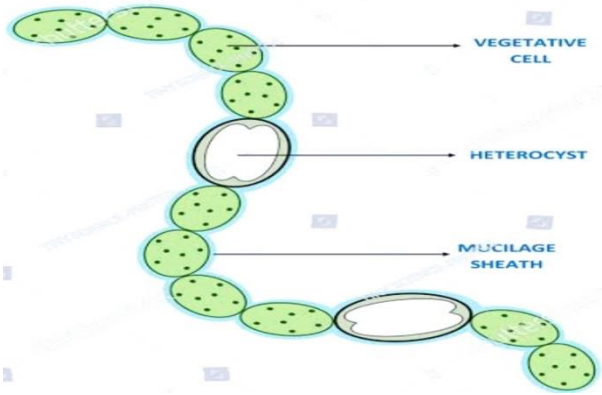


(d) *Rhizobium*

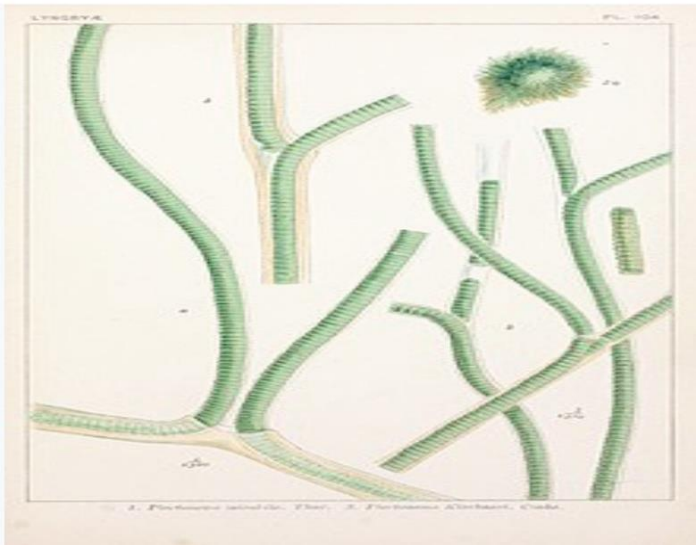
(e) *Clostridium*



(f) Cyanobacteria



(g)Nostoc



(h)Plectonema

(i)Oscillatoria

Fig.7.1: Different types of soil microbes, a to i.

Objectives

After study of the course of this unit, students will be able to understand-

- the microbial biofertilizers.
- the microbial inoculants and inoculants carriers.

the algal biofertilizers, cyanobacterial biofertilizers and phosphorus adding biofertilizers.

- the advantages and disadvantages of microbial biofertilizers.

7.2. Microbial Inoculants

Microbial inoculant is defined as a preparation containing live or dormant cells of efficient strains of nitrogen fixing, phosphate solubilizing, and cellulytic microorganisms, etc. Several microorganisms are commonly used as biofertilizers including nitrogen-fixing soil bacteria (*Azotobacter*, *Rhizobium*), nitrogen-fixing cyanobacteria (Nostoc, *Anabaena*), phosphate-solubilizing bacteria (P*seudomonas* sp.) and AM fungi.

Similarly, phytohormone (auxin)producing bacteria and cellulolytic microorganisms are also used in biofertilizer formulation. Microbial inoculants are gaining importance for attaining sustainable agricultural production systems. An increasing trend for the use of microbial bioinoculants to accomplish sustainable agriculture has been witnessed across the globe.

They have the ability to minimize the negative impact of inorganic chemical fertilizers and consequently increase the quantity and quality of agricultural products. Microbial inoculants are eco-friendly and deliver plant nutrients to plants in a more sustainable manner.

7.2.1.Various Types of Microbial Inoculants

There are various types of microbial inoculants. These are-

- **Nitrogen fixing bacteria**-*Azospirillum sp., Rhizobium sp., Diazotrophicus, Azorhizobium sp., Frankia sp., Allorhizobium sp., Bradyrhizobium sp., Herbaaspirillum sp.*
- **Phosphate solubilises**-*Pseudomonas sp., Bacillus sp., Rhodococcus sp., Serratia sp., Gordonia sp., Xanthomonas sp., Enterobacter sp., Klebsiella sp., Chryseobacterium sp.* Phosphate mobilizers – mycorrhizal fungi.
- **Biocontrol agents** –*Pseudomonas fluorescence, Bacillus subtilis, Trichoderma viride, T. Harzianum, Achromobacter kerstersii.*
- **Biopesticides –**

- Entomopathogenic bacteria- *B. thuringiensis* and *Paenibacillus popilliae*.
- Entomopathogenic fungi- *Beauveria bassiana, Metarhizium anisopliae, Isaria fumosorosea, Lecanicillium lecanii, Entomophaga sp.*
- Effective microorganisms (EM) – mixture of photosynthetic bacteria *(Rhodopseudomonas palustris), Lactobacillus casei, Sacchromyces cerevisiae.*
- S**iderozote** – mixture of siderphores producing rhizobacteria in phosphate buffer.
- **Mycodot –** mixture of mycophagous bacteria as formulate.
- **Fusostat** – mixture of chitinase producing rhizobacteria.

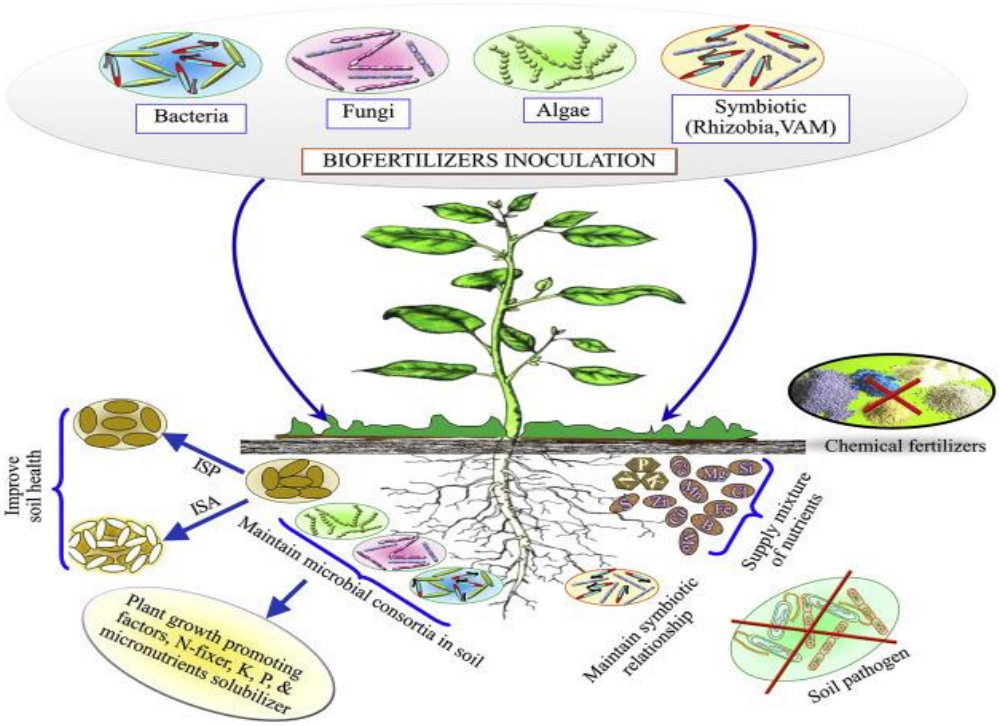


Fig.7.2: Biofertilizers inoculants

7.2.2. Different Types of Microbial Inoculant Carriers

The microbial inoculants carrier is a material, such as peat, lignite powder talc, rice bran, seed, charcoal, soil, rock phosphate pellet, paddy straw, compost, wheat bran,agro-industrial wastes, vermiculite clay, coir waste, perlite, calcium sulfate, and polysaccharides or a mixture of such materials, etc. This provides better shelf life and provides higher effectiveness to biofertilizers formulation.Carrier has been processed by mining, drying

milling.This is the expensive aspect of biofertilizer production.Mixture of microbial inoculent and microbial inoculent carriers is known as biofertilizers.

Table: 7.1. Different types of microbial inoculants carriers with their properties

Carrier	Organic matter %	Total %	Porosity %	Water holding capacity
Sedge peat	76	0.95	45	200
Farm yard manure	79	0.93	55	153
Compost	55	0.55	59	171
Liginite	75	0.31	35	198
Charcoal	22	0.01	72	200
Vermiculite clay	01	0.01	63	152

7.2.3. Qualities of Microbial Inoculant Carriers

The important qualities of a carrier material in legume inoculent production are:

1.Have good moisture absorption capacity,

2. Easy to dry and grind,

3. Nontoxic to rhizobia,

4. Free of abrasive minerals,

5. Low in content of soluble salts,

6. Easy to sterilize,

7. Available in adequate amounts at a reasonable cost,Fine,

8.Innert and free of lump forming material,

9.Have excellent buffering capacity.

7.2.4. Importance of Microbial Inoculant Carriers

Peat is the most commonly used solid carrier in making legume inoculants. It is also the most dependable, because rhizobia in a peat carrier remain viable for longer time, both

within the package and on the seed. However, good quality peat is not available in many countries. Chemical and physical analyses of carrier materials are helpful, but do not confirm the quality of a carrier. The quality can be determined only by placing viable rhizobia in the material and monitoring its growth and survival of rhizobia over a period of 6 months or more.

7.3. Production of Microbial Fertilizer

Bio-fertilizers are mixture of microbial inoculant and microbial inoculant carriers, containing live or latent cells of efficient strains of nitrogen fixing, mineral solubilizing or cellulolytic microbes, used for application to seed, soil or composting areas to increase the extent of availability of different essential nutrients in soil for growth of the plants. Biofertilizer has been extensively applied and accepted worldwide in agricultural crops, however, its application in forest tree species remains restricted.These microbes when applied in forest nurseries will ensure production of healthier seedlings, which may be more successfully established in the field. Its application in forest nurseries helps in improving soil fertility, nutrient uptake, controlling soil borne disease and early growth of seedling. Bio-fertilizers are cheap and eco-friendly alternative to inorganic chemical fertilizers.

The main roles of the formulation of inoculants are:

i. To provide a more suitable micro-environment for the microbial strain/s, combined with physical or chemical protection over a prolonged period, in order to avoid a rapid decrease of the cell viability during storage,

ii. to support the strain competition with the better-adapted native soil microflora, and

iii. to reduce losses due to the depredation by the micro-fauna after being introduced into soil.

The inoculants formulation is usually commercialized in one of three forms-

1) Powder in the form of processed sedge peat moss

2) Liquid

3) Granular

More recently, hard mineral based products have been also developed.

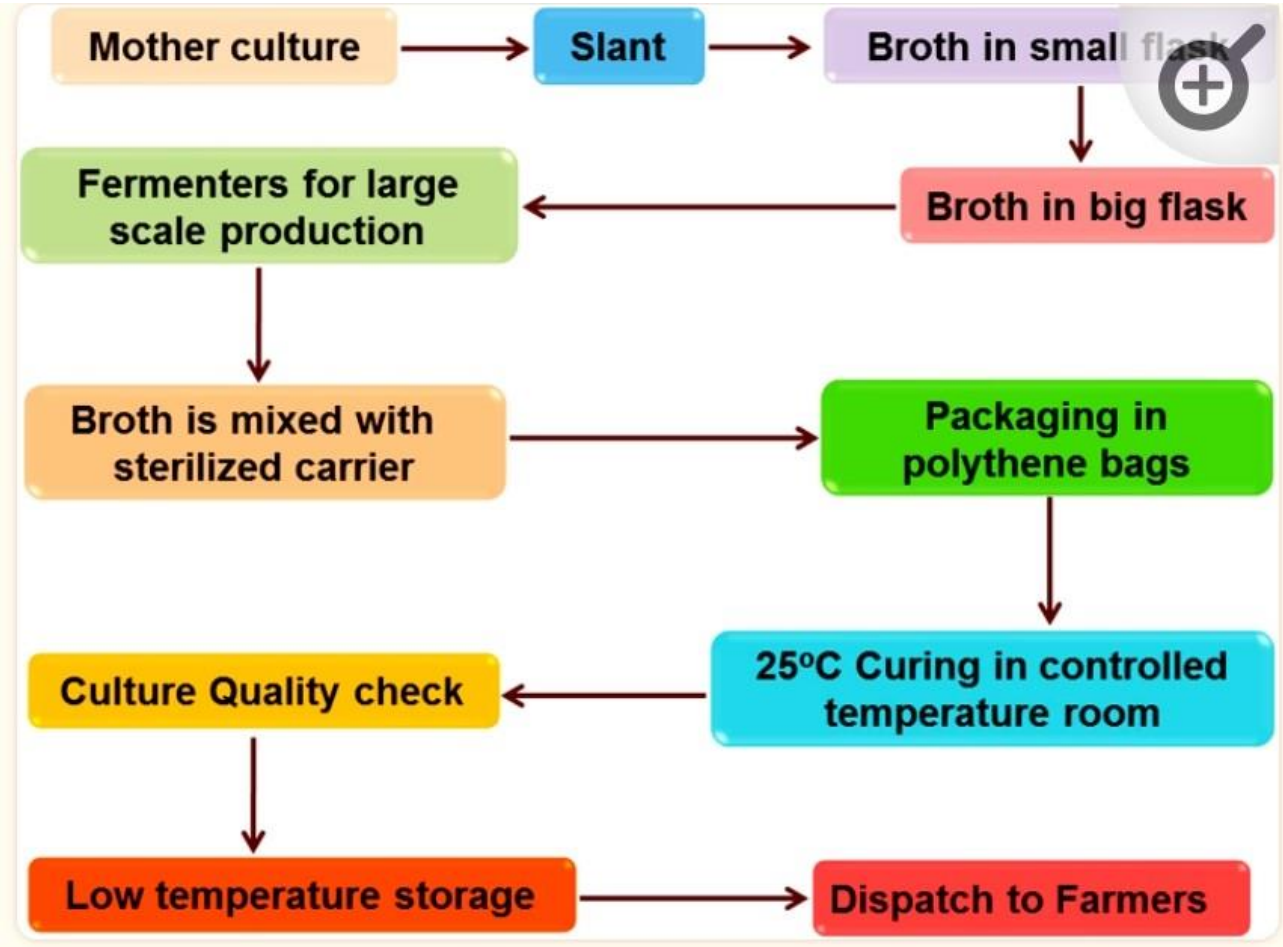


Fig.7.3.Represent the mass cultivation and their steps

7.3.1. Effect of Application of Microbial Fertilizers

- Improve plant nutrition and promotes plant growth.
- Stimulates the synthesis of plant growth promoting hormones (indole acetic acid and gibberellins)
- Manage soil structure and organic wastes.
- Manipulate the rhizosphere and phyllosphere region of plants.

- Induce induced systemic resistance (ISR), systemic acquired resistance (SAR) and integrated pest management(IPM).
- Enhances plant growth and production, and protection.
- Recycling of various vital elements through biogeochemical cycle.
- Suppress the soil borne pathogens.
- Reduces the concentration of toxic substances, including pesticides and heavy metals in soil.
- Enhances the photosynthesis process in plants.
- Promotes seed germination, and flowering and fruiting in plants.
- Solubilization of insoluble nutrients in soil.
- Mobilization of various soil nutrients in soil.
- Prevent plants from phytopathogens and insect pests.
- Produces polysaccharides to improve soil aggregation.
- Prevents plants from high temperature, salinity and desiccation.
- Application of bio-fertilizers in nursery will enhance the growth of seedlings in nursery and plantation. Seedlings supplemented with biofertilizer have better chances of establishment on unfertile barren lands.

7.4. Algal Biofertilizers

The agronomic potential of cyanobacterial N2 fixation in rice fields was recognised in India during 1939 by P.K. De, who attributed the natural fertility of tropical rice fields to Nitrogen fixing Blue green algae. The rice field ecosystem provides an environment favourable for the growth of blue green algae with respect to their requirements for light, water, high temperature and nutrient availability.Algal biofertilizers constitutes a perpetual source of nutrients and they do not contaminate ground water and deplete the resources. In addition to contributing 25-30 kg N of biologically fixed Nitrogen, they can also add organic matter to the soil, excrete growth promoting substances, solubilises insoluble phosphates and amend the physical and chemical properties of the soil. Algae are highly efficient producers of organic material, and they do not compete against agricultural production for arable soil. They release macronutrients as well as micronutrients. They are also known to contain high levels of potassium and nitrogen. This is the reason why algae

perform so well as biofertilizers. Freshwater algae can be grown in a variety of media, including sterile to contaminated wastewater. They only require water, nutrients, CO_2 and light. The species determine by environmental limitations, such as pH, growing temperature, and culture densities.

7.4.1. Production of Algal Biofertilizers

Step 1-let algae grow into clumps in a water body or lake. As the algae expand, it gets thicker and begins to form clusters. You can skim some of it and put it in the bucket.

Step 2-Clean the algae clumps using clear water. Algae has a high salt content. Rinsing can remove any additional salt.

Step 3-Now spread the washed algae clumps over the tary in a wide, sunny space. Break up any larger clusters of algae with your fingertips to help the speed drying process. The drying process will vary based on the prevalent weather conditions.

Step 4- now, break apart the dried clumps. Once it is dry, break chunks into the soil-like substance using the edge of a shovel.

Step 5-Incorporate dried algae into ready compost. Mix it into already decomposed compost. Mix the compost thoroughly and use it as fertilizer.

Avoid collecting algae during the blooming phase. The thin layer of algae is not nutritionally rich at this point.

7.4.2 Useful Aspects of Algal Biofertilizers

In the present time, when sustainability is a significant concern, algae is emerging as an alternative source of raw materials for various industries. The nutritional value of algae has other advantages in the form of biofertilizer. The nutrients present in algal biofertilizer improve the yield and growth of crops. Here are a few benefits of using algal biofertilizer:

- An alga is a renewable, sustainable, and eco-friendly biofertilizer. Because of their organic nature, algal biofertilizers are safer and greener than inorganic chemical fertilizers.

- When algae break down, it releases beneficial macronutrients and micronutrients into the soil,i.e., increase availability of nutrients in soil for plants.
- Dried algae are a soil humus and helps soil to increase its capacity to retain water. This reduces soil loss due to rain. Dried algae also increase soil aeration. The oxidized and fertilized soil helps plant roots to absorb nutrients better and develop more quickly.
- When algae are mixed in compost, the inorganic chemicals and nutrients released by it as speed up the process of decomposition. The nitrogen that algae releases help break down other components in the compost heaps.
- In reality, marine algae have long been utilized as a biofertilizer by farmers whose farms are near the ocean. Red and brown algae are extensively used as farmland fertilizers by farmers. They have a high content of potassium. Potassium helps in enhancing root growth and increases the drought-resistant in plants. Lack of potassium in soils can reduce plant growth (stunted plant growth) and lower crop yield.

Algae can be used for many purposes, including to produce fuel, oil or gas, direct combustion, food, feed, plastic, polymer compounds, and biofertilizer. Although, most of these uses are possible, many of them are not economically feasible.

7.5. Cyanobacterial Biofertilizers

They are the most abundant group of organisms on the earth, found in a diverse environment, especially in the marine and fresh water.Marine water is the ritchest source of nutrient for the cultivation of cyanobacteria.Cyanobacteria are associated with the periods of origin of plants.Green technology suggests the use of cyanobacteria as biofertilizer to improve the productivity of crop and soil fertility which are safe to human, plants, soil, other microorganisms of soil and environment.The cyanobacteria produces biomass of very high value which can also be used for the large scale production of food, energy, biofertilizers, secondary metabolites, cosmetics and medicines. Mostly Calothrix sp.,Tolypothrix sp., Anabaena variabilis, Nostoc linkia, Gloeotrichia, Plectonema, Aulosira fertilisima, Scytonema sp., Oscillatoria sp.,etc. are used in prepration of cyanobacterial biofertilizers.

7.5.1. Characterstics of cyanobacteria used as biofertizilers

1. They are photosynthetic and aquatic cyanobacteria, and some of them are able to fix nitrogen. They can be divided into two major groups, based on morphological growth habit.

a) Unicellular forms, example- *Cyanothece* strain BH63, *Cyanothece* strain BH68, both are aerobic, Nitrogen fixing and photosynthetic, evolved oxygen as by-product,decreasing the level of Co2.

b) Filamentous forms, example- *Nostoc* and *Anabaena*, have heterocyst in which free atmospheric molecular nitrogen is fixed by enzyme Nitrogenase in micro-anaerobic environment.

2. Nitrogen fixing species are from both groups, found in paddy fields, but the predominant ones are the heterocystous filamentous forms.

3. Cyanobacteria are not restricted to permanently wet habitats, as they are resistant to desiccation and hot temperatures, and can be abundant in upland soils. However, wet paddy soils and overlying flood waters provide an ideal environment for them to grow and fix nitrogen.

4. BGA are cosmopolitan in distribution and more widely distributed in tropical zone.

5. Anabaena is free living nitrogen fixing which can form symbiotic association with water fern, *Azolla*. *Azolla* is used as organic biofertilizer in rice field to increase nitrogen cotent of soil.

6. Heterocystous cyanobacteria formed less than 10% of the population of eukaryotic green algae and the abundance increased with the increase in amount of available phosphorus and the pH value between 4 – 6.5. In rice soil, population ranges from 10 – 107 cfu g-1 soil.

7. Beside blue green algae, *Chlorella vulgaris* unicellular member of green algae of the division Chlorophyta, most commonly used micro algae in biofertilizers.

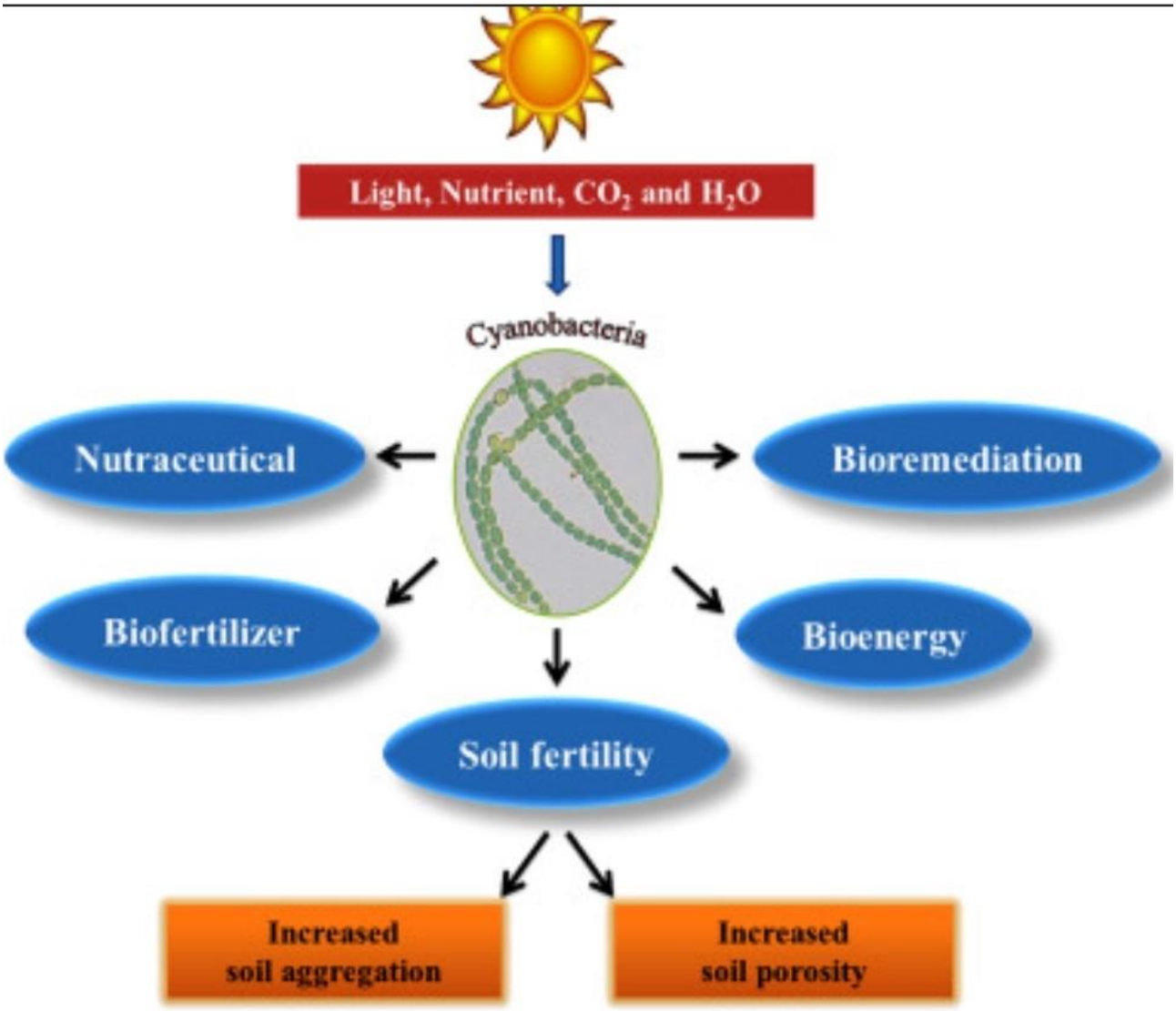


Fig.7.4.Cyanobacterial growth utilization in development of sustainable agricultural pratices

7.5.2. Production of Cyanobacterial Biofertilizers

The following four methods are used for mass production of cynobacterial biofertilizer:

(i) cemented tank method

(ii) shallow metal troughs method

(iii) polythene lined pit method

(iv) field method.

The polythene lined pit method is most suitable for small and marginal farmers to prepared algal biofertilizer. In this method, small pits are prepared in field and lined with thick polythene sheets. Mass cultivation of cyanobacteria is done by using any of the four methods under the following steps:

i. Prepare the cemented tanks, shallow trays of iron sheets or polythene lined pits in an open area. Width of tanks or pits should not be more than 1.5 m. This will facilitate the proper handling of culture.

ii. transfer 2 -3 kg soil (collected from open place, for lm 2 area of the tank) and add 100 g of superphosphate. Water the pit to about 10 cm height. Mix lime to adjust the pH 7. Add 2 ml of insecticide,e.g., malathion to protect the culture from mosquitoes. Mix well and allow to settle down the soil particles.

iii. when water becomes clear, sprinkle 100 g of starter inoculum on the surface of water.

iv. when temperature remains between 35-40°, during summer, optimum growth of cyanobacteria is achieved. Always maintain the water level to about 10 cm during this period,

v. after drying, the algal mat will get separated from the soil and forms flakes. During summer about 1 kg pure algal mat per m2 area is produced. These are collected, powdered, kept in sealed polythene bags and supplied to the farmers.

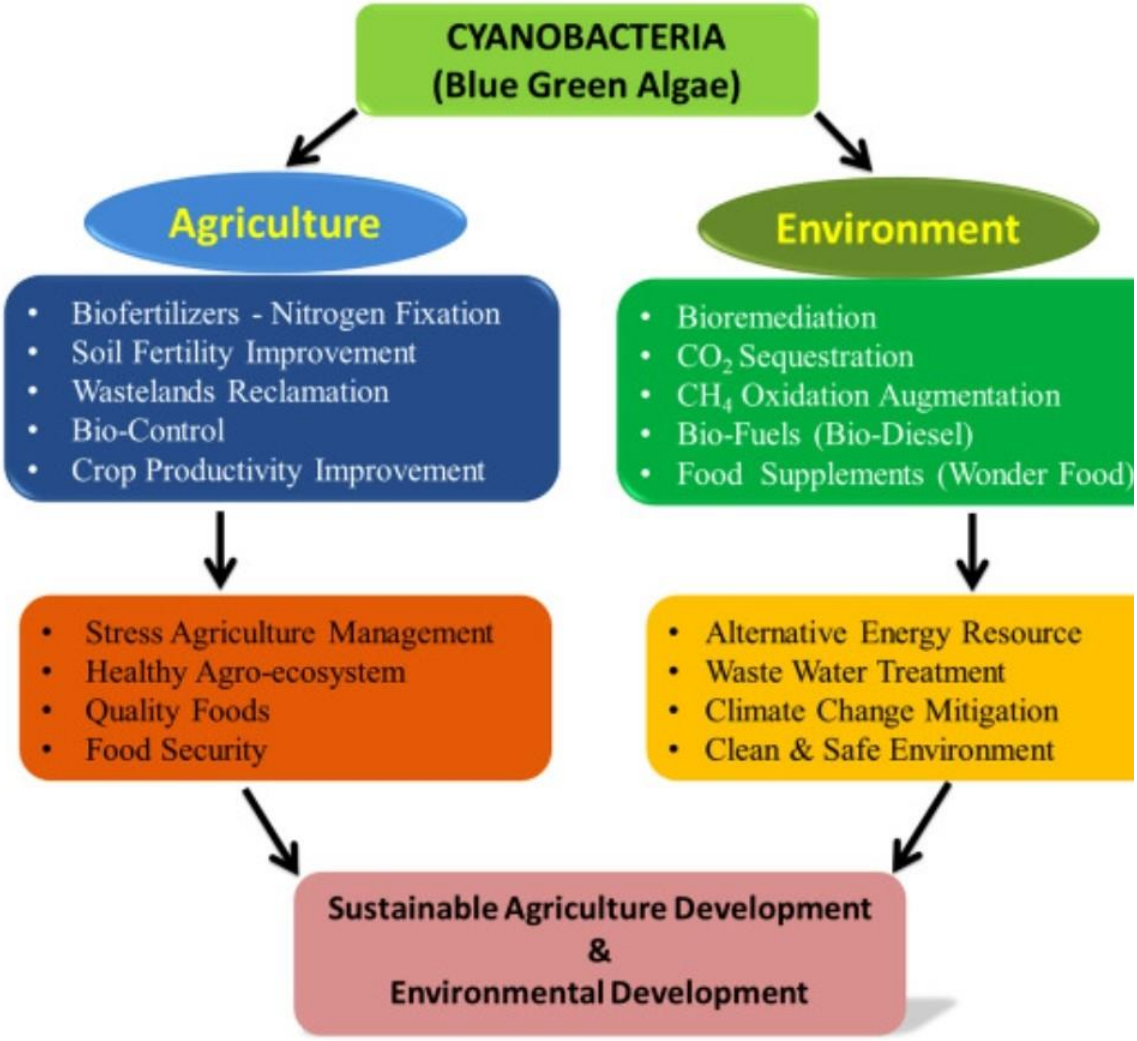


Fig.7.5.Potential fuctions of cyanobacterium in sustainable agriculture and environment development

7.5.3. Useful Aspects of Cyanobacterial Biofertilizers

The cyanobacterial biofertilizer increases rice yields by 5.03% to 20.06%. In rice fields. Cyanobacteria contribute significantly to soil fertility, and they grow on the surface of paddy soil and also in water logged in paddy fields.

The propagation of rice seedling was tremendously fast when treated with cyanobacteria,such as *Anabaena* and *Nostoc*.

An increase in 51% of plant height, 68%in root length, 56% in fresh root weight, 120% in dry root weight, 32% in soil moisture, 30% in soil porosity and 9.3% decrease in soil bulk density was noticed by the application of cyanobacterium *Nostoc*.

Cyanobacteria produces a wide range of bioactive compounds like amino acids, carbohydrates, vitamins and growth hormones like auxins, gibberellins, and cytokinin which are necessary for the growth of paddy plants.

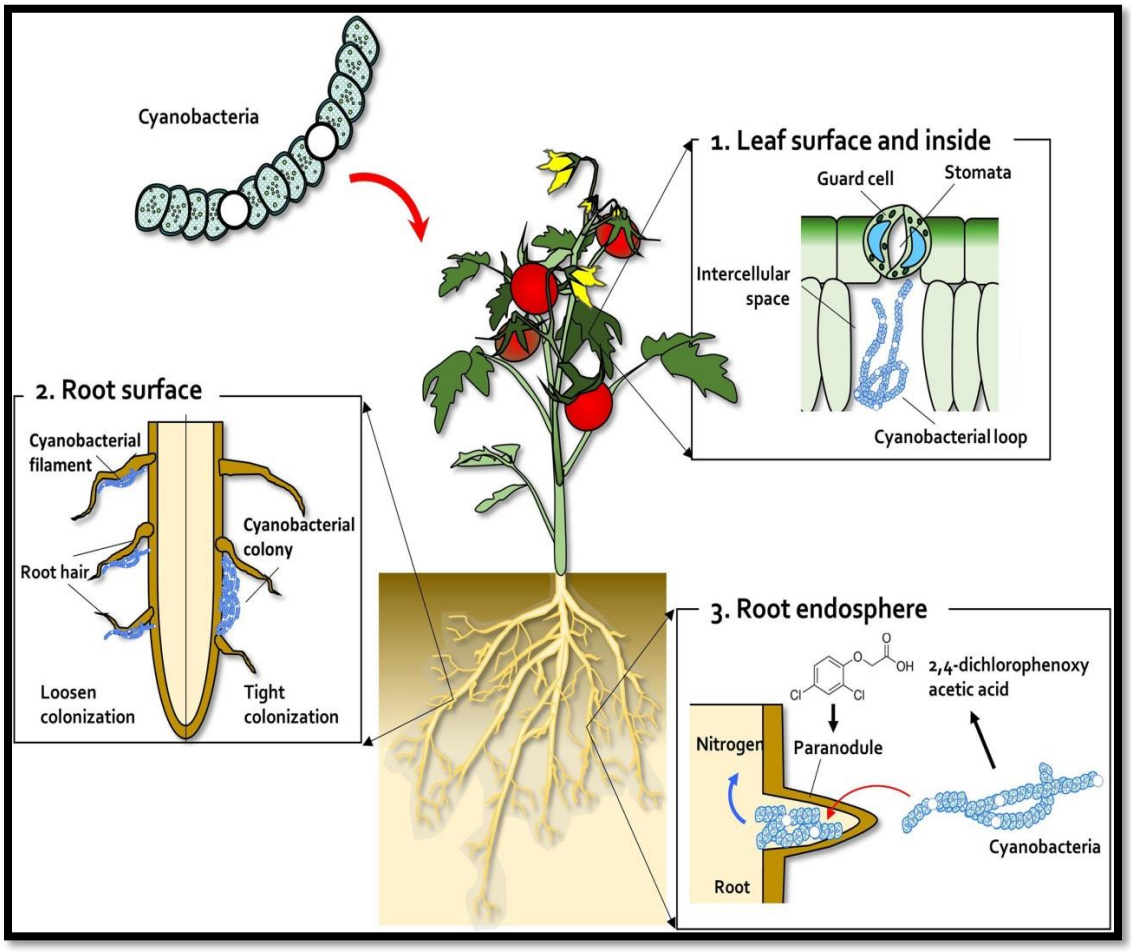


Fig.7.6. Activity of Cyanobacterial biofertilizers

7.6. Phosphorus adding biofertilizers

Though, most soil contain appreciable amount of inorganic phosphorus,but most of it being in insoluble forms, cannot be utilize by crops unless they are solubilized. Soils also contain organic phosphorus that also could not be utilize by plants directly. It is only utilizes when it is mineralized. Phosphate solubilizing microorganisms (PSM) not only able to solubilize insoluble forms of inorganic phosphorus but are also capable of mineralize organic forms of phosphorus present in soil, thus improving the availability of native soil phosphorus to plants. PSM also solubilize phosphorus from rock phosphate (RP), slag or bone meal, making their phosphorus available to plants. Thus,PSM biofertilizer are being economical and environmentally safe, offers a viable alternative to inorganic chemical phosphate fertilizers.

Many microorganisms can solubilize inorganic phosphates, which are then unavailable to plants. Various bacteria and fungi reported to solubilize different types of insoluble phosphates. They not only solubilize inorganic phosphates but also mineralize organic phosphorus compounds and release orthophosphates. Different mechanisms were suggested for the solubilization of inorganic phosphates and production of inorganic acids.

- Chelating effects
- Production of inorganic acids
- Hydrogen sulphide production(H_2S)
- Effect of carbon dioxide
- Siderophore production

The extent of PO_4 solubilization depends on the type of organisms involved. The genus *Bacillus* showed maximum activity followed by*Penicillium* and *Aspergillus.* Streptomyces was least effective.

A. awamori, A. niger, P. striata are effective in solubilization of phosphate.

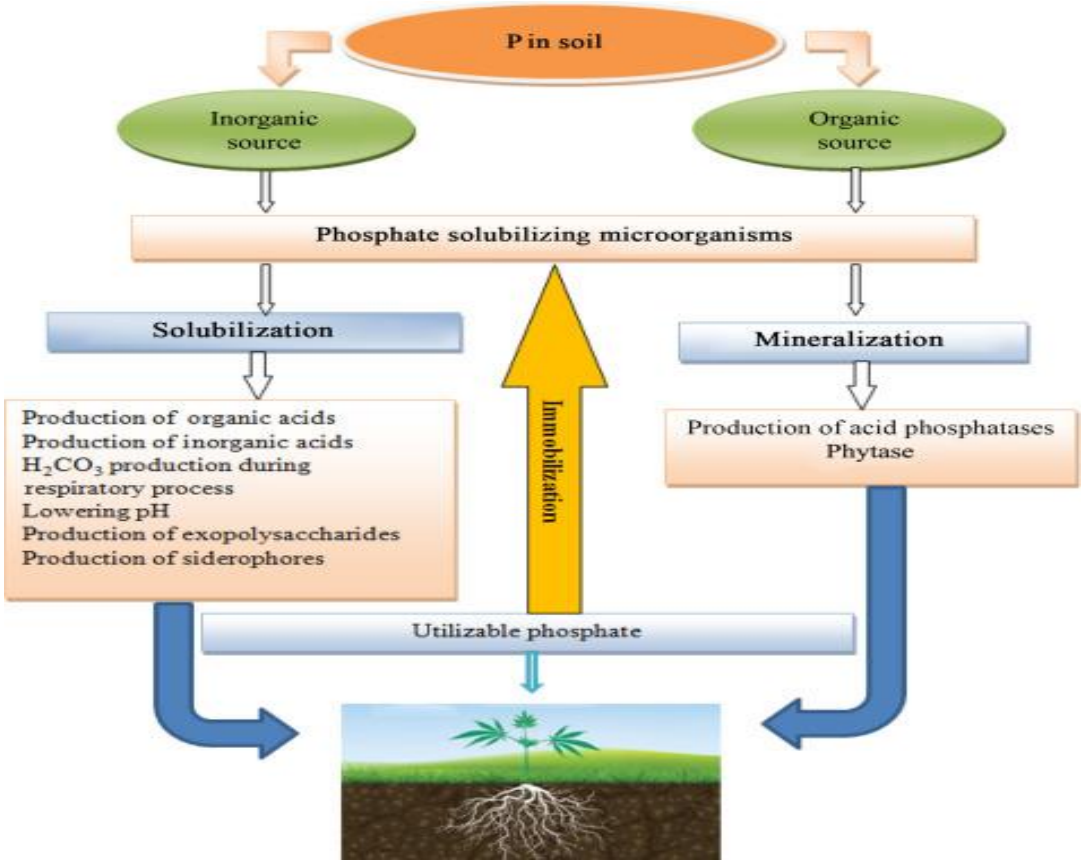


Fig.7.7. Phosphorus solubilizing microorganisms

7.7. Mycorrhizal Biofertilizers

Here the fertilizer contains mycorrhizae that colonize in the rhizosphere of roots and the interior of the plant roots when applied to seeds, plant surfaces, or soil, encourage the growth by increasing the supply or availability of primary nutrients to the host plant. The mycorrhizae obtain photosynthetic product in exchange, so both are mutually benefitted (symbiotic association). An arbuscular mycorrhiza, is a type of endo mycorrhiza, in which the fungus penetrates the cortical cells of the roots of a vascular plant. Arbuscular mycorrhizae (AM) are characterized by the formation of unique structures, such as arbuscules and vesicles by fungi of the phylum Glomeromycota (AM fungi). AM fungi (AMF) help plants to capture nutrients, such as phosphorus and micronutrients from the soil. It is believed that the development of the arbuscular mycorrhizal symbiosis played a crucial role in the initial colonisation of land plants and in the evolution of the vascular plants.

Ectomycorrhiza and endomycorrhiza are important in agriculture and forestry.In ectomycorrhiza, no intracellular penetration into epidermal or cortical cells occurs, but an extensive external network called the hartignet is formed between these cells. The fungi forming ectomycorrhizal association are coming under Basidiomycotina and Ascomycotina, e.g., *Laccaria laccata, Suillus, Rhizopogan, Amanita,* and more rarely from Zygomycota. They are less common, about 4%, found in roots of trees such as Pine, Spruce and Oak. This shows that ectomycorrhizal fungi develop primarily with conifers and hardwoods, and are essential for woody plants and trees.

Endomycorrhiza (vesicular arbuscular mycorrhiza; VA mycorrhiza; now known as arbuscular mycorrhiza, orchid mycorrhiza) produce an internal network of hyphae between cortical cells that extends out into the soil, where, the hyphae absorb mineral salts and water from soil. This fungus does not form an external mantle but lives within the root. In all forms, hyphae run between and inside the root cells, play a very important role on enhancing the plant growth and yield due to an increase supply of phosphorus to the host plant. Mycorrhizal plants can absorb and accumulate several times more phosphate from the soil or solution than non–mycorrhizal plants. Plants inoculated with endomycorrhiza have been shown to be more resistant to some root diseases. It is now generally recognized that they improve not only the phosphorus nutrition of the host plant but also its growth, which may result in an increase in resistance to drought stress and some diseases. Therefore, AM fungi offer a great potential for sustainable agriculture production, and the application of AM fungi to agriculture has been developed.

They are commonly found (about 80%) in roots of commercially cultivated plants such as Maize, wheat and Soyabeans. Other examples are most of vegetables, grasses, flowers, shrubs, fruits, trees and ornamentals.

In some species, both ecto and endo mycorrhizae occurs simultaneously, for example Salix, Populus, and Eucalypt. Members of Glomeromycota mostly participate in Endomycorrhiza.

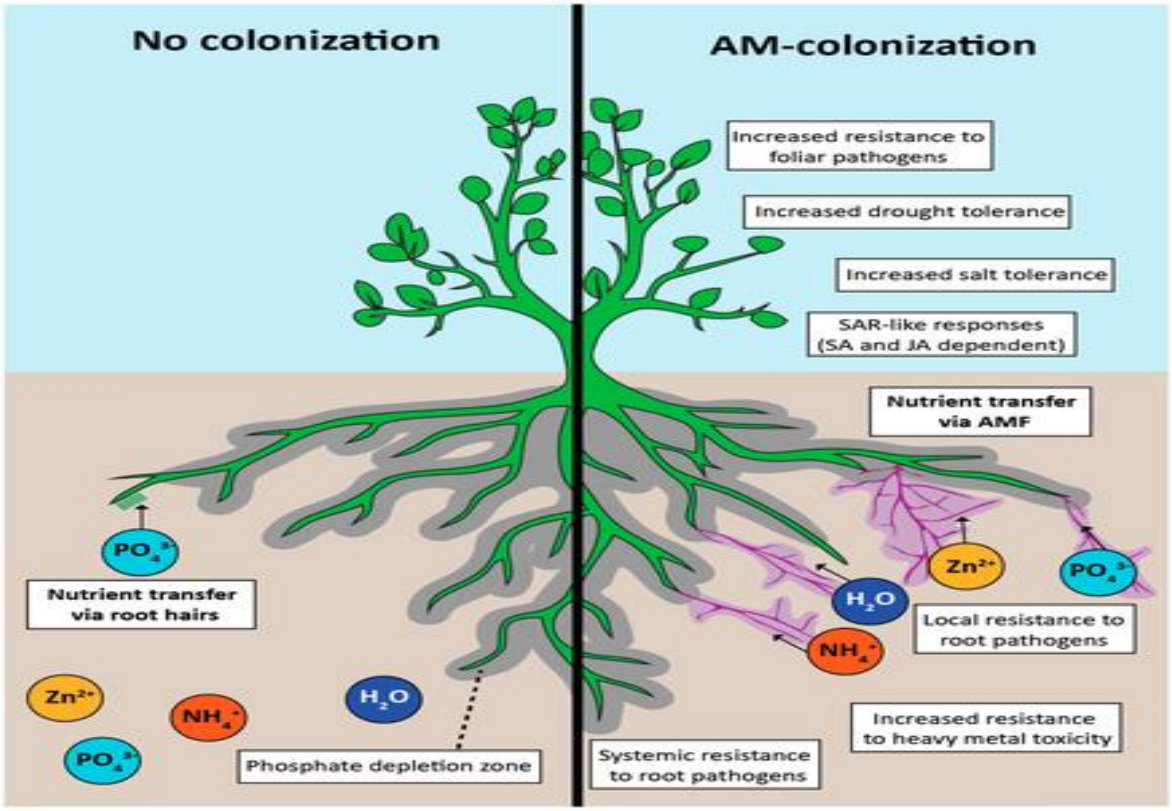


Fig.7.8. Effect of AM on growth of plant

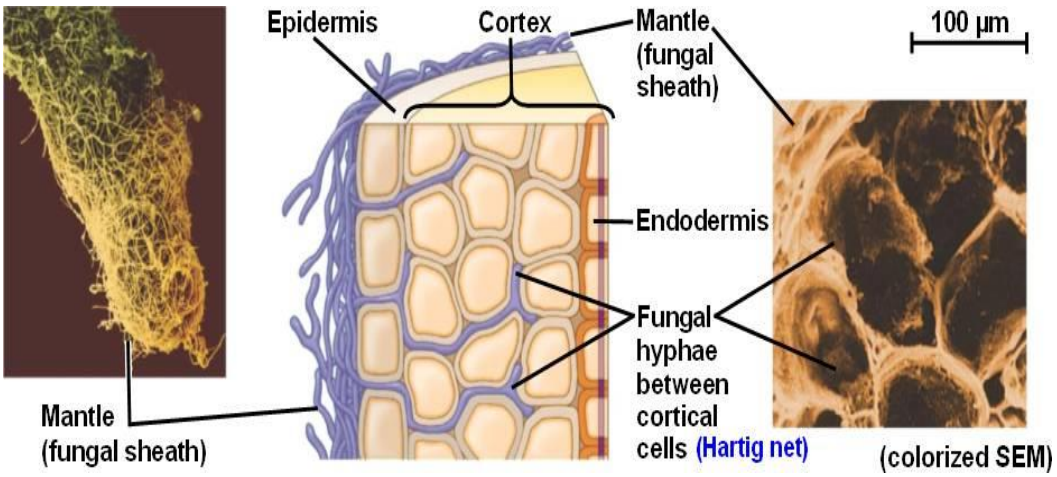


Fig.7.9. Ectomycorrhiza and Endomycorrhiza

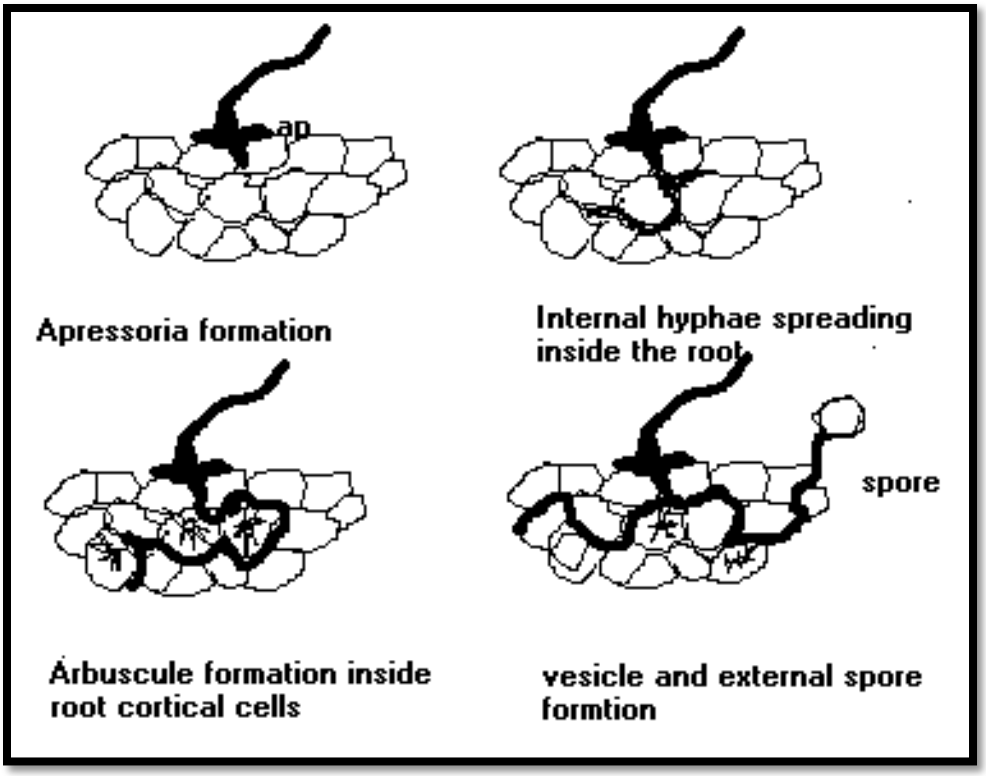


Fig.7.10. Mechanism of establishment of Endomycorrhizal symbiosis

7.7.1. Importance of Mycorrhizal Biofertilizers

- Mycorrhiza plays a very important role on enhancing the plant growth and yield due to an increase supply of mineral nutrients, mainly phosphorus to the host plant.
- Plants with mycorrhizal association will have higher efficiency for nutrients absorption, such as nitrogen, phosphorus, potassium, calcium, magnesium, zinc, and copper; and also increase plant resistance to drought.
- Mycorrhiza helps to absorb other organic substances that are not fully soluble for plants to use, and also help to absorb and dissolve other nutrients for plants by storage in the root.
- It leads to improvement in plant photosynthesis, nutrients translocation, and plant metabolism processes.
- Improve plant resistance to root rot and collar rot diseases.
- Mycorrhizal roots have elevated levels of phenols, while offers resistance to fungal hydrolytic enzymes.

- Mycorrhizal infection stimulates biosynthesis of phytoalexins.
- Increase yield and crop quality.
- Enhance flowering and fruiting.
- Increase plant establishment and survival at seedling or transplanting.

7.7.2 Production of Mycorrhizal Biofertilizers

- Ten to 50 g of freshly collected soil sample is put into 1 to 2 litters of plastic beakers.
- Soil is suspended with about 500 ml to 1 litter of tap water. Soil macro-aggregates should be crushed with hand.
- After 10-30 seconds of settling down of soil particles, the upper layer of soil suspension is poured into the sieving.
- The procedure should be repeated until the upper layer of soil suspension becomes transparent.
- The sieving on the fine mesh is collected into a small beaker and dispersed with ultra sonication.
- Weak sonication (i.e., 30w for 30 sec) is enough. Then the dispersed sample is again passed through the sieve.
- Depending on toughness of soil aggregate, the sonication process can be repeated.
- Usually AM fungal spores are collected on 100 µm sieve. Some small spores are on 50 µm sieve. To collect large spores of *Gigasporamargarita*, 250 µm sieve is required.
- Spores of am fungi have characteristic shapes and colours, and so it is not difficult to discriminate the spores in organic debris collected on the sieves.

7.8. Biofertilizers versus inorganic chemical fertilizers

Biofertilizers are preferred over inorganic chemical fertilizers, because they are not harmful to human, soil and environment as they contain organic materials. While, inorganic chemical fertilizers are made up of inorganic chemicals which are harmful to the plants, soil as well as to environment. Inorganic fertilizers usually contain only few nutrients, generally nitrogen, phosphorus, potassium, sulphur and sometime micro nutrients, either singly or in

combination. These nutrients are in a form readily available to plants. Fertilizers help in the development of the soil fertility quality of the field, by replenishing the necessary nutrients that are required by the plant for their growth. Let's see why biofertilizers are better than inorganic fertilizers (chemical fertilizers)-

Biofertilizers	**Inorganic chemical fertilizers**
They are part of organic farming and are best for sustainable agriculture.	They are not part of sustainable agriculture.
They are renewable nutritional resources mostly nitrogen-fixing microbes.	They are traditionally used on a large scale to obtain more crop yield.
They are free living and symbiotic bacteria, cyanobacteria, or fungi.	They are in the form of inorganic chemicals.
They enrich soil with nutrients. It is eco-friendly and not hazardous for our health.	Overutilization of chemical fertilizers causes pollution of soil &water it is hazardous for our health.
They provide almost of all the nutrients, but slowly.	They supply only the specific nutrients, but quickly.
Large scale application is possible.	Large scale application is possible but it will be costly.
Maintain a natural cycle of soil, build soil, organic matter and enrich soil fertility.	Over fertilization kills plants and degrades the renewable environment; contributing to the greenhouse gas effect.
They provide nutrients by natural processes to promote plant growth.	A high concentration of inorganic chemicals such as, nitrogen, phosphorus & potassium is required for plant growth.
Plant growth stimulation occurs through the synthesis of nutrient acquisition hormone production and biocontrol.	Plant growth stimulation occurs through the synthesis of inorganic material.
Eg;*Rhizobium, Azotobacter, Azospirillum*	Eg; ammonium nitrate, ammonium sulphate, urea, Diammonium phosphate, NPKA, Micronutrient fertilizers etc.

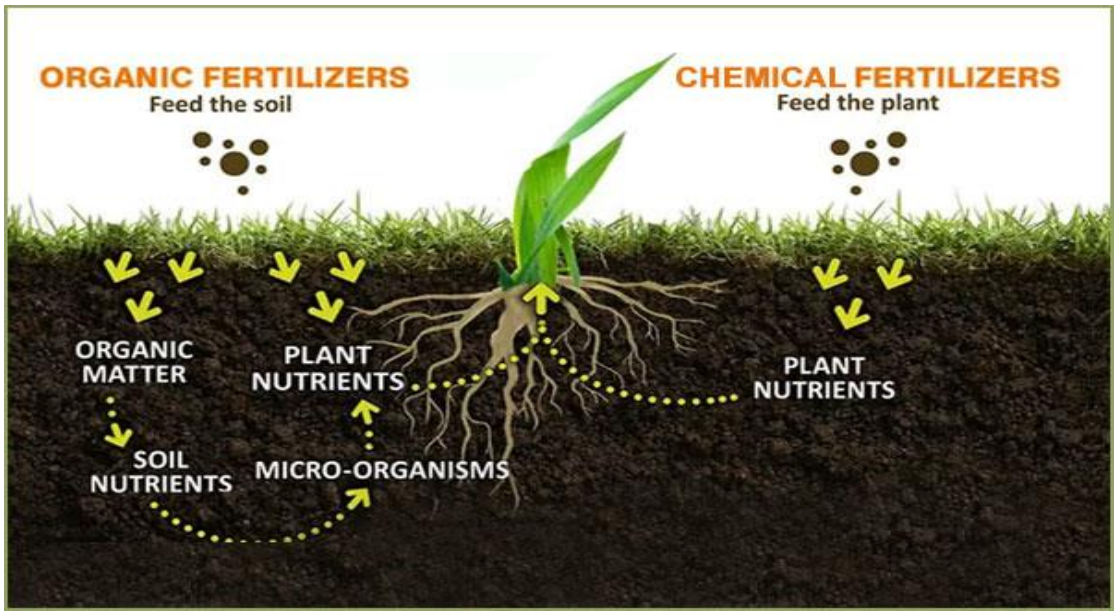


Fig.7.11. Supply of nutrients by organic and inorganic fertilizers to the plants

7.10. Summary

Continuous cropping resulting in decrease in soil fertility and decrease in yield in crop production. So, farmer use inorganic or organic fertilizers to increase soil fertility and crop production. However, excessive use of inorganic fertilizers decreases the fertility of soil, crop production, harden the soil, pollute the air and water (eutrophication), release the greenhouse gases, kills soil microorganisms, leads to acidification of soil and chemical burn to crop plants. Therefore, the farmers move towards organic farming. Organic farming is increasing the production of pollution-free crop. It involves the use of biofertilizers and biopesticides, which increases the nutrients availability to the crop and controls any kind of pest and pathogens, and develops good quality of crop with good yield. Biofertilizers are microorganisms that add the different types of nutrients to the soil. Bacteria, fungi, and algae are some of the beneficial microorganisms that help in improving the ability of the soil and increasing the yield of crop, and maintain the ecological balance and protect the environment.

Biofertilizers are the perfect alternative to inorganic chemical fertilizers. The chemical fertilizers not only harm the soil and its productivity but also harm the living

organisms, consuming the crops grown on that soil. Therefore, the scientists had discovered the use of microorganisms as biofertilizers.

They have gained recognition over the years of work and are being implemented on a large scale to increase agricultural productivity without harming human health, soil and environment. This is commonly known as organic farming. Different types of microorganisms are used in preparation of biofertilizers such as- Nitrogen fixing bacteria(*Rhizobium, Azospirillum, Frankia*), nitrogen fixing algae (Blue-green algae), phosphate solubilizing bacteria(*Pseudomonas, Bacillus, Xanthomonas*) and phosphate mobilizing mycorrhizal fungi. The different types of microbial organism carriers, commonly known as microbial inoculants carriers, also participate in the preparation of biofertilizers. They are sedge peat, farmyard manure, compost, lignite, charcoal, coir waste, rice bran, vermiculite clay, etc. The inoculants formulation is usually commercialized in one of three forms- 1.powder, 2.liquid, 3.granular. More recently, hard mineral waste products have been also developed.

Microbial fertilizers application has many useful effects such as- improve plant nutrients availability in soil, promotes plant growth, increase plant growth hormones synthesis (IAA), improve soil structure, participate in biogeochemical cycle and increase photosynthetic process etc.

Algal biofertilizers, cyanobacterial biofertilizers, participate in nitrogen fixation in rice field was recognize in India, during 1939, by P.K. De. There are four methods used for cultivation of cyanobacterial biofertilizers. They are- 1.cemented tank method, 2. Shallow metal troughs methods, 3. Polythene lined pit method, and 4. Field methods. Use of cyanobacterial biofertilizers increases plant growth and yield, soil moisture, and produces bioactive compounds, such as amino acids, carbohydrates, vitamins, and growth hormones like Auxin, Gibberellin and cytokinin.

Many microorganisms such as bacteria and fungi are able to solubilize inorganic phosphate and mineralizes organic phosphorus compound, thus improving the availability of phosphorus in soil for growth of plants.

Mycorrhiza is a type of symbiotic association between fungi and vascular plant root. It may be ectomycorrhiza (form an extensive external network, called Hartignet, between epidermal or cortical cells) or endomycorrhiza (fungi penetrate inside cortical cells of root, forms special structures known as arbuscles and vesicles , commonly known as VA mycorrhizae or AM). The mycorrhizal fungi play an important role in increasing growth of plant due to increase supply of mineral nutrients mainly phosphorus and water.

Biofertilizers are economical, effective and renewable sources of plant nutrients, harmless to human beings and ecofriendly to soil and environment.

The role of biofertilizers in agriculture production shows a special importance, in organic farming and sustainable agriculture development, particularly in the present context of the sky-rocketing cost of agriculture inputs.

Green technology supports the use of biofertilizers to increase the corp production.

7.11. Terminal questions

Q.1. What are biofertilizers? Explain their role in increasing soil fertility and sustainable crop production.

Answer----------

Q.2. Describe the production mechanism of microbial biofertilizers.

Answer----------

Q.3. What are algal biofertilizers and how it formed? Explain.

Answer----------

Q.4. Describe the phosphorus adding biofertilizers.

Answer----------

Q.5. Differentiate biofertilizers and inorganic chemical fertilizers.

Answer……

……

Q.6. Describe microbial inoculants and its importance in brief.

Answer----------

Q.7. What are mycorrhizal biofertilizers? Explain in detail.

Answer----------

7.12. Further suggested readings

1. Biofertilizers And Pesticides- H.C. Lakshman
2. Manures, Fertilizers And Pesticides- Amiitava Rakshit, Priyankar Raha And Nirmal DeFertilizers A Text Book- Ranjan Kumar Basak
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Unit-8: Microbial Biopesticides

Contents

8.1. Introduction

Pesticides are chemicals used by farmers to protect their crop plants from harmful or toxic to dangerous bugs, fungi and also to pathogen, in modern agriculture. These pesticides can be bad for human health and ecosystem, if they are toxic enough and amount that ends up in the environment is high enough. The scientists have detected certain bug-killing pesticides in water bodies near agricultural fields across the world. So chemical pest control agents, though extensively used in all countries of world, have been widely regarded as ecologically unacceptable. Another way of controlling pests is a use of biopesticides. The biopesticides are certain types of pesticides derived from natural materials, such as animals, plants, bacteria, fungi and certain minerals. For examples, Canola oil and baking soda have pesticidal applications and are considered as biopesticides. As of August 31, 2020, there were 390 registered biopesticides active ingredients. Therefore, there is an increased social pressure to replace these chemical pesticides gradually with biopesticides which are safe to humans, non-target organism, soil and enviroment.The integrated pest managemet(IPM) programs works to manage the crop to prevent the pests from becoming a threat.

Fig. 8.1: Intigrated Pest Management For Sustainable Agriculture and Enviroment

Objective

After studying the course of this unit, students will be able to

- understand the biopesticides and their types.
- know about the microbial herbicides and their control management.
- know about bacterial biopesticides(insecticides) with their advantages and disadvantages in their use.
- know about viral insecticides and their application, advantages and disadvantages of their use.

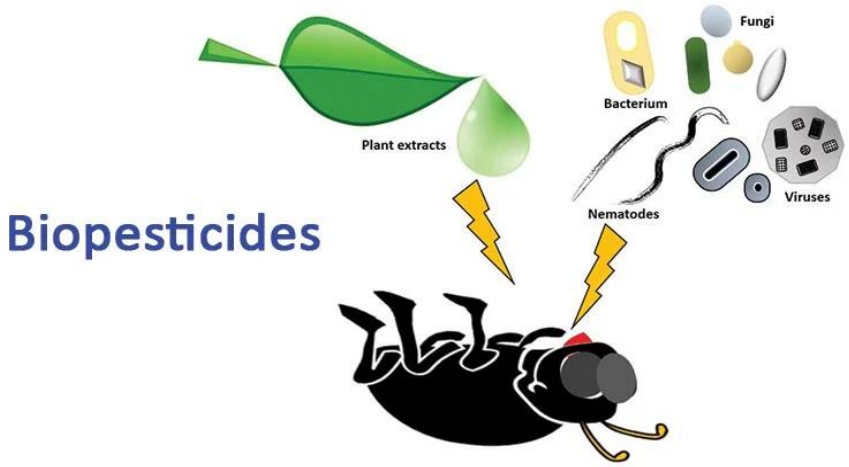

Fig.8.2: Different types of microorganisms used as biopesticides

8.2. Biopesticides and Their Classification

A biopesticides is a biological substance or organism that damages, kills or repels organisms seen as pests. Biological pest management intervention involves predatory, parasitic or chemical relationships.

The biopesticides and pollution prevention division of the environmental protection agency, which registers biopesticides in U.S., classified biopesticides into three categories-

- Biochemicals
- Microbials
- Plant- incorporated protectants

1. **Biochemicals-** obtained from naturally occurring substances such as plant extracts. This includes insectrepellents, insect attractants and repellents, insect sex pheromones, and non-pest management class- plant growth regulators.

   Examples-

   a. Azardiractin- broad spectrum insecticides
   b. Capsaicin- broad spectrum insecticides, nematicides and fungicides.
   c. Clove, rosemary and peppermint oil -all broad spectrum fungicides.

2. **Microbials-** microbials are products containing microorganism or their fermentation by-products. Microorganisms may be bacteria,fungi, virus or protozoans.These are-

   a. Bacteria

      i.Pseudomonaschlororaphis

      ii.Pseudomonasfluorescens

      iii.Bacillus thuringiensis- kills moth, flies and mosquito larvae.

   b. Fungus Beauveria bassiana- used against whiteflies, aphids and thrips control
   c. Virus- Baculovirus (BV)
   d. Protozoan pesticides- while bacterial pesticides are used widely, protozoan pesticides are comparatively less in number, though proved their potential in controlling a broad range of pests including caterpillar pests, grasshopper, corn earworm, army worms, European corn borer, cabbage looper and fall webworm.

3. **Plant- incorporated Protectants (PIPs**)-PIPs are pesticidal substances that are produce from genetic material, that are incorporated into plants as result of genetic manipulation. The scientists can take the gene for a specific Bt. pesticidal protein from *Bacillus thuringiensis*, and introduce the gene into the plant genetic material. Then the plant manufactures the pesticidal protein that control, the pest when it feeds on the plants. Both the protein and its genetic material are regulated by Environment Protection Agency (EPA), the plant itself is not regulated. PIPs have been approved for a number of crops, including corn, cotton, potato, soyabean, papaya and plum. Most PIPs (example Bt. varieties) produce substances for insect control while other protect against plant diseases. The pesticides also classified or grouped according to the types of pests which they kill-

   1. Insecticides- kills insects

2. Herbicides- kills weed plants
3. Rodenticides- kills rodents (rats and mice)
4. Bactericides- kills bacteria
5. Fungicides- kills fungi
6. Larvicides- kills larvae

Another way to classify these pesticides is based on how biodegradable they are-

1. Biodegradable pesticides- the biodegradable can be broken down by microbes and other living beings into harmless compounds.
2. Persistent pesticides- while, the persistent are those which may take months or years to break down.

The first known chemical pesticide was elemental sulphur dust, used in ancient time, about 4,500 years ago in ancient Mesopotamia. The Dichlorodiphenyltrichloroethane, commonly known as DDT, is the first modern commercially used synthetic inorganic insecticides, synthesized in 1940. The United States banned it in 1989 . India had produced it up to the year 2009, to control visceral leishmaniasis and insect born malarial disease. DDT is carcinogenic and is a persistant non-biodegradable inorganic pollutant that has a half-life of 22 days to 30 years in soil and upto150 years in aquatic environment.

Integrated Pest Management(IPM) is a sustainable approach to manage pests by combining biological, cultural, physical and chemical tools, in a way that minimize economic, health and environmental risks.

8.3. Microbial Herbicides

They are bioherbicides that can control the growth of weeds. Weeds are unwanted, persistet, harmful plants causing serious ecological problems and capable of altering the process of ecosystem, they may support populations of non-native animals and microbes, and hybridize with native species. Out of 30,000 species of weeds, 1900 species cause loss in yield by 9.5% of the total crop production every year in the world. Weeds also serve as reservoir for plant pathogens that may cause significant economic loss in crop production.

There are three types of control method of weeds. These are-

a.mechanical methods-weeds are removed from field by use of mechanical implement pulled by tractor to chop or uproot small weeds, or by hand removal. Tillage, primary and secondary. also contol the weeds.

b.chemical methods-chemicals that are used to kill unwanted plants or weeds are called hebicides (or weedicides), normally applied uniformally on soil surface or spray on foliage of plants (Atrazine, Butachlor, 2,4-dichlorophenoxyacetic acid, Isoproturon).

c.biological herbicides-weeds are killed with the help of livig organism, such as insects,fungi, bacteria (Xanthomonas, Pseudomonas) and viruses.

Selective herbicides, control specific weed species, while leaving the desired crop relatively unharmed, where as non-selective herbicides can be used to clear waste ground, industrial and construction sites, railways and railway embankments, as they kill all plant material with which they come to contact. Biological control is the deliberated use of living organism to control a pathogen or weed. Biological weed control practices have been developed for the sustainable use of biodiversity for economic benefits to mankind.

Table.8.1: Weeds fuction as reservoir for various pathogens

Name of the weed species	**Types of Infection**	**Name of the pathogen**
Chondrilla junces	Weed rust	*Puccinia chondrillina*
Rubus constrichus	Black berry rust	*Phragmidium violaceum*
Acacia saligna	Acacia gall rust	*Uromycladium teperium*
Carduus thoermeri weinm	Carduus rust	*Puccinia carduorum*
Centaurea diffusa	Centaurea rust	*Puccinia jaceae*
Ageratina riporia	Rust	*Cercosporella sp.*

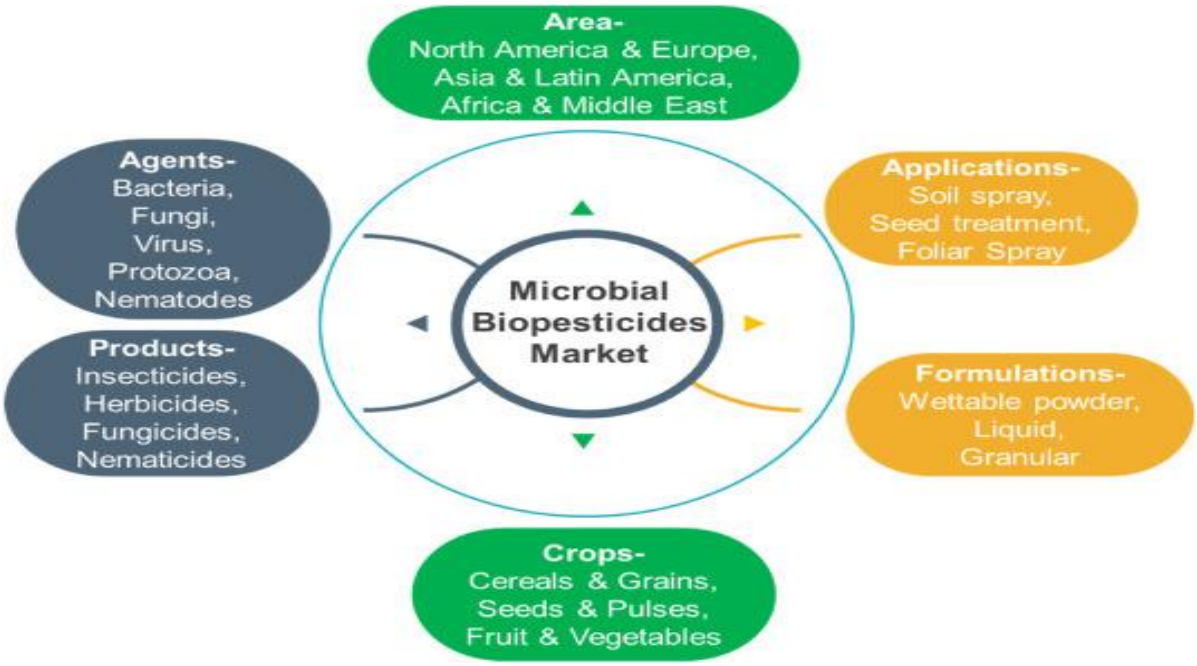

Fig.8.3: Microbial Biopesticides Market

Table: 8.2: Correction between colony pigmentation and P. Chlororaphis isolate classification

Name of P. Chlororaphis		
Aureofaciens	Green PCA	ATCC 9446
Aurantiaca	Orange/Yellow PCA, 2-OH PCA, 2-OH PHZ	ATCC 13985
Piscium	Yello/green, PCA and PCN	NCIMB 14478

8.4.1 Microbial Herbicides Control Management

A worldwide programme has been growing up to control the invasive weed species for the better crop production and stable ecosystem. Biological control is the deliberated use of living organism to control the weed. Biological weed control practices have been developed for the sustainable use of biodiversity for economic benefits successfully in bio-control of weeds for many years. Various successful weed control project has been initiated, one of them was the use of foliar smut fungus, *Cercosporella sp.* in Hawaiian forest

from Jamaica to control *Ageratina riporia*. It was estimated that more than 45,000 ha of pastureland (land used for grazing of animals) have been rehabilitated to their full potential due to the application of this pathogens. A major project has been undertaken for biological management of *Mimosa invisa (giant Mimosa),* a non-native invasive species that threaten to the Kaziranga National Park Assam in India. Insects, *Heteropsylla spinulosa, plant-parasitic hempterans,* causes extensive damage to weed *Mimosa invisa (now known as Mimosa diplotricha)* in various countries such as Australia, Fiji, and Brazil.

Many species of weeds were reported to acquire resistance against commercially available chemicals herbicides. There are about 307 herbicide resistant weeds biotype worldwide, 113 of these biotypes occurs in the United state alone. Today, it is possible to improve efficacy of plant pathogens by recombinant DNA Technology. Classical bio-control approach is not at all successful over the bio-herbicide approach. A number of microbial herbicides have been developed till date, but only a few of them are available in commercial form, due to several constraints in the formulation, application and commercialization. Biocontrol agents probably fail to be marketed internationally as these are living organisms and are fearful to introduce them from foreign countries. Screening and genetic modification of potent microbial species are highly encouraged for a better commercial mycoherbicide development.

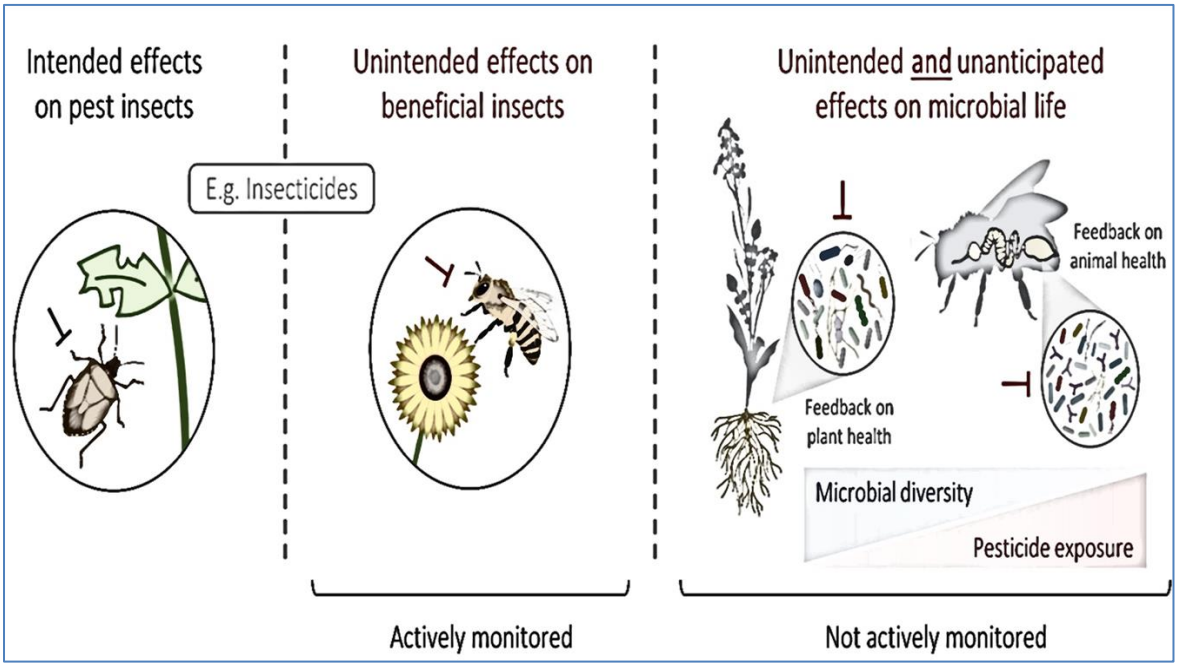

Fig.8.4: Effects on biopesticides upon pest insects

8.4. Microbial Insecticides

Microbial insecticides are those microorganisms or their products that are capable of attacking and killing pest insects. Microbial insecticides are comprised of microscopic living organisms (viruses, bacteria, fungi, protozoa, or nematodes) or the toxins produced by these organisms. They are formulated to be applied as conventional insecticidal sprays, dusts, or granules. Each product's specific properties determine the ways in which it can be used most effectively. The organisms used in microbial insecticides are essentially non-toxic and non-pathogenic to wildlife, humans, and other organisms not closely related to the target pest.

The safety offered by microbial insecticides is their greatest strength. If necessary, most of microbial insecticides can be used in combinatiom with synthetic chemical insecticides, because, in most cases, the microbial product is not deactivated or damaged by residues of conventional insecticides. These are sourced from entomopathogenic bacteria, entomopathogenic fungi, actinomycetes, viruses, protozoa or nematodes.

8.4.1 *Pseudomonas* as a Bacterial Biopesticides

Pseudomonas are rod-shaped, aerobic, gram-negative bacteria. Certain *Pseudomonas* species have previously been reported to have entomopathogenic properties and represent a promising source of insecticidal genes for use in GM crops. *Pseudomonas* including *P. chlororaphis* are ubiquitous in the environment, have a history of safe use in agriculture as seed treatments, foliar-applied biopesticides and as a gene source for GM crops, and lack known pathogenic, toxic or allergenic properties.

Pseudomonas chlororaphis isolates are being used in agriculture as biopesticides because they provide plant protection against an array of microbial pathogens, insects and nematodes. These isolates directly control microbial pathogens, insects, and nematodes through the production of an array of metabolites. In the rhizosphere, these metabolites are involved in direct antagonism of plant pests.

P. chlororaphis 06, like other biocontrol bacteria, releases an array of metabolites in the rhizosphere which includes-

i. Volatiles, such as hydrogen cyanide (HCN), butanediol, hydrocarbons, which would travel through air channels in the soil. HCN produced from amino acids glycine and have broad spectrum toxicity to fungi, nematodes, insects, etc.
ii. The water-soluble antagonistic chemicals, including several phenolics and siderophores, spread through water movement from the rhizosphere into the soil pore water.
iii. Insecticidal protein, toxic to insect, causes death of *Spodoptera* (army worm), *Heliothis* (tobacco bud worm) larvae.

The some *P. Chlororaphis* isolates for which direct pesticidal activity has been correlated with specific metabolites and some *P. chlororaphis* known to have biological pesticidal control activity but for which the specific metabolites have currently not been characterized (table 8.2.). This shows these bioactivate isolate exist globally and are associated with diverse plants. The genomes of several of those isolates are now available (table 8. 3.).

Pseudomonas fluorescens use for the management of bacterial pathogens, such as *Ralstonia solanouarum*, *Erwinia caratavora* as well as nematodes, like Root Knot nematodes, cyst nematodes, citrus nematodes and banana nematodes.

Table 8.3: Examples of *Pseudomonas chlororaphis* isolates with biological pasticidal activity and without designation of active metabolites.

Strain	**Source**	**Pathogens/disease controlled**
MA342	Swedish soils	Cereals diseases
Tx- 1	Turf grass	Sclerotinia, pythium
63-28	Canola	Pythium, Rhizoctonia
449	Maize rhizosphere	Sclerotinia, Rhizoctonia
M17	Tomato rhizosphere	Fusarium oxysporium
Pcho10	Wheat heads	Fusarium graminearum
YL-1	Soyabean roots	Bacterial and fungal diseases
HT66*	Rice rhizosphere	Phenazine producer

UFB2*	Soyabean soil	Bacterial tomato canker

Table 8.3: List of products of *Pseudomonas chlroraphis* strains commercialized as biopesticides.

Trade Name	Strain Name	Target pests of effects
AtEze	63-28	Stem and root rots in vegetables
BioJect	Tx-1	Dollar spot, anthracnose, Pythium, oink snow mold in turf
Cedomon	MA342	Seed borne pathogens in barley and oats
Cerall	MA342	Seed borne pathogens in wheat, rye and triticale
Cedress	MA342	Seed borne Ascochytain pea and Acrothecium carotae on carrots.
Nematokill	O6	Root knot nematodes
ItaEpi	O6	Aphids
Bastapa	O6	Plant growth-promoting microbial fertilizer for vegetables.
Biohelper	O6	Microbial fertilizer/inducer of disease resistance
Helper Plus	O6 mixed	Microbial fertilizer for vegetables

Understanding the mechanism involved in the efficacy of these metabolites will promote the use of these biochemicals as well as the microbes that synthesize these products, in formulations for agricultural practices aiming towards sustainability of soils as well as the quality and quantity of the crop.

The bacteria controls root rot and wilts diseases of banana, bean, cotton, groundnut, pigeon pea, soya, and tomato. *Pseudomonas* formulations are effective against the rice blast and sheath blight of paddy, and promote plant growth through the modulation of plant hormonal pathways. The activity is attributed to bioactive secondary metabolites, such as 2,4-diacetylphloroglucinol (DAPG), phenazines, pyrrolnitrin or alkylresorcinol.

This beneficial *Pseudomonas* sp. can be exploited as biological pesticides to reduce the use of chemical pesticides in agriculture. Biopesticides can be used either alone or in combination with chemicals to lower the doses of chemicals needed to obtain a profitable crop yield. In recent years, the production costs of new agrochemicals have increased and stricter safety rules on their use also require alternative pest control methods.

Biopesticides used for wheat (seed coating), potato, radish, sugar beet and fruits has indeed resulted in crop protection and increased crop yields.

Pseudomonas spp. are particularly suitable for application as agricultural biopesticidal control agents, since they have many properties-

1. can use many exudates compounds as a nutrient source.
2. are abundantly present in natural soils, in particular on plant root systems, which is indicative for their adaptive potential.
3. have a high growth rate relative to many other rhizosphere bacteria.
4. possess diverse mechanisms by which they can exert inhibitory activity towards phytopathogens and thereby mediate crop protection.
5. are easy to grow *in vitro.*
6. can subsequently be reintroduced into the rhizosphere by seed bacterization.

Pseudomonas aeruginosa- play a vital role in the biodegradation and bioremediation of the large number of toxic compounds, found in water and soil by utilizing the pesticides anti-carbon source and energy, thus producing secondary metabolites and biopolymers, making these strains useful in medicine, industries and environment.

These secondary metabolites show antibacterial and antifungal activity, and could be applied in the management of human, animals and plant disease.

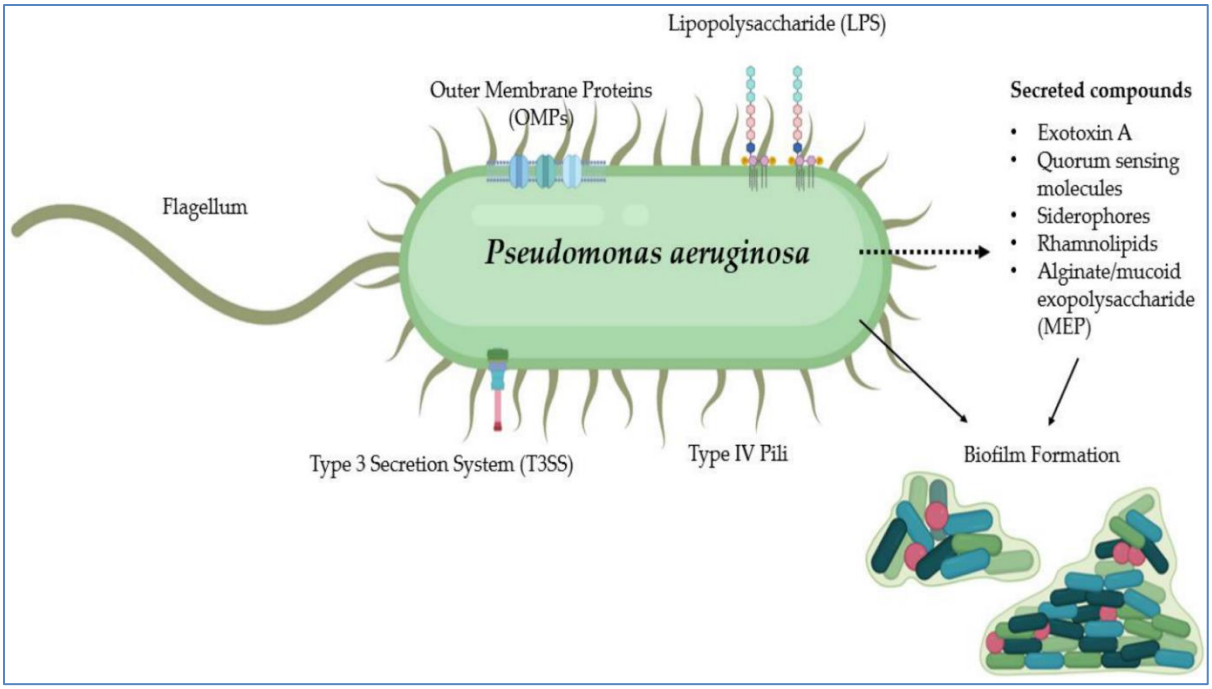

Fig.8.5: *Pseudomonasaeruginosa*

8.4.2 *Bacillus* sp. As Bacterial Insecticides

The bacteria, *Bacillus thuringiensis,* is an aerobic, gram positive and spore forming speces. It is commonly known as Bt. It is found in soil, water, insect faeces, up on insect and plants surfaces, etc. It is widely used as a bioinsecticide to control the infestation of butterfly caterpillars. Caterpillars eat the spores sprayed onto plants and get killed.

Bacillus thuringiensis (Bt.) produces an insecticidal protein during sporulation. The Bt.-toxin gene is introduced into plants to develop a variety of pest-resistant plants by genetic engineering, e.g., Bt. cotton, Bt. corn, etc.

Bacterial insecticides must be eaten to be effective. Insecticidal products comprised of a single *Bacillus* species may be active against an entire order of insects, or they may be effective against only one or a few species. For example, products containing *Bacillus thuringiensis* kills the caterpillar stage of a wide array of butterflies and moths. In contrast, *Bacillus papillae* (milky spore disease) kill Japanese beetle larvae but are not effective against the closely related annual white grubs. The microbial insecticides most widely used in the United States, since the 1960s. are preparations of the bacterium *Bacillus*

thuringiensis (abbreviated as BT.). Bt. products are produced commercially in large industrial fermentation tanks. As the bacteria live and multiply in the right conditions, each cell produces (internally) a spore and a crystalline protein toxin called an endotoxins. Most commercial Bt. products contain the protein toxin and spores, but some are cultured in a manner that yields only the toxin component.

When Bt. is ingested by a susceptible insect, the protein toxin is activated by alkaline conditions and enzyme activity in the insect's gut. The toxicity of the activated toxin is dependent on the presence of specific receptor sites on the insect's gut wall. This necessary match between toxin and receptor sites, determines the range of insect species killed by each Bt. subspecies and isolate. If the activated toxin attaches to receptor sites, it paralyzes and destroys the cells of the insect's gut wall, allowing the gut contents to enter the insect's body cavity and blood stream. Poisoned insects may die quickly from the activity of the toxin or may die within 2or 3 days from the effects of Septicaemia (blood poisoning). Although, a few days may elapse before the insect dies, it stops feeding (and therefore, stops damaging crops) soon after ingesting Bt. Cotton. Bt. Cotton does not colonize or cycle (reproduce and persist to infect subsequent generations of the pest) in the environment in the magnitude necessary to provide continuing control of target pests. The bacteria may multiply in the infected host, but bacterial multiplication in the insect does not result in production of abundant spores or crystalline toxins. The usual result is that few or no infective units are released into the environment when a poisoned insect dies. Consequently, Bt. products are applied much like synthetic insecticides. Bt. treatments are inactivated fairly rapidly (within one to a few days) in many outdoor situations, and repeated applications may be necessary for some crops and pests.

Bt. products that kill caterpillars are not effective against other types of pests, they will not control aphids, beetles, flies, or additional pests other than caterpillars. Even certain caterpillars are not effectively controlled by Bt., especially those that live in the soil or bore into plant tissues without consuming a significant amount of the Bt. applied to plant surfaces. Again, Bt. is a stomach poison that must be ingested to be effective. The peach tree borer in stone fruits, corn earworm in corn, and the cutworms that clip off field crops or garden plants are examples of caterpillars seldom controlled by Bt. treatments. Bt. is not

registered for the control of codling moth larvae that attack apples and pears, because these larvae do not feed much (if at all) on treated surfaces.

Where Bt. is applied to plant surfaces or other sites exposed to sunlight, it is deactivated rapidly by direct ultraviolet radiation. To maximize the effectiveness of Bt. treatments, sprays should thoroughly cover all plant surfaces, including the undersides of leaves. Treating in the late afternoon or evening can be helpful because the insecticide remains effective on foliage overnight before being inactivated by exposure to intense sunlight of the following day. Treating on cloudy (but not rainy) days provides a similar result. Production processes that encapsulated Bt. spores or toxins in a granular matrix (such as starch) or within killed cells of other bacteria, also provide protection from ultraviolet radiation. Registration and sale of products containing encapsulated Bt. are forthcoming. Some Bt. isolates (not those used in currently available insecticides) produce significant amounts of an additional toxin called thuringiensis, an exotoxin that is released outside the bacterial cell wall. Research is underway to develop commercial insecticides containing this toxin. Although. *thuringiensis* might be lauded as "natural" because it is produced by living organisms, it is nonetheless toxic to a wide range of animal species and humans.

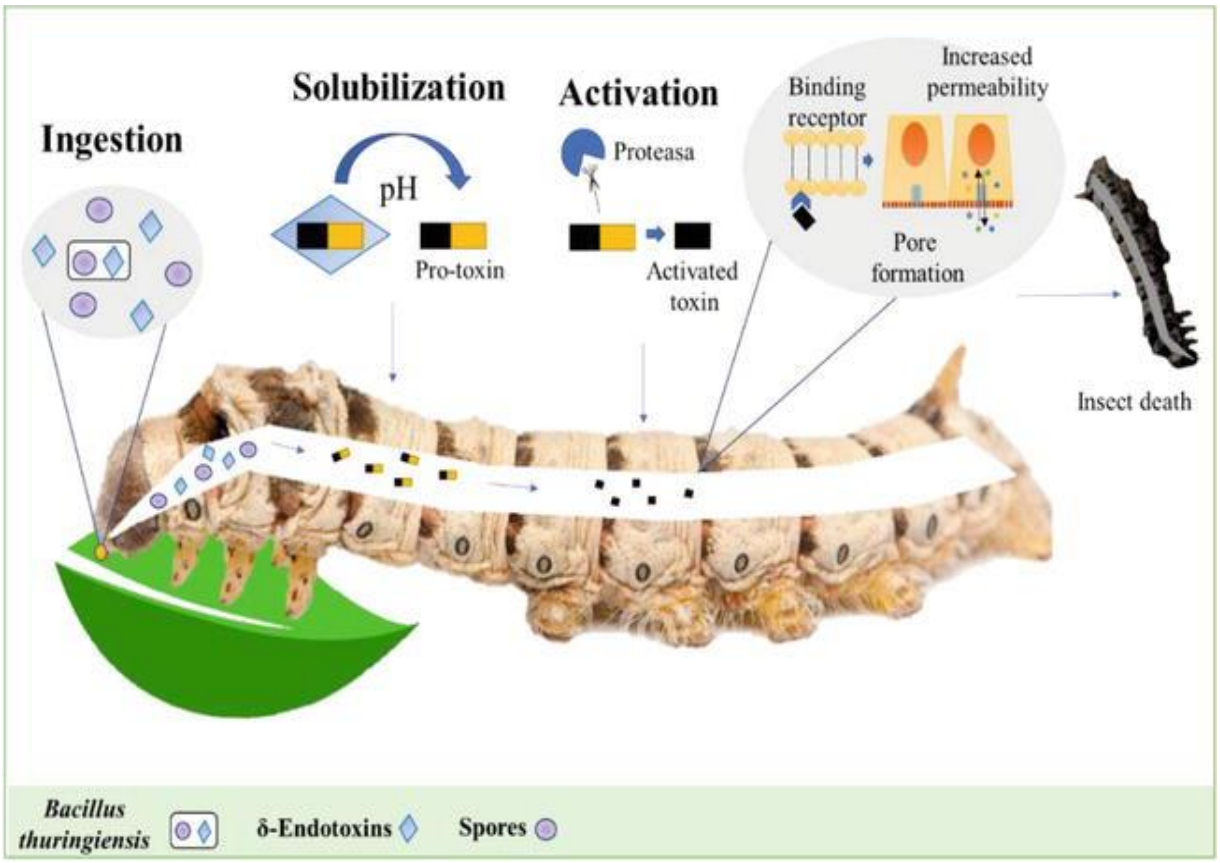

Fig.8.6: Action mechanism of Bt. Cotton inside the organism

8.4.3Advantages of Bacterial Insecticides

The organisms used in bacterial insecticides are essentially nontoxic and non-pathogenic to wildlife, humans, and other organisms. The safety offered by microbial insecticides is their great strength. The toxic action of microbial insecticides is often specific to a single group of insects, and the specificity means that most bacterial insecticides do not directly affect beneficial insects. If necessary, most bacterial insecticides can be used in conjunction with synthetic chemical insecticides, because in most cases, the product is not deactivated or damaged by residues of conventional insecticides. Because their residues present no hazards to human and animals, bacterial insecticides can be applied even when a crop is almost ready for harvest. They also enhance the root and plant growth by way of encouraging the beneficial soil micro flora. By this way, they take a part in the increase of the crop yield.

8.4.4Disadvantages of Bacterial Insecticides

- Because a single bacterial insecticide is toxic to only a specific species or group of insects, each application may control only a portion of the pests present in a field, garden, or lawn. If other types of pests are present in the treated area, they will survive and may continue to cause damage. Conventional insecticides are subject to similar limitations because they too are not equally effective against all pests. Nonetheless, the negative aspect of selectivity is often more noticeable for microbials insecticide.
- Heat, desiccation (drying out), or *exposure* to ultraviolet radiation reduces the effectiveness of several types of bacterial insecticides. Consequently, proper timing and application procedures are especially important for some products.
- Special formulation and storage procedures are necessary for some bacterial pesticides. Although, these procedures may complicate the production and distribution of certain products, storage requirements do not seriously limit the handling of bacterial insecticides that are widely available. (Store all pesticides, including bacterial insecticides, according to label directions.)
- Because several bacterial insecticides are pest-specific, the potential market for these products may be limited. Their development, registration, and production costs cannot be spread over a wide range of pest control sales. Consequently, some products are not widely available or are relatively expensive (for example several insect viruses insecticides).
- Bacterial pathogens used for insect control are spore-forming, rod-shaped bacteria in the genus *Bacillus*. They occur commonly in soils, and most insecticidal strains have been isolated from soil samples. Bacterial insecticides must be eaten to be effective, they are not contact poisons. Insecticidal products comprised of a single *Bacillus* species, may be active against an entire order of insects, or they may be effective against only one or a few species.

For example, products containing *Bacillus thuringiensis* kill the caterpillar stage of a wide array of butterflies and moths. In contrast, *Bacillus papillae* (milky spore disease) kills Japanese beetle larvae but is not effective against the closely related annual white grubs (masked chafers in the genus *Cyclocephala*), that commonly infest lawns in Illinois. Bt.

treatments are inactivated fairly rapidly (within one to a few days) in many outdoor situations, and repeated applications may be necessary for some crops and pests.

8.5. Virus Insecticides

Viruses are obligate disease-causing organisms that can only reproduce within a host. They can provide safe, effective and sustainable control of a variety of insect pests, although, they are most effective as part of a diverse integrated pest management program. Some viruses are produced as commercial products, most notably for fruit pests, but many others are naturally occurring and can initiate outbreaks without additional inputs. The most common and effective type of insect viruses are the baculoviruses (BV), extremely small and are composed of double stranded DNA, ranging from 80 to about 200 kbp in length, which as a group are known to infect over 600 insect species worldwide. Most baculoviruses of family *Baculoviridae* infect caterpillars, which are the immature form of moths and butterflies. Insect viruses are potent population regulators of many caterpillar pests. All are highly specific in their host range, usually limited to a single type of insect. Baculovirus pesticides was used in protection of soyabean fields in Brazil and shows that they may be an alternative to broad spectrum chemical insecticides. *Heliothis* NPV is first viral insecticides (1973), Elcar received a label in 1975.

8.5.1 Virus pest control mechanism

- Virus particles are usually found on the surface of plants or in the soil. Insects become infected during consuming plant material with viral particles on the surface, although some pests of low-growing plants can be infected by contact with the soil. Virus infection begins in the insect's digestive system but spreads throughout the whole body of the host in fatal infections. The body tissues of virus-killed insects are almost completely converted into virus particles. The digestive system is among the last internal organ system to be destroyed, so the insects usually continue to feed until they die. Infected insects look normal until just prior to death, when they tend to darken in colour and behave sluggishly. They often develop more slowly than uninfected individuals.

- Most virus-infected insects die attached to the plant on which they feed. Virus-killed insects break open and spill virus particles into the environment. These particles can infect new insect hosts. Because of the destruction of the internal tissues, dead insects often have a “melted” appearance. The contents of a dead insect can range from milky-white to dark brown or black.
- While natural virus outbreaks tend to be localized, virus particles can be spread by the movement of infected insects, the movement of predators such as other insects or birds that come into contact with infected insects, or non-biological factors like water run-off, rain-splash or air-borne soil particles. Many virus-infected insects also climb to higher positions on their host plant before they die, which maximizes the spread of virus particles after the insect dies and disintegrates.
- The number of virus infection cycles within a growing season depends heavily on the insect’s life cycle. Insect pests with multiple generations per season or longer life cycles can be more heavily impacted by virus outbreaks, since there is a greater opportunity for multiple virus infection cycles within a growing season.

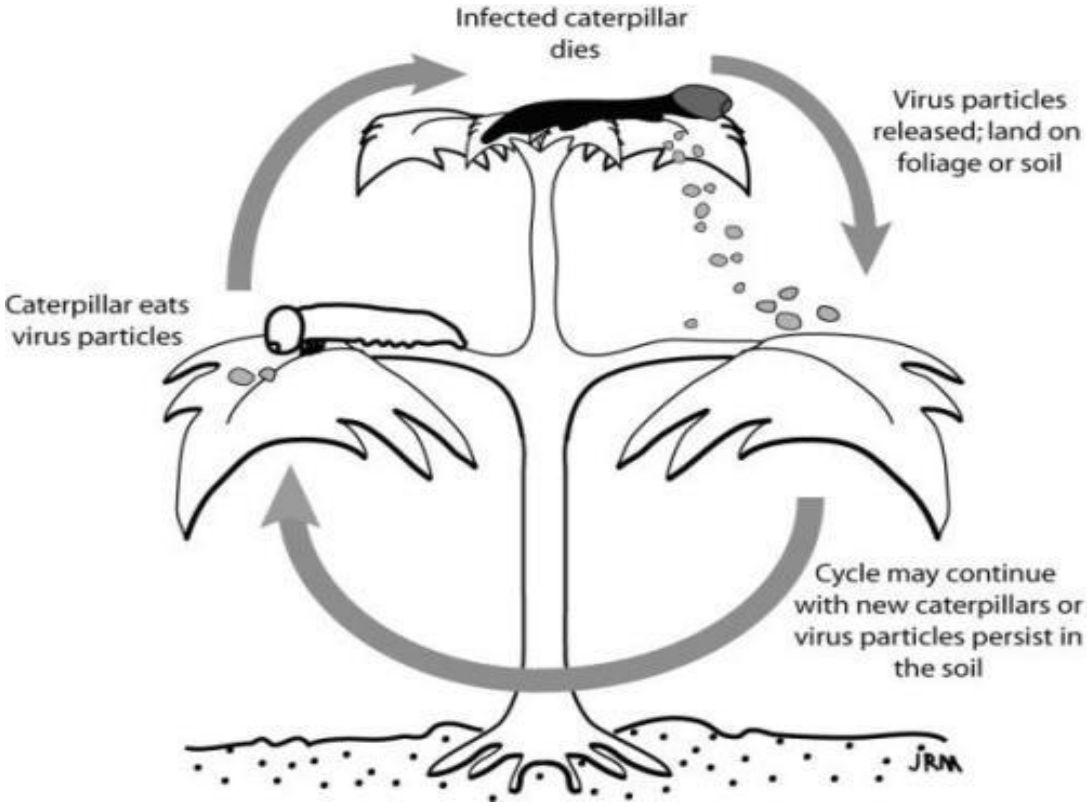

Fig.8.7: Virus action mechanism

8.5.2. Suggestions for application Of Insect Viruses

Viruses are usually not "stand alone" solutions to an insect pest problem, but are most effective in conjunction with other management strategies. Although, there are not many commercial products available in the United States right now, several are being developed and may become available in the near future. Not all commercially-available insect viruses are allowed for use in organic systems. Check with your certifier to determine if the product that you are planning to use is allowed.

If there is a viral product available, there are several things the grower can do to increase effectiveness such as:

1. Insect viruses are fairly specific, be sure that the target pest is correctly identified.
2. Carefully scout fields before application and apply virus when the target pests are young but actively feeding. Scouting can also help you discover natural viral or other disease outbreaks developing in your crop, which depending on their extent, could influence other control decisions.
3. Apply virus to maximize the longevity and effectiveness of virus particles:

   a. Thoroughly coat plants to maximize coverage. Young plants can even be dipped in a solution of virus particles to completely cover the leaf area and stem.

   b. Apply in the morning or evening or on cloudy days when degradation from sunlight is reduced.

   c. Avoid applying on rainy days, as rain will wash virus particles off the leaf surfaces.

   d. Use formulations with ultraviolet (UV) light blockers and sticking agents to increase longevity. Check carefully to make sure these formulations comply with organic standards.

   e. Using mixed cropping and reduce soil disturbance after application. These helps increase the persistence of virus particles in the system and may lead to better control within and between growing seasons.

8.5.3 Advantages of Viral Insecticides

- Insect viruses are unable to infect mammals, including humans, which makes them very safe to handle.
- Most insect viruses are relatively specific, so the risk of non-target effects on beneficial insects is very low.
- Many viruses occur naturally and may already be present in the environment. Even in these cases, where they are applied, successful infections can perpetuate the disease outbreak making repeat applications within a season unnecessary.
- They are safe to wildlife and their specificity is very narrow.

8.5.4 Disadvantages of Viral Insecticides

Most insect viruses take several days to kill their host insect, during which the pest is still causing damage to the crop plants. Insect death is also dose-dependent, and very high doses are often necessary for adequate control. As insects age, they can become less susceptible to virus infection, so viruses are usually only effective against early larval life stages of insects. Although, viruses can persist in the environment for months or years, as exposed virus particles, like those on the surface of plants, are quickly inactivated by direct sunlight or high temperatures, which can limit their persistence within a given season. Also, some agricultural practices can reduce persistence between seasons, such as tillage, which buries virus particles in the soil.

8.6. Summary

Pest problem is one of the major factors for achieving higher production in agriculture which suffers from loss of about 30% of its crops due to pests and diseases each year. To prevent this loss, pesticides are used by farmers to protect their crops from harmful or toxic to dangerous insects, nematodes, fungi, weeds and also to pathogen in modern agriculture. However, excessive use of pesticides not only leaves residues in soil, water and air, but also have adverse effects on the non- target organisms such as pollinators, parasites, predators and wild animals, and also human beings. This has

adversely affected the ecological balance resulted in pest resurgence, development to resistance in the pest species and environmental pollution. Therefore, there is the increased social pressure to replace these chemical pesticides gradually with biopesticides which are safe to human, non-target organism and environment.

A biopesticide is a biological substance or organisms that kills or repels organisms seen as pests.They are classified into three types-

1.Biochemicals-Azardiractin, Clove oil,Peppermint oil

2.Microbials or microorgsnisms-Bacteria,Fungi, Viruses,or protozoans

3.Plants Incorporated Protectants (PIPs)-Bt-Cotton.

They are also classified, according to type of pest which they kills-

a.Insecticides-kills insects

b.Herbicides-kill weeds

c.Rodenticides-kills rodents

d.Fungicides-kills fungi

e.Larvecides-kills larva

They may be biodegradable (biopesticides) or non- biodegradable(DDT).

The microbial insecticides are comprises of microscopic living organisms-viruses, bacteria, fungi, protozoa or nematodes, or toxins produced by them.They are applied as sprays, dusts or granules.Microbials insecticides are essentially non toxic and non – pathogenic to wild life,humans or other organisms, not closely related to target pest.Different species of bacteria (Pseudomonas, Bacillus,etc.) and viruses (Baculoviruses,BV) are used as microbial insecticides effectively in controlling insects, although,they haves some advantages and disadvantages also.

Weeds are unwanted plants growing with main crop, reducing its productivity, and causes serious ecological problems and capable of altering the process of ecosystem. Out of 30,000 species of weeds, 1900 species cause loss in yield by 9.5% of total crop production every year in the world.They also serve as reservoir (Ageratina riporia) for pathogens that

may cause significant loss in crop production. In these condition,it is essential for farmers to control weeds by application of hervicides.They may be selective(control specific weed) or non selective(kill all plant material with which they come in contact).Many chemical herbicides are available in market which are harmfull to humans, soil and ecosystem.The weeds also aquire resistence against chemical herbicides (about 307 biotypes),i,e., chemical herbicides becomes ineffective angainst these weeds.Therefore, a number of microbial herbicides have developed till date, but only few of them available in commercial forms,due to several reasons.The foliar smut fungus pathogen, Cercosporella sp., is used to control weed Ageratina riporia in Jamaica (45000 ha forest is cleaned) and insects , Hetropsylla spinulosa control weed Mimosa diplotricha in India(Kaziranga National Park,Assam, weeds found more on boundary of park), Australia, Fizi and Brazil. These finding are highly encouraged for a better commercial mycoherbicide development. IPM programs works to manage the crop to prevent the pests from becoming a threat.

8.7. Terminal Questions

Q.1: What are Microbial insecticides?

Answer----------

Q.2: Describe the importance of bacterial insecticides.

Answer----------

Q.3: Describe advantages and disadvantages of bacterial insecticides.

Answer----------

Q.4: Explain the viral insecticides and its action mechanism.

Answer----------

Q.5: Describe Bt. Cotton in brief.

Answer----------

---Q.6.What are bioherbicides? Discribe in short.

Answer

8.8. Further Suggested Reading

8. Biofertilizers And Pesticides- H.C. Lakshman

9. Manures, Fertilizers And Pesticides- Amiitava Rakshit, Priyankar Raha And Nirmal DeFertilizers A Text Book- Ranjan Kumar Basak

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Unit-9: Microorganisms of sewage degradation

Contents

9.1. Introduction

The domestic waterborne wastes including human and animal excreta, domestic washing waters, industrial water borne wastes, ground surface and atmospheric waters which enter the sewage system of a town or city is collectively known as sewage. It has minerals and organic nutrients in a dissociated state or dispersed in a solid condition. The variety of microorganisms such as bacteria, fungi, protozoa, algae, nematodes, amoeba and viruses are also perent in it. They may be aerobes, obligate anaerobes and facultative anaerobes. Most of them are soil and intestinal bacteria. Commonly coliform, *Streptococci*, *Clostridia*, *Micrococci*, *Proteus*, *Pseudomonas, Lactobacilli* etc. are present in sewage. Some pathogen causing various diseases such as amoebic dysentery, typhoid fever (*Salmonella typhi*), cholera (*Vibrio comma*), bacterial

dysentery, polio, hepatitis etc. In the beginning aerobic bacteria dominate and decompose organic material, and at the end anaerobic bacteria predominate (eg. methanogens) which produce methane, carbondioxide and hydrogen gases.Three tests are used to assess the amount of organic matter present in sewage water.These are BOD, COD andTOC.

Sewage treatment is of two types-

a. Small scale sewage treatment-It is used in villages mostly and treatment is done by Cesspools and Septic tanks.

2.Large scale sewage treatment-It is used in larger cities for larger populations by municipal bodies.It involves three basic treatment steps-

1.Primary treatment (physical treatment)-organic matter removed by physical method from sewage

2.Secondary treatment(biological treatment)-organic matter degradation take place by participation various microorganisms in aerobic (initial steps) as well as in anaerobic (later steps) condition. The BOD of sewage is decreased up to 90-95 %. Biological degradation is done by several methods-1.The oxidative pond2.The aerobic trickling filter3.The aerobic activated sludge4.The anaerobic digester-in this step fermentation, acetogenic reaction and methanogenesis take place, and results in formation og bio gas.

3.Tertiary treatment- remove non biodegradable organic material, heavy metals, xenobiotic substances (plastic, pesticides, PCBs,etc.), petroleum (oil and oil products), ,etc.,which remains after treatment of microorganisms of second step.The process commonly known as biodegradation or bioremediation.In this process, various species of algae,blue green algae, fungi, bacteria, actinomycetes participate in tertiary treatment.

9.2. Sewage

Sewage (or domestic sewage, domestic wastewater, municipal wastewater) is a type of wastewater that is produced by activity of a community of people. It is typically transported through a sewer system. Sewage consists of wastewater discharged from residences and from commercial, institutional and public facilities that exist in that locality. The composition of sewage varies from place to place and according to seasons. The sewage consists of 99% water and 1% inorganic and organic matter in suspended and soluble forms. The suspended forms

includes lignin,cellulose, hemicellulose, proteins, fats and various inorganic particulates, whereas, the soluble forms are sugars, fatty acids, alcohols, amino acids and inorganic ions. Sewage is the used water supply containing domestic water together with human excreta, wash water and industrial waste water, including acids, greases, oils, animal matter, vegetable matter and storms water. The basic principle in sewage treatment is that water is separated from the waste, while the solid organic matter is biodegraded by microorganisms to simple compounds like nitrates, sulphates, carbonates, carbon dioxide, methane etc. Salts of heavy metals, such as Zn, Cr, Ni, Pb etc. are also present in it. The amount of organic matter in domestic wastes determines the degree of biological treatment required.

Three tests are used to assess the amount of organic matter present in sewage water.These are :

1.total organic carbon (TOC),

2. biochemical oxygen demand (BOD)

3. chemical oxygen demand (COD)

The main objective of domestic waste treatment is the reduction of BOD.

Characteristic of sewage:

- Organic matter undergoes anaerobic or partial decomposition resulting in the production of gases like CH4, CO and H_2S which react with water and produce acids.
- Production of gases and acids makes the sewage acidic making it unfit for microbial life.
- Heavy metals such as Pb, Cr, N, Zn etc. present in high concentration in sewage affect growth of microorganisms.
- Reduced photosynthetic rate due to poor illumination cause death of oxygen dependent aerobic microorganism, plants and animals.
- Biological oxygen demand (BOD) and oxygen consumption values are extremely high in sewage.
- Dissolved oxygen becomes totally depleted in sewage water due to high oxygen demand and low photosynthesis.
- Sewage water has high specific conductivity due to presence of large amount of salts.

9.3. Sewage treatment and sewage degrading microorganism

There are two types of sewage treatment

A. Small scale sewage treatment:

a. Cesspools:

It is constructed underground within soil with concrete in such a way that it contains wall of cylindrical rings with pores. Its opening is near the ground level. Sewage enters the cesspool through the inlet pipe. Cesspool bottom remains open so that the suspended solid material falls on the bottom of cesspool and forms sludge after getting deposited in huge amount. Water passes out through the open bottom and pores into the surrounding soil.

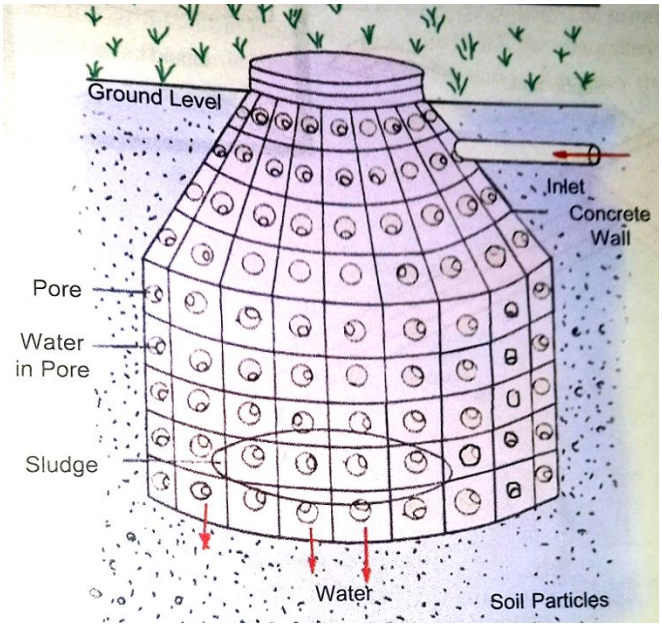


Fig.9.1: Cesspool

The organic material of the sludge is decomposed by anaerobic bacteria resulting in release and deposition of breakdown products on the ground.

Dried bacterial preparation of *Bacillus subtilis* and yeasts cells should be added which accelerates the decomposition of sludge deposited at the bottom of cesspool.

b. Septic Tanks:

It is used in rural areas due to lack of public sewers. Septic tank is a metallic or concrete tank which kept below the ground level somewhere near the homes. All the domestic wastes

flow through the inlet pipes into septic tank. The suspended organic materials are accumulated at the bottom of tank, whereas, the water flows through outlets to a distribution box which is connected with perforated pipes that open under the soil surface in the surrounding areas. Effluents passed to underground surface of soil from the tank.

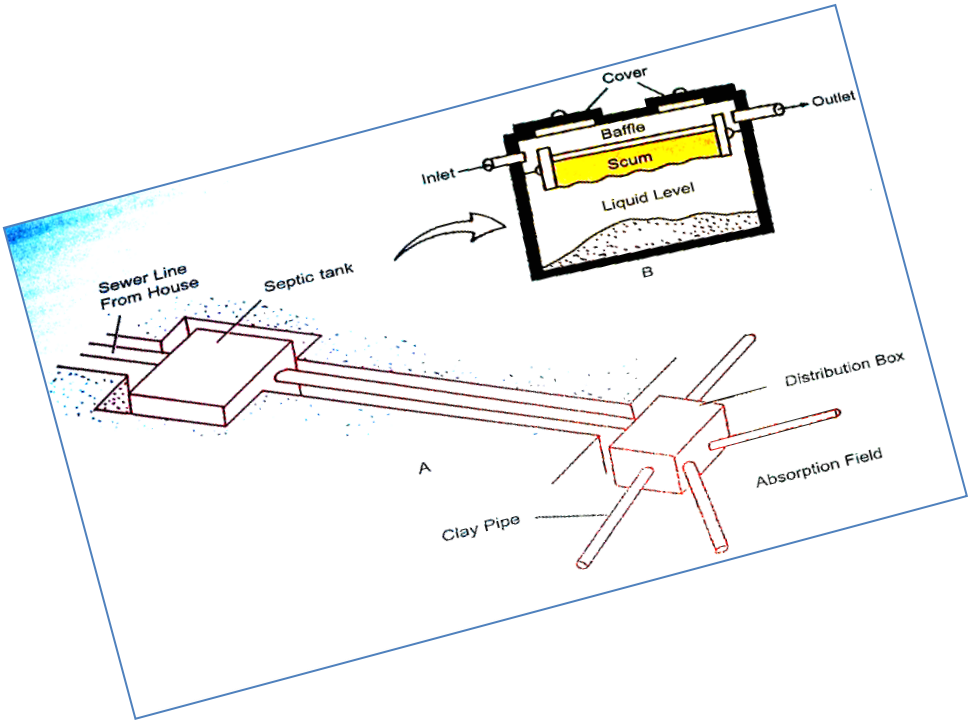


Fig.9.2: Septic Tank: A. Overall installation B. View of septic tank where water enters through inlet and solids accumulate as sludge

The organic materials accumulated in tank is decomposed by anaerobic bacteria releasing into water several by-products such as sugars, alcohols, organic acids, amino acids, fatty acids, glycerol and gases (H_2, H_2S, CH_4, CO_2 etc.). Sludge (undigested organic materials) is removed from the tank at certain intervals by pumping process. Sludge act as humus when applied in field.

B. Large scale sewage treatment:

Sewage treatment on a large scale of population of city is known as large scale sewage treatment. In cities sewage are treated by municipal plants

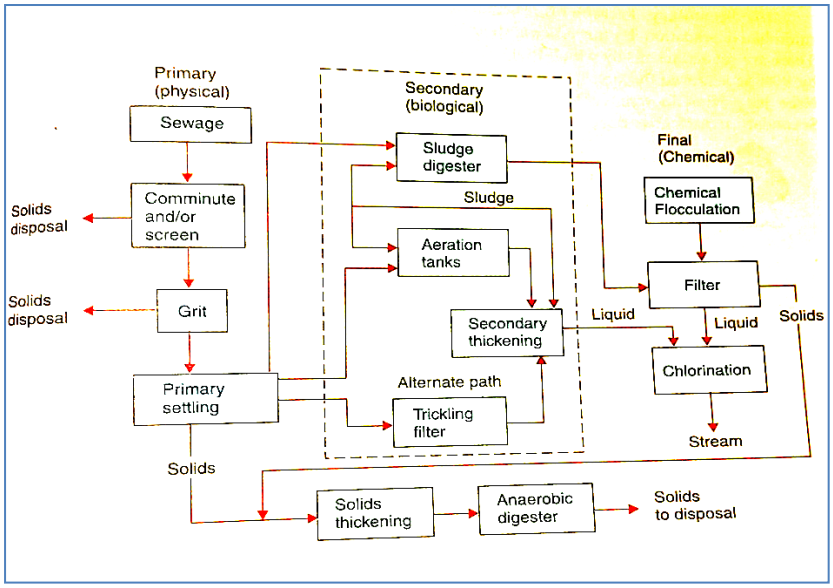


Fig. 9.3: Flow Chart of different stages of sewage treatment

Overall processes of conventional municipal sewage plant can be divided into three steps:

i. **Primary treatment**: It is the physical removal of 20-30% of organic materials present in sewage in particulate form. The particulate material is removed by screening, precipitation of small particulate and settling in basin or tanks where the raw sewage is piped into huge tanks. The solid material (sludge) is removed and kept in composting for anaerobic digestion. The liquid portion is piped into sludge tanks.

ii. **Secondary treatment:** It is also known as biological treatment or microbial degradation. By this process, about 90-95% of the BOD decresed and many pathogens are removed. The microbial activities may be aerobic or anaerobic. It is done by several methods:

a.The oxidation pond:

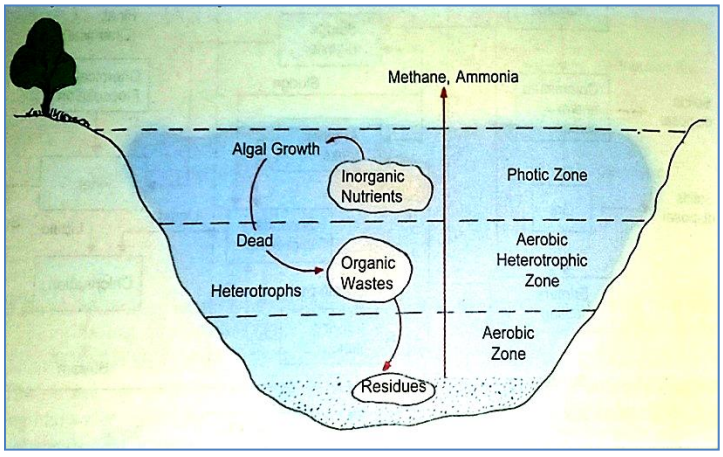


Fig. 9.4: Oxidation pond (provide aerobic condition)

Also called lagoons or stabilization ponds, permits the growth of algal forms on waste water effluent. Treatment through oxidation ponds is the aerobic sewage treatment device. The organic materials are degraded by heterotrophic bacteria into simpler forms that support the growth of algae. The algae growing in oxidation ponds are *Chlorella pyrenoidosa, C. ellipsoids, Scenedesmus acutus, S. quadricauda, Spirulina platensis* etc.

b.The aerobic trickling filter:

It is a simple sewage treatment device that consists of a bed of a crushed stone, slag or synthetic materials with drains made at the bottom of tanks. A revolving sprinkler is suspended over a bed of porous material which distributes the liquid sewage over it and collects the effluents at the bottom. Due to spraying process, sewage is saturated with the oxygen.

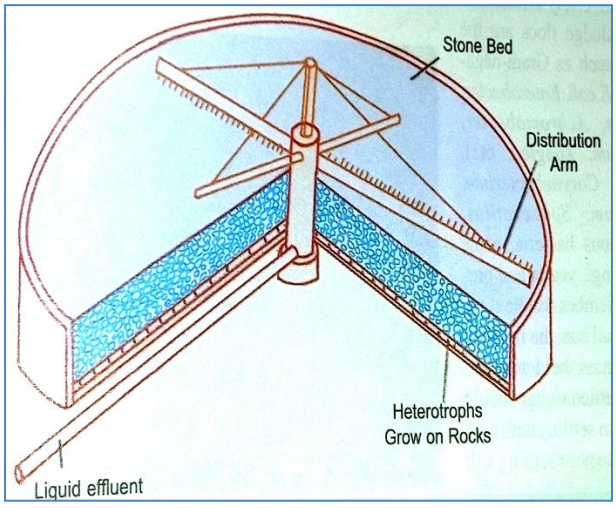


Fig. 9.5: Trickling filter

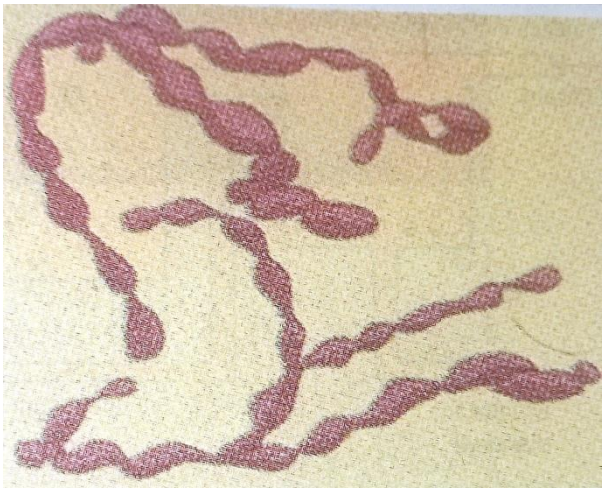


Fig. 9.6: *Pseudomonas* bacteria

The porous filter bed becomes coated with slimy bacterial growth mainly by *Zooglea ramigera* and other slime producing bacteria. The slime is colonized by heterotrophic microorganisms, e.g., bacteria such as *Beggiatoa alba*, *Sphaerotilus natons*, *Achromobacter* sp., species of *Pseudomonas* and *Flavobacterium*, fungi, nematodes, protozoa etc. These microorganisms forms a stationary microbial culture because of continuous supply of nutrients present in sewage and metabolizing the organic constituents into more stable product.

c.The aerobic activated sludge:

The sewage is passed into an aeration tank from primary settling tank. Sewage is aerated by mechanical stirring. Due to vigorous aeration of sewage, floc-formation occurs.

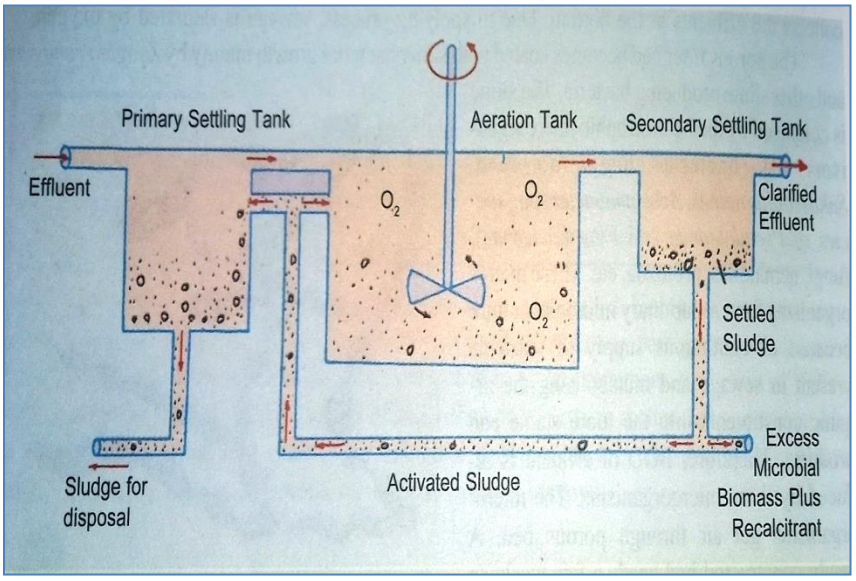


Fig. 9.7: Aerobic activated sludge

The colloidal and finely suspended matter of sewage form aggregates which are called floccules. The flocs are permitted to settle down in secondary settling tank. The particles of floc contain large amount of metabolising bacteria together with yeast, fungi and protozoa.

The microorganisms found in activated sludge flocs are the heterotrophs such as Gram-negative *E.coli*, *Enterobacter*, *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Zooglea* etc. *Arthobacter*, *Corynebacterium*, *Mycobacterium*, *Sphaerotilus*, large filamentous fungi, yeasts and protozoa.

d.Anaerobic digesters:

The sludge from aerobic sewage treatment together with the materials settled down in primary treatment are further treated in anaerobic digesters through the process of anaerobic digestion. These digesters are used only for processing of settled sewage sludge and the treatment of very high BOD industrial effluents.

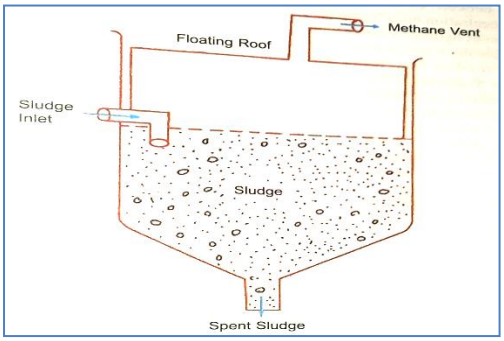
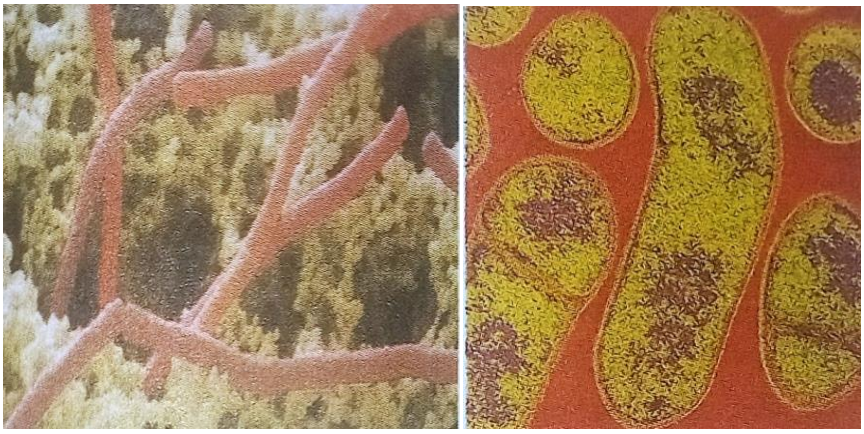


Fig. 9.8: Anaerobic sludge digester

Anaerobic digesters are large fermentation tanks designed to operate anaerobically with continuous supply of untreated sludge and removal of final, stabilized sludge product. The organics are decomposed by a number of anaerobic microorganisms whose population is found greater than the aerobes. Anaerobic digestion involves three steps:

1.Fermentation:

The fomentation take place by number of bacteria such as *Bacteroides*, *Clostridium*, *Peptostrptococcus*, *Eubacterium*, *L*actobacillus etc. The organic acids butyrate, propionate, lactate, succinate, acetate along with ethanol, H_2 and CO_2 are formed.



A **B**

Fig.9.9: *A. Lactobacillus B. Clostridium*

2.Acetogenic reactions:

The products (butyrate, propionate, lactate, succinate, acetate,ethanol) produced during fermentation are utilize as substrate by several acetogenic bacteria e.g. *Syntrophomonas*, *Syntrophobacter* and *Acetobacterium*. The products of this reactions are acetate, H_2 and CO_2.

3.Methanogenesis:

The products H_2 and O_2 are used as substrate by Methanogenic bacteria such as *Methanobrevibacte*r, *Methanomicrobium*, *Methanogenium*, *Methanobacterium*, *Methanococcus* and *Methanospirillum*. Methanogenesis is an anaerobic respiration and methane is final product of metabolism. H_2 is oxidised into H^+, and CO_2 is reduced into CH_4. The final product of anaerobic digestion is a mixture of gases 70% CH_4 ,30% CO_2 , microbial biomass and nonbiodegradable residues (heavy metals, polychlorinated biphenyls etc.).

The mixture of methane and carbon dioxide is known as bio gas. It is reneuable source of energy.Largest bio gas plant in india is CBG plant at Sangrur, Punjab.

iii.Tertiary treatment: this process remove non-biodegradable organic materials, heavy metals and minerals by using activated carbon filters- the organic pollutants can be removed, by adding

lime- the phosphorous is precipitated as calcium phosphate. Nitrogen can by removed by stripping volatilization as NH_3 at high pH values.

9.4. Microbial degradation of Petroleum

Petroleum is a complex mixture composed primarily of aliphatic, alicyclic and aromatic hydrocarbons. There are hundreds of individual compounds in every crude oil. Petroleum and its products are the hydrocarbons. It is a rich source of organic matter and is oxidised when comes in contact with air moisture. Some microorganisms like fungi, bacteria, cyanobacteria, yeast and algae cleave the hydrocarbons into simpler molecules. Oil and oil products are decompose by mainly fungi and bacteria. Methane is simplest hydrocarbon pollutant degraded by a specialized group of bacteria called methanotrophic bacteria.

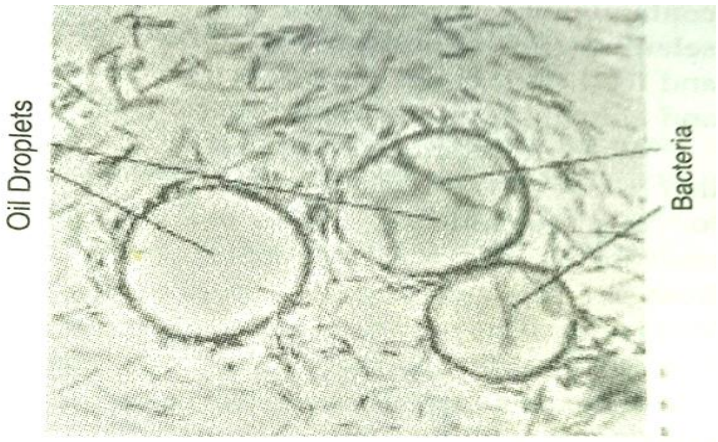


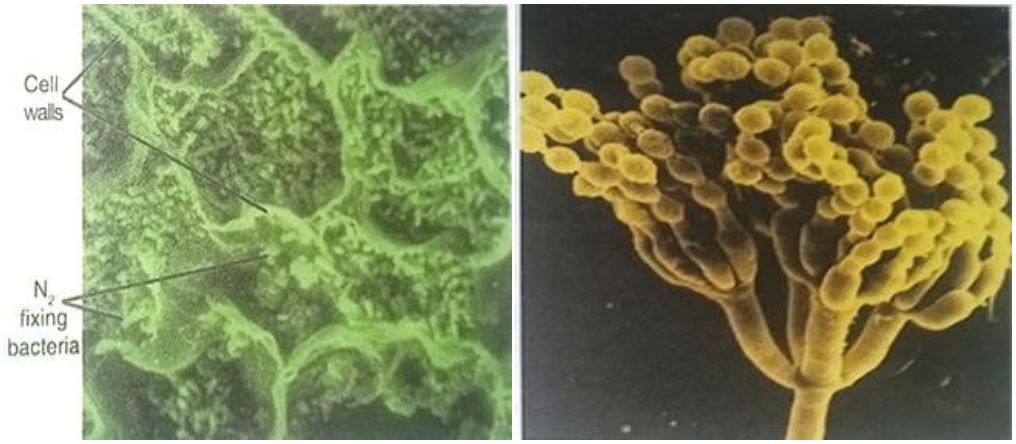
Fig. 9.10: Petroleum degrading bacteria in association with oil droplets

Oil is present both in absence of oxygen (anoxic) as well as presence of oxygen (toxic) environment. Oil is insoluble in water and is less dense, so floats on the surface of water and forms slicks or oil films. Hydrocarbon-oxidising microorganisms develop rapidly in such films. Many cyanobacteria, pseudomonads and mycobacteria are able for degradation of petroleum products. The non-volatile components are oxidised by bacteria and later certain fractions of branched chain and polycyclic hydrocarbons are degraded slowly. Bacteria and yeast can grow on several fractions of hydrocarbons, e.g., heptane, decane, hexadecane etc.

Structure and molecular weight of the hydrocarbon molecule determine its susceptibility to biodegradation. N-alkanes chain length (C_{10}-C_{24}) are degraded most rapidly. As the length of

chain increases, it resistance to biodegradation increases. Aromatic compounds are degraded slowly than alkanes.

The successful biodegradative removal of petroleum hydrocarbons from the sea depends on enzymatic capacities of microbes and various abiotic factors. Microbial degradation needs suitable temperature and available supplies of fixed forms of nitrogen, PO_4 and O_2. Low concentration of N_2 and PO_4 limits petroleum degradation in oceans.



B A

Fig.9.11: A. *Penicillium* B. *Rhizobium*

Pseudomonas putida known as Super bug (oil eating bug) a genetically engineerd strain that utilized complex chemical compounds created by Dr. Anand Mohan Chakraborty (India born American Scientist) and his coworkers in 1979. Chakraborty produced this super bug after introducing four plasmids from different strain into one single cell of *Pseudomonas putida*. This superbug degrades all the four types of substrates for which four separate plasmids were required.

i. OCT plasmid that degrades octane, hexane and decane
ii. XYL plasmid that degrades xylene and toluene
iii. CAM plasmid that decomposes camphor
iv. NAH plasmid which degrades naphthalene

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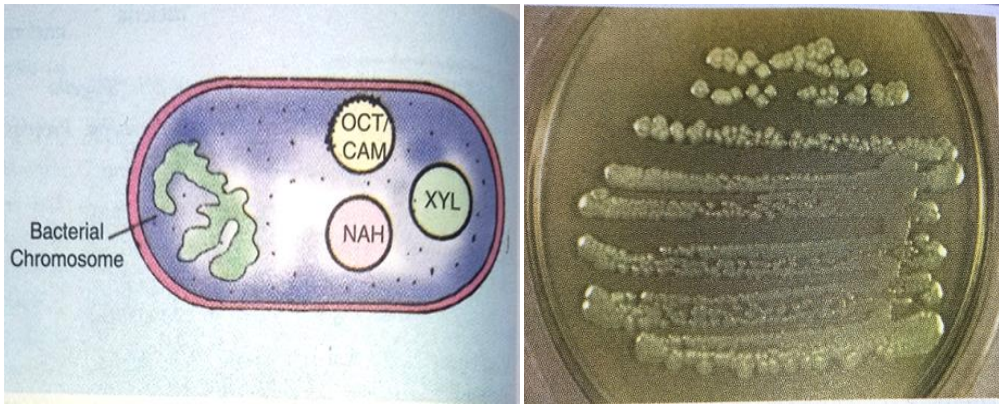

A **B**

Fig.9.12: A. Superbug containing four different plasmids, B. Genetically engineered pseudomonas strain help to degrade components of crude oil

9.5. Microbial degradation of xenobiotics compound

These are man-made, synthesized compounds, such as pesticides. These are chemicals which do not exist in nature. Pesticides are toxic chemicals which act by interfering with microbial reactions in the target organisms. They may affect those microorganisms which are important in maintaining soil fertility when added in soil. The xenobiotics have molecular structure and chemical bond sequences not recognized by the existing degradative enzymes. Recalcitrant molecules (resist biodegradation) are fossil organic matter (humus), polyaromatic compounds (tannins and lignins), persistent microorganisms (endospores and melanin rich fungi), synthetic molecules (fungicides, nematicides, herbicides, insecticides), polyhalogenated biphenyls (flame retardants and solvents), plastic and detergents. The persistence of xenobiotics ranges from days to years and minor alterations in biodegradable compounds can render them recalcitrant.

Table.9.1: Xenobiotic compound and its durability

Xenobiotic compound	**Duration for 75-100% disappearence**
Chlorinated insecticides	
DDT	4 Years
Aldrine	3 Years
Chlordane	5 Years
Heptachlor	2 Years
Lindane	3 Years

Organophosphate insecticides	
Diazonin	
Malathion	1 weeks
Parathion	1weeks
Herbicides	
2,4, D(2 4-dichlorophenoxyacetic acid)	4 weeks
2,4, D (2 4 5-trichlorophenoxyacetic acid)	20 weeks
Altrazine	40 weeks
Simazine	48 weeks
Propazine	1, 5 Years

Three major forces responsible for the breakdown of the compounds in the environment are antioxidation, degradation by sunlight and microbial action. Majority of the xenobiotics after their release go to the soil and aquatic sediments. There are diverse group of microorganisms (Fungi and bacteria) which are able to metabolize pesticides and herbicide. Pesticides are halocarbons that are used to control pests for better crop production. The widespread use of synthetic pesticides in agriculture and public health, that followed led to fears over the persistence of these toxic chemicals in the environment, bioaccumulation in the food chain and risks to non-target species. The pesticides are degraded by bacteria and fungi. Microorganisms produce dehalogenase enzyme which carry out reductive dehalogination. For example, *Phanerochaete chrysosporium* (white rot fungus) has enzymes that degrade lignin or cellulose and breakdown many recalcitrant chemicals including halogenated phenol ring compounds such as pentacholorophenol or DDT that are posing serious threats of environmental resistance. The microbial conversion of DDT occurs by two pathways:

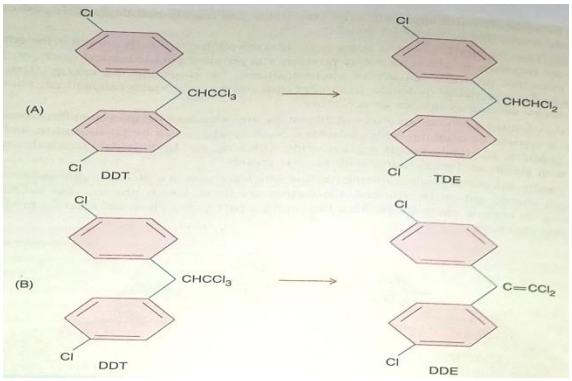

Fig. 9.13: A. Degradation of DDT into TDE through reductive dehydrogenase by *Aerobacter aerogenes* and DDE through dehydrogenation by *Trichoderma viride*

i. **Reductive dechlorination**: chlorinated compounds are used as terminal electron acceptors under anaerobic conditions.

ii. **Dehydrochlorination:** it occurs under aerobic conditions**.** This process is the removal of hydrogen and chlorine or hydrogen chloride. Several strains of *Trichoderma viride*, *Aspergillus flavus* and *A. parasiticus* metabolize DDT by producing various metabolites, such as DDD (dichlorodiphenyl dichloroethane, a break down product of DDT) and DDE (dichlorodiphenyldichlooethylene, a break down product of DDT).

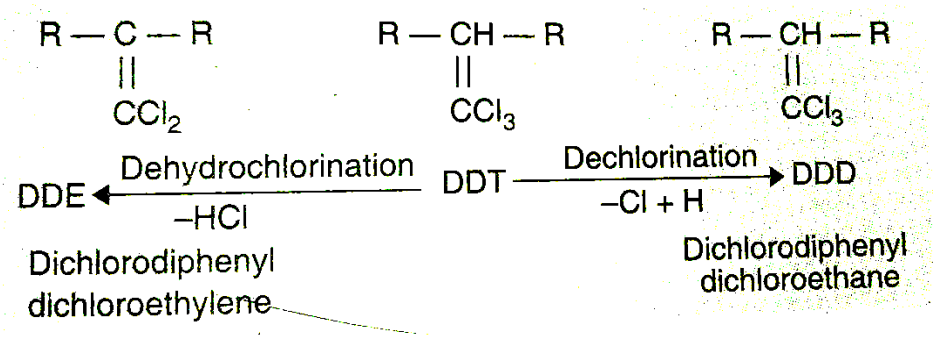

Fig. 9.14: Metabolism of DDT under aerobic and anaerobic conditions

Pesticides of the category chlorinated cyclodienes, include aldrin, heptachlor, halogenated phenoxyacetic acids and endosulphan.Fungal genera- *Aspergillus flavus*, *Penicillium notatum*, *P. chrysogenum* and *Trichoderma viride*, in association with bacteria degrade aldren by epoxidation (formation of cyclic ether with only three ring atoms) to form dialdrin. Heptachlor is oxidized to its epoxide form, chlordane by *Aspergillus niger* and *Pseudomonas urticae*. In the aerobic degradation of the pesticide 2,4,5-T (trichlorophenoxyacetic acid) following dechlorination, a dioxygenase enzyme breaks down the aromatic ring. This generates compounds that can be metabolized by the citric acid cycle. Recombinant strain of *Pseudomonas capacia* (isolated by Chakrabarty etal., 1981) which could degrade 2,4, 5-T.

Organophosphate pesticides like malathion is degraded by molds like *Trichoderma viride* along with *Pseudomonas spp.* into carboxylic acid. *Torulopsis utilis*, *Mucor plumbeus* and *Rhizopus arrhizus* have potential for degrading organophosphate such as phorate and dialkyl phenylphosphates.

Parathion degraded into aminoparthion by *Trichoderma viride and Penicillium walksmanii.Pesticide are widely used as insecticides, nematicides, herbicides and fungicides in the agriculture.*

Carbamates (ester derivatives of N-substituted carbamic acid). *Trichoderma viride* shown to degrade the carbaryl through the ring hydroxylation. The fungus *Gliocladium roseum* metabolizes carbaryl to napthyl, N-hydroxymethyl carbamate and 4 and 5-hydroxyl-1-napthylmethyl carbamate.

Helminthosporium, *Aspergillus niger*, *Trichoderma viride* degrade carbofuran to 3-hydroxycabofuran.

Polychlorinated biphenyls (PCBs), a class of organic compounds are used in adhesives, carbonless copy paper, insulation in transformers, high pressure hydraulic fuids, machine tool cutting oils, specialized lubricants, gasket sealers, plasticizers and protective coatings for wood, metal and concrete. PCB, are also degraded by aerobic and anaerobic microbial organism.

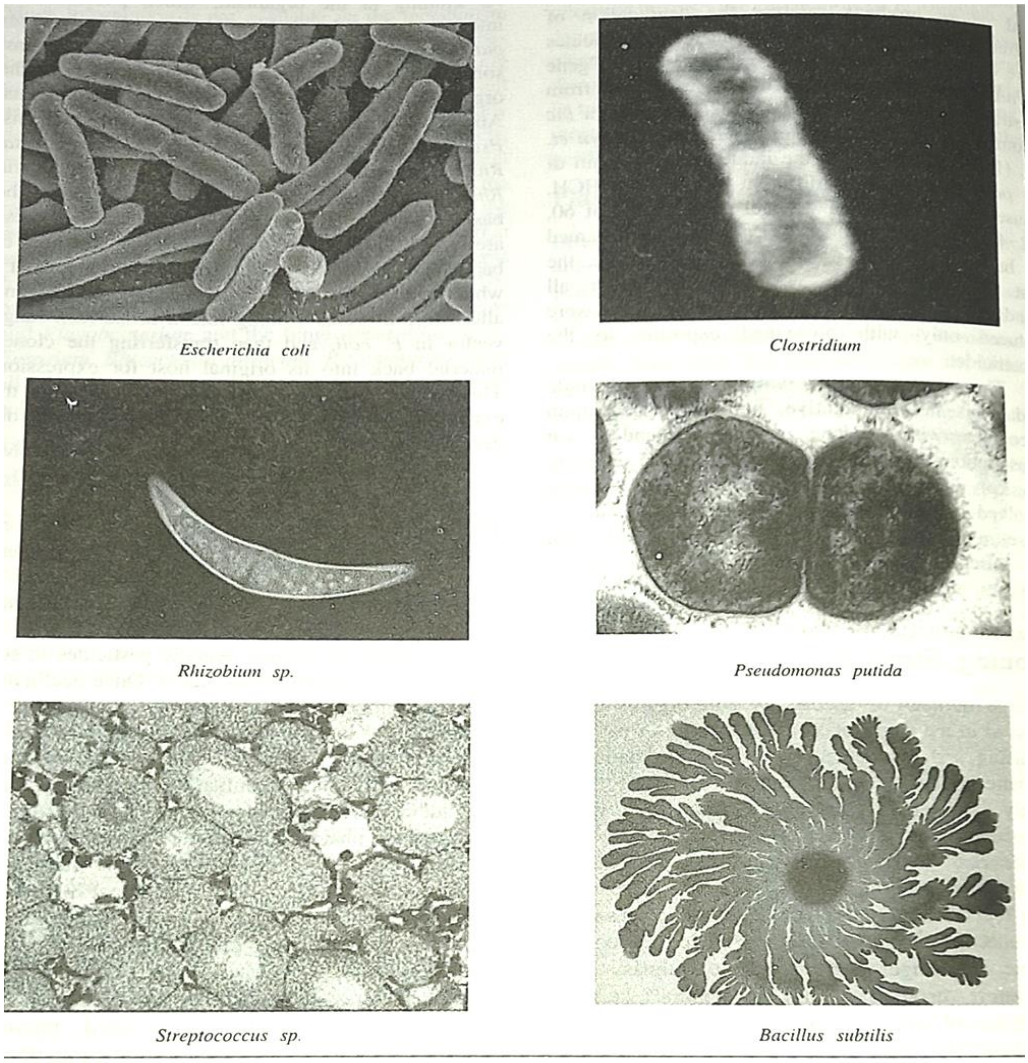

Fig. 9.15: Some microorganisms involved in biodegradation of xenobiotics

Many microorganisms such as *Rhizopus*, *Achromobacter*, *Nocardia*, *Pseudomonas*, *Alcaligene*s and *Acinetobacter* have been reported to degrade PCBs to some degree.

Plastics are the synthetic polymers which can be easily molded ino complex shapes, possess high chemical resistance and are more or less elastic. In the municipal garbage more than 90% of plastic materials consist of polyethylene, polyvinylchloride and polystyrene in roughly equal proportions. Plastics and related polymers cause aerial contamination, reduce the fertility of agricultural soil and destroy wildlife. The chemical constituents of polybag like vinylchloride and acrylonittile are categorized as environmental carcinogens. Resistance of plastics to biodegradation appears to be due to excessive molecular size, as it has been observed that biodegradation declines with increase in the molecular weight of constituent unit. Plastic

becomes susceptible to biodegradation if molecular weight is greatly reduced. Acrylonitrile fibres can be attack by *Aspergillus, Penicillium, Stachybotrys and Nigrospora*. The polycarpolactone is degraded by *Aspergillus Niger, A. flavus, Penicilium funiculosum, Chaetomium globosum* and *Pullularia pullulans. Aspergillus fumigates, Chaetomiumthermophile, Humicola lanuginose, Mucor pusillus and Torula thermophila* can degrade plastic polymers. N-alkanes, alkenes and other aliphatic hydrocarbons which are used in the synthesis of plastic polymers are readily utilized by several filamentous fungi and yeasts. Some of the important fungi capable of degrading these compounds are *Acremonium, Aspergillus, Penicillium, Mucor, Rhizopus, Candida, Helminthosporium, Monila and Saccharomyces.*

9.6. Microorganism in abatement of heavy metal pollution

Heavy metals are the metals in the periodic table that have a molecular weight greater than 55. Heavy metal contamination, from both natural and anthropogenic sources is recognized as a major environmental concern in marine ecosystems due to the pervasiveness and persistence of the contaminants. Most of the heavy metals, such as cadmium, vanadium, managanese, arsenic, lead, iron, mercury, zinc, nickel, chromium, copper, antimony, etc. are toxic to the biological system due to their inert nature and persistence long run cumulative effects. Some diseases, such as Itai Itai due to cadmium, Minimata due to mercury, miscarriage in women due to lead, skin cancer, lung cancer and liver cancer due to arsenic.

Some of these metals like Zn, Cu and Mo act as micronutrients in plant and animal growth as they function as cofactors in enzyme-catalyzed reactions. Exposure to heavy metals selects for resistance to heavy metals in the surviving microorganisms. There are several mechanisms of resistant to heavy metals: extracellular detoxification, altered permeability, pollutant removal from the cell environment, intracellular detoxification and abiotic binding or precipitation. Microorganisms may persist in soils contaminated with extremely high heavy metal concentrations because those heavy metals may be extractable but not biologically available.

Mercury is toxic to microorganisms and to survive in its presence, they transform Hg into methylated compounds such as CH_3Hg (methylmercury) and CH_3HgCH_3 (dimethyl mercury). These forms of Hg are volatile and harmless to microorganisms, however, they are readily

adsorbed and retained by human tissue and cause neurological disorders. The molds and bacteria (such as *Aspergillus*, *Neurospora*, *Bacillus*, *Clostridium*, *Mycobacterium* and *Pseudomonas*) have the ability to transform mercury. Along with methylation, these microorganisms release organically bound Hg during their heterotrophic activity.

Table.9.2: Microorganisms involved in metal removal from industrial waste water

Microorganism type	Species	Heavy metal removed	Environment type
	Bacillus cereus strain XMCr-6	Cr (VI)	Soil
	Kocuriaflave	Cu	Water
	Bacillus cereus	Cr (VI)	Water
Bacteria	*Sporosarcinaginsengisoli*	As (III)	Water and soil
	Pseudomonas veronii	Cd, Zn, Cu	Water
	Pseudomonas putida	Cr (VI)	Soil
	Enterobacter cloacae B2-DHA	Cr (VI)	Soil
	Bacillus subtilis	Cr (VI)	Soil
	Aspergillusversicolor	Ni, Cu	Water
Filamentous	*Aspergillus fumigates*	Pb	water
Fungi	*Gloeophyllumsepiarium*	Cr (VI)	Soil
	Rhizopusoryzae	Cr (VI)	soil
Yeast	*Saccharomyces cerevisiae*	Pb, Cd	Water
Algae	*Spirogyra* and *Cladophora*	Pb (II), Cu (II)	Water
	Spirulina and *Spirogyra*	Cr, Cu, Fe, Mn, Zn	Water
	Hydrodictylon	As	Water
	Oedogonium	As	Water

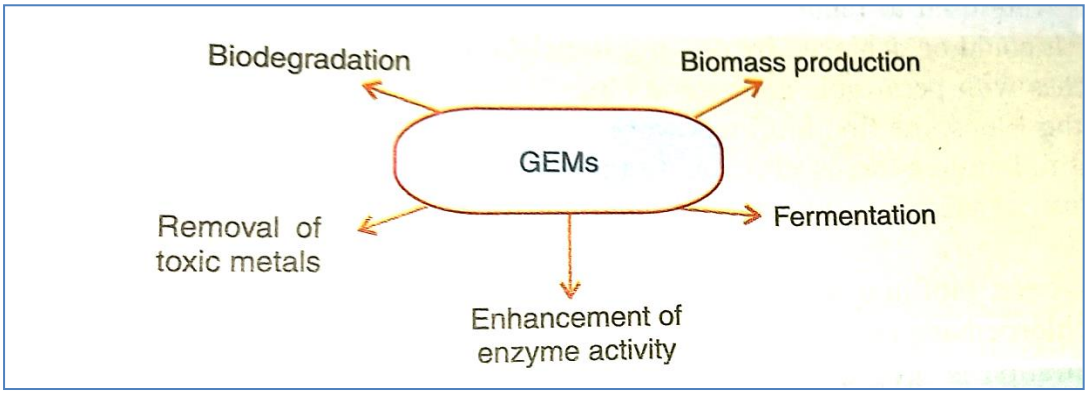

Fig.9.16: Genetically Engineered Microorganisms

9.7. Heavy metal tolerance in microbes

There are four main physiological mechanism of heavy metal resistance: inactivation, impermeability, by pass and altered target. Ashida (1965) reported that fungi tend to survive heavy metal stress either by adaptation or mutation. Few algae such as *Stigeoclonium tenue* can grow in a zinc rich effluent and showed slightly greater resistance under "in-vitro" conditions due to adaptation. Enzymatic flavoprotein play a key role in the mercury detoxification system of many bacteria. In bacteria, many plasmids were reported for heavy metal tolerance. Mediation of Penicillinase plasmid in importing resistance to divalent metal ions of mercury and cadmium is reported in *Staphylococcus aureus*.

Fungi, bacteria, algae, actinomycetes etc. are microorganisms reported to tolerate heavy metals universally occur in diverse ecosystems.

Algae: *Anabaena inequalis*, *Chlorella* sp., *Westiellopsis prolifica*, *Stigeoclonium tenue*, *Selenastrum capricornutum*, *Synechococcus* sps. etc.

Actinomycetes: *Actinomyces flavoviridis* and several species of *Streptomyces* exhibited high ability to absorb mercury and lead alongwith the other heavy metals from mixed metal solution of manganese, cobalt, nickel, copper, zinc, cadmium, mercury, lead and uranium.

Actinomycetes levoris and *S. virido* chromogenes were shown to accumulate a large amount of uranium from aqueous systems (Horikoslhi et al. 1981).

Bacteria: both Gram-positive and Gram-negative bacteria are resistance to mercury compounds. Bacterial genera such as Bacillus *cereus*, *Mycobacterium scrofulaceum*, *Streptococcus agalactiae*, *Streptomyces lividans*, *Thiobacillus ferrooxidans*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Staphylococcus aureus* etc. were reported to tolerate both cadmium and mercury. *Staphylococcus aureus* (Gram positive) intermediate in heavy metal sensitivity between *E. coli* and *Pseudomonas aeruginosa* (Gram negative). Nakajima and Sakaguchi (1986) reported species of *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Corynebacterium*, *Nocardia*, *Serratia* etc. absorb mercury and lead along with other heavy metals in the solution.

Alcaligenes faecalis tolerate zinc and cadmium when grown in nutrient broth (Prahalad and Seenayya).

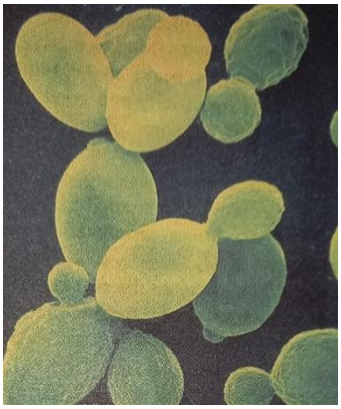

Fig. 9.17: *Saccharomyces*

Cyanobacteria:

Rai et. al., (1998) reported bio-absorption of cadmium and nickel by a capsulated nuisance cyanobacterium, *Microcystis*, both from field and in laboratory. Several species of microalgae including green alga, *Chlorella* (Aksu and Kutsal, 1991), blue green algae *Anabaena* (Mallick and Rai, 1994), marine algae (Holan et al., 1993) also shows absorption of some heavy metals.

Fungi:

Fungi accumulate heavy metals from dilute background concentrations. Lead and copper were more readily accumulated by fungi and actinomycetes in comparison to zinc, manganese, cobalt, nickel and cadmium. Yeasts are less sensitive to heavy metals (Avakyan, 1987), *Trichoderma viride, Aspergillus niger* and *A. giganteus* tolerate nickel concentration but showed prolonged growth and inhibited spore formation and spore germination.

Nakajima and Sakaguchi (1986) reported the selective absorption of mercury and lead from a mixed metal solution along with other heavy metals by *Aspergillus niger*, *A. oryzae*, *Chaetomium globosum*, *Fusarium oxysporium*, *Giberrella fujikuroi*, *Mucor hiemalis*, *Neurospora sitophila*, *Penicillium chrysogenum*, *P. lilacinum* and *Rhizopus oryzae* besides yeast species of the genera *Candida*, *Hensenula*, *Saccharomyces* and *Torulopsis*.

Macrophomina phaseolina tolerant to 500 ppm of cadmium in-vitro (Dubey and Dwivedi, 1985).

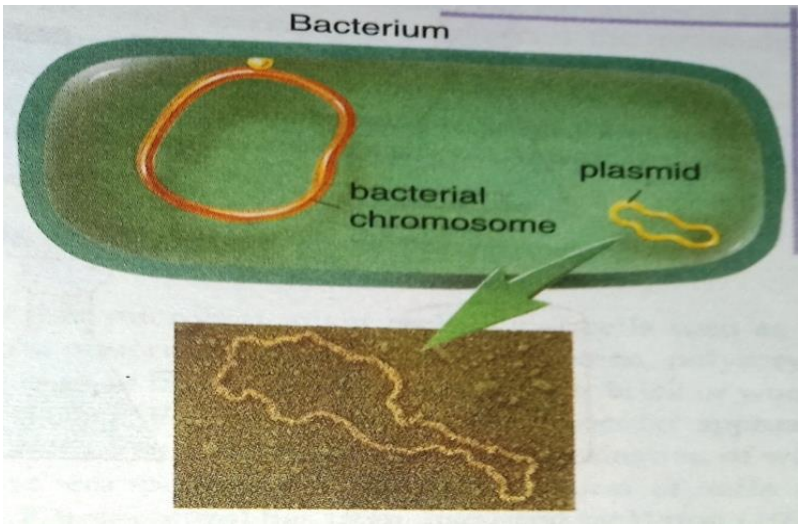

Fig. 9.18: Bacterial plasmids encode enzymes for biodegradation of specific compounds

9.8. Summary

The domestic waterborne wastes, including human and animal excreta, domestic washing waters, industrial water borne wastes, ground surface and atmospheric waters which enter the sewage system of a town or city is collectively known as sewage. Sewage is the used water supply containing domestic water together with human excreta and wash water and industrial waste, including acids, greases, oils, animal matter, vegetable matter and storms water. The basic principle in sewage treatment is that water is separated from the waste, while the solid organic matter is biodegraded by microorganisms to simple compounds like nitrates, sulphates, carbonates, carbon dioxide, methane etc. Salts of heavy metals such as Zn, Cr, Ni, Pb etc. are also present.

Three tests are used to assess the amount of organic matter:

1.total organic carbon (TOC),

2.biochemical oxygen demand (BOD)

3.chemical oxygen demand (COD)

The main objective of domestic waste treatment is the reduction of BOD. Commonly coliform, streptococci, clostridia, micrococci, Proteus, pseudomonas, Lactobacilli etc. are present in sewage. The organic material of the sludge is decomposed by anaerobic bacteria resulting in

release and deposition of breakdown products on the ground. In cities sewage are treated by municipal plants.

Sewage treatment is of two types-

a. Small scale sewage treatment-It is used in villages mostly and treatment is done by Cesspools and Septic tanks.

2.Large scale sewage treatment-It is used in larger cities for larger populations by municipal bodies.It involves three basic treatment steps-

1.Primary treatment (physical treatment)-organic matter removed by physical method from sewage

2.Secondary treatment(biological treatment)-organic matter degradation take place by participation various microorganisms in aerobic (initial steps) as well as in anaerobic (later steps) condition. The BOD of sewage is decreased up to 90-95 %. Biological degradation is done by several methods-1.The oxidative pond2.The aerobic trickling filter3.The aerobic activated sludge4.The anaerobic digester-in this step fermentation, acetogenic reaction and methanogenesis take place, and results in formation og bio gas.

3.Tertiary treatment- remove non biodegradable organic material, heavy metals, xenobiotic substances (plastic, pesticides, PCBs,etc.), petroleum (oil and oil products), ,etc.,which remains after treatment of microorganisms of second step.The process commonly known as biodegradation or bioremediation.

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Petroleum is a complex mixture composed primarily of aliphatic, alicyclic and aromatic hydrocarbons. There are hundreds of individual compounds in every crude oil. Some microorganisms like fungi, bacteria, cyanobacteria, yeast and algae cleave the hydrocarbons into simpler molecules. Oil and oil products are decompose by mainly fungi and bacteria. Methane is simplest hydrocarbon pollutant degraded by a specialized group of bacteria called methanotrophic bacteria. Many cyanobacteria, corynebacteria, pseudomonads and mycobacteria are able for degradation of petroleum products.

Pseudomonas putida, commonly known as oil eating bug or super bug, genetically enginered species of bacterium, synthesized by DR A. M. Chakraborty, have four different types of plasmids, able to degrade the petrolium products in soil and water.

Xenobiotics are man-made, synthesized compounds such as pesticides. Those are chemicals which do not exist in nature. They may affect those microorganisms which are important in maintaining soil fertility when added in soil. The xenobiotics have molecular structure and chemical bond sequences not recognized by the existing degradative enzymes.

Many microorganisms such as *Rhizopus*, *Achromobacter*, *Nocardia*, *Pseudomonas*, *Alcaligene*s and *Acinetobacter* have been reported to degrade Polychlorinated biphenyls (PCBs), a class of organic compounds which are used in adhesives, carbonless copy paper, insulation in transformers, high pressure hydraulic fuids, machine tool cutting oils, specialized lubricants, gasket sealers, plasticizers and protective coatings for wood, metal and concrete to some degree.

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9.9. Terminal questions

Q.1: What is sewage?Discribe its treatment.

Answer:----------

Q.2: Describe the biological treatment of sewage in detail.

Answer:----------

Q.3: Briefly describe different Xenobiotic substance and their bioremediation.

Answer:----------

Q.4: Write a account on biodegradation of heavy metals.

Answer:----------

Q.5: Write the brief account on biodegradation of Petroleum.

Answer:----------

Q.6: Write short notes on:

a. Cesspool and septic tank
b. Minimata disease
c. BOD
d. Super bug

Answer:----------

9.10. Further suggested readings

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Rajarshi Tandon Open University, Prayagraj

PGEVS-103N

Environmental Microbiology

Block- IV

Agriculture Microbiology

UNIT -10

Microbial Biofertilizers

UNIT-11

Microbial pesticides

UNIT-12

Microbiology degradation

PGEVS-103N

Rajarshi Tandon Open University, Prayagraj

Environmental Microbiology

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Block-4 *PGEVS-103N*

Introduction

This is the fourth block on microbes in organic matter and microbial diseases. It consists of following three units such as:

Unit-10: This unit covers the microorganism associated with organic matter decomposition-cellulose, hemicelluloses, lignin and proteins, carbon assimilation and immobilization, factor effecting microbial community in soil.

Unit-11: This unit covers the bacterial and virus diseases with reference to tuberculosis, cholera, AIDS, Rabies, food born diseases.

Unit-12: This unit covers the chemotherapy/antibiotics. The sewage antimicrobial agents, antibiotics, penicillins and cephalosporins and broad- spectrum antibiotics, sulfa drugs, antifungal antibiotics discussed here.

Unit 10: Microorganisms of Organic Matter Decomposition

Contents

10.1. Introduction

Soil organic matter has been increasingly in the news lately in part because the vast majority of terrestrial carbon is contained in soils. As such, soils play an important role in the global carbon cycle. In addition to serving as a key carbon store, soil organic matter is one of the central attributes of soils. Organic matter influences virtually all of soil properties and overall health, including its physical structure, nutrient status, and biodiversity.

Soil organic matter is composed of, both living and non-living components. The living component includes soil macro- and micro-fauna, and soil microbial communities, which may be active or dormant. The non-living portion of soil organic matter is derived from dead plant and animal parts, and excreta of human and animals such as urine or faeces.

Earthworms, mites, ants, etc., take part in the initial stages of physical (dead animals and plants found over surface of soil) decomposition. The smaller pieces of litter or the fragmented waste they release is called **detritus**. Earthworms, beetles, snails, millipedes etc., are known as **detrivores** that consume the detritus or decaying organic matter. After that, decomposers come into action

When a plant, animal or insect dies it breaks into tiny pieces and eventually, these small pieces become part of the soil. This process is known as decomposition. It is the process where organic substances break down into simpler matter. Bodies of any living organism

start decomposing shortly after their death. Animals such as worms help in decomposing the organic matter.

The decomposition (stabilization) of surface organic matter such as vegetable matter, animal dung, house waste and other organic refuse by microorganism to form fine stable organic material for use in soil amendments in farming (organic farming) to increase crop production is known as compost and process as composting. There are two processes, which yields compost-

1. Anaerobic process (reduction process)

2.Aerobic process (oxidative process)

The final product of aerobic decomposition is co_2 and that of anaerobic decomposition are hydrogen, ethyl alcohol (CH_4), various organic acids and carbon dioxide (CO_2).

Biogas is a mixture of gases, primarily consisting of methane (50-76%), CO_2, H_2S, water vapour and trace amounts of other gases which are produced by anaerobic decomposition of organic matter, such as agricultural waste, municipal waste, plant material, sewage, animal dung, waste water and food waste, etc. It is a renewable source of energy.

Soil microorganisms use organic matter as a source of energy and food. The process of decomposition is initially fast, but slows down considerably as the supply of readily decomposable organic matter gets exhausted.

Sugars, water-soluble nitrogenous compounds, amino acids, lipids, starchs and some of the hemicelluloses are decomposed first at rapid rate. While, insoluble compounds such as cellulose, hemicelluloses, lignin, proteins etc. ,which forms the major portion of organic matter are decomposed later slowly. Thus, the organic matter added to the soil is converted by oxidative decomposition to simpler inorganic substances (mineral nutrients) which are made available in latter stages for plant growth and the residue remains is transformed into amorphous organic substance known as humus. Humus make soil porous which allow water and air to penetrate deep underground, and improve soil fertility, water retention, CEC (carbon exchange capacity), nutrient availability and soil health.

Decomposition of organic matter take place by different microorganism, mainly fungi, bacteria, actnomycetes, protozoans, nematodes,etc. .

Different constituent of organic matter decomposed or hydrolized mostly by different types of enzymes , such as- cellulose by cellulase, cellubiose by cellubiase, hemicellulose by hemicellulase,chitin by chitinase, pectin by pectinase, protein by proteinase, and lignin by peroxidase and oxidase.These different enzymes synthesized by different microorganism organisms.

Number of soil factors affecting the organic matter decomposition in soil.These are-aeration/oxygen, moisture, soil texture, humus, temperature, inorganic minerals, and also particle size of organic matter.

The activity of microorganisms affected by number of soil factors. These are-moisture, organic and inorganic chemicals, organic matter, vegetation type, root exudates, phsical factors.

Fixation of CO_2 by photosynthetic plants in organic form is known as cabon assimilation (immobiligation of carbon), and decomposition of organic matter, which releases the minerals is known as mineralization. Both are important for ecosystem.

Objectives

After study of the course of this unit, students will be able to understand-

- About organic matter of soil.
- about the different microbes involved in the process of organic matter.
- about decomposition and their working mechanism.
- about the carbon assimilation and immobilization.
- about the factors affecting the microbial community in soil.
- about the decomposition of protein, cellulose. hemicellulose, chitin and lignin.

10.2. Organic Matter

Organic matter is anything that contains carbon compounds that were formed by living organisms. It covers a wide range of things like lawn clippings, leaves, stems,

branches, moss, algae, lichens, any parts of animals, manure, droppings, sewage sludge, sawdust, insects, earthworms and microbes. The main components of organic matter in soils are:

- Dead forms of organic material - mostly dead plant parts
- Living parts of plants - mostly roots
- Living microbes and soil animals
- Excreta of human and animals such as urine or faeces

By far, the largest component is the dead matter, it constitutes about 85% of all organic matter in soils. Living roots make up about another 10% and the microbes and soil animal excreta of human and animals make up the last few percent.

When organic matter is incorporated into soil, the larger organisms like mites and soil animals break it into smaller pieces. Then, the fungi and bacteria start to decompose it. They secrete enzymes to break up into the chemical compounds of *which it is made up of. When the enzymes break the molecules into smaller molecules,* the bacteria and fungi use some of energy or nutrients released for their own growth. Whereas, the remaining parts of nutrients that the microbes do not use, they will be available for growth of other soil organisms or plants. When microbes die, their cells are degraded and nutrients contained within them become available to plants and other soil organisms.

The dead fallen leaves in forest (and also in mango garden) upon surface of soil forms litter which is later on decomposed into soluble organic matter (SOM). SOM decomposed into minerals by microorganism. Conversion of organic matter into simple inorganic forms mainly by fungi and bacteria is called mineralization and it terminates with the formation of complex amorphous organic substance known as humus and process as humification. Humus so altered that it can no longer be recognised as plant material.

Earthworms, mites, ants, etc., take part in the initial stages of physical (dead animals and plants found over surface of soil) decomposition. The smaller pieces of litter or the fragmented waste they release is called **detritus**. Earthworms, beetles, snails,

millipedes etc., are known as **detrivores** that consume the detritus or decaying organic matter. After that, decomposers come into action.

The organic matter shows slow, active or passive nature during its decomposition.

Organic matter contains number of organic compounds such as cellulose, hemicellulose, chitin, proteins and nucleic acids. Decomposition of organic matters is an ecological process, which returns back the mineral nutrients to soil, which are later on available for plant growth and development. The decomposition is complex process in which both, macro and microorganism participate, and it is two steps process –In first step, mostly physical process, the soil macroinvertebrates organisms, commonly known as detritivores participate these detrivores feed (and chew) upon organic matter and convert it into detritus organic matter. Now, in second step, microorganism (bacteria and fungi) participate and they finally decompose it and mineral nutrients released in soil. The releasing process of minerals from organic matters is known as mineralization. These released minerals are taken up by plant and they become part of plant. This process is known as Immobilization, because, these mineral materials participate in formation of organic matter in plant and can not available for other plant utilization.

Sometime, under anaerobic conditions, organic matters upon surface of soil, such as vegetable matter, animal dung, house waste, etc., undergo in microbial decomposition and forms compost, which is used in organic farming to increase soil fertility and crop production. The dead fallen leaves in forest upon soil surface forms litter which later on forms soluble organic matter by decomposition.

Organic matter decomposition serves three functions for the microflora (1) providing energy for cell growth (2) supplying carbon for the formation of cell material, and providing other mineral nutrients elements needed for cell growth.

Biogas is a mixture of gases, mainly consists of methane (50-75%), CO_2, H_2S, water vapours and trace amounts of other gases which are produced by anaerobic decomposition of organic matter such as agricultural waste, municipal waste, sewage and animal dung etc. it is renewable source of energy.

The moisture, temperature, aeration (oxygen) and particle size of organic matter effect the rate of decomposition. Beside this, any activity which slow or halts microbial growth, also effect the decomposition. The different organic matter such as cellulose, hemicellulose, chitin, lignin and protein decompose by different micro-organisms.

Cellulose is polymer of glucose, in first step hydrolysed extracellularly into glucose by enzyme cellulase and cellubiase. This enzyme produced by fungus -Penicillium and Aspergillus, and bacteria- Pseudomonas and Streptomyces. Now, glucose enters into cell for further oxidation.

Hemicellulose is heteropolymer of five different sugars and uronic acid. It is second in bundance, after the cellulose. The hydrolysis of hemicellulose is carried out by number of hemicellulolytic enzymes known as Hemicellulases, excreted by microorganisms, and different types of monosaccharides are formed. These are xylose, arabinose, galactose and mannose which are further converted into organic acid, alcohols, CO_2 and H_2O, whereas, uronic acid is converted into pentose and H_2O. Microorganisms participate are bacteria (Bacillus subtilis), fungi (Aspergillus niger), actinomyces, protozoa (ciliates) and insects (termites and beetles). They all secretes enzyme hemicellulase.

Chitin is special homopolymer of N-acetyl D-glucosamine found in integuments of arthropods and cell wall of fungi. It is not easily decomposed and requires different types of enzymes for their decomposition. However, they hydrolysed by enzyme chitinases into smaller monomeric units, which is produced by number of microorganisms. These are chitinolytic bacteria (*Vibrio* and *Photobacterium*, *Bacillus*, *Streptomyces* etc.), chitinolytic fungi (members of Mucorales, Deuteromyces and Ascomyces), slime mold, protozoa (*Hartmanella*) and algae (Diatom, *Nitzchila*).

Lignin is third most abundant organic constituent of plant tissues which is composed of phenylpropane units linked together by various types of chemical linkages. It is one of the most resistance organic substances for the micro-organism to degrade, however, certain ascomycetes and basidiomycetes members are known to degrade lignin at a slow rate. Beside fungi, bacteria, cyanobacteria and actinomycetes also participate in the decomposition. In most of cases, microorganism produces peroxidases and oxidases.

Protein are complex organic homopolymer of amino acids. They are first hydrolysed into smaller peptides, and ultimately into nitrogenous compound amides and amino acids. These nitrogenous compounds undergo in ammonification and produce ammonia or ammonium. The ammonia or ammonium later on converted into nitrite and nitrate by nitrification process carried out by soil bacteria. Nitrate absorbed by most of plant root and becomes part of organic component of plant process known as Immobilization. Protein hydrolysis carried out by bacteria, actinomycetes, fungi and protozoa.

The decomposition of organic matter affected by various factors. These are- temperature (30-40^{0}C), soil pH (slightly acidic or neutral pH), inorganic mineral nutrients (mainly nitrogen), soil texture (clay retains larger amount of humus), aeration (oxygen required for aerobic microorganism), nature of plant organic matter and texture of soil (loose soil texture provide more space between soil particles for oxygen to collect, and decomposition will be faster by aerobic microorganism then tight soil).

Synthesis of organic substance (glucose) from atmospheric CO_2, by process of photosynthesis, in green plant in presence of sunlight is commonly known as CO_2 fixation or carbon assimilation or photosynthesis. It is an anabolic process. This fixed carbon becomes unavailable for use in the generation of new plant life and commonly known as immobilization. Whereas, CO_2 and inorganic minerals release by decomposition of this organic matter in the atmosphere (air, water and soil) and commonly known as Mineralization. CO_2 reaches to the atmosphere and mineral elements remain in soil and water, and again available for plant growth.

There are many factors which affect the microbial community in soil. These are soil moisture, organic and inorganic chemicals, soil pH, soil organic matter, types of vegetation and growth stage, regional and seasonal variation, root exudates and physical factors.

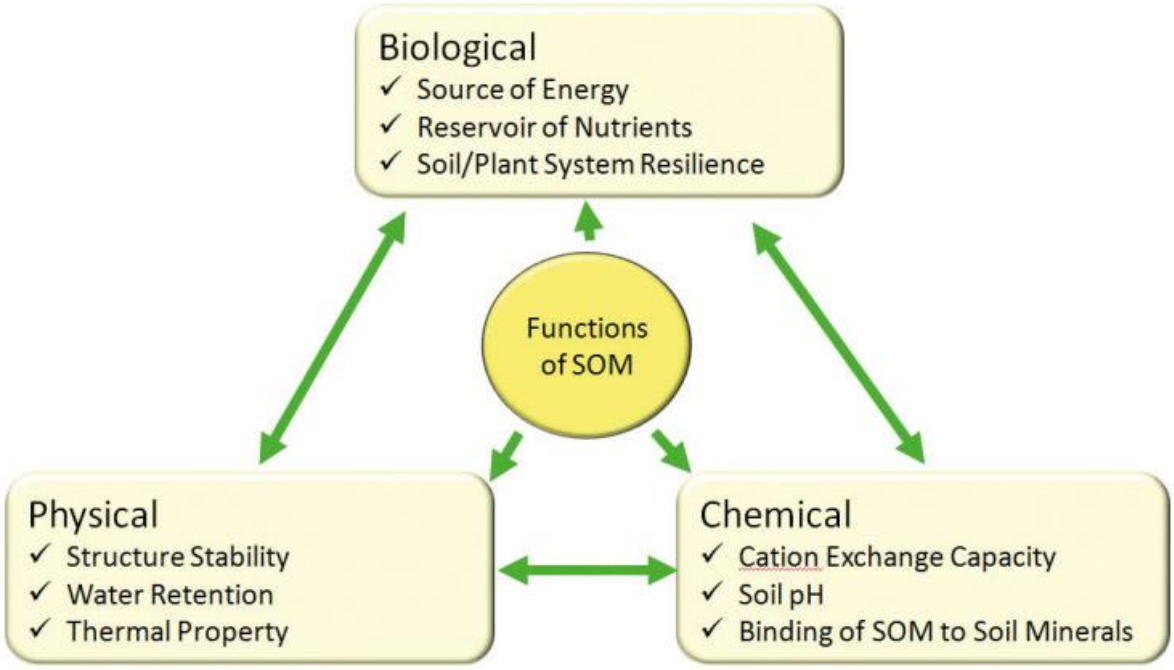

Fig. 10.1: Function of soil organic matter (SOM)

10.2.1. Decomposition of organic matter

Organic matter decomposition is part of an **ecosystem** that returns mineral nutrients to the soil. The decomposition process has two stages- first is upon the soil, and the second is below the ground. In the first stage, several terrestrial animals and insects ,above the ground, feed on dead leaves and animal residues left over the ground. Here, the nutrients and energy enter the **food web,** while some nutrients leach into the soil via chemical action but, the next stage is fundamentally a soil-based process. Different organisms take part in the successive digestion of the organic matter. These organisms are broadly classified into two main categories, based on their function.

- **Soil macrofauna,** soil invertebrates like earthworms, snails, mites, ants, beetles etc., function as detrivores.
- **Soil microflora** like heterotrophic bacteria and fungi are the typical decomposers.

Successive decomposition of the organic material produces carbon dioxide, water, mineral nutrients and humus. The products of decomposition contribute some

amount to the **SOM** (soil organic matter).The SOM influences soil's physical, chemical and biological properties.

. Earthworms, mites, ants, etc., take part in the initial stages of physical (dead animals and plants found over surface of soil) decomposition. The smaller pieces of litter or the fragmented waste they release is called **detritus**. Earthworms, beetles, snails, millipedes etc., are known as **detrivores** that consume the detritus or decaying organic matter. After that, decomposers come into action

Heterotrophic bacteria and saprophytic **fungi** primarily function as decomposers that allow a continuous flow of energy within an ecosystem, participate. They prominently feed upon plant and animal remains and secrete chemicals to digest the organic materials in detritus material. They get energy by breaking down chemical bonds present in the organic matter, such groups of organisms relying upon dead organic matter are called **saprophytes**. It releases carbon dioxide and reduces the organic matter into inorganic minerals like N, P, S, etc.

Thus, detrivores and saprophytes play a leading role in decomposing organic material and recycling soil nutrients.

The conversion of organic matter into simple inorganic forms, mineral nutrients, which are used by plants, is called mineralization, soil microorganisms, mainly bacteria and fungi participate in this conversion. The whole process of mineralization terminates into formation of complex amorphous substance known as humus and process called as humification. Humus consists of fulvic acid, humic acids and humin which make soil fertile.

Mineralization produces humus on one side and inorganic minerals, carbon dioxide and water in soil on other side, which are available for plant root absorption and reach within plant so unavailable for other plant. This commonly known as immobilization. Mineralization and immobilization are important processes of mineral recycling in an ecosystem in nature. Rainfall and sunlight are the external factors that favour in decaying of plant and animal wastes.

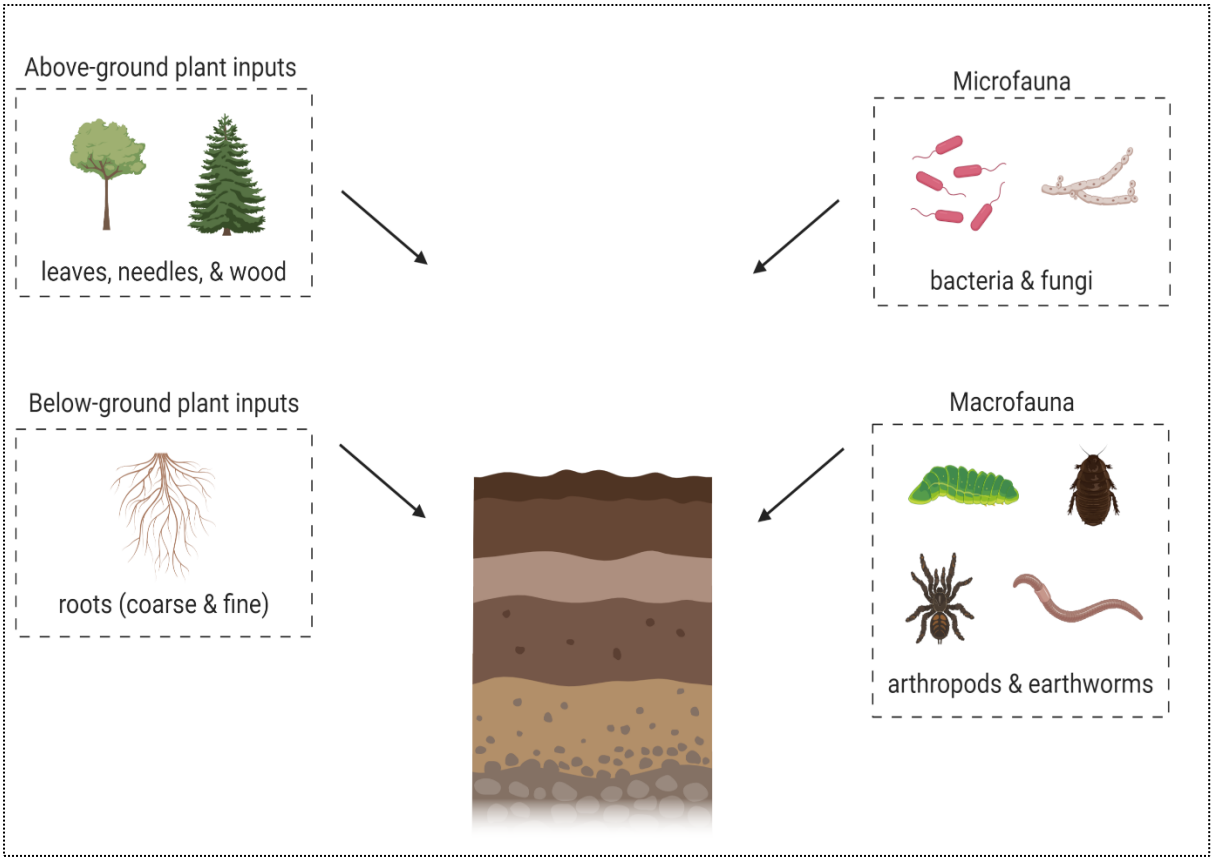

Fig. 10.2: Major sources of organic matter and their decomposes.

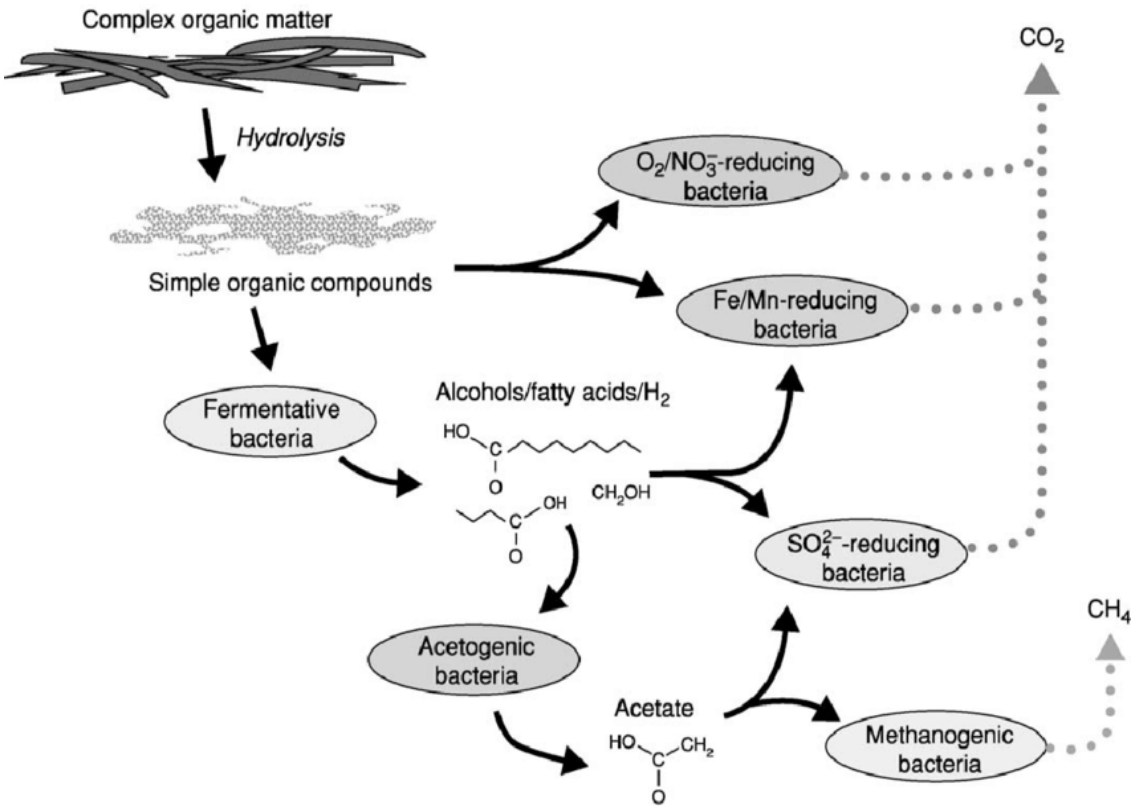

Fig. 10.3: Decomposition of organic matter

10.2.2. Microorganism associated with organic matter decomposition

Organic matter decomposition is primarily a microbiological process. Decomposition is carried out by heterotrophic micro flora and micro fauna comprising of bacteria, fungi, actinomycetes and protozoa. Besides the micro flora and micro fauna, many species of mesofauna (soil animals of intermediate size) such as earthworms, mites, arthropods, nematodes, molluscs, ants, sowbugs, spiders, beetles, flies and centipedes also play an important role in the initial breakdown of organic residues. Organic matter decomposition serves three functions for the micro flora: (i) providing energy for cell growth, (ii) supplying carbon for the formation of cell material, and (iii) providing other mineral nutrient, elements needed for cell growth.

The decomposition (stabilization) of surface organic matter such as vegetable matter, animal dung, house waste and other organic refuse by microorganism to form fine stable

organic material for use in soil amendments in farming (organic farming) to increase crop production is known as compost and process as composting. There are two processes, which yields compost-

1. Anaerobic process (reduction process)- take place in the absence of oxygen, less heat is generated than aerobic process. This is disadvantages as high heat or temperature is required for destruction of pathogen and parasites present in organic matter.

2.Aerobic process (oxidative process)- decomposition and stabilization take place in the presence of oxygen and more heat generated which kills the pathogen and parasite.

The final product of aerobic decomposition is co_2 and that of anaerobic decomposition are hydrogen, ethyl alcohol (CH_4), various organic acids and carbon dioxide (CO_2).

Biogas is a mixture of gases, primarily consisting of methane (50-76%), CO_2, H_2S, water vapour and trace amounts of other gases which are produced by anaerobic decomposition of organic matter, such as agricultural waste, municipal waste, plant material, sewage, animal dung, waste water and food waste, etc. It is a renewable source of energy.

Soil organisms use organic matter as a source of energy and food. The process of decomposition is initially fast, but slows down considerably as the supply of readily decomposable organic matter gets exhausted.

Sugars, water-soluble nitrogenous compounds, amino acids, lipids, starchs and some of the hemicelluloses are decomposed first at rapid rate. While, insoluble compounds such as cellulose, hemicelluloses, lignin, proteins etc. ,which forms the major portion of organic matter are decomposed later slowly. Thus, the organic matter added to the soil is converted by oxidative decomposition to simpler inorganic substances (mineral nutrients) which are made available in latter stages for plant growth and the residue remains is transformed into amorphous organic substance known as humus. Humus make soil porous which allow water and air to penetrate deep underground, and improve soil fertility, water retention, CEC (carbon exchange capacity), nutrient availability and soil health.

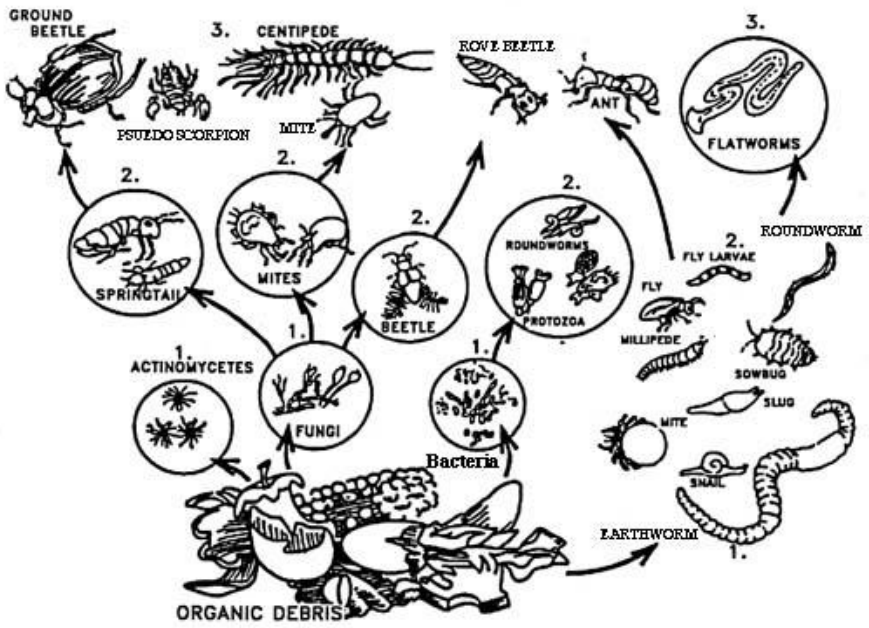

Fig. 10.4: Microorganisms involved in organic matter decomposition

10.2.3 Requirements for efficient decomposition of organic matter

Any activity which slows or halts microbial growth, also effect decomposition. Efficient decomposition effected by following factors-

A. **Aeration/oxygen**- oxygen is required for microbes during aerobic condition of decomposition. Due to smell (bad smell) problem, anaerobic composting is not recommended in a residential place (town and cities). The anaerobic condition takes 3 to 4 times longer time than aerobic decomposition to decompose organic matter due to very slow process in nature. In aerobic decomposition, oxygen level should be kept above at 5%, if it is decrease, then anaerobic decomposition started. More the oxygen (10-12%), the more quickly aerobic decomposition take place.

B. **Moisture**- moisture is essential for microbial activity. The dry organic matter will not decompose efficiently. However, excess water leads to anaerobic condition which slow down the decomposition.

C. **Temperature**- temperature is important factor for biological activity to take place. The low temperature in outside environment will slow down the activity of microorganism

and also decomposition. The warmer temperature speed up decomposition. The microorganisms fall into two categories in relation to temperature. These are

1. Mesophilic organisms- microorganisms live and function between 50 to 113^0F.
2. Thermophilic organisms- microorganisms lives and function between 110^0 to 160^0F.

The high temperature will also help to destroy weed seeds, egg and larva of diseases causing pathogenic nematodes and spores of pathogenic fungi.

D. **Particle size of organic matter**- the smaller the particle size of organic matter the more quickly it can be consumed by microorganism and greatly reduces the decomposition time.

10.3. Cellulose decomposition

cellulose is the most abundant structural polysaccharide present in plant residues/organic matter in nature which is polymer of glucose (homopolymer). When cellulose is associated with pentosans (Xylans & mannans), it undergoes rapid decomposition, but when associated with lignin, the rate of decomposition is very slow. The decomposition of cellulose occurs in two stages:

1) In the first stage, the long chain of cellulase is broken down into cellubiose and then into glucose by the process of hydrolysis in the presence of enzymes cellulase and cellubiase.
2) In second stage, glucose is then transported into the cell for energy generation (catabolism) or production of biomass (anabolism). During catabolism CO_2 and H_2O is produced beside energy. Fungi, such as *Penicillium* and *Aspergillus,* and bacteria such as *Streptomyces* and *Pseudomonas* are important participants in the extracellular cleavage of cellulose. The most extensively studied sources of cellulolytic enzymes have been obtained from the fungi *Trichoderma* and *Phanerochaete,* and the bacteria *Cellulomonas* (an aerobe) and *Clostridium thermocellum* (an anaerobe).

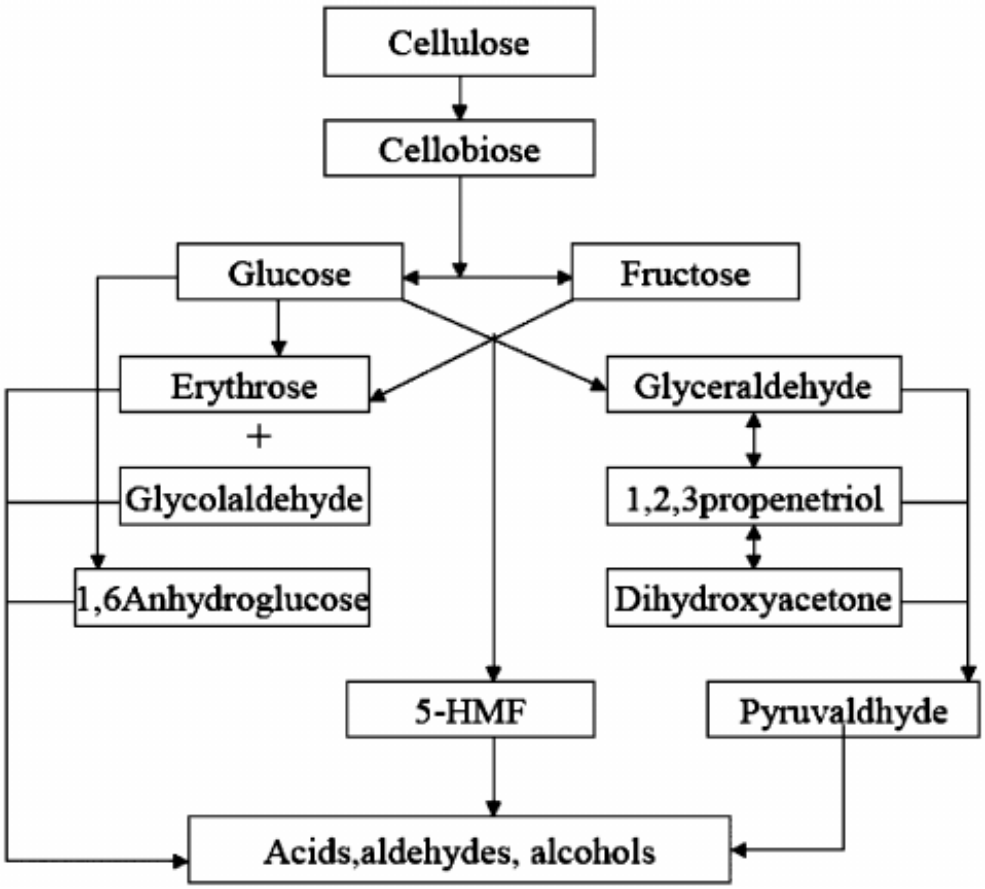

Fig. 10.5: Cellulose decomposition pathway

10.3.1 Microorganisms involved in decomposition of cellulose

Cellulose may be decomposed by fungi or bacteria.

Cellulolytic fungi

- Cellulase-producing fungi are widespread among fungi and include species from the ascomycetes (*Trichoderma reesei*), basidiomycetes (*Fomitopsis palustris*) with few anaerobic species.
- The soft rots activity hydrolyses cellulose by production of cellulase by fungi, such as, *Aspergillus niger, Fusarium oxysporum*, *Neurospora crassa,* etc.
- The brown rot actively hydrolyses cellulose during the earlywood decay as they lack exoglucanases. Some of the common examples of these fungi include *Poria placenta*, *Lenzites trabea, Coniophora puteana,* and *Tyromyces palustris*.

- The white rot, in turn, mostly lignocellulose degradation takes place with fungi, for examples like *Phanerochaete chrysosporium, Sporotrichum thermophile,* and *Trametes versicolor*.
- Among the anaerobic cellulolytic fungi, most studied are with the *Neocallimastix frontalis, Piromyces (piromonas) communis,* and *Orpinomyces* species.

Cellulolytic bacteria

- Most of the bacterial cellulolytic enzymes are reported from *Bacillus, Acinetobacter, Cellulomonas,* and *Clostridium*.
- Bacteria are also known to produce cellulases that can degrade the cell wall components, such as *Fibrobacter succinogenes, Ruminococcus albus, Pseudomonas, Proteus,* and *Staphylococcus*.
- Some thermophilic bacteria like *Anoxybacillus* sp, *Geobacillus* sp, and *Bacteroides* also exhibit cellulase activity.

10.4. Hemicellulose decomposition

Hemicelluloses are water-soluble polysaccharides which is a heteropolymer of five different sugars. It contains two five carbon sugars (D-xylose and L-arabinose), three six carbon sugars (D-galactose, D-glucose and D-mannose) and uronic acids, and are the major plant constituents, second only in quantity to cellulose, and is sources of energy and nutrients for soil micro flora. The hydrolysis is brought about by number of hemicellulolytic enzymes known as "hemicellulases" excreted by the microorganisms. On hydrolysis, hemicelluloses are converted into soluble monosaccharide/sugars, e.g, xylose, arabinose, galactose and mannose, which are further convened to organic acids, alcohols, CO_2 and H_2O, and uronic acids is broken down to pentose and CO_2. Various microorganisms including fungi, bacteria and actinomycetes, both aerobic and anaerobic are involved in the decomposition of hemicelluloses.

10.4.1. Microorganisms involved in decomposition of hemicellulose

Microorganisms play an important role in the degradation of hemicellulose in natural environment. Some of the microorganisms involved in decomposition are-

Bacteria: many bacteria are able to produce hemicellulases that can break down hemicellulose into its constituent sugars. Examples *Bacillus subtilis, Clostridium thermocellum, and Cellvibrio japonicus.*

Fungi: fungi are also important decomposers of plant material, including hemicellulose. Some fungi, such as Aspergillus niger and Trichoderma reesei, are known to produce hemicellulases that can break down hemicellulose.

Actinomycetes: actinomycetes are a group of bacteria that are commonly found in soil and are known for their ability to produce a wide range of enzymes, including hemicellulases.

Protozoa: some protozoa, such as ciliates, are able to break down hemicellulose in the digestive tracts of animals that consume plant material.

Insects: some insects, such as termites and certain beetles, are able to break down hemicellulose in the wood and plant material that they consume. They have specialized symbiotic microorganisms in their gut that produce hemicellulases.

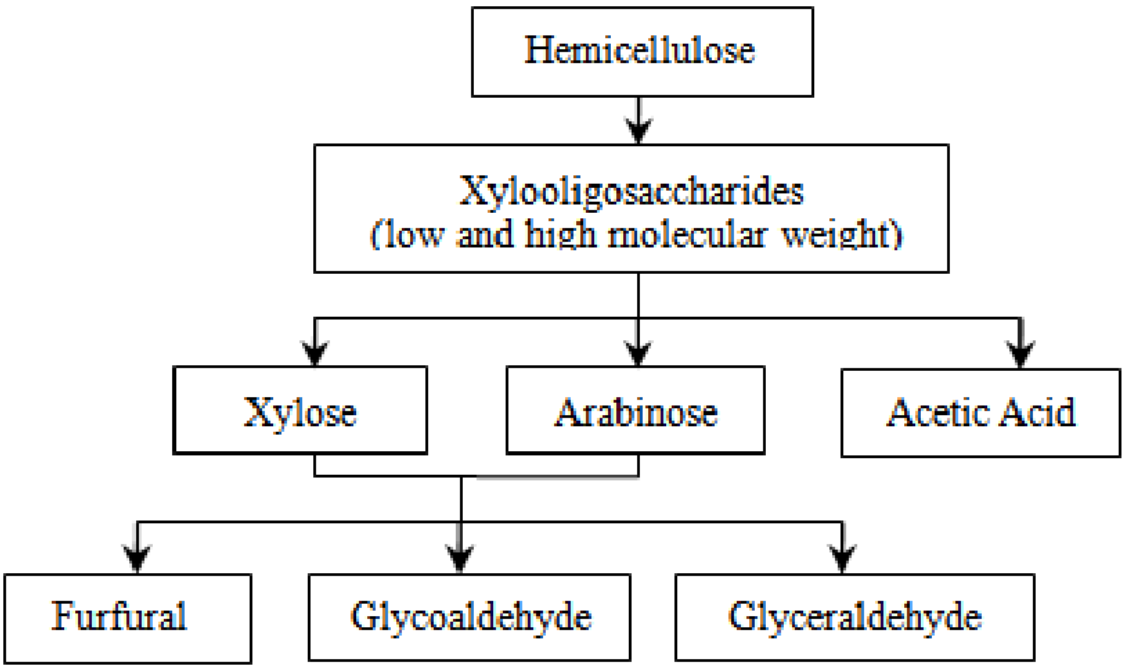

Fig. 10.6: Decomposition of hemicellulose

10.5. Chitin decomposition

Chitin is a special compound which is found in the integument of arthropods and cell wall of fungi. The polymer is not easily degraded, and requires a variety of enzymes for their decomposition process of chitin and chitosan. Chitin is similar to cellulose in that it is also indigestable by vertebrate animals due to the lack of enzyme system required for its degradation. Chitin is a β 1,4-linked linear homopolymer of N-acetyl D-glucosamine derivative of glucose, and It shares close structural similarity to cellulose. In chitin, the alcoholic oh group of the second carbon atom of β-glucose units is replaced by an n-acetylamino group. Chitin is insoluble in both aqueous and non-polar solvent, despite the presence of charges at the acetyl groups. Chitin has a three-dimensional α-helix configuration. The stability of the α-helix chitin structure is brought about by the hydrogen bonding of the N-acetyl side chains.

In nature, however, the chitin polymers bind extracellularly by intermolecular hydrogen bonding that forms a crystalline microfibril structure. Chitinases works by degrading the β-1,4-linkages that exist between n-acetyl glucosamine units to reduce the length of the polymer, ultimately leading to the formation of monomeric units. They are

smaller with low molecular weight and known as chitooligomers, which have industrial, agricultural, and medical functions.

10.5.1. Microorganisms involved in decomposition of chitin

Bacteria, fungi, algae and protozoans participate in chitin decomposition-

1 **Chitinolytic bacteria**

Chitinolytic bacteria are widely distributed in different habitats, and chitinases are produced by many genera of gram-negative and gram-positive bacteria, but not by Archaebacteria. Chitinolytic bacteria of the genera *Vibrio* and *Photobacterium* are associated with zooplankton and particulate matter that are found in a sea associated with zooplanktons and carapaces.

Bacterial species of *Vibrio, Photobacterium, Aeromonas, Cytophaga, Streptomyces, Bacillus, Clostridium*and *Chromobacterium* are well-known chitinolytic bacteria.

These are specialized utilizers of diacetylchitobiose, and the accumulation of N-acetylglucosamine indicates non-utilizable monomers during random hydrolysis of chitin oligomers.

Chitinolytic bacteria are also abundant in freshwaters, characteristic genera are *Serratia, Chromobacterium, Pseudomonas, Flavobacterium*, and *Bacillus*, with *Cytophaga johnsonae*.

2. Chitinolytic fungi

The primary habitat of chitinolytic fungi is the soil where the chitinolytic activity of fungi might even exceed to that of bacteria.

The most common fungal species involved in chitinolysis include members of mucorales like *Mortierella* sp, and deuteromycetes and ascomycetes-*Aspergillus*, *Verticillium, Thielavia, Trichoderma, Penicillium,* and *Humicola*.

The chitinolytic system in these fungi is inducible, and the activity increases with the increase in the chitin-rich substrate.

Freshwater species, *Chrytriomyces* and *Karlingia* are obligate chitinophilic that degrade chitin to fulfil their nutritional requirement.

3. Slime mold, protozoa, and algae

- Myxomycetes (true slime molds) like *Physarum polycephalum* are a rich source of lytic enzymes that produce a complex of extracellular chitinases.
- Soil protozoa like *Hartmanella* and *Schizopyrenus*, along with slime mold *Plasmodium* are also known to produce chitinases that participate in the digestion of chitinous food particles engulfed by these invertebrates.
- A colourless heterotrophic algae diatom, *Nitzchia alba*, is the only known diatom to digest chitin.

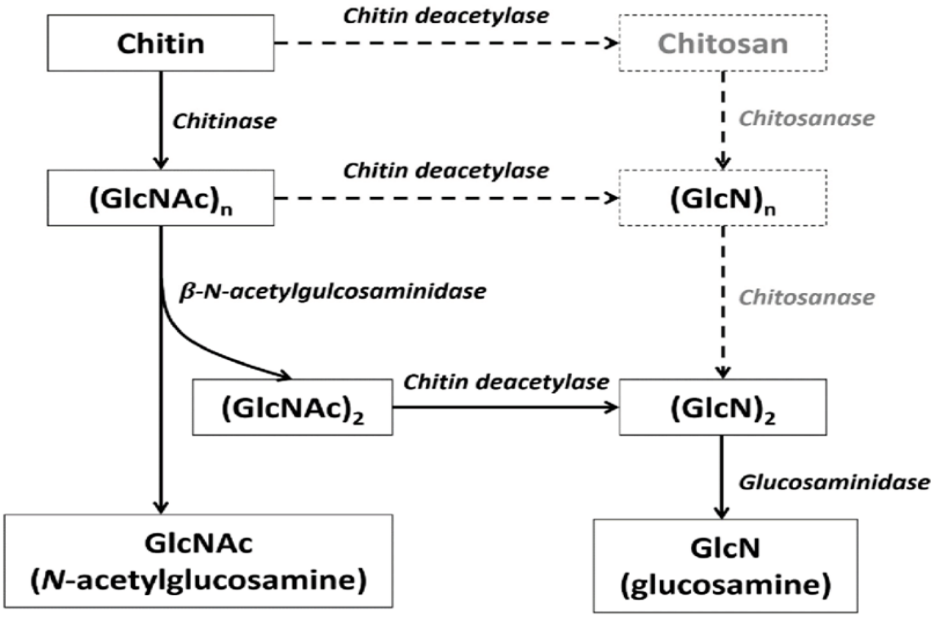

Fig. 10.7: Decomposition of chitin

10.6. Lignin decomposition

Lignin is the third most abundant organic constituent of plant tissues, and accounts about 10-30 percent of the dry matter of mature plant materials that has found application in a variety of industries from construction of paper to bioethanol production. Lignin content of young plants is low and gradually increases as the plant grows old. Lignin is

composed of phenylpropane units linked together by the chemical linkages of alkyl-alkyl, alkyl-aryl, and aryl-aryl groups.

The precursors of lignin synthesis in nature include p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. Besides, important structural characteristics of lignin, it include the functional groups, alcohol hydroxyl group, carbonyl group, carboxyl group, phenolic hydroxyl group, methoxyl, and sulfonic acid.

It is one of the most resistant organic substances for the microorganisms to degrade. However, certain Ascomycetous and basidiomycetous fungi are known to degrade lignin naturally at slow rates. Beside fungi, bacteria, cyanobacteria and actinomycetes also participate in the decomposition. The final cleavages of these aromatic compounds yield organic acids, carbon dioxide, methane and water. Lignin is the main component of wood in trees. Lignin has a varied, unique, and complicated chemical structure which contains many aromatic rings. These aromatics can be released from the lignin structure by fungal enzymes, such as peroxidases and oxidases. These enzymes utilize H_2O_2 and OH radicals to break the bonds in the lignin. The lignin degrading enzyme produced by microbial species, especially white rot fungi (*Phanerochaete chrysosporium*), brown rot fungi and soft rot fungi, and bacteria.

10.6.1. Microorganisms involved in lignin decomposition

The decomposition of lignin take place by bacteria, cyanobacteria, actinomycetes and fungi.

1. Lignin-degrading bacteria

- The occurrence of lignin-degrading enzymes has been observed in *Mycobacterium tuberculosum, M. avium, Pseudomonas syringae, P. aeruginosa, P. putida, Bordetella pertussis, Xanthomonas campestris, Escherichia coli, Caulobacter crescentus, Rhodobacter capsulatus, Yersinia pestis, Campylobacter jejuni*, and *Aquifex aeolicus.*

- Rumen bacteria like *Fibrobacter succinogenes, Ruminococcus albus*, and *Flaavefaciens* are major degraders of plant fibre cell walls and have an emerging role for bacteria in lignin degradation and bio-product formation.

2. Lignin-degrading actinomycetes

- Lignin-degrading enzymes have been observed in five different species of *Streptomyces, Streptomyces antibioticus, S. Griseus, S. coelicolor, S. cyaneus,* and *S. lavendulae*.
- Peroxidase and polyphenol oxidase produced by the thermophilic Streptomyces isolates and mesophilic Streptomyces shows lignin breakdown.

3. Lignin-degrading fungi

- *Ganoderma applanatum* and *Heterobasidion annosum* that preferentially remove lignin without a substantial loss of cellulose and cause white-pocket or white-mottled type of rot.
- Other ascomycetes like *Rhizoctonia solani, Aspergillus nidulans, Podospora anserina, Neurospora crassa, Gaeumannomyces graminis var. tritici* and *Trichoderma reesei* have also been described to produce laccase and other lignocellulolytic enzymes.
- Wood-decaying fungi such as *Hypholoma* sp. *(Nematoloma* sp.*), Pleurotus sp*., and *Armillaria* are also capable of colonizing in soil in contact with wood debris and causing lignin degradation.
- Litter-decomposing fungi (e.g., *Stropharia rugosoannulata*) are able to grow on the straw that is usually favoured only by wood-decay fungi.
- *Agaricus bisporus* is also a litter-degrading fungus which secretes laccases and manganese peroxidases, and its ability to break down lignin and cellulose enables it to function as a typical white-rot fungus.
- Cyanobacteria- marine cyanobacterial species such as *Phormidium*, *Oscillatoria* and *Anabaena azollae* are able to degrade lignin in coir fibre.

10.7. Protein decomposition

Proteins are complex organic homopolymer, made up of basic amino acid units, containing nitrogen, phosphorus, and sometimes sulphur in addition to carbon, hydrogen and oxygen. They synthesized upon mRNA and sequence of amino acids in protein is control by it. During the course of decomposition, proteins are first hydrolysed (process proteolysis) to a number of intermediate peptides, collectively known as polypeptides. In this process, hydrolysis of peptide bond take place. The intermediate products so formed are then hydrolysed and broken down ultimately to individual amides and amino acids, or ammonia. The process of hydrolysis of nitrogenous organic compound into ammonia or ammonium carried out in water and soil is known as Aminization or ammonification, which is brought about by certain microorganism (bacteria and fungi), known as ammonifiers, including *Streptomyces, Bacillus, Clostridium, Pseudomonas,* etc. After ammonification, nutrification take place, and nitrite and nitrate are formed. The nitrates absorbed by plant root.

Natural hydrolysis of protein (uncatalyzed) requires hundreds of years. However, it is typically catalysed by cellular enzyme called proteases, but my also occur by intra-molecular digestion. Hydrolysis controlled by moisture, temperature and bacteria.

10.7.1. Microorganisms involved in the decomposition of protein

Protein decomposition commonly known as proteolysis, in which hydrolysis of peptide bonds takes place, carried out by proteolytic enzymes, bacteria are rich source of these enzymes. Beside bacteria, actinomycetes, fungi and protozoa are also participate. Protein gradually decomposed into smaller peptides, then into amides and amino acids by decomposition. These nitrogenous organic compounds latter on converted into ammonia or ammonium by ammonifying microorgamisms(bacteria), the process is known as ammonification.Example of ammonifying bacteria are *Bacillus, Clostridium, Proteus, Pseudomonas and Streptomyces.* After ammonification, nitrification take place. In this process, ammonia or ammonium compounds are converted into nitrates and nitrites by bacterium *Nitrosomonas* and *Nitrobacter*, respectively. The released nitrate absorbed by most of higher green plants, as source of nitrogen and now enter into plant metabolism and incorporated into biomass(immobilization).

10.8. Soil factors affecting the decomposition of organic matter

The most important factors that affect the rate of decomposition are-

1 **Soil temperature:** cold periods retard the organic matter decomposition and there will be more accumulation of organic matter on the top soil, compared to that of warm climates. The most suitable temperature is 30-40^{0}C for proper decomposition.

2 **Soil moisture:** near or slightly wetter than field capacity of moisture condition is most favourable for decomposition. About 60-75 % water holding capacity (WHC) is optimum. Moisture plays a critical role in determining the activity of microorganisms in decomposition.

3 **Soil pH:** slightly acidic pH (6-8) or neutral pH is required for optimum growth of most of microorganisms. For example, actinomycetes are more at pH 8 -10, Bacteria at 6 - 7 pH, algae at pH of 5.5 - 7.5, fungi at pH 4.0, protozoa at pH 5.4-7.8.

4 **Soil aeration:** good aeration increases the rate of decomposition by aerobic microorganisms. In the pores of soil, sufficient amount of oxygen is present which is required by aerobic flora. In water-logged soil conditions, where O_2 becomes a limiting factor, aerobic microorganisms will be absent, and only anaerobic microorganisms will grow and decompose the organic matter. Soil texture affects aeration and the later affects growth of microorganisms.

5 Soil inorganic mineral nutrients: the concentration of already available inorganic nutrients substances, also affects the rate of decomposition of organic matter in soil. Lack of nutrients, particularly N reduces microbial growth and it slows decomposition. Addition of N fertilizers (urea) increases the speed of decomposition.

6 In addition, after decomposition of humus, the nutrient elements N, P, K, Na, Mg, Ca, etc. are released in soil (process known as mineralization). Some amount of it, is taken up by the growing microorganisms and the remaining amount is available to plant growth. Plant absorbs these mineral elements which later on becomes the part of organic matter of plant (process known as Immobilization).

7 Nature of plant organic matter: composition, age of plants, and types of vegetation much affects the decomposition. It is fast in young, tender, and juicy material, but it is slow in plants organic matter with more cellulose, hemicelluloses and lignin content.

8 Soil texture: like all living organisms, the creatures of soil need oxygen to live. Oxygen comes from the air above the soil, so there must be a means for air to penetrate into the soil. Soil with a loose structure allows for ample spaces between soil particles for oxygen to collect. In such soils, organic matter will decompose faster by aerobic decomposes. Compacted or "tight" soils, such as soils with a high clay content, do not provide adequate space for air to collect, causing less biological activity and a slower organic matter break down.Clay soils have more humus which provide bettter nutients to microorganisms and plants.

10.9. Carbon assimilation and immobilization

The most important element in the biological realm and substance that serve as the cornerstone of the cell structure is carbon. It constitutes about 40-50% of all living organisms, yet the ultimate sourceof this carbon is the CO_2 that exist in a very low concentration, only 0.03%, in the earth's atmosphere. Carbon is a common constituent of all organic matter in plant and animal. This carbon is continually being fixed (assimilation) into organic form, by anabolic process, photosynthesis in chlorophyll containing plant under the influence of sunlight and once bound, this carbon becomes unavailable for use in the generation of new plant life. Therefore, it is essential for the carbonaceous materials to be decomposed and returned the CO_2 with other elements to the atmosphere for the survival of the higher organisms. The decomposition of plant and animal remains in soil constituents a basic biological process in that carbon is recirculated to the atmosphere as CO_2, nitrogen (N) is made available as NH_4 and NO_3 ,and other associated nutrient elements like P, S, Fe, Mn, Cu and Zn etc, appear in available forms for plants. In this process, part of the nutrient elements is assimilated by microorganisms and incorporated into microbial tissues (soil biomasses).

The conversion of organic forms of C, N, P and S into inorganic or mineral forms is called mineralization and the conversion of inorganic forms of these elements to their organic forms is known as immobilization.

The process of converting inorganic carbon substrate to protoplasmic organic carbon is known as assimilation. Under aerobic conditions 20-40% of the substrate carbon

is assimilated, the remainder is released as CO_2. Fungi are more efficient, in their metabolism, since they convert carbon into cell carbon as filaments and release less of CO_2, 30-40% of which is used to form new mycelium during the decomposition. Compared to fungi, bacteria are less efficient further, aerobic bacteria are less efficient than anaerobic bacteria.

During the decomposition of organic matter, three separate simultaneous processes participate. These are-

1. Plant and animal constituents disappear under the influence of enzymes
2. Synthesis of new microbial cells, so that proteins, polysaccharides and nucleic acids, typical of bacteria and fungi appear.
3. Certain end products of the breakdown are released, excreted into surroundings atmosphere. Where, they are either accumulate or to be further metabolized in living microorganism.

In immobilization, inorganic nutrients are taken up by soil microbes and become unavailable for plant uptake. Immobilization is therefore a biological process controlled by bacteria that fix inorganic atmospheric nitrogen, process known as biological nitrogen fixation and form amino acids ultimately and which is a biological-macromolecules of nitrogen or organic forms. This latter on forms protein by translation. Immobilization and mineralization are continuous processes that occur concurrently, for example the free inorganic atmospheric nitrogen is converted into fix organic nitrogen state by immobilization in organism and from a fix organic to an inorganic free nitrogen state by decay and mineralization of dead organism.

Whether, nitrogen is mineralized or immobilized depends on the C/N ratio of the plant residues. For example, incorporating materials high in carbon to nitrogen ratio, such as saw dust and straw, will stimulate soil microbial activity, increase demand for nitrogen, leading to immobilization. This is known as priming effect. In general plant residues entering the soil have too little nitrogen than the carbon in their cells for the soil microbial population to convert all of the carbon into their cells. If the C:N ratio of the decomposing plant material is above 30:1, the nitrogen demand increases, therefore, the

soil microbial population may take nitrogen in mineral form (e.g, nitrate) from soil. This mineral nitrogen is said to be immobilized. Furthermore, both microorganisms and plants complete for NH_{4+} and NO_{3-} during immobilization, and plants can easily become nitrogen deficient.

When the C:N ratio of organic matter falls below 25:1, microbial demand for mineral nitrogen is decreased. Further, decomposition of organic matter, result in simultaneous mineralization of nitrogen which is in excess that required by the microbial population for their growth.

When decomposition is virtually completed, soil mineral nitrogen will be higher than it was initially due to mineralization of the plant organic residue nitrogen.

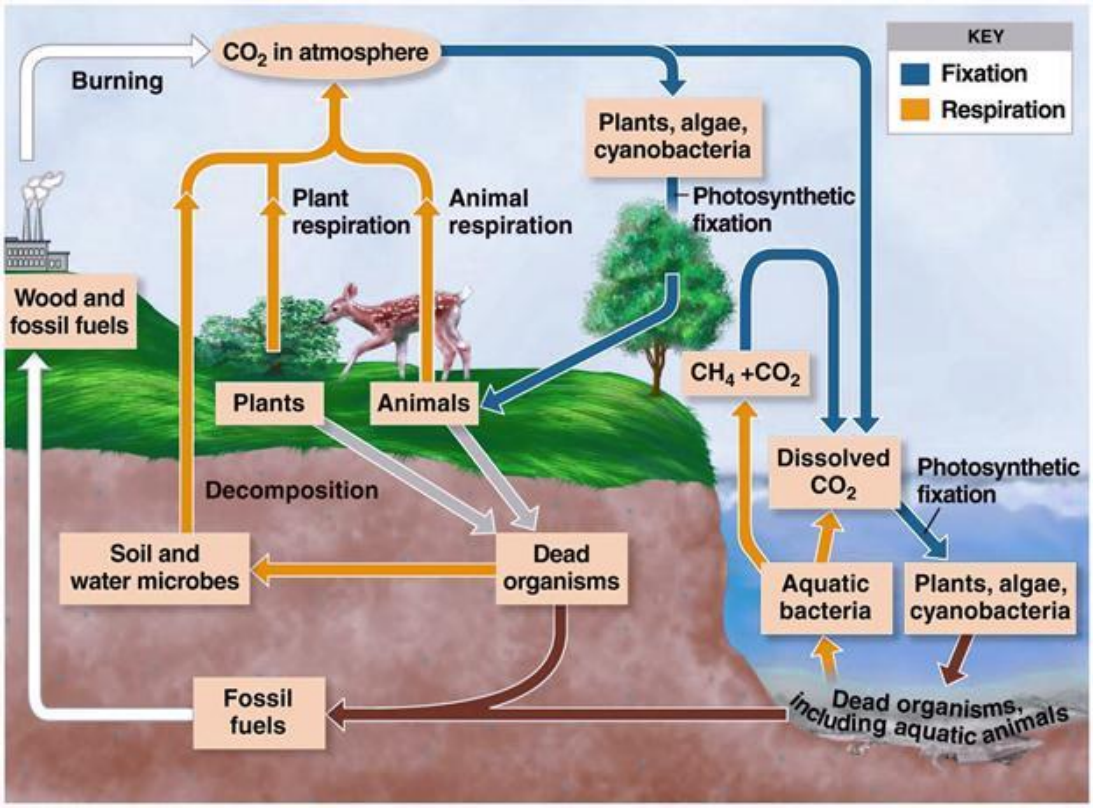

Fig. 10.8: carbon cycle

10.10. Soil factors affecting microbial community in soil

The major soil factors that influence the microbial community in soil are:

- Soil moisture
- Soil organic and inorganic chemical substances
- Soil organic matter
- Soil vegetation tye and its growth stages
- Soil regional and seasonal variation
- Soil root exudates
- Soil phsical factors

1. **Soil moisture:**

Soil Moisture is the water content of soil. The main source of soil water is rain water. The amount of soil water increases with increase in porosity of soil. Pore-size depends on soil texture, i.e., composition of sand, silt and clays. Moreover, soil moisture is affected through irrigation, drainage or management practices like tillage or crop rotation that enhance the intake and transmission of water in soil. Soil moisture needed for growth of microorganism.

2. **Soil organic and inorganic chemical substances:**

The chemicals are very important for microorganisms as these provide nutrition for their growth, activity and survival of microorganisms in ecologically deficient niches in soil. The chemical factors are gases, acids, micro- and macro-elements, clay minerals, etc. In the soil solution, gases (oxygen, methane and carbon dioxide) and microorganisms are dissolved.

However, the dissolved components are in constantly in shifting equilibrium with the solid phase, soil air, and moisture as well as with soil organisms, and plant root activity. It has been found that low potassium and high nitrogen favour cotton wilt by *Fusarium vasinfectum*.

3. **Soil pH**-Soil-borne fungi are sensitive to pH. As a result of pH range for vigour and growth, they are more destructive at acid and neutral than at alkaline conditions. For example, *Plasmodiophora brassicae* favours best in acid soil, and the disease produced

by it is uncommon or mild in soil of pH more than 7.5. Acidophilic natives of *Trichoderma viride* increased in soil on addition of sulphur, carbon disulphide, and methyl bromide due to lowering down of pH to about 4.0

4. Soil organic matter:

The dead organic material of plant and animal origin, and excretory product of living beings constitute the total soil organic matter, which later is subjected to microbial colonization and decomposition. However, upon incorporation of green manures, crop residues, etc. in soil, the community size of microorganisms gets increased.

At the same time, application of these organic matter, alters the composition of soil microflora, microfauna, and relative dominance of antagonistic bacteria, actinomycetes, fungi, amoebae, etc.

5. Soil vegetation type and its growth stage:

The dominance of one or the other group of microorganisms in particular soil is related to the type of vegetation and growth stage of a plant growing in the particular soil.

This selective action of plants is attributed to microbial growth response, either to specific root-exudates or chemical constituents of sloughed-off tissues that undergo in decomposition.

6. Soil regional and seasonal variation:

The amount of plant available nutrients is governed by the number and activity of microorganisms. They remain in constant dynamic state in soil where microbial community is greatly influenced by physio -chemical and biological factors. Changes in microbial community are known in soils of tropical, sub-tropical and temperate regions.

Maximum number of fungal taxa and average number of bacteria and fungi (per gram soil) were recorded in rainy season, and minimum in summer season in banj-oak and chir-pine forest soils of Himalaya.

Extreme cold and heat are unsuitable for normal decomposition of organic matter. In cooler parts, decomposition rate increases in spring, whereas, warmer and rainy conditions favours the activity of microorganisms.

7. Soil root exudates:

In the soil, where plants are growing, the root exudates also effect the distribution, density and activity of soil microorganisms. Root exudates and sloughed off material of root surfaces provide an abundant source of energy and nutrients, and thus directly or indirectly influence the quality as well as quantity of soil microorganisms in the rhizosphere region. Root exudates contains sugars, organic acids, amino acids, sterols, vitamins and other growth factors which have the profound effect on soil microbes.

8. Soil physical factors:

The various soil physical factors are temperature (high temp. denatures protein and enzyme), oxygen level, pH, osmotic pressure, and radiation.

10.11. Summary

Organic matter is one of the most important attributes of soils. It decreases soil bulk density, and increases its water retention capacity, porosity of soil ,cation exchange capacity and buffering capacity. Organic matter decompositio is central to the biogeochemical cycling of many plant essential nutrients and support the growth of huge biodiversity present in soils.

Soil organic matter is comprised of both, living and non-living components. The living component includes soil macro- and micro-fauna, and soil microbial communities, which may be active or dormant. The non-living portion of soil organic matter is derived from dead plant and animal parts mainly. Plant residues are the major substrate for soil organic matter formation. Soil organic matter is composed of these residues at various stages of decomposition, and also contains large amount of dead fauna in some cases and microbial biomass, and products of microbial synthesis, animal waste, human and animal excreta.

Earthworms, mites, ants, etc., take part in the initial stages of physical (dead animals and plants found over surface of soil) decomposition. The smaller pieces of litter or the fragmented waste they release is called **detritus**. Earthworms, beetles, snails, millipedes

etc., are known as **detrivores** that consume the detritus or decaying organic matter. After that, decomposers come into action.

The dead fallen leaves in forest upon soil surface forms litter which later on forms soluble organic matter by decomposition.

When a plant, animal or insect dies, it breaks into tiny pieces and eventually, these small pieces become part of the soil. This process is known as decomposition. It is the process where organic substances break down into simpler matter. Bodies of any living organism start decomposing shortly after their death. Animals such as worms help in decomposing these organic matter.

The decomposition (stabilization) of surface organic matter, such as vegetable matter, animal dung, house waste and other organic refuse in anaerobic condition by microorganism to form fine stable organic material for use in soil amendments in farming (organic farming) to increase soil fertility and crop production is known as compost and process as composting. There are two processes, which yields compost-

1. Anaerobic process (reduction process)- take place in the absence of oxygen, less heat is generated than aerobic process. This is disadvantages as high heat or temperature is required for destruction of pathogen and parasites present in organic matter.

2.Aerobic process (oxidative process)- decomposition and stabilization take place in the presence of oxygen and more heat generated which kills the pathogen and parasite.

The final product of aerobic decomposition is co_2 and that of anaerobic decomposition are hydrogen, ethyl alcohol (CH_4), various organic acids and carbon dioxide (CO_2).

Biogas is a mixture of gases, primarily consisting of methane (50-76%), CO_2, H_2S, water vapour and trace amounts of other gases which are produced by anaerobic decomposition of organic matter, such as agricultural waste, municipal waste, plant material, sewage, animal dung, waste water and food waste, etc. It is a renewable source of energy.

Soil organisms use organic matter as a source of energy and food. The process of decomposition is initially fast, but slows down considerably as the supply of readily decomposable organic matter gets exhausted.

Sugars, water-soluble nitrogenous compounds, amino acids, lipids, starchs and some of the hemicelluloses are decomposed first at rapid rate. While, insoluble compounds such as cellulose, hemicelluloses, lignin, proteins etc. ,which forms the major portion of organic matter are decomposed later slowly. Thus, the organic matter added to the soil is converted by oxidative decomposition to simpler inorganic substances (mineral nutrients), which are made available in latter stages for plant growth and the residue remains is transformed into amorphous organic substance known as humus. Humus make soil porous which allow water and air to penetrate deep underground, and improve soil fertility, water retention, CEC (carbon exchange capacity), nutrient availability and soil health.

Organic matter decomposition serves three functions for the microflora-

(1) providing energy for cell growth

(2) supplying carbon for the formation of cell material

(3) providing other mineral nutrients elements

Decomposition of organic matter take place by different microorganism, mainly fungi, bacteria, actnomycetes, protozoans, nematodes,etc. .

Different constituent of organic matter decomposed or hydrolized mostly by different types of enzymes , such as- cellulose by cellulase, cellubiose by cellubiase, hemicellulose by hemicellulase,chitin by chitinase, pectin by pectinase, protein by proteinase, and lignin by peroxidase and oxidase.These different enzymes synthesized by different microorganism organisms.

Number of soil factors affecting the organic matter decomposition in soil.These are-aeration/oxygen, moisture, soil texture, humus, temperature, inorganic minerals, and also particle size of organic matter.

The activity of microorganisms also affected by number of soil factors. These are- moisture, organic and inorganic chemicals, organic matter, vegetation type, root exudates, and phsical factors.

Fixation of CO2 by photosynthetic plants in organic form is known as cabon assimilation (immobiligation of carbon), and decomposition of organic matter, which releases the minerals is known as mineralization. Both are important for ecosystem.

10.12. Terminal questions

Q.1. What is organic matter? Describe its various components.

Answer----------

Q.2. Describe the process of decomposition of organic matter in brief.

Answer----------

Q.3. Explain the decomposition process of cellulose and which microorganisms involved in this process.

Answer----------

Q.4. Describe the decomposition of chitin in brief.

Answer----------

Q.5. What is carbon assimilation and immobilization?

Answer----------

Q.6. Describe the various factors affecting decomposition of organic matter.

Answer----------

Q.7. What are various factors affecting the microbial community in soil.

Answer----------

Q.8. Write short notes on-

a) **Hemicelluloses decomposition**

b) **Protein decomposition**

Answer----------

10.13. Further suggested readings

15. Biofertilizers And Pesticides- H.C. Lakshman

16. Manures, Fertilizers And Pesticides- Amiitava Rakshit, Priyankar Raha And Nirmal DeFertilizers A Text Book- Ranjan Kumar Basak

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19. K Vijaya Ramesh , Environmental Microbiology, MJP Publication-2019.

20. P.K. Mahapathra, A Textbook of Environmental Microbiology, I K International Publishing House Pvt. Ltd-213.

21. Barbara Kołwzan et al., Introduction To Environmental Microbiology, academic teachers from Wroclaw University of Technology, Poland

Unit-11: Microbial Diseases

Contents

11.1 Introduction

Microbial diseases are caused by microorganisms, such as bacteria, viruses, fungi, and parasitic protozoa, and they pose significant health threats to humans, animals and plants. Some microbes are useful in day-to-day life, while others are harmful for human health. The harmful disease causing microorganisms are collectively known as **pathogens.**

These microscopic invaders can enter the body through various routes and disrupt normal bodily functions, leading to illness. They can cause a wide range of infections, including respiratory infections, gastrointestinal illnesses, sexually transmitted diseases, food poisoning and more. Microbial diseases can spread rapidly through person-to-person contact, intake of contaminated food or water, insect bites, or other means. Prevention measures such as vaccination, proper hygiene practices, and sanitation are crucial in controlling the spread of these diseases and protecting public health.

The bacteria are prokaryotic organism, i.e., with true nucleus. The bacterial cell structure have slime layer (outer to cell wall), cell wall, plasma mwmberane, cytoplasm, bacterial chromosome, mesosome (infolding of plasma membrane), plasmids (extra chromosomal DNA) , ribosome (70S,for protein synthesis),flagella (organ of locomotion in motile form) ,bacterial chlorophyll (in photosynthetic bacterium) and pili (organ of attachment). Howerever,they lack membrane bound cell organelles.

The main source of bacterial infection in human is by consumption of contaminated food, water and air. They cause cholera, tuberclosis, salmonellosis, dysentery, etc. The most common types of dysentery are the **amoebic dysentery or intestinal amoebiasis**, is caused by a single-

celled microscopic parasite living in the large bowel and the other type **bacillary dysentery** is caused by invasive bacteria. These infections include shigella, campylobacter, *E. coli* and *salmonella* species of bacteria.

Virus is a cellular in nature and has very simple structure, and made up of nucleic acid (either DNA or RNA, but not both) and protein coat. The protein coat is protective in nature, while, nucleic acid is infective in nature. They are considered as connecting link between the living and non-living organisms. The diseases caused by them are – influenza, AIDS, Covid-19, rabies, measles, hepatitis, Ebola virus disease, Zika fever or Zika virus disease, etc. Viral diseases are transmitted through various methods-by contact, by blood transfusion, by insects' vector, by sexual, by medical needle, etc.

Food borne diseases are caused by taking food and water having pathogenic microorganisms. These are Botulism, diarrhea, gastroenteritis, E-coli infection, salmonellosis, campylobacteriosis, etc.

Mycotoxin food poisoning caused by taking food having mycotoxin. These toxins are naturally occurring secondary metabolite produced by various fungi, and is capable of causing disease and death in both, humans and animals. These microscopic fungi growing on various food products, such as-grains of various plants- maize, cotton seeds, peanuts, coffee beans (Aspergillus flavus and parasiticus, produces aflatoxin which is also carcinogenic in nature) and up on animal fodder (egotism, caused by ascomycetus fungus Claviceps purpuria which produces hard, dark and elogated sclerotium in wheat, barley, oat, rye).

Objectives:

After study of the course this unit, you will be able to known:

- the different types of bacterial and viral diseases
- the different types of food born disease
- the different diseases- tuberculosis, cholera, AIDS and Rabies.

11.2. Structure of Bacteria

Bacteria are single-celled microorganisms that are classified as prokaryotes. They lacking a membrane-bound nucleus and other membrane-bound cell organelles found in eukaryotic cells. The bacteria possess a well-defined simple structure that allows them to carry out all essential

life processes. The typical structure of a bacterium consists of several components. The cell envelope encompasses the cell and includes the cell wall, cell membrane, and sometimes an outer membrane. The cell wall provides shape, rigidity, and protection to the bacterium. Chemically, it is made up of polysaccharide called mucopeptide. The mucopeptide is made up of glucosamine and N-acetyl muramic acid. The bacteria are of two types, gram-positive and gram-negative. The cell wall of gram-positive bacteria is thick and contains fewer lipids, whereas of gram-negative bacteria is thin and have more lipids.

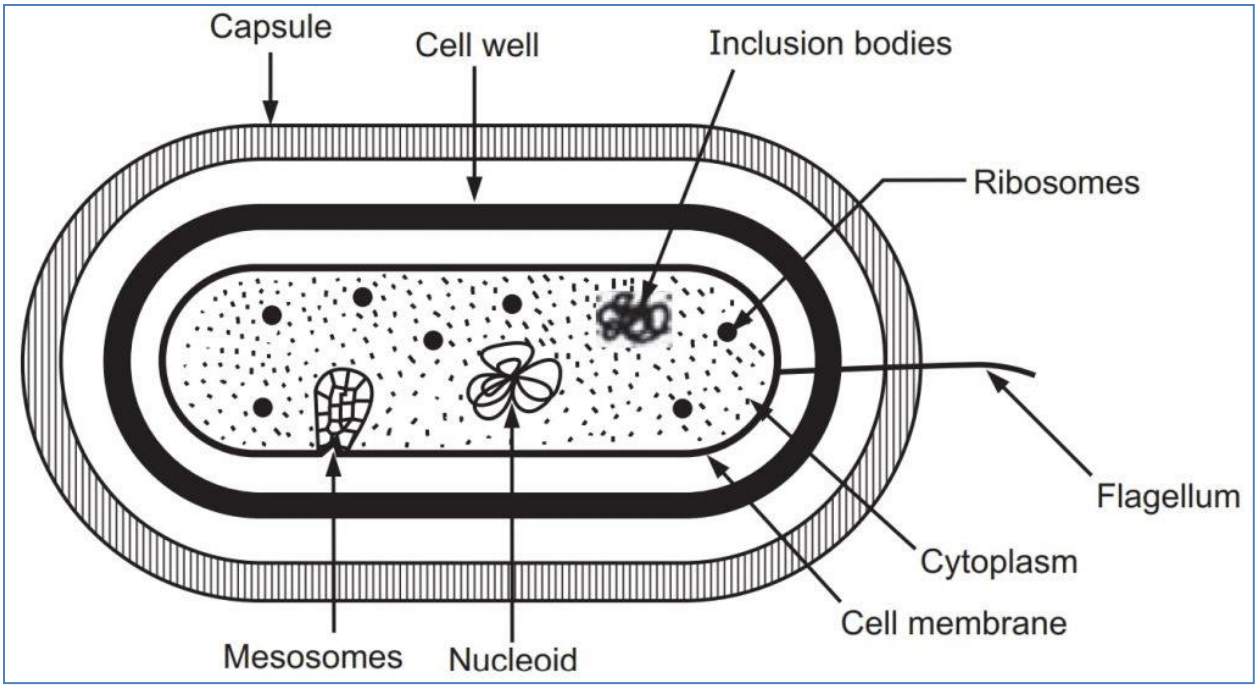
External to cell wall is a gelatinous slime layer. This slime layer is secreted by protoplast of the cell. In pathogenic bacteria, the slime layer becomes thick and called capsule. The capsule protects the bacteria from desiccation, viral attack and antibiotics. The capsulated bacteria are generally none flagellated.

Inside the cell envelope, the cytoplasm contains the genetic material and essential cellular machinery. Bacterial DNA is typically organized into a single circular chromosome, which carries the genes responsible for the bacterium's characteristics and functions. In addition to the chromosome, bacteria may contain plasmids, small circular pieces of DNA that can confer additional traits like antibiotic resistance. Ribosome (70S) responsible for protein synthesis, which scattered throughout the cytoplasm. Bacterial ribosomes are smaller than eukaryotic ribosome’s and have a different composition, making them potential targets for antibiotics.

The respiratory enzymes are found on the inner surface of plasma membrane. In some gram-positive bacteria infoldings are present. These are called mesosomes, which contains respiratory enzymes. The function of mesosome is in respiration, synthesis of wall and secretion of intracellular substances from inside to outside the cell. Long thread like flagella is attached to the cell membrane, which helps in movement of bacterial cell in motile bacterium.

In some gram-negative bacteria, straight hair like minute appendages is found. These are called pili, which helps in the attachment of bacterial cell to the host cell. The extra chromosomal genetic material found in bacterial cell known as plasmid or F factor or fertility factor. This can automatically reproduce. When plasmid gets integrated with bacterial DNA, it is called episome.

On the basis of number and mode of attachment of flagella, the bacteria are of following types:-



Source: Ultra Structure of Bacteria - Solution Parmacy (solutionpharmacy.in)

Fig.11.1 Structure of a bacterial cell.

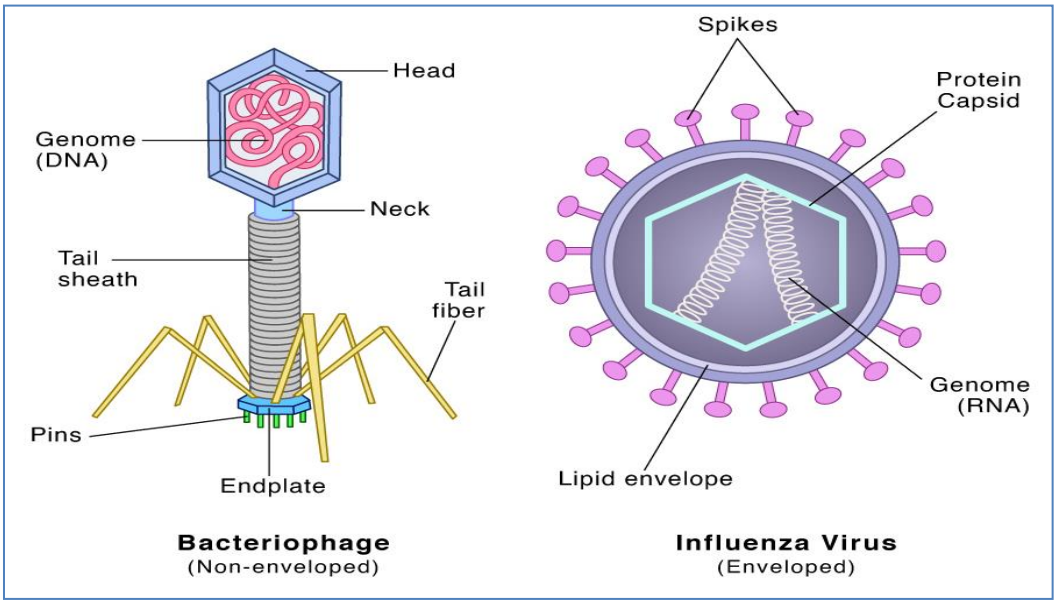
- **Monotrichous:** Single flagellum is attached to one end of bacterial cell.
- **Amphitrichous:** One flagellum is at each pole of bacterial cell.
- **Cephalotrichous:** Two or more flagella are present in bunch at one pole of the bacterial cell.
- **Lophotrichous:** Two or more flagella are present at both the poles of the bacterial cell.
- **Peritrichous:** Large number of flagella are evenly distributed all over the surface of bacterial cell.
- **Atrichous:** Bacterial cell lack flagella are called atrichous.

11.3. Structure of virus

Viruses are infectious agents that are much smaller and simpler than bacteria. The study of viruses is known as virology, a subspeciality of microbiology. Viruses exist in the form of independent particles or *virions*, consisting of (i) the genetic material, i.e. long molecules of DNA

or RNA that encode the structure of the proteins by which the virus acts; (ii) a protein coat, the capsid, which surrounds and protects the genetic material.

The structure of a virus can be described in several key components.



Source: Virus – Definition, Parts, Structure, Characteristics, Diagram (sciencefacts.net)

Fig. 11.2: Structure of Virus

Capsid: The capsid is the protein shell, which surrounds the viral genetic material. It is composed of repeating subunits called capsomeres, which come together to form a symmetrical structure. The Capsid provides protection to the viral genome and helps in the recognition and attachment to host cells.

Genetic Material: Viruses can have either DNA or RNA as their genetic material, but not both. *It is infectious in nature*. The genetic material carries the instructions necessary for the virus to replicate inside a host cell. It can be single-stranded or double-stranded, linear or circular, depending on the type of virus.

Envelope (optional): Some viruses have an additional outer layer called the envelope. The envelope is derived from the host cell's membrane and contains viral proteins. It helps the virus in evading the host's immune system and facilitates entry into host cells. Enveloped viruses are more fragile and susceptible to environmental conditions than non-enveloped viruses.

Spike Proteins: Many viruses, especially those with envelopes, have spike proteins on their surface. These proteins play a crucial role in host cell recognition and attachment, allowing the virus to bind to specific receptors on the host cell surface. Spike proteins are particularly important for viral entry and are often targeted by the host immune response.

Tail Fibers or Tail Sheath (bateriophage only): Bateriophage is viruses that infect bacteria. They possess a tail structure composed of tail fibers or a tail sheath, along with a baseplate. These components allow the bateriophage to attach to specific receptors on the bacterial cell surface and inject its genetic material into the host.

A virus is microscopic infectious agent that replicates only inside the living cells of an organism known as host. Viruses transmission take place through various pathways. Viruses are often transmitted from plant to plant by insects known as vectors that feed on plant sap, such as aphids; and viruses in animals can be carried by blood sucking insects. Influenza viruses spread in the air by coughing and sneezing. Norovirus and rotavirus, common causes of viral gastroenteritis, are transmitted by the fecal-oral route, passed by hand-to-mouth contact or in food or water. Some viruses including those that cause AIDS, HPV infection and viral hepatitis, evade these immune responses and result in chronic infections.

Microbes can also cause:

- Acute infections, which are short-lived.
- Chronic infections, which can last for weeks, months, or a lifetime.

The virus is called the link between living and non living organism because it has special characteristics such as

Non-living or Inanimate characters of Virus:

- They have no complete cellular structure (No cell membrane, cell wall).
- Viruses have no independent existence. They are active only inside the host cell.
- They lack cellular metabolism.
- They can be crystallized like simple chemical substances. The living organism cannot be crystallized.
- They can be precipitated like chemical substances.

Living or Animate characters of Virus:

- They have definite shape and have genetic material either DNA or RNA.
- All viruses attack specific host and causes disease.
- They show mutation.
- Virus show irritability and respond to environmental conditions.
- Virus multiplies inside the host.

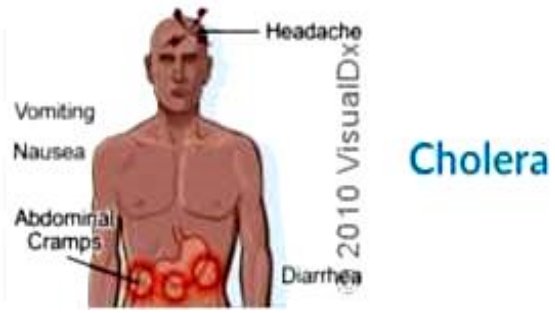
Thus, viruses have some characters of living organism and some characters of non living organism thus, form a bridge between non living and living organisms. They are non living when, free existing and living when, present inside the host energy provided by host. Thus, viruses are exceptionally simple microbes and complex chemicals.

11.4. Bacterial Diseases

Bacterial diseases are illnesses caused by pathogenic bacteria that invade the body and disrupt its normal functioning. Bacteria can cause a wide range of diseases, affecting various organ systems and leading to different symptoms.

- **Cholera:**

Cholera is an acute diarrheal disease caused by the bacterium Vibrio cholerae. It is typically transmitted through contaminated water or food. Cholera infection can lead to severe dehydration and electrolyte imbalance, resulting in profuse watery diarrhea, vomiting, and muscle cramps. Cholera hase a significant global health concern, particularly in areas with inadequate sanitation and hygiene practices. Cholera infection can range from mild to severe. The majority of infected individuals exhibit no or mild symptoms. However, in severe cases, the disease can progress rapidly and cause profuse watery diarrhea, vomiting, and dehydration. Severe dehydration can lead to electrolyte imbalances, muscle cramps, and shock, potentially resulting in death if left untreated. Cholera bacteria are typically found in fecally contaminated water sources, and transmission occurs through the consumption of contaminated food or water. Contaminated seafood, raw or undercooked, is another potential source of infection. Cholera is a major public health concern in regions with inadequate access to clean water, sanitation, and hygiene facilities.



The immediate treatment of the disease is the oral rehydration therapy with NaCl plus glucose to estimate water uptake by the intestine. The antibiotics of choice are tetracycline and ciprofloxacin. The killed vaccine of cholera can be taken as prophylactic control measure.

Incubaton Period

The incubation period varies from less than 24 hours to about five days. The clinical illness may begin slowly with mild diarrhea and vomiting in 1-3 days or abruptly with sudden massive diarrhea.

Pathogenesis- Natural infection with cholera occurs in human and not in animals. A number of animal models have been developed which helped in understanding the pathogenic mechanisms in cholera.

In human infection, the Vibrios enter orally through contaminated water or food. Vibrios are highly susceptible to acid and gastric acidity provides an effective barrier against small doses of cholera Vibrios. In the small intestine vibrios are enabling to cross the protective layer of mucus and reach the epithelial cells by chemotaxis, motility, mucinase and other proteolytic euzymes. A hemagglutinin protease (formerly known as cholera lectin) cleaves mucus and fibronectin. It also helps in realeasing vibrios bound to bowel mucosa, facilitating their spread to other parts of the intestine and also their fecal shedding. Adhesion to the epithelial surface and colonization may be facilitated by special fimbria such as the toxin coregulted pilus (TCP). Throughout the course of infection, the vibrios remain attached to the epithelium, but do not damage or invade the cells. The changes induced are biochemical rather than histological.

Epidemiology

Cholera is an exclusively human disease. Infection originates from the patient or the carrier. Carrier may be incubatory, convalescent healthy or chronic.

- Incubatory carriers shed vibrios only during the brief incubation period of 1-5 days.
- Convalescents may excrete then for 2-3 weeks.
- The healthy carrier who has had subclinical infection usually sheds the vibrios for less than 10 days.
- The chronic carriers continue to be active for months or years, the longest duration recorded being 10 years.

Infection is acquired through fecally contaminated water or food. Direct person-to-person spread by contact may not be common, but hand contamination of stored drinking water has been shown to be an important method of domestic spread of infection. Large scale movement of persons as occurs during fairs and festivals has traditionally been associated with the spread of cholera.

Cholera is both, an epidemic and endemic disease. The epidemicity and endemicity of a disease will depend on the characteristics of the agent, and those of the system (environment). Characteristics of the agent which influence its distribution include its ability to survive in a given environment, its virulence, the average number of organisms required to cause infection, etc. Characteristics of the system, which affect the distribution of the agent include the number of susceptible, and the opportunities it provides for transmission of the infection.

Epidemics of cholera are characteristically abrupt and often create an acute public health problem. They have a high potential to spread fast and cause deaths. The epidemic reaches a peak and subsides gradually as the “force of infection” declines. Often-times, by the time control measures are instituted the epidemic has already reached its peak and is waning. Thus cholera epidemic in a community is self-limited.

The “force of infection” is composed of 2 components, namely the force of infection through water and the force of infection through contacts. It is well-known that the elimination of contaminated water does not immediately bring an outbreak to an end, but a so-called “tail” of the epidemic is produced. This is due to the continuation of transmission through contacts.

In areas where cholera is endemic it does not show a stable endemicity like typhoid fever. It undergoes seasonal fluctuations as well as epidemic outbreaks. The seasonal variation differs between countries and even between regions of the same country.

Health Education

The most effective prophylactic measure is perhaps health education. It should be directed mainly to (a) the effectiveness and simplicity of oral rehydration therapy (b) the benefits of early reporting for prompt treatment (c) food hygiene practices (d) hand washing after defecation and before eating, and (e) the benefit of cooked, hot foods and safe water. Since cholera is mainly a disease of the poor and ignorant persons, these groups should be tackled first.

Diarrhoeal Diseases Control Programme

During the year 1980-81, strategy of the National Cholera Control Programme has undergone changes. It is now termed as Diarrhoeal Diseases Control Programme. Oral Rehydratin Therapy Programme was started in 1986-87 in a phased manner. The main objective of the programme is to prevent diarrhea associated deaths in children due to dehydration. The training programme and health education material highlight the management of diarrhea in children, including increased intake of home available fluids and breast feeding. ORS is promoted as first line of treatment. In the child Survival and Safe Motherhood programme in districts, ORS is being supplied as a part of the sub-centre kits.

- **Tuberculosis (TB)**

Tuberculosis is a contagious bacterial infection caused by the bacterium Mycobacterium tuberculosis. It primarily affects the lungs but can also spread to other parts of the body. Pulmonary tuberculosis the most important form of different types of tuberculosis which affects the man. It can also affects intestine, meninges, bones and joints, lymph glands, skin and other tissues of the body. The diseases also affect animals like cattle, this is known as ***"bovine tuberculosis"*** which may sometimes be communicated to man.

Mycobacterium is slender rods that sometimes show branching filamentous forms resembling fungal mycelium. In liquid cultures they form a mold like pellicle. Hence the name "mycobacterium" meaning fungus like bacteria is given. They do not stain readily, but once stained resist decolourisation with dilute mineral acids. Mycobacterium is therefore called acid fast bacilli, or AFB. They are aerobic, nonmotile, noncapsulated and nonsporing bacterium. Growth is generally slow. The genus includes obligate parasites opportunistic pathogens and saprophytes.

The first member of this genus to be identified was the leptra bacillus discovered by Hansen in1868. Koch (1882) isolated the mammalian tubercle bacillus and proved its causative role in tuberculosis by satisfying Koch's postulates.

Tuberculosis in humans was subsequently shown to be caused by two types of bacillus – the human and bovine types, designated *mycobacterium tuberculosis* and *mycobacterium bovis* respectively.

Fig.11.3 Tuberculosis infection in lungs

Symptoms include persistent cough, chest pain, fatigue, weight loss, and night sweats. The infection typically progresses in several stages, which can vary in duration and severity. Here are the primary stages in the progression of tuberculosis:

Latent TB Infection: In this stage, individuals are infected with M. tuberculosis, but the bacteria are in an inactive state and do not cause any symptoms. Latent TB infection is not contagious, and most people with this stage of infection do not feel sick or show any signs. However, they have the potential to develop active TB disease in the future if their immune system weakens.

Primary TB Infection: When a person with a latent TB infection, experiences a weakened immune system, the bacteria can become active and cause primary TB infection. This usually occurs soon after the initial infection. In primary TB, the bacteria multiply rapidly and spread within the lungs, leading to the formation of small, localized areas of infection called granulomas. Symptoms may include a mild cough, fatigue, fever, night sweats, and weight loss.

Latent TB Reactivation: In some cases, the primary infection does not progress to active disease but remains dormant in the body. However, it can reactivate later in life if the immune system becomes compromised, such as due to aging, malnutrition, HIV infection, or certain medications. Reactivation TB often affects the lungs, but it can also spread to other organs and tissues.

Active TB Disease: Active TB disease occurs when the bacteria overcome the immune system's defenses and multiply unchecked. This stage is characterized by the presence of clinical symptoms and signs of illness. Active TB can affect the lungs (pulmonary TB) or other parts of the body (extrapulmonary TB). Common symptoms include a persistent cough, chest pain, coughing up blood, fatigue, fever, night sweats, and unintentional weight loss. If left untreated, active TB can lead to severe complications and even death.

Morphology

M. tuberculosis is a straight or slightly curve rod, about $3\mu m \times 0.3\mu m$, occurring singly, in pairs as small clumps. The size depends on conditions of growth and long filamentous club shaped and branching forms may be sometimes seen. M. bovis is usually straighter, shorter and stouter.

Mode of transmission

Tuberculosis is transmitted mainly by droplet infection and droplet nuclei generated by sputum of positive patients with pulmonary tuberculosis. Coughing generates the largest number of droplets of all sizes. Tuberculosis is not transmitted by fomites, such as dishes and other articles used by the patients. Patients with extra pulmonary tuberculosis or smear negative tuberculosis constitute a minimal hazard for transmission of infection.

Incubation Period: The time from receipt of infection to the development of a positive tuberculin test, ranges 3 to 6 weeks, and thereafter, the development of diseases depends upon the closeness of contact, extent of the disease and sputum costiveness of the source case (dose of infection) and host parasite relationship. Thus the incubation period may be weeks, months or years.

Classification of Tuberculosis

Depending on the time of infection and the type of response, tuberculosis may be classified as;

(1) Primary tuberculosis

(2) Post primary tuberculosis

Primary Tuberculosis- Primary tuberculosis is the first infection by tubercle bacilli in a host. Which an individual acquires by inhalation of air droplets and the bacteria are phagocytosed by macrophages inside the lungs forming small, hard nodules called tubercles (the characteristics of tuberculosis). In endemic countries like India this usually occurs in young children. In them the bacilli engulfed by alveolar macrophages multiply and give rise to a subpleural focus of tuberculous pneumonia, commonly located in the lower lobe or the lower part of the upper lobe (Ghon focus). The hilar lymph nodes are involved. The Ghon focus together with the enlarged hilar lymph node constitutes the "primary complex"

Post-primary Tuberculosis

The Post-primary (secondary or adult) type of tuberculosis is due to reactivation of latent infection (post-primary progression, endogenous reactivation) or exogenous reinfection and differs from the primary type in many respects. It affects mainly the upper lobes of the lungs, the lesions undergoing necrosis and tissues destruction, spread of pathogen to other parts of the body and ultimately cause the death.

The national Tubeculosis Programne (NTP)- The national tuberculosis programme has been in operation since 1962. It is essentially a permanent country wide programme, integrated with the general health services at both, the rural and urban levels. The long-term of the NTP is to reduce the problem of tuberculosis in the community, sufficiently and quickly to the level, where it causes no public health problem.

The District Tuberculosis programme

The district tuberculosis programme (DTP) is the backbone of the national tuberculosis programme. It was evolved by the national tuberculosis institute, Bangalore, and was accepted by the Government of India for implementation, which started in 1962. The district tuberculosis centre (DTC) is the nucleus of the DTP. The function of the DTC is to plan, organize and implement the DTP, in the entire district, in association with general health services.

Prevention and control of tuberculosis needs rapid specific therapy. In many countries, individuals particularly children are vaccinated with BCG (bacilli calmette-Guerin) vaccine.

- **Lyme disease:**

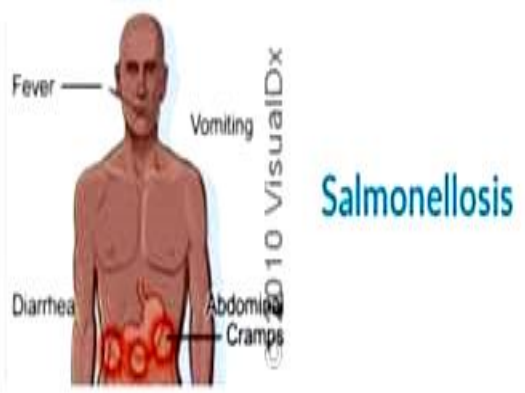
  Lyme disease is caused by the bacterium Borrelia burgdorferi, transmitted to humans through the bite of infected black-legged ticks. It primarily affects the skin, joints, and nervous system. Symptoms include a characteristic "bull's eye" rash, fatigue, fever, headache, muscle and joint aches, and neurological problems if left untreated.

- **Staphylococcal Infections:**

  Staphylococcus aureus is a bacterium commonly found on the skin and in the nasal passages. It can cause various infections, including skin infections (such as boils and impetigo), wound infections, urinary tract infections, and bloodstream infections. Methicillin-resistant Staphylococcus aureus (MRSA) is a strain of bacteria that is resistant to many antibiotics, making it more challenging to treat.

- **Salmonellosis:**

  Salmonella bacteria are a common cause of foodborne illness. Ingestion of contaminated food or water contaminated with Salmonella can lead to symptoms such as diarrhea, abdominal cramps, fever, and vomiting. Severe cases may require medical intervention, especially in young children, the elderly, or individuals with weakened immune systems.



11.5 Viral Diseases

Viruses can cause a wide range of diseases in humans, animals, and plants.

- **Influenza:**

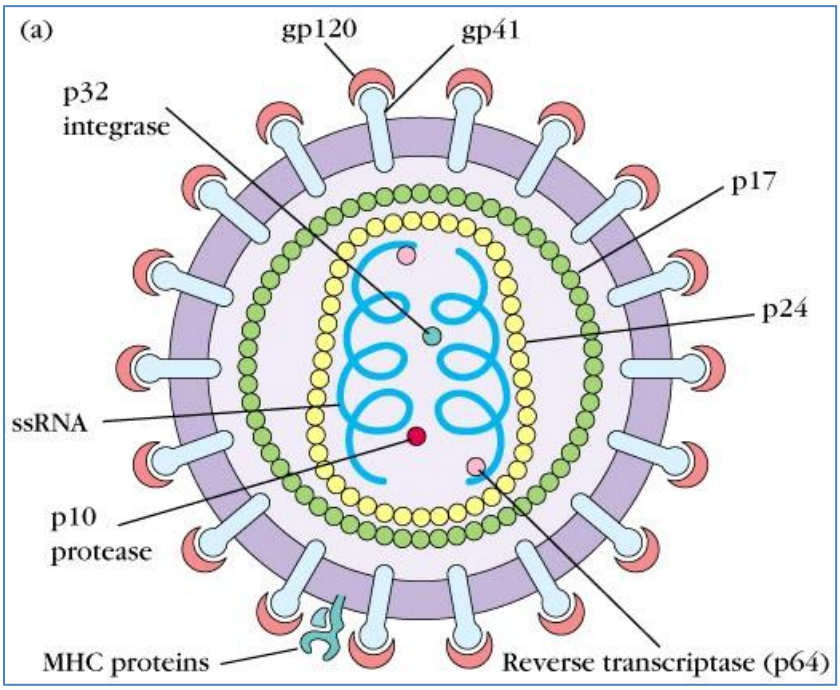
Influenza**,** commonly known as the flu, is caused by the influenza virus. It is a highly contagious respiratory illness characterized by symptoms such as fever, cough, sore throat, body aches, fatigue, and sometimes respiratory complications.

- COVID-19:

  COVID-19 is a disease caused by the novel coronavirus SARS-CoV-2. It emerged in late 2019 and quickly spread worldwide. COVID-19 primarily affects the respiratory system and can cause symptoms ranging from mild cold-like symptoms to severe pneumonia and acute respiratory distress syndrome (ARDS).

- **AIDS (acquired immunodeficiency syndrome)**

  Human immunodeficiency virus (HIV) is a retrovirus that attacks the immune system. If left untreated, it can lead to acquired immunodeficiency syndrome (AIDS). Human immunodeficiency virus or HIV is the virus that causes AIDS. The emergence and pandemic spread of the acquired immuno deficiency syndrome (AIDS) have posed the greatest challenge to public health in modern times. HIV is a virus that attacks cells on the immune system (the body's natural defence against illness). HIV destroys an important kind of the cell in immune system (called T cell). The diagrammatic representation of AIDS Virus (HIV) is as shown below.



Source: Structure of HIV (ucsb.edu)

Fig.11.4 Structure of HIV

The HIV virus transmitted from following ways. HIV is spread only by three modes-

1. sexual contact with infected person (heterosexual or homosexual),

2. by blood and blood products

3.from infected mother to babies (intrapartum, perinatal, postnatal).

There is no evidence of HIV transmission by other means including casual contact or through insects.

- **Vertical transmission-** HIV can be spread to babies born to or breastfed by mother infected with the virus.
- **Sexual transmission-** In adults and adolescents, HIV is spread most commonly by sexual contact with an infected partner.
- HIV may also spread through contact with infected blood.
- HIV is frequently spread by sharing needles, syringes, or drug use equipment with some one who is infected with this virus. Transmission from patient to health care worker or vice versa, through accidental sticks with contaminated needles or other medical instruments is rare.

When the virus was identified, it was called lymphadenopathy associated virus (LAV) by the French scientists researchers, and called human T-cell lymphotropic virus in USA. The international committee on the taxonomy gave it a new name, human immuno deficiency virus (HIV) in May, 1986.

Blood Contact

AIDS is also transmitted by contaminated blood- transfusion of whole blood cells, and platelets. There is no evidence that transmission ever occurred through blood products such as albumin, immune-globulins. Contaminated blood is highly infective when introduced in large quantities directly in to the blood stream. The risk of contracting HIV infection from transfusion of a unit of infected blood is estimated to be over 95 percent. Since the likelihood of HIV transmission through blood, depends on the “dose” of virus injected. The risk of getting infected through a contaminated needle, syringe or any other skin-piercing instrument is very much lower than the transfusion. As a result, needle sharing by drug users is a major cause of AIDS in many countries, both, developed and developing, and in some, it is the predominant cause.

Any skin piercing (including injection, etc) can transmit the virus if the instruments used have not been sterilized properly and have previously been used on an infected person.

Maternal-foetal transmission (Mother-to-child transmission)

HIV may pass from an infected mother to her foetus, through the placenta or to her infant during delivery or by breast feeding. About one third of the children of HIV positive mothers, get infected through above route. The risk of infection transmission are higher, if the mother is newly infected or if she has already developed AIDS. There is no evidence that HIV is transmitted through mosquitoes or any insects, casual social contact with infected persons, even within households or by food or water. There is no evidence of spread to health care workers in their professional contact.

The Mechanism of Infection

HIV infection normally occurs first in macrophages, an antigen-presenting cell that has a very low level of CD4 proteins on its surface. The gp 120 protein of HIV binds to the CD4 protein molecule of the macrophage at the cell surface. The viral protein gp 120 then interacts with another macrophage protein (CCR5), which acts as a coreceptor for HIV and together with CD4 forms the docking site, where the HIV envelop fuses with the host cell membrane allowing insertion of the viral nucleocapsid. After HIV has infected the macrophage, a different form of gp 120 protein is made which in turn binds to a different coreceptor called CXCR4 on T-cells (T-lymphocytes). HIV then enters and destroys the T-cells. Thus, HIV first infects macrophages and then to T-cells. The net result of HIV infection is the systematic destruction of macrophages and T-cells resulting in a catastrophic breakdown of immunity in the body of the victim.

Counseling is an essential part of voluntary and confidential HIV anti-body testing programmes. It also plays a very valuable role in HIV prevention and care on its own, without testing. Issues such as reducing the risk of infection, family planning, relationships, sexuality and sexual problems, are all important areas for discussion. Counseling is a vital part of caring for people who are dying, and supporting those who are taking care of them. The impact of HIV goes far beyond HIV positive individuals. It has great implication for their sexual partners and family members- including future children. Coping with HIV can be easier, if people choose to share counseling sessions with those close to them. Many counseling services are now setting up self-help or discussion groups run by and for people who are HIV positive.

Control of AIDS

There are four basic approaches to the control of AIDS

1. **Education**:

The cure for AIDS is found the only means at present available is health education to enable to make life-saving choices. All mass media channels should be involved in educating the people on AIDS, its nature, transmission and prevention, this includes international travelers also.

2. **Prevention of blood borne HIV transmission**:

People in high risk groups should be urged to refrain from donating blood, body organs, sperm or other tissues. Strict sterilization practices should be ensured in hospitals and clinics. Presterilized disposable syringes and needles should be used as far as possible. One should avoid injections unless they are absolutely necessary.

3. **Specific prophylaxis**- At present, there is no vaccine or cure for treatment of HIV infection, AIDS. However, several researchers are working on drugs to interfere with HIV's production cycle at one stage or the other.

4. **Primary health care**- Because of its wide ranging health implications. AIDS touches all aspects of primary health care, including mother and child health, family planning and education. It is important therefore that AIDS control programmers are not developed in isolation. Integration in countries primary health care system is essential.

- **Rabies**

Rabies is a viral disease that affects the central nervous system and is usually transmitted through the bite or scratch of an infected animal. The symptoms of rabies can be divided into two forms: the "prodromal" stage and the "acute" stage. Rabies also known as hydrophobia,in which extreme or irrational fear of water,specially as sytem of rabies in humans. is nervous system, It is primarily a zoonotic disease of warm-blooded animals, particularly, carnivorous such as dogs, cats, Jackals and wolves. The source of infection to man is the saliva of rabid animals. In dogs and cats, the virus may be present in the saliva for 3-4 days, (Occasionally 5-6 days), before the onset of clinical symptoms and during the course of illness till death.

Mode of transmission

i. **Animal bites:-**In India, most of the human rabies causes, have been resulted from dog-bites. Transmission to man is particularly through rabid dog bites. In the transmission, the saliva of the dogs (or the biting animal) must contain the virus at the time of bite.

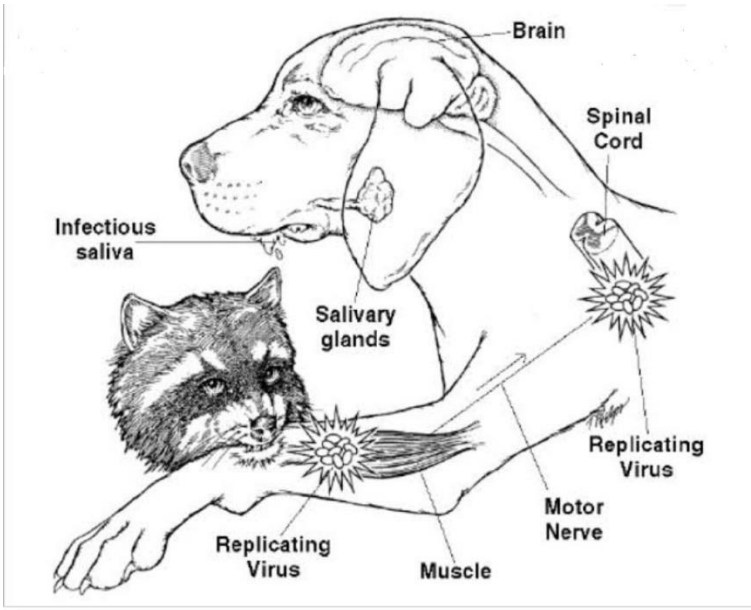


Fig. 5 Rabies infection by dog biting

i. **Licks:** Licks on abraded skin and mucosa (abraded or unabraded) can transmit the disease. Dogs have the habit of licking. In rare instances, the disease may be caused by accidental injury with bone splinter or other object contaminated by the saliva of a rabid animal.

ii. **Aerosols:** Aerosols (respiratory) transmission has been observed in nature only in certain caves harbouring rabies infected bats and in the laboratory, where aerosols created during homogenization of infected animals, brains can infect lab workers.

iii. **Person to Person:** Man to man transmission although rare is possible. A case of a child biting its parents is on record. There are also reports of transmission of rabies in corneal and organ transplants.

Rabies in humans is similar to that in animals**.** Symptoms include depression, headache, nausea, seizures, anorexia, muscle, stiffness and increased production of saliva.

- **Measles:**

Measles is caused by the measles virus and is highly contagious. It presents with a characteristic rash, high fever, cough, runny nose, and red, watery eyes. In severe cases, it can lead to complications such as pneumonia, encephalitis, and even death.

- **Ebola virus disease:**

  Ebola virus disease (EVD), is a severe and often fatal illness caused by the Ebola virus. It is characterized by symptoms such as fever, severe headache, muscle pain, weakness, vomiting, diarrhea, and in some cases, internal and external bleeding, organ failure and lead to death

- Hepatitis:

  Hepatitis is inflammation of the liver, and several viruses can cause it, including hepatitis A, B, C, D, and E viruses. Each type of viral hepatitis has different modes of transmission and can lead to liver damage, cirrhosis, or liver cancer.

- **Zika Virus disease or Zika fever:**

  Zika virus is primarily transmitted by mosquitoes and can cause symptoms such as mild fever, rash, joint pain, and conjunctivitis. Of particular concern is its association with birth defects when pregnant women are infected.

11.6 Food Born Diseases

Foodborne diseases, also known as foodborne illnesses or food poisoning, are caused by consuming contaminated food or beverages. Here are some common foodborne diseases and their associated pathogens:

- **Salmonellosis**: Caused by Salmonella bacteria, commonly found in raw or undercooked eggs, poultry, meat, and contaminated dairy products. Symptoms include diarrhea, abdominal cramps, fever, and vomiting.
- **Campylobacteriosis:** Caused by Campylobacter bacteria, often found in undercooked poultry, unpasteurized milk, and contaminated water. Symptoms include diarrhea (sometimes bloody), abdominal pain, fever, and nausea.
- **E. coli Infections:** Certain strains of Escherichia coli (E. coli) bacteria can cause foodborne illnesses. Contamination can occur through undercooked ground beef, unpasteurized milk, contaminated milk products, and contaminated water. Symptoms include severe abdominal cramps, diarrhea (often bloody), vomiting, and sometimes fever.

- **Listeriosis:** Caused by the bacterium Listeria monocytogenes, commonly found in unpasteurized dairy products, deli meats, and certain fruits and vegetables. Symptoms may include fever, muscle aches, headache, confusion, stiff neck, and gastrointestinal symptoms. It can be particularly dangerous for pregnant women, newborns, and individuals with weakened immune systems.
- **Gastroenteritis and Diarrhea**

  Many pathogenic bacteria transmitted by feces-contaminated food or water can cause ***gastroenteritis,*** an acute inflammation of the gastrointestinal tract (particularly the small intestine and or the large intestine). One of the most common symptoms of gastroenteritis is *diarrhea*, which is characterized by increased water content in the feces (watery stools). Diarrhea is ultimately the result of either (1) a decreased absorption of fluid from the intestinal tract or (2) an increased secretion of fluid derived from the patient's blood into the intestinal tract, which is a reversal of the normal process of water absorption.

- **Norovirus Infections:** Norovirus is a highly contagious virus that can spread through contaminated food, water, or person-to-person contact. Symptoms include nausea, vomiting, diarrhea, abdominal pain, and sometimes fever and body aches.
- **Hepatitis A:** Caused by the hepatitis A virus, it can be transmitted through contaminated food and water. Symptoms include fatigue, loss of appetite, nausea, abdominal discomfort, jaundice (yellowing of the skin and eyes), and dark urine.
- **Botulism**

  Botulism is caused by the bacterium *Clostridium botulism,* which produces an exotoxin that is the most potent of all known poisons. Botulism occurs in three forms; *food poisoning botulism, infant botulism and wound botulism.* Of these, infant botulism is the most common form.

  Food Poisoning Botulism

  The name botulism comes from the Latin botulus (sausage). In the eighteenth century the disease was first associated with the consumptions of sausages. Since then, many other foods have been found to cause this type of food poisoning. This is because foods can easily become contaminated by *C. botulinum* endospores, which occur widely in soil and in some marine and lake sediments.

Botulinum toxin causes paralysis by its action as a nerve poison, or ***neurotoxin***. It affects the ability of nerves to stimulate muscles. Normally, for muscle contraction to occur, a nerve impulse travels along a nerve fiber to the *neuromuscular junction* the area of contact between the end of the nerve fiber and the muscle, to be stimulated. There the end of the nerve fiber secretes a chemical called *acetylcholine*, which initiates muscle contraction. In patients with botulism the neurotoxin binds to the nerve fiber near the neuromuscular junction and prevents the fiber from secreting acetylcholine, thus the muscle cannot contract and paralysis occurs. The toxin causes the muscle of the chest and diaphragms are affected causing great difficulty in breathing or even death from respiratory failure.

Wound Botulism

The rare type of botulism known as *wound botulism* is also an infection. If C.botulinum spores enter a wound and if appropriate anaerobic conditions exist, then the spores may germinate and enough toxin can be made to cause the symptoms of botulism.

Other Types of Food Poisoning:

Bacillus cereus Food Poisoning

Some strains of Bacillus cereus can cause a "short-incubation" type of food poisoning that resembles staphylococcal food poisoning. Patients experience nausea and vomiting within 1 to 6 hours, after consuming the poisoned food, diarrhoea is usually absent. As in staphylococcal food poisoning, the symptoms are caused by a heat stable enterotoxin. *Bacillus cereus* food poisoning is associated most often with consumption of fried or boiled rice, but it has been linked to other foods as well, such as mashed potatoes and spaghetti.

Mycotoxin Food Poisoning

Various fungi can produce poisonous substances called *mycotoxins,* which can produce severe disease symptoms. Poisoning caused by consumption of certain mushrooms, such as *Amanita virosa* is a familiar example, Poisonous substances can also be produced by microscopic fungi growing on food products such as grains, peanuts, coffee beans, tobacco.

Foodborne and Waterborne Infections Caused by Bacteria

In foodborne or waterborne infection caused by bacteria, the microorganisms enter the body through consumption of contaminated food or water by fecal matter from humans or

animals. Foodborne and waterborne diseases are usually diseases of the intestinal tract, although other areas of the body may be affected. *Salmonella gastroenteritis* is of particular interest because of its widespread occurrence, the variety of sources of contamination, and the antigenic complexity of the organisms. Another bacterial infection called *typhoid fever*, is a serious disease with special features not found in salmonella gastroenteritis. *Campylobacter gastroenteritis* is now as the most frequent kind of bacterial gastroenteritis worldwide, and the causative bacteria have **and** unusual physiological features that complicate their isolation from patients.

Salmonella Gastroenteritis Typhoid Fever

Salmonella gastroenteritis is caused by bacteria of the genus *Salmonella*. Typhoid fever is caused by one particular salmonella serotype, *Salmonella typhi*.

Transmission of Salmonellas.

Humans are infected by salmonellas almost exclusively through the consumption of contaminated food or water. The foods most commonly involved are cream containing pastries, ground meats, sausages, poultry, commercially prepared beef roasts and eggs.

Humans can spread salmonellas to other human. Asymptomatic carriers and ill persons may excrete salmonellas in their feces, and the salmonellas may contaminate their hands. If person with contaminated hands is involved in food. If the food is stored in a warm place for several hours, the bacteria may multiply to numbers high enough to cause disease in those who eat the food. Human feces may also contaminate water supplies and cause *Salmonella* infections. The main source of many salmonellas is animals not humans.

Treatment of Salmonella Infections

Most patients with *Salmonella* gastroenteritis require no treatment. However, if diarrhea is severe, intravenous administration of fluids and salts may be necessary to prevent dehydration.

Patients with typhoid fever are treated with ampicillin, chloramphenicol, or amoxicillin. Prolonged antibiotic treatment is needed to cure the disease, because antibiotics have difficulty in reaching typhoid bacilli inside the macrophages. Typhoid bacilli can be eliminated from chronic typhoid carriers by treatment with ampicillin, but in some instances surgical removal of the gallbladder may be the only effective measure.

Prevention of Salmonella Infections

Since most cases of *Salmonella* gastroenteritis result form the ingestion of contaminated food. The following measures can prevent infection:

1. Adequate cooking of food from animal sources to kill salmonellas that may be present.
2. Suitable refrigeration temperatures for holding food, so that salmonellas do not multiply to high numbers.
3. Protection of food from contamination by rodents, flies and other animals that may carry salmonella contaminated materials.
4. Periodic analysis of stool samples from food handlers by public health personnel to detect carriers
5. Periodic inspection of food processing plants by public health personnel to detect contamination of food products by salmonellas.
6. Good personal sanitary and hygienic practices

Once a case of foodborne salmonella infections is discovered, it should be reported to public health authorities, so that suitable measures can be implemented to prevent an epidemic.

Campylobacter and Helicobacter Infections

Campylobacter bacteria are among those microorganisms that can cause great discomfort in the human digestive system. *Campylobacter jejuni* had long been suspected as a cause of diarrhea in humans.

Traansmission and Pathogenicity of Campylobacter jejuni and Helocobacter pylori.

C. jejuni is transmitted by contaminated food or water and animal feces are the major source of contamination. C jejuni is a part of normal intestinal flora of cattle, sheep, dogs, cats, poultry and other animal, and it is likely that outbreaks of infections occur when feces from these animals reach food or water supplies. Several epidemics have been traced to contaminated water. *C. jejuni* is often present on poultry carcasses and undercooked poultry is an important source of infection.

Most *C. jejuni* gastroenteritis occurs 2 to 4 days after ingestion of contaminated food or water. Patients experience fever, diarrhea, and abdominal pain. In many cases, the diarrhoeic stools contain blood.

Mycotoxins are toxins produced by fungi among which aflatoxin is one of the most potent toxin produced by isolates of *Aspergillus flavus.* The aflatoixn occurrence was first reported in 1960 when more than 1,00,000 tukery's and ducklings were died in England due to consumption of mouldy peanut meals important from Brazil. On the basis of animal studies, it seems that aflatoxins are potential danger to human health. They may be one of the factors responsible for the higher incidence of liver cancer in tropical Africa and Asia.

Total 18 aflatoxins are known of which aflatoxins B1 is most common and most potent carcinogen. If cattle or dairy animals consume aflatoxin contaminated feeds, aflatoxin appears in the milk and dairy products. Aflatoxin B_1 and B_2 after ingestion by dairy animals are modified into the aflatoxin M_1 and M_2 in the animal body. The aflatoxins have also been found in cocoa, raisins, peanut butter, Soyabean meal and beer.

The toxin producing moulds infect our food and feed stuffs and produce toxin in them. The consumption of such toxin producing mould infected grain results health hazards in humans and animals, because toxin affects liver and kidneys. The aflatoxin functions as potent toxin, a carcinogen, a teratogen and mutagen. Person having hepatitis B disease have a 30 times higher risk of liver cancer when exposed to aflatoxin the healthy person.

Animals feeding on sclerotia infested fooder suffer from ergotism disease. The severe attack of ergotism results in toes and feet gangrene in animals.

11.7. Detection of Food-Borne Pathogens

The food borne illnesses emphasize, the need to protect the public from microbial contamination in the food supply is paramount. Several guiding principles must be considered in developing the technologies, protocols, and policies used to keep foods safe. These include (1) specificity and sensitivity for the given pathogen, (2) speed and (3) simplicity (e.g. foods should be tested without the need for a lot of sample preparation). Ideally "testing to prevent," that is to confirm food is safe before it leaves the farm, is the goal. When this is unreasonable due to logistics and cost, then the next level is "testing to protect," which involves analysis before the food is accessible for consumption. These strategies are designed to avoid "testing to recover," when an outbreak has occurred and the origin of the contaminated food must be identified.

Despite the use of molecular techniques in other branches of microbiology, food borne pathogen is still most commonly identified by standard culture techniques.

Molecular methods are valuable for a number of reasons. These include the ability to detect (1) the presence of specific pathogen; (2) viruses that cannot be grown conveniently; (3) microbes that present in very small. Foodborne and waterborne infections affect the intestinal tract.

Food Poisoning

Food poisoning occurs, when people consume food containing a toxin made by a microorganism. There are several kinds of food poisoning caused by microbes, the most familiar example is staphylococcal food poisoning and botulism.

Staphylococcal Food Poisoning

Staphylococcal food poisoning is one of the most common types of food poisoning. Many thousands of cases occur each year in the United states, most of which could be prevented easily by using simple precautions in preparing and storing food. Human carriers are responsible for contaminating food with an enterotoxins producing strain of *S. aureus.* The staphylococcal food poising is usually as follows:

1. The hands of the carrier become contaminated with nasal secretions.
2. The carrier's hands inoculate the food during its preparation.
3. The food is stored for several hours without being properly refrigerated. During this period the staphylococci multiply and produce the enterotoxins.

The food is consumed, raw or cooked. Cooking does not destroy the enterotoxins. It is heat stable and can withstand boiling for 30 minutes or more.

The foods most likely to be involved in this type of food poisoning are milk products, custard, processed meat spreads, cream puff fillings, sandwich spreads, poultry stuffing and potato salad. Symptoms occur within 1 to 6 hours after consumption of the food, they include severe nausea, vomiting, and moderate diarrhea, but usually no fever.

11.8 Summary

- AIDS, the acquired immune deficiency syndrome (sometimes called slim disease) is a newly described, usually fatal illness caused by retrovirus known as the human immune deficiency virus (HIV).
- AIDS, breakdown the body's immune system, AIDS can be called our modern pandemic affecting both industrialized and developing countries.
- Viruses spread in many ways. One transmission pathway is through disease-bearing organisms known as vectors; for example, viruses are often transmitted from plant to plant by insects that feed on plant sap, such as aphids.
- Viruses in animals can be carried by blood sucking insects. Influenza viruses spread in the air by coughing and sneezing.
- Norovirus and rotavirus, common causes of viral gastroenteritis, are transmitted by the faecal-oral route, passed by hand-to-mouth contact or in food or water.
- Some viruses including those that cause AIDS, HIV infection and viral hepatitis, evade these immune responses and result in chronic infections.
- Tuberculosis is a specific infectious disease caused by *Mycobacterium tuberculosis*.
- Cholera is severe water borne diarrheal disease frequently occurring in developing countries. It is caused by *Vibrio cholera*, a gram-negative, curved, bacillus bacterium, transmitted almost exclusively via contaminated water, food etc.
- Many pathogenic bacteria transmitted by feces, contaminated food or water, can cause ***gastroenteritis*** an acute inflammation of the gastrointestinal tract (particularly the small intestine and the large intestine).

11.9 Terminal Questions

Q.1. What are symptoms of rabies in people and animals and how rabies is transmitted?

Answer:----------

Q.2. What is the main cause of Cholera and how is cholera prevented?

Answer:----------

Q.3. How is Cholera spread from one person to another?

Answer:----------

Q.4. What is the history of HIV? And what is the difference between HIV and AIDS.

Answer:----------

Q.5. How is HIV transmitted?

Answer:----------

Q.6. What are symptoms of tuberculosis? And how is tuberculosis spread?

Answer:----------

Q.7. What are the signs and symptoms associated with food borne disease?

Answer:----------

11.10 Further readings

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13. Ian L. Pepper, Charles P. Gerba, Terry J. Gentry, A Microbiology, Academic Press-2015.

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Unit- 12: Chemotherapy and Antibiotics

Contents

12.1. Introduction

Chemotherapy is a widely used treatment approach in the field of oncology (cancer treatment) that involves the use of powerful drugs to destroy cancerous cells or slow down their growth activity. It is a systemic treatment, meaning that the drugs travel throughout the body via the bloodstream, targeting both, cancerous and normal cells.

The term *chemotherapy* has come to connote non-specific usage of intracellular poisons to inhibit mitosis (cell division) or induce DNA damage, which is why inhibition of DNA repair can augment chemotherapy. The connotation of the word chemotherapy, excludes more selective agents that block extracellular signals (signal transduction). The development of therapies with specific molecular or genetic targets, which inhibit growth-promoting signals from classic endocrine hormones (primarily estrogens for breast cancer and androgens for prostate cancer) are now called hormonal therapies. By contrast, other inhibitors of growth-signals like those associated with receptor tyrosine kinases are referred to as targeted therapy.

The main goal of chemotherapy is to eliminate cancerous cells, control the growth of tumors, and prevent the spread of cancer to other parts of the body. It can be used as the primary treatment for certain types of cancer, as an adjuvant therapy to eliminate any remaining cancer cells after surgery or radiation, or as a palliative treatment to relieve symptoms and improve the quality of life in advanced stages of cancer.

Chemotherapy drugs work by disrupting the life cycle of rapidly dividing cells, which includes cancer cells. However, because normal cells in the body also divide quickly, they can be affected by chemotherapy, leading to its side effects. Commonly affected cells include those in the bone marrow (responsible for producing blood cells), hair follicles, gastrointestinal tract, and reproductive organs. The specific chemotherapy drugs and treatment regimens used depend on various factors, including the type and stage of cancer, the patient's overall health, and the treatment goals. Chemotherapy can be administered in different ways, including intravenously (through a vein), orally (in the form of pills or liquids), or topically (applied directly to the skin). Chemotherapy treatments are usually given in cycles, with each cycle consisting of a period of treatment followed by a period of rest to allow the body to recover. The total duration of chemotherapy treatment varies depending on the type and stage of cancer. While, chemotherapy can be an effective treatment for many types of cancer, it can also cause a range of side effects.

These side effects can vary widely from person to person and depend on the specific drugs used. Common side effects include fatigue, nausea and vomiting, hair loss, decreased blood cell counts, and increased susceptibility to infections.

In recent years, there have been advancements in chemotherapy research, leading to the development of targeted therapies and immunotherapy that specifically attack cancer cells, while, minimizing damage to healthy cells. These advancements have improved treatment out comes and reduced some of the side effects associated with traditional chemotherapy. The traditional chemotherapeutic agents are cytotoxic by means of interfering with cell division (mitosis), but cancer cells vary widely in their susceptibility to these agents. To a large extent, chemotherapy can be thought of as a way to damage or stress cells, which may then lead to cell death if apoptosis is initiated. Many of the side effects of chemotherapy can be traced to damage to normal cells that divide rapidly, and are thus sensitive to anti-mitotic drugs, cells in the bone marrow, digestive tract and hair follicles.

This results in the most common side-effects of chemotherapy: myelosuppression (decreased production of blood cells, hence also immunosuppression), mucositis (inflammation of the lining of the digestive tract), and alopecia (hair loss). Because of the effect on immune cells (especially lymphocytes), chemotherapy drugs often find use in a host of diseases that result from harmful over activity of the immune system against self (so-called autoimmunity). These include rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, vasculitis and many others.

The number of chemotherapeutic agents are used in treatment these are-1.Alkylating agent 2.Antimetabolites 3.Topoisomerase inhibitors 4.Anthracycline 5.Taxanes 6.Targeted therapies.

The different types of chemotherapy are as under-i.Adjuvent chemotherapy ii. Neo-adjuvent chemotherapy iii. Palliative chemotherapy iv.Continuation chemotherapy v. High dose chemotherapy vi. Targeted chemotherapy vii. Immuno chemotherapy viii.Hormonal chemotherapy.

Chemotherapic drugs given,intraveinously, orally, intramuscularly, intraartrial, intraperitonial.The drug, dose and treatment type depends upon various factors, actually, these are decided by an oncologist.

Antibiotics are drug synthesized one microorganism and able to kill or decrease the growth of other pathogenic bacteria microorganism, and curing the disease. These are used to treat the different bacterial diseases, such as-pneumonia, urinary tract infection, respiratory infection, bacterial meningitis, cholera, tuberculosis, measles, skin infection, gonorrhia, wound infection, throat infection,etc.Commonly used antibiotics are-Penicillins, cephalosporins,tetracyclines,etc.

Antibiotics are classified to two-

1.Narrow- spectrum antibiotic-only able to kill or inhibit the growth of limited number of bacteria, e,g., fidaxomicin and sarecycline.

2.Broad-spectrum antibiotic-they are able to kill or inhibit growth of number of pathogenic bacteria,e.g.,penicillins, cephalosporins (have five generations), azithromycin, amoxicillin, tetracycline, ofloxacin, chloramphenicol and quinolones.Hower,some time, use of antibiotic develop antibiotic resistance in pathogenic bacterium against antimiotic and patient remains uncured, this is due to over use or misuse of antibiotic. Some time irregular treatment (mismanaged, patient do complete their full course) develop antibiotic drug resistance in patient, e.g.,MDR TB, XDR TB (rare tye).In these cases,TB disease again develop in patient, after having taken TB medicine in the past.

Antimicrobial anents are broader category of drugs (medecines) used against broader category of disease causing microorganisms, such as bacteria, virus, fungi, parasites.According to micoorganism they infect, these are classified as-

1.Antibiotics-effective against bacterial infection

2.Antiviral agent-effective against virus infection, herpes, HIV, influenza, hepatitis

3.Antifungal agent-effective against fungal infection (mycosis), ringworm, candidiansis, athletes foot.The common antifungal drugs are amphotericin- B, griseofulvin, allylamine, azoles, polyene.

4.Antiparasitic agent- effective againt parasite (protozoa, helminths) causing malaria, giardiasis.

5.Antiseptic and disinfectants-effective against a germ microorganism present up on skin, wounds or inanimate objects. Usually, alcohal and hydrogen peroxide are used.

Sulfa drugs are synthetic broad-spectram antibiotics used against number bacterial infection in humans.They are bacteriostatic in nature, i.e., inhibit the growth of bacterium but do not kill them. Sulfa powder or sulfanilimide is used in second world war to treat wounds of soldiers.

Objectives

After study of content of this unit ,students will we able to known-

- about the concept of chemotherapy and its types.
- about antibiotics and antifungal agents and their types.
- about the molecular mechanism of drug resistance.
- the sulpha drugs

12.2. Concept of chemotherapy

Chemotherapy is a treatment approach that utilizes powerful drugs to destroy cancer cells or slow down their growth. It is based on the concept that cancer cells have a higher rate of division and growth compared to normal cells. By targeting these rapidly dividing cells, chemotherapy aims to eliminate or control the spread of cancer throughout the body. The concept of chemotherapy revolves around the administration of specific drugs that have cytotoxic (cell-killing) properties. These drugs work by interfering with the processes that are necessary for cancer cell growth and division. They can disrupt DNA synthesis, inhibit cell replication, or interfere with the proteins and enzymes that cancer cells rely on for survival. Chemotherapy drugs are typically designed to be toxic to cancer cells, but they can also affect normal cells in the body that divide rapidly. This accounts for the side effects associated with chemotherapy, as normal cells in the bone marrow, hair follicles, gastrointestinal tract, and reproductive organs can be affected. The choice of chemotherapy drugs and the treatment regimen depend on several factors, including the type of cancer, its stage, the patient's overall health, and the treatment goals. The drugs can be administered in different ways, such as intravenously, orally, or topically, depending on the specific needs of the patient. Chemotherapy is often used in combination with other treatment modalities, such as surgery and radiation therapy, to maximize the effectiveness of cancer treatment. It can be used before surgery to shrink tumors, after surgery to eliminate any remaining cancer cells, or in combination with radiation therapy to enhance its effects.

12.3. Chemotheraptic agents

Chemotherapeutic agents, also known as chemotherapy drugs or anticancer drugs, are medications, specifically designed to treat cancer. These agents are the cornerstone of chemotherapy and are used to destroy cancer cells or inhibit their growth. Chemotherapeutic agents can target cancer cells throughout the body, even if the cancer has spread to multiple sites. Chemotherapy drugs can be classified into several categories based on their mode of action and chemical structure. Here are some common types of chemotherapeutic agents:

- **Alkylating agents:** These drugs work by directly damaging the DNA of cancer cells, preventing them from dividing and growing. Examples include cyclophosphamide, cisplatin, and temozolomide.
- **Antimetabolites:** These drugs interfere with the normal metabolic processes of cancer cells by acting as false building blocks for DNA or RNA. They disrupt the synthesis of nucleic acids and inhibit cell division. Methotrexate, 5-fluorouracil (5-FU), and gemcitabine are examples of antimetabolites.
- **Topoisomerase inhibitors:** These drugs target enzymes called topoisomerases, which are essential for DNA replication and repair. By inhibiting these enzymes, topoisomerase inhibitors prevent cancer cells from dividing. Examples include etoposide and irinotecan.
- **Anthracyclines:** This class of drugs includes doxorubicin and daunorubicin. Anthracyclines work by intercalating into DNA and interfering with its replication. They also generate free radicals that can cause DNA damage.
- **Taxanes**: Drugs such as paclitaxel and docetaxel interfere with the normal function of microtubules, which are essential for cell division. By stabilizing microtubules, taxanes prevent cancer cells from dividing properly.
- **Targeted therapies**: These drugs specifically target certain molecules or pathways involved in the growth and survival of cancer cells. Examples include tyrosine kinase inhibitors (such as imatinib), monoclonal antibodies (such as trastuzumab), and proteasome inhibitors (such as bortezomib).

12.4. Types of chemotherapy

There are several types of chemotherapy, each with its own specific purpose and approach. The choice of chemotherapy type depends on the type and stage of cancer, the treatment goals, and individual patient factors.

- **Adjuvant chemotherapy:** This type of chemotherapy is given after primary treatment, such as surgery or radiation therapy. Its purpose is to destroy any remaining cancer cells that may not be visible or have the potential to spread. Adjuvant chemotherapy aims to reduce the risk of cancer recurrence.
- **Neoadjuvant chemotherapy:** This type of chemotherapy is administered before the main treatment, usually surgery or radiation therapy. The goal is to shrink tumors and make them more manageable for subsequent treatments. Neoadjuvant chemotherapy is commonly used in the treatment of breast cancer and certain other types of cancer.
- **Palliative chemotherapy:** Palliative chemotherapy is aimed at relieving symptoms and improving the quality of life for patients with advanced or metastatic cancer. The primary goal is not to cure the cancer but to control its growth, manage symptoms, and prolong survival.
- **Combination chemotherapy:** Combination chemotherapy involves the use of multiple chemotherapy drugs in a treatment regimen. This approach aims to target cancer cells in different ways, reduce the risk of drug resistance, and increase treatment effectiveness. Different drugs may be given simultaneously or sequentially in a carefully planned schedule.
- **High-dose chemotherapy:** High-dose chemotherapy involves the administration of chemotherapy drugs at higher doses than conventional doses. This approach is often used in the treatment of certain cancers, such as lymphoma and leukemia, and is sometimes followed by stem cell transplantation to replenish the bone marrow.
- **Targeted therapy:** While, not strictly considered chemotherapy, targeted therapies are an important class of drugs used in cancer treatment. These drugs specifically target molecular abnormalities or specific pathways in cancer cells, blocking their growth or promoting their destruction. Targeted therapies have the advantage of being more selective and causing fewer side effects compared to traditional chemotherapy.
- **Immunotherapy**: Similarly, immunotherapy is a type of treatment that uses the body's immune system to fight cancer. It enhances the immune response against cancer cells and can be used as a standalone treatment or in combination with chemotherapy or other therapies.

12.5. How does chemotherapy treat cancer

Doctor (oncologist) use chemotherapy in different ways at different times. These include:

- Before surgery, radiation therapy to shrink tumors. This is called neoadjuvant chemotherapy.
- After surgery, radiation therapy to destroy any remaining cancer cells. This is called adjuvant chemotherapy.
- As the only treatment. For example, to treat cancers of the blood or lymphatic system, such as leukemia and lymphoma.
- For cancer that comes back after treatment, called recurrent cancer.
- For cancer that has spread to other parts of the body, called metastatic cancer.

The goals of chemotherapy

The goals of chemotherapy depend on the type of cancer and how far it has spread. Sometimes, the goal of treatment is to get rid of all the cancer and keep it from coming back. If this is not possible, you might receive chemotherapy to delay or slow cancer growth. Delaying or slowing cancer growth with chemotherapy also helps to manage symptoms caused by the cancer. Chemotherapy given with the goal of delaying cancer growth is sometimes called palliative chemotherapy.

Chemotherapy plan

There are many drugs available to treat cancer. A doctor who specializes in treating cancer with medication, called a medical oncologist, will prescribe your chemotherapy. You may receive a combination of drugs, because this sometimes works better than only one drug. The drugs, dose, and treatment schedule depend on many factors. These include:

- The type of cancer
- The tumor size, its location, and if or where it has spread. This is called the stage of cancer.
- Your age and general health

- Your body weight
- How well you can cope with certain side effects
- Any other medical conditions you have
- Previous cancer treatments

Where is chemotherapy given?

Your health care team may give you chemotherapy at the clinic, doctor's office, or hospital. Some types of chemotherapy are given by mouth, and these can be taken at home.

How long does chemotherapy take?

Chemotherapy is often given for a specific time, such as 6 months or a year. you might receive chemotherapy for as long as it works. Side effects from many drugs are too severe to give treatment every day. Doctors usually give these drugs with breaks, so you have time to rest and recover before the next treatment. This lets your healthy cells heal. For example, you might get a dose of chemotherapy on the first day and then have 3 weeks of recovery time before repeating the treatment. Each 3-week period is called a treatment cycle. Several cycles make up a course of chemotherapy. A course usually lasts in 3 months or more.

Some cancers are treated with less recovery time between cycles. This is called a dose-dense schedule. It can make chemotherapy more effective against some cancers. But it also increases the risk of side effects. Talk with your health care team about the best schedule for you, before start of chemotherapy treatment.

How is chemotherapy given?

Chemotherapy may be given in several different ways, which are discussed below.

Intravenous (IV) chemotherapy

Many drugs require injection directly into a vein. This is called intraveinous or IV chemotherapy. Treatment takes a few minutes to a few hours. Some IV drugs work better if you

get them over a few days or weeks. You take them through a small pump you wear or carry. This is called continuous infusion chemotherapy.

Oral chemotherapy

We can take some drugs by mouth. They can be in a pill, capsule, or liquid. This means that you may be able to pick up your medication at the pharmacy and take it at home. Oral treatments for cancer are now more common. Some of these drugs are given daily, and others are given less often. For example, a drug may be given daily for 4 weeks, followed by a 2-week break.

Injected chemotherapy

This is when you receive chemotherapy as a shot. The shot may be given in a muscle or injected under the skin. We may receive these shots in the arm, leg, or abdomen. Abdomen is the medical word for your belly.

Chemotherapy into an artery

An artery is a blood vessel that carries blood from your heart to another part of our body. Sometimes, chemotherapy is injected into an artery that goes directly to the cancer. This is called intra-arterial or IA chemotherapy.

Chemotherapy into the peritoneum or abdomen

For some cancers, medication might be placed directly in your abdomen. This type of treatment works for cancers involving the peritoneum. The peritoneum covers the surface of the inside of the abdomen and surrounds the intestines, liver, and stomach. Ovarian cancer, is one type of cancer that frequently spreads to the peritoneum. We can take some types of chemotherapy in a cream and put on the skin. We can get your medication at the pharmacy and take it at home.

Other drug treatments for cancer

The traditional drugs used for chemotherapy are an important part of treatment for many cancers. The drugs affect both cancer cells and healthy cells. But scientists have designed newer drugs that work more specifically to treat cancer cells. These treatments cause different side

effects. Doctors may use these newer cancer drugs as the only drug treatment. But they are often added to traditional chemotherapy. These types of treatment include:

Hormonal therapy

These treatments change the amount of hormones in your body. Hormones are chemicalsand your body makes them naturally. They help to control the activity of certain cells or organs. Doctors use hormonal therapy, because hormone levels control several types of cancers. These include some breast and prostate cancers.

Targeted therapy

These treatments target and disable genes or proteins found in cancer cells, that the cancer cells need them to grow.

Immunotherapy

This type of treatment helps your body's natural defenses fight against the cancer. Immunotherapy is now an important part of treatment for several types of cancer and will play an increasingly important role in treatment in the future.

12.6. Antibiotics

An antibiotic is a type of medication used to treat bacterial infections. Antibiotics are synthesized by particular microorganism and able to kill or slow down the growth of the pathogenic microorganism, and curing inffection It is a powerful tool in the field of medicine, helping to combat a wide range of bacterial diseases and improve patient outcomes. Antibiotics work by targeting bacteria, and either killing them or inhibiting their growth, allowing the body's immune system to effectively eliminate the infection.

The discovery of antibiotics revolutionized medicine and has saved countless lives. Before their introduction, bacterial infections were a major cause of illness and death. Antibiotics have played a crucial role in treating common infections such as pneumonia, urinary tract infections, skin infections, and strep throat, as well as more severe conditions like sepsis and bacterial meningitis.

Antibiotics can be classified into different groups based on their mechanism of action and the bacteria they target. Some commonly used classes of antibiotics include penicillins,

cephalosporins, macrolides, tetracyclines, and fluoroquinolones. Each class of antibiotics has specific properties and may be more effective against certain types of bacteria.

It is important to note that antibiotics are ineffective against viral infections, such as the common cold, flu, or most cases of bronchitis. Viral infections are caused by viruses, which are fundamentally different from bacteria. Using antibiotics to treat viral infections can contribute to the development of antibiotic resistance and has no therapeutic benefit.

Antibiotic resistance is a major concern in healthcare today. Overuse and misuse of antibiotics can lead to the emergence of antibiotic-resistant bacteria, making infections more difficult to treat. It is essential to use antibiotics judiciously, following prescribed dosage and duration, and only use when they are truly necessary. Healthcare professionals play a critical role in prescribing antibiotics appropriately and educating patients about their use.

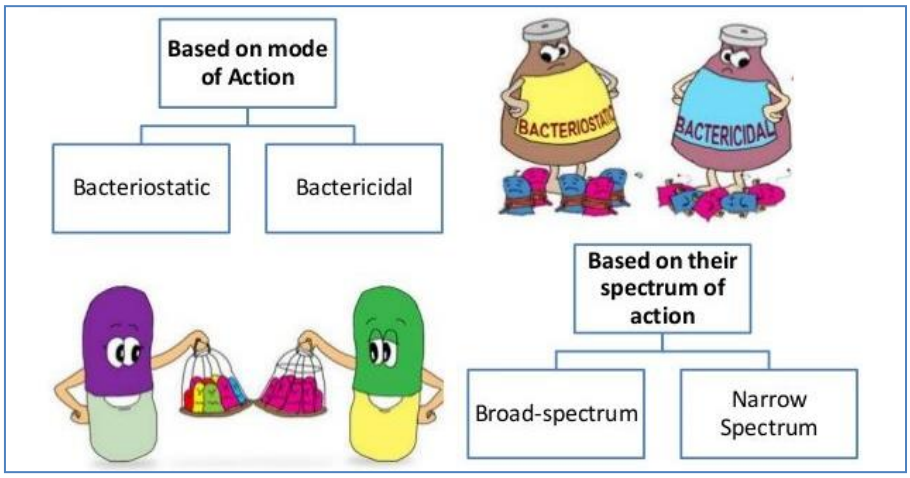

Fig. 12.1: Classification of antibiotics

Antibiotics may be given as a preventive measure and this is usually limited to at-risk populations such as those with a weakened immune system (particularly in HIV cases to prevent pneumonia), those taking immunosuppressive drugs in cancer patients, and those having surgery. Their use in surgical procedures is to help in prevent infection of incisions. They have an important role in dental antibiotic prophylaxis, where, their use may prevent bacteremia and consequent infective endocarditis. Antibiotics are also used to prevent infection in cases of neutropenia, particularly cancer-related. The use of antibiotics for secondary

prevention of coronary heart disease is not supported by current scientific evidence, and may actually increase cardiovascular mortality, all-cause mortality and the occurrence of stroke.

Routes of administration

There are many different routes of administration for antibiotic treatment. Antibiotics are usually taken by mouth. In more severe cases, particularly deep-seated systemic infections, antibiotics can be given intravenously or by injection. Where, the site of infection is easily accessed, antibiotics may be given topically in the form of eye drops onto the conjunctiva for conjunctivitis or ear drops for ear infections and acute cases of swimmer's ear. Topical use is also one of the treatment options for some skin conditions including acne and cellulitis. Advantages of topical application include achieving high and sustained concentration of antibiotic at the site of infection, reducing the potential for systemic absorption and toxicity, and total volumes of antibiotic required are reduced, thereby also reducing the risk of antibiotic misuse.

Topical antibiotics applied over certain types of surgical wounds have been reported to reduce the risk of surgical site infections. However, there are certain general causes for concern with topical administration of antibiotics. Some systemic absorption of the antibiotic may occur, the quantity of antibiotic applied is difficult to accurately dose, and there is also the possibility of local hypersensitivity reactions or contact dermatitis occurring. It is recommended to administer antibiotics as soon as possible, especially in life-threatening infections. Many emergency departments, stock antibiotics for this purpose.

Global consumption

Antibiotic consumption varies widely between countries. The WHO report on surveillance of antibiotic consumption, published in 2018, analyzed 2015 data from 65 countries. As measured in defined daily doses per 1,000 inhabitants per day. Mongolia had the highest consumption with a rate of 64.4 and Burundi had the lowest at 4.4. Amoxicillin and combination of amoxicillin and clavulanic acid were the most frequently consumed.

Side effects

Health advocacy messages, such as this one encourage patients to talk with their doctor about safety in using antibiotics. Antibiotics are screened for any negative effects, before their

approval for clinical use, and are usually considered safe and well tolerated. However, some antibiotics have been associated with a wide extent of adverse side effects, ranging from mild to very severe, depending on the type of antibiotic used, the microbes targeted, and the individual patient. Side effects may reflect the pharmacological or toxicological properties of the antibiotic or may involve hypersensitivity or allergic reactions. Adverse effects range from fever and nausea to major allergic reactions, including photodermatitis and anaphylaxis. Safety profiles of newer drugs are often not as well established as for those that have a long history of use.

Common side-effects include diarrhea, resulting from disruption of the species composition in the intestinal flora, resulting, for example, in overgrowth of pathogenic bacteria, such as *Clostridium difficile*. Taking probiotics during the course of antibiotic treatment, can help to prevent side-effect in antibiotic-associated diarrhea. Antibacterials can also affect the vaginal flora, and may lead to overgrowth of yeast species of the genus *Candida* in the vulvo-vaginal area. Additional side effects can result from interaction with other drugs, such as the possibility of tendon damage from the administration of a quinolone antibiotic with a systemic corticosteroid.

12.7. Antimicrobial agents

Antimicrobial agents are a broader category of medications that encompass both, antibiotics, which specifically target bacteria, and other types of drugs that can act against a broader range of microorganisms, including bacteria, viruses, fungi, and parasites. They are used to treat various types of infections caused by these microorganisms.

More recently, microbiologists such as Louis Pasteur and Jules Francois Joubert observed antagonism for some *bacteria,* and discussed the merits of controlling these interactions in medicine. In 1928, Alexander Fleming became the first to discover a natural powerful antimicrobial fungus known as Penicillium Rubens. The substance extracted from the fungus he named Penicillin, and in 1942, it was successfully used to treat a Streptococcus infections. But nowadays, all over the world treatment of using antimicrobial agents is currently facing its own limitation, due to the development of resistance by the microbes over the period of time. *Bacteria* are involved in many aspects of ecology and health. It likely seems that all species have both, benefit and suffer from interactions with *bacteria*. For example, we use *bacteria* for making yoghurt, curd, cheese and other fermented foods and also large number

of *bacteria* lives on the skin and in the digestive tract. The human gut contains more than 1000 *bacterial species* which are beneficial.

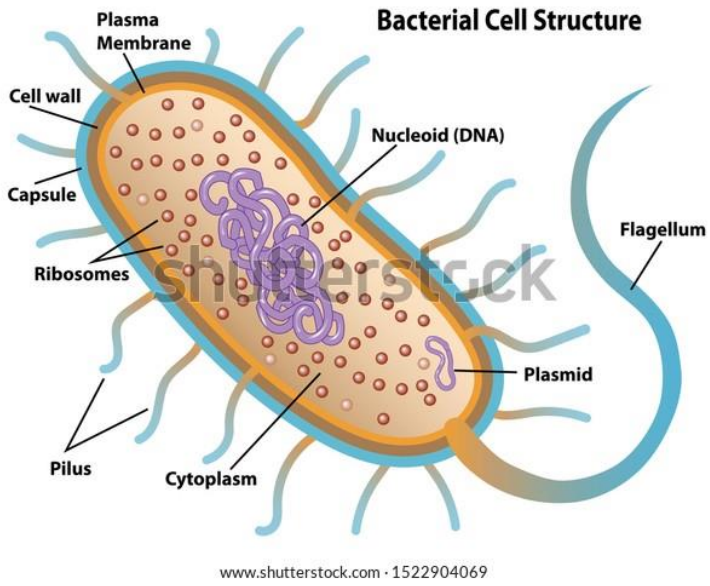

Fig. 12.2: Bacterial cell structure

Gut *bacteria* synthesize vitamins, such as Folic Acid, Vitamin K and biotin, and they ferment the complex, indigestible carbohydrates. Other useful *bacteria* in the gut flora include Lactobacillus species, which convert milk sugar into Lactic Acid. Also *bacteria* play very important role in the medicine, such as vaccine component, and in the production of antibiotic drugs, hormones, and antibodies. On the other hand, a pathogenic bacterium causes an enormous level of spoilage, suffering and death through the infection. The bacterial cells differs dramatically in structure and function as compared to mammalian cells. The bacterial cytoplasm is separated from the external environment by a plasma membrane.

1. Antibiotics:

Antibiotics are medications specifically designed to kill or inhibit the growth of bacteria. They can be further categorized based on their mechanism of action and chemical structure, such as penicillins, cephalosporins, macrolides, tetracyclines, and fluoroquinolones. Antibiotics are crucial in the treatment of bacterial infections, but it's important to use them judiciously to prevent antibiotic resistance.

a) **Penicillins**

Penicillins are a class of antibiotics that are derived from the fungus Penicillium. They are among the most widely used and important antibiotics in medical practice, primarily effective against bacterial infections. Penicillins work by interfering with the synthesis of bacterial cell walls, leading to the destruction of the bacteria. Penicillin was first discovered by Sir Alexander Fleming, in 1928, when he noticed that a mold, Penicillium notatum, produced a substance that killed bacteria. Penicillins exert their antibacterial effect by inhibiting the enzyme responsible for building the bacterial cell wall, called transpeptidase or penicillin-binding protein (PBP). By interfering with cell wall synthesis, penicillins weaken the bacterial cell structure, causing it to rupture and its death.

Penicillins have a broad spectrum of activity, meaning they can target a wide range of bacteria. However, their effectiveness may vary depending on the specific type of penicillin. Some penicillin, such as penicillin G and penicillin V, are more effective against Gram-positive bacteria, while others, like ampicillin and amoxicillin, have extended coverage against certain Gram-negative bacteria. Penicillins are commonly used to treat various bacterial infections, including respiratory tract infections (such as strep throat and pneumonia), skin and soft tissue infections, urinary tract infections, and certain sexually transmitted infections. Penicillins are generally well-tolerated, but like any medication, they can have side effects. Common side effects include gastrointestinal disturbances (such as nausea, vomiting, and diarrhea), allergic reactions (ranging from mild rash to severe anaphylaxis), and rarely, serious conditions like Stevens-Johnson syndrome.

How do penicillins work?

Penicillins are a group of antibacterial drugs that attack a wide range of bacteria. They were the first drugs of this type that doctors used. The discovery and manufacture of penicillins

have changed the face of medicine, as these drugs have saved millions of lives. *Penicillium* fungi are the source of penicillin, which people can take orally or via injection. People across the globe, now widely use penicillins to treat infections and diseases.

Fast facts on penicillin

- Penicillins were the first antibiotic that doctors used.
- There are several antibiotics in the penicillin class.
- Experts credit Alexander Fleming with discovering penicillins.
- Penicillin works by interfering with bacterial cell walls.
- Less than 1 percent of people are dangerously allergic to penicillin.

Functions

Drugs in the penicillin class, work by indirectly bursting bacterial cell walls. They do this by acting directly on peptidoglycans, which play an essential structural role in bacterial cells. Peptidoglycans create a mesh-like structure around the plasma membrane of bacterial cells, which increases the strength of the cell walls, and prevents external fluids and particles from entering the cell.

When a bacterium multiplies, small holes open up in its cell walls, as the cells divide. Newly-produced peptidoglycans then fill these holes to reconstruct the walls. Penicillins block the protein structure, that link the peptidoglycans together. This prevents the bacterium from closing the holes in its cell walls. As the water concentration of the surrounding fluid is higher than that inside the bacterium, water rushes through the holes into the cell and the bacterium bursts.

Resistance

Contrary to popular opinion, it is not the person who develops resistance to penicillins but the bacteria itself. Bacteria have been around for billions of years. During this time, they have endured extreme environments and as a result, are highly adaptable. They also regenerate very rapidly, making relatively quick genetic changes possible across a population. There are three common ways, in which bacteria can develop immunity to penicillin:

- Penicillinase: Bacteria are sometimes able to produce penicillinase, an enzyme that degrades penicillins. This ability can spread throughout the bacterial population via a small ring of

DNA, in a process called conjugation. This is the bacterial sexual reproduction, where individual organisms share new genetic information between them.

- Altered bacterial structure: Some bacteria can subtly change the format of the penicillin-binding proteins in their peptidoglycan wall so that penicillins can no longer bind to it.
- Penicillin removal: Other bacteria develop systems to export penicillins. Bacteria have efflux pumps that they use to release substances from the cell. The repurposing of some of these pumps can allow the cell to dispose of penicillins.

b) Cephalosporins

Cephalosporins are a class of antibiotics that are widely used to treat bacterial infections. They belong to the beta-lactam group of antibiotics, similar to penicillins, and share some structural and functional similarities with penicillin. Cephalosporins are derived from a fungus called Cephalosporium. Cephalosporins are classified into different generations, based on when they were developed and their spectrum of activity. There are five generations of cephalosporins, with each subsequent generation, generally having an expanded spectrum of activity against various bacteria. Cephalosporins are used to treat various bacterial infections, including respiratory tract infections, skin and soft tissue infections, urinary tract infections, bone and joint infections, sepsis, meningitis, and others. Cephalosporins are generally well-tolerated, but they can have side effects. Common side effects include gastrointestinal disturbances (such as nausea, vomiting, and diarrhea), allergic reactions (ranging from mild rash to severe anaphylaxis), and in rare cases, kidney toxicity.

a) First-generation cephalosporins (e.g., cephalexin, cefazolin) are effective against many Gram-positive bacteria and some Gram-negative bacteria.
b) Second-generation cephalosporins (e.g., cefuroxime, cefoxitin) have a broader spectrum of activity, including better coverage against some Gram-negative bacteria.
c) Third-generation cephalosporins (e.g., ceftriaxone, cefotaxime) have even broader coverage against Gram-negative bacteria, including some that are resistant to earlier generations.
d) Fourth-generation cephalosporins (e.g., cefepime) have an extended spectrum of activity against both Gram-positive and Gram-negative bacteria, including some resistant strains.
e) Fifth-generation cephalosporins (e.g., ceftaroline) have expanded coverage against Gram-positive bacteria, including methicillin-resistant Staphylococcus aureus (MRSA).

Similar to penicillins, cephalosporins work by interfering with bacterial cell wall synthesis. They inhibit the formation of the bacterial cell wall by binding to and inactivating the enzyme responsible for cross-linking the peptidoglycan layers, which are essential for the bacterial cell wall's structural integrity.

Mechanism of Action

Bacteria synthesize a cell wall that is strengthened by cross-linking peptidoglycan units via penicillin-binding proteins (PBP, peptidoglycan transpeptidase). Initially derived from the fungus *Cephalosporium sp.*, cephalosporins are a large group of bactericidal antimicrobials that work via their beta-lactam rings. The beta-lactam rings bind to the penicillin-binding protein and inhibit its normal activity, unable to synthesize a cell wall, the bacteria die.

Staphylococcus aureus that is initially susceptible to cephalosporins, can develop resistance by changing the structure of the penicillin-binding proteins. *S. aureus* does this by having a gene that encodes and modified penicillin-binding protein, this prevents the cephalosporins beta-lactam rings to inactivate the protein. The bacterium that develops this mechanism of resistance is called methicillin-resistant *Staphylococcus aureus* (MRSA). As indicated above, out of the five generations of cephalosporin, only the fifth generation ceftaroline has coverage against methicillin-resistant *Staphylococcus aureus*. Another very important mechanism of resistance is by producing the enzyme beta-lactamase, which cleaves the beta-lactam ring preventing it from attaching to the penicillin-binding proteins, e.g., peptidoglycan transpeptidase. Beta-lactamase inhibitors can be co-formulated with cephalosporins to increase their spectrum of activity, e.g., ceftazidime avibactam, and ceftolozane tazobactam.

Adverse Effects

Cephalosporins have low toxicity and are generally safe. The most common adverse reactions from cephalosporins are nausea, vomiting, lack of appetite, and abdominal pain. The less common adverse reaction includes:

Hypersensitivity Reaction

A hypersensitivity reaction to cephalosporin is infrequent and is more common in first and second-generation cephalosporins. Common allergic reaction to cephalosporin includes rash, hives, and swelling. Rarely, will the hypersensitivity reaction result in anaphylaxis. Patients who

are allergic to penicillin might show a hypersensitive reaction to cephalosporins as well. This cross-reactivity is once again more common in first and second-generation cephalosporins, because they have R-groups more similar to penicillin G. Third generation and beyond show minimal cross-reactivity.

Drug-induce Immune Hemolytic Anemia (DIIHA)

The proposed mechanism of action of DIIHA is that the drug binds to the red blood cell membrane, this causes no harm to the red blood cell itself nor the patient. However, if the patient starts making IgG antibodies against the drug, the antibody will bind the red blood cell. The immune system will react with the abnormal red blood cell resulting in hemolysis. Cefotetan and ceftriaxone are the two cephalosporins most likely to cause DIIHA.

Disulfiram-like Reaction

Cephalosporins containing a methyltetrazolethiol side chain can inhibit the aldehyde dehydrogenase enzyme resulting in the accumulation of acetaldehyde. Cefamandole, cefoperazone, and moxalactam are the most common cephalosporin to present with this reaction.

Vitamin K Deficiency

Certain cephalosporins can inhibit vitamin K epoxide reductase, preventing the production of the reduced(active) vitamin K. Therefore, there is a decreased synthesis of coagulation factors and the patient is predisposed to hypoprothrombinemia.

2. **Antiviral agents**:

Antiviral agents are used to treat viral infections by inhibiting the replication of viruses or targeting specific viral enzymes or proteins. They are available for the treatment of various viral infections, including influenza, herpes viruses, HIV, hepatitis, and others. Antiviral agents can help to reduce symptoms, prevent viral spread, and manage chronic viral infections.

3. **Antifungal agents**:

Antifungal agents are used to treat fungal infections. They can be categorized into several classes, including azoles, polyenes, echinocandins, and allylamines. These medications work by targeting specific components of fungal cells, disrupting their growth, and eliminating the infection. Antifungal agents are used to treat conditions like candidiasis, ringworm, athlete's foot, and systemic fungal infections.

Clinically, fungal infections are best categorized, first according to the site and extent of the infection, then the route of acquisition, and finally, the virulence of the causative organism. These classifications are essential, when determining the most effective treatment regimen for a particular mycosis. Mycosis classify as local (superficial, cutaneous, subcutaneous) or systemic (deep, bloodborne). The acquisition of the fungal infection is either an exogenous (airborne inhalation, cutaneous exposure, percutaneous inoculation) or endogenous process (normal flora or reactivated infection). The virulence of the organism classifies as either a primary infection (disease arising in a healthy host) or opportunistic infection (disease arising in human hosts, that have a compromised immune system or other defenses).

Antifungal drugs represent a pharmacologically diverse group of drugs that are crucial components in the modern medical management of mycoses. While, antimycotic pharmacology has advanced significantly, particularly in the last three decades, common invasive fungal infections still carry a high mortality rate: *Candida albicans* (approximately 20 to 40% mortality), *Aspergillus fumigatus* (approximately 50 to 90%), *Cryptococcus neoformans* (approximately 20 to 70%). Amphotericin B deoxycholate, a polyene antibiotic, was the first antimycotic agent introduced in 1958, to treat systemic mycoses. While, this drug is an effective agent, the demand for other efficacious topical, oral, and intravenous was apparent. Griseofulvin was introduced in 1959, representing a second class of antifungals. The next significant introduction would not take place until 1971, when the antimetabolite drug flucytosine entered the market. Azoles first became available in 1973, with the arrival of clotrimazole; with additional azoles that have the pharmaceutical industry has rolled out over the past five decades: miconazole (1979), ketoconazole (1981), fluconazole (1990), itraconazole (1992), voriconazole (2002), posaconazole (2006), and most recently (isavuconazole) sulfate water solubility producing, 2015.

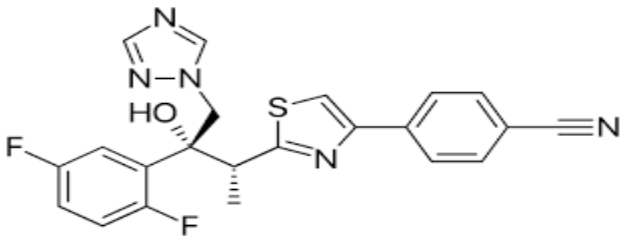

Fig.12.3: Molecular structure of isavuconazole

Terbinafine, an allylamine antifungal, was approved in 1996, by FDA, but has indications for the treatment of local, non-systemic fungal infections. The next break through in systemic therapy, would have a basis in amphotericin B lipid formulations, which have more favourable side effect profiles. Following lipid formulations of azoles, a new class of antifungal agents that are highly effective in treating some systemic mycoses, are the recently developed echinocandins class. While, the echinocandins demonstrate less renal toxicity than amphotericin B, they cause significant hepatotoxicity and are more expensive than azoles, this effectively relegates this class to being second or third-line agents. Mechanistically, antifungal agents are diverse, yet due to the alarming and rapid increase in drug-resistant systemic fungal infections, new agents are necessary more than ever. This discussion will focus on the currently available antifungal agents.

Table 12.1: List of medically relevant fungal infections

Fungal Infection	*Typical causative organisms*
Aspergillosis	*Aspergillus fumigatus, A. flavus*
Blastomycosis	*Blastomyces dermatitidis*
Candidiasis	*Candida albicans, C. glabrata, C. krusei, C. parasilosis, C. tropicalis*
Chromoblastomycosis (Chromomycosis)	*Cladosporium carrionii, Phialophora verrucosa, Fonsecaea pedrosoi*
Coccidioidomycosis	*Coccidioides imitis, C. posadasii*
Cryptococcosis	*Cryptococcus neoformans, C. gattii*
Dermatophytosis (Tinea)	*Microsporum spp., Epidermophytum spp., Trichophyton spp*
Fusariosis	*Fusarium oxysporum, F. proliferatum, F. verticillioides*
Histoplasmosis	*Histoplasma capsulatum*
Mucormycosis (Zygomycosis)	*Mucor spp., Rhizopus spp*
Paracoccidioidomycosis	*Paracoccidioides brasiliensis*
Pneumocystis pneumonia	*Pneumocystis jirovecii* (formerly called *P. carinii*)

12.8. Mechanism of action antifungal and antibiotics

The mechanism of action of different antifungal and antibiotics varies widely, such as:

- **Polyene** antifungals (e.g., amphotericin B) bind to ergosterol, a steroid-alcohol unique to Fungi. The polyene-ergosterol complex creates pores in the fungal cell membrane, ultimately leading to electrolyte leakage, cell lysis, and ultimately cell death.

- **Azole** (e.g., miconazole) antifungal compounds are non-competitive inhibitors of the fungal enzyme, lanosterol 14-alpha-demethylase, which is a rate-limiting enzyme in the fungal biosynthetic pathway of ergosterol. This action destabilizes the fungal cell membrane, causing cell content leakage, lysis, and eventually death of cell.
- **Allylamines** (e.g., terbinafine) inhibit the rate-limiting enzyme squalene epoxidase, responsible for synthesizing precursors to ergosterol. This type of drug is another antifungal compound whose mechanism of action is based on the loss of cell membrane integrity.
- **Echinocandins** (e.g., caspofungin) inhibit the fungal, beta-(1, 3)-D-glucan synthase, which is the enzyme responsible for synthesizing beta-(1,3)-D-glucan, a key component of fungal cell walls. Losing this cell wall component leads to osmotic instability and cell death.
- **Griseofulvin** is a mitotic inhibitor, which binds to polymerized fungal microtubules, thereby inhibiting the de-polymerization and leading to the failure of the fungal cell replication.
- **Flucytosine** is an antimetabolite compound, absorbed into fungal cells via cytosine permease. Within the fungal cell, flucytosine gets converted to 5-fluorouracil, which interferes with fungal RNA biosynthesis.
- **Ciclopirox** has a poorly understood mechanism of action, but, it is believed to interfere with the structural integrity of the fungal cell membrane.
- **Quinoline** antifungal (e.g., clioquinol) compound derivatives also have a poorly understood mechanism of action.
- **Potassium Iodide** exerts its effects directly on *Sporothrix sp.*, yet the exact mechanism of action remains unproven. Leading theories, suggest that human polymorphonuclear cells, convert potassium iodide to iodine via the action of myeloperoxidase. Iodine inhibits fungal germination and reduces structural integrity through the intracytosolic destruction of structural components.
- **Zinc Pyrithione** has a poorly understood, antifungal mechanism of action. Still, leading theories suggest this agent modifies fungal cellular membrane transport, leading to decreased concentrations of critical metabolic substrates, inhibiting protein synthesis, and limiting ATP

synthesis. These metabolic changes are likely due to an increase in intracellular copper and iron-sulfur clusters, which lead to protein damage.

4. **Antiparasitic agents**:

Antiparasitic agents are used to treat infections caused by parasites, including protozoa (very small microparasite) and helminths (large macroparasite). They can be further divided into antiprotozoal agents and antihelmintic agents. Antiparasitic medications are used to treat conditions like malaria, amoebiasis, giardiasis, leishmaniasis, and various parasitic worm infections.

5. **Antiseptics and disinfectants**:

These are substances used to kill or inhibit the growth of microorganisms on the skin, wounds, or inanimate objects. They are commonly used for wound care, hand hygiene, and surface disinfection. Examples of antiseptics include alcohol, iodine, hydrogen peroxide, and chlorhexidine.

12.9. Broad-spectrum antibiotics

Broad-spectrum antibiotics have activity against a wide range of bacteria, including many Gram-positive bacteria (such as Staphylococcus and Streptococcus species) and Gram-negative bacteria (such as Escherichia coli, Klebsiella, and Pseudomonas species). They are designed to cover a diverse array of bacterial pathogens. Broad-spectrum antibiotics are often used in situations, where the specific bacterial pathogen causing the infection is unknown or when there is a risk of multiple bacteria being involved. They are commonly employed in the treatment of severe infections, such as complicated urinary tract infections, pneumonia, intra-abdominal infections, and sepsis. Some examples of broad-spectrum antibiotics include fluoroquinolones (such as ciprofloxacin and levofloxacin), third-generation cephalosporins (such as ceftriaxone and ceftazidime), carbapenems (such as imipenem and meropenem), and certain penicillins (such as amoxicillin-clavulanate). While, broad-spectrum antibiotics have a wide range of activity,but their use comes with certain risks. They can disrupt the normal balance of microbial flora in the body, leading to an overgrowth of drug-resistant bacteria or opportunistic infections, such as

Clostridium difficile-associated diarrhea. Additionally, the widespread use of broad-spectrum antibiotics can contribute to the development of antibiotic resistance.

12.10. Molecular mechanism of drug resistance

With the advent of technological advancements, the rising scientific era witnessed the emergence of infectious diseases. This led to a sharp increase in global mortality and morbidity rate. Hence, the research community held up the war against these pathogens by investigating deep into their molecular mechanisms, their host–pathogen interaction, and their epidemiology for the discovery of fine effective antimicrobial measures for host survival and safety. The researchers treated the pathogenic ailments with useful inventions for long-term medication. Drug generally implies to foreign elements or agents that have some medicinal properties for common therapeutic usage. They can be used for bacterial infections, even as antifungal or antiparasitic agents, for cancer treatments, etc. The discovery of antibiotics was the greatest medical intervention affecting, human survivability and health regime.

However, indiscriminate usage had dramatically introduced new biological problems that are hard to confront with the present-day scientific solutions. Hence, failure of medications did set the dawn of a post-antimicrobial era. At the time of the Second World War, had limited access to these expensive, rare, systemic medications (sulfonamides, penicillin, etc.). With time, simplified production of formulations, eased the use of such treatments. Gradually, these antimicrobial agents, mostly antibiotics, became the elixir for the ailments from time then.

Moreover, the discoverer of penicillin by Sir Alexander Fleming, warned the surfacing of resistant, forms of *Staphylococcus aureus*, due to improper penicillin usage, which would cause serious host complications. Few years later, resistant forms emerged with 50 % of susceptible strains, becoming resistant to the drug. Similar trend was observed in many other microbial species switching their drug sensitivity approach to a severe resistance mechanism, thereby affecting healthy non-vulnerable population. This section will discuss in detail, the emergence of drug-resistance in microbial populations, and the factors that govern their drug-resistant feature. The major focus of this segment will high light the molecular, cellular, clinical, and genetic factors, that bring about this severe cause of drug resistance. Beginning from the natural microbial resistance to the evolutionary alteration in the pathogen’s genome, this chapter will cover the idea of how dealing with the conventional drug resistance mechanisms in the twenty-

first century, will create new frontiers for innovative therapeutic development. The problems and the complex challenge of dealing the multidrug resistance (MDR) mechanism at the molecular level, will enable strategies for futuristic drug development for combating fungal, bacterial, and viral resistance mechanism.

12.10.1.Emergence of Drug Resistance

Adaption is a very essential condition for survival as well as sustenance. All living organisms, nurture themselves with crucial components from their living system. In addition to fundamental requirements, adaption against the toxic agents also requires armors of endurance. The adage "survival of the fittest" also applies to the environmental sustenance of microbes. This microbial tolerance has enabled the mechanism of resistance, as one of the means to combat the harmful environmental effects. This results in conferring multiple drug resistance within pathogens against idle treatments. The first drug resistance occurred against penicillin and sulfonamides against *S. aureus*.

The discovery of antibiotics led to the emergence of antibiotic resistance in the following two or more decades. The pathogens in the hospitals were not only reported to be resistant to the therapeutics, but also remained viable for further infecting the vulnerable individuals with weakened immune system. The nineteenth century had an impressive pattern of increased tolerance mechanism, among the pathogens from sulfonamide and penicillin-resistant *S. aureus* to multidrug-resistant *M. tuberculosis*.

Some gastroenteric pathogens like *Shigella, Salmonella, V. cholera, E. coli, P. aeruginosa*, etc., also developed resistance against many antimicrobials during the course of time. Some strains, also enabled community-dependent infection spread like *Streptococcus* developing resistance to penicillin, and *S. aureus* and *Enterococcus* developing resistance to vancomycin.

12.10.2.Factors for Drug Resistance Development

Antibiotic resistance is a serious global issue that has seized the roots of development. Antimicrobial resistance affects host immune profile, modulates with pathogen's fitness cost, and influences the genetic co-selection of resistant species with their frequency of reversibility potential. The biologic mechanisms of the microbe are mostly responsible for such a resistant

feature to fight the environmental toxic conditions. The inherent property of the pathogen, i.e., the natural resistance of the microbe, is a reason of resistance emergence. The major causative factor of resistance development is also the frequency of appearance of resistant bacteria due to genetic mutations or evolutionary horizontal gene transfer.

12.10.3.General Mechanism of Drug Resistance

Drug development still forms the top headed research enterprise globally due to unsuccessful therapeutic reign of potent drugs over microbial weapons. The term “drug” is generally applied to all foreign chemicals, including antibiotics, herbicides, and therapeutic agent, used against virus, parasites, cancer, etc. The host–microbe warfare has led to the compromise of clinical interventions and rise of multidrug-resistant species (*Streptomyces*). Resistance to seven or more antibiotics has even led to a resistance phenotype for around 20 drugs. Such mechanisms have made the environment emerge into a reservoir of pathogen tolerance.

The emergence of new infectious agents causing AIDS, SARS, etc., has modulated the resistance standards with raised clinical challenges. The fast-growing drug resistance mechanism will become the signature of potent microbes inhabiting the environment with new emerging diseases, and higher tolerance level causing mortality and morbidity. Understanding of microbial genetics and gene manipulation modes, will give a greater insight and provide a new dimension into fighting the resistance mechanisms.

12.10.3.1.Intrinsic Resistance

Intrinsic resistance is defined as the ability of an organism to resist the antimicrobial or chemical compounds using a characteristic feature, which is an inherent or integral property developed by virtue of evolution. This can also be referred to as “insensitivity” due to the invulnerable nature of the organism toward that particular drug. The natural resistance feature, though less prevalent, sometimes undergoes spontaneous genomic alterations due to the absence of antibiotic-based selective pressure. However, mostly the antibacterial-based microecological pressure triggers the stimulus for pathogen adaptation by the development of drug resistance. Mutations or evolutionary competition enables drug resistance gene uptake. It can arise due to certain events as outlined in Fig. 1 and mentioned below:

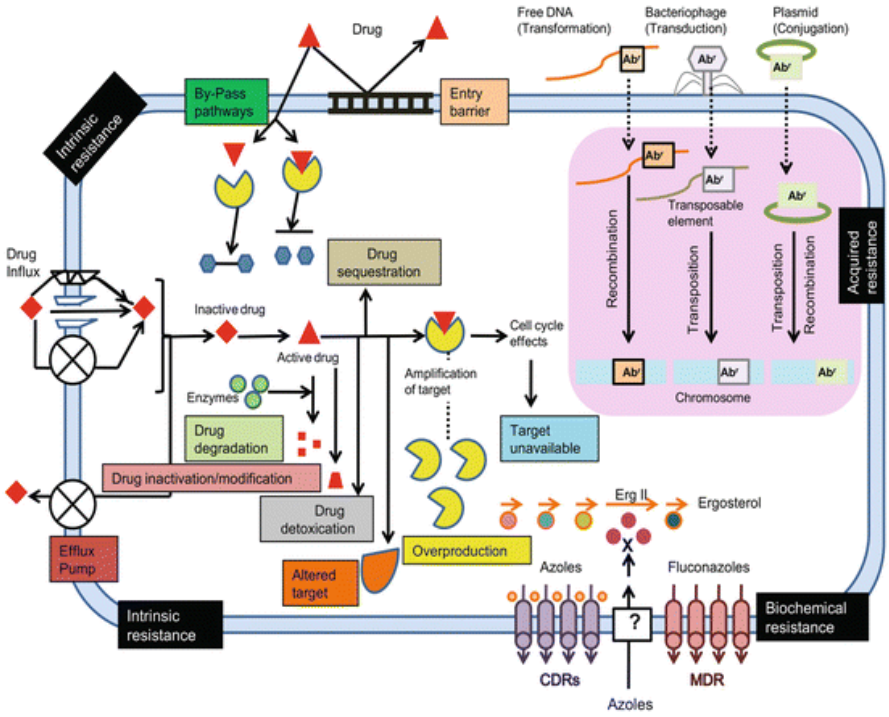

Fig. 4 Schematic presentation of multiple diverse molecular mechanism of microbial resistance

12.10.3.2.Absence/Modification of Target Site

Microbial uptake of an antimicrobial drug is essential for a target-oriented action. Porins serve as the passageways for the drugs to cross the outer membrane of the bacterial cell. Some bacteria have the ability to manipulate their cell wall or membrane in order to protect themselves from foreign drugs. For example, certain Gram-negative bacteria can significantly lessen the uptake of certain antibiotics, like amino glycosides, by altering the membrane porin frequency, size, and selectivity. On the other hand, in some bacteria, the modification in the PBP (penicillin-binding protein) site led to the insensitivity toward the β-lactam antibiotics.

12.10.3.3.Species-Specific Structure of Target Site

Although, the mode of action of antibiotics is almost similar across the same community of bacteria. However, species specificity has been detected in some cases. This is due to the lack

of affinity of the drug to its target site. Different species under a single genus of a bacterium can alter the binding site of the drug, by presenting various structural motifs for the same target, thus developing resistance. For example, the crystal structures of the large ribosomal subunit, in *Staphylococcus aureus*, showed specific structural motifs (short segment of protein structure) and binding modes for different antibiotics of same function as well as for a particular drug against different species of the bacteria.

12.10.3.4.Inactivation of Antimicrobial Agents via Modification/Degradation

Destroying or manipulating the active component of the antimicrobial drug has always been considered as one of the effective techniques adopted by microbes for protection. For example, in penicillins and cephalosporins, the bacterial enzyme beta-lactamase, hydrolyzes and deactivates the beta-lactam ring producing inactive penicilloic acid. It is then unable to bind to the PBPs, thereby maintaining the cell wall synthesis of the bacteria. This kind of inactivation has been observed in many Gram-negative and Gram-positive bacteria against chloramphenicol, aminoglycosides, etc., via acetylation, phosphorylation, and adenylation.

12.11. Sulfa drugs

Sulfa drug, also called **sulfonamide**, is member of a group of synthetic antibiotics containing the sulfanilamide molecular structure. Sulfa drugs were the first chemical substances systematically used to treat and prevent bacterial infections in humans. Their use has diminished, because of the availability of antibiotics that are more effective and safer and because of increased instances of drug resistance. Sulfonamides are still used, but largely for treating urinary tract infections and preventing infection of burns. They are also used in the treatment of certain forms of malaria.

The antibacterial effects of sulfonamides were first observed in 1932, when German bacteriologist and pathologist Gerhard Domagk noted the effects of the red dye P rontosil on *Streptococcus* infections in mice. It was later proved by French researchers that the active agent of Prontosil was sulfanilamide, or *para*-aminobenzene sulfonamide, a product of the body's metabolism of Prontosil. By the 1940s, sulfanilamide was a widely used drug. During World War II, white sulfanilamide powders became standard in first-aid kits for the treatment of open wounds, and sulfanilamide tablets were taken to fight intestinal infections.

Though, the medicine was relatively safe, allergic reactions such as skin rashes, fever, nausea, vomiting, and even mental confusion were common. With the introduction of less-toxic derivatives and especially with the mass production of penicillin, its use declined.

Many other sulfa drugs were derived from sulfanilamide in the 1940s, including sulfathiazole (systemic bacterial infections), sulfadiazine (urinary tract and intestinal tract infections), and sulfamethazine (urinary tract infections). However, all sulfa drugs induced some of the side effects listed above, and bacteria developed resistant strains after exposure to the drugs. Within a few decades many of the sulfa drugs had lost favour to more-effective and less-toxic antibiotics. Trisulfapyrimidine (triple sulfa), a combination of sulfadiazine, sulfamerazine, and sulfamethazine, is used in the treatment of vaginal infections, and several sulfa drugs are used in combination with antibiotics to treat a wide range of conditions, from skin burns to malaria to pneumonia in HIV/AIDS patients.

Sulfa drugs are bacteriostatic, i.e., they inhibit the growth and multiplication of bacteria but do not kill them. They act by interfering with the synthesis of folic acid (folate), a member of the vitamin B complex present in all living cells. Most bacteria make their own folic acid from simpler starting materials. However, humans and other higher animals must obtain folic acid in the diet. Thus, sulfa drugs can inhibit the growth of invading microorganisms without harming the host.

When trimethoprim (a dihydrofolate reductase inhibitor) is given with sulfamethoxazole, the sequential blockage of the pathway produced by the two drugs achieves markedly greater inhibition of folic acid synthesis. As a result, this combination is valuable in treating urinary tract infections and some systemic infections. The sulfones are related to the sulfonamides and are inhibitors of folic acid synthesis. They tend to accumulate in skin and inflamed tissue, and are retained in the tissue for long periods. Thus, sulfones, such as dapsone are useful in treatment of leprosy.

12.12. Summary

Chemotherapy refers to the use of drugs to treat cancer. It works by targeting and destroying rapidly dividing cancer cells, either by directly killing them or by inhibiting their growth. Chemotherapeutic agents can be administered orally, intravenously, or through other

methods, and they circulate throughout the body to reach cancer cells in various organs and tissues.

Chemotherapy can be used as a stand alone treatment or in combination with other treatments, such as surgery or radiation therapy. It is important to note that chemotherapy drugs can also affect healthy cells that divide rapidly, leading to side effects such as hair loss, nausea, and lowered immune function. This results in the most common side-effects of chemotherapy: myelosuppression (decreased production of blood cells, hence also immunosuppression), mucositis (inflammation of the lining of the digestive tract), and alopecia (hair loss). Because of the effect on immune cells (especially lymphocytes), chemotherapy drugs often find use in a host of diseases that result from harmful over activity of the immune system against self (so-called autoimmunity). These include rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, vasculitis and many others.

The number of chemotherapeutic agents are used in treatment these are-1.Alkylating agent 2.Antimetabolites 3.Topoisomerase inhibitors 4.Anthracycline 5.Taxanes 6.Targeted therapies.

The different types of chemotherapy are as under-i.Adjuvent chemotherapy ii. Neo-adjuvent chemotherapy iii. Palliative chemotherapy iv.Continuation chemotherapy v. High dose chemotherapy vi. Targeted chemotherapy vii. Immuno chemotherapy viii.Hormonal chemotherapy.

Chemotherapic drugs given,intraveinously, orally, intramuscularly, intraartrial, intraperitonial.The drug, dose and treatment type depends upon various factors, actually, these are decided by an oncologist.

Antibiotics are drug snthesized one microorganism and able to kill or decrese the growth of other pathogenic bacterial microorganism, and curing the disease.These are used to treat the different bacterial diseases, such as-pneumonia, urinary tract infection, respiratory infection, bacterial meningitis, cholera, tuberculosis, measls, skin infection, gonorrhia, wound infection, throat infection,etc.Commonly used antibiotics are-Penicillins, cephalosporins,tetracyclines,etc.

Antibiotics are classified to two-

1.Narrow- spectrum antibiotic-only able to kill or inhibit the growth of limited number of bacteria, e,g., fidaxomicin and sarecycline.

2.Broad-spectrum antibiotic-they are able to kill or inhibit growth of number of pathogenic bacteria,e.g.,penicillins, cephalosporins (have five generations), azithromycin, amoxicillin, tetracycline, ofloxacin, chloramphenicol and quinolones.Hower,some time, use of antibiotic develop antibiotic resistance in pathogenic bacterium against antimiotic and patient remains uncured, this is due to over use or misuse of antibiotic. Some time irregular treatment (mismanaged, patient do complete their full course) develop antibiotic drug resistance in patient, e.g.,MDR TB, XDR TB (rare tye).In these cases,TB disease again develop in patient, after having taken TB medicine in the past.

Antimicrobial anents are broader category of drugs (medecines) used against broader category of disease causing microorganisms, such as bacteria, virus, fungi, parasites.According to micoorganism they infect, these are classified as-

1.Antibiotics-effective against bacterial infection

2.Antiviral agent-effective against virus infection, herpes, HIV, influenza, hepatitis

3.Antifungal agent-effective against fungal infection (mycosis), ringworm, candidiansis, athletes foot.The common antifungal drugs are amphotericin- B, griseofulvin, allylamine, azoles, polyene.

4.Antiparasitic agent- effective againt parasite (protozoa, helminths) causing malaria, giardiasis.

5.Antiseptic and disinfectants-effective against a germ microorganism present up on skin, wounds or inanimate objects. Usually, alcohal and hydrogen peroxide are used.

Sulfa drugs are synthetic broad-spectram antibiotics used against number bacterial infection in humans.They are bacteriostatic in nature, i.e., inhibit the growth of bacterium but do not kill them. Sulfa powder or sulfanilimide is used in second world war to treat wounds of soldiers.

Antifungal agents are medications used to treat fungal infections. They specifically target fungal pathogens, either by disrupting their cell membranes, inhibiting cell wall synthesis, or interfering with key metabolic processes. Antifungal agents can be classified into different classes, including polyenes, azoles, echinocandins, allylamines, and pyrimidine analogs, each with a specific mechanism of action. Fungal infections can affect various parts of the body,

including the skin, nails, mouth, throat, lungs, and internal organs. Proper diagnosis and treatment selection are crucial for effective antifungal therapy, as different antifungal agents may be more effective against specific types of fungi. Chemotherapy, antibiotics, and antifungal agents are powerful classes of medications used to treat cancer, bacterial infections, and fungal infections, respectively. They have different mechanisms of action and target specific types of cells of microorganisms. Proper usage, adherence to prescribed treatments, and monitoring for potential side effects or drug resistance are important considerations in their use. It is always recommended to consult with healthcare professionals for accurate diagnosis and appropriate treatment options.

12.13. Terminal questions

Q.1: Describe chemotherapy and its side effects on living systems.

Answer:----------

Q.2: What are the antimicrobial agents? Describe it.

Answer:----------

Q.3: Describe antibiotics with their history.

Answer:----------

Q.4: Describe penicillin and how do penicillin works?

Answer:----------

Q.5: Explain medical uses of antibiotics in brief.

Answer:----------

Q.6: What are the broad spectrum antibiotics?

Answer:----------

12.14. Further readings

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