COURSE INTRODUCTION

The objective of this course is to cover the process of microbiology and toxicology. The discussion of microbial growth and development has been carried out in this book. The microbial nomenclature, classification and basic characteristic of microbes is briefly discussed. The principles of microbial nutrition, construction of culture media, isolation and culture of aerobic and anaerobic bacteria are discussed briefly. The Microbial nutrition reveals modes of nutrition in bacteria. Microbes act as antibiotic agent and its role in environment discussed in this chapter.

Block 1 covers the Microbial diversity and culture

Block 2 deals the microbial nutrition and chemotherapy

Block 3 describes in brief on microbial diseases and environmental toxicology

Rajarshi Tandon Open University, Prayagraj

Microbiology and Toxicology

Course Design Committee

Dr. (Prof.) Ashutosh Gupta,
Chairman

School of Science, UPRTOU, Prayagraj

Prof. Prof. Umesh Nath Tripathi **Member**

Department of chemistry

Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Prof. S.I. Rizvi **Member**

Department of Biochemistry

University of Allahabad, Prayagraj

Prof. Dinesh Yadav **Member**

Department of Biotechnology

Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Prof. Sharad Kumar Mishra **Member**

Department of Biotechnology

Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur

Dr. Ravindra Pratap Singh **Member**

Academic Consultant (Biochemistry)

School of Science, UPRTOU, Prayagraj

Dr. Dharmveer Singh **Course Coordinator**

Academic Consultant (Biochemistry)

School of Science, UPRTOU, Prayagraj

Course Preparation Committee

Dr. Saroj Ahirwar **Author** **Block-1,&2** (Unit: 1&3)
Assistant Professor
Department of Industrial Microbiology,
(JIBB), SHUATS

Dr. Arun Kumar Pandey **Author** **Block-1&6 (Unit:4)**
Department of Zoology
PSMPG College,
Anand Nagar Maharaj Ganj

Dr. Sushma Chauhan **Author** **Block-1 (Unit: 2)**
Academic consultant
School of Science, UPRTOU, Prayagraj

Dr. Deepa Chaubey **Author** **Block-3 (Unit: 5)**
Academic consultant
School of Science, UPRTOU, Prayagraj

Dr. Dharmveer Singh **Author** **Block-3 (Unit:6)**
Academic consultant
School of Science, UPRTOU, Prayagraj

Dr. **Ayodhya Prasad Verma** **Editor** **Block-1&2 (Unit: 1,2,3,5)**
Associate Professor,
Department of Botany
Bappa Sri Narain Vocational Post Graduate College,
Lucknow

Dr. **Pramode Kumar Pandey** **Editor** **Block-2&3 (Unit:4-5)**
Associate Professor,
Department of Zoology
PSMPG College,
Anand Nagar Maharaj Ganj

Dr. Dharmveer Singh
(Course Coordinator)
School of Sciences, UPRTOU, Prayagraj

Block- I

Microbial diversity and culture

UNIT -1

Microbial diversity and systematic

UNIT-2

Methods in Microbiology

Introduction

This is the first block on microbial diversity and culture. It consists of following two units:

Unit-1: This unit covers the general introduction of microbial diversity and systematic. The microbial taxonomic based of modern approach has been clearly defined. In addition, the characteristic of primary domains and of taxonomic groups belonging to bacteria, archaea and Eukarya is also discussed. Nomenclature and outline of bacterial classification as per Bergey's Manual describes briefly. Accessing microbial diversity using molecular methods such as Denaturing Gradient Gel Electrophoresis (DGGE) also discussed in this unit.

Unit-2: Sterilization play important role in microbial growth and development. In this unit the theory and practices of sterilization is described. In addition some cultural techniques and their role in microbial growth mentioned here. Apart from this, the principles of microbial nutrition, construction of culture media, isolation and culture of aerobic and anaerobic bacteria is discussed briefly. The culture collection, preservation and maintenance of microbial cultures mentioned in this unit.

Unit-1: Microbial diversity and systematic

1.1. Introduction

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 microbiology or 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 bacteria is also comes under the toxicology is called polyphasic because it is based on 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. With in 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: Large organisms have many easily distinguished features (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 arrange of units.

Identification, once is known by characterization and by their special features

Nomenclature once 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 relatedness. The bacterial classification was firstly done by Mueller, 1786; Ehrenberg, 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 based. 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 biology, with high form of life, a species unit constitutes a stage of evolution, with a characteristic morphology. But do 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.

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 fist 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 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 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.

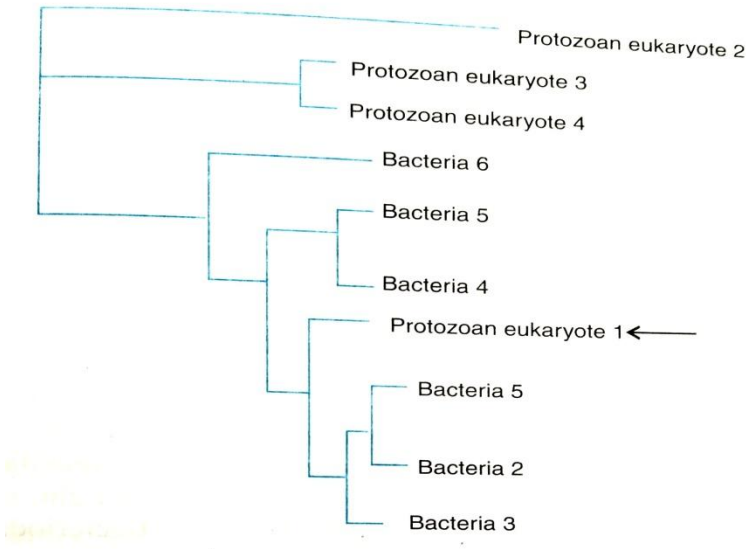


Fig. 1.1: Phylogenetic tree

This 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 and widely accepted by scientists, The three domains are

1. Bacteria
2. Archaea
3. Eukarya

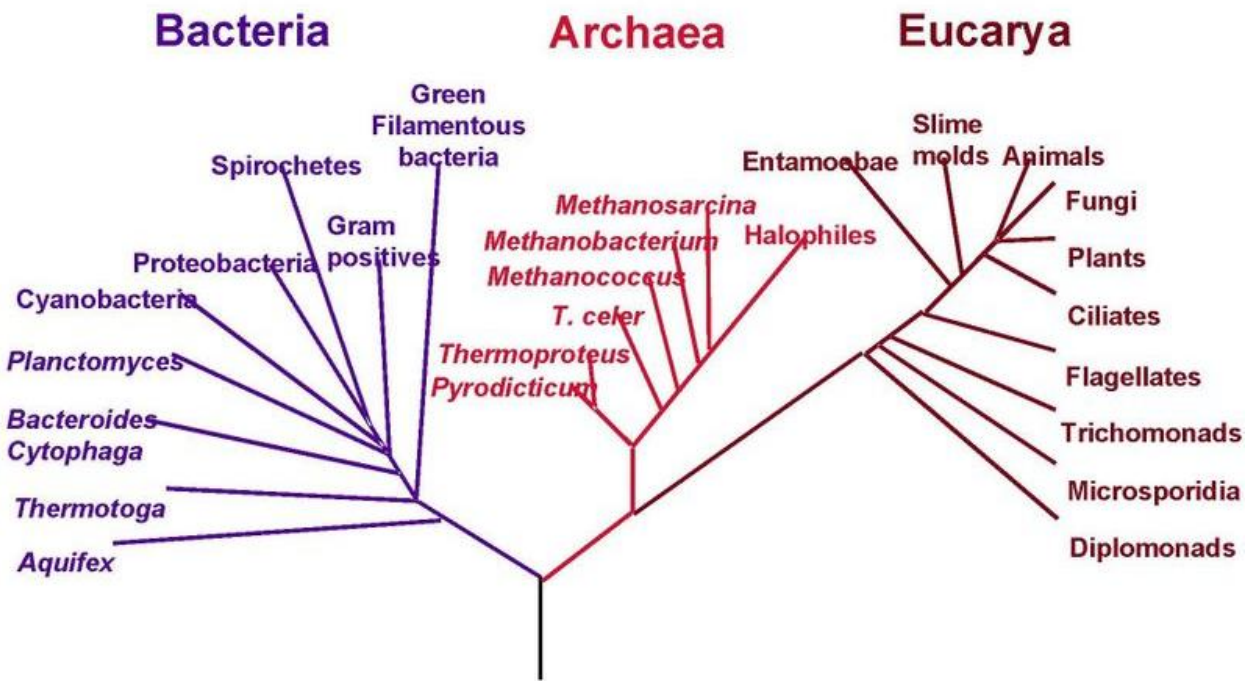


Fig.1.1: Demonstration of phylogenetic tree of life.

The Bacteria (Eubacteria)

Eubacteria are prokaryotic microorganisms consisting of a single cell lacking a nucleus and containing DNA is a single circular chromosome. Eubacteria, or "true" bacteria, are single-celled prokaryotic microorganisms that have a range of characteristics and are found in various 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 ribosome's. The example of Eubacteria is E. *coli*, *Lactobacilli,* and *Azospirillum.*

The Eubacteria has following three characteristics such as

i. The bacteria are unicellular microorganisms of a prokaryotic cell.

ii. They contain 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 nucleus and cell organelles present in eukaryotic cells.

vi. Their DNA is not inside a nucleus

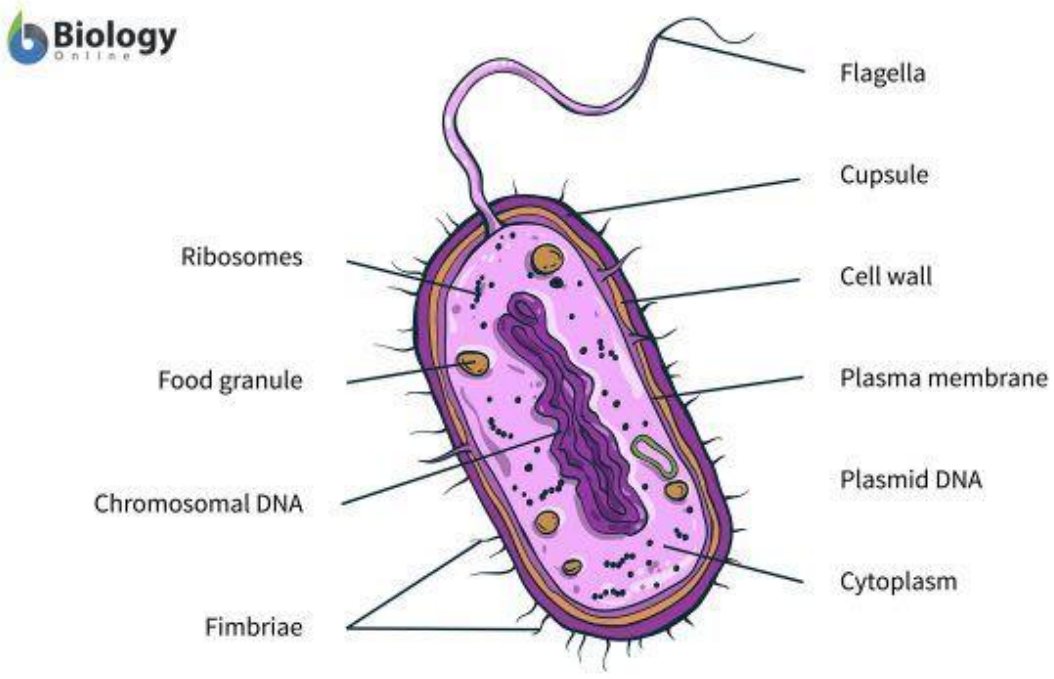


Fig.1.3: Image of Eubacteria

The Archaea (archaebacteria)

The Archaea are primitive, single-celled microorganisms that are prokaryotes with no true nucleus (early nucleolus, primitive nucleolus). 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 (4) in all cases known so far, their occurrence only in unusual habitats.

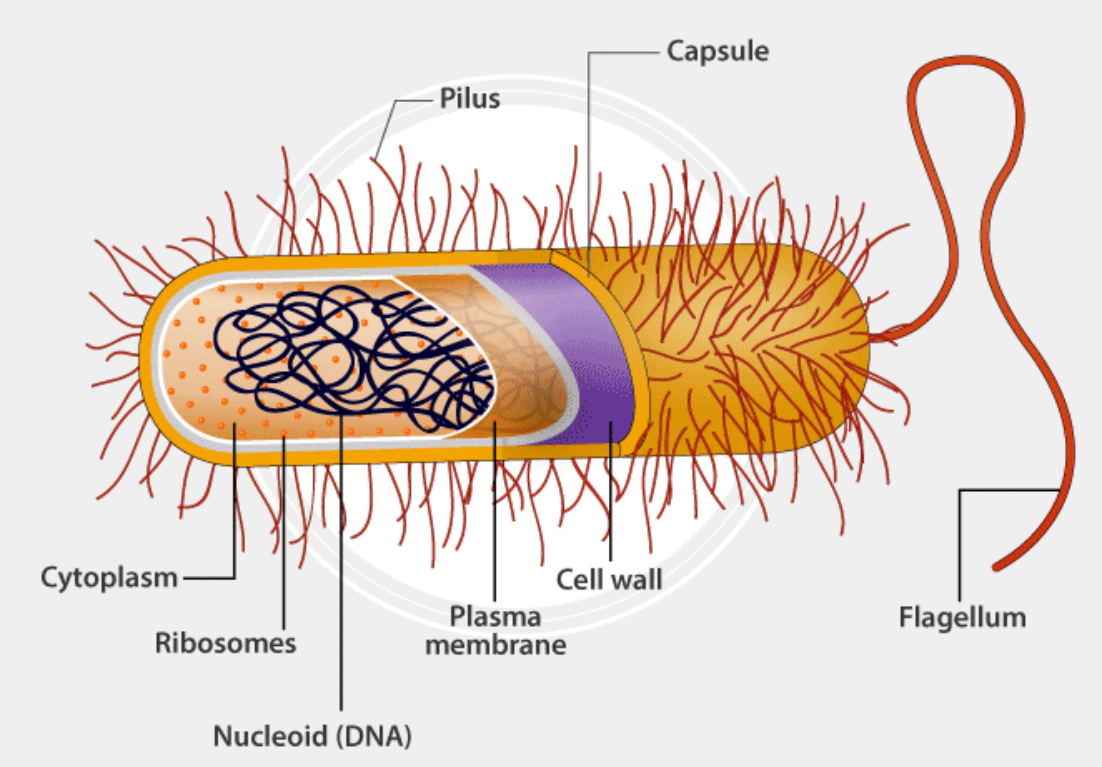


Fig.1.2: Morphology of archaebacteria

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 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. The cells of eukaryotes divide by mitosis and meiosis. While, mitosis gives rise to two *daughter cells* and meiosis gives rise to four daughter cells. The cells from meiosis will be haploid after two consecutive divisions. The cell is an entire organism capable of performing all the fundamental

functions (e.g. ingestion, respiration, excretion, osmoregulation, homeostasis, etc.) that different systems do 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.

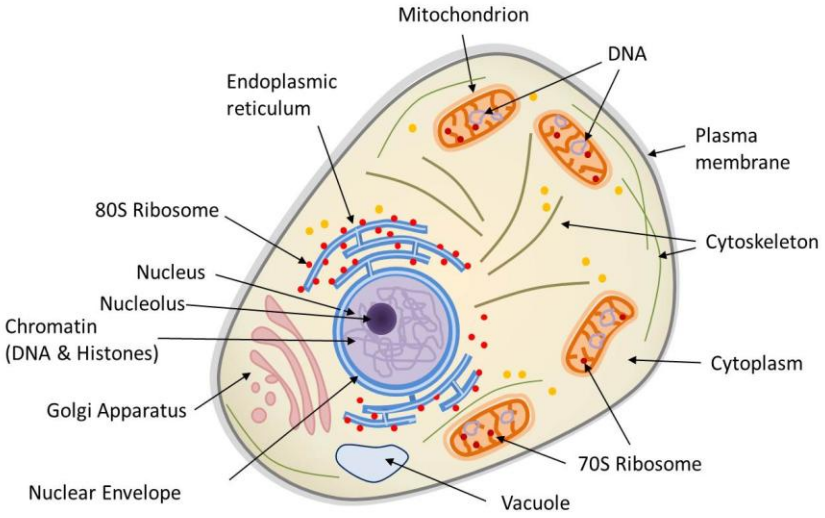


Fig.1.4: Cellular structure of eukaryotes

In modern sense, bacteria, cynobacteria, Actinomycetes are distributed in the modern domain bacteria. The methanogens, extremely hemophilic 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 of 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. There is some characteristic feature of taxonomy such as

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 biochemical properties, pathogenicity, antibiotic sensitivity, serological relationships etc. Taxonomically important morphological, cultural and physiological- biochemical characteristics are shown in Table 3.1, 2 and 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 : Taxonomically useful physiological and biochemical properties	
Characters	**Variations**
Nutritional type	Chemoorganotrophic, chemolithotrophic, photolithotrophic, photoorganotrophic.
Mode of energy metabolism	Respiration, fermentation, photosynthesis, oxidation of inorganic substrates, nitrate and sulfate respiration.
Nitrogen source utilization	Molecular nitrogen, ammonium salts, nitrate, organic nitrogenous compounds.
Carbon source utilization	CO_2, sugars, sugar acids, sugar alcohols, organic acids, polysaccharides.
Pathway for glucose dissimilation	Embden-Meyerhof, Pentose phosphate cycle, Enter-Doudoroff

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

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 things. It was developed by the Swedish botanist Carolus Linnaeus, who lived during the 18th Century, and his system of classification 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 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. 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 modern characterization techniques such as restriction fragment length polymorphism (RELP), DNA-DNA hybridization and 16S rRNA sequencing make the studies easier. These techniques create interest in bacterial toxicology.

Bacteria are scientifically recognized, using a binomial nomenclature 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 process. For example *Escherichia* coli, caused diarrhea, hemorrhagic colitis, dysentery like illness similar to *Shigella* infection. After that Carl Linnaeus, who gave classification system, which was already attempt 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 on 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. Bacterial classification as per Bergey's Manual

Bergey's manual, which first appeared in 1923 and, at present, is in its 9th edition under the title "Bergey's Manual of Systematic Bacteriology" is a major taxonomic treatment of bacteria (prokaryotes). The bergey's manual of systematic bacteriology has four volumes, that contain the internally recognized names and descriptions of bacterial species. The four volumes, their year of publication, and the sections and the groups of bacteria included in each of them are the following:

Vol. I: 1984 (sections 1-11): Gram-negative bacteria of general, medical, or industrial importance.

Vol. II: 1986 (sections 12-17): Gram-positive bacteria other than Actinomycetes.

Vol. IlI: 1989 (sections 18-25): Gram-negative bacteria with distinctive properties, cyanobacteria, and archaea.

Vol. IV: 1991 (sections 26-33): Actinomycetes (Gram-positive filamentous bacteria).

All prokaryotes were retained in this edition in a single kingdom Prokaryotiae, divided into four divisions called Gracilicutcs, Firmicutes, Tenericutes, and Mendosicutes.

Gracilicutes (thin skin): it possesses gram-negative cell wall;

Firmicutes (thick and strong skin); it have gram-positive cell wall

Tenericutes (soft or tender skin): it is lack cell wall and represented by mycoplasmas

Mendosicutes (skin with faults): it accommodating archaebacteria that lack conventional peptidoglycan.

1.8. Accessing microbial diversity using Molecular methods

The variety and variability among individual of organism is called biodiversity. The individual of organism are some time has various similar feature or morphological level but it would be different at genetical level. Due to this genetic variations individual of organism makes their colony and they seems similar in their colony. In nature, if you see small patch of earth, you will get a number of species like plants, animals and insects are existed in this place but they all are different. They may be differing on morphological and genetically level. Biosphere is a store house of various microorganisms. The structure and dynamics of microbial communities in several ecosystems are found due to their little fraction of microbial diversity. The bacterial community represented by the morphological and nutritional criteria failed to provide a natural taxonomic order according to the evolutionary relationship. Genetic diversity among the isolates recovered from mushroom compost has not been widely studied. Species diversity consists of:

1. Species richness
2. Total no. of species
3. Species evenness
4. Distribution of species

METHODS OF STUDYING MICROBIAL DIVERSITY

There are two method is existed to understand the microbial diversity.

1. BIOCHEMICAL METHODS
    - Plate Count
    - Community physiological profiling (CLPP)
    - Fatty acid methyl ester analysis (FAME)
2. MOLECULAR BASED METHODS

Based on the basic unit of life: nucleic acids Methods incl.:

- Guanine & cytosine (G+C) content
- Nucleic acid re-association and hybridization

- DNA microarrays
- DNA cloning & sequencing
- PCR-based methods

1.9. Denaturing Gradient Gel Electrophoresis (DGGE)

We know the microbial diversity is the verity and variability among the microbial organism where different kinds of unicellular organisms, bacteria, archaea, protists, and fungi are presents. Various different microbes thrive throughout the biosphere, defining the limits of life and creating conditions conducive for the survival and evolution of other living beings. The different kinds of microbes are distinguished by their differing characteristics of cellular metabolism, physiology, and morphology, by their various ecological distributions and activities, and by their distinct genomic structure, expression, and evolution. The study of microbial diversity and their function in contaminated soil creates a serious problem, because they observed significant limitations in methodology and taxonomy of this group. Methodology for the determination of bacterial diversity does not include their function in the soil and other environment areas. Microbes are known for their catabolic activity in bioremediation, but changes in microbial communities are still unpredictable. Denaturing gradient gel electrophoresis (DGGE) is a commonly used molecular technique for rapid fingerprint analysis of microbial community composition, diversity, and dynamics. DGGE, since it was introduced into microbiology by Muyzer and colleagues, has been used to analyze the composition of a range of microbial groups, including viruses and microbial eukaryotes. This method is rapid and affordable, allowing multiple samples to be processed simultaneously. The protocol provides a background to the theory and progress in DGGE techniques, and offers a detailed step-by-step procedure for laboratories employing DGGE for the analysis of bacterial populations from environmental samples. Potential sources of bias are highlighted in addition to a detailed troubleshooting section that helps to overcome common problems associated with DGGE analyses. In DGGE, DNA fragments with the same length but different sequences (even with one base-pair difference) can be separated in that they have different melting temperatures and the electrophoretic mobility of a partially melted DNA molecule will be decreased DGGE is PCR-based and can only detect dominant microbial species (commonly relative abundance > 0.1%) in environments.

1.10. 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 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.

1.11. Terminal questions

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

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

Q.2: Discuss the modern approach of taxonomy.

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

Q.3: Discuss the role of Denaturing Gradient Gel Electrophoresis (DGGE) in microbial study.

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

Q.4: What do you mean by nomenclature, discuss the role of primary domain in nomenclature?

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

Q.5: Discuss the bacterial classification as per Bergey's Manual.

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

Q.6: What do you understand by Polyphasic classification?

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

1.12. Suggested further 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. Foundations in Microbiology, By Kathleen Park Talaro and Barry Chess, 10 edition
6. Microbiology: An Introduction, 13th Edition by Gerard J. Tortora, Berdell R. Funke and Christine L. Case.

Unit-2: Methods in Microbiology

Structure

2.1 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 eye. Thus microbiology means study of small living things i.e. microbes. 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 septic 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.

Objectives:-

After studying this unit you will be able to know:

- Various methods of sterilization used in microbiology.
- Methods of culturing microbes and their nutritional requirement.
- Construction of various types of culture media and enriched culture techniques.
- Isolation and culture of aerobic and anaerobic bacteria
- Methods of preservation and maintenance of pure culture.

2.2 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 glass wares e.g. peridish 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 over. Therefore precaution is taken to put off the switch as soon as the required temperature reaches.

Inoculating loops and needles are used for inoculation. They 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 organisms present upon the surface.

b) Moist Heating: In sterilization moist heating is more 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 the process has three incubation periods which allow heat resistant spores surviving 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 an laboratory apparatus made up of tough double walled chamber designed for sterilization of media as well as apparatus. The autoclave works under the same principle as the pressure cooker where water boils at increased atmospheric pressure i.e. > 100^0C. For sterilization of liquid media a pressure of 15lb/in^2 (121^0C) is given for 10-15 minutes. Thus the high temperature is sufficient to kill vegetative cells and spores 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 bacteria only while the membrane filters retain all forms of microorganisms.

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 Petridishes.

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. Scince 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 methods like mercuric chloride and organomercurials are used for sterilization of surface of the bench tops and other inanimate objects.
- 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 vapours of ethylene oxide are used under pressure by special equipment. It's vapours are highly toxic to viruses, bacteria, fungal cells, heat resistant spores and endospores. Ethylene oxide is widely used in hospitals for disinfecting chemical respirators, heart-lung machines, ophthalmoscope etc.

SAQ 1.

a. All microbial life is destroyed by ……..….. Process.
b. Pertridishes are sterilized by dry heating in ……..……..
c. Culture media are sterilized in ……..….
d. In autoclave a pressure of 15lb/in^2 is given for ……
e. Autoclave works under the same principle as the ……

2.3 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 inoculums taken from natural habitat is grown in culture medium a large number of crowded diverse colonies develop there. The colonies loose their individuality therefore colonies are picked up and grown separately for detailed study. Several methods are in use for obtaining pure cultures. Some of them are common methods like streak plate method, pour plate method, spread plate method and serial dilution method.

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 inoculums sufficiently thin out in successive streaks and microorganisms are also separated from each other. now the plates are incubated to allow the growth of colonies. 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. 2.1).

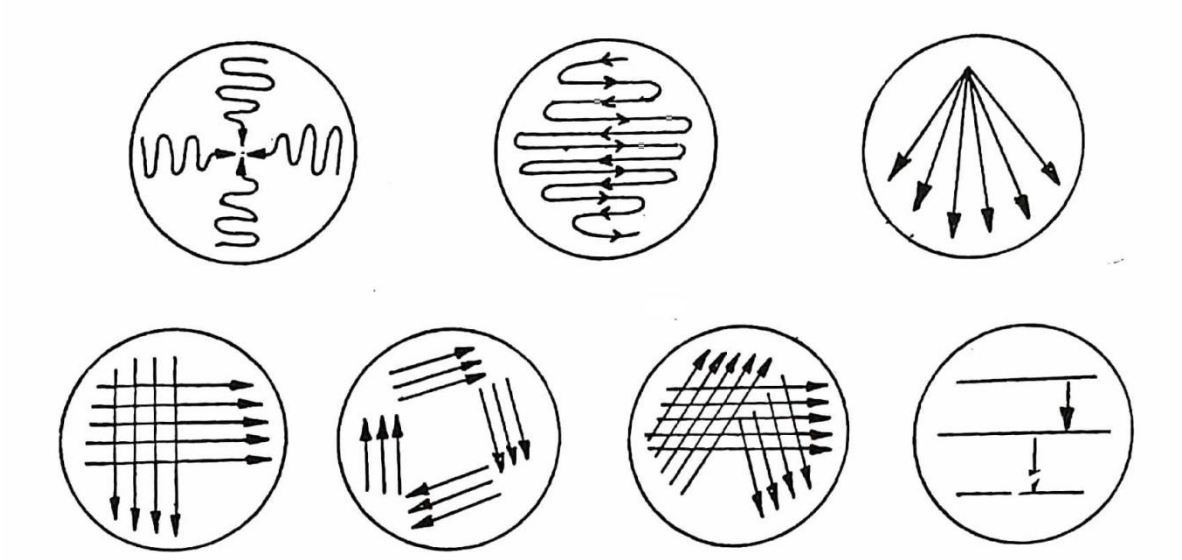


Fig. 2.1. Different methods of streaking.

Source Microbiology by R.P. Singh

Pour Plate Method:

In this method diluted samples of inoculums mixed with melted agar medium is plated in petridishes. The main principle of 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 42-45^{0}C because agar solidifies below 42^{0}C. Now the contents of each tube are poured in to pertridishes, allow solidifying. The inoculated petridishes 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 purety of the culture. The pour plate method has some disadvantages like (i) The picking up of surface colonies needs digging them out of agar medium and (2) The isolated microorganism must be able to with stand 42-45^{0}C temperature of liquid medium. Due to which this technique is unsuitable for the isolation of psychrophilic (Fig. 2.2) microorganisms.

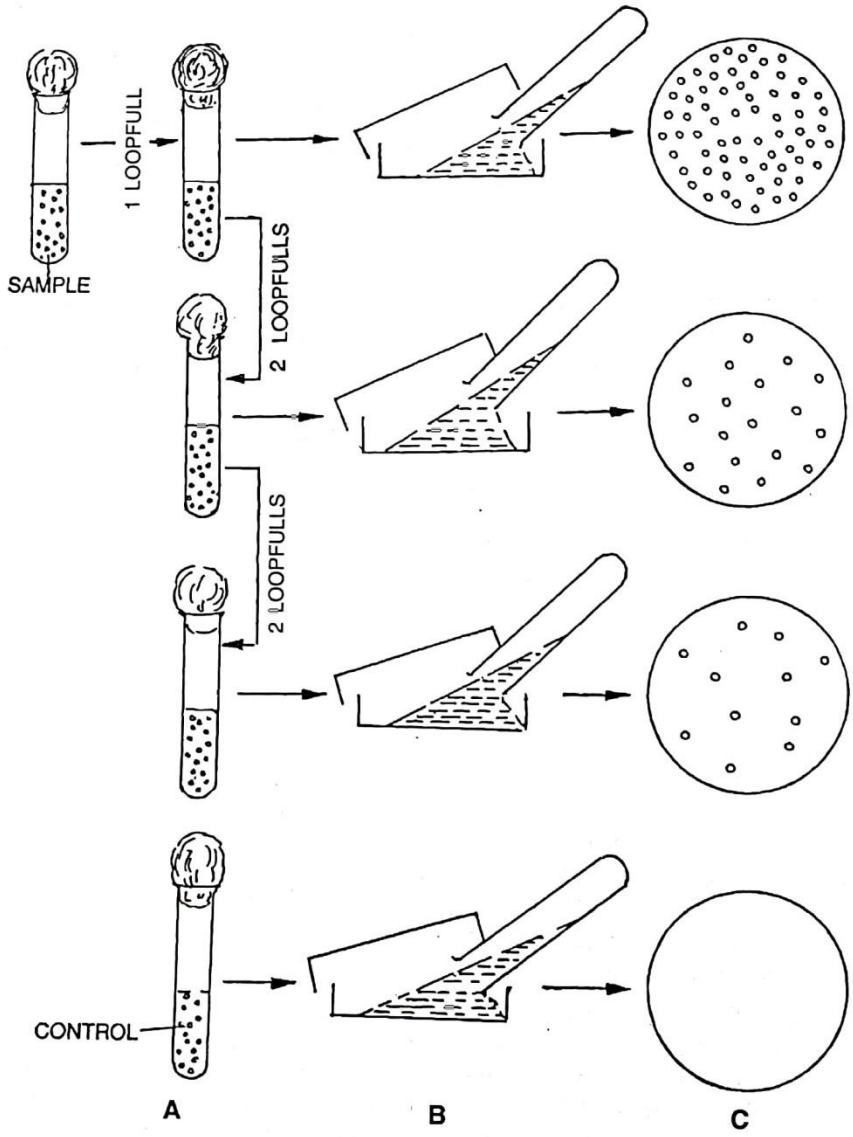


Fig. 2.2. Pour plate method. **A.** Media/dilution; **B.** pouring of the plate; and **C.** colony development after incubation. Control consists of the sterilized plating medium alone.

Source Microbiology by R.P. Singh

3. Spread Plate Method:

The mixed culture of microorganism is diluted in a series of tubes having sterile distilled water. Now a drop of so diluted liquid form each tube is placed on the centre of sterilized petridish having agar medium. The drop is spread evenly over the surface with the help of sterilized bend glass rod. Now the peridish is incubated for the growth of isolated microbial

colonies. The isolated colonies are picked up and transferred into fresh medium to ensure their purity.(Fig. 2.3)

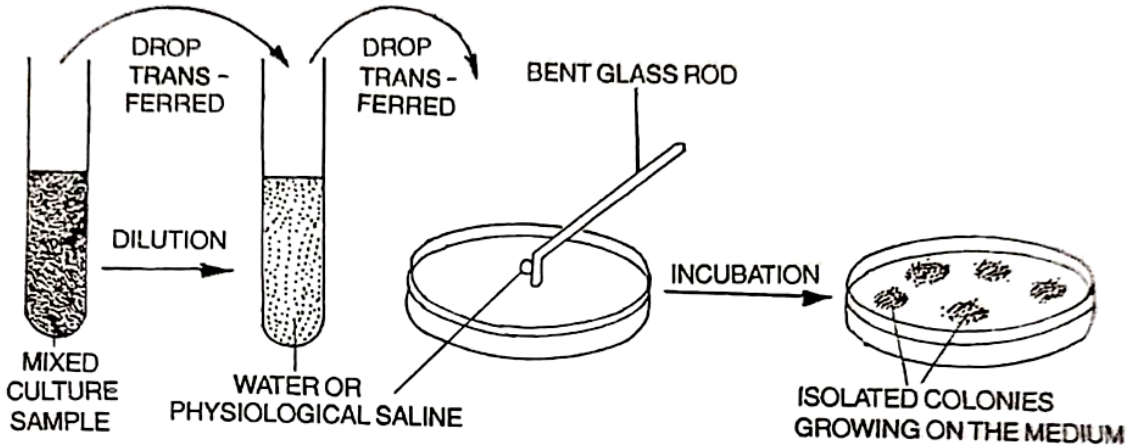


Fig.2.3. Spread plate method.

Source Microbiology by R.P. Singh

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 another 1ml medium from the tube of step 2. Which contains 10 microorganisms and mix it in a fresh tube having 9ml medium? Now this tube will have 10ml medium with 10 microorganisms.

Step 4: Take out again 10ml 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 10ml 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 microorganism.

SAQ 2

a) In nature microorganism occur as ……..population.
b) In streak plate method culture is ……. across the surface of the agar medium.
c) In pour plate method mixed culture of bacteria is diluted in ……..agar medium.
d) In spread plate method mixed culture of microorganism is diluted in sterile ……
e) The microorganism which grow only in liquid medium are isolated by …… method.

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 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 dissolved form. Carbon is needed to synthesize the organic molecules from which organisms are built. 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 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, calcium** and **iron**.

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 cobalt, copper, manganese, molybdenum, nickel, selenium, tungsten, vanadium and zinc in small amount. These elements are **minor** (micro) **nutrients** or trace elements. Besides the mineral nutrients, the microorganisms also need some organic compounds like **amino acids, purines, pyrimidines,** and **vitamins**. These are the constituents of proteins and nucleic acids respectively. 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 chemical substances (inorganic) as sources of energy and carbon dioxide as the main source of carbon.
2. **Chemoheterotrophs** those that use chemical substances (organic) as sources of energy and organic compounds as the main source of carbon.
3. **Photoautotrophs** those that use 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 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 Construction of Culture Media and Enrichment Culture Technique

Culture medium has nutrient or combination of nutrients and 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 (i) 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, yeast cells or any of a number of highly notorious 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 are :

(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 issued 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. e.g. 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 said pH is adjusted (6.8-7.2) and is dispensed in tubes or other contains, 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 to water and heated 60^0C to promote solubilization of the ingredients. It is 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 (or 121^0C temperature) for 15 minutes.

(c) **Trypticase Soy Agar:** This is solid medium for a general purpose. It favours 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): The 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±

Enrichment Culture Techniques:

Enrichment culture 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. Which is physically impossible. The medium of known composition and specific conditions of incubation favors the growth of desired microorganisms but 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.

1) Modifying Physical Conditions of the Culture Medium:

In this technique the physical condition of the culture medium is modified to provide growth conditions favorable for the organism of interest and unfavorable for other competing organisms. e.g. To isolate a thermopile bacterium (grow at high temperature i.e. 55^0C), the inoculated sample is incubated at high temperature. The organisms that cannot tolerate high temperature will die or fail to grow, while Thermophiles will grow. They increase in number and become a larger population in the sample. Thus physical culture favors growth of thermopile bacterium.

(2) Modifying the Nutrient Content of the Culture Medium

Enrichment can also be achieved by modifying the nutrient content of the culture medium. e.g. A variety of bacteria are present in soil which convert the atmospheric nitrogen into ammonia. 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 create their own nitrogenous nutrients for growth and increase in number in the enriched culture where other's cannot.

SAQ 3:-

a) Non synthetic media do not have ……chemical.

b) Synthetic media have pure chemical in …….. Concentration.

c) Nutrient broth is a …… medium.

d) Potato dextrose- agar medium is a …….. solid medium.

2.6. 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 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. Shake cultures in Veillon tubes were in use formely but are now obsolete. This consists of a glass tube upon 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 *N. meningitides* and *N. catarrhalis* 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 taken from the other. 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. *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 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 are 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

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

Anaerobic media

Anaerobic bacteria need special media for growth because need low system oxygen content, 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 *Cl. 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 the 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 bacteria.

- **Chemical or biological method:** This method, first introduced by Buchner (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, 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.

2.7 Culture Collection, Preservation and Maintenance

Culture Collection: For collection of culture we need culture tube having microorganism, culture tube having sterilized agar slant, inoculating needle, burner, absolute alcohol and cotton.

First of all, clean the working table, inoculation needle, hands and all exposed surface with absolute alcohol. Burn the sprit lamp and keep it at the centre of table where inoculation is to be performed. Inoculation is done using following procedure:

- Hold the culture tubes (one having microorganism and other having agar slant) in your left hand between the fingers and inoculating needle in the right hand.
- Both the tubes are opened by removing cotton plug with the fingers of right hand.
- Dip the inoculation needle in absolute alcohol and burn to sterilize.
- Now take out a pinch of microbial culture and transfer it into the tube having agar slant.
- The open mouths of tubes and cotton plugs are sterilized over flame and tubes are immediately pluged.
- The inoculated tube is incubated under suitable temperature to favour rapid growth of microorganisms.

Following precautions should be taken during culture collection:

(i) Inoculation should be done in clean and sterilized inoculation chamber.

(ii) The exposed surfaces and instruments should be sterilized properly.

(iii) During inoculation mouth of culture tube and inoculation tube should be pointed towards flame.

(iv) Inoculums should be transferred at the centre of agar slant.

Preservation and Maintenance of microbial culture:

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 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.

2.8 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, vapours 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, calcium, iron as macro nutrients and cobalt, copper, manganese, molybdenum etc. as micro nutrients along with water.
- On the basis of nutrition source of carbon, microorganism may be chemoautotrophs, chemoheterotrophs, photoautotroph's and photoheterotrophs. There are lithotrophs and organotrophs also.
- For isolation of microorganisms, there are non synthetic or complex and synthetic or defined media.
- Enrichment culture technique is used to enhance the population density of a particular group of microorganism.
- Numerous culture media has been in practice for development of bacteria but anaerobic bacteria special oxygen free or low oxygen content media are required.
- 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.

2.9 Terminal Questions

Long Answer Questions:

Q.1: Describe chemical methods of sterilization.

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

Q.2: What are pure culture techniques?

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

Q.3: Describe principles of microbial nutrition.

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

Q.4: Describe different media used for culture of bacteria in general.

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

Q.5: Describe methods used for isolation of aerobic and anaerobic bacteria.

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

Q.6: How will you collect culture? Describe maintenance and preservation of culture.

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

Q.7: **Write Short Notes on:**

- **a)** Pour plate method
- **b)** Serial dilution method
- **c)** Mineral nutrients
- **d)** Non synthetic media
- **e)** Synthetic media
- **f)** Enrichment culture technique

g) Culture methods for anaerobic bacteria

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

2.10 Answers

SAQ 1:

a) Sterilization

b) Oven

c) Autoclave

d) 10-15 minute

e) Pressure cooker

SAQ 2:

a) Mixed

b) Streaked

c) Melted

d) Distilled water

e) Serial dilution

SAQ 3:

a) Exact

b) Definite

c) liquid

d) Liquefiable

2.11. Further suggested readings

7. Prescott' microbiology, eighth edition by By Joanne Willey and Kathleen Sandman and Dorothy Wood.

8. A textbook of Microbiology, R.C. Dubey and D.K. Maheshwari,, S Chand & Company P Ltd, New Delhi

9. Text book of microbiology by Ananthanarayan and paniker's, Seventh edition, Orient longman private limited.

10. Foundations in Microbiology, By Kathleen Park Talaro and Barry Chess, 10 edition

11. Microbiology: An Introduction, 13th Edition by Gerard J. Tortora, Berdell R. Funke and Christine L. Case.

Block- II

Microbial Nutrition and Chemotherapy

UNIT -3

Microbial Diversity among Microorganism

UNIT-4

Chemotherapy/Antibiotics Antimicrobial agents

Introduction

This is the first block on microbial nutrition and chemotherapy. It consists of following two units:

Unit-3: This unit covers the microbial Diversity among microorganism. Types and mode of modes of nutrition in bacteria and its growth is briefly discussed. The microbial growth has been defined by mathematical expression of growth and growth curve. The measurement of growth and growth yields were also discussed. The synchronous growth and continuous culture is briefly discussed in this unit. The details associated with each growth curve (number of cells, length of each phase, rapidness of growth or death, overall amount of time) will vary from organism to organism or even with different conditions for the same organism. But the pattern of four distinct phases of growth will typically remain

Unit-4: Chemotherapy is the use of drugs to destroy cancer cells. It usually works by keeping the cancer cells from growing, dividing, and making more cells. Because cancer cells usually grow and divide faster than normal cells, chemotherapy has more of an effect on cancer cells. However, the drugs used for chemotherapy are powerful, and they can still cause damage to healthy cells. Chemotherapy is most often used to treat cancer, since cancer cells grow and multiply much more quickly than most cells in the body. Many different chemotherapy drugs are available. Chemotherapy drugs can be used alone or in combination to treat a wide variety of cancers. Though chemotherapy is an effective way to treat many types of cancer, chemotherapy treatment also carries a risk of side effects. Some chemotherapy side effects are mild and treatable, while others can cause serious complications.

Unit-3: Metabolic Diversity among Microorganism

3.1. 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 present on the earth 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 would. Microorganisms vary with regard to the sources of energy they use for assembling macromolecules and other cellular components from smaller molecules. Phototrophs obtain their energy from light; chemotrophs use chemicals as energy sources. Many organisms use organic compounds as sources of energy; these are 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 of cellular

metabolism, physiology, and morphology, by their various ecological distributions and activities, and by their distinct genomic structure, expression, and evolution.

3.2. Microbial nutrition

Nutrients are materials that are acquired from the environment, and are used for growth and metabolism. There are two categories of essential nutrients: macro-nutrients and micro-nutrients. Macro-nutrients usually help maintain the cell structure and metabolism. Micro-nutrients help 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" it 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 acquired from the environment and are used for growth and metabolism. 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. There are two categories of essential nutrients: macro-nutrients and micro-nutrients. Macro-nutrients usually help maintain the cell structure and metabolism. Micro-nutrients help in enzyme function and maintain protein structure. The microbial cell is made up of several elements such as carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron. 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 small level which are known as microelements or micronutrients or traces elements. These nutrients include 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 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* is 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 synthesis by the use of nitrogen. However, all organism require nitrogen is same form while bacteria are prefers to versatile in utilization of nitrogen. Some bacteria use atmospheric nitrogen by the process of nitrogen fixation. Nitrogen is the part of various enzymes and synthesis of different nitrogenous compounds such as nitrates, nitrites or ammonium salts.

Phosphorous

Phosphorous is essential for the synthesis of nucleic acids and adenosine triphosphate (ATP). ATP is the main energy compound 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, sulfur and phosphorous

Apart from carbon and nitrogen some other elements such as hydrogen, oxygen, sulfur and phosphorous as also essential for microorganism. The hydrogen and oxygen are the part of many compounds. Sulfur is needed for the biosynthesis of the amino acids, cysteine and methionine. 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.

3.3. 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 (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 energy are called *chemotrophs*; those that use light as their energy sources 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 four groups such as some phototrophic bacteria (*Rhodospirillum rubrum*) can also grow as chemotrophs. In the absence of oxygen, they depend on light as it 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 3.1.

Table 3.1

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

3.4. 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 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 density. 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 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 survival and derive their food from them. They may or may not be disease causing. Disease causing is referred as pathogenic which may cause disease by attacking host cells or releasing toxins. Toxins may be further sub divided into exotoxins and endotoxins.

3.5. Extremophiles

Extremophiles" are organisms with the ability to thrive in extreme environments such as hydrothermal vents, for example they are able to survive in hot springs or thermal vents, at low temperatures in glaciers or in high levels of salt or radiation etc. They often belong to the domain of Archaea and Bacteria. Extremophiles used unique enzymes called extremozymes,, that is useful for functioning of such forbidding environments. These creatures hold great promise for genetically based medications and industrial chemicals and processes. There are many classes of extremophiles that range all around the globe; each corresponding to the way its environmental niche differs from mesophilic conditions. These classifications are not exclusive. Many extremophiles fall under multiple categories and are classified as polyextremophiles. For example, organisms living inside hot rocks deep under Earth's surface are thermophilic and piezophilic such as Thermococcus barophilus. The various types of extremophiles and their corresponding environmental conditions are tabulated below in Table 3.2.

Table 3.2: Classification of extremophiles

Barophile or Piezophile	Extreme pressure	*Halomonas sp.*
Thermophile	High temperature (hot springs, thermal vents)	*Pyrococcus sp. Sulfolobus sp.*
sychrophile	Extreme low temperatures	*Arthrobacter sp., Psychrobacter sp.*
Radiophiles	Extreme radiation	*Deinococcus radiodurans*
Acidophile	Low ph conditions	*Thiobacillus sp., Acetobacter aceti*
Alkaliphil	High ph conditions	*Halorhodospira*
Metallophil	High metal ions concentrations	*Ferroplasma sp.*

Halophile	Extreme salinit	*Salinibacter ruber, Halobacterium sp*
Osmophile	High sugar concentration	*Saccharomyces sp*
Xerophil	Extremely dry, desiccating conditions	*Trichosporonoides nigrescens*

It is now known that for inhabiting such environments, extremophiles have suitably adapted themselves to growing under such extreme conditions by developing mechanisms to allow their cellular machinery to function optimally. In this context, the various biomolecule, including enzymes which are involved in the cellular machinery of above microorganisms are attuned to function under such harsh and extreme conditions in which these microbes thrive. The adaptations in extremophilic proteins do not seem to follow any generic trend but are specific to the extremophile and the surrounding environmental condition in which it is thriving. Extremophiles are therefore perceived to be one excellent a potentially valuable resource for novel and stable enzymes which can be explored for catalysis of industrial process and development of novel biotechnological processes. This is because, owing to their own adaptations, the enzymes are endowed with inherent ability to function optimally under extreme conditions like extremes of temperature, salt, pressure, radiation etc which are often found in industrial processes. Further in this course, we will learn about some of the various kinds of extremophilic enzymes, their adaptations and applications.

3.6. Microbial growth

The growth is defined as an orderly increase in cellular components. Microbes grow in a variety of physical and chemical environments. Provided with the right conditions (food, correct temperature, etc) microbes can grow very quickly. Depending on the situation, this could be a good thing for humans or a bad thing. It's important to have knowledge of their growth, so we can predict or control their growth under particular conditions. Bacteria are mostly unicellular organisms that multiplying rapidly under favorable 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 stems 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, in a process called binary fission. Providing no event occurs, the resulting daughter cells are genetically identical to the original cell. Both daughter cells from the division do not necessarily survive. However, if the number of surviving exceeds unity on 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 stages 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 every 12 to 15 minutes. However, some are 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. 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).

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 such as

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.

2. Nutrients requirement for Growth:

Water is main component require for microbial growth. Apart from that 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 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.

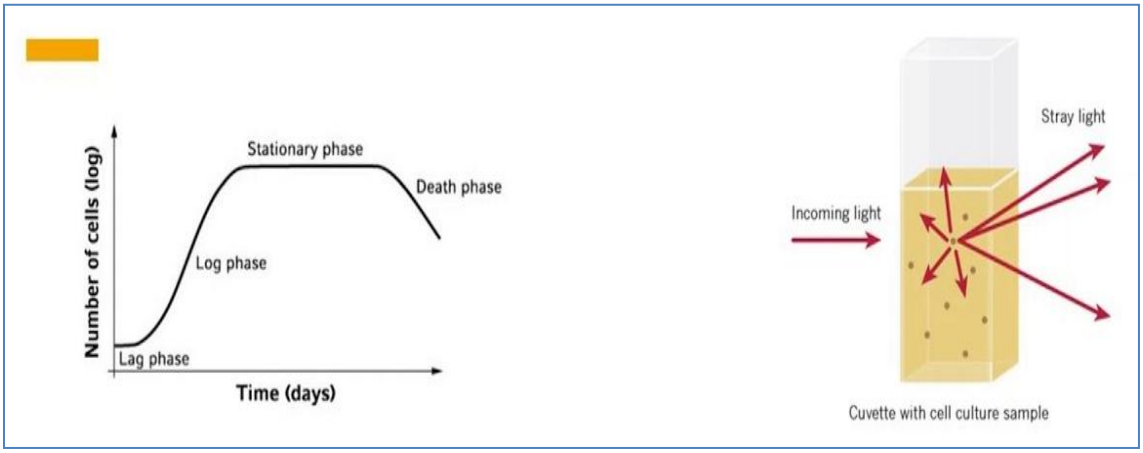


Fig.3.1: Microbial OD measurement

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.

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. In this chamber includes a glass slide, a cover slip which is formed and kept 1/50 mm above the slid. Where bacteria suspension is present in each ruled square of the slide.

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 are 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.

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 count 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 an 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.

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 3.3.

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. 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:

$$\mathrm{logb} = \mathrm{logB} + \mathrm{nlog2}$$

$$n = \frac{logb - logB}{log_2}$$

$$n = \frac{logb - logB}{0.301}$$

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

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

Solve for G

$$G = \frac{t}{\frac{3.3\ logb}{B}}$$

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

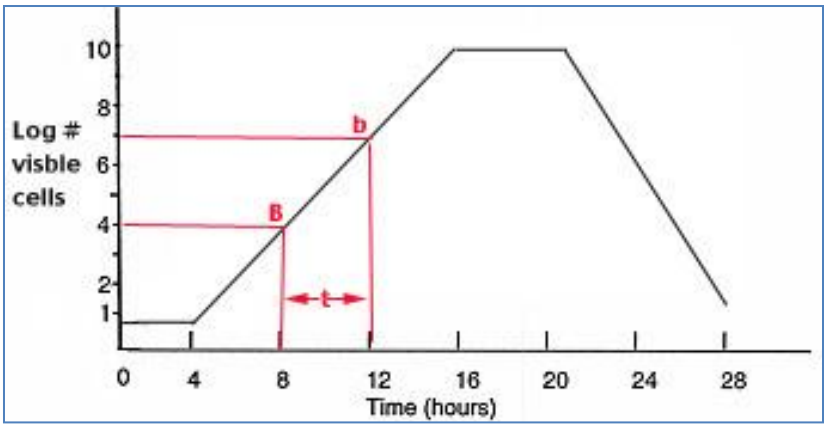


Fig: 3.2: Graph number of visible cell vs time

$$\mathrm{G} = \frac{\mathrm{t}}{\frac{3.3\ \mathrm{logb}}{\mathrm{B}}}$$

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

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

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

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.

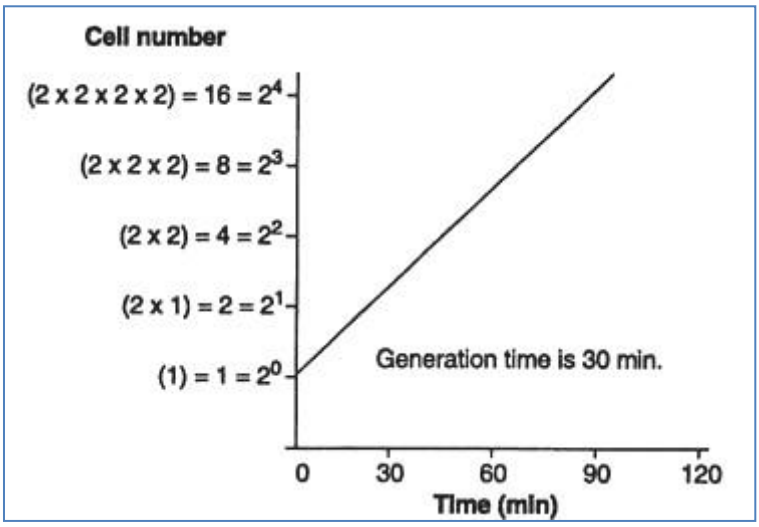


Fig. 3.3: Relationship of generation time and growth

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

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.

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) its 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

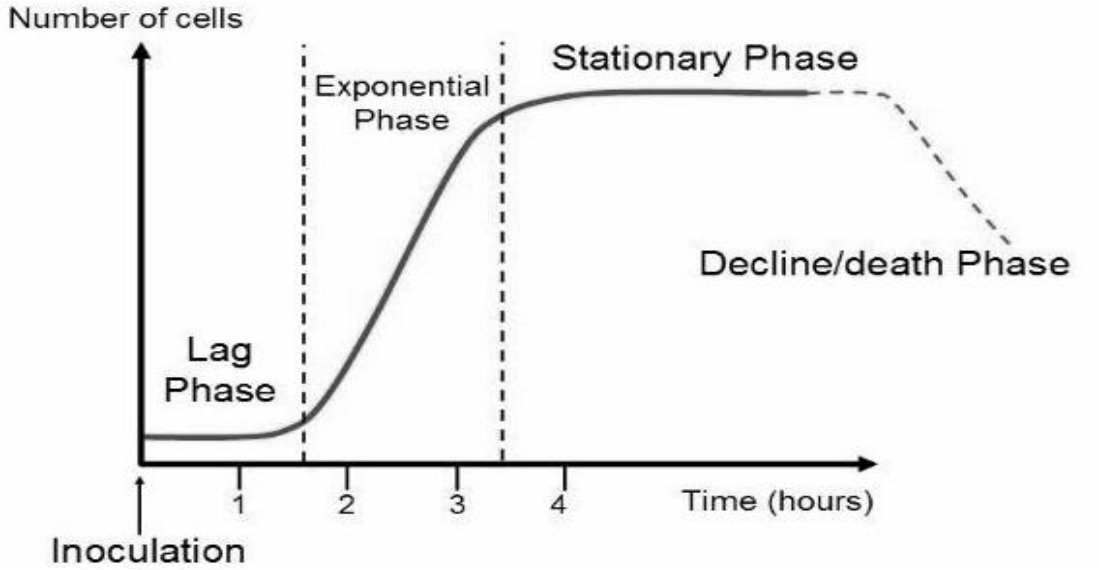


Fig.3.4: Bacterial Growth Curve-'S'shape

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 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

- Cellar 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.

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 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 condition.

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.

- 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, sporulation occur as the bacteria enter stationary phase.

Phase of decline: This is the phase occurs when the population decreased due to cell death. The death phase cause 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.

3.10. 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.

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.

3.11. 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, it this 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. 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.

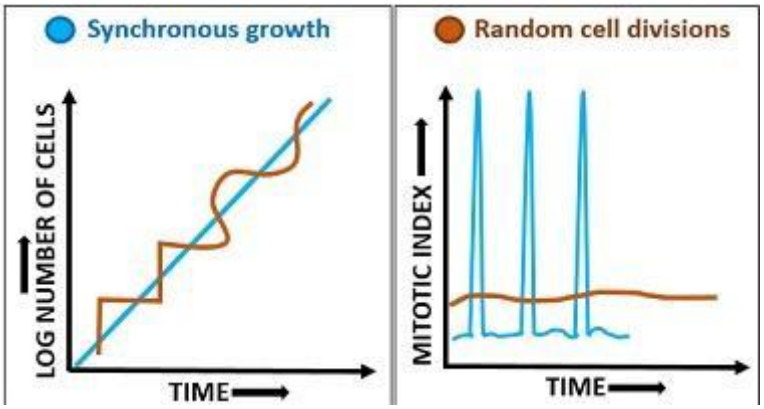


Fig.3.5: Synchronous growth of bacteria

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 it genetics and metabolic activity.

3.12. Summary

Microorganisms exist everywhere and can manipulate in all the environments in which they live. They were present on Earth 3.5 to 4 billion years ago, and have been evolving and expanding into new environments ever since. Biological diversity means the variability among living organisms from all sources and the ecological complexes in which they are present. 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 and 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), 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 that 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. The most common way to assess microbial growth in solution is the measurement of the optical density at 600 nm, or short OD_{600}. The method is based on absorbance detection mode and basically determines which portion of light passes through a sample, more specifically through a suspension of microorganisms. Synchronous cultures can be obtained in several ways. External conditions can be changed, so as to arrest growth of all cells in the culture, and then changed again to resume growth. The newly growing cells are now all starting to grow at the same stage, and they are synchronized. For example, for photosynthetic cells light can be eliminated for several hours and then re-introduced.

3.13. Terminal questions

Q.1: What do you learn from microbial growth?

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

Q.2: Discuss the role of nutrients in microbial growth.

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

Q.3: Discuss about mathematical expression for microbial growth and culture.

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

Q.4: What do know about microbial yields, discuss briefly?

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

Q.5: What is synchronous culture and continuous growth discus briefly?

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

Q.6: Mention the different between the growth rate and generation time of a microorganism.

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

3.14. 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. Foundations in Microbiology, By Kathleen Park Talaro and Barry Chess, 10 edition
6. Microbiology: An Introduction, 13th Edition by Gerard J. Tortora, Berdell R. Funke and Christine L. Case.

Unit- 4: Chemotherapy/Antibiotics

Contents

4.1. Introduction

Chemotherapy is a type of cancer treatment that uses one or more anti-cancer drugs (chemotherapeutic agents) as part of a standardized chemotherapy regimen. Chemotherapy may be given with a curative intent (which almost always involves combinations of drugs), or it may aim to prolong life or to reduce symptoms (palliative chemotherapy). Chemotherapy is one of the major categories of the medical discipline specifically devoted to pharmacotherapy for cancer, which is called *medical oncology*.

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 inhibitions of growth-signals like those associated with receptor tyrosine kinases are referred to as targeted therapy.

Importantly, the use of drugs (whether chemotherapy, hormonal therapy or targeted therapy) constitutes *systemic therapy* for cancer in that they are introduced into the blood stream and are therefore in principle able to address cancer at any anatomic location in the body. Systemic therapy is often used in conjunction with other modalities that constitute *local therapy* (i.e. treatments whose efficacy is confined to the anatomic area where they are applied) for cancer such as radiation therapy, surgery or hyperthermia therapy.

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 overactivity of the immune system against self (so-called autoimmunity). These include rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, vasculitis and many others.

Objectives

This is the second block on Microbial Nutrition and Chemotherapy. It consists of following two units. Under second unit (Chemotherapy/Antibiotics, Antimicrobial agents; Antibiotics) we have following objectives. These are as under:

- To know the concept of chemotherapy.
- To know about different types of chemotherapy.

- To know about antibiotics and their types.
- To know about drugs and their types.
- To know the mechanism of action of drugs.
- To know about transformation, conjugation, transduction and recombination.

4.2. Different types of chemotherapy

Treatment with these powerful drugs is called standard chemotherapy, traditional chemotherapy, or cytotoxic chemotherapy.

How does chemotherapy treat cancer?

Doctors use chemotherapy in different ways at different times. These include:

- Before surgery or radiation therapy to shrink tumors. This is called neoadjuvant chemotherapy.
- After surgery or 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 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 1 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. Or 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 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.

How is chemotherapy given?

Chemotherapy may be given in several different ways, which are discussed below.

4.2.1. Intravenous (IV) chemotherapy

Many drugs require injection directly into a vein. This is called intravenous 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.

4.2.3. Oral chemotherapy

You 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.

4.2.4. Injected chemotherapy

This is when you receive chemotherapy as a shot. The shot may be given in a muscle or injected under the skin. You may receive these shots in the arm, leg, or abdomen. Abdomen is the medical word for your belly.

4.2.5. Chemotherapy into an artery

An artery is a blood vessel that carries blood from your heart to another part of your body. Sometimes chemotherapy is injected into an artery that goes directly to the cancer. This is called intra-arterial or IA chemotherapy.

4.2.6. 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. You can take some types of chemotherapy in a cream you put on your skin. You get your medication at the pharmacy and take it at home.

4.2.7. 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. 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:

4.2.8. Hormonal therapy

These treatments change the amount of hormones in your body. Hormones are chemicals your body makes naturally. They help 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.

4.2.9. Targeted therapy

These treatments target and disable genes or proteins found in cancer cells that the cancer cells need to grow.

4.2.10. Immunotherapy

This type of treatment helps your body's natural defenses fight 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.

4.3. Antibiotics

An antibiotic is a type of antimicrobial substance active against bacteria. It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in the treatment and prevention of such infections. They may either kill or inhibit the growth of bacteria. A limited number of antibiotics also possess antiprotozoal activity. Antibiotics are not effective against viruses such as the common cold or influenza; drugs which inhibit viruses are termed antiviral drugs or antiviral rather than antibiotics. Different classes of antibiotics are as given below.

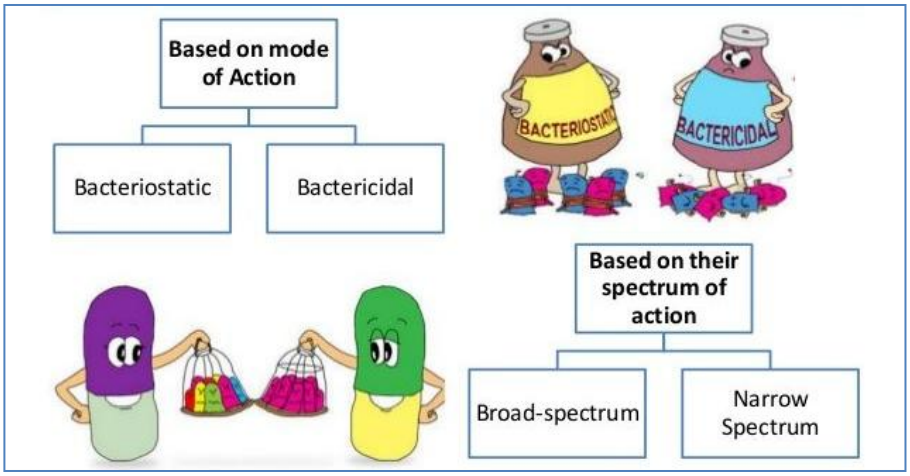


Fig. 1 Classification of antibiotics

Sometimes, the term *antibiotic*—literally opposing life, from the Greek roots *anti*, "against" and *bios*, "life"—is broadly used to refer to any substance used against microbes, but in the usual medical usage, antibiotics (such as penicillin) are those produced naturally (by one microorganism fighting another), whereas nonantibiotic antibacterials (such as sulfonamides and antiseptics) are fully synthetic. However, both classes have the same goal of killing or preventing the growth of microorganisms, and both are included in antimicrobial chemotherapy. Antibacterials include antiseptic drugs, antibacterial soaps, and chemical disinfectants, whereas antibiotics are an important class of antibacterials used more specifically in medicine and sometimes in livestock feed.

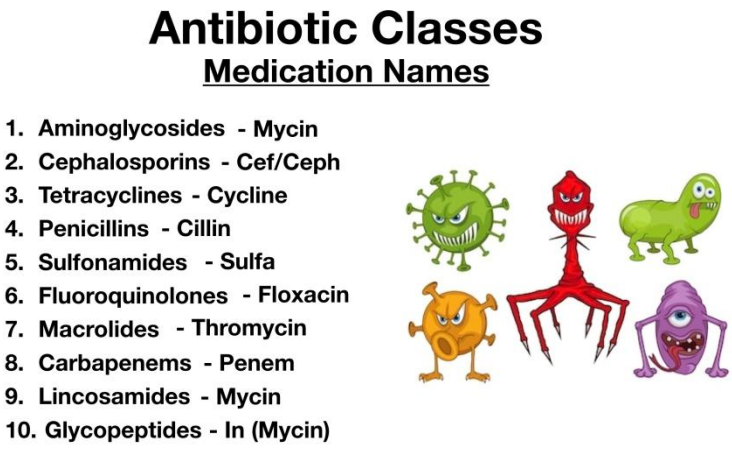


Fig. 2

Antibiotics have been used since ancient times. Many civilizations used topical application of mouldy bread, with many references to its beneficial effects arising from ancient Egypt, Nubia, China, Serbia, Greece, and Rome. The first person to directly document the use of molds to treat infections was John Parkinson (1567–1650). Antibiotics revolutionized medicine in the 20th century. Alexander Fleming (1881–1955) discovered modern day penicillin in 1928, the widespread use of which proved significantly beneficial during wartime. However, the effectiveness and easy access to antibiotics have also led to their overuse and some bacteria have evolved resistance to them. The World Health Organization has classified antimicrobial resistance as a widespread "serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country".

4.3.1. Medical uses

Antibiotics are used to treat or prevent bacterial infections, and sometimes protozoan infections. (Metronidazole is effective against a number of parasitic diseases). When an infection is suspected of being responsible for an illness but the responsible pathogen has not been identified, an empiric therapy is adopted. This involves the administration of a broad-spectrum antibiotic based on the signs and symptoms presented and is initiated pending laboratory results that can take several days.

When the responsible pathogenic microorganism is already known or has been identified, definitive therapy can be started. This will usually involve the use of a narrow-spectrum antibiotic. The choice of antibiotic given will also be based on its cost. Identification is critically important as it can reduce the cost and toxicity of the antibiotic therapy and also reduce the possibility of the emergence of antimicrobial resistance. To avoid surgery, antibiotics may be given for non-complicated acute appendicitis.

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, cancer patients, and those having surgery. Their use in surgical procedures is to help 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.

4.3.2. 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.

4.3.3. Global consumption

Antibiotic consumption varies widely between countries. The WHO report on surveillance of antibiotic consumption' published in 2018 analysed 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. Burundi had the lowest at 4.4. Amoxicillin and amoxicillin/clavulanic acid were the most frequently consumed.

4.3.4. 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 prevent 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.

Some antibiotics may also damage the mitochondrion, a bacteria-derived organelle found in eukaryotic, including human, cells. Mitochondrial damage cause oxidative stress in cells and has been suggested as a mechanism for side effects from fluoroquinolones. They are also known to affect chloroplasts.

4.3.5. Correlation with obesity

Exposure to antibiotics early in life is associated with increased body mass in humans and mouse models. Early life is a critical period for the establishment of the intestinal microbiota and for metabolic development. Mice exposed to subtherapeutic antibiotic treatment – with either penicillin, vancomycin, or chlortetracycline had altered composition of the gut microbiota as well as its metabolic capabilities. One study has reported that mice given low-dose penicillin (1 μg/g body weight) around birth and throughout the weaning process had an increased body mass and fat mass, accelerated growth, and increased hepatic expression of genes involved in adipogenesis, compared to control mice.

In addition, penicillin in combination with a high-fat diet increased fasting insulin levels in mice. However, it is unclear whether or not antibiotics cause obesity in humans. Studies have found a correlation between early exposure of antibiotics (<6 months) and increased body mass (at 10 and 20 months). Another study found that the type of antibiotic exposure was also significant with the highest risk of being overweight in those given macrolides compared to penicillin and cephalosporin. Therefore, there is correlation between antibiotic exposure in early

life and obesity in humans, but whether or not there is a causal relationship remains unclear. Although there is a correlation between antibiotic use in early life and obesity, the effect of antibiotics on obesity in humans needs to be weighed against the beneficial effects of clinically indicated treatment with antibiotics in infancy.

4.4. Antimicrobial agents

Use of substances with antimicrobial properties is known to have been common practice for at least 2000 years. Ancient Egyptians and ancient Greeks used specific molds and plant extracts to treat infection. 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 seems likely that all species 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.

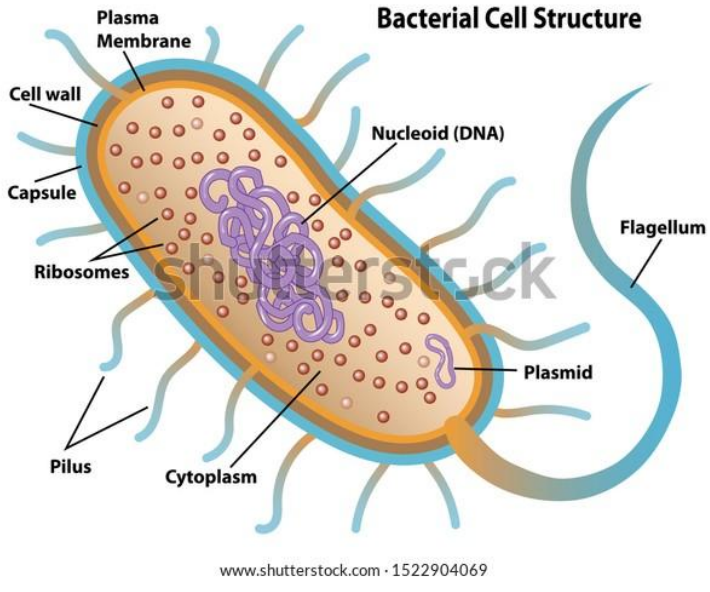


Fig. 3 Bacterial cell structure

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 antibiotics, 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 compared to mammalian cells. The bacterial cytoplasm is separated from the external environment by a

4.4.1. Penicillins

History of penicillins

People generally attribute the discovery of penicillins to Alexander Fleming. The story goes that he returned to his laboratory one day in September 1928 to find a Petri dish containing *Staphylococcus* bacteria with its lid no longer in place. The dish had become contaminated with a blue-green mold called *Penicillium notatum*. Fleming noted that there was a clear ring surrounding the mold where the bacteria had been unable to grow. By discovering this mold and recognizing its use, Fleming set the wheels in motion to create one of the most useful drugs in medical history. In March 1942, Anne Miller became the first civilian to receive successful treatment with penicillin. She narrowly avoided death following severe infection after a miscarriage. Although Fleming technically discovered the first antibiotic, scientists had to do a lot of work before penicillins could become available for general use.

Scientists with a superior laboratory and a deeper understanding of chemistry than Fleming carried out the bulk of the work. Howard Florey, Norman Heatley, and Ernst Chain performed the first in-depth and focused studies on the drug. In Fleming's Nobel Prize acceptance speech, he warned that the overuse of penicillins might, one day, lead to bacterial resistance.

How do penicillins work?

- Quick facts
- Function
- History
- Resistance

- Side effects
- Risks
- Takeaway

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 bacteria 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 struts 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 equivalent of 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.

The most commmon side effects of taking penicillins include:

- Diarrhea
- Nausea
- A headache
- Skin rashes and hives

Less common side effects include:

- Shortness of breath or irregular breathing
- Joint pain
- Sudden lightheadedness and fainting
- Puffiness and redness of the face
- Scaly, red skin
- Vaginal itching and discharge, due to either a yeast infection or bacterial vaginosis
- Sore mouth and tongue, sometimes with white patches
- Abdominal cramps, spasms, tenderness, or pain

Rare side effects include:

- Anxiety, fear, or confusion
- A sense of impending doom
- Hallucinations
- Yellowing of the eyes and skin
- A sore throat
- Unusual bleeding

- Diarrhea and reduced urination
- Convulsions

Risks

Although the use of penicillins is widespread, some issues or contraindications can occur, as with any drug:

- **Breast-feeding:** People who are breast-feeding may pass small amounts of penicillin to the child. This can result in the child experiencing allergic reactions, diarrhea, fungal infections, and skin rash.
- **Interactions:** Some other drugs can interact with penicillins. Checking with a doctor before taking multiple medications is vital.
- **Bleeding problems:** Some penicillins, such as carbenicillin, piperacillin, and ticarcillin, can make pre-existing bleeding problems worse.
- **Oral contraceptives:** Penicillins can interfere with birth control pills, increasing the risk of unwanted pregnancy.
- **Cystic fibrosis:** People with cystic fibrosis are more prone to fever and skin rashes when taking piperacillin.
- **Kidney disease:** Individuals with kidney disease have an increased risk of side effects.
- **Methotrexate:** Methotrexate disrupts cell growth and can treat several conditions, including leukemia and some autoimmune diseases. Penicillins prevent the body from disposing of this drug, potentially leading to severe complications.
- **Phenylketonuria:** Some stronger, chewable amoxicillin tablets contain high levels of aspartame that the body converts to phenylalanine. This is dangerous for anyone with phenylketonuria.
- **Gastrointestinal problems:** Patients with a history of stomach ulcers or other intestinal diseases might be more likely to develop colitis when taking penicillins.

Check your knowledge…

Q. 1 Define chemotherapy.

Q. 2 What do you mean by antibiotics?

Q. 3 Define penicillin with examples.

4.4.2. Cephalosporins

Cephalosporins are antimicrobials grouped into five generations based on their spectrum of coverage against gram-positive and gram-negative bacteria as well as their temporal discovery. First-generation cephalosporins have coverage against most gram-positive cocci as well as gram-negative bacteria, e.g., *Escherichia coli (E. coli), Proteus mirabilis,* and *Klebsiella pneumoniae*. Second-generation cephalosporins have coverage against *Haemophilus influenza (H. influenza), Moraxella catarrhalis,* and *Bacteroides spp.* Third-generation cephalosporins have less coverage against most gram-positive organisms but have increase coverage against Enterobacteriaceae, *Neisseria spp.*, and *H. influenza*. Fourth-generation cephalosporins have similar coverage as third-generation cephalosporins but with additional coverage against gram-negative bacteria with antimicrobial resistance, e.g., beta-lactamase. Fifth-generation cephalosporins have coverage against methicillin-resistant staphylococci and penicillin-resistant pneumococci.

First-generation cephalosporins include cefazolin, cephalothin, cephapirin, cephradine, cefadroxil, and cephalexin. First-generation cephalosporins have active coverage against most gram-positive cocci such as *staphylococci spp.*and *streptococci spp.*while having minimal coverage against gram-negative bacteria. Gram-negative bacteria that are more susceptible to first-generation cephalosporins are *Proteus mirabilis, E. coli,* and *Klebsiella pneumoniae*. Oral first-generation cephalosporins are commonly prescribed to use against uncomplicated skin and soft tissue infections such as cellulitis and abscesses commonly due to a *staphylococci spp.* or *streptococci spp.* infection. Additionally, clinicians can also use them for bone, respiratory tract, genitourinary tract, biliary tract, bloodstream infection, otitis media, and surgical prophylaxis. In fact, cefazolin is the cephalosporin of choice for surgical prophylaxis. One of the non-FDA approved indication is to use first-generation cephalosporins for endocarditis prophylaxis for those who are susceptible and undergoing a dental or respiratory procedure.

Second-generation cephalosporins divide into two subgroups: the second-generation and the cephamycin subgroup. Some of the second-generation subgroups include cefuroxime and cefprozil. The cephamycin subgroup includes cefmetazole, cefotetan, and cefoxitin. Within the first subgroup, cefuroxime has increase coverage against *H. influenza*. Indications for cefuroxime also include Lyme disease in pregnant women and children. The cephamycin subgroup has increased coverage against *Bacteroides* species. Second-generation cephalosporins have less

activity against gram-positive cocci compared to the first-generation cephalosporins but have increase activity against gram-negative bacilli. They are often prescribed to treat respiratory infections such as bronchiolitis or pneumonia. Other indications for second-generation cephalosporins are similar to first-generation indications (bone, respiratory tract, genitourinary tract, biliary tract, bloodstream infection, otitis media, and surgical prophylaxis). In addition to the gram-negative bacteria covered by first-generation cephalosporins, second-generation cephalosporins also have coverage against *H. influenza, Enterobacter aerogenes, Neisseria* species, and *Serratia marcescens*.

Third-generation cephalosporins include cefotaxime, ceftazidime, cefdinir, ceftriaxone, cefpodoxime, and cefixime. This generation has extended gram-negative bacteria coverage often used to treat gram-negative infection resistant to the first and second generation or other beta-lactams antimicrobials. When given IV, third-generation can penetrate the blood-brain barrier and cover bacteria in the cerebral spinal fluid, especially ceftriaxone and cefotaxime. Ceftriaxone can be given to treat meningitis caused by *H. influenza, Neisseria meningitidis,* or *Streptococcus pneumoniae.* Ceftriaxone is also used to treat gonorrhea and disseminated Lyme disease. Ceftazidime, very importantly, has *Pseudomonas aeruginosa* coverage.

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 a modified penicillin-binding protein; this prevents the cephalosporin's 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.

4.4.3.Broad-spectrum antibiotics

A broad-spectrum antibiotic is an antibiotic that acts on the two major bacterial groups, Gram-positive and Gram-negative, or any antibiotic that acts against a wide range of

disease-causing bacteria. These medications are used when a bacterial infection is suspected but the group of bacteria is unknown (also called empiric therapy) or when infection with multiple groups of bacteria is suspected. This is in contrast to a narrow-spectrum antibiotic, which is effective against only a specific group of bacteria. Although powerful, broad-spectrum antibiotics pose specific risks, particularly the disruption of native, normal bacteria and the development of antimicrobial resistance. An example of a commonly used broad-spectrum antibiotic is ampicillin.

Antibiotics are often grouped by their ability to act on different bacterial groups. Although bacteria are biologically classified using taxonomy, disease-causing bacteria have historically been classified by their microscopic appearance and chemical function. The morphology of the organism may be classified as cocci, diplococci, bacilli (also known as "rods"), spiral-shaped or pleomorphic. Additional classification occurs through the organism's ability to take up the Gram stain and counter-stain; bacteria that take up the crystal violet dye stain are referred to as "gram-positive," those that take up the counterstain only are "gram-negative," and those that remain unstained are referred to as atypical. Further classification includes their requirement for oxygen (ie, aerobic or anaerobic), patterns of hemolysis, or other chemical properties. The most commonly encountered groupings of bacteria include gram-positive cocci, gram-negative bacilli, atypical bacteria, and anaerobic bacteria. Antibiotics are often grouped by their ability to act on different bacterial groups. For example, 1st-generation cephalosporins are primarily effective against gram-positive bacteria, while 4th-generation cephalosporins are generally effective against gram-negative bacteria.

4.4.4. Sulfa drugs

Sulfa drug, also called **sulfonamide**, any 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 Prontosil 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; humans and other higher animals, however, 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.

4.4.5. Antifungal antibiotics

Fungi are unicellular or multi-cellular eukaryotic organisms that exist in all environments worldwide. From fungi visible to the naked eye, such as mushrooms, to microscopic yeasts and molds, they exist in a multitude of forms. While most fungi do not play a significant role in human disease, there are several hundred fungi that do, resulting in fungal infection or disease. Fungal infections (mycoses) range from common benign infections like 'jock itch' to serious, life-threatening infections such as cryptococcal meningitis. The term 'antifungals' encompasses all chemical compounds, pharmacologic agents, and natural products used to treat mycoses.

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. Mycoses 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 isavuconazonium.

Terbinafine, an allylamine antifungal, was FDA approved in 1996 but has indications for the treatment of local, non-systemic fungal infections. The next breakthrough in systemic therapy would have a basis in amphotericin B lipid formulations, which have more favorable 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. Common, medically relevant fungal infections include, but are not limited to, the following (**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*)

4.4.5.1. Mechanism of action

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 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 eventual death.

- **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 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 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 spp.*, 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 production. These metabolic changes are likely due to an increase in intracellular copper and iron-sulfur clusters, which lead to protein damage.

4.4.5.2. 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. 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 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-resistant microbial populations and the factors that govern their drug-resistant feature. The major focus of this segment will highlight 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.

Check your knowledge…

Q.1: Define antifungal antibiotics.

Q.2: What do you mean by cephalosporins?

Q.3: Define sulpha drugs with examples.

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.

History of Antibiotic Resistance Development

The emergence of drug resistance has always been a major concern worldwide right after the introduction of drugs for common use. The crucial role of microbes in causing diseases led to

the discovery of antimicrobial drugs. Penicillin was the first of its kind as mentioned before to be introduced by Alexander Fleming in 1928. Its effectiveness was against the Gram-positive bacteria, especially *Staphylococcus aureus* followed by few more antibiotics including streptomycin, tetracycline, chloramphenicol, vancomycin, macrolides, nalidixic acid, etc. However, different drug-resistant microorganisms also started to show up with due time course.

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.

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 agents 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.

Intrinsic Resistance

Intrinsic resistance is defined as the ability of an organism to resist the antimicrobial/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:

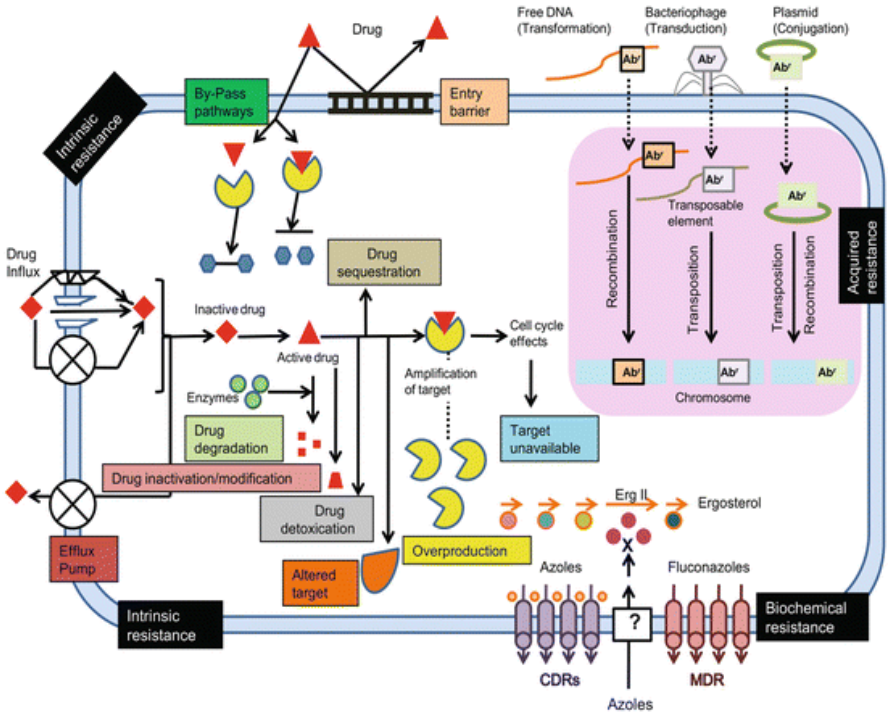


Fig. 4 Schematic presentation of multiple diverse molecular mechanism of microbial resistance

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 aminoglycosides by altering the membrane porin frequency, size, and selectivity. On the other hand, the modification in the PBP (penicillin-binding protein) site led to the insensitivity toward the β-lactam antibiotics.

Species-Specific Structure of Target Site

Although the mode of action of antibiotics is almost similar across the same community of bacteria, 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 and binding modes for different antibiotics of same function as well as for a particular drug against different species of the bacteria.

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.

4.5. Bacterial genetics

Bacterial genetics is the subfield of genetics devoted to the study of bacteria. Bacterial genetics are subtly different from eukaryotic genetics, however bacteria still serve as a good model for animal genetic studies. One of the major distinctions between bacterial and eukaryotic genetics stems from the bacteria's lack of membrane-bound organelles (this is true of all prokaryotes. While it is a fact that there are prokaryotic organelles, they are never bound by a

lipid membrane, but by a shell of proteins), necessitating protein synthesis occurs in the cytoplasm.

Like other organisms, bacteria also breed true and maintain their characteristics from generation to generation, yet at the same time, exhibit variations in particular properties in a small proportion of their progeny. Though heritability and variations in bacteria had been noticed from the early days of bacteriology, it was not realised then that bacteria too obey the laws of genetics. Even the existence of a bacterial nucleus was a subject of controversy. The differences in morphology and other properties were attributed by Nageli in 1877, to bacterial pleomorphism, which postulated the existence of a single, a few species of bacteria, which possessed a protein capacity for a variation. With the development and application of precise methods of pure culture, it became apparent that different types of bacteria retained constant form and function through successive generations. This led to the concept of monomorphism.

4.5.1.Transformation

In molecular biology and genetics, transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane(s). For transformation to take place, the recipient bacterium must be in a state of competence, which might occur in nature as a time-limited response to environmental conditions such as starvation and cell density, and may also be induced in a laboratory.

Transformation is one of three processes for horizontal gene transfer, in which exogenous genetic material passes from one bacterium to another, the other two being conjugation (transfer of genetic material between two bacterial cells in direct contact) and transduction (injection of foreign DNA by a bacteriophage virus into the host bacterium).[1] In transformation, the genetic material passes through the intervening medium, and uptake is completely dependent on the recipient bacterium.

As of 2014 about 80 species of bacteria were known to be capable of transformation, about evenly divided between Gram-positive and Gram-negative bacteria; the number might be an overestimate since several of the reports are supported by single papers. Transformation may also be used to describe the insertion of new genetic material into nonbacterial cells, including animal and plant cells; however, because "transformation" has a special meaning in relation to

animal cells, indicating progression to a cancerous state, the process is usually called "transfection".

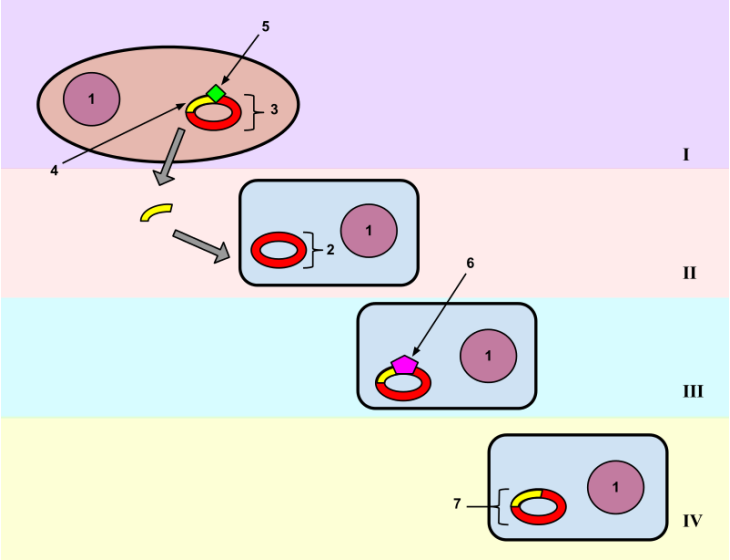


Fig. 5 In the figure, a gene from bacterial cell 1 is moved to bacterial cell 2. This process of bacterial cell 2 taking up new genetic material is called transformation.

Transformation in bacteria was first demonstrated in 1928 by the British bacteriologist Frederick Griffith. Griffith was interested in determining whether injections of heat-killed bacteria could be used to vaccinate mice against pneumonia. However, he discovered that a non-virulent strain of *Streptococcus pneumoniae* could be made virulent after being exposed to heat-killed virulent strains. Griffith hypothesized that some "transforming principle" from the heat-killed strain was responsible for making the harmless strain virulent. In 1944 this "transforming principle" was identified as being genetic by Oswald Avery, Colin MacLeod, and Maclyn McCarty. They isolated DNA from a virulent strain of *S. pneumoniae* and using just this DNA were able to make a harmless strain virulent. The results of Avery et al.'s experiments were at first skeptically received by the scientific community and it was not until the development of genetic markers and the discovery of other methods of genetic transfer by Joshua Lederberg that Avery's experiments were accepted.

It was originally thought that *Escherichia coli*, a commonly used laboratory organism, was refractory to transformation. However, in 1970, Morton Mandel and Akiko Higa showed that *E. coli* may be induced to take up DNA from bacteriophage λ without the use of helper phage after treatment with calcium chloride solution. Two years later in 1972, Stanley Norman Cohen, Annie Chang and Leslie Hsu showed that CaCl

$_2$ treatment is also effective for transformation of plasmid DNA. The method of transformation by Mandel and Higa was later improved upon by Douglas Hanahan. The discovery of artificially induced competence in *E. coli* created an efficient and convenient procedure for transforming bacteria which allows for simpler molecular cloning methods in biotechnology and research, and it is now a routinely used laboratory procedure.

Transformation using electroporation was developed in the late 1980s, increasing the efficiency of in-vitro transformation and increasing the number of bacterial strains that could be transformed. Transformation of animal and plant cells was also investigated with the first transgenic mouse being created by injecting a gene for a rat growth hormone into a mouse embryo in 1982. In 1897 a bacterium that caused plant tumors, *Agrobacterium tumefaciens*, was discovered and in the early 1970s the tumor-inducing agent was found to be a DNA plasmid called the Ti plasmid. By removing the genes in the plasmid that caused the tumor and adding in novel genes, researchers were able to infect plants with *A. tumefaciens* and let the bacteria insert their chosen DNA into the genomes of the plants. Not all plant cells are susceptible to infection by *A. tumefaciens*, so other methods were developed, including electroporation and micro-injection. Particle bombardment was made possible with the invention of the Biolistic Particle Delivery System (gene gun) by John Sanford in the 1980s.

4.5.2. Conjugation

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. This takes place through a pilus. It is a parasexual mode of reproduction in bacteria**.** It is a mechanism of horizontal gene transfer as are transformation and transduction although these two other mechanisms do not involve cell-to-cell contact. Classical *E. coli* bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction or mating since it involves the exchange of genetic material. However, it is not sexual reproduction, since no exchange of gamete occurs, and indeed no generation of a new organism: instead an existing organism is transformed. During classical *E. coli* conjugation the *donor* cell provides a conjugative or mobilizable genetic element that is most often a plasmid or transposon.

Most conjugative plasmids have systems ensuring that the *recipient* cell does not already contain a similar element. The genetic information transferred is often beneficial to the recipient.

Benefits may include antibiotic resistance, xenobiotic tolerance or the ability to use new metabolites. Other elements can be detrimental and may be viewed as bacterial parasites. Conjugation in *Escherichia coli* by spontaneous zygogenesis and in *Mycobacterium smegmatis* by distributive conjugal transfer differ from the better studied classical *E. coli* conjugation in that these cases involve substantial blending of the parental genomes.

History

The process was discovered by Joshua Lederberg and Edward Tatum in 1946.

Mechanism

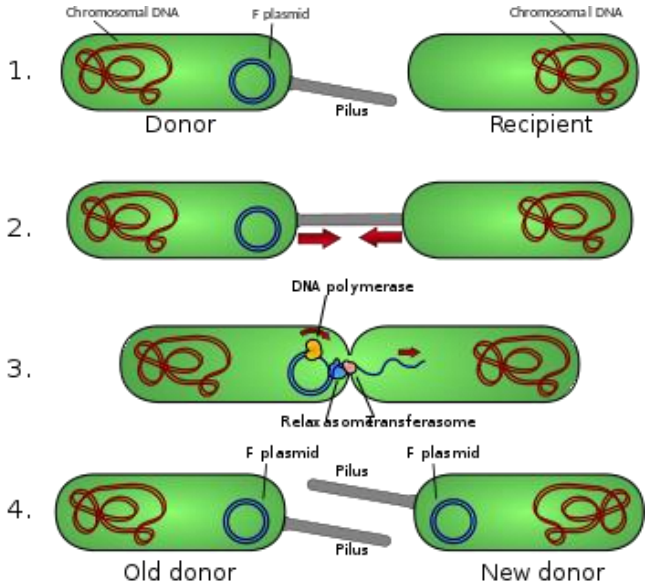


Fig. 6 Schematic drawing of bacterial conjugation.

Conjugation diagram

1. Donor cell produces pilus.
2. Pilus attaches to recipient cell and brings the two cells together.
3. The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell.
4. Both cells synthesize a complementary strand to produce a double stranded circular plasmid and also reproduce pili; both cells are now viable donor for the F-factor.

The F-plasmid is an episome (a plasmid that can integrate itself into the bacterial chromosome by homologous recombination) with a length of about 100 kb. It carries its own origin of replication, the *oriV*, and an origin of transfer, or *oriT*.[4] There can only be one

copy of the F-plasmid in a given bacterium, either free or integrated, and bacteria that possess a copy are called *F-positive* or *F-plus* (denoted F^+). Cells that lack F plasmids are called *F-negative* or *F-minus* (F^-) and as such can function as recipient cells.

Among other genetic information, the F-plasmid carries a *tra* and *trb* locus, which together are about 33 kb long and consist of about 40 genes. The *tra* locus includes the *pilin* gene and regulatory genes, which together form pili on the cell surface. The locus also includes the genes for the proteins that attach themselves to the surface of F^- bacteria and initiate conjugation. Though there is some debate on the exact mechanism of conjugation it seems that the pili are not the structures through which DNA exchange occurs. This has been shown in experiments where the pilus are allowed to make contact, but then are denatured with SDS and yet DNA transformation still proceeds. Several proteins coded for in the *tra* or *trb* locus seem to open a channel between the bacteria and it is thought that the traD enzyme, located at the base of the pilus, initiates membrane fusion.

When conjugation is initiated by a signal the **relaxase** enzyme creates a nick in one of the strands of the conjugative plasmid at the *oriT*. Relaxase may work alone or in a complex of over a dozen proteins known collectively as a **relaxosome**. In the F-plasmid system the relaxase enzyme is called TraI and the relaxosome consists of TraI, TraY, TraM and the integrated host factor IHF. The nicked strand, or *T-strand*, is then unwound from the unbroken strand and transferred to the recipient cell in a 5'-terminus to 3'-terminus direction. The remaining strand is replicated either independent of conjugative action (vegetative replication beginning at the *oriV*) or in concert with conjugation (conjugative replication similar to the rolling circle replication of lambda phage). Conjugative replication may require a second nick before successful transfer can occur. A recent report claims to have inhibited conjugation with chemicals that mimic an intermediate step of this second nicking event.

If the F-plasmid that is transferred has previously been integrated into the donor's genome (producing an Hfr strain ["High Frequency of Recombination"]) some of the donor's chromosomal DNA may also be transferred with the plasmid DNA. The amount of chromosomal DNA that is transferred depends on how long the two conjugating bacteria remain in contact. In common laboratory strains of *E. coli* the transfer of the entire bacterial chromosome takes about 100 minutes. The transferred DNA can then be integrated into the recipient genome via homologous recombination.

A cell culture that contains in its population cells with non-integrated F-plasmids usually also contains a few cells that have accidentally integrated their plasmids. It is these cells that are responsible for the low-frequency chromosomal gene transfers that occur in such cultures. Some strains of bacteria with an integrated F-plasmid can be isolated and grown in pure culture. Because such strains transfer chromosomal genes very efficiently they are called Hfr (high **f**requency of **r**ecombination). The *E. coli* genome was originally mapped by interrupted mating experiments in which various Hfr cells in the process of conjugation were sheared from recipients after less than 100 minutes (initially using a Waring blender). The genes that were transferred were then investigated.

Conjugal transfer in mycobacteria

Conjugation in *Mycobacteria smegmatis*, like conjugation in *E. coli*, requires stable and extended contact between a donor and a recipient strain, is DNase resistant, and the transferred DNA is incorporated into the recipient chromosome by homologous recombination. However, unlike *E. coli* Hfr conjugation, mycobacterial conjugation is chromosome rather than plasmid based. Furthermore, in contrast to *E. coli* Hfr conjugation, in *M. smegmatis* all regions of the chromosome are transferred with comparable efficiencies. The lengths of the donor segments vary widely, but have an average length of 44.2kb. Since a mean of 13 tracts are transferred, the average total of transferred DNA per genome is 575kb. This process is referred to as "Distributive conjugal transfer." Gray et al. found substantial blending of the parental genomes as a result of conjugation and regarded this blending as reminiscent of that seen in the meiotic products of sexual reproduction.

Inter-kingdom transfer

Fig. 7 Agrobacterium tumefaciens gall at the root of Carya illinoensis.

Bacteria related to the nitrogen fixing *Rhizobia* are an interesting case of inter-kingdom conjugation. For example, the tumor-inducing (Ti) plasmid of *Agrobacterium* and the root-tumor inducing (Ri) plasmid of *A. rhizogenes* contain genes that are capable of transferring to plant cells. The expression of these genes effectively transforms the plant cells into opine-producing factories. Opines are used by the bacteria as sources of nitrogen and energy. Infected cells form crown gall or root tumors. The Ti and Ri plasmids are thus endosymbionts of the bacteria, which are in turn endosymbionts (or parasites) of the infected plant.

The Ti and Ri plasmids can also be transferred between bacteria using a system (the *tra*, or transfer, operon) that is different and independent of the system used for inter-kingdom transfer (the *vir*, or virulence, operon). Such transfers create virulent strains from previously avirulent strains.

Genetic engineering applications

Conjugation is a convenient means for transferring genetic material to a variety of targets. In laboratories, successful transfers have been reported from bacteria to yeast, plants, mammalian cells, diatoms and isolated mammalian mitochondria. Conjugation has advantages over other forms of genetic transfer including minimal disruption of the target's cellular envelope and the ability to transfer relatively large amounts of genetic material. In plant engineering, *Agrobacterium*-like conjugation complements other standard vehicles such as tobacco mosaic virus (TMV). While TMV is capable of infecting many plant families these are primarily herbaceous dicots. *Agrobacterium*-like conjugation is also primarily used for dicots, but monocot recipients are not uncommon.

4.5.3. Transduction

Transduction is the process by which foreign DNA is introduced into a cell by a virus or viral vector. An example is the viral transfer of DNA from one bacterium to another and hence an example of horizontal gene transfer. Transduction does not require physical contact between the cell donating the DNA and the cell receiving the DNA (which occurs in conjugation), and it is DNase resistant (transformation is susceptible to DNase). Transduction is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome (both bacterial and mammalian cells).

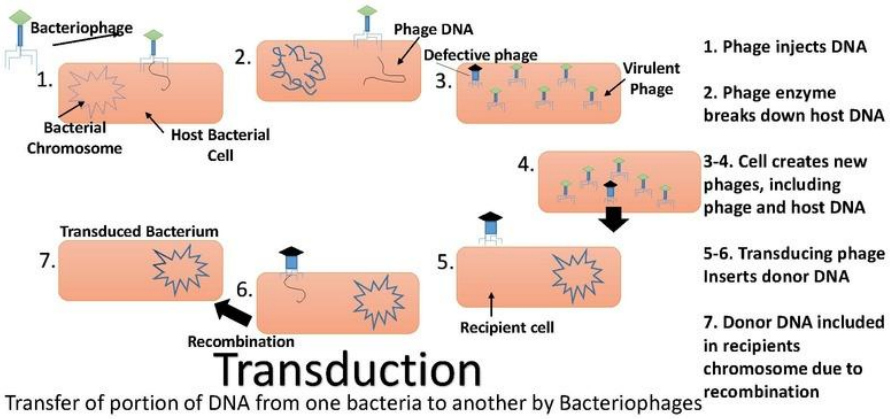


Fig. 9 Transduction

Discovery (bacterial transduction)

Transduction was discovered by Norton Zinder and Joshua Lederberg at the University of Wisconsin–Madison in 1952 in Salmonella.

In the lytic and lysogenic cycles

Transduction happens through either the lytic cycle or the lysogenic cycle. When bacteriophages (viruses that infect bacteria) that are lytic infect bacterial cells, they harness the replicational, transcriptional, and translation machinery of the host bacterial cell to make new viral particles (virions). The new phage particles are then released by lysis of the host. In the lysogenic cycle, the phage chromosome is integrated as a prophage into the bacterial chromosome, where it can stay dormant for extended periods of time. If the prophage is induced (by UV light for example), the phage genome is excised from the bacterial chromosome and initiates the lytic cycle, which culminates in lysis of the cell and the release of phage particles. Generalized transduction (see below) occurs in both cycles during the lytic stage, while specialized transduction (see below) occurs when a prophage is excised in the lysogenic cycle.

Method for transferring genetic material

Transduction by bacteriophages

The packaging of bacteriophage DNA into phage capsids has low fidelity. Small pieces of bacterial DNA may be packaged into the bacteriophage particles. There are two ways that this can lead to transduction.

Generalized transduction

Generalized transduction occurs when random pieces of bacterial DNA are packaged into a phage. It happens when a phage is in the lytic stage, at the moment that the viral DNA is packaged into phage heads. If the virus replicates using 'headful packaging', it attempts to fill the head with genetic material. If the viral genome results in spare capacity, viral packaging mechanisms may incorporate bacterial genetic material into the new virion. Alternatively, generalized transduction may occur via recombination. Generalized transduction is a rare event and occurs on the order of 1 phage in 11,000.[3]

The new virus capsule that contains part bacterial DNA then infects another bacterial cell. When the bacterial DNA packaged into the virus is inserted into the recipient cell three things can happen to it:

1. The DNA is recycled for spare parts.
2. If the DNA was originally a plasmid, it will re-circularize inside the new cell and become a plasmid again.
3. If the new DNA matches with a homologous region of the recipient cell's chromosome, it will exchange DNA material similar to the actions in bacterial recombination.

Specialized transduction

Specialized transduction is the process by which a restricted set of bacterial genes is transferred to another bacterium. The genes that get transferred (donor genes) flank where the prophage is located on the chromosome. Specialized transduction occurs when a prophage excises imprecisely from the chromosome so that bacterial genes lying adjacent to it are included in the excised DNA. The excised DNA is then packaged into a new virus particle, which then delivers the DNA to a new bacterium. Here, the donor genes can be inserted into the recipient chromosome or remain in the cytoplasm, depending on the nature of the bacteriophage. When the partially encapsulated phage material infects another cell and becomes a prophage, the partially coded prophage DNA is called a "heterogenote". An example of specialized transduction is λ phage in Escherichia coli.

Lateral transduction

Lateral transduction is the process by which very long fragments of bacterial DNA are transferred to another bacterium. So far, this form of transduction has been only described in Staphylococcus aureus, but it can transfer more genes and at higher frequencies than generalized and specialized transduction. In lateral transduction, the prophage starts its replication in situ before excision in a process that leads to replication of the adjacent bacterial DNA. After which, packaging of the replicated phage from its pac site (located around the middle of the phage genome) and adjacent bacterial genes occurs in situ, to 105% of a phage genome size.

Successive packaging after initiation from the original pac site leads to several kilobases of bacterial genes being packaged into new viral particles that are transferred to new bacterial strains. If the transferred genetic material in these transducing particles provides sufficient DNA for homologous recombination, the genetic material will be inserted into the recipient chromosome. Because multiple copies of the phage genome are produced during in situ replication, some of these replicated prophages excise normally (instead of being packaged in situ), producing normal infectious phages.

4.5.4. Recombination

Genetic recombination (also known as genetic reshuffling) is the exchange of genetic material between different organisms which leads to production of offspring with combinations of traits that differ from those found in either parent. In eukaryotes, genetic recombination during meiosis can lead to a novel set of genetic information that can be passed on from the parents to the offspring. Most recombination is naturally occurring.

During meiosis in eukaryotes, genetic recombination involves the pairing of homologous chromosomes. This may be followed by information transfer between the chromosomes. The information transfer may occur without physical exchange (a section of genetic material is copied from one chromosome to another, without the donating chromosome being changed) (see SDSA pathway in Figure); or by the breaking and rejoining of DNA strands, which forms new molecules of DNA (see DHJ pathway in Figure).

Recombination may also occur during mitosis in eukaryotes where it ordinarily involves the two sister chromosomes formed after chromosomal replication. In this case, new combinations of alleles are not produced since the sister chromosomes are usually identical. In

meiosis and mitosis, recombination occurs between similar molecules of DNA (homologous sequences). In meiosis, non-sister homologous chromosomes pair with each other so that recombination characteristically occurs between non-sister homologues. In both meiotic and mitotic cells, recombination between homologous chromosomes is a common mechanism used in DNA repair.

Mechanism

Genetic recombination is catalyzed by many different enzymes. Recombinases are key enzymes that catalyse the strand transfer step during recombination. RecA, the chief recombinase found in *Escherichia coli*, is responsible for the repair of DNA double strand breaks (DSBs). In yeast and other eukaryotic organisms there are two recombinases required for repairing DSBs. The RAD51 protein is required for mitotic and meiotic recombination, whereas the DNA repair protein, DMC1, is specific to meiotic recombination. In the archaea, the ortholog of the bacterial RecA protein is Rad A.

Bacterial recombination

In Bacteria there are:

- Regular bacterial recombination, as well as noneffective transfer of genetic material, expressed as
- Unsuccessful transfer or abortive transfer which is any bacterial DNA transfer of the donor cell recipients who have set the incoming DNA as part of the genetic material of the recipient. Abortive transfer was registered in the following transduction and conjugation. In all cases, the transmitted fragment is diluted by the culture growth.

Gene conversion

In gene conversion, a section of genetic material is copied from one chromosome to another, without the donating chromosome being changed. Gene conversion occurs at high frequency at the actual site of the recombination event during meiosis. It is a process by which a DNA sequence is copied from one DNA helix (which remains unchanged) to another DNA helix, whose sequence is altered. Gene conversion has often been studied in fungal crosses where the 4 products of individual meioses can be conveniently observed. Gene conversion events can be distinguished as deviations in an individual meiosis from the normal 2:2 segregation pattern.

Nonhomologous recombination

Recombination can occur between DNA sequences that contain no sequence homology. This can cause chromosomal translocations, sometimes leading to cancer.

In B cells

B cells of the immune system perform genetic recombination, called immunoglobulin class switching. It is a biological mechanism that changes an antibody from one class to another, for example, from an isotype called IgM to an isotype called IgG.

Genetic engineering

In genetic engineering, recombination can also refer to artificial and deliberate recombination of disparate pieces of DNA, often from different organisms, creating what is called recombinant DNA. A prime example of such a use of genetic recombination is gene targeting, which can be used to add, delete or otherwise change an organism's genes. This technique is important to biomedical researchers as it allows them to study the effects of specific genes. Techniques based on genetic recombination are also applied in protein engineering to develop new proteins of biological interest.

Recombinational repair

DNA damages caused by a variety of exogenous agents (e.g. UV light, X-rays, chemical cross-linking agents) can be repaired by homologous recombinational repair (HRR). These findings suggest that DNA damages arising from natural processes, such as exposure to reactive oxygen species that are byproducts of normal metabolism, are also repaired by HRR. In humans, deficiencies in the gene products necessary for HRR during meiosis likely cause infertility In humans, deficiencies in gene products necessary for HRR, such as BRCA1 and BRCA2, increase the risk of cancer (see DNA repair-deficiency disorder).

In bacteria, transformation is a process of gene transfer that ordinarily occurs between individual cells of the same bacterial species. Transformation involves integration of donor DNA into the recipient chromosome by recombination. This process appears to be an adaptation for repairing DNA damages in the recipient chromosome by HRR. Transformation may provide a benefit to pathogenic bacteria by allowing repair of DNA damage, particularly damages that occur in the inflammatory, oxidizing environment associated with infection of a host.

When two or more viruses, each containing lethal genomic damages, infect the same host cell, the virus genomes can often pair with each other and undergo HRR to produce viable progeny. This process, referred to as multiplicity reactivation, has been studied in lambda and T4

bacteriophages, as well as in several pathogenic viruses. In the case of pathogenic viruses, multiplicity reactivation may be an adaptive benefit to the virus since it allows the repair of DNA damages caused by exposure to the oxidizing environment produced during host infection.

4.6. Bacterial genetic map with reference to Ecoli

Macrorestriction mapping is often the first step toward a thorough physical and genetic characterization of a bacterial genome. The problem of deducing the order of partially or completely digested macrorestriction fragments to yield a physical genome map may readily be solved by applying twodimensional pulsed-field gel electrophoresis (2D-PFGE) techniques. These powerful methods are quick and technically easy to perform; specifically, they are independent of DNA probes and should therefore be applicable to any bacterial species irrespective of its prior genetic characterization. Other topics include preparation of bacterial genomic DNA, screening for suitable rare-cutting restriction enzymes and determination of optimal running conditions. Accompanied by many notes, these protocols are meant to offer the novice a sound and rapid access to these important methods.

Several methods have revealed that the genetic map of the main chromosome of E. coli is an un-branched circle with a circumference of 100 minutes as in given figure which corresponds to the transfer time during conjugation. About 650 genes have been mapped. Some important features of the genetic map have been noted. In many cases functionally related genes occur together and form clusters.

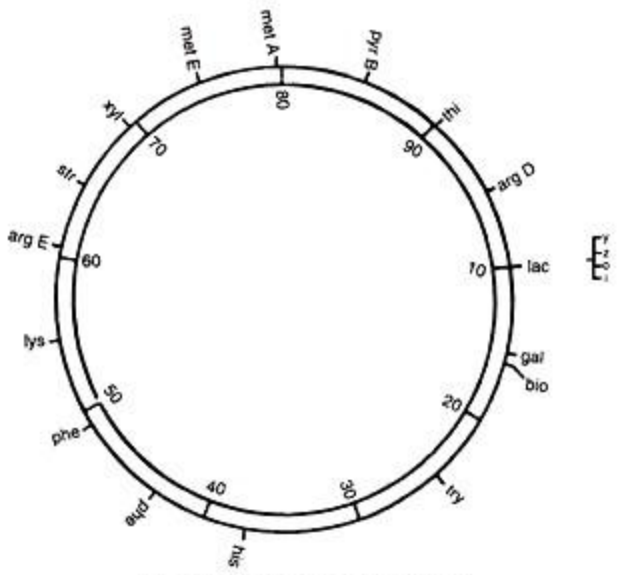


Fig. 17.5 The genetic map of *E. coli*.

Fig.10 Genetic map of E. coli.

For example the genes involved in the catabolism of lactose and synthesis of amino acids tryptophan and histidine are all clustered together. On the contrary, some functionally related genes are placed far apart. For example, the genes related with catabolism of arabinose are present at three sites, and those concerned with biosynthesis of leucine occur at several different sites. The orientation of all genes is not the same. Genes are arranged in both clockwise and anticlockwise manner.

4.7. Summary

Under this unit we are discussing concept of chemotherapy, antibiotics and molecular mechanism of drug resistance. The word antimicrobial was derived from the Greek words anti (against), micro (little) and bios (life) and refers to all agents that act against microbial organisms. Antimicrobial agent is a general term that is mainly concerned with antibiotics, antibacterials, antifungals, antivirals and anti*protozoans*. Antimicrobial agents are drugs, chemicals or other substances that are capable of acting by two modes either kill (*microbiocidal*) or slow the growth of microbes (*microbiostatic*). Antimicrobial medicines can be classified according to the microorganisms they act primarily against. For example, antibacterials are used against *bacteria* and antifungals are used against *fungi*.

Chemotherapy is a drug treatment that uses powerful chemicals to kill fast-growing cells in your body. Chemotherapy is most often used to treat cancer, since cancer cells grow and multiply much more quickly than most cells in the body.

Many different chemotherapy drugs are available. Chemotherapy drugs can be used alone or in combination to treat a wide variety of cancers. Though chemotherapy is an effective way to treat many types of cancer, chemotherapy treatment also carries a risk of side effects. Some chemotherapy side effects are mild and treatable, while others can cause serious complications

Resistance to chemotherapy limits the effectiveness of anti-cancer drug treatment. Tumours may be intrinsically drug-resistant or develop resistance to chemotherapy during treatment. Acquired resistance is a particular problem, as tumours not only become resistant to the drugs originally used to treat them, but may also become cross-resistant to other drugs with different mechanisms of action. Resistance to chemotherapy is believed to cause treatment failure in over 90% of patients with metastatic cancer, and resistant micrometastic tumour cells may also reduce the effectiveness of chemotherapy in the adjuvant setting. Clearly, if drug resistance could be overcome, the impact on survival would be highly significant. This review focuses on molecular mechanisms of drug resistance that operate to reduce drug sensitivity in cancer cells.

Antibiotics, also known as antibacterials, are medications that destroy or slow down the growth of bacteria. They include a range of powerful drugs and are used to treat diseases caused by bacteria. Antibiotics cannot treat viral infections, such as cold, flu, and most coughs. This unit will explain what antibiotics are, how they work, any potential side effects, and antibiotic resistance. Antibiotics are no longer routinely used to treat infections because:

- Many infections are caused by viruses, so antibiotics are not effective
- Antibiotics are often unlikely to speed up the healing process and can cause side effects
- The more antibiotics are used to treat trivial conditions, the more likely they are to become ineffective for treating more serious conditions

Both the NHS and health organizations across the world are trying to reduce the use of antibiotics, especially for health problems that are not serious.

Drug resistance can occur at many levels, including increased drug efflux, drug inactivation, alterations in drug target, processing of drug-induced damage, and evasion of apoptosis. Advances in DNA microarray and proteomic technology, and the ongoing

development of new targeted therapies have opened up new opportunities to combat drug resistance. We are now able to characterize the signalling pathways involved in regulating tumour cell response to chemotherapy more completely than ever before. This will facilitate the future development of rational combined chemotherapy regimens, in which the newer targeted therapies are used in combination with cytotoxic drugs to enhance chemotherapy activity. The ability to predict response to chemotherapy and to modulate this response with targeted therapies will permit selection of the best treatment for individual patients.

4.8. 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:----------

Q.7: Write a short note on the followings.

(i) Transduction

(ii) Conjugation

(iii) Transformation

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

4.9. Further readings

1. Biochemistry- Lehninger A.L.
2. Biochemistry –J.H.Weil.
3. Biochemistry fourth edition-David Hames and Nigel Hooper.
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Block- III

Microbial diseases and Environmental Toxicology

UNIT -5

Microbial diseases

UNIT-6

Introduction to Environmental Toxicology

Introduction

This is the first block on Microbial diseases and Environmental Toxicology. It consists of following two units:

Unit-5: This unit covers the general introduction of microbial diseases. A variety of microorganisms are responsible to cause disease in human being. Pathogenic organisms are of five main types: viruses, bacteria, fungi, protozoa, and worms. An infection is the invasion and multiplication of pathogenic microbes in an individual or population. Disease is when the infection causes damage to the individual's vital functions or systems. In this unit, the bacterial and virus diseases with reference to tuberculosis, cholera, AIDS and Rabies are discussed in this unit. Foodborne illness is caused by consuming contaminated foods or beverages. Most foodborne diseases are infections caused by a variety of bacteria, viruses, and parasites.

Unit-6: this unit covers the basic introduction of environmental toxicology. Environmental toxicology that is concerned with the assessment of toxic substances in the environment such as bioconcentration, bioaccumulation, and biomagnifications. Toxicokinetics is the study of kinetics of absorption, distribution, metabolism, and excretion of xenobiotic under the conditions of toxicity evaluation. The toxicokinetics studies of xenobiotics compounds, Biotransformation of Xenobiotics compounds are briefly discussed in this unit.

Unit-5 Microbial Diseases

Contents

5.1 Introduction

The Microbes or microorganisms are minute unicellular organism that cannot be seen by naked eyes. Some microbes are useful in day-to-day life, while others are harmful for health. The harmful microorganism is called **pathogens.**

The diseases causing microorganisms include bacteria, viruses, fungi and parasites. Those that can cause disease are collectively known as pathogens. Viruses are the smallest, followed by bacteria, fungi and parasites. Viral disease is caused by viruses. These include both acute and infectious disease like the common cold, to chronic disease like AIDS. A part from these acute disease viruses are also responsible for mumps, polio, rabies etc, diseases caused by bacteria, include diphtheria, typhoid, cholera etc.

The main source of bacterial infection is contaminated food and water. 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. While viral diseases spread mainly through the air.

Objectives:

After studying this unit you will be able to known:

- Describe the different types of bacterial and viral diseases
- Describe the different types of food born disease
- Describe tuberculosis, cholera, AIDS and Rabies.

5.2 Structure of Bacteria and Virus

A virus is microscopic infectious agent that replicates only inside the living cells of an organism. 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; and in some cases (iii) a outside envelope of lipids.

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; 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.

Bacterial and viral infections can cause similar symptoms such as coughing and sneezing, fever, inflammation, vomiting, diarrhea, fatigue, and cramping -- all of which are ways the immune system tries to rid the body of infectious organisms. But bacterial and viral infections are dissimilar in many other important respects, most of them due to the organisms' structural differences.

Bacteria have a rigid cell wall which protects the protoplast and provide definite shape to the cell. 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 less lipid whereas of gram-negative bacteria are thin and have more lipid.

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 non flagellated.

Next to cell wall is plasma membrane made up of phospholipids, proteins and polysaccharides. 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 some gram-negative bacteria straight hair like minute appendages are found. These are called pili, which helps in the attachment of bacterial cell. Inside the plasma membrane is cytoplasm, which has a mixture of protein, carbohydrates, lipids, minerals, nucleic acid, enzymes and coenzymes. It also contains ribosome, RNA and nuclear body called nucleoid. The true nucleus is absent in bacteria. Only DNA is present, which is called nucleoid. The DNA is double stranded. Reserve food is in the form of oil globule and volutin granules. Other cellular organelles like mitochondria, endoplasmic reticulum, plastids are absent.

Plasmid- Besides nucleotide extra chromosomal genetic material is 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 no and mode of attachment of flagella the bacteria are of following types:-

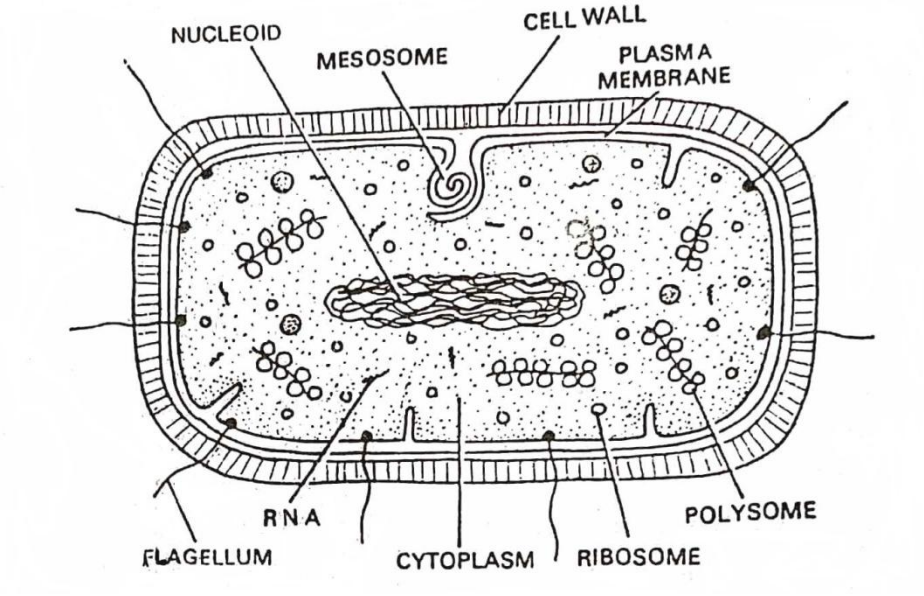


Fig. 1 Structure of a bacterial cell.

1. **Monotrichous:** Single flagellum is attached to one end of bacterial cell.
2. **Amphitrichous:** One flagellum is at each pole of bacterial cell.
3. **Cephalotrichous:** Two of more flagella are present in bunch at one pole of the bacterial cell.
4. **Lophotrichous:** Two or more flagella are present at both the poles of the bacterial cell.
5. **Peritrichous:** Large no of flagella are evenly distributed all over the surface of bacterial cell.
6. **Atrichous:** Bacteria which lack flagella are called atrichous.

Nature of Virus/Biological Status of Virus:

The biological status of virus is still not certain as some of the characters of virus are similar to the nonliving one and others are similar to the living one.

Non-living or Inanimate characters of Virus: The non living characters are as follows:

1. They have no complete cellular structure (No cell membrane, cell wall).
2. Viruses have no independent existence. They are active only inside the host cell.
3. They lack cellular metabolism.
4. They can be crystallized like simple chemical substances. The living organism cannot be crystallized.
5. They can be precipitated like chemical substances.

Living or Animate characters of Virus: The characters on the basis of which viruses are considered as living are as follows:

1. They have definite shape and have genetic material either DNA or RNA.

2. All viruses attack specific host and causes disease.
3. They show mutation.
4. Virus show irritability and respond to environ mental conditions.
5. 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. Thus, viruses are exceptionally simple microbes and complex chemicals.

5.3. Bacterial Diseases

Tuberculosis:

Tuberculosis is a specific infectious disease caused by *Mycobacterium tuberculosis*. The disease primarily affects lungs and causes pulmonary tuberculosis. 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. Pulmonary tuberculosis the most important form of tuberculosis which affects the man.

Mycobacteria are slender rods that sometimes show branching filamentous forms resembling fungal mycelium. In liquid cultures they form a mold like pellicle. Hence the name "mycobacteria" meaning fungus like bacteria. They do not stain readily, but once stained resist, decolourisation with dilute mineral acids. Mycobacteria are therefore called acid fast bacilli, or AFB. They are aerobic, nonmotile, noncapsulated and nonsporing. 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. 2 Tuberculosis infection in lungs

SAQ-1

Q.1: Tuberculosis is a serious ……..

Q.2: Tuberculosis is caused by a bacterium called ……..

Q.3: TB bacteria are spread through the …… form one person to another.

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 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 extrapulmonary 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 positive 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 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 quickly to the level where it caeses to be a 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 plane 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.

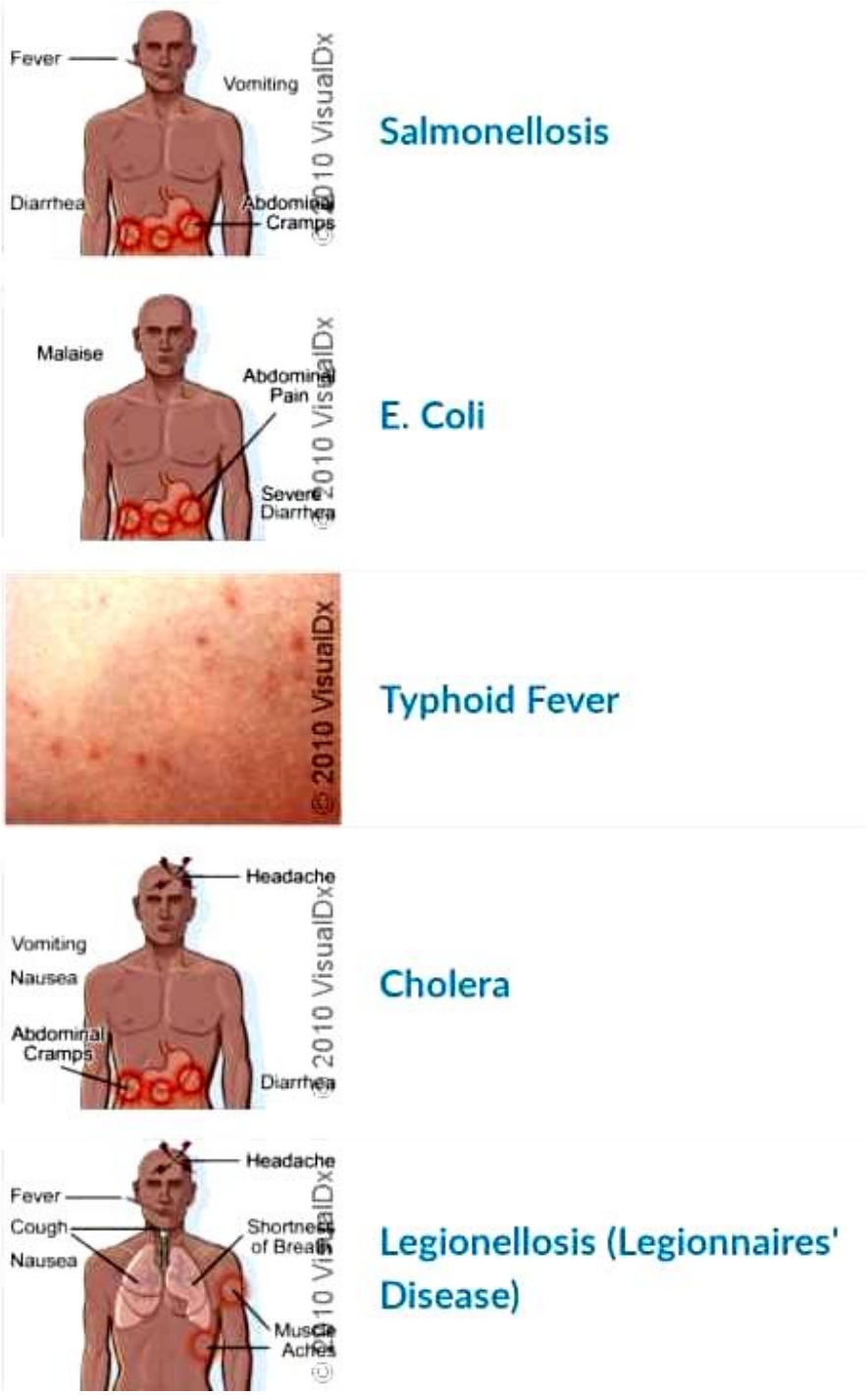


Fig. 3

Cholera:

Cholera is a severe water borne diarrheal disease frequently occurring in developing countries. It is caused by *Vibrio cholerae* a gram-negative curved bacillus bacterium transmitted almost exclusively via contaminated water, food etc. vibrio cholerae multiply in the small

intestine and produce the enterotoxin called choleragen which binds irreversibly to epithelial cells of small intestine and stimulates cyclic AMP within the mucosa cells. The activation of cyclic AMP initiates excessive secretion of water and electrolytes (chlorine ions) into the intestine and inhibits absorption of sodium ions. The majority of infections are mild or asymptomatic. Typical cases are characterized by the sudden onset of profuse, effortless, watery diarrhea followed by vomiting, rapid dehydration muscular cramps and suppression of urine.

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 ciprofloxacin. The killed vaccine of cholera can be taken as prophylactic.

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 with have 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 enable 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 shed's 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, 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 districts, ORS is being supplied as a part of the sub-centre kits.

SAQ-2

Q.1: Cholera is caused by ……. bacteria

Q.2: Cholera is an acute ……..

Q.3: The acute diarrheal infection caused by ……with the bacterium vibrio cholera.

5.4 Viral Diseases

AIDS (acquired immunodeficiency syndrome)

Human immunodeficiency virus or HIV is the virus that causes AIDS. The emergence and pandemic spread of the acquired immunodeficiency 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.

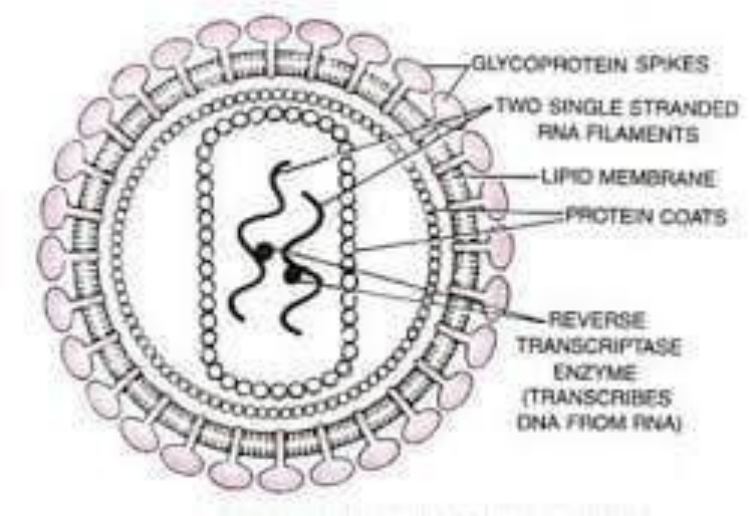


Fig. 4 Diagrammatic representation of AIDS Virus (HIV)

The HIV virus transmitted from following ways. HIV is spread only by three modes sexual contact with infected person (heterosexual or homosexual), by blood and blood products and 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 the 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 in USA called it "human T-cell lymphotropic virus. In may 1986, the international committee on the taxonomy gave it a new name:- human immuno deficiency virus (HIV)

Blood Contact

AIDS is also transmitted by contaminated blood- transfusion of whole blood cells, 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 with transfusion. As a result needle sharing by drug users is a major causes of AIDS in many countries, both developed and developing, and in some it is the predominant causes.

Any skin piercing (including injection, etc) can transmit the virus if the instruments used have not been sterilized 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 or HIV positive mothers get infected through this 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.

Counselling 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. Counselling 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 counselling sessions with those close to them. Many counselling 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.

2. **Prevention of bloodborne 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 to countries primary health care system is essential.

SAQ-3

Q. Write the full name of these words-

(1) HIV …….

(2) AIDS ……...……...

(3) Which virus causes AIDS ……..

Rabies

Rabies is a viral disease that causes inflammation of the brain in human and other mammals. Rabies is an enzootic and epizootic disease of worldwide importance. Rabies also known as hydrophobia is nervous system, caused by lyssavirus. 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 maybe 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 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.

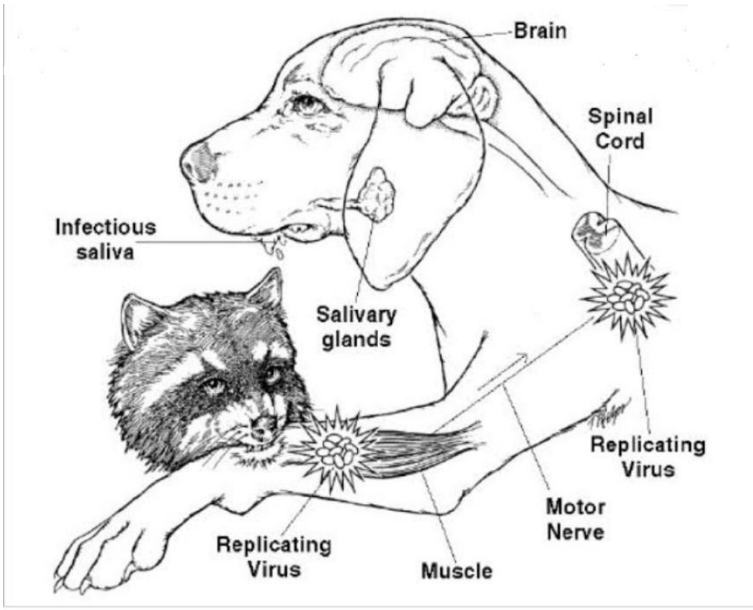


Fig. 5 Rabies infection by dog biting

ii. **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.

iii. **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 infected lab workers.

iv. **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 by 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.

SAQ -4

1. Rabies is caused by a ……
2. Rabies is viral infection that affects the …… of mammals.

5.5 Different types of Food Born Diseases

Types of Food-Borne Disease

Food-borne illnesses impact the entire world. There are two primary types of food related diseases; food borne infection and food intoxications. All of these food borne disease are associated with poor hygienic practices.

Food Borne Infection

A food borne infection involves the ingestion of the pathogen, followed by growth in the host, including tissue invasion or the release of toxins. Food borne infections can cause serious outbreaks with wide ranging consequences. More recently other types of food have been found to be contaminated with E-coli.

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 are 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 a specific pathogen; (2) viruses that cannot be grown conveniently; (3) microbes that present in very small. Foodborne and waterborne infections affect the intestinal tract.

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 an 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.

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 are 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 enterotoxin producing strain of *S. aureus.* The staphylococcal food poising are 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 enterotoxin.

The food is consumed, raw or cooked. Cooking does not destroy the enterotoxin. 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 diarrhoea, but usually no fever.

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 diaphragm 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 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. Only a few examples of the many kinds of foodborne and waterborne infections are included. *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 unusual physiological features that complicate their isolation from patients.

Salmonella Gastroenteritis and 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.

SAQ-5

- The most common symptom of food borne illness is ……..
- Food poisoning is a food borne illness caused by eating contaminated with …….

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 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 effects liver and kidneys. The aflatoxin function as potent toxin, a

carcinogen, a teratogen and mutagen. Person having hepatisis 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.

5.6 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).
- Which 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, HPV 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 a severe water borne diarrheal disease frequently occurring in developing countries. It is caused by *Vibrio cholerae* 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 or the large intestine).

5.7 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:----------

5.8 Answers

SAQ-1

1. Bacterial diseases.
2. Mycobacterium tuberculosis
3. Air

SAQ-2

1. Vibrio cholera
2. Diarrheal disease
3. Ingestion of food or water contaminated

SAQ-3

1. Human immuno deficiency virus

2. Acquired immune deficiency Syndrome.
3. HIV

SAQ-4

1. Virus
2. Nervous system

SAQ-5

1. Diarrhea.
2. Bacteria, viruses or parasites.

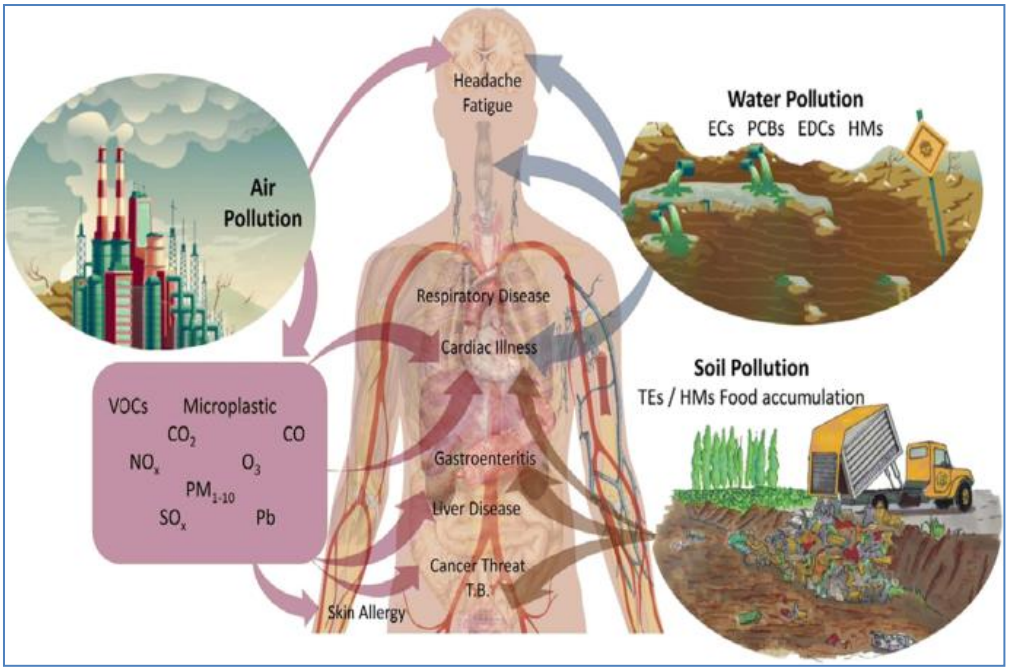
Further readings

1. Biochemistry- Lehninger A.L.
2. Biochemistry –J.H.Weil.
3. Biochemistry fourth edition-David Hames and Nigel Hooper.
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.
6. Microbiology by Michael Pelczar.
7. Microbiology by Prescott's.

Unit-9: Introduction to environmental toxicology

9.1. Introduction

Environmental toxicology refers to toxicity of environmental contaminants associated with health of living beings. It is produce from the anthropogenic as well as natural activity. Due this anthropogenic activity, our environment is surrounding by number of toxicant in different form. Toxicant contaminated (The term "**contaminant**" means hazardous substances, pollutants, pollution, and chemicals) the environmental components, like air, water and atmosphere. Thus these toxicant alter the quality of these component temporarily or permanently. The term environmental toxicology is also describes the management of environmental toxins and toxicity, and the development of protections for humans and the environment. Role of different metals, pesticide and different organic complexes has been clearly identified that are responsible for contamination of surrounding environments. The natural activity that is directly or indirectly affected form human activity is also responsible for contamination of environmental components. Before going to more details about environmental toxicology, we should know about toxicant.



Objectives

- To discuss about environmental toxicity and their effects
- To understand bioaccumulation, biotransformation, biomagnifications and bioremediation
- To know the xenobiotics compounds and their Toxicokinetics

9.2. Environmental toxicity

Environmental toxicology is a multidisciplinary field of science concerned with the study of the harmful effects of various chemical, biological and physical agents on living organisms. Rachel Carson is considered the mother of environmental toxicology, as she made it as a distinct field within toxicology in 1962 with the publication of her book Silent Spring, which covered the effects of uncontrolled pesticide use. The toxicant may be any chemical or physical agent that produces adverse responses in the biological system when they interact with them. The response may be of long term or with short period of time. Sometime, this response may be long term or permanent. At the basis of response of exposure of toxicant the, acute and chronic effects is determined.

An acute effect of a contaminant is one that occurs rapidly after exposure to a large amount of that substance. A chronic effect of a contaminant results from exposure to small amounts of a substance over a long period of time. In such a case, the effect may not be immediately obvious.

Chronic effect is difficult to measure, as the effects may not be seen for years. Long-term exposure to cigarette smoking, low level radiation exposure, and moderate alcohol use are all thought to produce chronic effects.

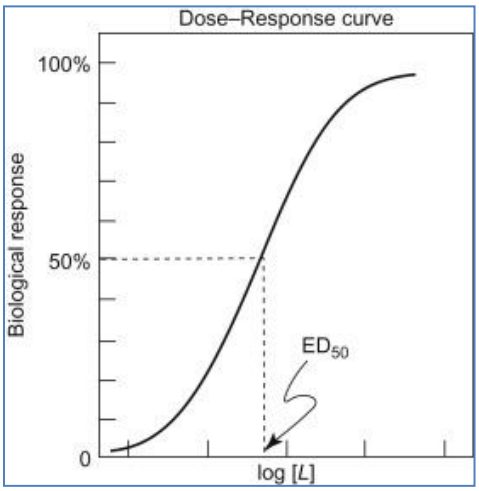
In our body, the toxicant enters through number of routes like through drink, breath, food, medicine or through accidental or occupational exposure. During toxicant exposure, some of chemical alter when enter into the body because the human body physiological function attempt to eliminate these toxicant through the excretory path way. The toxicant not only affects the human health but also cause serious threat on survivability of different biota. In order to cause health problems, chemicals must enter in our body. There are three main "routes of exposure," or ways, a chemical can get into your body.

- **Breathing (inhalation):** Breathing in chemical gases, mists, or dusts that are in the air.
- **Skin or eye contact**: Getting chemicals on the skin, or in the eyes. They can damage the skin, or be absorbed through the skin into the bloodstream.
- **Swallowing (ingestion):** This can happen when chemicals have spilled or settled onto food, beverages, cigarettes, beards, or hands.

When metal are introduce in the aquatic system through the natural or manmade activity, they alter the water quality that creates number of water born disease in human being and aquatic organism. Due to non degradable nature of metals, it persist long time in air, water and soil. In addition the pesticide and herbicide are also non biodegradable nature and make more complexes molecule when inter within living organism and resultant creates multi diseases or failure of organs, like kidney failure, lungs damage and skin disorder. Heavy metals are considered to be more toxic element due to having density relative to water which is greater than 5.

The toxicity test is determine by the dose response relationship where consistence mathematical relationship describes the proportion of test organisms responding for given dosage interval for a given exposure period. When dosage is described to be lethal (LD), means it in test the response is mortality. When dosage is described to be effective (ED), means it in test the

response is desirable, while toxic dose (TD) is said to be create undesirable toxicity other than death.



On the basis of response of dosage-response curve we would determine the LD50, LC50 and LT50. In addition, the dosage response curve also use full in determination of toxicity of chemical substance and threshold limits of toxicity. A dose that is lethal to 50% of a population of test animals is called the lethal dose-50% or LD-50. However, a dose that causes 50% of a population to exhibit any significant response is referred to as the effective dose-50% or ED-50. The overall dose–response curve depends on the kinetics of hormone binding to its receptor and to the kinetics of post receptor events (**Figure 9.2**).

There are some factors that directly and indirectly affect the dosage response curve

- Physical and chemical properties of substances
- Duration and condition of exposure
- Genetic makeup of test system (living being)
- Environmental conditions

The substances/chemicals may be in different forms such as solids, liquids, dusts, vapors, gases, fibers, mists and fumes. A chemical can also change forms. For example, liquid solvents can evaporate and give off vapors that you can inhale.

Contaminants are often chemicals found in the environment in amounts higher than what would found naturally. We can be exposed to these contaminants from a variety of residential, commercial, and industrial sources.

Types of contaminants	**Means and example of contaminant s**
Carcinogen	Any substance that may cause cancer, either by

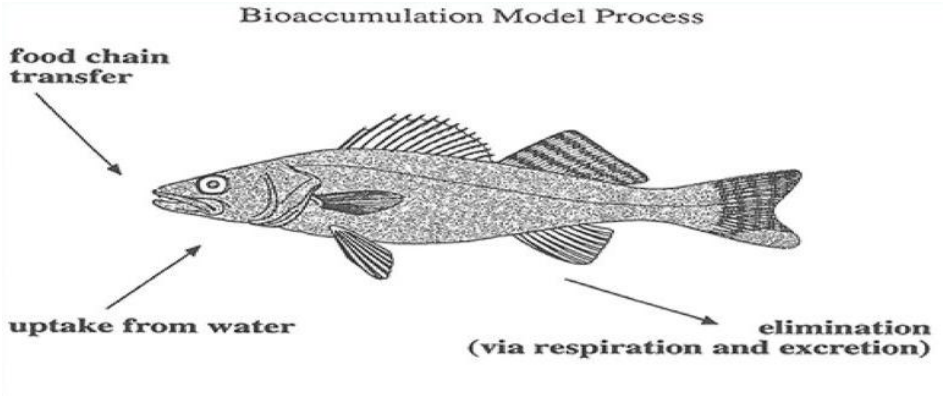
	itself or in conjunction with another substance. Examples: formaldehyde, asbestos, radon, vinyl chloride, and tobacco.
Teratogen	Any chemical which can cause physical defects in a developing embryo. Examples: alcohol and cigarette smoke.
Mutagen	Those agent which creates genetic changes (mutations) in the DNA. Examples: radioactive substances, x-rays and ultraviolet radiation.
Neurotoxicant	A substance that can cause an adverse effect in nervous system. Examples: lead and mercury heavy metals
Endocrine disruptor	A chemical that may interfere with the body's endocrine (hormonal) system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife. Example: pharmaceuticals, dioxin and dioxin-like compounds, arsenic, polychlorinated biphenyls (PCBs), DDT and other pesticides,

Apart from above contaminants some, other common contaminants are also listed here, such as Arsenic, Lead, Mercury, Radon, Bisphenol A (BPA) , Phthalates, Formaldehyde and Radiation etc.

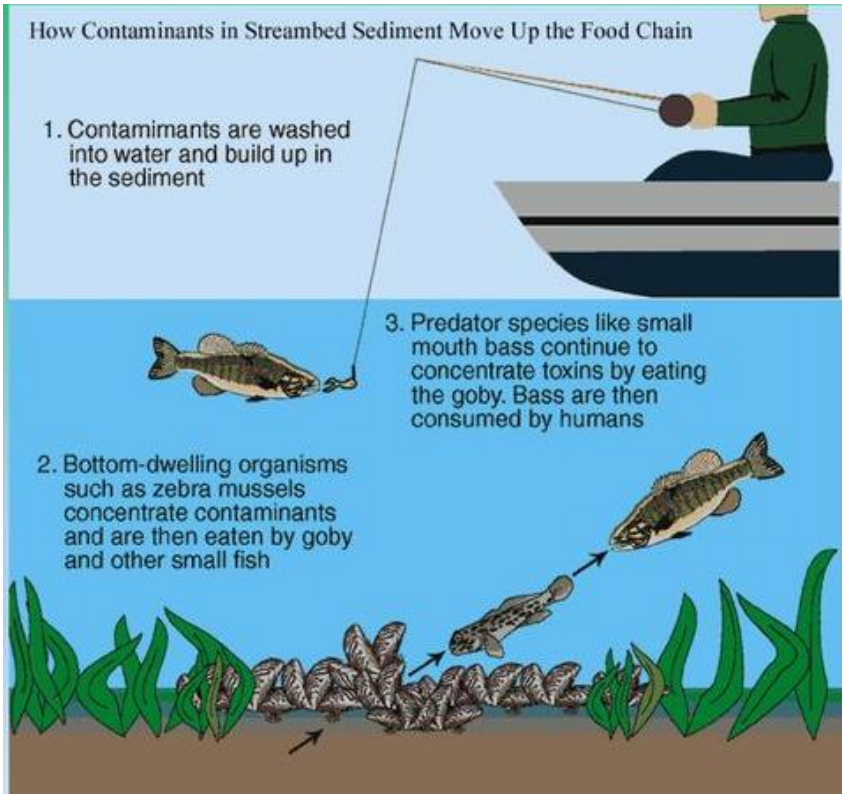
9.3. Bioconcentration

The term Bioconcentration is in simple means, to intake of chemicals by tissue of organism more than their surrounding environment. It is most popular term used for describing the process by which pesticide or other chemicals enter directly to organism through the gills or through epithelial tissues. For examples, fish intake comical from water through their gill. In Bioconcentration, the organism has ability of intake the contaminates from external environment

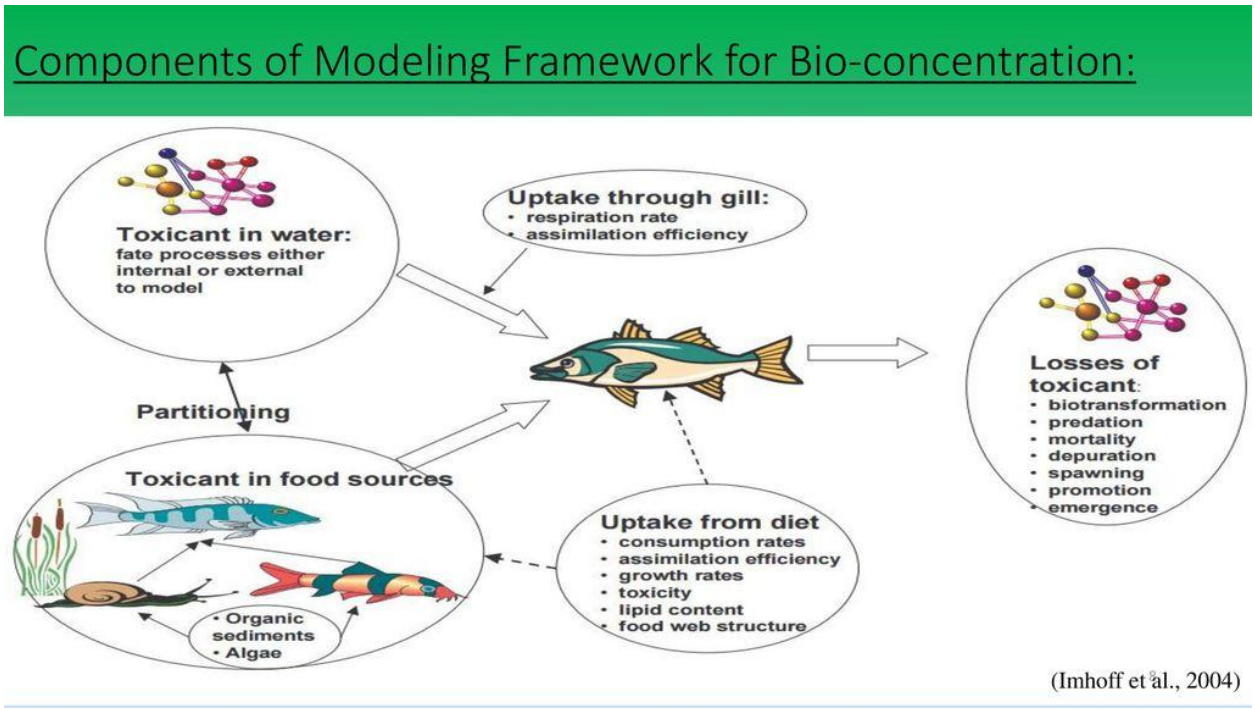
get intake in organism through food or other direct sources. In other world, the Bioconcentration is the process of direct partitioning of chemicals between the water and the organism, leading to elevated concentrations in the latter, when exceeds the level of concentration of chemical in aquatic organism than the concentration level of chemical in water. Bioconcentraion is the process by which chemical contamination in organism increase with each steps in the food chain and food web.



Bioaccumulation = Bio-concentration + food chain transfer – (elimination + growth dilution)



Thus we can say that the Bioconcentration is the ability of an organism to accumulate a chemical from the ambient environment. When the levels of a pesticide, accumulated by organisms, are concentrated through two or more trophic levels in a food web, the process is referred to as bio-magnification.



Bioconcentration is quantitatively expressed in terms of the bioconcentration factor (BCF) which is the ratio of the concentration of the chemical in the organism to its concentration in the ambient environment. The BCF is a measure of the extent of chemical sharing between an organism and the surrounding environment. It can be viewed as the product of the BCF and a 'multiplier' dependent on the BAF of the diet and the ratio of the rates of dietary uptake and respiratory uptake. Bioconcentration depends the properties of chemicals such as hydrophobicity, lipophilicity, and resistance to degradation; environmental factors such as salinity, temperature concentration of other organic chemicals, and redox potential; biotic factors such as the organism's mode of feeding and metabolism and bioavailability such as current chemical inputs, transport mechanisms, and degree of contamination.

The uptake of contaminants and their accumulation can be hazardous to the organism as well as to other aquatic life utilizing the test species as a food source. Bioconcentration tests are usually conducted with single chemicals and single species of algae, fish, and bivalve mollusks.

A variety of fish have been used, including the fathead minnow, bluegill, rainbow trout, sheepshead minnow, and several species of oysters, scallops, and mussels. There are several test designs that can be used to estimate the Bioconcentration potential of a compound such as uptake and depuration phase. The uptake phase is carried out for 28 days or united the steady state is obtained. However, the depuration period lasts until the concentration in the test species is 10% of the steady-state concentration in the tissue. During both phases, the test water and test species are analyzed daily for the test chemical.

Long term effect of Bioconcentration, because of persistent chemical can be seen as damage to organisms and decline in population of species. Heavy metals such as organic and biomethylated mercury, lead, cadmium, and organic tin can cause environment degradation in local level. The effects of organo-chlorine compounds are more widespread.

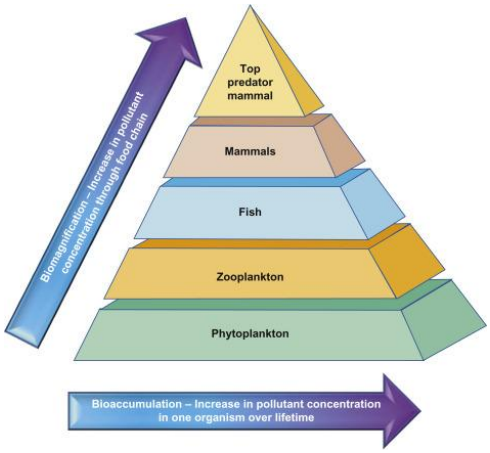
9.4. Bioaccumulation

Bioaccumulation and biomagnifications are two terms commonly used for metal toxicity. Bioaccumulation refers to how pollutants (metals) enter in a food chain and results to the accumulation of contaminants, in biological tissues of aquatic organisms, from sources such as water, food, and particles of suspended sediment. Biotransformation can strongly modify bioaccumulation of chemicals in an organism

In other, world bioaccumulation is the process by which the concentration of pesticide or other chemicals in an aquatic organism achieves a level that exceeds the level in the water, because of chemical uptake through all possible routes (e.g., dietary absorption, transport across the respiratory surface, dermal absorption, and inhalation). Bioaccumulation is a combination of chemical bioconcentration and biomagnification. Biomagnification is increase in concentration of a pollutant from one link in a food chain to another. In order for biomagnification to occur, the pollutant must be long-lived, mobile, soluble in fats and biologically active. In bioaccumulation, the concentration of pollutant increases to be in first organism of food chain. Organism at the any level of their tropic level is capable for the accumulation. It is the net result of the interaction of uptake, storage and elimination of chemicals. Accumulation is measured in parts per million (ppm). This refers to one particle of a given substance mixed with 999999 other particles. For examples oysters can concentrate DDT from 0.001 ppm in sea-water to 700ppm in their bodies.

The metals that are non degradable in nature, are absorbed by the biotic system are accumulate at higher order in different biota of ecosystem. The chemical continues to accumulate

until it eventually becomes deadly to the living organism. Through the food chain, chemicals like PCBs, DDT, dioxins, and mercury build up in the bodies of the fish. People are at the top of this food chain. When you eat a lot of fish that have chemicals in them, these chemicals accumulates up in our body, too. While the chemicals in fish won't make you sick right away, they could cause health problems someday like cancer or diabetes, in some people. Not everyone will get sick, though. Some people may be fine after years of eating fish with these chemicals in them.. Persistent chemicals that are very stable and don't break down over long period time. Chemicals such as PCBs, DDT, dioxins, and mercury are all persistent chemicals.



Transport and biological magnification of some heavy metals in sewage fed aquatic ecosystem

Kind of bioaccumulation

1. Organismal bioaccumulation
2. Trophic transfer
3. Soil accumulation

1. Organism bioaccumulation:

In this type of bioaccumulation, chemical present in organism environment may concentrate in the body over time. For example fish which swim in contaminated water may builtup pollutant in their fatty tissues.

2. Trophic transfer:

In this process, the accumulated chemical or contaminant transfer from one trophic level to another, where the concentration of these substance increases at each trophic level. For

example top carnivores or herbivores in ecosystem such as human or lion can receive the highest concentration.

3. Soil accumulation

In soil, toxic substances move down from the topsoil and get accumulated or bind with soil particles and persist until they are removed through erosion or uptake by plants or microorganism.

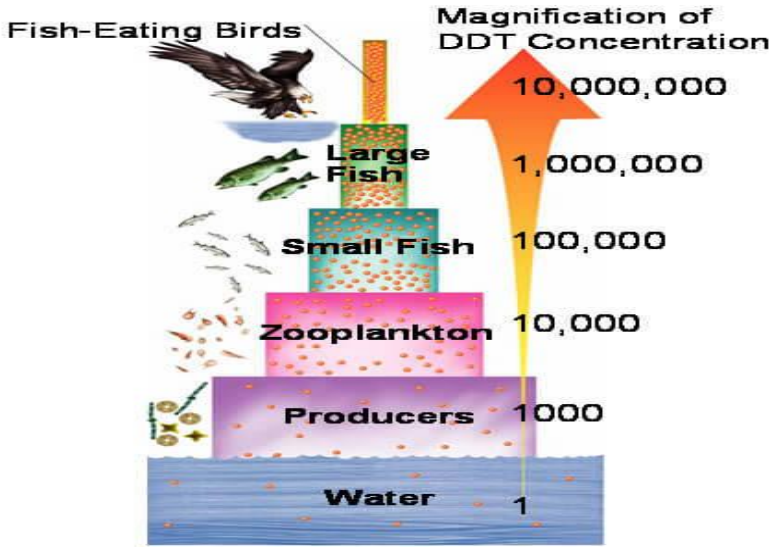
Factors on which bioaccumulation depends are following

- Uptake of substance
- Storage of substance, storage capacity
- Elimination of substance
- Hydrophobicity
- Concentration of pollutant in water
- Age, sex and type of organism

9.5. Biomagnification

The tendency of pollutants to concentrate as they move from one organism to the next is called biomagnifications. It is the increase in concentration of a pollutant from one link in a food chain to another, in which pollutants not only accumulate, but also become more concentrated at each trophic level in food chain. The term food web biomagnification is used to describe trophic enrichment of contaminants within food webs and refers to the progressive increase in chemical concentrations with increasing animal trophic status.

Food-web biomagnification alone can produce a 10000-100000 fold increase in lipid-normalized concentration of a bioaccumulative substance. Biomagnification is of ecotoxicological significance because it can cause organisms at higher trophic levels to be exposed to high concentrations.



A number of mechanisms have been proposed to account for biomagnification. The gastrointestinal magnification model and recent amendments to this model are outlined as well as alternative, non dietary, mechanisms that can lead to similar and potentially confounding observations of biomagnification. Biomagnification is simply increase in concentration of substance in a food chain, not in organism. Conservative pollutants are not metabolized. Therefore, when an organism containing a pollutant is eaten, they are simply passed on to predator and accumulate in its tissue. In aquatic environments, chemicals that are accumulated through biomagnification may eventually become toxic to higher organisms as well. The lowest substrate concentration that is required to sustain growth of a species is generally referred to as 'threshold' concentration.

Pathway for biomagnification

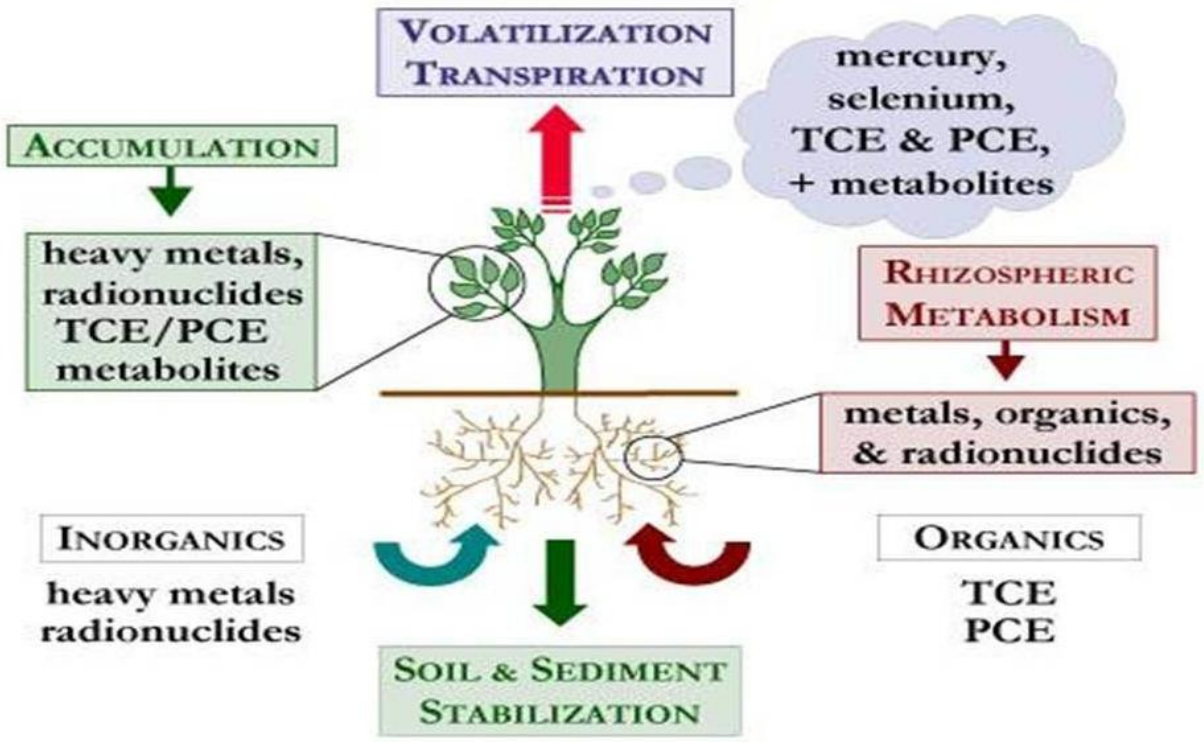
Pollutants enter organisms via different routes such as though the mouth and digestive tract or across gill surfaces. Small aquatic organisms absorb most toxins directly from the water.

Some properties of biomagnification

In order for biomagnification to occur, the pollutant must have a long biological half-life (long-lived), must not be soluble in water but must be soluble in fats. E.g. DDT. If the pollutant is soluble in water, it will be excreted by the organism. Pollutants that dissolve in fats are retained for a long time. Hence it is traditional to measure the amount of pollutants in fatty tissues of organisms such as fish. In mammals, milk produced by females is tested for pollutants since the milk has a lot of fat.

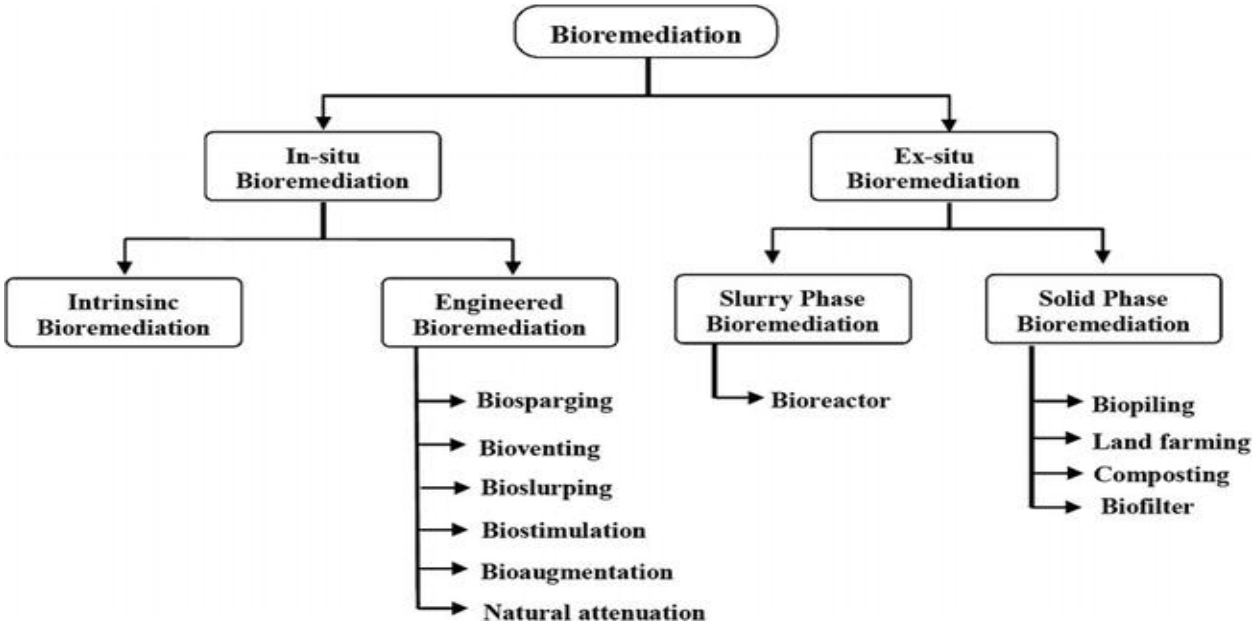
9.6. Bioremediation

Bioremediation is a 'treatment techniques' that uses naturally occurring organisms to break down harmful materials into less toxic or non-toxic materials. It is a waste management technique that involves the use of organisms such as plants, bacteria, and fungi to remove or neutralize pollutants from a contaminated site. Bioremediation is highly involved in degradation, eradication, immobilization or detoxification of diverse chemical wastes and physical hazardous materials from the surrounding through the all-inclusive and action of microorganisms. The main principle is degrading and converting pollutants to less toxic forms.



Concept of bioremediation

Bioremediation is widely used to treat human sewage and has also been used to remove agricultural chemicals (pesticides, herbicide and fertilizers) that leach from soil into groundwater. Certain toxic metals, such as selenium and arsenic compounds, can also be removed from water by bioremediation. Mercury is an example of a toxic metal that can be removed from an environment by bioremediation. It is an active ingredient of some pesticides and is also a byproduct of certain industries, such as battery production. Mercury is usually present in very low concentrations in natural environments but it is highly toxic because it accumulates in living tissues. Several species of bacteria can carry out the biotransformation of toxic mercury into nontoxic forms. These bacteria, such as *Pseudomonas aeruginosa*, can convert Hg^{2+} to Hg, which is less toxic to humans. Bioremediation is promoted by adding inorganic nutrients that help bacteria already present in the environment to grow. Hydrocarbon-degrading bacteria feed on the hydrocarbons in the oil droplet, breaking them into inorganic compounds. In the case of oil spills in the ocean, ongoing, natural bioremediation tends to occur, inasmuch as there are oil-consuming bacteria in the ocean prior to the spill. Bioremediation is the most effective, economical, eco-friendly management tool to manage the polluted environment. All bioremediation techniques have its own advantage and disadvantage because it has its own specific applications. Bioremediation can be carried out ex-situ and in-situ, depending on several factors, which include but not limited to cost, site characteristics, type, and concentration of pollutants.



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Bioremediation involve digging of pollutants from polluted sites and successively transporting them to another place for their treatment.

In-situ bioremediation, pollutant are treated at the same site by using biological system. Whereas in ex-situ bioremediation, they are treated in some other place from the original site.

In-situ remediation, technology are attractive convenient and sustainable option in comparison to ex situ bioremediation.

Ex-situ bioremediation techniques are regularly considered based on the depth of pollution, type of pollutant, degree of pollution, cost of treatment and geographical location of the polluted site. Performance standards also regulate the choice of ex-situ bioremediation techniques. Solid-phase bioremediation is an ex-situ technology in which the contaminated soil is excavated and placed into piles.

Slurry-phase bioremediation is a relative more rapid process compared to the other treatment processes. Contaminated soil is combined with water, nutrient and oxygen in the bioreactor to create the optimum environment for the microorganisms to degrade the contaminants which are present in soil.

Intrinsic bioremediation also known as natural reduction, is an in-situ bioremediation technique, which involves passive remediation of polluted sites, without any external force (human intervention). Genetically Engineered microorganisms used in the in-situ bioremediation accelerate the degradation process by enhancing the physic-chemical conditions to encourage the growth of microorganisms.

Bioventing techniques involve controlled stimulation of airflow by delivering oxygen to unsaturated (vadose) zone in order to increase activities of indigenous microbes for bioremediation.

Bioslurping used to remediate soils which are contaminated with volatile and semi-volatile organic compounds. The method uses a “slurp” that spreads into the free product layer, which pulls up liquids from this layer. Phytoremediation is depolluting the contaminated soils. This technique based on plant interactions like physical, chemical, biological, microbiological and biochemical in contaminated sites to diminish the toxic properties of pollutants. Pollutants like heavy metals and radionuclides are commonly removed by extraction, transformation and

sequestration. Biostimulation and bioaugmentation approaches speed up microbial activities in polluted sites.

9.7. Xenobiotics Compounds

Xenobiotic is defined as a chemical or molecule that is foreign to and exerts a variety of effects on the biological system. Xenobiotics are manmade substances that are foreign to complete biological system which are not generated by the body itself and did not exist previously before they were generated synthetically by humans. In human body, xenobiotics compound may be beneficial, in the case of drugs, or deleterious, in the case of poisons. Xenobiotics are any chemical or synthetic complexes that are found in any living organism but are unknown to that organism as it does not usually produce the compound or consume it as a part of its diet. Xenobiotics are mostly lipohilic in nature due to which they are easily absorbed through the skin, lungs, or gastrointestinal tract and cannot be easily eliminated from the body. Detoxification is the process of transforming and removing xenobiotics that are potentially harmful for the body.

Properties of xenobiotics compounds

1. They are extremely stable in nature and insoluble in water.
2. Degradative microorganisms do not recognize them as substrate.
3. They are extremely toxic and contain large molecular weight that checks entrance into microbial cells.

Biotransformation of Xenobiotics

Biotransformation reaction occurs by two phases of reactions

Phase I Reactions:

Phase I reaction is a catabolic process that breaks down the toxicant into various components. This reaction alters the xenobiotics complex from lipophilic to polar so as to commence individual groups into the preliminary compound: -OH, -NH2, -COOH, or –SH. Phase I reactions are non-synthetic in nature and in general produce a more water-soluble and less active metabolites. This reaction involves following reactions:

By oxidation reaction:

Benzene - (aromatic hydroxylation) epoxide. Then any of two probable reactions takes place:

a) epoxide -(non enzymatic rearrangement) phenol

b) epoxide -(epoxide hydrolyase) 1,2-dihydro-1,2-diol also 1,2-dihydro-1,2-diol

By Reduction*:*

It is supported by anaerobic conditions that most commonly take place in mammalian tissue wherever oxygen concentration is short. Nitro reduction (nitro reductase) and azo reduction (azo reductase) are the two types of reductions in which nitro reduction involves three chief enzyme systems that are

a) Cytochrome P450 (e.g., in liver). Hindered by CO

b) DT-diaphorase: cytosolic flavoprotein (in liver) = NAD(P)H quinone oxido reductase

c) Bacterial intestinal enzymes

Table: Phase I biotransformation reaction and location where the reaction occurs in the body.

REACTION	**ENZYME**	**LOCALIZATION**
Hydrolysis	Esterase	Microsomes, cytosol, lysosomes,
	Peptidase	blood Blood, lysosomes
	Epoxide hydrolase	Microsomes, cytosol
Reduction	Azo- and nitro-reduction	Microflora, microsomes, cytosol
	Carbonyl reduction	Cytosol, blood, microsomes
	Disulfide reduction	Cytosol
	Sulfoxide reduction	Cytosol
	Quinone reduction	Cytosol, microsomes
	Reductive dehalogenation	Microsomes
Oxidation	Alcohol dehydrogenase	Cytosol
	Aldehyde dehydrogenase	Mitochondria, cytosol
	Aldehyde Oxidase	Cytoso
	Xanthine oxidase	Cytosol
	Monoamine oxidase	Mitochondria
	Diamine oxidase	Cytosol
	Prostaglandin H synthase	Microsomes
	Flavin-monooxygenases	Microsomes
	Cytochrome P450	Microsomes

Consequences of Phase I reactions:

- Active drug may be converted into inactive metabolite. Active parent drug inactivation mayterminate biological activity.
- Active drug may be converted into active metabolite. E.g. morphine is converted into more active• metabolite.
- Prodrug may be converted into active metabolite
- Active drug may be converted into toxic metabolite e.g. halothane used in general anesthesia, is converted into trifluoroacetylated compound or trifluoroacetic acid, leading to hepatic toxicity. Biotransformation of xenobiotics to mutagenic or carcinogenic agents.
- Conversion of xenobiotics into harmless compound.

Phase II Reactions:

Phase I metabolites or parent xenobiotic compound which contains appropriate reactions go through conjugation reactions, amid the substrates to capitulate conjugates. Basically, conjugates are polar in nature which is readily excreted.

Glucuronidation: ts reaction distinctiveness is low attraction, high capability for catalysis and availability of proficient feedstock conjugation at high substrate concentrations

Sulfation: The main reaction characteristics are high-affinity, little capability for catalysis and availability of competent feedstock conjugation at low concentrations. The end product released after sulfate conjugation entails the relocation of sulfonate (SO_3^-), not sulfate (SO_4^-) from phosphoadenosinephosphosulfate (PAPS) to the xenobiotic.

Acetylation: Two types of acetylation reactions take place. One engaged xenobiotic compound and an activated conjugating intermediate, acetyl CoA. Enzymes involved in acetylation are two cytoplasmic N-acetyltransferases, NAT_1 and NAT_2 which are found in human beings.

Methylation: Methylation is an ordinary but usually an insignificant pathway of xenobiotic metabolism. A methylation phase II reaction usually reduce the water solubility of xenobiotics compounds and covers functional groups or else are conjugated by other phase II enzymes.

Table: Phase I biotransformation reaction and location where the reaction occurs in the body

ENZYME	**LOCALIZATION**

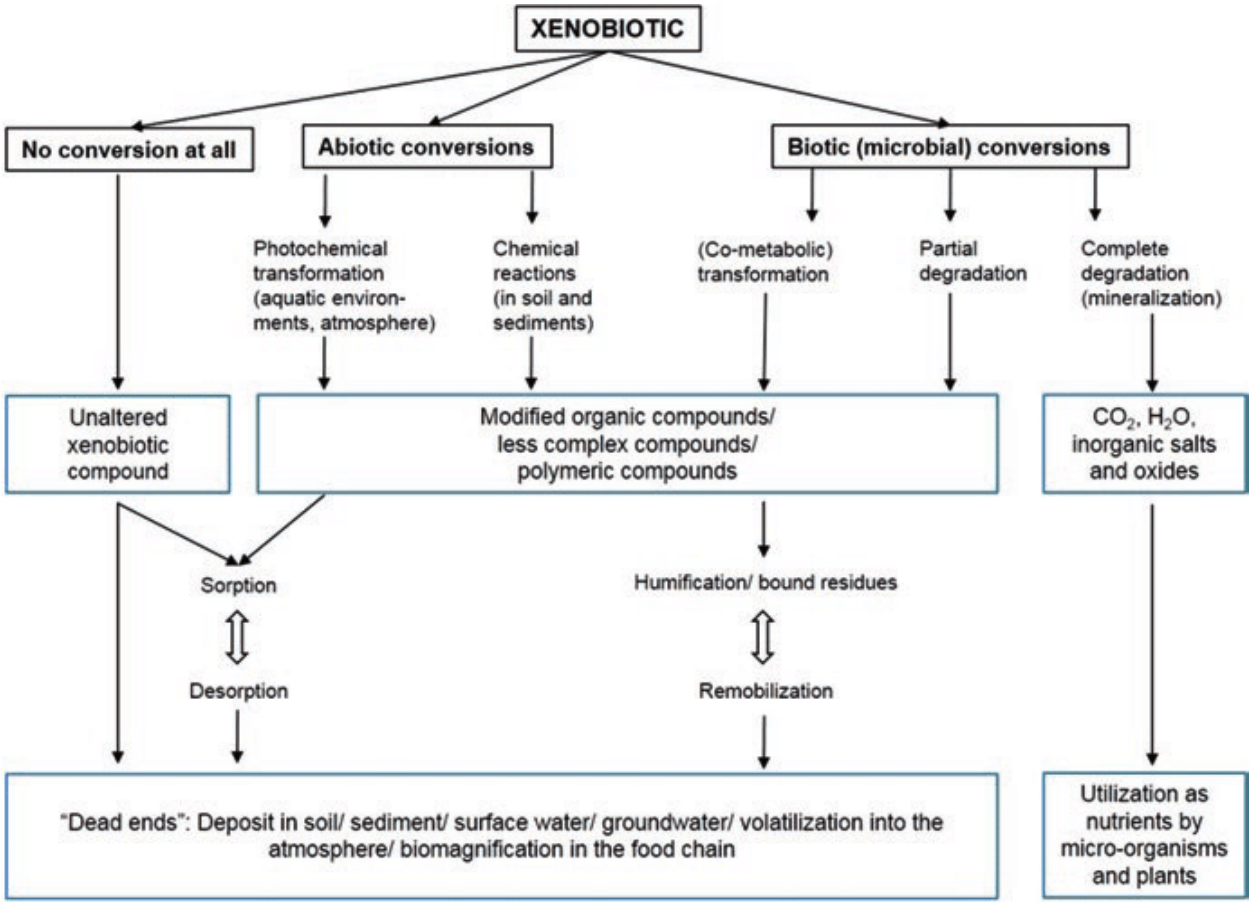
Glucuronide conjugation	Microsomes
Sulfate conjugation	Cytosol
Glutathione conjugation	Cytosol, microsomes
Amino acid conjugation	Mitochondria,microsomes Mitochondria,
Acylation	cytosol
Methylation	Cytosol, microsomes, blool

9.8. Environmental xenobiotics

Environmental xenobiotics are any chemicals of synthetic origin which are not normally expected to present in an organism. There are several environmental xenobiotics that impose adverse impacts, both on ecology and human beings. Pesticides, toxic heavy metals, polychlorinated biphenyls and persistent organic pollutants are environmental xenobiotics, for which, there are ample scientific evidences showing the obnoxious health impacts that these chemicals are imposing on humans. Environmental contamination by xenobiotics is a worldwide phenomenon, as a result of human activities, resulting from rise in urbanization and population growth. Xenobiotics are biologically active synthetic chemicals, many of which compromise human health. Environmental xenobiotics are substances which did not exist in nature before their synthesis by humans.It use as food additives, drugs or pesticides. Xenobiotics are are harmful towards nature and health as environmental pollutants like dioxins, furans and carcinogen.

Structural features of xenobiotic compounds:

1. Instead of hydrogen, halogen is present in the molecule which needs additional energy for cleavage.
2. Other groups like sulphonate, nitro, amino, methoxy, etc. are present.
3. Cycloalkanes, aromatic compounds, and heterocyclic compounds are more recalcitrant.
4. Branched linear chains are resistant to biodegradation.



Source: 1 Possible environmental fate of a xenobiotic compound | Download Scientific Diagram (researchgate.net)

Xenobiotics are basically categorized into six types:

Halocarbons: they are halogen congaing xenobiotic compounds found their application in cosmetics, paints and solvents such as chloroform. They use in cooling systems (freons, CCl_3 F, CCl_2F_2 etc.) and as insecticide (DDT, BHC, lindane etc.) and herbicide such as dalapon.

Polychlorinated biphenyls (PCBs): utilized as coolants in transformer or as exchange fluids for heat.

Synthetic polymers: These are polyethylene, polyvinyl chloride, polystyrene, etc.

Alkylbenzyl Sulphonates: They are surface-active detergents. The sulphonate ($-SO_3^-$) group at one end opposes microbial degradation.

Oil Mixtures: Oil is recalcitrant basically because it is not soluble in water and due to lethal nature of some of its compounds. It is naturally produced that contains many components which are biodegradable in nature.

Other xenobiotics: It includes aliphatic compounds, cyclic compounds like nitro, sulphonates, amino, methoxy, or carbamyl groups other than halogen group.

9.9. Toxicokinetics

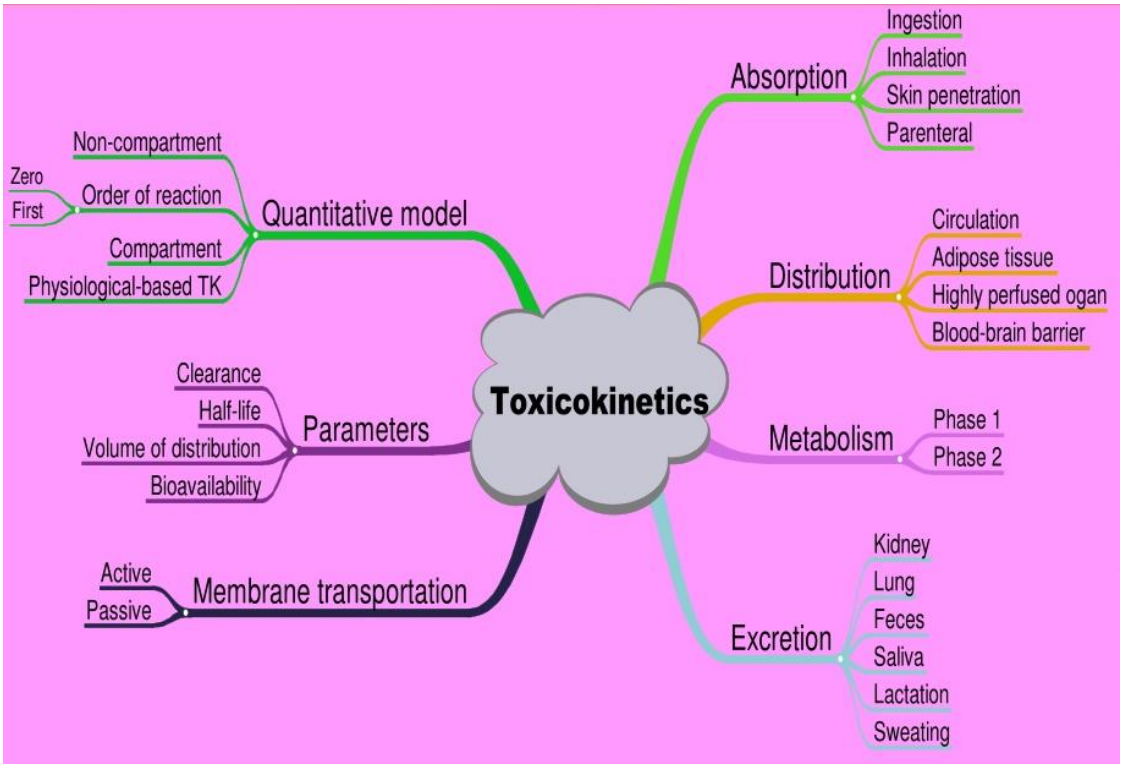
Toxicokinetics is the study of kinetics of absorption, distribution, metabolism, and excretion of xenobiotics or pharmaceutical products under the conditions of toxicity evaluation. Toxicokinetics is the quantitative study of the movement of an exogenous chemical from its entry into the body, through its distribution to organs and tissues via the blood circulation, and to its final disposition by way of biotransformation and excretion. However, it is useful in the derive pharmacokinetics data and interpretation of drug safety evaluation studies. Toxicokinetics is also defined as the rates of all metabolic processes related to the expression of toxicological end points. The human body can be open to a range of toxicants that may be existent in different environmental media such as air, soil, water or food. However, just merely being exposed to these dangerous chemicals does not certainly transform into a toxicological response. As the toxicological response is often associated to the exposed dose, relations between the toxicant and the body's barriers and defense mechanisms will have an effect on toxicant movement in the body, and eventually control the degree and magnitude of toxicant absorption and distribution to the target tissue.

Toxicokinetics has significance role in the pharmacokinetics studies. Because the toxicokinetics study provide information on fate of the xenobiotics, following exposure by a define route, which is useful in the determination of dose-response curve. However, the principle of toxicokinetics study involved in may environmental and biological process through absorption, distribution and elimination of xenobiotic substance. Toxicokinetics data have the potential to define:

i) Internal exposure of parent compound or metabolites forms plasma or blood.

ii) The relationship between plasma or blood concentrations and those at the site of toxicity

iii) It's defined the dose response curve after the administration of trace dose.



a. Absorption

Absorption is the process by which the chemical enters in the body from surrounding environment. These chemical, in the human body, enter by different routes. Even our body has number of defense mechanisms and membrane barriers to check the entry of contaminants. In order for a xenobiotic to reach its site of action, it must pass across various body membranes, that is, cells of skin, cells of lung, gastrointestinal tract, erythrocyte membrane, etc. As soon as the toxicant has been absorbed, the toxicant molecules can move around the body in two ways:

i. By bulk flow transfer (i.e., in the blood stream)

ii. By diffusional transfer (i.e., molecule-by-molecule over short distances)

There are following types of absorption routs in our boy

Oral route – the GIT is the most important route of absorption, as most acute poisonings involve ingestions.

Dermal route – lipid solubility of a substance is an important factor affecting the degree of absorption through the skin

Inhalational route – toxic fumes, particulate and noxious gases may be absorbed through the lungs.

Bioavailability

It is the fraction of unchanged drug reaching the systemic circulation following of non-vascular administration. Therefore, a portion of the chemical fails to reach the systemic circulation in original form after oral administration.

b. Distribution

After absorption of toxicants into the blood, its distribution takes place. In the distribution process, the absorb toxicant or xenobiotics compounds reached to other tissues where various types of effects causes. The distribution is dependent on various physiological factors and physicochemical properties of the drug. This process is therefore a reversible movement of the toxicant between blood and tissues or between extracellular and intracellular compartments. There are, however, several complicating factors that can influence the distribution of a toxicant. Volume of distribution (Vd) is calculated from the dose taken and the resulting plasma concentration:

$$V_d = \frac{\text{dose}}{\text{plasma concentraion}}$$

c. Biotransformation

Biotransformation or metabolism of chemical is happened after the distribution of chemical or xenobiotics compounds. Biochemical is process which leads to a metabolic transformation of foreign compounds (xenobiotics) in the body. It is a process by which the body transforms a chemical and makes it more water soluble so the chemical can be eliminated more rapidly via the kidney into the urine. Biotransformation follows a complex series of chemical reactions that are enzymatically facilitated, and are ordinarily irreversible reactions that are actively positive, resulting in a reduction in the Gibbs free energy of the system. The liver is the foremost place of biotransformation for many chemicals & other organs that are involved are lungs, kidneys, skin etc. There are two phases of biotransformation

Phase I – In this phase reactions introduce new functional group into the molecule by oxidation, reduction or hydrolysis.

Phase II – a drug or its metabolite is conjugated with an endogenous substance e.g. glucuronide conjugate

The biotransformation are also catalyzed by a large number of enzymes, it is to be expected that they will vary among species. Most biotransformations are detoxication reactions, many oxidative reactions produce reactive metabolites.

d. Excretion

Excretion is the final means of chemical elimination, either as metabolites or unchanged parent chemical. The ability to efficiently eliminate toxic materials is critical to the survival of a species. Three main routes of elimination culminate in the specialized organs of elimination, the liver, kidneys, and lungs. Excretion through the lungs is the major route for gaseous substances; and in the case of non-volatile water – soluble drugs, the kidneys are the most important routes of excretion. Additional routes include sweat, saliva, tears, nasal secretions, milk, bile and feces etc.

9.10. Summary

Toxicant contaminated (The term "contaminant" means hazardous substances, pollutants, pollution, and chemicals) the environment components like air, water and atmosphere, thus these toxicant alter the quality of these component temporarily or permanently. Bioconcentration is the intake and retention of a substance in an organism entirely by respiration from water in aquatic ecosystems or from air in terrestrial ones. Bioaccumulation is the intake of a chemical and its concentration in the organism by all possible means, including contact, respiration and ingestion. Bioremediation is highly involved in degradation, eradication, immobilization, or detoxification diverse chemical wastes and physical hazardous materials from the surrounding through the all-inclusive and action of microorganisms. The main principle is degrading and converting pollutants to less toxic forms. A xenobiotic is a chemical substance found within an organism that is not naturally produced or expected to be present within the organism. . Environmental xenobiotics include pesticides, polycyclic aromatic hydrocarbons (PAHs), pharmaceutical active compounds (PhACs), personalcare products (PCPs) .Toxicokinetics is defined as the rates of all metabolic processes related to the expression of toxicological end points. It is the method of the uptake of possibly toxic substances by the body, the biotransformation they go through, the distribution of the substances and their metabolites in the tissues, and the elimination of the substances and their metabolites from the body.

9.11. Terminal questions

Q.1: What is toxicity? Discuss toxicant, contaminant and toxicology.

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

Q.2: What are heavy metals? Discuss the role of heavy metal in environmental toxicity.

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

Q.3: What are biomagnifications and biotransformation.

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

Q.4: What are xenobiotics compounds? Discuss about environmental xenobiotics compounds.

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

Q.5: Discuss the role of bioremediation in ecosystem.

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

Q.6: Write about Toxicokinetics?

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

9.12. Further suggested reading

1. Environmental Science, by Subhas Chandra Santra
2. Environmental Toxicology, Kees van Gestel, Vrije Universiteit, Amsterdam
3. Copyright Year: 2020
4. Environmental Toxicology, Third Edition, By Sigmund F. Zakrzewski, Oxford University Press
5. Chemistry And Environmental Toxicity By Neelam Richhariya, Pragati Prakashan