STUDY MATERIAL FOR MICROBIOLOGY

BASIC MICROBIOLOGY

I YEAR

SEMESTER – I

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Prepared by

MICROBIOLOGY DEPARTMENT

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INDEX

UNIT – I

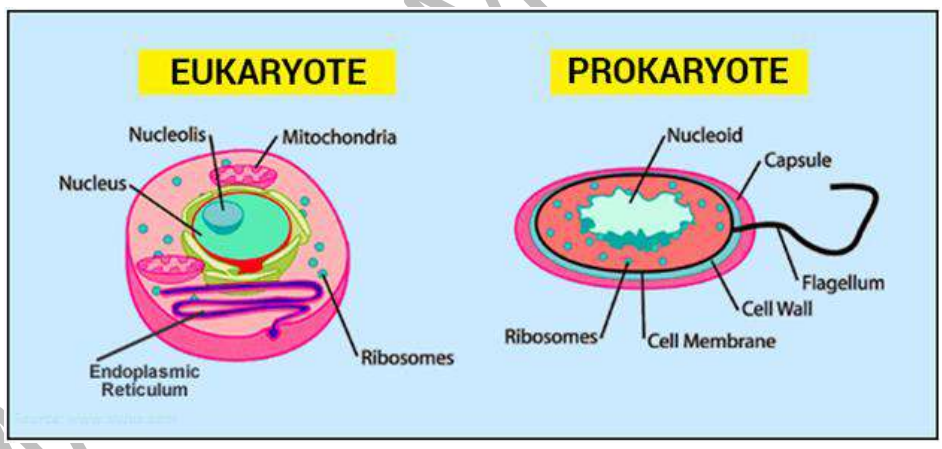
PROKARYOTES AND EUKARYOTES

Cells fall into one of two broad categories: prokaryotic and eukaryotic. The single-celled organisms of the domains Bacteria and Archaea are classified as prokaryotes (pro = before; kerion– = nucleus). Animal cells, plant cells, fungi, and protists are eukaryotes (eu = true).

A prokaryotic cell is a simple, single-celled (unicellular) organism that lacks a nucleus, or any other membrane-bound organelle. We will shortly come to see that this is significantly different in eukaryotes. Prokaryotic DNA is found in the central part of the cell: a darkened region called the nucleoid.

Eukaryotic Cells

A eukaryotic cell is a cell that has a membrane-bound nucleus and other membrane-bound compartments or sacs, called organelles, which have specialized functions. The word eukaryotic means “true kernel” or “true nucleus,” the presence of the membrane-bound nucleus in these cells. The word “organelle” means “little organ,” and, as we learned earlier, organelles have specialized cellular functions, just as the organs of your body have specialized functions.



MICROBES:

Microbes are very diverse and represent all the great kingdoms of life. In fact, in terms of numbers, most of the diversity of life on Earth is represented by microbes. Here is an outline of the major groups of microorganisms:

- Viruses
- Bacteria
- Algae
- Fungi
- Protozoa

Prokaryotes	Eukaryotes
Circular DNA (in cytosol)	Linear DNA (in nucleus)
No organelles	Several membrane bound organelles
Nucleoid (not membrane bound)	Nucleus (membrane bound)
Single chromosome	Several chromosomes
Plasma membrane typically lacks receptors	Plasma membrane with receptors (sterols and carbohydrates)
Chemically complex cell wall (may contain peptidoglycan)	Chemically simple cell walls (cellulose (plants) and chitin (fungi))
DNA transctription and mRNA translation occurs simultaneously (in cytosol)	DNA transctription in nucleus, and mRNA translation in cytosol
Flagellum (if present) Simple, built from two proteins	Flagellum (if present) Complex, built from microtubules
May have pili and fimbriae	May have cilia
Haploid genome (only one copy of each gene)	Diploid genome (more than one copy of each gene)
May have plasmids (DNA outside chromosome)	Plasmid DNA not common
Compact genome (little repetitive DNA)	Usually large amounts of non-coding and repetitive DNA
May have a glycocalyx cover	Glycocalyx only if no cell wall
Small ribosomes	Large ribosomes in cytosol/nucleus small ribosomes in organelles
No histones in chromosome	DNA "wound" around histones
Lacks cytoskeleton	Cytoskeleton (actin, microtubules)
Mycolaginous capsule	No mycolaginous capsule
Cell size range 0.5–100 µm	Cell size range 10–150 µm
Asexual reproduction (binary fission)	Sexual reproduction (meiosis and mitosis)

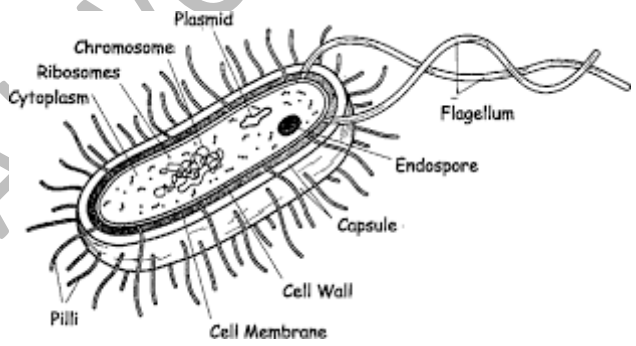
BACTERIA

Bacteria are a type of biological cell.

- They constitute a large domain of prokaryotic microorganisms.
- Bacteria inhabit soil, water, acidic hot springs, radioactive waste, and the deep portions of Earth's crust.
- Bacteria also live in symbiotic and parasitic relationships with plants and animals.
- Most bacteria have not been characterised, and only about 27 percent of the bacterial phyla have species that can be grown in the laboratory .
- The study of bacteria is known as bacteriology, a branch of microbiology.
- The word bacteria is the plural of the New Latin bacterium, meaning "staff, cane".

CELL STRUCTURE OF BACTERIAL CELL:

- The bacterial cell is surrounded by a cell membrane which is made of phospholipids.
- This membrane encloses the contents of the cell and acts as a barrier to hold nutrients, proteins and other essential components of the cytoplasm within the cell.
- Unlike eukaryotic cells, bacteria usually lack large membrane-bound structures in their cytoplasm such as a nucleus, mitochondria, chloroplasts and the other organelles present in eukaryotic cells.



- A bacterial cell shows a typical prokaryotic structure.
- The cytoplasm is enclosed by three layers, the outermost slime or capsule, the middle cell wall and inner cell membrane. The major cytoplasmic contents are nucleoid, plasmid, ribosome, mesosome etc., and the cell is devoid of endoplasmic reticulum, mitochondria, centrosome and Golgi bodies.

FLAGELLA OF BACTERIA:

- Most of motile bacteria (e.g., Spirochetes) possess long (5-20 µm), thin (12-30 nm), helical appendages, called flagella.
- Electron microscopy shows that the flagellum consists of three distinct regions — filament, hook and basal body.
- Filament is attached at one end through the cell wall to the cell membrane by the hook, which, in turn, is attached to the basal body.
- The rings of basal body remain attached to the cell membrane and cell wall. The filament lies external to the cell.
- Filament is made up of identical subunits (3 or more), arranged helically along the axis to give a hollow tube.
- These subunits are made up of protein molecule, called flagellin. Each subunit is about 4.5 nm thick.
- The filament ends with a capping protein. Some bacteria have sheath surrounding the flagella (e.g., Vibrio cholerae has a sheath of lipopolysaccharide).
- Structurally, the hook and basal body are quite different from the filament (Fig. 2.14). The hook is slightly wider than the filament and made up of protein subunits.
- The structure of basal body is quite different in gram (-ve) and gram (+ve) bacteria. Gram (-ve) bacteria like E. coli and others have 4 rings connected to a central rod. The outer L arid P- rings are associated with the lipopolysaccharide and peptidoglycan layers.
- The inner two rings, i.e., S and M rings, are associated with plasma membrane (Fig. 2.14). On the other hand, in gram (+ve) bacteria like Clostridium sporogens, Bacillus subtilis and others, have only two rings attached with the plasma membrane. The structural differences of flagella between gram (-ve) and gram (+ve) bacteria.

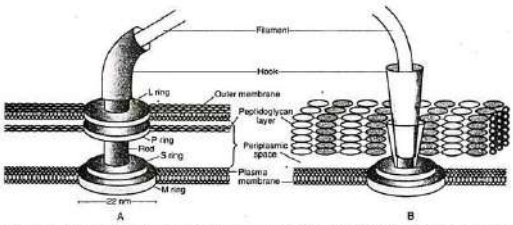


Fig. 2.14 : The ultrastructure of bacterial flagella showing flagellar basal bodies and hooks. A. Gram-negative bacteria and B. Gram-positive bacteria

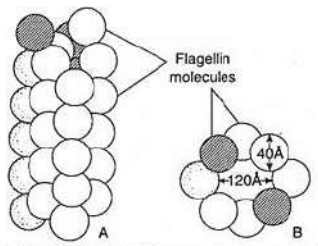


Fig. 2.15 : Structure of filament. A. Arrangement of flagellin molecules showing hollow cylinder and B. T.S. of a filament

- Some bacteria are devoid of flagella and are non-motile, called atrichous e.g., Cocci, Lactobacillus, etc.

The number and arrangement of flagella on a cell are useful for identification and classification of bacteria. On the basis of arrangement of flagella, the bacteria are categorised into the following types:

i. Polar flagellation:

(a) Monotrichous. Single fla- gellum at one pole of the cell, e.g., Vibrio cholerae.

(b) Amphitrichous Each single flagellum is attached at both ends, e.g., Alkaligenes faecalis, Nitrosomonas.

(c) Cephalotrichous Or Amphitrichous: Two or more flagella at one end only, e.g., Pseudomonas fluorescens.

(d) Lophotrichous A tuft of flagella at both ends, e.g., Spirillum volutans.

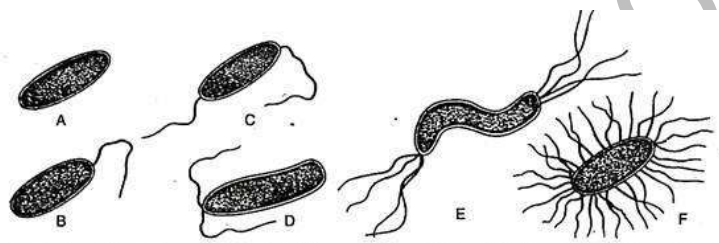


Fig. 2.16 : Different types of flagellation : A. Atrichous, B. Monotrichous, C. Amphitrichous, D. Cephalotrichous, E. Lophotrichous, and F. Peritrichous

ii. Non-polar flagellation:

Peritrichous (Fig. 2.16F). Numerous flagella are distributed all over the surface of the cell e.g., Bacillus typhosus, Clostridium.

FIMBRIAE OR PILI:

- The term fimbriae (sing, fimbria) was introduced by Duguid et al. (1955) and pili (sing. pilus) by Brinton (1959).
- Fimbriae are observed mostly in Gram-negative rods (Salmonella typhi, typhoid fever; Shigella dysen- teriae, bacillary dysentery) and also in cocci (Neisseria gonorrhoeae, gonorrhoea).
- Gram- positive bacilli like Corynebacterium renale also have fimbriae or pili.
- These are extremely thin and short, filamentous, non-flagellar appendages projecting peritrichously from cell surface.
- Their number is 100-500 per cell and measure 0.5-20 µm in length and 3-25 nm in diameter. They are made up of subunits of protein, the pilin, arranged helically and form hollow filament.

- They are antigenic. Pili are of 3 types: Common pili, sex pili or F (fertility) pili and col I (colicin) pili.
- The common pili are responsible for adhesion; the sex pili (1-5 per cell) help in the transfer of genetic material during conjugation and the-col I for colicin or hemolysin production.

BACTERIAL CELL WALL:

- The bacterial cell wall is tough and rigid due to the presence of strong fibres composed of heteropolymers called mucopeptides, peptido- glycans, mucocomplex, murein etc.
- The peptidoglycan is composed of alternate units of N-acetyl muramic acid and N-acetyl glucosamine residues, cross-linked with tetra-peptide subunits.

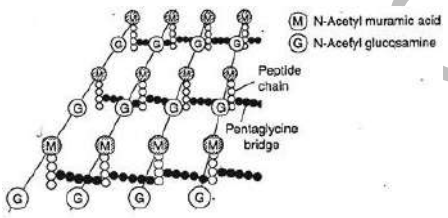


Fig. 2.7 : Chemical structure of cell wall of Gram-positive bacteria

- The peptide linked with muramic acid varies in their composition, but everywhere it contains a minimum of three amino acids viz., glutamic acid, alanine and either lysine or diaminopimelic acid.

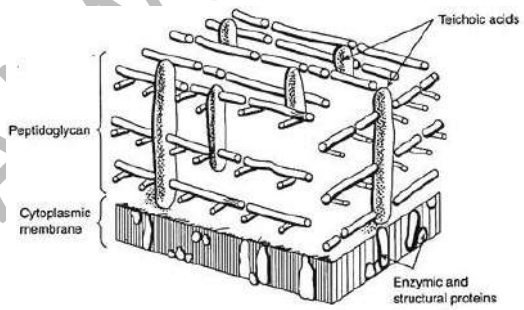


Fig. 2.8 : Three-dimensional representation of Gram-positive bacterial cell wall

The structural constituents of wall vary in Gram- negative and Gram-positive bacteria

(a) Gram-negative cell wall (Salmonella, Escherichia):

It is a complex structure with three components outside the peptidoglycan layer:

(i) LIPOPOLYSACCHARIDE (LPS):

It consists of a complex lipid with attached polysaccharide. LPS is the endotoxin, whose toxicity is associated with lipid region and polysaccharide confers antigenic specificity.

(ii) PHOSPHOLIPID:

This layer consists of phospholipid bilayer and specific proteins. The specific proteins form porins and hydrophilic molecules are transported through it. Other proteins are target sites for antibiotic and phages.

(iii) PERIPLASMIC SPACE:

It is situated in between peptidoglycan and phospholipid layer. This space contains a number of important proteins (as enzyme and binding protein for specific substrate) and oligosaccharides (help in osmo-regulation).

(b) GRAM-POSITIVE CELL WALL (STAPHYLOCOCCUS, BACILLUS):

- The peptidoglycan layer present just outside the cell membrane is about 16-80 nm thick.
- Special components like teichoic acid and teichuronic acid (as much as 50% of dry weight of cell wall) are present.
- They maintain the level of divalent cations outside the cell membrane. Teichoic acids constitute the major surface antigens. The periplasmic space is absent in Gram-positive cell wall.

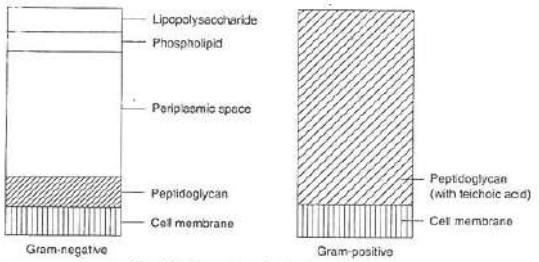
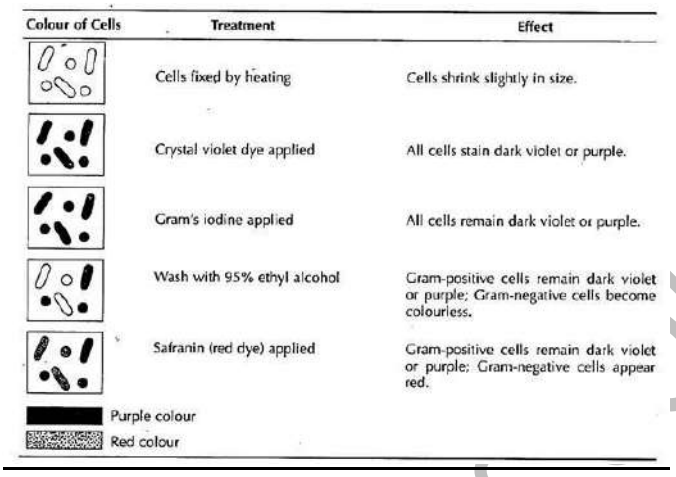


Fig. 2.9 : Comparison of cell walls of bacteria

Gram Staining:

- This technique was developed by a Danish physician, Hans Christian Gram, in 1884; which is very useful in taxonomic grouping of bacteria.
- The basic distinction between Gram(+ve) and Gram(-ve) bacteria is in the difference of cell wall structure, along with some other characteristics.
- The crystal violet, iodine, ethyl alcohol (95%) and safranin are used for staining. First, the cells are stained with crystal violet, then with Gram's iodine and finally washed in 95% ethanol which differentiate two groups: one which retains stain and becomes dark-violet or purple coloured i.e., Gram-positive and the other one which loses stain i.e., Gram-negative.

- The slides are washed with distilled water, followed by counter-stain with safranin for 30 sec. and finally the slides are again washed with distilled water. Gram-positive bacteria remain dark-violet or purple coloured, but Gram-negative bacteria become red (pink)



CYTOPLASMIC MEMBRANE OF BACTERIA:

- The cytoplasmic layer is the boundary layer of the protoplast, situated beneath the cell wall. It is thin (5-10 nm), elastic and semipermeable layer.
- it appears as a triple-layered structure consisting of a bilayer region of phospholipid molecules, with polar heads on the surface and fatty-acyl chains towards the inner side.
- The proteins are found embedded in the lipid bilayer.

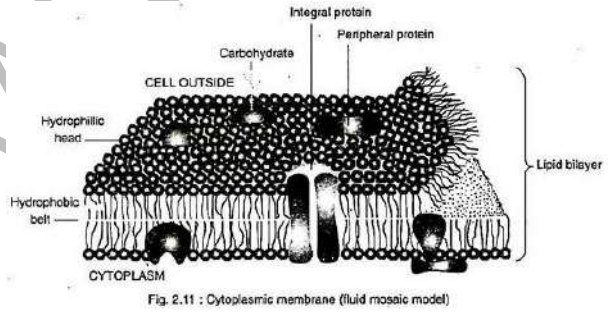


Fig. 2.11 : Cytoplasmic membrane (fluid mosaic model)

Functions

(i) Transport:

(a) Active:

Being the site of many enzymes like oxidase, polymerase etc., it is involved in the active transport of selective nutrients. It is impermeable to ionised substances and macromolecules.

(b) Passive:

The passive transport of fat soluble micromolecular solutes takes place by diffusion.

(ii) Energy production:

It is the site of electron flow in both respiration and photosynthesis leading to phosphorylation and, therefore, the membrane is the site of carriers and enzymes in these reactions.

(iii) Polymer production:

Cell membrane is the site of polymerising enzymes necessary for synthesis of cell wall.

CYTOPLASM OF BACTERIA:

- The cytoplasm is a colloidal system containing both organic and inorganic substances.
- It lacks mitochondria, endoplasmic reticulum, centrosome and golgi bodies. It contains many ribosomes, few mesosomes, soma inclusions and vacuoles.

Ribosome:

- It is a complex substance of 10-20 nm size and of 70S (S = sedimentation coefficient) type having two subunits, 50S and 30S.
- They are the sites of protein synthesis. The ribosomes are held together on m-RNA (messenger RNA) strands, known as polysomes or polyribosomes.

Mesosomes (Chondroids):

These are convoluted multi-laminated localised infoldings of the cytoplasmic membrane into the cytoplasm (Fig. 2.12). Their number is usually 2-4, but often found to be more in cells with high respiratory activity, e.g., Nitrosomonas.

- It serves to accommodate more spaces for respiration.
- In photosynthetic bacterial (Rhodopseudomonas), they are the site of photosynthetic pigments.
- The mesosomes are of two types — septal mesosome and lateral mesosome.
- The septal mesosomes are involved in DNA segregation and in the formation of transverse septum during cell division.

Chromatophores:

- These are the pigment- bearing structures, found in photosynthetic bacteria.
- They are found in all members of Chromatiaceae and Rhodospirillaceae in different forms.
- Such as membranes, vesicles, tubes, bundle tubes etc. or as thylakoids, as found in Cyanobacteria.

Cytoplasmic inclusion:

These are the sources of stored energy, characteristic of different species of bacteria such as lipid (poly (3 hydroxybutyrate), volutin (polymetaphosphate), starch or glycogen (polysaccharide) and granules of sulphur.

GENETIC MATERIAL OF BACTERIA:

The genetic material is present both in nucleoid and plasmid.

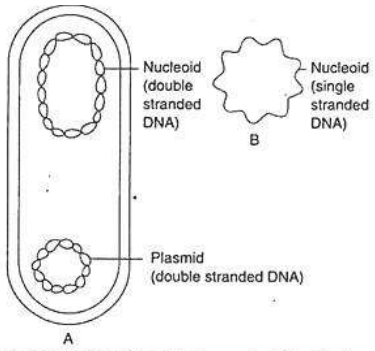


Fig. 2.13 :A. Bacterial cell having nucleoid (double stranded DNA) and plasmid (extrachromosomal carrier of gene). B. Nucleoid (single stranded DNA)

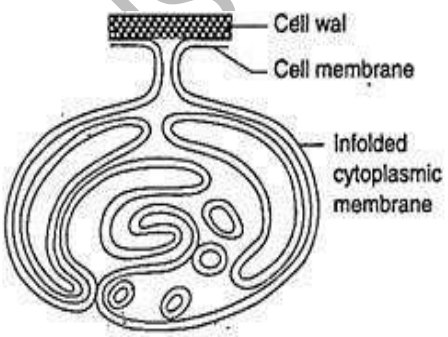


Fig. 2.12 : Single mesosome

Nuclear material:

Under light microscope nuclear body cannot be differentiated in the cytoplasm, but it is differentiated only under electron microscope as a central area of lower electron dense region than rest of the cytoplasm.

- The bacterial nuclear body is devoid of nuclear membrane, nucleolus and nuclear sap and is known as genophore or nucleoid.
- The genophore is composed of a single or double stranded circular DNA.
- When straightened the DNA measures 1000 µm.
- It has approximately 5 x 106 base pairs and a molecular weight of about 3 x 109. It is devoid of any basic protein.

- The DNA is haploid — it undergoes semiconservative replication by simple fission and maintains genetic characteristics.

Plasmid:

- Bacterial cytoplasm may contain some genetic material excepting the genophore, called plasmid or episomes.
- Lederberg (1952) termed as plasmid those extragenophoral genetic materials.
- Plasmids are ring-like double stranded DNA molecules which may contain about 100 genes having the molecular weight range from 5 x 107 to 7 x 107 or less.
- The replication of plasmid seems self-controlled. They contain different non-essential characters. Based on host properties, the plasmids are classified into different types.

These are:

(i) “F-factor” for fertility

(ii) “Col-factor” — for colicinogeny

(iii) “R-factor”—for resistance

(iv) Tumor inducing plasmid (e.g., Agrobacterium tumit’aciens),

(v) Degradative plasmid (e.g., Pseudomonas),

(vi) Pathogenecity to mammals,

(vii) Penicillase plasmid (e.g., Staphylococcus),

(viii) Mercury resistance, and

(ix) Cryptic plasmids.

SPORE

- Bacterial spores are highly resistant, dormant structures (i.e. no metabolic activity) formed in response to adverse environmental conditions. They help in the survival of the organisms during adverse environmental conditions; they do not have a role in reproduction in bacteria.
- The endospore consists of the bacterium's DNA, ribosomes and large amounts of dipicolinic acid. Dipicolinic acid is a spore-specific chemical that appears to help in the ability for endospores to maintain dormancy. This chemical accounts for up to 10% of the spore's dry weight. Endospores can survive without nutrients.

- Spores are inhaled and deposited into the lung tissue, where they proceed to germinate and spread through lymph nodes, rapidly causing systemic disease, massive tissue damage, shock and death.

STAPHYLOCOCCI

Characteristics:

- Gram positive non spore-forming non-motile, spherical cells, arranged in grape-like clusters
- Single cocci, pairs, tetrads and chains
- Young cocci stain strongly gram-positive, on aging many cells become gram-negative
- The three main species of clinical importance.
    - Staphylococcus aureus
    - Staphylococcus epidermidis
    - Staphylococcus saprophyticus
- Can readily grow in ordinary media under aerobic and microaerophilic conditions grow most rapidly at 370C but form pigment best at room temperature of 20-25oC.
- Colonies in solid media are round, smooth, raised and glistening.
- Some of them are normal flora of the skin and mucus membrane of human; others cause suppuration abscess formation and fatal septicemia.
- Produce catalase, which differentiate them from the streptococci.
- Relatively resistant to drying, heat, and 9% NaCl, but readily inhibited by 3 % hexachlorophene

Antigenic structure:

- **Peptidoglycan (Mucopeptide):** Polysaccharide polymer which provide the rigid exoskeleton of the cell wall. It is important in the pathogenesis of infection like eliciting production of cytokines and opsonic antibodies; chemoattractant for polymorphs; and activates complement.
- **Teichoic acid:** Polymer of glycerol or ribitol phosphate
- **Protein A:** Important in immunologic diagnostic test (co agglutination test).
- **Capsule:** Anti-phagositic property
- **Enzymes:** Catalase- Produced by staphylococci Converts H202 into H20 and 02

Catalase test differentiates staphylococci (catalase-positive) from streptococci (catalase-negative)

Coagulase and clumping factor

- Coagulase clots oxidated or citrated plasma.
- Coagulase maydeposit fibrin on the surface of organism and alter ingestion byphagocytic cells.

Clumping factor:

- Fibrinogen and fibrin. It determines Invasive potential of the organism.
- Coagulase test differentiates S.aureus (coagulasepositive) from S.epidermidis (coagulase-negative)

Hyaluronidase-Spreading factor: Proteinases and lipases

Staphylokinase- Fibrinolysin

B-lactamase-Provides resistance of staphylococcus to β-lactamantibiotic like penicillin.
Dnase: Deoxyribonucleotidase, Nuclease

Toxins

Exotoxins (α, β, γ, δ)
Enterotoxin-Produced by S.aureus when grown in carbohydrate and protein foods.

Clinical features:

Folliculitis: Infection of one hair follicle.
Curbuncle: Infection of multiple hair follicle and surroundingskin.
Cellulitis: Infection of skin and subcutankeous tissue.
Abscess formation: focal suppuration
Mastitis: Infection of breast, especially in lactating mother
Bulous impetigo: Crusted superficial skin lesion
Pneumonia: Infection of lung parenchyma.
Empyema: Accumulation of pus in pleural space
Osteomyelitis: Infection of bone
Endocarditis and meningitis: Infection of heart tissue and leptomeninges respectively.
Food poisoning: Caused by enterotoxin produced by S.aureus

Toxic shock syndrome:

- Produced by S.aureuscharacterized by abrupt onset of high fever, vomiting, diarrhea, and myalgia, scarlatiform rashand hypotension with cardiac and renal failure in the most severe disease. Occurs within 5 days after the onset of menses in young women who use tampons.

- **Staphylococcal scalded skin syndrome:** Caused by exfoliative toxin produced by S.aureus.

  S. saprophyticus: Relatively common cause of urinary tract infections in young women

  S. epidermidis: occasional cause of infection often associated with implanted appliances and devices

Laboratory Diagnosis:

- **Specimen:** Surface swabs, pus, blood, sputum, cerebrospinal fluid
- **Smear:** Gram positive cocci in clusters, singly or in pairs.
- **Culture:** Grow well aerobically and in a CO2 enriched ordinary media at an optimal temperature of 350C -370C.

Colony appearance:

- **S.aureus:** characteristically golden colonies. Frequently non-pigmented after over-night incubation. Hemolytic on blood agar plate.
- **S.epidermidis:** white colonies, non-hemolytic.
- **S.saprophyticus:** may be white or yellow, non-hemolytic.

Biochemical reaction

Catalase test

Active bubbling……. Catalase producing Bacteria (Staphlococci)
No active bubbling……Non-catalase producing bacteria (Streptococci)

Coagulase test

Slide test: To detect bound coagulase
Clumping within 10 seconds…… S.aureus
No clumping within 10 seconds……CONS (Coagulase negative Staphylococci)

Tube test: To detect free coagulase
Fibrin clot…….S.aureus
No fibrin clot……CONS

Sensitivity testing:

Novobiocin sensitive……. S.aureus and S.epidermidis
Novobiocin resistant…….S.saprophyticus

Treatment

- Penicillin sensitive staphylococci……penicillin, ampicillin
- Penicillin resistant staphylococci……cloxacillin, Nafcillin
- Methicillin resistant staphylocicci…… Vancomycin

Prevention and Control

- Source of infection is shedding human lesions, the human respiratory tract and skin
- Contact spread of infection occur in hospitals
- Treatment of nasal carriers with topical antiseptics or rifampin and anti-staphylococcal drug

Clostridium

General:

a. obligate anaerobic Gram positive rods, "Spore-forming"
b. Location of spore in the bacterium may aid in species identification (e.g. terminal spores=Clostridium tetani)
c. catalase negative
d. oxidase negative

Species:

a. **Clostridium botulinum** - noninvasive, causing botulism (can be agent of bioterrorism)

b. **Clostridium tetani**- generally noninvasive (very limited invasion potential), causative agent of tetanus

c. **Clostridium difficile** - noninvasive, secreted toxin causing pseudomembranous enterocolitis (a cause of antibiotic-mediated diarrhea)

d. **Clostridium perfringens** - very invasive pathogen, gas gangrene

Pathogenesis: Ability of these organisms to synthesize a variety of extracellular toxins
1. botulism toxin - cause of flaccid paralysis

2. tetanus toxin - cause of tetanus (locked jaw)

3.Exotoxins A and B - cause of diarrhea in pseudomembranous colitis due to Clostridium difficile

4. Alpha toxin - lecithinase which lysed host cell membrane- in combination with other degradative enzymes as the cause for "Gas Gangrene" due to Clostridium perfringens

Clostridium botulinum – Botulism

Clostridium botulinum, Gram + rod with subterminal oval spores

Epidemiology

- Very low incidence (123 cases documented from 1976-1984)
- Soil organism, spores very resistant to physical and chemical agents;

Contrary to the C.botulinum spores, botulism toxin is very heat-labile

- C. botulinum spores found in contaminated food, under anaerobic conditions (canned goods), germinate, grow and produce botulism toxin in 2-3 days.

A. **Food botulism** found in canned vegetables (e.g. green beans, peppers and mushrooms), smoked fish and preserved fruits

B. **wound associated botulism** – requires spore germination in the wound

C**. infant botulism** (honey as a source) – this requires ingestion of spore, germination and intestinal colonization in the infant

Pathogenesis

- Botulism toxin - extremely potent (1 mg killed 200,000 mice)
- heat-labile (inactivated by boiling for 10 min)
- Not destroyed by stomach acid
- 7 types (toxins A to G), with toxins A, B and E being most common
- two subunit toxins (A and B subunits)

Mechanism of action

- upon absorption in intestine, carried via blood to peripheral nerve synapses, acting as a neurotoxin
- binding of toxin to a receptor on the nerve synapse, toxin enters cell, blocking release of acetylcholine from the cell by interfering with proteolytic processing (i.e. the toxin is a protease) and hence release of the acetylcholine.
- flaccid paralysis, “No fever”, “Normal mental status”

Clinical manifestations

- incubation period 18-36 h
- weakness or flaccid paralysis of peripheral nerves including cranial nerves, symmetrical in distribution
  dysphagia, diplopia, dry throat, diluted pupils
- “no sensory deficit”
- may affect respiratory muscles

Diagnosis

- usually a clinical diagnosis
- Do not rely on culturing the microorganisms (preformed toxin is the culprit)
- detecting botulism toxin in the serum, vomitus or feces
- detecting bacterial toxin in food with serological tests
- EMG (electromyography) - diminished action potential of the peripheral nerves, results suggestive not diagnostic

Differential Diagnosis

- Myasthenia gravis
- Guillain-Barre syndrome

Therapy

- Removal of toxin from stomach (if detected early) with lavage
- treatment with antitoxin, remember the antitoxin (from horse serum) is toxin specific
- Supportive care, may require respiratory support
- 12% fatality rate

Prevention

- Canned food must be cooked (100ºC for 10 min to inactivate the toxin)

There are two clinical variants of botulism (also due to Clostridium botulinum and its toxin) besides food botulism

NEISSERIA

Characteristics:

- They are non-motile, gram-negative intracellular diplococcic
- Rapidly killed by drying, sunlight, heat, and disinfectants
- Ferment carbohydrate producing acid but not gas
- Each cocci is kidney-shaped with adjacent concave sides
- Grow best on complex media under aerobic conditions containing 5%CO2
- Oxidase positive.

The main species of medical importance are:

- N. meningitides
- N.gonorrhoea.

Neisseria meningitidis

Characteristics:

- Gram-negative intra cellular diplococci.
- Present in the nasopharynx in 5-10% of healthy people.

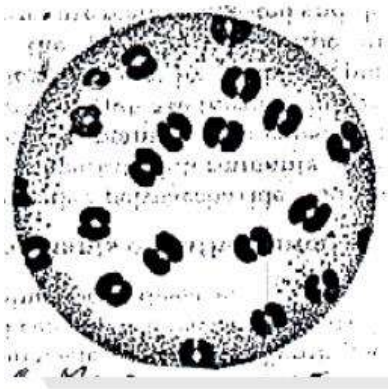


Fig. 3.5 *Neisseria meningitidis*

Antigenic structure:

Capsular carbohydrate

- It is important for serogrouping of meningococci and there are 13 serogroups. The most important serogroups associated with disease in humans are A, B, C, Y and W135.

Outer membrane protein

- Analogous to pore protein of gonococci and responsible for the formation of pore in the meningococcal cell wall
- 20 known serotypes
- It is responsible for serotype specificity of

meningococci.
Lipopolysaccharide

Responsible for the toxic effects found in meningococcal disease

Clinical manifestation:

- Meningococcal meningitis
- Meningococcemia: Meningococcal septicemia

Laboratory diagnosis:

- **Specimen:** Cerebrospinal fluid, blood

  Smear: Gram-negative intracellular diplococci

- **Culture:** Transparent or grey, shiny, mucoid colonies in chocolate agar after incubation at 35-37OC in a CO2 enriched atmosphere.

Biochemical reaction: Oxidase positive. Ferment glucose and maltose in carbohydrate utilization test.

Serology: Latex agglutination test/ Hemmagglutination test.

Treatment: Penicillin

- Penicillin-allergic patients are treated with third generation cephalosporins or chloramphenicol

Prevention and control

- Chemoprophylaxis (Rifampin or minocycline) for households or close contacts
- Avoidance of overcrowding.
- Vaccination with polyvalent conjugate vaccine to high risk groups

ESCHERICHIA COLI

Characteristics:

- Normal flora in human and animal gastrointestinal tract.
- Found in soil, water and vegetation.
- Most are motile; some are capsulated.

Clinical features:

- Urinary tract infection- cystitis, pyelonephritis
- Wound infection- appendicitis, peritonitis
- Neonatal septicemia and meningitis

E.coli-associated diarrheal disease

1. Enteropathogenic E.coli (EPEC)

- causes outbreaks of self-limiting infantile diarrhea
- they also cause severe diarrhea in adults
- antibiotic treatment shorten the duration of illness and cure diarrhea

2. EnteroinvasiveE.coli(EIEC)

- Non-motile, non-lactose fermenting
- E.coli invade the mucosa of the ileum and colon, and causes shigellosis-like dysentery in children in developing countries and travellers to these countries

3. Enterotoxigenic E.coli (ETEC)

- Colonization factor of the organism promote adherence to epithelial cells of small intestine followed by release of enterotoxin which causes toxin-mediated watery diarrhea in infants and young adults.
- It is an important cause of traveller's diarrhea
- Antibiotic prophylaxis can be effective but may increase drug resistance.

4. EnterohaemorrhagicE.coli(EHEC)

Cytotoxic verodoxin producing E.coli serotypeO157:H7 causes haemorrhagic colitis (severe form of diarrhea), and hemolytic uremic syndrome characterized by acute renal failure, hemolytic anemia and low platelet count.

5. EnteroaggressiveE.coli(EAEC)

- Adhere to human intestinal mucosal cells and produce ST-like toxin and hemolysin, and causes acute and chronic diarrhea in persons in developing countries.
- Produce food-borne illness in developed countries

Laboratory diagnosis:

- **Specimen:** Urine, pus, blood, stool, body fluid
- **Smear:** Gram-negative rods
- **Culture:** Lactose-fermenting mucoid colonies on Mac Conkey agar and some strains are hemolytic on blood agar. Pink, circular, convex colonies.

Biochemical reaction:

- Produce indole from tryptophan containing peptone water.
- Reduce nitrate to nitrite.

Serology: For serotyping (Epidemiologic information)

Treatment: Base on antibiotic sensitivity pattern.

MICROBIAL CELL DIVISION

Mitosis and meiosis are the two processes that are involved in the process of cell division. When people talk about "cell division," they're usually referring to mitosis, the process of forming new body cells. The form of cell division that produces egg and sperm cells is called meiosis. Mitosis is an essential part of existence.

Microbiology is the study of viruses, bacteria, algae, fungi, slime molds and protozoa which are tiny organisms. The techniques used to investigate and handle these tiny, mostly unicellular animals are not used in most other scientific investigations.

Cell Division Types

The first is vegetative division, in which each daughter cell replicates the parent cell. The second is mitosis in which each daughter cell duplicates the parent cell. Meiosis, which creates four haploid daughter cells is the second.

Mitosis: It is the process through which cells double themselves. Mitosis can be found in practically every cell in the body, including those in the eyes, skin, hair and muscles.

Meiosis: Instead of identical daughter cells, sperm or egg cells are created in this type of cell division.

Binary Fission: Single-celled organisms such as bacteria reproduce by replicating themselves. Binary fission is used by prokaryotes like bacteria to reproduce. Unicellular organisms can only create new individuals by cell division. In both prokaryotic and eukaryotic cells, the process of cell reproduction results in the generation of two daughter cells that are genetically indistinguishable from the parent cell.

Binary Fission:

Binary fission, or prokaryotic cell division, is less difficult and faster than eukaryotic cell division. Due to the velocity with which bacteria divide their cells, bacterial populations can quickly grow. Bacteria's single circular DNA chromosome is not encased in a nucleus, but rather occupies a specific position within the cell called the nucleoid. The nucleoid's DNA is linked to proteins that help package the molecule into a small size, just like in eukaryotes. However, some of the proteins involved in chromosomal compaction in eukaryotes are related to the packing proteins of bacteria. The origin, or start of replication,

is near to the chromosome's binding location to the plasma membrane. DNA replication is bidirectional, with both strands of the DNA loop traveling outward from the origin at the same time. Each origin point advances away from the cell-wall attachment toward opposing ends of the cell as additional double strands are created. The expanding membrane aids in the transport of the chromosomes as the cell lengthens. Cytoplasmic separation begins once the chromosomes have cleared the midpoint of the elongated cell. From the cell's perimeter to its core, a septum forms between the nucleoids. The daughter cells separate once the new cell walls are in place.

Budding in bacteria:

Some Planctomycetes, Cyanobacteria, Firmicutes (a.k.a. Low G+C Gram-Positive Bacteria) and Proteobacteria have been found to be budding. Although the eukaryotic yeast Saccharomyces cerevisiae has been extensively investigated for budding, the molecular principles of bud production in bacteria are unknown. Below is a schematic illustration of budding in a Planctomyces species.

Intracellular offspring production by some Firmicutes

Multiple intracellular progeny are produced by Epulopiscium spp., Metabacterium polyspora, and the Segmented Filamentous Bacteria (SFB). This appears to be the only means for some of these bacteria to reproduce. Endospore creation in Bacillus subtilis is similar to intracellular offspring development in these bacteria.

Conclusion:

Virtually all cells divide as part of their life cycle. One cell divides into two new cells in the process of cell division. Binary fission is the process by which most bacterial cells divide. Cell division occurs in eukaryotes in two stages: mitosis and cytokinesis. Microbiology is the scientific study of microorganisms, which include protozoans, algae, molds, bacteria, and viruses, among others. Microbiology is concerned with the structure, function, and categorization of these organisms, as well as methods for managing and exploiting their activity.

UNIT – II

IMPORTANCE OF MICROBIOLOGY

There are several ways in which microorganisms benefit the earth. Apart from those that threaten us, there are those who play a vital role in maintaining the health of our ecosystem. Here are some of these important benefits explained.

Importance of Microbiology in the Pharmaceutical Industry

One of the most significant contributions of microbiology to the pharmaceutical industry is the discovery of antibiotics. Microorganisms produce antibiotics as a metabolic by product. Another significant microbiological discovery is the vaccine, which prevents viral infection. For instance, the polio vaccine helps in the eradication of polio worldwide.

Another pharmaceutical item produced by microbes is steroids. Other significant advancements in the field of microbiology include the prevention of microbial contamination of medications, injectables, eye drops, nasal sprays, and inhalation products.

Importance of Microbiology in Medicine and Science

Cells in both humans and animals can profit from and be harmed by microorganisms. Viruses, bacteria, fungi, and parasites are some of these microbes. Microbiology in medicine is significant for a number of reasons.

Microbiologists are able to recognise, isolate, diagnose, and prevent harmful bacteria due to their expertise in medical microbiology. They can also create antibacterial medications by genetically engineering advantageous microbes.

A good example of medical microbiology that assists in the prompt detection of pathogens in tissue specimens is fluorescent fusion.

Importance of Microbiology in the Field of Biotechnology

There are many applications for microorganisms in biotechnology. Microbes are used in fermentation to break down complicated organic materials to produce organic acids, ethanol, vinegar, and fermented meals.

In molecular biology and recombinant DNA technology, microbes, such as viruses, are utilised as a source of molecular vectors like plasmids, phagemids, and cosmids.

In bioremediation, organic wastes are broken down by microbes to eliminate hydrocarbons and organic compounds from sewage water.

Bioleaching and biomining are two processes that microbiologists use to extract metals or heavy metals from their ore.

Enzymes, organic acids, vitamins, amino acids, antibiotics, and polysaccharides are additional metabolic products produced by microorganisms for commercial purposes.

Importance of Microbiology in the Food Industry

Bacteria, yeasts, and moulds are a few of the microbes that are involved in food microbiology. Bacteria primarily cause food poisoning and food deterioration, which leads to various disorders affecting the human gastrointestinal tract.

A variety of foods and dairy products are produced using different bacterial strains. These bacterial strains include Lactobacillus Bulgaricus, Bifidobacterium sp. and Streptococcus Thermophiles.

A few microorganisms, such as bacteria and viruses, are used to control pests that damage agricultural crops. Consequently, they are known as natural pesticides. They are so particular to pests or insects and do not affect humans, animals, plants, or other living things.

Nisin, an antibacterial substance used in cheese, meats, and beverages to prolong shelf life by inhibiting the growth of undesirable bacteria, is an example of microbiology employed in the food sector.

Importance of Microbiology in the Environment

Environmental microbiology is the study of the composition and biology of microbial communities in natural environments. It is applied to the degradation of oil. Although it is difficult to address oil spills in coastal areas and the open sea, a significant amount of the oil can be removed by the hydrocarbon-degrading activities of microbial populations, particularly the Hydrocarbon clastic bacteria (HCB). These species can contribute to the ecological restoration of maritime environments that have been affected by oil contamination.

It is also used to degrade aromatic compounds. Isolated environmental Acinetobacter strains are capable of degrading a variety of aromatic compounds.

Importance of Microbiology in Chemical Substances

Microorganisms are used to manufacture a wide range of industrial chemical products.

Acetaldehyde, acetoacetic acid, acetic acid, ethanol, butanol, galactose, fructose, glycerol, mannitol, lactic acid, mannose, sorbose, succinic acid, and pyruvic acid are some of these products.

These compounds are produced by various microorganisms, including Aerobacter aerogenes, lactic acid bacteria, acetic acid bacteria, butyric acid bacteria, propionic acid bacteria, and E. coli.

Importance of Microbiology in Biofuel

Biomethane can be produced by anaerobic digestion of microalgae biomass, and ethanol can be produced by fermenting carbohydrates. Biofuel can also be produced from extracted microalgae oil.

A significant source of oil content for the manufacturing of biodiesel is microalgae. Additionally, they have higher levels of lipids, which are used as an initial point for the synthesis of biodiesel.

Large amounts of cellulose, starch, mannitol, agar, and laminarin found in microalgae are fermented to alcohol (ethanol and butanol). Chlorella, Chlamydomonas, Spirulina, are among the microalga in this group.

Importance of Microbiology in Everyday Life

In our daily lives, microbiology is used and has a significant impact. Microbiology is used in many aspects of daily life, including food production, biodegradation, the manufacture of commercial goods and genetic engineering. They are required in a variety of dishes. Microorganisms, for instance, are required for the production of curd and cheese.

The lactose sugar in milk is converted to lactic acid by a bacterium known as Lactobacillus, turning milk into curd. Yeast can also be used to make bread, while bacteria are necessary when manufacturing yoghurt. Additionally, only the microorganisms in the human body can manufacture vitamin K.

In order to improve and broaden our fundamental understanding of microorganisms, the science of microbiology investigates their morphology, physiology, metabolism, reproduction, and genetics. This is how microbiology contributes significantly to several industries. In the coming years, we will witness a wide range of further applications of microbiology that will be extremely advantageous for us in every way.

Branches of Microbiology

Due to the importance of microbiology and the ease with which it may be studied, the field is divided into several areas, including Parasitology, Mycology, Bacteriology, Virology, and Microbial Genetics.

The specific disciplines of microbiology have great similarities with one another and with other academic fields, and some of its elements may go beyond the traditional parameters of microbiology. Cellular microbiology is a subfield of microbiology that focuses entirely on research.

UNIT – III

MICROBIAL TAXONOMY

Microbial taxonomy is a means by which microorganisms can be grouped together. Organisms having similarities with respect to the criteria used are in the same group, and are separated from the other groups of microorganisms that have different characteristics. There are a number of taxonomic criteria that can be used. For example, numerical taxonomy differentiates microorganisms, typically bacteria, on their phenotypic characteristics. Phenotypes are the appearance of the microbes or the manifestation of the genetic character of the microbes. Examples of phenotypic characteristics include the Gram stain reaction, shape of the bacterium, size of the bacterium, where or not the bacterium can propel itself along, the capability of the microbes to grow in the presence or absence of oxygen, types of nutrients used, chemistry of the surface of the bacterium, and the reaction of the immune system to the bacterium.

Numerical taxonomy invokes a number of these criteria at once. The reason for this is that if only one criterion was invoked at a time there would be a huge number of taxonomic groups, each consisting of only one of a few microorganisms. The purpose of grouping would be lost. By invoking several criteria at a time, fewer groups consisting of larger number of microorganisms result.

The groupings result from the similarities of the members with respect to the various criteria. A so-called similarity coefficient can be calculated. At some imposed threshold value, microorganisms are placed in the same group.

A well-known example of taxonomic characterization is the kingdom, division, class, family, genus, and species and strain divisions. Such a "classical" bacterial organization, which is typified by the Bergey's Manual of Determinative Bacteriology, is based on metabolic, immunological, and structural characteristics. Strains, for example, are all descended from the same organism, but differ in an aspect such as the antigenic character of a surface molecule.

Microbial taxonomy can create much order from the plethora of microorganisms. For example, the American Type Culture Collection maintains the following, which are based on taxonomic characterization (the numbers in brackets indicate the number of individual organisms in the particular category): algae (120), bacteria (14400), fungi (20200), yeast (4300), protozoa (1090), animal viruses (1350), plant viruses (590), and bacterial viruses (400). The actual number of microorganisms in each category will continue to change as new microbes are isolated and classified. The general structure, however, of this classical, so-called phenetic system will remain the same.

The separation of the microorganisms is typically represented by what is known as a dendrogram. Essentially, a dendrogram appears as a tree oriented on a horizontal axis. The

dendrogram becomes increasingly specialized—that is, the similarity coefficient increases—as the dendrogram moves from the left to the right. The right hand side consists of the branches of the trees. Each branch contains a group of microorganisms.

The dendrogram depiction of relationships can also be used for another type of microbial taxonomy. In this second type of taxonomy, the criterion used is the shared evolutionary heritage. This heritage can be determined at the genetic level. This is termed molecular taxonomy.

Molecular microbial taxonomy relies upon the generation and inheritance of genetic mutations that is the replacement of a nucleotide building block of a gene by another nucleotide. Sometimes the mutation confers no advantage to the microorganism and so is not maintained in subsequent generations. Sometimes the mutation has an adverse effect, and so is actively suppressed or changed. But sometimes the mutation is advantageous for the microorganism. Such a mutation will be maintained in succeeding generations.

Because mutations occur randomly, the divergence of two initially genetically similar microorganisms will occur slowly over evolutionary time (millions of years). By sequencing a target region of genetic material, the relatedness or dissimilarity of microorganisms can be determined. When enough microorganisms have been sequenced, relationships can be established and a dendrogram constructed.

For a meaningful genetic categorization, the target of the comparative sequencing must be carefully chosen. Molecular microbial taxonomy of bacteria relies on the sequence of ribonucleic acid (RNA), dubbed 16S RNA, that is present in a subunit of prokaryotic ribosomes. Ribosomes are complexes that are involved in the manufacture of proteins using messenger RNA as the blueprint. Given the vital function of the 16S RNA, any mutation tends to have a meaningful, often deleterious, effect on the functioning of the RNA. Hence, the evolution (or change) in the 16S RNA has been very slow, making it a good molecule with which to compare microorganisms that are billions of years old.

Molecular microbial taxonomy has been possible because of the development of the technique of the polymerase chain reaction. In this technique a small amount of genetic material can be amplified to detectable quantities

The use of the chain reaction has produced a so-called bacterial phylogenetic tree. The structure of the tree is even now evolving. But the current view has the tree consisting of three main branches. One branch consists of the bacteria. There are some 11 distinct groups within the bacterial branch. Three examples are the green non-sulfur bacteria, Gram-positive bacteria, and cyanobacteria.

The second branch of the evolutionary tree consists of the Archae, which are thought to have been very ancient bacteria that diverged from both bacteria and eukaryotic organisms billions of years ago. Evidence to date places the Archae a bit closer on the tree

to bacteria than to the final branch (the Eucarya). There are three main groups in the archae: halophiles (salt-loving), methanogens, and the extreme thermophiles (heat loving).

Finally, the third branch consists of the Eucarya, or the eukaryotic organisms. Eucarya includes organisms as diverse as fungi, plants, slime molds and animals (including humans).

BERGEYS MANUAL OF SYSTEMATIC BACTERIOLOGY

Bergey's Manual of Systematic Bacteriology is a manual referring to the taxonomy of prokaryotic bacteria. It was prepared by the American bacteriologist, David Hendricks Bergey in 1923. It is a manual that deals with the identification of bacteria. It has been published in 9 editions.

The first eight editions were published under the name 'Bergey's Manual of Determinative Bacteriology'. In the 9th edition, it was renamed as 'Bergey's Manual of Systematic Bacteriology' and was published in four volumes in 1984, 1986, 1989 and 1991.

It is highly regarded by bacteriologists as this manual is continuously updated with successive editions and helps in bacterial taxonomy and research.

The manual classifies bacteria on the basis of their functional and structural attributes and arranges the organisms into familial orders. In recent years, empirical evidence has also been considered in this classification.

Note: Since 2015, the manual has been replaced with Bergey's Manual of Systematic of Archaea and Bacteria and is available online.

Organisation

The 1980 edition of the manual took into consideration the relationship between organisms along with an expanded scope in bacterial taxonomy. The set of four volumes contains:

Volume I: It talks about all Gram-negative bacteria and considers them important for medicinal and industrial purposes.

Volume II: It includes all the information about Gram-positive bacteria.

Volume III: It includes information about the remaining Gram-negative bacteria and about Archaea as well.

Volume IV: It talks about filamentous actinomycetes and similar types of bacteria.

The second edition has been published in five volumes, the details of which are given below:

Volume I: It was published in 2001 and talks about the archaea and the branching phototrophic bacteria.

Volume II: It was published in 2005 and gives details about the proteobacteria.

Volume III: It was published in 2009 and gives details about the firmicutes.

Volume IV: It was published in 2011. It mentions the Spirochaetes, Bacteroidetes, Tenericutes (Mollicutes), Chlamydiae, Acidobacteria, Verrucomicrobia, Fusobacteria, Dictyoglomi, Fibrobacteres, Gemmatimonadetes, Lentisphaerae, and Planctomycetes.

Volume V: It was published in 2012 and talks about the actinobacteria.

The First Edition

In the first edition, Bergey classified the kingdom Prokaryotae in four divisions:

- **Gracilicutes:** they have a gram-negative cell wall.
- **Firmicutes:** they have a gram-positive cell wall.
- **Tenericutes:** they do not have a cell wall.
- **Mendosicutes:** they lack peptidoglycan in their cell wall and are similar to Archaea.

This classification was entirely based upon gram staining, presence of endospore, general shape, motility, morphology and mode of energy production. While the first edition of Bergey's manual is entirely phenetic, the second edition was based on phylogenetic characters such as its DNA, RNA and protein.

In the current 9th edition, the manual is designed for identification of bacteria that is very different from the previous editions. In this edition, the bacteria are divided into 35 groups in the four major divisions.

The first division includes groups 1 to 16 (example: spirochete, sulphur-reducing bacteria, chlamydia and rickettsia), the second division includes groups 17 to 29 (example: gram-positive cocci, endospore forming, gram-positive cocci and rods, gram-positive, non-sporing rods), the third division includes group 30 such as Mycoplasma and the last division includes groups 31 to 35 (example: methanogens, halophiles and archaebacteria).

UNIT – IV

GENERAL METHODS FOR ISOLATION AND IDENTIFICATION OF BACTERIA

- Diagnosis of infectious disease sometimes involves identifying an infectious agent either directly or indirectly.
- In practice most minor infectious diseases such as warts, cutaneous abscesses, respiratory system infections and diarrheal diseases are diagnosed by their clinical presentation and treated without knowledge of the specific causative agent.
- Diagnosis of infectious disease is nearly always initiated by medical history and physical examination.
- Culture allows identification of infectious organisms by examining their microscopic features, by detecting the presence of substances produced by pathogens, and by directly identifying an organism by its genotype.
- Other techniques (such as X-rays, CAT scans, PET scans or NMR) are used to produce images of internal abnormalities resulting from the growth of an infectious agent.

Symptomatic diagnostics

Some signs are specifically characteristic and indicative of a disease and are called pathognomonic signs; but these are rare. Not all infections are symptomatic.

Microbial culture

- Microbiological culture is a principal tool used to diagnose infectious disease.
- Most pathogenic bacteria are easily grown on nutrient agar, a form of solid medium that supplies carbohydrates and proteins necessary for growth of a bacterium, along with copious amounts of water and selective media also available.
- A single bacterium will grow into a visible mound on the surface of the plate called a colony, which may be separated from other colonies or melded together into a "lawn".
- The size, color, shape and form of a colony is characteristic of the bacterial species, its specific genetic makeup (its strain), and the environment that supports its growth.
- In the absence of suitable plate culture techniques, some microbes require culture within live animals. Bacteria such as *Mycobacterium leprae* and *Treponema pallidum* can be grown in animals, although serological and microscopic techniques make the use of live animals unnecessary.
- Viruses are also usually identified using alternatives to growth in culture or animals. Some viruses may be grown in embryonated eggs.
- Another useful identification method is Xenodiagnosis, or the use of a vector to support the growth of an infectious agent. Chagas disease is the most significant example, because it is difficult to directly demonstrate the presence of the causative agent, *Trypanosoma cruzi* in a patient, which therefore makes it difficult to definitively make a diagnosis.

Microscopy & Staining:

- Another principal tool in the diagnosis of infectious disease is microscopy. Microscopy may be carried out with simple instruments, such as the compound light microscope, or with instruments as complex as an electron microscope.
- Samples obtained from patients may be viewed directly under the light microscope, and can often rapidly lead to identification.
- Microscopy is often also used in conjunction with biochemical staining techniques, and can be made exquisitely specific when used in combination with antibody based techniques.
- For example, the use of antibodies made artificially fluorescent (fluorescently labeled antibodies) can be directed to bind to and identify a specific antigens present on a pathogen. A fluorescence microscope is then used to detect fluorescently labeled antibodies bound to internalized antigens within clinical samples or cultured cells. This technique is especially useful in the diagnosis of viral diseases, where the light microscope is incapable of identifying a virus directly.
- The Gram stain and the acid-fast stain, are the standard approaches used to classify bacteria and to diagnosis of disease.

Biochemical tests

- Biochemical tests used in the identification of infectious agents include the detection of metabolic or enzymatic products characteristic of a particular infectious agent.
- Since bacteria ferment carbohydrates in patterns characteristic of their genus and species, the detection of fermentation products is commonly used in bacterial identification.
- Acids, alcohols and gases are usually detected in these tests when bacteria are grown in selective liquid or solid media.
- The isolation of enzymes from infected tissue can also provide the basis of a biochemical diagnosis of an infectious disease.
- For example, humans can make neither RNA replicases nor reverse transcriptase, and the presence of these enzymes is characteristic, of specific types of viral infections.

Serological methods are highly sensitive, specific and often extremely rapid tests used to identify microorganisms.

- These tests are based upon the ability of an antibody to bind specifically to an antigen.
- For example, "Strep throat" is often diagnosed within minutes, and is based on the appearance of antigens made by the causative agent, *S. pyogenes*, that is retrieved from a patient's throat with a cotton swab.
- Some serological methods are extremely costly, although when commonly used, such as with the "strep test", they can be inexpensive.
- Complex serological techniques have been developed into what are known as Immunoassays.

PCR-based diagnostics

- Technologies based upon the polymerase chain reaction (PCR) method will become nearly ubiquitous gold standards of diagnostics of the near future, for several reasons.
- The diagnosis of a few diseases will not benefit from the development of PCR methods, such as some of the clostridial diseases (tetanus and botulism).

Metagenomic sequencing

Metagenomics is currently being researched for clinical use, and shows promise as a sensitive and rapid way to diagnose infection using a single all-encompassing test. This test is similar to current PCR tests;

Indication of tests

There is usually an indication for a specific identification of an infectious agent only when such identification can aid in the treatment or prevention of the disease, or to advance knowledge of the course of an illness prior to the development of effective therapeutic or preventative measures. For example, in the early 1980s, prior to the appearance of AZT for the treatment of AIDS, the course of the disease was closely followed by monitor0020ring the composition of patient blood samples, even though the outcome would not offer the patient any further treatment options. In part, these studies on the appearance of HIV in specific communities permitted the advancement of hypotheses as to the route of transmission of the virus.

TYPING METHODS:

A single isolate with distinctive characteristic[s] may also represent a strain. Members of the same species that have small differences between them can be distinguished by additional methods. These species is then subdivided into subspecies, subgroups, biotypes, serotypes, variants etc.

The process of differentiating strains based on their phenotypic and genotypic differences is known as 'typing'.

These typing methods are useful in hospital infection control, epidemiological studies, and understanding the pathogenesis of infection.

In hospital settings they may be used to:

- determine whether a set of isolates obtained from one patient represents a single infecting strain or multiple contaminants.
- determine whether a series of isolates obtained over time represents relapse of an infection due to single strain or separate episodes of disease due to different strains.

Types of typing methods:

1. Phenotypic techniques, those that detect characteristics expressed by the microorganism and
2. Genotypic techniques, those that involve direct DNA-based analysis of chromosomal or extrachromosomal genetic elements.

PHENOTYPIC METHODS:

- Phenotypic properties are properties like shape, size, staining properties, biochemical properties, antigenic properties that can be measured without reference to the genome.
- These phenotypic methods are limited by:
- Capacity of microorganisms to alter the expression of genes, which may occur spontaneously or in
- Response to environmental stimuli. Cells, which are genetically indistinguishable, may have quite distinct phenotypes/ phenotypic properties if growing under different conditions.
- Point mutations that may not bring considerable change in genotype can result in abnormal regulation or function responsible for a particular phenotype.

1. Bio typing

Bio typing makes use of the pattern of metabolic activities expressed by an isolate, colonial morphology and environmental tolerances. Strains are referred to as "biotypes". Bio typing may be performed manually or using automated systems.

Examples of bio typing methods:

- Sugar fermentation
- Amino acid decarboxylation/deamination
- Standard enzymatic tests such as IMViC, Citrate, urease
- Tolerance to pH, chemicals and dyes
- Hydrolysis of compounds
- Haemagglutination
- Hemolysis

Advantages:

Most strains are typeable. The techniques are reproducible with relatively ease in performance and interpretation.

Disadvantages:

They have poor discriminatory power. Variation in gene expression is the most common reason for isolates that represent single strain to differ in one or more biochemical reactions. Point mutation too contributes to this problem.

2. Phage Typing

Strains can be characterised by their pattern of resistance or susceptibility to a standard set of bacteriophages. This relies on the presence or absence of particular receptors on the bacterial surface that are used by the virus to bind to the bacterial wall. This method is used to type isolates of *Staphylococcus aureus* and *Salmonella sps*. Such stains are referred as 'phage types'.

Advantages:

This technique has fair amount of reproducibility, discriminatory power and ease of interpretation.

Disadvantages:

This technique requires maintenance of biologically active phages and hence is available only at reference centres. Even for the experienced worker, the technique is demanding. Many strains are non-typeable.

3. Bacteriocine Typing

An isolate is assessed for susceptibility to a set of bacterial peptides (bacteriocine) produced by certain bacteria. Bacterocines produced by a particular strain are usually only active against other strains of the same species. It has been used to type stains of *Pseudomonas aeruginosa*, E. coli, *Yersinia pestis* etc.

Advantages:

This technique has fair amount of reproducibility, discriminatory power and ease of interpretation.

Disadvantages:

This technique is available only at reference centres. Even for the experienced worker, the technique is demanding. Many strains are non-typeable.

4. Serotyping

Serotyping is based on fact that strains of same species can differ in the antigenic determinants expressed on the cell surface. Surface structures such as lipopolysaccharides, membrane proteins, capsular polysaccharides, flagella and fimbriae exhibit antigenic variations. Strains differentiated by antigenic differences are known as 'serotypes'. Serotyping is used in several gram negative and gram-positive bacteria. Serotyping is performed using several serologic tests such as bacterial agglutination, latex agglutination, co-agglutination, fluorescent and enzyme labelling assays.

Advantages:

Most strains are typeable. They have good reproducibility and ease of interpretation though some have ease of performance.

Disadvantages:

Some auto agglutinable (rough) strains are untypeable. Some methods of serotyping are technically demanding. There is dependency on good quality reagent from commercial sources.

In-house reparation of reagents is difficult process. Serotyping has poor discriminatory power due to large number of serotypes, cross reaction of antigens and untypeable nature of some strains.

5. Antimicrobial Susceptibility Typing (Antibiogram):

- This typing technique involves comparison of different isolates to a set of antibiotics. Isolates differing in their susceptibilities.

Advantages:

Almost all strains are typeable. The technique has ease of performance and interpretation with fair amount of reproducibility.

Disadvantages:

As a consequence of various genetic mechanisms, different strains may develop similar resistance pattern thus reducing the discriminating power. The susceptibility pattern of isolates taken over a period of time that represents the same strain may differ for one or more antibiotics due to acquisition of resistance.

6. Protein Typing

- Protein typing relies on major or minor differences in the range of proteins made by different strains.
- Variations in the types and structures of the proteins expressed by bacteria can be detected by several methods.
- The proteins, glycoproteins or polysaccharides are extracted from a culture of the strain, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained to compare with those of other strains. More-similar organisms display more-similar protein patterns.
- In another method termed immunoblotting, the electrophoresed products are transferred to nitrocellulose membrane and then exposed to antisera raised against specific strain. The bound antibodies are then detected by enzymelabelled anti-immunoglobulins.
- These methods are currently employed for epidemiological studies of Staphylococcus aureus and Clostridium difficile.

Advantages:

Almost all strains are typeable and techniques have good reproducibility and ease of interpretation.

Disadvantages:

Since the patterns detected are very complex, comparisons among multiple strains are difficult and the interpretation becomes difficult. Methods employed are technically demanding and equipment are costly and hence are not available in all laboratories.

7. Multilocus Enzyme Electrophoresis (MLEE)

Here, the isolates are analysed for differences in the electrophoretic mobilities of a set of metabolic enzymes. Cell extracts containing soluble enzymes are electrophoresed in starch gels.

Variations in the electrophoretic mobility of an enzyme, referred to as 'electro morph', typically reflect amino acid substitution that alter the charge of the protein.

Advantages:

Almost all strains can be typed. The technique has excellent reproducibility and ease of interpretation.

Disadvantages:

It is only moderately discriminatory for the epidemiological analysis of clinical isolates. It requires techniques and equipments that are not available in most laboratories.

GENOTYPIC METHODS:

These methods involve the study of the microbial DNA, the chromosome and plasmid, their composition, homology and presence or absence of specific genes. Originally performed only in research laboratories have now found their way into diagnostic laboratories. Due to their complexities and cost involved, they are however limited to few laboratories only.

1. Plasmid analysis:

The number and sizes of plasmids carried by an isolate can be determined by preparing a plasmid extract and subjecting it gel electrophoresis.

Advantages:

- Most strains are typeable and have good ease of interpretation.

Disadvantages:

- Reproducibility of this method suffers due to the existence of plasmid in different molecular forms such as supercoiled, nicked or linear, each of which migrate differently on electrophoresis.
- Since plasmids can be spontaneously lost or readily acquired, related strains can exhibit different plasmid profiles.
- Since certain genes are contained in transposons that can be readily acquired or deleted, the composition of plasmid DNA can change rapidly.
- Clinical isolates lacking plasmids are untypeable.
- Those strains with one or two plasmids provide poor discriminatory powers.

2. Restriction Endonuclease Analysis (REA) of Chromosomal DNA

A restriction endonuclease enzymatically cuts DNA at a specific nucleotide recognition sequence. The number and sizes of restriction fragments are influenced by the recognition sequence of enzyme and composition of DNA. Bacterial DNA is digested with endonucleases that have relatively frequent restriction sites, thereby generating hundreds of fragments ranging from ~0.5 to 50 kb in length. Such fragments can be separated by size using agarose gel electrophoresis. The pattern stained by ethidium bromide and examined under UV light. Different strains of the same species have different REA profiles because of variations in their DNA sequences.

Advantages:

All the strains can by typed with good reproducibility.

Disadvantages:

The complex profile consists of hundreds of bands that may be unresolved or overlapping thus making comparison difficult. The pattern may consist of bands generated from digestion of plasmids too. These reduce the ease of interpretation and discriminatory power.

3. PFGE of Chromosomal DNA

This technique overcomes the limitations of REA. It is a variation of agarose gel electrophoresis in which the orientation of the electric field across the gel is changed periodically. This modification enables large fragments to be effectively separated by size.

Advantages:

All the strains can by typed with good reproducibility. Restriction profiles are easily read and interpreted.

Disadvantages:

The process takes 2-4 days and requires costly reagents and equipment besides being labour intensive.

4. Southern blot analysis of RFLPs

In contrast to REA of DNA, southern blot analyses detect only the particular restriction fragment. The DNA is digested by endonuclease, the fragments are separated by gel electrophoresis and the fragments transferred to nitrocellulose membranes. The fragments containing specific sequences are then detected by labelled DNA probes.
Variations in the number and sizes of the fragments detected are referred to as restriction fragment length polymorphism (RFLP).

Ribotyping is the blotting of restriction enzyme digestion of 16s rRNA, 25s rRNA and one or more tRNAs. As the 16SrRNA is so highly conserved it is a very useful molecule for comparing relatedness of organisms over the course of evolution. Organisms are classified

as separate species if their sequences show less than 98% homology and are classified as different genera if their sequences show less than 93% identity.

Advantages:

All strains carrying loci homologous to probe are typeable. They are reproducible, have good ease of interpretation.

Disadvantages:

The discriminatory power depends on the choice of probes. The process requires costly reagents and equipment besides being labour intensive.

5. Nucleotide Sequence Analysis:

Genotype information at highest precision may be determined as DNA (or RNA) nucleotide-base sequences. RNA's are often sequenced either by converting the RNAs into DNA or by sequencing the DNA gene that gives rise to the RNA. By using Polymerase Chain Reaction (PCR) to amplify a known DNA segment and automated techniques to sequence the amplified product, it is possible to compare multiple isolates.

Advantages:

This technique can apply on all strains; results are reproducible with ease in interpretation.

Disadvantages:

The process requires costly reagents and equipment besides being labour intensive.

UNIT – V

IMPORTANT MICROBES IN SOIL, WATER, AIR, FOOD

Microorganisms are present everywhere in our environment, in soil, air, food and water. Also called microbes, microorganisms are living organisms, generally observable only through a microscope. Bacteria live in almost any warm, moist environment, and there are thousands of different kinds. They are single-cell organisms that can reproduce very quickly and can spread through food, air or blood.

The transfer of contamination through the airborne route, soil and water body is one of the most significant areas of high-care food production. The food industry specially the manufacturing of the chilled meat products strive for lower levels of the air contamination, therefore lot of experimental and numerical studies considers the concentration of airborne particulate contaminants, such as different species of food spoilage microorganisms. The risk is higher when air is contaminated with eventually foodborne pathogen microorganisms and spores.

Airborne microbes are biological airborne contaminants (also known as bio aerosols) like bacteria, viruses or fungi as well as airborne toxins passed from one victim to the next through the air, without physical contact, causing irritation at the very least. This usually happens when an infected subject sneeze, coughs, or just plain breathes. It is hard to prevent such a method of transmission. Airborne microbes are a major cause of respiratory ailments. Mainly Bacillus, Micrococcus, and Staphylococcus are the microorganisms which are present in air.

Soil and water are common sources of important pathogenic and spoilage microorganisms, which is why it is important to thoroughly wash raw foods with good quality water. Air and dust are important sources of microorganisms during food processing and can influence food quality. Bacillus subtilis is mostly present in soil. Apart from this nitrogen-fixing bacteria, nitrifying bacteria, denitrifying bacteria and actinomycetes are also present in a huge quantity in soil.

Although most soil and water borne microbes will contaminate plants, very few types actually persist on them. Those that persist, such as lactic acid bacteria and some yeasts, must be able to adhere to the plant material and to utilize it for growth. Everyone has their own natural microorganisms that live on, in and around their own bodies. These bacteria are known as natural flora and our own bodies recognize that they are good for us. We, as humans, would not survive without such creatures.

2. Airborne microorganisms:

Bacteria have no active mechanisms for becoming airborne. They are dispersed on dust particles disturbed by physical agencies, in minute droplets of water generated by any

process which leads to the formation of an aerosol. Many actinomycetes, especially those in the genus Streptococcus undergo this process. Air is mainly transport medium for microorganisms. They occur in small numbers in air when compared with soil or water.

The bacteria in the air consist both "good" (non-pathogenic) and "bad" (pathogenic) bacteria, but most of them are good bacteria, and the levels of bad bacteria are low. The microflora of air can be studied under two headings; outdoor and indoor microflora.

Environmental factors that affect air microflora include atmospheric temperature (There is a progressive increase in the death rate with an increase in temperature from -18°C to 49°C), humidity (Low and high relative humidity cause the death of most microorganisms) and air current.

The majority of the airborne bacteria belonged to the genera Bacillus, Micrococcus, and Staphylococcus, but a total of 37 different genera were identified in the air. These results suggest that anthropogenic sources are major contributors to airborne bacteria: gram positive bacilli include:

- Clostridium species
- Corny bacterium species

Gram negative bacilli include:

- Salmonella species
- Escherichia species
- Pseudomonas species
- Bacteroides species

Various Micrococcus like:

- Micrococcus antarcticus; M. luteus; M. lylae; M. roseus; M. sp.

Concentration of airborne microorganisms:

The air around us is filled with microbes. Bacteria, fungi, algae, protozoa, and viruses float in air currents. The numbers of microbes in the air range from 10 to 10,000 per cubic meter. They are found easily up to 3000 meters and as high as six miles into the air. According to a study, in Marseilles, concentrations of airborne viable microorganisms averaged 791 ± 598 bacteria m−3 (with a geometric mean of 536 ± 103 bacteria m−3) and 92 ± 92 fungi m−3 (with a geometric mean of 63 ± 15 fungi m−3). Airborne microflora, which increased a log-normal distribution in Marseilles, was shown to have a large variability. Airborne bacteria increase with temperature and wind velocity whereas airborne fungi increase with temperature and varied with wind direction in urban and natural areas. Partial identification of bacteria in Marseilles Island showed that geographical location Outdoor

airborne microflora was investigated in urban and natural areas, the city of Marseilles had qualitative as well as quantitative influence on airborne microflora, this was illustrated by an increase of global airborne microorganisms, and more particularly Gram-negative bacteria, in the urban area.

Morphology:

The word bacillus may be used to describe any rod-shaped bacterium, and such bacilli are found in many different taxonomic groups of bacteria. Bacilli are usually solitary, but can combine to form diplobacilli, streptobacilli, and palisades.

Micrococci have Gram-positive spherical cells ranging from about 0.5 to 3 micrometers in diameter and typically appear in tetrads. They are catalase positive, oxidase positive, indole negative and citrate negative. Micrococcus has a substantial cell wall, which may comprise as much as 50% of the cell mass. The genome of Micrococcus is rich in guanine and cytosine (GC), typically exhibiting 65 to 75% GC-content.

Micrococci can grow well in environments with little water or high salt concentrations. Most are mesophiles; some, like Micrococcus antarcticus (found in Antarctica) are psychrophiles. Though not a spore former, Micrococcus cells can survive for an extended period of time: unprotected cultures of soil micrococci have been revived after storage in a refrigerator for 10 years. Recent work by Greenblat et al. demonstrates that Micrococcus luteus has survived for at least 34,000 to 170,000 years on the basis of 16S Rrna analysis, and possibly much longer.

Soil borne microorganisms:

There is enough evidence in the literature to believe that microorganisms were the earliest of the living things that existed on this planet. Man depends on crop plants for his existence and crop plants in turn depend on soil and soil microorganisms for their nutrition. Scientists, from the beginning, studied the microorganisms from water, air, soil etc. and recognized the role of microorganisms in natural processes and realized the importance of soil microorganisms in growth and development of plants. Soil is considered to be the main source of scavenging the organic wastes through microbial action and is also a rich store house for industrial micro flora of great economic importance. Soil microbiology emerged as a distinct branch of soil science during first half of the 19th century. Some of the notable contributions made by several scientists in field of soil microbiology are highlighted. J. B. Boussingault (1838) showed that leguminous plants can fix atmospheric nitrogen and increase nitrogen content in the soil. S. N. Winogradsky discovered the autotrophic mode of life among bacteria and established the microbiological transformation of nitrogen and sulphur. Isolated for the first-time nitrifying bacteria and demonstrated role of these bacteria in nitrification (l890), further he demonstrated that free-living Clostridium pasteuriamum could fix atmospheric nitrogen (1893). He is known as “Father of soil microorganism”. B. Frank, i) discovered (1880) an actinomycetes “Frankia” (Actinorhizal

symbiosis) inducing root nodules in non-legumes tress of genera Alnus sp and Casurina growing in temperate forests, ii) coined (1885) the term " Mycorrhiza" to denote association of certain fungal symbionts with plant roots (Mycorrhiza-A symbiotic association between a fungus and roots of higher plants. Renamed the genus Bacillus as Rhizobium (1889).

Bacteria in soil

Various microorganisms present in soil:

- **Nitrogen-fixing bacteria** form symbiotic associations with the roots of legumes like clover and lupine, and trees such as alder and locust. Visible nodules are created where bacteria infect a growing root hair. The plant supplies simple carbon compounds to the bacteria, and the bacteria convert nitrogen (N2) from air into a form the plant host can use. When leaves or roots from the host plant decompose, soil nitrogen increases in the surrounding area. For example: - Cyanobacteria, Rhizobia.

- **Nitrifying bacteria** change ammonium (NH4+) to nitrite (NO2-) then to nitrate (NO3-), a preferred form of nitrogen for grasses and most row crops. Nitrate is leached more easily from the soil, so some farmers use nitrification inhibitors to reduce the activity of one type of nitrifying bacteria. Nitrifying bacteria are suppressed in forest soils, so that most of the nitrogen remains as ammonium. Nitrifying bacteria involves, Nitrosomonas, Nitrobacter, Nitroso coccus.

- **Denitrifying bacteria** convert nitrate to nitrogen (N2) or nitrous oxide (N2O) gas. Denitrifies are anaerobic, meaning they are active where oxygen is absent, such as in saturated soils or inside soil aggregates. For example: - Thiobacillus denitrificans, Micrococcus denitrificans, Paracoccus denitrificans.

- **Actinomycetes** are a large group of bacteria that grow as hyphae like fungi. They are responsible for the characteristically "earthy" smell of freshly turned, healthy soil. Actinomycetes decompose a wide array of substrates, but are especially important in degrading recalcitrant (hard-to-decompose) compounds, such as chitin and cellulose, and are active at high pH levels. Fungi are more important in degrading these compounds at low pH.

A number of antibiotics are produced by actinomycetes such as Streptomyces.

Example of nitrogen fixing bacteria

Rhizobia are one of the groups of microorganisms living in soil. They are single celled bacteria, approximately one thousandth of a millimetre in length. Rhizobia belong to a family of bacteria called Rhizobiaceae. There are a number of groups of bacteria in this family.

Rhizobia belong to a specific group of bacteria that form a mutually beneficial association, or symbiosis, with legume plants. These bacteria take nitrogen from the air (which plants cannot use) and convert it into a form of nitrogen called ammonium (NH4+), which plants can use. The nitrogenase enzyme controls the process, called nitrogen fixation, and these bacteria are often called "nitrogen fixers".

Rhizobia are found in soils of many natural ecosystems. They may also be present in agricultural areas where they are associated with both crop legumes (like soybean) and pasture legumes (like clover). Usually, the rhizobia in agricultural areas have been introduced at sowing by applying an inoculum to the exterior of the seeds as liquid formations or pellets

The presence of numerous genera of spoilage bacteria, yeasts and molds, and an occasional pathogen on fresh produce has been recognized for many years. Several outbreaks of human gastroenteritis have been linked to the consumption of contaminated fresh vegetables and, to a lesser extent, fruits. Salads containing raw vegetables have been identified as vehicles of traveler's diarrhea, an illness sometimes experienced by visitors to developing countries. Enterotoxigenic Escherichia coli is the most common cause of this illness. Outbreaks of salmonellosis in humans have been attributed to consumption of contaminated tomatoes, mustard cress, bean sprouts, cantaloupe, and watermelon.

Fungi in soil:

Soil fungi are considered to be an important food source for earthworms. Fungal species (Cladosporium cladosporioides, Rhizoctonia solani, Mucor sp., Trichoderma viride, Fusarium nivale, Phlebia radiata, Glaeophyllum trabeum, Coniophora puteana, Coriolus versicolor), followed by fast-growing species such as Mucor sp. and R. solan.

Most natural antibiotics come from soil fungi and bacteria:

Many microorganisms have been playing a significant role long before they were discovered by man. Today, soil is considered to be the main source of scavenging the organic wastes through microbial action and is also a rich store house for industrial microflora of great economic importance. Soil bacteria and fungi live by digesting and recycling dead plant material such as leaves and seed. This material is typically carbon-rich and nitrogen-poor. Most common antibiotics are carbon-rich polymers made by enzymes that strongly resemble those that normally make saturated fats. The building blocks of these polymers are often exactly the same as those used to make saturated fats. Antibiotics are not that easy to find in microbes. Bacteria of the Bacillus genus occur mainly in soil and produce many widely studied antibiotic compounds. For example, Bacillus subtilus produces more than seventy-five known antibiotics consisting predominantly of small, cyclic peptides, but also including phospholipid, lipopeptide, and amino sugar antibiotics. It was hypothesized that antibiotic-producing bacteria would be of the genera Bacillus or

Streptomyces and that the antibiotics would be peptides that inhibited the growth of Gram-positive bacteria.

4. **Waterborne microorganisms:**

There are many types of watery environments ranging from freshwater ponds, streams, puddles, lakes, rivers, and swamps to salty seas with three times the salt concentration of the ocean. Microbes live in overgrown slime, on pipes and in open oceans with few nutrients to support microbial life. Microbes thrive in streams with lots of oxygen to murky bogs that have no oxygen. In ponds there is a rich thriving ecosystem of microbial life including green and purple bacteria and algae, sulfate reducers, methane producers, and others. Many microbes live in the bottom of lakes and rivers in sediments. Many microbes cannot survive except in the presence of high concentrations of salt. The largest watery place on earth is the ocean. Oceans cover 71% of the Earth's surface and are responsible for producing about half of the world's organisms, which includes the plants, animals, fungi, and microbes. Most life in the oceans lives at the sunlit ocean surface. Below 25 meters there is little light in the ocean, and life productivity decreases. As well as little light, deeper waters are cooler, which supports less life. Below 50 meters, the temperature is less than 10 degrees Celsius.

Microorganisms found in water: water is also a habitat for various types of microorganisms, such as, bacteria, viruses, fungi and protozoans. Campylobacter, Cholera, Cryptosporidium, Escherichia coli, Giardia, Hepatitis, Legionella, Protozoan parasite, Salmonella, Shigella are commonly found in water.

Morphology:-

Campylobacter: These are gram negative, non-spore forming, microaerophilic bacteria. They are found in spiral and coccoid form and are distinguished from others due to their darting motility. Spiral form is found in young cultures and coccoid form is found in old cultures.eg: C. jejuni, C.coli, C. laridis

Vibrio cholera: It is a gram- negative, , comma-shaped bacterium. V. cholerae is facultatively anaerobic and has a flagellum at one cell pole. V. cholerae was first isolated as the cause of cholera by Italian anatomist Filippo Pacini in 1854.

Cryptosporidium: There are two species which are morphologically indistinguishable by light microscope examination namely Cryptosporidium hominis, Cryptosporidium parvum. Oocysts size ranges from 4-6 µm. It is round or oval in shape.

Giardia: It has two morphological stages: the trophozoite and the cyst.

- The trophozoite is pear shaped, with a broad anterior and much attenuated posterior. It is 1012µm long and 5-7µm wide, bilaterally symmetrical, and has two nuclei. It is also relatively flattened, with a large sucking disk on the anterior ventral side, which serves as the parasite's method of attachment to the mucosa of the host.

The trophozoite also has two median bodies and four pairs of flagella (anterior, caudal, posterior and ventral).

- The G. lamblia cyst is egg-shaped, and measures 8-14µm by 7-10µm. After encystation, each organelle duplicates, so each cyst contains four nuclei, four median bodies, eight pairs of flagella--although these organelles are not arraigned in any clear pattern. Upon excystation, each cyst produces two trophozoites.

Hepatitis: Among the smallest and structurally simplest of the RNA animal viruses. The virion is non enveloped and, with a diameter of 27-32 nm, it is composed entirely of viral protein and RNA. Electron microscopy analyses show particles with icosahedral symmetry although no structural details could be discerned. Morphologically, Hepatitis A virus particles are indistinguishable from other picornaviruses.

5. ungi: These are a diverse group of organisms belonging to the kingdom Eumycota. This kingdom comprises five phyla, namely Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota, and Glomeromycota. As a practical approach to classification, fungi have been divided into groups, such as the filamentous fungi, also called moulds, the yeasts, and the mushrooms. Some fungi are primarily adapted to aquatic environments, and will, therefore, naturally be found in water. These fungi are zoosporic, and many belong in phyla Chytridiomycota. Fungi belonging to the other phyla in Eumycota are primarily adapted to terrestrial environments.

Morphology:

Fungi are composed of filaments called hyphae; their cells are long and thread-like and connected end-to-end. Because of this diffuse association of their cells, the body of the organism is given the special name mycelium, a term which is applied to the whole body of any fungus. When reproductive hyphae are produced, they form a large organized structure called a sporocarp, or mushroom. This is produced solely for the release of spores, and is not the living, growing portion of the fungus.

In addition to being filamentous, fungal cells often have multiple nuclei. In the chytrids and zygomycetes, the cells are coenocytic, with no distinction between individual cells. Another feature of fungi is the presence of chitin in their cell walls. The chitin adds rigidity and structural support to the thin cells of the fungus, and makes fresh mushrooms crisp. Most members of the kingdom Fungi lack flagella; the structures are completely absent in all stages of their life cycle.

Ascomycota: Ascomycota is phylum of the kingdom fungi and, together with the Basidiomycota, form the sub kingdom Dikarya. Its members are commonly known as the sac fungi. They are the largest phylum of Fungi, with over 64,000 species. The defining feature of this fungal group is the "ascus", a microscopic in which nonmotile spores, called ascospores, are formed. The ascomycetes are a monophyletic group.

Zygomycota: The unique characteristic of the Zygomycota is the zygospore. Zygospores are formed within a zygosporangium after the fusion of specialized hyphae called gametangia during the sexual cycle. Single zygospore is formed per zygosporangium. Because of this one-to-one relationship, the terms are often used interchangeably. The mature zygospore is often thick-walled and undergoes an obligatory dormant period before germination. Most Zygomycota are thought to have a zygotic or haplontic life cycle. Thus, the only diploid phase takes place within the zygospore. Nuclei within the zygospore are believed to undergo meiosis during germination, but this has only been demonstrated genetically within the model eukaryote Phycomyces blakesleeanus.

Microorganisms play a key role in maintaining life on earth, fixing gases and breaking down dead plant and animal matter into simpler substances that are used at the beginning of the food chain. Biotechnologists can also exploit the activities of microbes to benefit humans, such as in the production of medicines, enzymes and food. They are also used to breakdown sewage and other toxic wastes into safe matter. However, the above discussed pathogens and the types of disease caused by them makes clear that they are also agents of different diseases and these infectious agents not only spread through contaminated water and spoiled food but also through the omnipresent air and people with weak immune system becomes the first victims of these diseases. Control of these air, soil and water borne pathogens is important, and today many organisations like WHO is taking care of it. Many resistant bugs are found and controlling them is a new challenge for science. Strict adhesion to regulations and other standards set for basic hygiene maintenance and antibiotic use is a necessity

Role of microorganisms in nature and in foods

The food of humans which is of plant and animal origin are naturally associated with microorganisms of several kinds. Microorganisms in their natural habitat play an important role in cycling of nutrients in the ecosystem. In the process of performing their primary role in nature, the microflora associated with food cause spoilage of foods meant for human consumption. Thus, the knowledge on types of microorganisms naturally associated with plant and animal foods helps to predict the microbial types that could be present at later stages of handling, storage and preservation of food.

Depending on the nature of food and its habitat a variety of microorganisms are expected in the food and these may often affect the safety of food. Therefore, information on factors such as total number and types of microorganisms' naturally present, types of microorganisms present in specific food, and ones which are not natural to the food becomes necessary. This information becomes valuable in ascertaining the safety of food during different stages of processing, handling and storage.

Microorganisms in aquatic environment

All surface waters such as ponds, lakes, rivers and oceans differ in their physical, chemical and biological characters. Depending on the nutrient status of water body, the microbial load varies with higher numbers encountering in eutrophic waters. Ground waters or subterranean waters generally have very low microbial load because of filtration effect of soil layers.

Categories of microorganisms in natural waters

The natural waters contain a variety of microorganisms. These include,

- Natural flora: Microorganisms natural to the water body and
- Transient flora: Microorganisms entering the water body from outside environment like from soil, air and through pollutants.

Microorganisms in natural aquatic environment play an important role in nutrient recycling, and as primary producers and decomposers of organic matter. All the microorganisms present in a water body can be seen as surface flora of inhabiting organisms. These not only include spoilage organisms but also human pathogenic microorganisms especially in sewage contaminated waters.

Primary source of microorganisms found in food

The foods of plants and animal origin carry several microorganisms associated with their natural habitat. Plants carry typical micro-flora on their surface and also get contaminated from outside sources. Animals carry microorganisms on their surface and intestine, and also contain contaminants from surrounding environment. Through their excretions and secretions animals release microorganisms in to surrounding environment. Besides, both plants and animals carry pathogenic microorganisms capable of causing human illness. The food associated microorganisms are influenced by the availability of specific nutritional requirements and the environmental parameters. The primary sources of entry of microorganisms in to foods are from the soil, water, air, during handling, processing transportation and storage of foods.

Soil

Soil being the rich source of several kinds of microorganisms immediately contaminates the plants and edible plant parts, and the surface of animals with the soil associated microorganisms. As the soil particles are carried in to aquatic environment through wind, rain and other means contamination of water takes place with several soil micro-flora. Therefore, it is not uncommon to find several microorganisms both in soil and water environment. These soil derived microorganisms form part of the the microbial flora involved in spoilage of foods of plant and animal source. Thus, there is a need to reduce the

load of soil microorganisms in foods which can be achieved by removing the soil by washing the surface of foods with good quality water, and by avoiding contact with soil/ dust.

Water

Natural waters not only contain several microorganisms native to the aquatic environment but also from soil, raw/treated sewage and pollutants entering the water body. The microbial numbers and types vary in different water bodies depending on the nutrient status. Thus, all kinds of microorganisms found in water are likely to be associated with the aquatic organisms as surface flora. Use of such water for food processing will add microorganisms from water to food.

Sewage waters containing human pathogenic microorganisms contaminate foods when such waters are used without proper treatment. The water used in food processing should meet agreeable chemical and bacteriological characteristics.

Air

Air contains several microorganisms which may get deposited on the food being processed and handled. Though the air does not contain natural flora of microorganisms, whatever microorganisms encountered are those associated with the suspended solid material and water droplets. The sources of microorganisms to air are from dust, dry soil, and water spray from natural surface waters, droplets of moisture from coughing, sneezing and talking by food handlers, from sporulating moulds growing on walls, ceilings, floor, foods and food ingredients. Thus, it is likely that the microorganisms persisting in air get deposited on the food being processed and contribute for microbial load and subsequent spoilage of food.

The number of microorganisms present in air depends on factors such as extent of movement of air, sunshine, humidity, location and amount of suspended dust in air. Quiet air allows settling of microorganisms but the moving air brings in microorganisms and keeps them suspended. Thus, the number of microorganisms in air is increased by air currents caused by movement of people, by ventilation and by breeze. The rain or snow removes microorganisms from the air.

Micro-flora of food processing facility

The nature of micro-flora in a food processing facility varies depending on the nature of food being processed. Hence characteristic microbial populations are encountered in different processing units. Also variations may be observed in microbial numbers from one area of processing plant to another. The microbial types present inside the processing plant are related to quality of air outside the plant and the microbial population levels are related to the level of activity of workers.

Reducing microbial load in processing area

There is a need to reduce microbial load in the processing area. This can be achieved by by installing filtration, chemical treatment and heat or electrostatic precipitation units, and taking measures in preventing the build up after reducing the microorganisms. The build-up of microorganisms in the processing area can be prevented by maintaining the positive pressure in food process area, installing filters in ventilating systems that prevent spread of microorganisms from one part of a plant to another and installing UV- irradiated air locks at doors to reduce the number of organisms carried by workers.

Handling and processing

Foods grown/cultured in natural environment containing specific groups of microorganisms are further contaminated by several microorganisms during harvesting, handling and processing. Further, addition of microorganisms to food may take place from;

- All food contact surfaces including equipments coming in contact with foods, packaging material, and from food handlers. Foods are also prone for microbial contamination during transportation and storage.
- Use of sewage contaminated water for washing foods being processed contaminates it with human pathogenic microorganisms
- All the microorganisms associated with food handlers enter the food during handling of food from hands, garments, body surface, hair etc under poor personnel hygiene practices.

Significance of microorganisms in foods

Microorganisms associated with food derive energy from food for those cell growth, maintenance and reproduction. Based on their function microorganisms associated with foods may be divided in to three general groups;

- Those causing spoilage or undesirable changes in the food
- Those producing desirable changes
- Those producing disease

Based on the extent of stability to microbial invasion foods may be classified as;

- Perishable foods Ex. Fish and meat
- Semi-perishable foods Ex. Potatoes. Tomato
- Stable foods Ex. Cereals, Flour and Sugar

The stable or semi-stable foods become unstable or perishable when the moisture content increases.

Factors affecting microorganisms in foods

The survival and activity of microorganisms in foods depends on several factors namely, numbers and types of microorganisms present, type of food, treatments to which the food has been exposed, processing or storage treatments that the food receives, whether the food is to be consumed as it is or heated.

The food associated microorganisms may have useful function, cause spoilage, cause health hazard and play no role or remain inert. The cases of spoilage, food-borne illnesses or useful activity results due to the growth and multiplication of the microorganisms. The inert microorganisms are those which do not find food environment favourable for their growth, and remain dormant without causing any changes in food.

Causes for spoilage of food

Spoilage of food usually occurs due to,

- Undesirable changes brought about by the microorganisms in the odour, colour, taste, texture and appearance of the food.
- Some microorganisms may not directly involve in spoilage but bring about changes in food that will facilitate growth of spoilage organisms. Ex. Bacteriophage attacking useful organisms and facilitating growth of undesirable organisms leading to spoilage.

Microorganisms associated with food

The presence of small numbers of microorganisms associated with foods may not cause any problem, but their unrestricted growth can result in spoilage or deterioration of the food making it unfit for consumption. The wide variety of microorganisms associated with foods is mainly saprophytic. They cannot be avoided in food as these are derived from the environment in which the food is prepared or processed, and also difficult to eliminate completely. However, it is possible to reduce the number or decrease their activities by altering the environmental conditions.

A variety of bacteria, molds and yeasts are important as food spoilage organisms. Important microorganisms involved in spoilage of fish are:

Bacteria Gram Negative Bacteria

Acinetobacter, Aeromonas, Alkaligens, Enterobacter, Flovobacterium, Moraxella, Photobacterium, Pseudomonas Vibrio etc

Gram Positive Bacteria

Bacillus, Corynebacterium, Enterococcus, Listeria, Microbacterium, Clostridium, Staphalococcus, etc.