I B.SC BIOTECHNOLOGY

APPLIED MICROBIOLOGY

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PG & RESEARCH DEPARTMENT OF BIOTECHNOLOGY

SUBMITTED BY

Dr. J.JEYASREE

ASSISTANT PROFESSOR

PG & RESEARCH DEPARTMENT OF BIOTECHNOLOGY

BHARATH COLLEGE OF SCINCE AND MANAGEMENT

THANJAVUR

UNIT I -Fermentation

Fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes. In biochemistry, it is narrowly defined as the extraction of energy from carbohydrates in the absence of oxygen. Fermentation is the primary means of producing adenosine triphosphate (ATP) by the degradation of organic nutrients anaerobically. Humans have used fermentation to produce foodstuffs and beverages since the Neolithic age. For example, fermentation is used for preservation in a process that produces lactic acid found in such sour foods as pickled cucumbers, kombucha, kimchi, and yogurt, as well as for producing alcoholic beverages such as wine and beer. Fermentation also occurs within the gastrointestinal tracts of all animals, including humans.

Isolation Preservation and improvement of strains.

Ideal Characteristics of Strain -Rapid growth -Genetic stability - Non-toxicity to humans - Ability to use cheaper substrates - Elimination of the production of compounds that may interfere with downstream processing - To improve the use of carbon and nitrogen sources. -Reduction of cultivation cost -Shorter fermentation time.

Purpose of Strain Improvement

Increase the productivities -Regulating the activity of the enzymes - Introducing new genetic properties into the organism by Recombinant DNA technology / Genetic engineering.

Approaches for Strain Improvement

- Mutant Selection
- Recombination
- Recombinant DNA Technology

MUTANT SELECTION

- A MUTATION is a Sudden and Heritable change in the traits of an organism.
- Application of Mutagens to Induce mutation is called MUTAGENESIS.
- Agents capable of inducing mutations are called MUTGENS
- Chemical mutagens–Alkylating agents, Acridine Dyes, etc.
- Mutation occurring without any specific treatment are called " Spontaneous Mutation."
- Mutation are resulting due to a treatment with certain agents are known as "Induced Mutation."
- Isolation of mutants

The following points highlight the four methods to detect and isolate mutants.

The methods are:

1. Replica Plating Technique
2. Resistance Selection Method
3. Substrate Utilization Method
4. Carcinogenicity Test.

1. **Replica Plating Technique**:

Lederberg and Lederberg (1952) have given replica plating technique. This technique is used to detect auxotrophic mutants which differentiates between mutants and wild type strains on the basis of ability to grow in the absence of an amino acid.

2. **Resistance Selection Method**:

It is the other approach for isolation of mutants. Generally the wild type cells are not resistant either to antibiotics or bacteriophages. Therefore, it is possible to grow the bacterium in the presence of the agent (antibiotics or bacteriophage) and look for survivors.

3. **Substrate Utilization Method**:

This method is employed in the selection of bacteria. Several bacteria utilize only a few primary carbon sources. The cultures are plated onto medium containing an alternate carbon sources. Any colony that grows on medium can use the substrate and are possibly mutants. These can be isolated.

4. **Carcinogenicity Test**:

To identify the environmental carcionogens that cause mutation and induce cancer in organisms. It's based on detecting potential of carcinogens and testing for mutagenicity in bacteria.

Novel genetic technologies :

Metabolic engineering & Genome shuffling

Metabolic engineering-

The existing pathways are modified, or entirely new ones are introduced through the manipulation of the genes so as to improve the yields of the microbial product, eliminate or reduce undesirable side products or shift to the production of an entirely new product. It has been used to over-produce the amino acid isoluecine in Corynebacterium glutamicum, & ethanol by E. coli and has been employed to introduce the gene for utilizing lactose into Corynebacterium glutamicum thus making it possible for the organism to utilize whey which is plentiful and cheap.

Genome Shuffling

It is a novel technique for strain improvement that allows for recombination between multiple parents at each generation and several rounds of recursive genome fusion were carried out resulting in the final improved strain involving genetic trait from multiple initial strains.

Handling and development of inoculum for various fermentation process

Definition of inoculum :- Inoculum is the mixture of cultured microbes Along with media in which it is growing.

Constituent of Inoculum media :-

- Chemical composition :- The Inoculum media must have a suitable Chemical composition. Generally, the medium should contain a source of carbon, a source of Nitrogen, growth factors and Mineral salts. Buffering Capacity :- Maintenance of the PH in the optimum range is necessary for making the process successful. In order the control the PH of the medium, buffers (e.g. CaCo) Should be added to the medium.
- Avoidance of foaming :- 1.Foaming is a serious problem in a fermentation Industry. 2. Hence, defoamers (e.g. oil mixed with Octadecanol for penicillin fermentations) should be used for controlling foam. Consistency :- 1. Proper airation and agitation.

Stepes Involving Inoculum development :-

1. Compostions of Bennett's medium used in the prepartion of Inoculum for vitamin productions :- Component Amount (g./Litre) - Yeast extract 1.0 gm Beef extract 1.0 gm N-Z-Amine A 2.0 gm Glucose 10.0 gm Distilled water 15.0 ml.

2.(Bacterial Insecticides) mass production of Bacillus thuringiensis :-
Name of Component Amount (%) Beet molusses 1.00 Corn-steep solids 0.85 $CaCO_3$ 1.00

Upstream process

Upstream processing includes formulation of the fermentation medium, sterilization of air, fermentation medium and the Fermenter, inoculum preparation and inoculation of the medium. The fermentation medium should contain an energy source, a carbon source, a nitrogen source and micronutrients required for the growth of the microorganism along with water and oxygen, if necessary. A medium which is used for a large scale fermentation, in order to ensure the sustainability of the operation, should have the following characteristics;

1. It should be cheap and easily available

2. It should maximize the growth of the microorganism, productivity and the rate of formation of the desired product

3. It should minimize the formation of undesired products

Usually, waste products from other industrial processes, such as molasses, lignocelluloses wastes, cheese whey and corn steep liquor, after modifying with the incorporation of additional nutrients, are used as the substrate for many industrial fermentations. Sterilisation is essential for preventing the contamination with any undesired microorganisms. Air is sterilised by membrane filtration while the medium is usually heat sterilised. Any nutrient component which is heat labile is filter-sterilised and later added to the sterilised medium. The fermenter may be sterilised together with the medium or separately.

Inoculum build up is the preparation of the seed culture in amounts sufficient to be used in the large Fermenter vessel. This involves growing the microorganisms obtained from the pure stock culture in several consecutive Fermenter. This process cuts down the time required for the growth of microorganisms in the Fermenter, thereby increasing the rate of productivity. Then the seed culture obtained through this process is used to inoculate the fermentation medium.

Inoculum preparation procedure

Test tube Pure culture under good condition Test tube Contain seed culture In Suitable conditions Conical flask Containing culture for large scale fermentation Fermenter contain medium and culture for mass culture contain suitable conditions Fermenter

. • The degradation of carbon compounds by cells or organisms under anaerobic conditions. Carbon compounds degraded Microorganisms Complex into simple one

Process •

Three cultural fe**rmentation system used in bioreactors**

• Batch culture fermentation

• Continuous culture fermentation

• Feed batch culture fermentation

• Batch culture fermentation • It is closed system filled fresh medium and inoculated • It is refilled for the next process of fermentation

. **• Continuous reaction •** Fresh medium added continuously • Shut down less frequently then batch system.

Feed batch

• Feed continuously added until maximum liquid fermenter volume reached. • Then fermenter may be allowed to continue or completed partially or completely depending on the process

• Define different modes of fermentation and known their limitation. • Develop a suitable medium and perform a material balance. • Deter-mind fermentation productivity and yields. • Fermentation take place in absence of oxygen. • The science of fermentation is know as zymology.

when fermentation is over, the desired product is recovered from the growth medium.

Media for industrial fermentation

Medium formulation is essential stage in manufacturing process Carbon & Nitrogen other Energy + sources + O_2 + nutrients

Elemental composition of microorganisms may be taken as guide Biomass + products + CO_2 +H_2O +heat

Elemental composition

CARBON SOURCE

 A carbon source is required for all biosynthesis leading to reproduction, product formation and cell maintenance. In most fermentations it also serves as the energy source.

 Molasses
 malted barley
 Starch and Dextrins
 Sulphite Waste Liquor
 Alkanes and Alcohols n-Alkanes
 Oils and fats Factors influencing the carbon source –

Cost of the product - rate at which it is metabolized - geographical locations - government regulations - cellular yield coefficient

Nitrogen Sources

 Most industrial microbes can utilize both inorganic and organic nitrogen sources.

 Inorganic nitrogen may be supplied as ammonium salts, often ammonium sulphate and diammonium hydrogen phosphate, or ammonia. Ammonia can also be used to adjust pH of the fermentation.

 Organic nitrogen sources include amino acids, proteins and urea.

 Corn Steep Liquor Yeast Extracts Peptones Soya Bean Meal

Minerals

 All microorganisms require certain mineral elements for growth and metabolism. In many media, magnesium, phosphorous, potassium, sulphur, calcium and chlorine are essential components and must be added. Others such as cobalt, copper, iron, manganese, molybdenum and zinc are present in sufficient quantities in the water supplies and as impurities in other media ingredients.

Chelators

 Many media cannot be prepared without precipitation during autoclaving. Hence some chelating agents are added to form complexes with metal ions which are gradually utilised by microorganism

 Examples of chelators: EDTA, citric acid, polyphosphates etc.,

 It is important to check the concentration of chelators otherwise it may inhibit the growth.

 In many media these are added separately after autoclaving Or yeast extract, peptone complex with these metal ions

Vitamins and Growth Factors

 Many bacteria can synthesize all necessary vitamins from basic elements. For other bacteria, filamentous fungi and yeasts, they must be added as supplements to the fermentation medium.

 Most natural carbon and nitrogen sources also contain at least some of the required vitamins as minor contaminants

Precursors

 Precursors are defined as “substances added prior to or simultaneously with the fermentation which are incorporated without any major change into the molecule of the fermentation product and which generally serve to increase the yield or improve the quality of the product”.

 They are required in certain industrial fermentations and are provided through crude nutritive constituents, e.g., corn steep liquor or by direct addition of more pure compounds.

Inducers and Elicitors

 If product formation is dependent upon the presence of a specific inducer compound or a structural analogue, it must be incorporated into the culture medium or added at a specific point during the fermentation.

 The majority of enzymes of industrial interest are inducible. Inducers are often substrates such as starches or dextrins for amylase.

 In plant cell culture the production of secondary metabolites, such as flavanoids and terpenoids can be triggered by adding elicitors.

Inhibitors

 Inhibitors are used to redirect metabolism towards the target product and reduce formation of other metabolic intermediates

 others halt a pathway at a certain point to prevent further metabolism of the target product.

 An example of an inhibitor specifically employed to redirect metabolism is sodium bisulphite

WATER

 All fermentation processes, except SSF, require vast quantities of water.

 Not only is water a major component of all media, but it is important for ancillary services like heating, cooling, cleaning and rinsing.

 A reliable source of large quantities of clean water, of consistent composition, is therefore essential.

 Assessing suitability of water - pH - dissolved salts - effluent contamination Reuse of water is important - It reduces water cost by 50% - Effluent treatment cost by 10 fold

Oxygen

 Depending on the amount of oxygen required by the organism, it may be supplied in the form of air containing about 21% (v/v) oxygen or occasionally as pure oxygen when requirements are particularly high.

 The organism's oxygen requirements may vary widely depending upon the carbon source. For most fermentations the air or oxygen supply is filter sterilized prior to being injected into the fermenter.

Antifoams

 Antifoams are necessary to reduce foam formation during fermentation. Foaming is largely due to media proteins that become attached to the air-broth interface where they denature to form a stable foam "skin" that is not easily disrupted

 An ideal antifoam should have the following properties

 Disperse readily and have fast action

 Active at low concentrations

 Long acting in preventing new foam

 Should not be metabolized

 Should not be toxic to m.o, humans etc

 Cheap, should not cause problem in fermentation

Types of media

COMMON BROADLY-DEFINED CULTURE MEDIA

- Nutrient media
- Minimal media
- Selective media
- Differential media
- Transport media
- Enriched media

Mannitol Salt Agar (MSA): Mannitol salt agar is both a selective and differential media used for the isolation of pathogenic Staphylococci from mixed cultures. Ingredients per liter of deionized water

MacConkey's Agar (MAC): MacConkey's Agar is both a selective and differential media; it is selective for Gram negative bacteria and can differentiate those bacteria that have the ability to ferment lactose. Ingredients per liter of deionized water

Composition of Nutrient Agar •0.5% Peptone It is an enzymatic digest of animal protein. Peptone is the principal source of organic nitrogen for the growing bacteria. •0.3% beef extract/yeast extract It is the water-soluble substances which aid in bacterial growth, such as vitamins, carbohydrates, organic nitrogen compounds and salts. •1.5% agar It is the solidifying agent. •0.5% NaCl The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is similar to the cytoplasm of the microorganisms. •Distilled water Water is essential for the growth of and reproduction of micro-organisms and also provides the medium through which various nutrients can be transported. •pH is adjusted to neutral (7.4) at 25 °C.

Potato dextrose agar Potato infusion 200 gm Dextrose 20 gm Agar 20 gm Distilled water 1 liter Potato Dextrose Agar (PDA) is used for the cultivation of fungi. Potato Dextrose Agar (PDA) is a general purpose medium for yeasts and molds that can be supplemented with acid or antibiotics to inhibit bacterial growth. It is recommended for plate count methods for foods, dairy products

and testing cosmetics. PDA can be used for growing clinically significant yeast and molds.

UNIT II – MICROBIAL ENERGETIC

Microbial metabolism is the means by which a microbe obtains the energy and nutrients (e.g. carbon) it needs to live and reproduce. Microbes use many different types of metabolic strategies and species can often be differentiated from each other based on metabolic characteristics. The specific metabolic properties of a microbe are the major factors in determining that microbe's ecological niche, and often allow for that microbe to be useful in industrial processes or responsible for biogeochemical cycles

1. How the organism obtains carbon for synthesizing cell mass:

autotrophic – carbon is obtained from carbon dioxide (CO2)

heterotrophic – carbon is obtained from organic compounds

mixotrophic – carbon is obtained fromboth organic compounds and by fixing carbon dioxide

2. How the organism obtains reducing equivalents (hydrogen atoms or electrons) used

either in energy conservation or in biosynthetic reactions:

lithotrophic – reducing equivalents are obtained from inorganic compounds

organotrophic – reducing equivalents are obtained from organic compounds

3. How the organism obtains energy for living and growing:

phototrophic – energy is obtained from light

chemotrophic – energy is obtained from external chemical compounds

chemolithoautotrophs obtain energy from the oxidation of inorganic compounds and carbon from the fixation of carbon dioxide. Examples: Nitrifying bacteria, sulfur-oxidizing bacteria, ironoxidizing bacteria, Knallgas-bacteria

photolithoautotrophs obtain energy from light and carbon from the fixation of carbon dioxide,using reducing equivalents from inorganic compounds. Examples:Cyanobacteria (water (H2O)
as reducing equivalent = hydrogen donor), Chlorobiaceae, Chromatiaceae (hydrogen sulfide(H2S) as hydrogen donor), *Chloroflexus* (hydrogen (H2) as reducing equivalent donor

chemolithoheterotrophs obtain energy from the oxidation of inorganic compounds, but cannot fix carbon dioxide (CO2). Examples: some *Thiobacilus*, some *Beggiatoa*, some *Nitrobacter* spp., *Wolinella* (with H2 as reducing equivalent donor), some Knallgas-bacteria,some sulfate-reducing bacteria

chemoorganoheterotrophs obtain energy, carbon, and hydrogen for biosynthetic reactionsfrom organic compounds. Examples: most bacteria, e. g. *Escherichia coli*, *Bacillus* spp.,*Actinobacteria*

photoorganoheterotrophs obtain energy from light, carbon and reducing equivalents forbiosynthetic reactions from organic compounds. Some species are strictly heterotrophic, manyothers can also fix carbon dioxide and are mixotrophic. Examples: *Rhodobacter*,*Rhodopseudomonas*, *Rhodospirillum*, *Rhodomicrobium*, *Rhodocyclus*, *Heliobacterium*,*Chloroflexus* (alternatively to photolithoautotrophy with hydrogen)

Photosynthesis

Overview of Photosynthesis a) Light-dependent Reactions:

• Light energy is harvested by photosynthetic pigments and transferred to special reaction center (photosystem) chlorophyll molecules.

• The light energy is used to strip electrons from an electron donor (the electron donor goes from a reduced to an oxidized state).

• The electrons are shuttled through a series of electron carriers from high energy state to a low energy state.

• During this process, ATP is formed.

• In the cyclic pathway of electron transport, electrons are returned to the electron transport chain

• In the noncyclic pathway, the electrons are used to reduce NAD (or NADP) to NADH (or NADPH)

Photosynthesis

b) Light-independent Reactions: • ATP and NADH (NADPH) from the light-dependent reactions are used to reduce CO2 to form organic carbon compounds (carbon fixation). • The reduced organic carbon is usually converted into glucose or other carbohydrates.

2. Oxygenic photosynthesis

a) Found in cyanobacteria (blue-green algae) and eukaryotic chloroplasts

b) Electron donor is H2O: Oxidized to form O2

c) Two photosystems: PSII and PSI

d) Major function is to produce NADPH and ATP for the carbon fixation pathways

3. Anoxygenic photosynthesis

a) Found in: • Green sulfur bacteria (e.g. Chlorobium) • Green nonsulfur bacteria (e.g. Chloroflexus) • Purple sulfur bacteria (e.g. Chromatium) • Purple nonsulfur bacteria (e.g. Rhodobacter) the green and purple sulfur bacteria • H2 or organic compounds in the green and purple nonsulfur bacteria c) Only one photosystem • In green bacteria, the photosystem is similar to PSI • In purple bacteria, the photosystem is similar to PSII d) Primary function is ATP production, chiefly via cyclic photophosphorylation

Chemolithotrophy

1. Features of Chemolithotrophy

a) Electrons are removed from a reduced inorganic electron donor

b) The electrons are passed through a membrane- bound electron transport pathway, often coupled to the synthesis of ATP and NADH

c) The electrons are ultimately passed to a final electron acceptor

d) ATP and NADH may be used to convert CO2 to carbohydrate

. **Examples of electron donors**

a) Ammonia (NH_4^+) → Nitrite (NO_2^-) in Nitrosomonas

b) Nitrite (NO_2^-) → Nitrate (NO_3^{2-}) in Nitrobacter

c) Hydrogen sulfide (H_2S) → Sulfur (So) in Thiobacillus and Beggiatoa d) Sulfur (So) → Sulfate (SO_4^{2-}) in Thiobacillus e) Hydrogen (H_2) → Water (H_2O) in Alcaligenes

Examples of electron acceptors

a) Oxygen (O_2) → Water (H_2O) in many organisms

b) Carbon dioxide (CO_2) → Methane (CH_4) in the methanogenic bacteria

The Nitrogen Cycle

1. Mineralization: Organic nitrogen (mostly amino acids) → NH_4^+ (All organisms)

Nitrification: NH_4^+ → NO_2^- (Nitrosomonas) NO_2^- → NO_3^{2-} (Nitrobacter) 1. Denitrification NO_3^- → N_2O N_2O → N_2 (Several species, including certain Pseudomonas and Bacillus)

Assimilatory Nitrate Reduction NO_3^{2-} → Organic Nitrogen (Many microbial species and plants)

N2 fixation N_2 → NH_4^+ Free-living nitrogen fixers eg Azotobacter and Azospirillum Symbiotic nitrogen fizers eg Rhizobium and Bradyrhizobium Cyanobacteria attached to the cordgrass plant Spartina in salt marshes

UNIT III – FOOD MICROBIOLOGY

Food poisoning General definition Illness caused by the infection with microorganisms and ingestion of toxins produced, and chemical poisoning.

Causes

• Bacteria and their toxins • Viruses • Chemicals • Vegetable poisoning

Foods contaminated with pathogenic microorganisms usually do not look bad, taste bad, or smell bad.

• It is impossible to determine whether a food is contaminated with pathogenic microorganisms without microbiological testing.

Classification of food borne diseases Food borne diseases are classified into:

1. Food borne infections
2. Food borne intoxications

Foodborne infection • is caused by the ingestion of food containing live bacteria which grow and establish themselves in the human intestinal tract.

Foodborne intoxication • is caused by ingesting food containing toxins formed by bacteria which resulted from the bacterial growth in the food item. • The live microorganism does not have to be consumed.

FOOD BORNE DISEASES

Food borne diseases are as a result of ingestion of food stuffs infected with microorganism or chemicals

 The contamination of food may occur at any stage in the process from production to consumption

- Food infections - Contaminated food acting as Food that serve as culture carrier of microorganisms medium for growth of (Infections include

typhoid, pathogen(Infection include cholera, dysentery, hepatitis) salmonellosis, shigellosis,gastroenteristis)

- Food infection can be due to Bacteria (Eg., Salmonellosis,Gastroenteritis, Shigellosis) Viruses (Eg., Poliomyelitis, Infectious hepatitis) Parasites(Eg., Ameabiasis, trichionosis, tapeworm infection)

SALMONELLOSIS:

Salmonellosis is a food borne caused by the Salmonella sps. Optimum condition for growth: 10-45˚C, pH 4-9, water activity(0.93) Incubation: 12 to 36 hrs Sources: Unrefrigerated meat and poultry Symptoms: Nausea, vomiting, greenish foul stools, fever, weakness and drowsiness Prevention: Holding food at 66˚C for 12 min to kill organisms, eliminating contaminated food, cooking food for sufficient time and proper storing by cooling

GASTROENTERITIS:

Disease caused due enterotoxin released in gut by Clostridium perfringens Optimum conditions: 20 to 55˚C, pH(5-9), heat resistant Incubation: 10 to 24 hrs Sources: Raw foods and food that are cooled slowly and held sometime before consumption Symptoms: Abdominal pain, diarrhoea, gas formation Prevention: heating above 60˚C followed by rapid cooling of cooked meat ad other foods, reheating leftovers and personal hygiene

Disease also caused by *Bacillus cereus*

Optimum conditions: 10 to 49˚C and pH(4.9 to 9.3)

Incubation: 2 to 8hrs

Sources: Contaminated foods, cereals, mashed potatoes, vege sprouts, meat loaf, puddings Symptoms: Nausea, abdominal cramps, vomiting, diarrhoea Prevention: Holding foods above 65˚C and reheating leftovers for 72˚C and personal hygiene

Most *E.coli* are harmless unless they are Enteropathogenic *E.coli* (EEC)

 Incubation: 8 to 24 hrs

 Symptoms: Vomiting, dehydration, fever, headache, abdominal cramps, diarrhoea

 Prevention: cooking food properly, rapid chilling of foods, use of protected water, ensuring personal hygiene

It is a bacterial dysentery caused due to *Shigella sps.*

Sources: Moist foods like milk, potato, shrimp, tuna, turkey, apple, cider

Symptoms: Fever, abdominal cramps, chills, mucus or pus, bloody stools, headache, nausea and dehydration

Prevention: Personal hygiene, Control of flies, use of purified water, sanitary conditions

viral infections -

- Norovirus (most common viral food borne illness, which causes gastroenteritis, a medical condition characterised by diarrhoea, vomiting and abdominal pain), Hepatitis A and E (which cause inflammation of the liver), Rotavirus (particularly associated with gastroenteritis in children)
- Viruses spread through Contamination of food by infected food handlers due to poor hygienic practices, Contact of food with animal waste, human sewage or sewage- polluted water, Consumption of products of animal origin contaminated with viruses (e.g. meat, fish etc.)
- Foods associated with viral infection include Shellfish (e.g. Oysters, mussels), crustaceans and their products which are farmed and/or harvested in waters near human sewage outlets (e.g. waste-water treatment plants), Fruit/vegetables grown on land fertilised with animal waste or irrigated with contaminated water, Undercooked meats such as pork.
- **Prevention** - can be by Training and awareness in good hygiene practices (e.g. hand washing, washing and proper handling of fruits and

vegetables, adequate storage of food in the refrigerator, thorough cooking of pork meat). This is particularly important where food is prepared for sick or vulnerable people in hospitals for example, Employees suffering from illness should be restricted from food service work,

- Use of clean water to irrigate crops, particularly ready to eat crops, Avoiding the use of animal manures on crops, particularly ready to eat crops, Farming of shellfish in clean seawater protected from sewage contamination

Infections include **Amebiasis -** caused by *Endamoeba histolytica* due to sewage contaminated in water

Trichinosis: caused by Nematods due to raw, contaminated pork

Tapeworm Infection: caused due to tapeworm infected pork, fish and beef

Prevention: Proper cooking, quick freezing, purified water and personal hygiene

Personal hygiene - Washing hands properly before and after consuming foods

Purify water before consuming

DETERMINATION OF FOOD IN MICROORGANISM

- Culture, Microscopic and Sampling methods
- Chemical methods
- Immunological methods
- Molecular genetic methods
- Physical methods

- The detection and enumeration of pathogens in food and on surfaces that come into contact with food are an important component of any integrated program to ensure the safety of foods throughout the food supply chain.
- Traditional methods of detecting foodborne pathogenic bacteria are often time-consuming because of the need for growth in culture media, followed by isolation, biochemical and/or serological identification, and

in some cases, sub-specific characterization. o Advances in technology have made detection and identification faster, more sensitive, more specific, and more convenient than traditional assays.

- It is important to determine the safety and quality of food.

 Rapid detection methods are important, particularly in food industry, as they are able to detect the presence of pathogens in raw and processed foods immediately.

 Rapid methods are also sensitive enough to detect pathogens that present in low numbers in the food.

Types of methods

Culture, Microscopic and Sampling methods

The four basic methods employed for "total" numbers are as follow:

a. Standard plate counts (SPC) or Aerobic plate counts (APC) for viable cells or colony forming units.

b. The most probable numbers (MPN) method as a statistical determination of the viable cells.

c. Direct microscopic counts (DMC) for both viable and non-viable cells.

d. Dye reduction techniques to estimate numbers of viable cells that possess reducing capacities.

Conventional Standard Plate Count By the conventional SPC method:

- Portions of food samples are blended or homogenized Serially diluted in an appropriate diluents Plated in or onto a suitable agar medium Incubated at an appropriate temperatures for a given time After that all visible colonies are counted by use of a Quebec or electronic counter This SPC is far the most widely used method for determining the numbers of viable cells or colony-forming-units (cfu) in a food products.

- When total viable counts are reported for a product, the counts/numbers should be viewed as a function of at least some of the following factors:
  - Sampling methods employed
  - Nature of the food biota
  - Nature of the food material
  - The pre-examination history of the food product
  - Nutritional adequacy of the plating medium employed
  - Incubation temperature and time used
  - pH, water activity and oxidation-reduction potential of the plating medium
  - Type of diluents used
  - Relative number of organisms in the food
  - Existence of other competing or antagonistic organisms

The spiral plater

- The spiral plater is a mechanical device that distributes the liquid inoculums on the surface of a rotating plate containing a suitable poured and hardened agar medium.
  - The dispensing arm moves from the near centre of the plate toward the outside, depositing the sample in an Archimedes spiral.
  - The attached special syringes dispense a continuously decreasing volume of a sample so that a concentration range of up to 10,000:1 is effected on a single plate.
  - Following incubation at an appropriate temperature, colony development reveals a higher density of deposited cells near the centre of the plate, with progressively fewer toward the edge.

Advantages:

- Less agar is used
- Fewer plates, dilution banks and pipettes are required
- Three to four times more samples per hour can be examined

Disadvantage: • Food particles may cause the blocking in the dispensing stylus.

Membrane filters

• Membranes with a pore size that will retain bacteria but allow water or diluents to pass are used.

• Following the collection of bacteria upon filtering a given volume, the membrane is placed on an agar plate or an absorbent pad saturated with the culture medium of choice and incubated appropriately.

• Following growth, the colonies are enumerated Cellulose filters were among the earliest used, however, polycarbonate Nucleopore filters offer the advantage of retaining all bacteria on the filter.

Direct Microscopic Count (DMC) In this method:

- The organisms are collected on the membranes are viewed and counted microscopically following appropriate staining, washing and treatment of the membrane to render it transparent. These methods are especially suited for samples that contain low number of bacteria.

Direct Epifluorescent Filter Technique (DEFT)

• This technique is viewed as an improved modification of the basic method. • Employs fluorescent dye and fluorescent microscopy.

• DEFT has been employed successfully to estimate numbers of microorganisms on meat, poultry and on food contact surfaces.

A diluted food homogenate is filtered through a 5-µm nylon filter Filtrate is collected and treated with 2 ml of Triton X-100 and 0.5 ml of trypsin Reagents are used to lyse the somatic cells and to prevent clogging of filters After incubation, the treated filtrate is passed through a 0.6 µm Nucleopore polycarbonate membrane Filter is stained with acridine orange Stained cells are enumerated by epifluorescent microscopy Number of cells per gram is calculated by multiplying the average number per field by the microscope factor Results can be obtained in 25-30 minutes and the numbers as low as around 6,000 cfu/g can be obtained from meats and milk products.

Microcolony- DEFT

- DEFT allows for the direct microscopic determination of cells whereas microcolony-DEFT is variation that allows one to determine viable cells only.

- Food homogenates are filtered through DEFT membranes Placed on the surface of an appropriate culture media Incubated for microcolony development 3-hour incubation can be used for Gram-negative bacteria 6-hour incubation is used for Gram-positive bacteria Developed microcolonies viewed with microscope For coliforms, pseudomonas and staphylococci, as few as 103/g could be detected within 8 hours.

Hydrophobic Grid Membrane Filters (HGMF)

- The method employs a specially constructed filter that consists of 1600 wax grids on a single membrane filter that restricts growth and colony size of individual grids.
- On one filter, from 10-9 x 104 cells can be enumerated by an MPN procedure and enumeration can be automated. o Method can detect as few as 10 cells per gram and results can achieved in 24 hours or so. o It can be used to enumerate all cfus or specific groups such as indicator organisms, fungi, salmonella and yeast and molds. For use: 1 ml of 1:10 homogenate is filtered through a filter membrane Place membranes on suitable agar medium Incubate overnight to allow colonies to develop Grids that contain colonies are enumerated and the MPN is calculated

Microscopic colony count

1. Spreading 0.1 ml of milk-agar mixture over 4-cm2 area on a glass slide Incubation, drying and staining Microcolonies developed with the aid of a microscope

2. 2 ml of melted agar are mixed with 2 ml of warmed milk After mixing, 0.1 ml of inoculated agar is spread over a 4-cm2 area Staining with thionin blue Slide is viewed with the 16-mm objective of a wide-field microscopic

This method involve the counting of micro-colonies that develop in agar layered over microscopic slides

Agar droplets method

The food homogenate is diluted in tubes of melted agar (45°C) For each food sample, three tubes of agar are used First tube being inoculated with 1 ml of food homogenate After mixing, a sterile capillary pipette (delivering 0.033 ml/drop) is used to transfer a line of 5 x 0.1 ml droplets to the bottom of an empty petri dish With the same capillary pipette, three drops (0.1 ml) from the first 9 ml tube are transferred to the second tube After mixing, another line of 5 X 0.1 ml droplets is placed next to the first This step is repeated for third tube Petri plates containing agar droplets are incubated for 24 hrs Colonies are enumerated with the aid of a 10x viewer Method is three time faster and 24 hours incubation gave counts equal to those obtained after 48 hours by the conventional plate count. Dilution blanks are not required and only one Petri dish per sample is needed.

Dry film and related methods

• A rehydrated dry film method consisting of two plastic films attached together on one side and coated with culture medium ingredients and a cold-water-soluble jelling agent.

• The method can be used with non-selective ingredients to make aerobic plate counts (APC) and with selective ingredients, certain specific groups can be detected. For use: 1 ml of diluent is placed between the two films Spread over the nutrient area by pressing with a special flat-surface device Incubation Micro-colonies appear red on the non-selective film because of the presence of a tetrazolium dye in the nutrient phase

Redigel plating

It is a plating medium that does not use agar as a solidifying agent .

- It is employed by inoculating pre- sterilized ingredients with food homogenates by mixing and holding to allow for solidification which occurs in about 30 minutes.

- It is attractive for enumerating psychrotrophic organisms. Sim plating o It is a culture method based on the activity of several enzymes common to many food born organisms.
- The special plates have holes or wells (in two sizes-84 and 198 incubation wells). o It does not allow for the characterization of colony features.

Most Probable Number

- In this method, dilutions of food samples are prepared as for the SPC o Three serial aliquots or dilutions are then planted into 9 or 15 tubes of appropriate medium for the three or five-tube method, respectively o Numbers of organisms in original sample are determined by use of standard MPN tables o The method is statistical in nature and MPN results are generally higher than SPC results.

Advantages include:

 Relatively simple

 Specific groups of organisms can be determined by use of appropriate selective and differential media

 It is method of choice for determining fecal coliform densities

Disadvantages:

 Large volume of glassware required

 Lack of opportunity to see the morphology of the colonized organism

 Lack of precision

Presence of acids and carbon dioxide indicates the positive MPN test

FOOD PRESERVATION

- Food Preservation is the process of treating and handling food to stop or slow down food spoilage, loss of quality, edibility, or nutritional value and thus allow for longer food storage.

- Preservation usually involves preventing the growth of bacteria, fungi (such as that the food treated that way will go bad (spoil from bacteria) later that if it had not been treated that way.
- For thousands of years, humans have used methods of preserving food, so that they can store food to eat later.

ADVANTAGES

 Food preservation prevents the food from being spoiled by the action of enzymes and microorganisms.

 Food preservation increases the safe storage period of foodstuffs.

 It increases the availability of out of season foodstuffs.

 It increases the availability of various foodstuffs even at distant and not easily approachable places.

METHODS OF FOOD PRESERVATION TRADITIONAL METHODS

 Drying

Cooling

 Freezing

 Boiling

Heating

 Salting

Sugaring

 Smoking

Pickling

 Lye

 Canning

Jellying

 1Jugging

INDUSTRIAL MODERN METHODS

 Pasteurization

Vacuum packing

 Artificial food additives

- Irradiation
- Pulsed electric field electroporation
- Modified atmosphere
- Nonthermal plasma
- High-pressure food preservation
- Biopreservation
- Hurdle technology

Drying

- (dehydrating) food is one of the oldest and easiest methods of food preservation.
- Dehydration is the process of removing water or moisture from a food product. Removing moisture
- Dehydrated foods are ideal for backpacking, hiking, and camping because they weigh much less than their non-dried counterparts and do not require refrigeration.
- Drying food is also a way of preserving seasonal foods for later use.

How dehydration preserves foods?

- Foods can be spoiledby food microorganisms or through enzymatic reactions within the food. Bacteria, yeast, and molds must have a sufficient amount of moisture around themto grow and cause spoilage.
- Reducing the moisture content of foodprevents the growthof these spoilage-causing microorganisms and slows down enzymatic reactions that take place within food.
- The combination of these events helps to prevent spoilage in dried food.

Pasteurisation is a process that kills bacteria in liquid food. In this method moderately high (62°C to 100°C) temperatures are used (for about 15 to 30 minutes) to inactivate certain enzymes and kill certain other microorganisms specially in milk. Since all pathogens are not killed at these temperatures,

pasteurized products need refrigeration after exposure to air. It was invented by French scientist Louis Pasteur during the nineteenth century.

Purpose of Pasteurization

• To increase milk safety for the consumer by destroying disease causing microorganisms (pathogens) that may be present in milk.

• To increase keeping the quality of milk products by destroying spoilage microorganisms and enzymes that contribute to the reduced quality and shelf life of milk.

Canning

The process of applying heat to food that's sealed in a jar in order to destroy any microorganisms that can cause food spoilage.

Proper canning techniques stop this spoilage by heating the food for a specific period of time and killing these unwanted microorganisms.

During the canning process, air is driven from the jar and a vacuum is formed as the jar cools and seals.

Vacuum Packing

Vacuum Packingis a method of packaging that removes air from the package prior to sealing. This method involves (manually or automatically) placing items in a plastic film package, removing air from inside, and sealing the package. Shrink film is sometimes used to have a tight fit to the contents

PURPOSE OF VACUUM PACKING

• To remove oxygen from the container to extend the shelf life of foods and, with flexible package forms, to reduce the volume of the contents and package.

• It is also commonly used to store dry foods over a long period of time, such as cereals, nuts, cured meats, cheese, smoked fish,coffee, and potato chips

• On a more short term basis, vacuum packing can also be used to store fresh foods, such as vegetables, meats, and liquids, because it inhibits bacterial growth.

SUGARING & SALTING

- Sugaringis the process of desiccating a food by first dehydrating it, then packing it with pure sugar.
- This sugar can be crystalline in the form of table or raw sugar, or it can be a high sugar density liquid such as honey, syrup or molasses.
- The purpose of sugaring is to create an environment hostile to microbial life and prevent food spoilage.
- Sugaring is commonly used to preserve fruits as well as vegetables such as ginger. From time to time sugaring has also been used for non- food preservations.

Salting –

- The preservation of food with dry edible salt .It is related to pickling (preparing food with brine, that is, salty water) and is one form of curing. It is one of the oldest methods of preserving food.
- Salting is used because most bacteria, fungi and other potentially pathogenic organisms cannot survive in a highly salty environment, due to the hypertonic nature of salt.
- Any living cell in such an environment will become dehydrated through osmosis and die or become temporarily inactivated.

FOOD ADDITIVES

What are food additives?

Food additives are substances added to products to perform specific technological functions. These functions include preserving, increasing shelf-life or inhibiting the growth of pathogens, or adding colouring and flavouring to food for interest and variety. It is also a substance or a mixture of substance other than basic foodstuffs, which is present in food as a result of production, processing, or packing

• Maintaining the nutritional quality of the food;

• Enhancing the keeping quality or stability of food thereby reducing food wastage;

• Making food attractive to consumers in a manner which precludes deception; and

• Providing essentials aids in food processing

Food Additives are substances added to food to improve its: • Storage properties, • Appearance, • Flavor, and • Nutritional value.© Food – a fact of life 2009

- Additives are very important for the processed food industry. Natural additives are came from plants and animals while other additives are artificial sing various chemicals.

Common types of additives are:

• Preservatives,

• Coloring,

• flavoring,

• Nutrient

The FDA makes sure that food additives used by food manufacturers safe and approved for regulated use.

Types of additives Additives may be:

• natural – found naturally, such as extracts from beetroot juice , used as a colouring agent;

• manmade versions – synthetic identical copies of substances found naturally, such as benzoic acid (E210),

used as a preservative; • artificial – produced synthetically and not found naturally, used as a preservative in some dairy products and in semolina and tapioca puddings.

. Manmade additives may prove more efficient at preserving, and some natural colours fade in some product.

Classification of Food Additives

1. Preservatives

• prevent the growth of micro-organisms which could cause food spoilage and lead to food poisoning; • extend the shelf-life of products, so that they can be distributed and sold to the consumer with a longer shelf-life

Examples of selected preservatives

a. Salt, sugar, vinegar, and pepper(common household preservatives)

b. Saltpeter or sodium nitrate (used for meat curing)

c. Sulfur dioxide (sulfurous acid) and sulfide (inhibit discoloration of cut fruits and serve as anti- browning agent)

d. Benzoic acid or Sodium benzoate (for fruit juices, jellies, margarine, and catsup)

e. Citric and Tartaric Acids (provide the acid for flavor improvement in syrups, drinks, and jellies)

f. Alum and soaked lime or apog (used as a firming agent for pickles and fruit preserves)

Antioxidants

• prevent food containing fat or oil from going rancid due to oxidation, developing an unpleasant odour or flavour;

• prevent the browning of cut fruit, vegetables and fruit juices (and so increase shelf life and appearance).

For example, vitamin C, also known as ascorbic acid, or E300, is one of the most widely used antioxidants.

• Very-beneficial in preventing spoilage in animals fat caused by oxidation.© Food – a fact of life 2009

. **Sequestrants** • Is a chemical which combines with a substance and sets aside so it can be removed from the food.

• Used to inactivate a substance which interferes with the processing of a food. They are frequently used to keep the minerals from settling out of beverages and making them cloudy. Sorbital and Phosporic Acid are used as sequestrants.© Food – a fact of life 2009

Humectants

• Prevent food from drying out

• Glycerine, Sorbital, and Monitol are called humectants and are used in foods such as coconut and certain confections to help retain moisture

Bleaching and Maturing Agents, starch Modifier

• Chemicals such as Chlorine Dioxide, Bromate and Iodate, and Chlorine are used in bleaching and maturing agents for flour. The use of these materials reduces the time required for natural aging of flour therefore is economically important.

• Bleaching agents are also used in manufacturing of certain cheese to impart a white color.

• The bleaching agent used is Benzoyl Peroxide, Hydrogen Peroxide is used to bleach tripe, a variety meat.

Emulsifiers, stabilisers, gelling agents and thickeners

• Emulsifiers help mix ingredients together that would normally separate, e.g. Lecithins .

• Stabilisers prevent ingredients from separating again, e.g. locust bean gum . • Emulsifers and stabilisers give food a consistent texture, e.g. they can be found in low-fat spreads.

• Gelling agents are used to change the consistency of a food, e.g. pectin , which is used to make jam.

Surface Active Agents

• Lecithin is an emulsifier, an example of surface- active agents. When added to baked goods, it facilitates in machining of dough and improves resulting bread appearance.

Anti – foaming agents

• Anti-caking agents ensure free movement or flow of particles, e.g. in dried milk or table salt.

• Anti-foaming agents prevent or disperse frothing, e.g. in the production of fruit juices.

• Calcium Phospate, Silica Gel in curing mixes and Stearate are examples of anti-caking agent.

Colours

• restore colour lost during processing or storage, e.g. marrowfat peas; • ensure that each batch produced is identical in appearance or does not appear 'off';

• reinforces colour already in foods, e.g. enhance the yellowness of a custard;

• give colour to foods which otherwise would be colourless (e.g. soft drinks) and so make them more attractive.© Food – a fact of life 2009

Flavour enhancers Flavour enhancers bring out the flavour in foods without imparting a flavour of their own, e.g. monosodium glutamate (E612) is added to processed foods.

For example some soups, sauces and sausages. Flavourings, on the other hand, are added to a wide range of foods, usually in small amounts to give a particular taste. These do not have E numbers because they are controlled by different food laws. Ingredients lists will say if flavourings have been used, but individual flavourings might not be named

Acids, bases and buffers Acids, bases and buffers control the acidity or alkalinity of food, for safety and stability of flavour

Glazing agents Glazing agents provide a protective coating or sheen on the surface of foods, e.g. confectionary (for appearance and shelf-life).

UNIT IV – MEDICAL MICROBIOLOGY

BACTERIAL DISEASES

Staphylococcus aureus

Family: Micrococceae (consists of Gram positive cocci, arranged in tetrads, clusters)

• Genus : Staphylococcus

• Term "staphylococcus" derived from Greek :Staphyle = bunch of grapes and Kokkos = berry, meaning bacteria occurring in grapelike clusters or berry.

Classification

• Based on pathogenecity:

Pathogenic:- includes only one i.e., *S.aureus*

Non-pathogenic:- includes *S.epidermidis, S.saprophyticus, S.albus, S. citrus, S.hominis*,etc.

• Based on coagulase production: –

Coagulase positive: *S. aureus*

Coagulase negative: *S. epidermidis, S. saprophyticus S. albus , S. aureus , S. citrus* on Nutrient Agar

•Based on pigment production: •*S.aureus* :-golden-yellow pigmented colonies

•*S.albus* :- white colonies •*S.citrus* :-lemon yellow colonies

S.aureus • Natural habitat:-Nostril and skin

Morphology:- – Gram-positive, cocci, 0.5-1.5µm in diameter; occur characteristically in group, also singly and in pairs – Form irregular grapelike clusters (since divide in 3 planes) – Non-motile, non- sporing and few strains are capsulated

Culture • Aerobes and facultative anaerobes

• Opt. Temp. For growth= 37°C

• Opt. pH for growth= 7.5

• On Nutrient agar, – golden yellow and opaque colonies with smooth glistening surface, 1-2 mm in diameter

Virulence Factors

Cellwall associated structures • Peptidoglycan • Capsule • proteinA • Clumping factor (bound coagulase) Extracellular toxins • Haemolysin • Leukocidin • Enterotoxin • TSST • Exfoliatin toxin Coagulase • staphylokinase • DNAase • Phosphatase • lipase • Phospholipase • hyaluronidase • serokinase • protease

Pathogenesis

• Adhere to damaged skin, mucosa or tissue surfaces – At these sites, they evade defence mechanisms of the host, colonize and cause tissue damage •

S.aureus produces disease by – Multiplying in tissues – Liberating toxins, – Stimulating inflammation

Clinical Syndromes

18. Clinical Syndromes

1. Cutaneous infections – Folliculitis – Boils/furuncles – Carbuncle – Impetigo – Wound infections

2. Deep infections – Osteomyelitis – Periostitis – endocarditis

3. Exfoliative diseases

4. Toxin shock syndrome

5. Staphylococcal food intoxication

Cutaneous Infections

• Folliculitis: It is inflammation of the hair follicles. • A small red bump or pimple develops at infection sites of hair follicle.

• A sty is folliculitis affecting one or more hair follicles on the edge of the upper or lower eyelid.

• Furuncle/boils: Furuncle is deep seated infection, originating from folliculitis,(if infection extends from follicle to neighbour tissue)

• **Causes** redness, swelling, severe pain • Commonly found on the neck, armpit and groin regions • Carbuncle: Carbuncle is an aggregation of infected furuncles. Carbuncles may form large abscesses.

• It is a large area of redness, swelling and pain, punctuated by several sites of drainage pus.

- **Cutaneous Infections**
- • **Impetigo**: a very superficial skin infection common in children, usually produces blisters or sores on the face, neck, hands, and diaper area.
- • It is characterized by watery bristles, which become pustules and then honey coloured crust impetigo with vesicles, pustules, and sharply demarcated regions of honey-colored crusts.

Deep Infections • Osteomyelitis: inflammation of bone • Bacteria can get to the bone – Via bloodstream – Following an injury Clinical features: pain,

swelling, deformity, defective healing, in some case pus flow, Diagnosis: X-ray, MRI, bone aspirates

Deep Infections

• Periostitis: inflammation of periosteum

• Clinical features: fever, localised pain, leucocytosis

• **Diagnosis**: needle aspiration of subperiosteal fluid

Deep Infections:

• Endocarditis: It is an inflammation of the inner layer of the heart, the endocardium • Endocarditis occurs when bacteria enter bloodstream, travel to heart, and lodge on abnormal heart valves or damaged heart tissue.

)Exfoliative Disease • (Exfoliate= scaling off tissues in layers)

• Also known as 'Staphylococcal skin scalded syndrome' • previously called dermatitis exfoliativa, pemphigus neonatorum, Lyell's disease and Ritter's disease

• Epidermal toxin produced by *S.aureus* at skin and is carried by bloodstream to epidermis , where it causes a split in a cellular layer i.e., this toxin separates outer layer of epidermis from underlying tissue

Toxic Shock Syndrome • Caused when Toxin shock syndrome toxin (TSST) liberated by S.aureus enters bloodstream • It is a multisystem illness, characterized by: Vomiting Diarrhoea Skin rashes Kidney failure High Fever Headache Conjunctival reddening Hypotension

Staphylococcal Food Poisoning • Caused when consuming food in which S.aureus has multiplied and formed endotoxin

• Symptoms: – Nausea – Vomiting – Severe abdominal cramp – Diarrhoea – Sweating – Headache,etc.

Mode Of Transmission :Person with lesions Airborne droplets Asymptomatic carrier Cross-infection Mode of transmission

Prevention Wash your hands Keep wounds covered Reduce tampon risks Avoid sharing personal care items Cooking and storing food properly

Treatment and Drugs Antibiotic therapy Wound drainage Device removal Removal of dead tissue

Laboratory Diagnosis

A. Haematological Investigation: 1. TLC (Total leukocyte count): Normal: 4000-10000 cells/mm^3 In case of infection: > 10000 cells/mm^3 2. DLC (Differential leukocyte count): Normal neutrophil : 80% In case of infection: > 80%

B. Bacteriological Investigation: • Specimens: – Pus: from wound or abscess or burns] – Nasal Swab: from suspected carrier – Food: to diagnose staphylococcal intoxication – Blood: to diagnose endocarditis and bacteremia – Sputum: to diagnose lower respiratory tract infection

• Culture and isolation: – Specimens are cultured on BA plate and are incubated @ 37 °C for 24 hours – After incubation, BA plate is observed for significant bacterial growth (> 2mm in diameter) – Then, Gram-staining is performed of the isolated organisms – Then, subcultured on NA plate for further biochemical tests

• Tube coagulase test: – i. Mix 0.5ml of human plasma with 0.1ml of an overnight broth culture of S.aures – ii. Incubate the mix in a water bath @ 37°C for 3-6 hours – Result: plasma clots and doesn't flow if the tube is inverted

34. MRSA

• Most strains of S.aureus, even those acquired in community, are penicillin resistant – Resistance is attributable to beta-lactamase production due to genes located on extrachromosomal plasmids.

• Some are resistant to the newer beta-lactamase resistant semisynthetic penicillins, such as methicillin, oxacillin, nafcillin.

Resistance is due to presence of unusual penicillin-binding protein(PBP)in the cellwall of resistant strains

• Infection with MRSA is likely to be more severe and require longer hospitalization, with incumbent increased costs than infection with a methicillin susceptible strain.

Coagulase Negative Staphylococci that are commonly implicated as pathogens include

• *Staphylococcus epidermidis*: causes infection of native heart valves and intravascular protheses.

• *Staphylococcus saprophyticus*: causes urinary tract infections, mainly in sexually active women.

• That are less commonly implicated as pathogens include: *S.hominis, S.haemolyticus, S.cohnii, s.lugdunensis, S.saccharolyticus, S.schleiferi, S.simulans and S. warneri*

MYCOPLASMA

Nocard and Roux contributed for Mycoplasma discovery In 1896 Nocard and Roux reported the

 cultivation of the causative agent of contagious bovine pleuropneumonia (CBPP), which was at that time a grave and widespread disease in cattle herds. The work of Nocard and Roux represented the first isolation of a mycoplasma species.

Basic Characters of Mycoplasma

Prokaryotic microbes

 Size of 150-250 nm

 Lack of a cell wall

Sterol-containing cell

 membrane Fastidious growth

 requirements Fried-egg or mulberry

 colonies on agar

- Mycoplasma are cell wall deficient microorganisms Cross-section of Mycoplasma bacteria, a common cause of atypical pneumonia. This bacteria is unusual in that it lacks a cell wall.
- Culturing Mycoplasma Mycoplasma can be
- cultured on liquid or solid medium Growths optimally at 35 to 37^0c Medium of growth should

- □ be enriched with 20% horse or human serum. The colonies appears as □ fried egg appearance

Characters of Mycoplasma

They are prokaryotes but lack a cell wall.

□ However, they have a unique cell membrane that contains sterols, which are not present in either bacteria or viruses.

Mycoplasma organisms are small (150-250 nm) and have deformable membranes. The name Mycoplasma refers to the plasticity of the bacterial forms resembling fungal elements.

Important Species in Mycoplasma Scientists have isolated at least 17 species □ of Mycoplasma from humans, 4 types of organisms are responsible for most clinically significant infections that may come to the attention of practicing physicians. These species are *Mycoplasma pneumoniae, Mycoplasma hominis, Mycoplasma genitalium, and Ureaplasma species*

- Mycoplasma Pneumonia Mycoplasma pneumonia is most often seen in children and young people. Up to 15 % of all cases of pneumonia in patients younger than 40 years are caused by mycoplasma pneumoniae. Most mycoplasma infections are manifested clinically as bronchitis and/or Pharyngitis. Pneumonia develops in between 3 and 10% of the patients
- **Diagnosis**
- Biochemical Characters of Mycoplasma The metabolism of □ Mycoplasma are fermentative Most species utilize □ glucose or arginine Urea is hydrolyzed by □ Ureaplasma only
- Diagnosis of Urogenital Infections Material from urethra, cervical, or vaginal □or centrifuged deposit of urine is added to separate vials with liquid mycoplasmal medium containing phenol red and 0.1% glucose, arginine or urea □The Ureaplasmal urease also breaks down urea to ammonia

- Newer methods in Diagnosis Phylogeny based rapid identification of urogenital Mycoplasmas and ureaplasmas based on amplification of part of 165rRNA gene by PCR is available

E.coli

- Enterobacteriaceae Commonly present in large intestine Non sporing , Non Acid fast, Gram – bacilli. A complex family of organisms, Some are non pathogenic A few are highly Pathogenic, Some commensals turn out to be pathogenic. as in UTI after catheterization.
- **Characters of Enterobacteriaceae**

  All Enterobacteriaceae Gram-negative rods Ferment glucose with acid production Reduce nitrates into nitrites Oxidase negative Facultative anaerobic Motile except Shigella and Klebsiella Non-capsulated except *Klebsiella* Non-fastidious Grow on bile containing media (MacConkey agar)

- Classification

Domain: Bacteria

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gamma Proteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: Escherichia

Species: Escherichia coli (E. coli)

E. coli Infections

 Neonatal meningitis – is the leading cause of neonatal meningitis and septicemia with a high mortality rate. Usually caused by strains with the K1 capsular antigen. Gastroenteritis – there are several distinct types of E. coli that are involved in different types of gastroenteritis

Antimicrobial therapy- E. coli is usually susceptible to a variety of chemotherapeutic agents, though drug resistant strains are increasingly prevalent. It is essential to do susceptibility testing.

Treatment of patients with EHEC infections is not recommended because it can increase the release of shiga-like toxins and actually trigger HUS Escherichia coli as a Genetic tool. The study of Escherichia coli and its plasmids and bacteriophages has provided a vast body of genetical information, much of it relevant to the whole of biology. This was true even before the development of the new techniques, for cloning and analysing DNA, that have revolutionized biological research during the past decade.. Much of the background of knowledge necessary for the cloning and expression of genetically engineered information, as well as the techniques themselves, came from work with this organism.

VIRAL DISEASES

Hepatitis refers to an inflammatory condition of the liver. It's commonly caused by a viral infection, but there are other possible causes of hepatitis. These include autoimmune hepatitis and hepatitis that occurs as a secondary result of medications, drugs, toxins, and alcohol. Autoimmune hepatitis is a disease that occurs when your body makes antibodies against your liver tissue.

Your liver is located in the right upper area of your abdomen. It performs many critical functions that affect metabolism throughout your body, including:

bile production, which is essential to digestionfiltering of toxins from your body excretion of bilirubin (a product of broken-down red blood cells), cholesterol, hormones, and drugsbreakdown of carbohydrates, fats, and proteins activation of enzymes, which are specialized proteins essential to body functionsstorage of glycogen (a form of sugar), minerals, and vitamins (A, D, E, and K)

synthesis of blood proteins, such as albumin ,synthesis of clotting factors

According to the Centers for Disease Control and Prevention (CDC)Trusted Source, approximately 4.4 million Americans are currently living with chronic hepatitis B and C. Many more people don't even know that they have hepatitis.

Treatment options vary depending on which type of hepatitis you have. You can prevent some forms of hepatitis through immunizations and lifestyle precautions.

The 5 types of viral hepatitis

- Viral infections of the liver that are classified as hepatitis include hepatitis A, B, C, D, and E. A different virus is responsible for each type of virally transmitted hepatitis.

- Hepatitis A is always an acute, short-term disease, while hepatitis B, C, and D are most likely to become ongoing and chronic. Hepatitis E is usually acute but can be particularly dangerous in pregnant women.

Hepatitis A

Hepatitis A is caused by an infection with the hepatitis A virus (HAV). This type of hepatitis is most commonly transmitted by consuming food or water contaminated by feces from a person infected with hepatitis A.

Hepatitis B

Hepatitis B is transmitted through contact with infectious body fluids, such as blood, vaginal secretions, or semen, containing the hepatitis B virus (HBV). Injection drug use, having sex with an infected partner, or sharing razors with an infected person increase your risk of getting hepatitis B.

It’s estimated by the CDCTrusted Source that 1.2 million people in the United States and 350 million people worldwide live with this chronic disease.

Hepatitis C

Hepatitis C comes from the hepatitis C virus (HCV). Hepatitis C is transmitted through direct contact with infected body fluids, typically through injection drug use and sexual contact. HCV is among the most common bloodborne viral infections in the United States. Approximately 2.7 to 3.9 million AmericansTrusted Source are currently living with a chronic form of this infection.

Hepatitis D

Also called delta hepatitis, hepatitis D is a serious liver disease caused by the hepatitis D virus (HDV). HDV is contracted through direct contact with infected blood. Hepatitis D is a rare form of hepatitis that only occurs in conjunction with hepatitis B infection. The hepatitis D virus can’t multiply without the presence of hepatitis B. It’s very uncommon in the United States.

Hepatitis E

Hepatitis E is a waterborne disease caused by the hepatitis E virus (HEV). Hepatitis E is mainly found in areas with poor sanitation and typically results from ingesting fecal matter that contaminates the water supply. This disease is uncommon in the United States. However, cases of hepatitis E have been reported in the Middle East, Asia, Central America, and Africa.

Signs and symptoms of acute hepatitis appear quickly.

They include:

- fatigue
- flu-like symptoms
- dark urine
- pale stool
- abdominal pain
- loss of appetite
- unexplained weight loss
- yellow skin and eyes, which may be signs of jaundice
- Chronic hepatitis develops slowly, so these signs and symptoms may be too subtle to notice

How hepatitis is diagnosed

History and physical exam

To diagnose hepatitis, first your doctor will take your history to determine any risk factors you may have for infectious or noninfectious hepatitis.During a physical examination, your doctor may press down gently on your abdomen to see if there's pain or tenderness. Your doctor may also feel to see if your liver is

enlarged. If your skin or eyes are yellow, your doctor will note this during the exam.

Liver function tests

Liver function tests use blood samples to determine how efficiently your liver works. Abnormal results of these tests may be the first indication that there is a problem, especially if you don't show any signs on a physical exam of liver disease. High liver enzyme levels may indicate that your liver is stressed, damaged, or not functioning properly.

Other blood tests

If your liver function tests are abnormal, your doctor will likely order other bloodtests to detect the source of the problem. These tests can check for the viruses that cause hepatitis. They can also be used to check for antibodies that are common in conditions like autoimmune hepatitis.

Ultrasound

An abdominal ultrasound uses ultrasound waves to create an image of the organs within your abdomen. This test allows your doctor to take a close at your liver and nearby organs. It can reveal:fluid in your abdomen,liver damage or enlargementliver tumors abnormalities of your gallbladder

Liver biopsy

A liver biopsy is an invasive procedure that involves your doctor taking a sample of tissue from your liver. It can be done through your skin with a needle and doesn't require surgery. Typically, an ultrasound is used to guide your doctor when taking the biopsy sample.

This test allows your doctor to determine how infection or inflammation has affected your liver. It can also be used to sample any areas in your liver that appear abnormal

POLIO

Polio (also known as poliomyelitis) is a highly contagious disease caused by a virus that attacks the nervous system. Children younger than 5 years old are more likely to contract the virus than any other group.

According to the World Health Organization (WHO), 1 in 200 polio infections will result in permanent paralysis. However, thanks to the global polio eradication initiative in 1988, the following regions are now certified polio-free:

- Americas
- Europe
- Western Pacific
- Southeast Asia

spread the virus and cause infection in others.

SYMPTOMS

Non-paralytic polio -Signs and symptoms of non-paralytic polio can last from one to 10 days. These signs and symptoms can be flu-like and can include:

Fever, sore throat, headache. Vomiting. Fatigue,meningitis

Non-paralytic polio is also known as abortive polio.

Paralytic polio

About 1 percent of polio cases can develop into paralytic polio. Paralytic polio leads to paralysis in the spinal cord (spinal polio), brainstem (bulbar polio), or both (bulbospinal polio).

Initial symptoms are similar to non-paralytic polio. But after a week, more severe symptoms will appear. These symptoms include:

- loss of reflexes
- severe spasms and muscle pain
- loose and floppy limbs, sometimes on just one side of the body
- sudden paralysis, temporary or permanent
- deformed limbs, especially the hips, ankles, and feet

FUNGAL DISEASES

- Fungi are eukaryotes with cell walls that give them their shape. • Fungal cells can grow as a multicellular filaments called moulds Or as single cells or chains of cells called yeast.
- Most yeasts reproduce by budding. Some yeasts such as Candida albicans produce buds that fail to detach and become elongated, producing a chain of elongated yeast cells called pseudo hyphae.

Structure • The main body of most fungi is made up of fine, branching, usually colourless threads called hyphae. • An individual fungus filament is called hypha • Several of these these hyphae, all intertwining to make up a tangled web called the mycelium • One major difference is that most fungi have cell walls that contain chitin, unlike the cell walls of plants, which contain cellulose. **Fungal spores enters through respiratory tract**

MORPHOLOGICAL CLASSIFICATION

• a) Moulds-they are hyphae in form eg..ringworm or dermatophytes .

• b) yeasts-single cell that bud to reproduce eg .cryptococcus neoformans.
• c) yeast like –form pseudo hyphae eg.. candida albicans.

• d) Dimorphic fungi-fungi having yeast form in tissue and mould in culture eg..blastomyces dermatitides.

• Fungal diseases are classified into 4 groups:

• ACCORDING TO PATHOGENICITY:

• Superficial mycoses • Mucocutaneous mycoses • Subcutaneous mycoses • Deep mycoses

Superficial And Cutaneous Mycoses!! common and limited to the very superficial or keratinized layers of skin, hair, and nails. • Piedra – colonization of the hair shaft causing black or white nodules • Tinea nigra – brown or black superficial skin lesions • Tinea capitis – folliculitis on the scalp and eyebrows

SUPERFICIAL MYCOSES • Favus – destruction of the hair follicle. • Pityriasis : • – dermatitis characterized by redness of the skin and itching:

CUTANEOUS AND MUCOCUTANEOUS MYCOSES • Associated with: • Skin • Eyes • Sinuses • Oropharynx and external ears • Vagina• Ringworm – skin lesions characterized by red margins, scales and itching. • onychomycosis – chronic infection of the nail bed Commonly seen in toes • Hyperkeratosis – extended scaly areas on the hands and feet

• Mucocutaneous candidiasis – colonization of the mucous membranes

• Caused by the yeast Candida albicans • Often associated with a loss of immunocompetence • Thrush – fungal growth in the oral cavity. • Vulvovaginitis – fungal growth in the vaginal canal • Can be associated with a hormonal imbalance

SUBCUTANEOUS MYCOSES • Localized primary infections of subcutaneous tissue:involve lymphatics and rarely desseminate Can cause the development of cysts and granulomas.: • Sporotrichosis – traumatic implantation of fungal organisms. • Paranasal conidiobolae mycoses – infection of the paranasal sinuses • Causes the formation of granulomas. • Zygomatic rhinitis – fungus invades tissue through arteries • Causes thrombosis • Can involve the CNS.

• Deep mycoses Usually seen in immunosuppressed patients with: • AIDS • Cancer • Diabetes • Can be acquired by: • Inhalation of fungi or fungal spores • Use of contaminated medical equipment • Deep mycoses can cause a systemic infection – disseminated mycoses • CAN DISSEMENIATE TO SKIN!!!! – caused by genus Coccidioides. • Primary respiratory infection. • Leads to fever, erythremia, and bronchial pneumonia. • Usually resolves spontaneously due to immune defense. • Some cases are fatal.

- Histoplasmosis – caused by Histoplasma capsulatum • Often associated with immunodeficiency. • Causes the formation of granulomas. • Can necrotize and become calcified. • If disseminated can be fatal.

• Aspergillosis – caused by several species of Aspergillus • Associated with immunodeficiency. • Can be invasive and disseminate to the blood and lungs • Causes acute pneumonia • Mortality is very high. • Death can occur in weeks.

Candidiasis Etioliogical agent:

- Candida albicans –Dimorphic fungus of the class Deuteromycetes .

  Grows as yeast or pseudohyphae

  Spread by contact; often part of normal flora

 Opportunistic infections common. Vulvovaginitis

  Oral candidiasis (thrush)

  Intestinal candidiasis

.**Candidiasis** •Residing normally in the skin, mouth, gastrointestinal tract, and vagina.

•DIABETICS AND BURN PATIENTS susceptible to superficial candidiasis.

•Severe disseminated candidiasi.

•commonly occurs in patients who are neutropenic due to Leukemia, Chemotherapy, Or Bone Marrow Transplantation, and may cause

Types of candidiasis

Oral candidiasis (Thrush). Perlèche (Angular cheilitis). Candidal vulvovaginitis . Diaper candidiasis. Congenital cutaneous candidiasis . Perianal candidiasis . Candidal paronychia . Erosio interdigitalis . Chronic mucocuntaneous candidiasis . Systemic candidiasis. Antibiotic candidiasis (Iatrogenic candidiasis)

Treatment : Non pharmacologic therapy: Sunlight accelerates repigmentation of hypopigmented areas . Pharmacological treatment: Topical treatment: selenium sulfide2.5% suspension, applied daily for 10mts for 7 consecutive days. Antifungal topical agents: miconazole.

PARASITIC INFECTIONS

ENTAMOEBA HYSTOLYTICA

 Causes amoebic dysentery

 About 50 million people worldwide are affected

 Carried asymptomatically in the digestive tracts of humans

 No animal reservoir exists

1CAUSES :Infection occurs most often by drinking water contaminated with feces containing cysts; can be transmitted through contaminated food. MODE OF TRANSMISSION: Ingestion of fecally contaminated water and food (raw vegetables), by fecally contaminated hands of foodhandlers INCUBATION PERIOD: Variable, from a few days to several months; usually 2-4 weeks

SYMPTOMS: Abdominal pain, diarrhea, fatigue, fever, vomiting, bloody stool, weight loss

TREATMENT: Susceptible to metronidazole, tinidazone, ornidazole, deloxanide furoate, chloroquine, tetracycline

 Found in intestinal tracts of animals and in the environment Causative agent of giardiasis Common gastrointestinal disease in the world. Range from asymptomatic infection to gastrointestinal disease The parasite multiplies in the small intestines Symptoms: diarrhea, abdominal cramps, vomiting, fever Prevention: use of filtered water

TRANSMISSION :

Giardia infection can occur through ingestion of dormant microbial cysts in contaminated water, food, or by the faecal-oral route .

INCUBATION PERIOD: 1 to 3 weeks after exposure to the parasite.

TREATMENT : Human infection is conventionally treated with metronidazole, tinidazole or nitazoxanide. Although metronidazole is the current first-line therapy.

Malaria Plasmodium

 Causative agent of malaria

 Around 216 million cases of malaria worldwide; 1.2 million deaths.(WHO 2012)

 Four species cause malaria P. falciparum, P. vivax, P. ovale, and P. malariae

 Malaria is endemic throughout the tropics and subtropics Mosquitoes (Anapholes) are vector for Plasmodium

 The Plasmodium life cycle has three prominent stages

Plasmodium

 Some genetic traits increase malaria resistance in endemic areas Sickle-cell trait Sickle-shaped cells resist penetration by Plasmodium Hemoglobin C Two genes for hemoglobin C protect against malaria

Malaria

 Symptoms of malaria depend on cycle of parasite:

  High fever, joint pain, vomiting, weakness, renal failure, confusion, seizures

 Cerebral malaria results in tissue death in the brain *(P. falciparum)*

  Immunity develops if victim survives acute stage Periodic episodes become less severe over time

Treatment - Various antimalarial medication – type depends on severity of case Prevention - Avoid getting bitten - Use anti-malaria medication (prophylaxis) – chloroquine, mefloquine, primaquine.

UNIT V - ENVIRONMENTAL AND AGRICULTURAL MICROBIOLOGY

Waste management' shall mean "the collection, transport, recovery and disposal of waste, including the supervision of such operations and aftercare of disposal sites" ….European Union Directive on waste • However the newer concepts of 'Waste management' talk about 'Reduce, Reuse and Recycle of waste' over and above waste disposal.

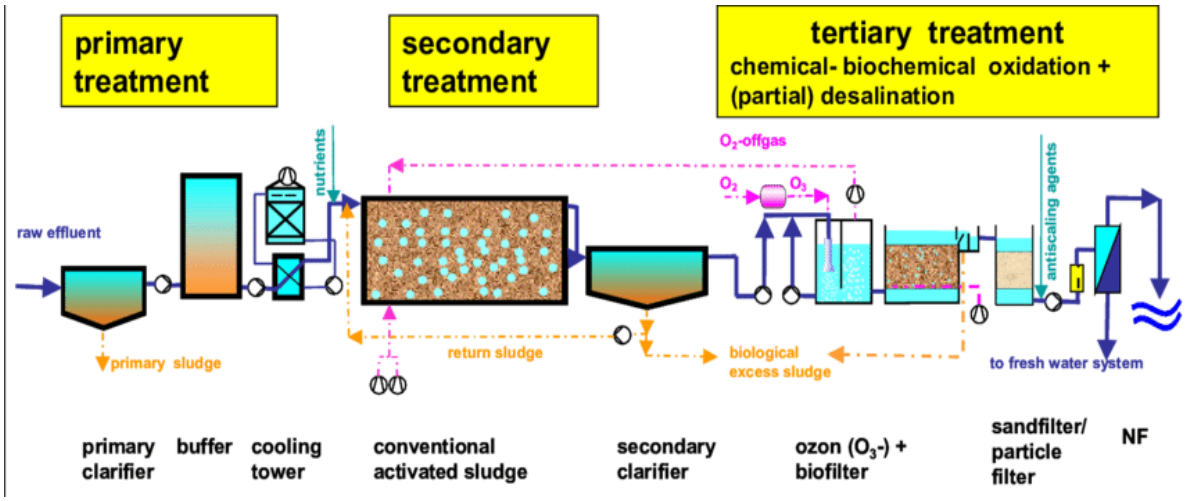
THE 3 R's – 3. Reuse • Reuse envelopes. • Reuse single-sided paper for scratch paper. • Reuse foam peanuts and other packaging material. • Use remanufactured or surplus office equipment. • Use rechargeable batteries. • Use rechargeable fax and printer cartridges. • Compost grass clippings and food waste. • Donate toys and other items to charity.

Waste water treatment

Wastewater treatment • A process to convert wastewater - which is water no longer needed or suitable for its most recent use - into an effluent that can be either returned to the water cycle with minimal environmental issues or reused.

Levels of Treatment Primary – removal by physical separation of grit and large objects (material to landfill for disposal) – Sedimentation and screening of large debris Secondary – Biological and chemical treatment – aerobic microbiological process (sludge) organic matter + O_2

Treatment stages



Primary treatment • typical materials that are removed during primary treatment include – fats, oils, and greases – sand, gravels and rocks – larger settle-able solids including human waste, and – floating materials

Methods used in primary treatment • Bar screens • Grinding • Grit Chamber • Sedimentation Tank- primary Settling tank • Chlorination of effluent

Sedimentation Tank- primary Settling tank – Remove grease, oil – Fecal solid settle, floating material rise to the surface – Produce a homologous liquid for later biological treatment – Fecal sludge are pumped to sludge treatment plant

Secondary treatment • Biological treatment – activated sludge – trickling filter – oxidation ponds

Activated sludge process • Primary wastewater mixed with bacteria-rich (activated) sludge and air or oxygen is pumped into the mixture • Both aerobic and anaerobic bacteria may exist • Promotes bacterial growth and decomposition of organic matter • BOD removal is approximately 85% • Microbial removal by activated sludge • 80-99% removal of bacteria • 90-99% removal of viruses

physical components • Aeration tank • oxygen is introduced into the system • Aeration source • ensure that adequate oxygen is fed into the tank • provided pure oxygen or compressed air • Secondary clarifiers • activated-sludge solids separate from the surrounding wastewater • Activated sludge outflow line • Pump activated sludge back to the aeration tank • Effluent outflow line • discharged effluent into bay or tertiary treatment plant

Aeration and rapid mixing Settling collects sludge on bottom Secondary process air diffuser Fromprimaryprocess Totertiaryprocess

• Trickling filters are beds made of coke (carbonized coal), limestone chips or specially fabricated plastic media • Optimize their thickness by insect or worm grazing • The primary wastewater is sprayed over the filter and microbes decompose organic material aerobically. • Low pathogen removal - Bacteria, 20-90% - Viruses, 50-90% - Giardia cysts, 70-90% Trickling filters

Stabilization or oxidation ponds • Oxidation ponds are a few meters deep, and up to a hectare in size. • They are low cost with retention times of 1 to 4

weeks. • Odor and mosquitoes can be a problem • Pathogen removal: - Bacteria, 90-99% - Virus, 90-99% - Protozoa, 67-99% • Mechanisms include the long detention time, high pH (10- 10.5) generated by photosynthesis, predation, sunlight, temperature

When the treatment is done… • Effluent back to stream after – a final carbon filtration and – chlorination/de-chlorination • Sludge – very nutrient rich – applied directly to land as fertilizer – incinerated (good fuel after drying) – composted

- Sludge Treatment Processes Thickening (water removal) Digestion (pathogen inactivation and odor control) Conditioning (improved dewatering with alum and high temp, 175-230o C) Dewatering (pathogen inactivation and odor control) Incineration (volume and weight reduction) Final disposal

• Septic Tanks • Constructed Wetlands • Composting Wastewater Treatment Alternatives

ORGANIC COMPOST

Composts commonly contain about 2 percent nitrogen, 0.5–1 percent phosphorus, and about 2 percent potassium. Nitrogen fertilizers and manure may be added to speed decomposition. The nitrogen of compost becomes available slowly and in small amounts, which reduces leaching and extends availability over the whole growing season. Because of their fairly low nutrient content, composts are usually applied in large amounts.

Compost can be prepared on a small scale for home gardens, usually in a simple pile of yard waste and kitchen scraps, though compost bins and barrels are also used. Aeration is important for proper decomposition, so

piles are usually mixed every few days. When properly prepared, compost is free of obnoxious odours. A compost pile with the right ratio of carbon to nitrogen (30:1) and with adequate moisture will produce enough heat during decomposition to kill many pathogens and seeds, though it is advisable to avoid adding diseased plant matter and weeds that have gone to seed. Some municipalities collect household yard waste for large-scale composting, which reduces the amount of organic matter in landfills.

Methods of composting

- **In Coimbatore method**, composting is done in pits of different sizes depending on the waste material available. A layer of waste materials is first laid in the pit. It is moistened with a suspension of 5-10 kg cow dung in 2.5 to 5.0 I of water and 0.5 to 1.0 kg fine bone meal sprinkled over it uniformly. Similar layers are laid one over the other till the material rises 0.75 m above the ground level. It is finally plastered with wet mud and left undisturbed for 8 to 10 weeks. Plaster is then removed, material moistened with water, given a turning and made into a rectangular heap under a shade. It is left undisturbed till its use.
- In the Indore method of composting, organic wastes are spread in the cattle shed to serve as bedding. Urine soaked material along with dung is removed every day and formed into a layer of about 15 cm thick at suitable sites. Urine soaked earth, scraped from cattle sheds is mixed with water and sprinkled over the layer of wastes twice or thrice a day. Layering process continued for about a fortnight. A thin layer of well decomposed compost is sprinkled over top and the heap given a turning and reformed. Old compost acts as inoculum for decomposing the material. The heap is left undisturbed for about a month. Then it is thoroughly moistened and given a turning. The compost is ready for application in another month.

In the Bangalore method of composting, dry waste material of 25 cm thick is spread in a pit and a thick suspension of cow dung in water is sprinkled over for moistening. A thin layer of dry waste is laid over the moistened layer. The pit is filled alternately with dry layers of material and cow dung suspension till it rises 0.5 m above ground level. It is left exposed without covering for 15 days. It is given a turning, plastered with wet mud and left undisturbed for about 5 months or till required.

- In Coimbatore method, there is anaerobic decomposition to start with, following by aerobic fermentation. It is the reverse in Bangalore method. The Bangalore compost is not so thoroughly decomposed as the Indore compost or even as much as the Coimbatore compost, but it is bulkiest.
- Compost is a rich source of organic matter. Soil organic matter plays an important role in sustaining soil fertility, and hence in sustainable agricultural production. In addition to being a source of plant nutrient, it improves the physico-chemical and biological properties of the soil. As a result of these improvements, the soil:

(i) becomes more resistant to stresses such as drought, diseases and toxicity;

(ii) helps the crop in improved uptake of plant nutrients; and

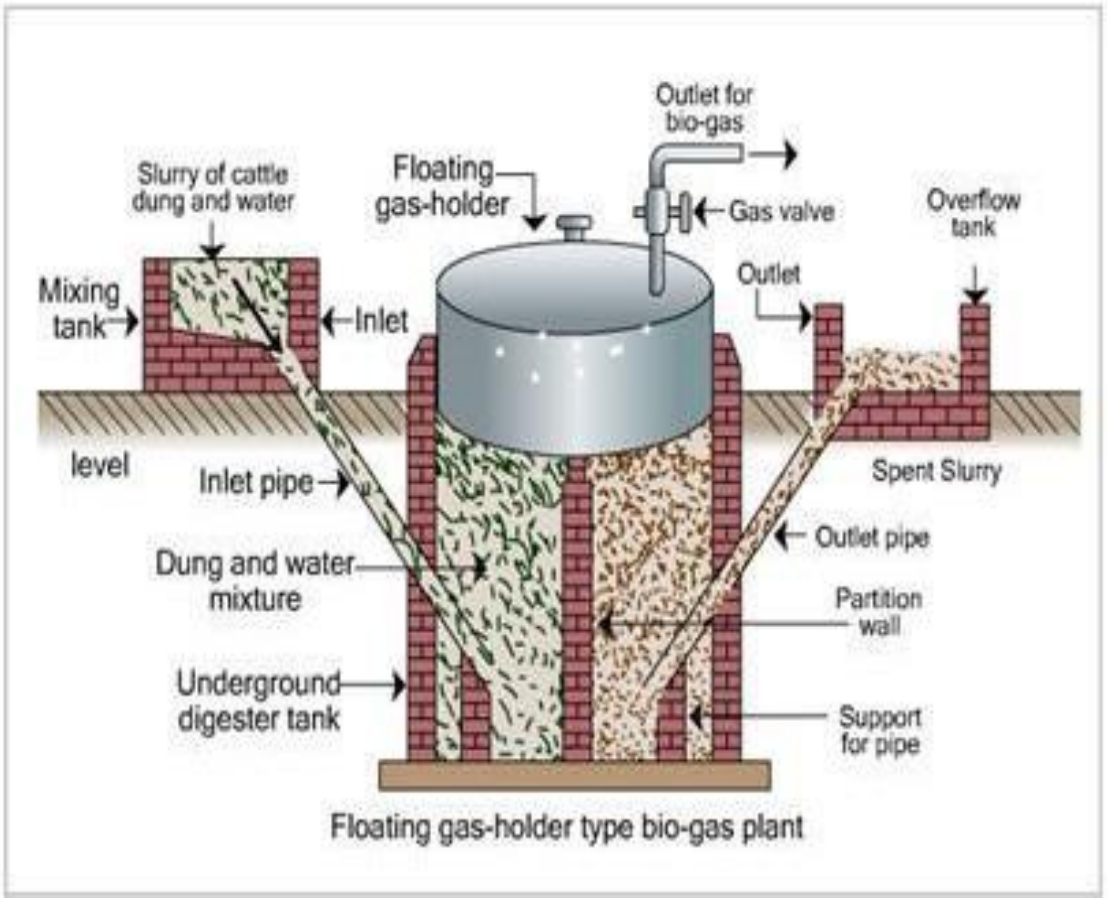
(iii) possesses an active nutrient cycling capacity because of vigorous microbial activity.

BIOGAS PRODUCTION:

Biogas production is an eco-friendly strategy for energy production from biomass and the residue can be used as a soil conditioner. Biogas is produced by the anaerobic biological breakdown of organic matter. It primarily consists of methane and carbon dioxide.

Biofuels: how Bio Gas is Generated.

Floating gas holder type of plant: The diagram below shows the details of a floating gas holder type of bio gas plant.



BIOLEACHING

Bioleaching is the extraction of metals from their ores through the use of living organisms. This is much cleaner than the traditional heap leaching using cyanide. Bioleaching is one of several applications within biohydrometallurgy and several methods are used to recover copper, zinc, lead, arsenic, antimony, nickel, molybdenum, gold, silver, and cobalt

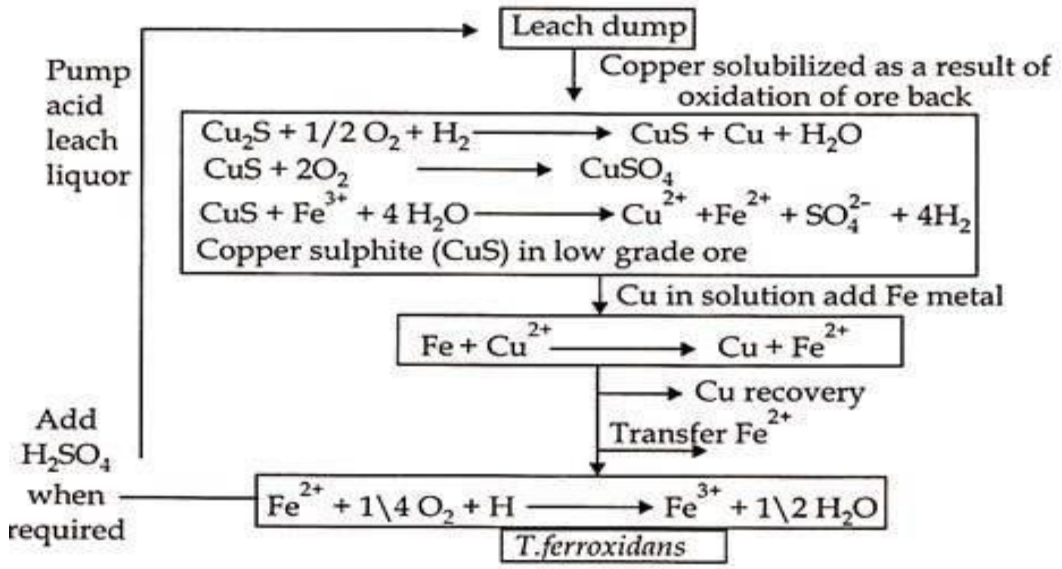


Fig. 12.2: Microbial leaching of copper

- The most commonly used microorganisms in bioleaching are *Thiobacillus thiooxidants o Thiobacillus ferrooxidants other microorganisms which may also be used are; Bacillus Licheniformis, B. luteus, B megaterium, B polymyxa, B leptospirillum ferrooxidants, Pseudomonas flurescens, Sulfolobus acidocaldarius,* etc

BIOFERTILIZER

A biofertilizer is a substance which contains living microorganisms, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances

TYPES OF BIOFERTILIZERS

Bacterial Fungal Algal Aquatic fern Earthworms VAM fungi

. Bacteria: Symbiotic nitrogen fixers. Rhizobium, Azospirillum spp Free living nitrogen fixers. Azotobacter, Klebsiella etc.,

Algal biofertilizers: BGA in association with Azolla Anabena, Nostoc, Ocillatoria

Phosphate solubilising bacteria: Pseudomonas, Bacillus megaterium

Fungal biofertilizer VAM Earthworms

Bacterial biofertilizers

The live cells of bacteria used as a biofertilizers These microbes contains unique gene called as Nif-Gene which make them capable of fixing nitrogen. The nitrogen fixing bacteria work under two conditions, Symbiotically Free living bacteria (non-symbiotic). The symbiotic bacteria make an association with crop plants through forming nodules in their roots. The free living bacteria do not form any association but live freely and fix atmospheric nitrogen

Symbiotic nitrogen fixers

Most important symbiotic Nitrogen fixing bacteria is Rhizobium and Azospirillum. Rhizobium:

- Rhizobium lives in the root hairs of the legumes by forming nodules
- Plant root supply essential minerals and newly synthesized substance to the bacteria
- The name Rhizobium was established by Frank in 1889.
- This genus has seven distinct species based on "Cross Inoculation Group Concept"

Azospirillum:

It mainly present in cereal plants.

inhabits both root cells as well as surrounding of roots

forming symbiotic relation and increasing nitrogen fixing potential of the cereal plant.

Azospirillum is recognized as a dominant soil microbe nitrogen in the range of 20- 40 kg/ha in the rhizosphere in non-leguminous plants such as cereals, millets, Oilseeds, cotton etc.

Considerable quantity of nitrogen fertilizer up to 25-30 % can be saved by the use of Azospirillum inoculant.

These species have been commercially exploited for the use as nitrogen supplying Bio-Fertilizers.

Vesicular Arbuscular Mycorrhiza (VAM)

- The term mycorrhiza was taken from Greek language meaning 'fungus root'. term was coined by Frank in 1885 The mycorrhiza is a mutualistic association between fungal mycelia and plant roots. VAM is an endotrophic (live inside) mycorrhiza formed by aseptated phycomycetous fungi. VAM help in nutrient transfer mainly of phosphorus, zinc and sulfur.
- Mycorrhizae is the symbiotic association between plant roots and soil fungus of the 7 types of mycorrhizae, VAM plays a great role in inducing plant growth. VAM are symbiotic entophytic soil fungi, which colonize the roots of approximately 80% plants. The VAM hyphae also help is retaining moisture around the root zone of plants It increases the resistance to root borne or soil borne pathogens and Nematodes.

- They also mobilize different nutrients like Cu(copper), K(potassium), Al(aluminum), Mn(manganese), Fe (iron)and Mg (magnesium) from the soil to the plant roots. They posses vesicles (sac like structure) for storage of nutrients and arbuscular for funneling them into root system. Morphology External hyphae Arbuscles Vesicles External hyphae vesicles Arbuscles

- Mechanism of Action

  The VAM forms an association with plant roots. It penetrates in the root cortex and spreads around the roots of the plant. As the name indicates, they posses sac like structure called vesicules which stores phosphorus as phospholipids. The other structure called arbuscule helps bringing the distant nutrients to the vesicules and root.

  Bio - fertilizers application methods There are three ways of using these N-fixing/P.S.M. bacteria. Seed treatment Root dipping Soil applications

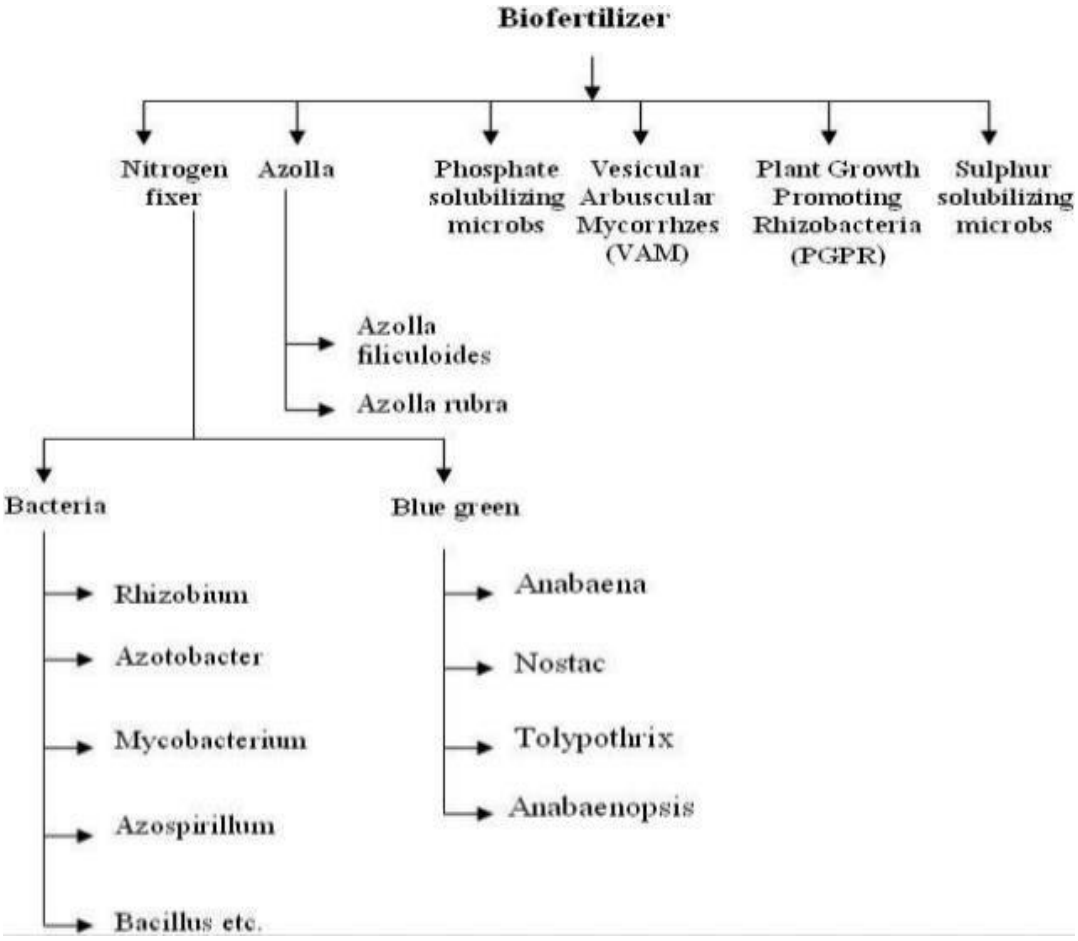
Seed Treatment

- Seed treatment is a most common method adopted for all types of inoculant. The seed treatment is effective and economic. Seed treatment with Rhizobium, Azotobacter, Azospirillum along with P.S.M. seed treatment can be done with any of two or more bacteria. no side effect. important things has the seeds must be coated first with Rhizobium or Azotobacter or Azospirillum when each seeds get a layer of above bacteria then the P.S.M. inoculant has to be treated on outer layer of the seeds.

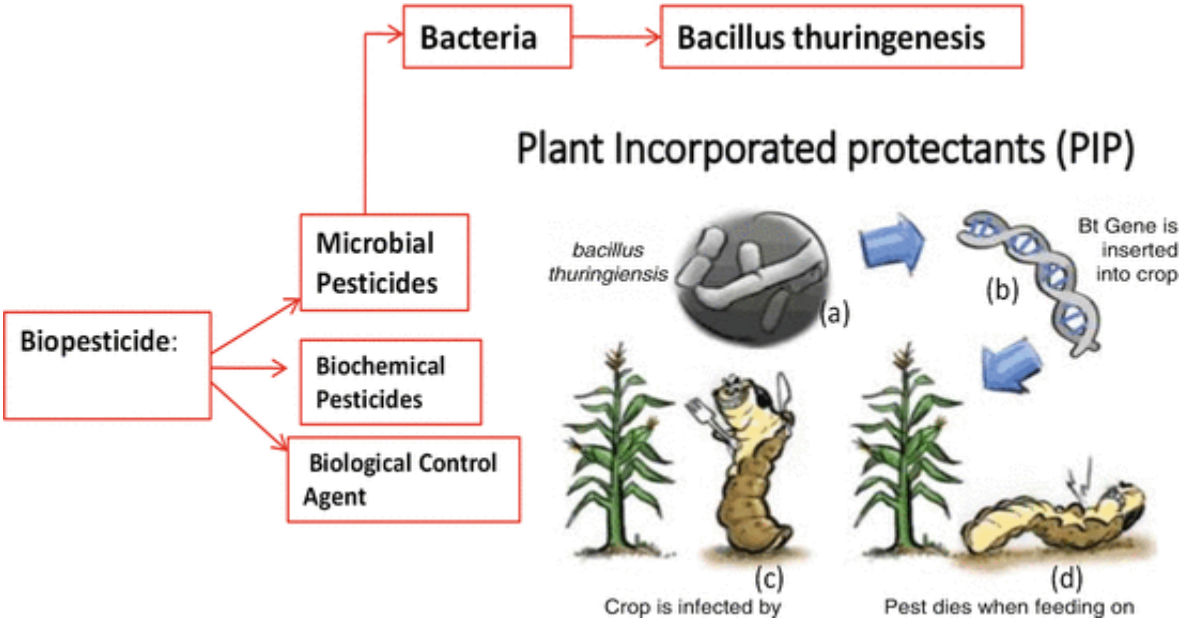
- This method will provide maximum number of population of each bacteria required for better results. Mixing the any of two bacteria and the treatment of seed will not provide maximum number of bacteria of individuals.

- Root dipping Application of Azospirillum with the paddy/vegetable plants this method is needed. The required quantity of Azospirillum has to be mixed with 5-10 ltr of water at one corner of the field and all the plants have to kept for minimum ½ an hour before sowing .

- Soil application P.S.M. has to be used as a soil application use 2 kgs of P.S.M. per acre. Mix P.S.M. with 400 to 600 kgs of Cowdung along with ½ bag of rock phosphate if available. The mixture of P.S.M., Cowdung and rock phosphate have to be kept under any tree shade or celling for over night and maintain 50% moisture. Use the mixture as a soil application in rows or during leveling of soil.



BIOPESTICIDE



Biopesticide is a formulation made from naturally occurring substances that controls pests by non toxic mechanisms and in ecofriendly manner. Biopesticides may be derived from animals (e.g. nematodes), plants (Chrysanthemum, Azadirachta) and micro-organisms (e.g. Bacillus thuringiensis, Trichoderma, nucleopolyhedrosis virus), and include living organisms (natural enemies) etc.

 However, biopesticides are generally less toxic to the user and are non-target organisms, making them desirable and sustainable tools for disease management.

Types of biopesticides

• Microbial pesticides

• Plant-incorporated-protectants (PIPs)

• Biochemical pesticides

• Botanical pesticides

• Biotic agents (parasitoids and predators)

Bacillus thuringiensis

• Discovered in Japan in early 20th century and first become a commercial product in France in 1938. • Control lepidopterous pests like American bollworm in cotton and stem borers in rice. Fig: Bacillus thuringiensis • When ingested by pest larvae, Bt releases toxins which damage the mid gut of the pest, eventually killing it. • Main sources for the production of Bt preparations are the strains of the subspecies kurstaki, galeriae and dendrolimus

Agrobacterium radiobacter (Agrocin)

•Agrobacterium radiobacter is used to treat roots during transplanting, that checks crown gall. •Crown gall is a disease in peaches, grapevine, roses and various plants caused by soil borne pathogen Agrobacterium tumefaciensm. •The effective strains of A. radiobacter posses two important features: They are able to colonize host roots to a higher population density. They produce an antibiotic, agrocin, that is toxic to *A. tumefaciens*

Pseudomonas fluorescens (Phenazine)

•This bacteria is used to control damping off caused by *Pythium sp., Rhizoctonia solani, Gaeumannomyces graminis.* •It has ability to grow quickly in the rhizosphere d. Trichoderma Fig: Pseudomonas

•Trichoderma is a fungicide effective against soil born diseases such as root rot. •This is also used against Necteia galligena, that causes silver leaf disease of fruit trees by entering through pruning wounds.

Plant-incorporated-protectants (PIPs)

• Pesticidal substances that plant produce from the genetic material that has been added to the plant. • As the pest feed on such plants they will eventually die. Botanical pesticides: • These are naturally occurring plant material that may be crude preparation of the plant parts ground to produce a dust or powder that can be used in full strength or dilute form in a carrier such as clay, talc or diatomaceous earth. • “Azadirachtin” effects the reproductive and digestive procees of pest. • Several plant based insecticides as nicotinoids, natural pyrethrins, rotenoids, neem products etc are used.