Semester – V

15MBU503 FOOD AND AGRICULTURAL MICROBIOLOGY 5H – 5C

Instruction Hours / week: L: 5 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

This paper adds information about the role of microorganisms in many food, beverage and pharma industries both in production and spoilage processes. Provides detailed idea about biofertilizer, production and plant disease.

OBJECTIVES

To encode the importance of the role of microorganisms in food industries both in beneficial and harmful ways. To obtain a good understanding of food microbiology and become qualified as microbiologist in food industries. To know the role of microbes which make crop output more and increase the fertility of crops.

UNIT – I

Food and microorganisms – Important microorganisms in food – Fungi, Bacteria; Intrinsic and extrinsic parameters of food affecting microbial growth – Sources of contamination of food.

UNIT – II

Food preservation – principles – Food preservation using low temperature – characteristics of psychrotrophs – high temperature food preservation – characteristics of thermophiles – preservation of foods by drying chemicals and radiation – limitations – commercial application.

UNIT – III

Food borne diseases - Food poisoning - Food borne infection and Intoxication- Food control agencies- Microbiological criteria for food, microbial quality control and food laws, HACCP.

UNIT – IV

Biological Nitrogen fixation -symbiotic and non-symbiotic microorganisms, Root nodule formation - Nitrogen fixers – Hydrogenase – Nitrogenase – *Nif* gene – regulation - Biochemistry of Nitrogen fixation, Interaction of microbes with plants.

UNIT – V

Biofertilizer - Rhizobium, Azospirillum, Azotobacter, phosphobacteria, Plant Growth Promoting Rhizobacteria (PGPR) - Blue Green Algae (BGA) and Azolla - Production and quality control of biofertilizers., field application and crop response.

TEXT BOOKS

1. Adams, M.R. and M.O. Moss, 2003. Food Microbiology. New Age International (P) Limited Publishers. New Delhi.
2. Banwart, G.J., 2004. Basic Food Microbiology. 2nd Edition. CBS Publishers and Distributors New Delhi.
3. Jay, J.M., 2000. Modern Food Microbiology. CBS Publishers and Distributors. New Delhi.
4. Motsara, M.R., P. Bhattacharyya and B. Srivastava, 1995. Biofertilizer - Technology, Marketing and Usage. Fertilizer Development and Consultant Organization, New Delhi.
5. Subba Rao, N.S., 1999. Biofertilizers in Agriculture and Agroforestry. Oxford and IBH, New Delhi.
6. Rao, N.S., 1995. Soil Microorganisms and plant growth. Oxford and IBH Publishing Co., New Delhi.
7. Wallace, R.B, Oria, M, 2010. Enhancing food safety-the role of the food and drug administration. Washington; National Academic Press 2010
8. James, M. JAY, 2012. Modern Food Microbiology. 13th Edition Spring,US.
9. Pinam. M.Fratamico, Arun Kabhunia and James L. Smith,2015. Food Borne Pathogen and microbiology and Molecular Biology, Caistern Academic Press.
10. Forsythe, P.R and Hayes, 2013. Food Hygiene and Microbiology and HACCP. 3rd Edition. Springer Science plus Buisness media, Newyork.
11. Jain, N 2013.Agricultural Microbiology and Biotechnology. Centrum Press.
12. Sathyanarayanan, T, Bhavdish Narain Johri and Anil Prakash, 2012. Springer Science Plus Buisnss Media, London, Newyork.

REFERENCES

1. Atlas, R.M. and R. Bartha, 1992. Microbial Ecology- Fundamentals and Applications. 3rd Edition. Red Wood City. Benjamin/ Cumming Science Publishing Co., New Delhi.
2. Doyle, M.P., L. R. Beuchat and T. J. Montuille, 2001. Food Microbiology – Fundamentals and Frontiers. ASM Press, U.S.
3. Frazier, W.C. and D. C. Westhoff, 1995. Food Microbiology. Tata McGraw- Hill Publishing Company limited. New Delhi.
4. Gould, G.W., 1996. New Methods of Food Preservation. Blackie Academic and Professional, Madras.
5. Rangaswami, G. and D.J. Bhagyaraj, 2001. Agricultural Microbiology. 2nd Edition. Prentice Hall, New Delhi.

Lecture plan

FOOD AND AGRICULTURAL MICROBIOLOGY (15MBU503)

SEMESTER – V **5H – 5C**

UNIT I

S. No	Duration	Topic	Reference
1	1	Food and microorganisms - Introduction	R1: 17
2	1	Important microorganisms in food	R1:17
3	1	General characteristics of fungi	R1: 17-34
4	1	Bacteria	R1: 42-51
5	1	Intrinsic parameters of food affecting microbial growth	R1: 3-4
6	1	Hydrogen ion concentration (pH)	R1: 4-5
7	1	Moisture requirement	R1: 5-9
8	1	Oxidation – reduction potential	R1: 10-11
9	1	Nutrient content	R1: 11-14
10	1	Sources of contamination of food	R1: 59-67
11	1	Green plants, fruits, animals and sewage	R1: 59-61
12	1	Soil, water, air, handling processing	R1: 62-67
13	1	Tutorial hour	
14	1	Unit Revision	
Total Hrs: 14			

R1: Frazier, WC and DC. Westhoff, 1995. Food: Microbiology. Tata McGraw – Hill Publishing Company limited. New Delhi.

2017

FOOD AND AGRICULTURAL MICROBIOLOGY

Prepared by – Ms. P. Akilandeswari, Asst Professor, Dept of Microbiology, KAHE.

UNIT- 1

Microorganisms important in food microbiology

Molds

Mold growth on foods, with its fuzzy or cottony appearance, sometimes colored, is familiar to everyone, and usually food with a moldy or "mildewed" food is considered unfit to eat. Special molds are useful in the manufacture of certain foods or ingredients of foods. Thus, some kinds of cheese are mold-ripened, e.g., blue, Roquefort, Camembert, Brie, Gammelost, etc., and molds are used in making Oriental foods, e.g., soy sauce, miso, sonti, and other discussed later. Molds have been grown as food or feed and are employed to produce products used in foods, such as amylase for bread making or citric acid used in soft drinks.

General characteristics of molds

The term "mold" is a common one applied to certain multicellular filamentous fungi whose growth on foods usually is readily recognized by its fuzzy or cottony appearance. Colored spores are typical of mature mold of some kinds and give color to part or all of the growth. The thallus, or vegetative body, is characteristic of thallophytes, which lack true roots, stems, and leaves.

Morphological Characteristics

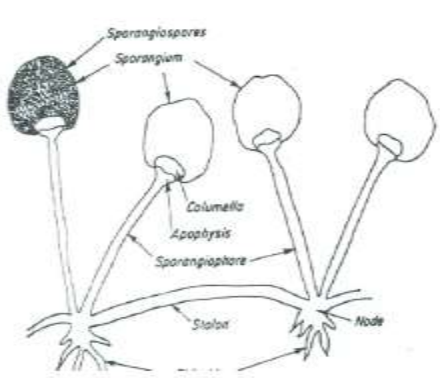
Hyphae and Mycelium: The mold thallus consists of a mass of branching, intertwined filaments called hyphae (singular hypha), and the whole mass of these hyphae is known as the mycelium. Molds are divided into two groups: septate, i.e., with cross walls dividing the hypha into cells; and noncoenocytic, septate with the hyphae apparently consisting of cylinders without cross walls. The non-septate hyphae have nuclei scattered throughout their length and are considered multicellular. Special, mycelial structures or parts aid in the identification of molds. Examples are the rhizoids, or "holdfasts," of *Rhizopus* and *Absidia*, the foot cell in *Aspergillus*, and the dichotomous, or Y-shaped, branching in *Geotrichum*.

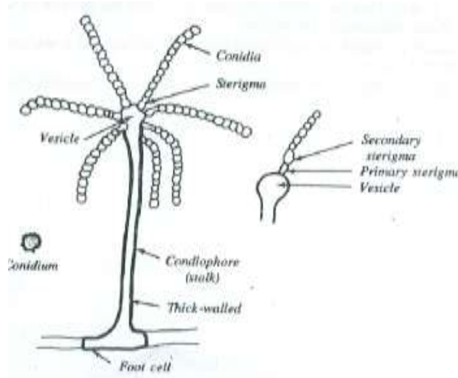
Reproductive Parts or Structures

Reproduction of molds is chiefly by means of asexual spores. Some molds also form sexual spores. Such molds are termed “perfect’’ and are classified as either Oomycetes or Zygomycetes if non-septate, or Ascomycetes or Basidiomycetes if septate, in contrast to "imperfect" molds, the Fungi Imperfecti (typically septate), which have only asexual spores.

Asexual Spores

The asexual spores of molds are produced in large numbers and are small, light, and resistant to drying. They are readily spread through the air to alight and start new mold thallus where conditions are favorable. The three principal types of asexual spores are (1) conidia (singular conidium), (2) arthrospores or oidia (singular oidium), and (3) sporangiospores. Conidia are cut off, or bud, from special fertile hyphae called conidiophores and usually are in the open, i.e., not enclosed in any container, in contrast to the sporangiospores, which are in sporangium (plural sporangia), or sac, at the tip of a fertile hypha, the sporangiophore. Arthrospores are formed by fragmentation of a hypha, so that the cells of the hypha become arthrospores. A fourth kind of asexual spore, the chlamydospore, is formed by many species of molds when a cell here and there in the mycelium stores up reserve food, swell, and forms a thicker wall than that of surrounding cells.





Sexual Spores: The molds which can produce sexual spores are classified on the basis of the manner of formation of these spores and the type produced. The non-septate molds (Phycomycetes) that produce.

1. *Oospores* are termed Oomycetes. These molds are mostly aquatic; however, included in this group are several important plant pathogens. The oospores are formed by the union of a small male gamete and a large female gamete.
2. *Zygospores*: Zygomycetes form zygospores by the union of the tips of two hyphae which often appear similar and which may come from the same mycelium or from different mycelia. Both Oospores and zygospores are covered by a tough wall and can survive drying for long periods.
3. *Ascospores*: The Ascomycetes (septate) form sexual spores known as ascopores, which are formed after the union of two cells from the same mycelium or from two separate mycelia. The ascospores, resulting from cell division after conjugation, are in an ascus, or sac, with usual eight spores per ascus.
4. *Basidiospores*: The Basidiomycetes, which include most mushrooms, plant rusts, smuts, etc.,form a fourth type of sexual spore, the basidiospore.

Physiological characteristics

Moisture Requirements In general most molds require less available moisture than do most yeasts and bacteria. Approximate limiting total moisture content of a given food for mold growth can be estimated, and therefore it has been claimed that below 14 to 15 percent total moisture in flour or some dried fruits will prevent or greatly delay mold growth.

Classification and identification of molds

Molds are plants of the kingdom Myceteae. They have no roots, stems, or leaves and are devoid of chlorophyll. They belong to the Eumycetes, or true fungi, and are subdivided further to subdivisions, classes, orders, families, and genera.

The following criteria are used chiefly for differentiation and identification of molds:

1 Hyphae septate or non-septate
2 Mycelium clear or dark (smoky)
3 Mycelium colored or colorless
4 Whether sexual spores are produced and the type: oospores, zygospores, or ascospores
5 Characteristics of the spore head.
a) Sporangia: size, color, shape, and location
b) Spore heads bearing conidia: single conidia, chains, budding conidia, or masses; shape and arrangement of sterigmata or phialides; gumming together of conidia.

6 Appearance of sporangiophores or conidiophores: simple or branched, and if branched the type of branching; size and shape of columella at tip of sporangiophore; whether conidiophores are single or in bundles.

7 Microscopic appearances of the asexual spores, especially of conidia: shape, size, color; smooth or rough; one-, two-, or many-celled.

8 Presence of special structures (or spores): stolons, rhizoids, foot cells, apo-physis, chlamydospores, sclerotia, etc.

Molds of Industrial Importance

Mucor: *Mucor* are involved in the spoilage of some foods and the manufacture of others. Widely distributed species is *M. racemosus*; *M. rouxii* is used in the "Amylo" process for the saccharification of starch, and mucors help ripen some cheese, (e.g., Gammelost) and are used in making certain Oriental foods.

Zygorrhynchus: These soil molds are similar to *Mucor* except that the zygo-spore suspensors are markedly unequal in size.

Rhizopus: *Rhizopus stolonifer*, the so-called bread mold, is very common and is involved in the spoilage of many foods: berries, fruits, vegetables, bread, etc.

Absidia: Similar to *Rhizopus*, except that sporangia are small and pear-shaped.

Thamnidium: *Thamnidium elegans* is found on meat in chilling storage, causing "whiskers" on the meat.

Aspergillus: The aspergillus is very widespread. Many are involved in the spoilage of foods, and some are useful in the preparation of certain foods.

Penicillium: P. expansum, the blue-green-spored mold, causes soft rots of fruits. Other important species are *P. digitatum*, with olive, or yellowish-green conidia, causing a soft rot of citrus fruits; *P. italicum*, called the “blue contact mold” with blue green conidia, also rotting citrus fruit; *P.camemberti*, with grayish conidia, useful in the ripening of Camembert cheese; and *P. roqueforti*, with bluish-green conidia, aiding in the ripening of blue cheeses, e.g., Roquefort.

Trichothecium: The common species, *T. roseum*, is a pink mold which grows on wood, paper, fruits such as apples and peaches, and vegetables such as cucumbers and cantaloupes.

Yeasts and yeast like fungi

Like mold, the term "yeast" is commonly used but hard to define. It refers to those fungi which are generally not filamentous but unicellular and ovoid or spheroid and which reproduce by budding or fission.

Yeasts may be useful or harmful in foods. Yeast fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar, and surface ripened cheese, and yeasts are grown for enzymes and for food. Yeasts are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups; molasses, honey, jellies, meats, wine, beer, and other foods.

Bacteria

Morphological characteristics important in food bacteriology

One of the first steps in the identification of bacteria in a food is microscopic examination to ascertain the shape, size, aggregation, structure, and staining reactions of the bacteria present. The following characteristics may be of special significance.

Encapsulation

The presence of capsules or slime may account for sliminess or ropiness of a food. In addition, capsules serve to increase the resistance of bacteria to adverse conditions, such as heat or chemicals. To the organism they may serve as a source of reserved nutrients. Most capsules are polysaccharides of dextrin, dextran, or levan.

Formation of Endospores

Bacteria of the genera Bacillus, Clostridium, Desulfotomaculum, Sporolactobacillus (rods), and Sporosarcina (cocci) share the ability to form endospores. Bacillus - aerobic and some facultative anaerobic and Clostridium - anaerobic. Endospores are formed at an intracellular site, are very refractile, and are resistant to heat, ultraviolet light, and desiccation.

Formation of Cell Aggregates

It is characteristic of some bacteria to form long chains and of others to clump under certain conditions. It is more difficult to kill all bacteria in intertwined chains or sizable clumps than to destroy separate cells.

Cultural characteristics important in food bacteriology

Bacterial growth in and on foods often is extensive. Pigmented bacteria cause discolorations on the surfaces of foods; films may cover the surfaces of liquids; growth may make surfaces slimy; or growth throughout the liquids may result in undesirable cloudiness or sediment.

Physiological characteristics important in food bacteriology

These changes include hydrolysis of complex carbohydrates to simple ones; hydrolysis of proteins to polypeptides, amino acids, and ammonia or amines; and hydrolysis of fats to glycerol and fatty acids. O-R reactions, which are utilized by the bacteria to obtain energy from foods (carbohydrates, other carbon compounds, simple nitrogen-carbon compounds, etc.), yield such products as organic acids, alcohols, aldehydes, ketones, and gases.

Genera of bacteria important in food bacteriology

Genus *Acetobacter*

These bacteria oxidize ethyl alcohol to acetic acid. They are rod-shaped and motile and are found on fruits, vegetables, souring fruits, and alcoholic beverages. They are a definite spoilage problem in alcoholic beverages.

Genus *Aeromonas*

These are gram-negative rods with an optimum temperature for growth of 22 to 28 °C. They are facultative anaerobes and can be psychrophilic. They are frequently isolated from aquatic environments. A. hydrophila can be a human pathogen; it is also pathogenic to fish, frogs, and other mammals.

Genus *Alcaligenes*

As the name suggests, an alkaline reaction usually is produced in the medium of growth. *A. viscolactis* causes ropiness in milk, and *A. metalcaligenes* gives a slimy growth on cottage cheese. These organisms come from manure, feeds, soil, water, and dust. This genus also contains organisms which were formerly classified in the genus *Achromobacter*.

Genus *Alteromonas*

Several former species of *Pseudomonas* are now classified as *Alteromonas*. They are marine organisms that are potentially important in sea foods.

Genus *Bacillus*

The endospores of species of this aerobic to facultative genus usually do not swell the rods in which they are formed. Different species may be mesophilic or thermophilic, actively proteolytic, moderately proteolytic, or non proteolytic, gas-forming or not, and lipolytic or not. In general the spores of the mesophiles, e.g., *B. subtilis*, are less heat-resistant than spores of the thermophiles. Spores of the obligate thermophiles, e.g., *B. stearothermophilus*, are more resistant than those of facultative thermophiles, e.g., B. coagulans. The actively proteolytic species usually may also sweet-curdle milk; B. cereus is such a species. The two chief acid- and gas-forming species, *B. polymyxa* and *B. macerans*, sometimes are termed "aerobacilli."

Genus *Brevibacterium*

B. linens are related to *Arthrobacter globiformis* and may be synonymous.

Genus *Brochotrix*

These are gram-positive rods which can form long filamentous like chains that may fold into knotted masses. The optimum temperature for growth is 20 to 25 °C, but growth can occur over a temperature range of 0 to 45 °C depending on the strain.

Genus *Campylobacter*

These bacteria were originally classified in the genus *Vibrio*. Several strains of *C. fetus* subsp. *jejuni* have been associated with gastroenteritis in humans.

Genus *Clostridium*

The endospores of species of this genus of anaerobic to microaerophilic bacteria usually swell the end or middle of the rods in which they are formed. Different species may be mesophilic or thermophilic and proteolytic or non-proteolytic. *Clostridium thermosaccharoolyticum* is an example of a saccharolytic obligate thermophile; this organism causes gaseous spoilage of canned vegetables. Putrefaction of foods often is caused by mesophilic, proteolytic species, such as *C. lentoputrescens* and *C. putrefaciens*.

Genus *Corynebacterium*

The diphtheria organism, *C. diptheriae*, may be transported by foods. *C. bovis*, with the slender, barred, or clubbed rods characteristic of the genus, is commensally on the cow's udder, can be found in aseptically drawn milk, and may be a cause of bovine mastitis.

Genus *Erwinia*

The species of this genus are plant pathogens that cause necrosis, galls, wilts, or soft rots in plants and therefore damage the plants and vegetable and fruit products from them. *E.carotovora* is associated with the market disease called "bacterial soft rot." *E. carotovora* subsp. *carotovora* causes rotting in a large number of plants. *E. carotovora subsp. atroseptica* produces a black rot in potatoes. *E. carotovora* subsp. *betavasculorum* causes soft rot in sugar beets.

Genus *Escherichia*

Found in feces, a predominant gram-negative rod isolated from the intestinal tract of warm-blooded animals and widely distributed in nature. One of the "*coliform* groups," the genus is divided into many biotypes and serotypes, some of which can be pathogenic to humans.

Genus *Flavobacterium*

The yellow to orange-pigmented species of this genus may cause discolorations on the surface of meats and be involved in the spoilage of shellfish, poultry, eggs, butter, and milk. Some of the organisms are psychrotrophic and have been found growing on thawing vegetables.

Genus *Klebsiella*

Many are capsulated. Commonly associated with the respiratory and intestinal tracts of humans. *K. pneumoniae* is the causative organism for a bacterial pneumonia in humans.

Genus *Lactobacillus*

The lactobacilli are rods, usually long and slender, that form chains in most species. They are microaerophilic, (some strict anaerobes are known), are catalase-negative and gram-positive, and ferment sugars to yield lactic acid as the main product.

FACTORS AFFECTING THE GROWTH AND SURVIVAL OF MICROORGANISMS IN FOODS

INTRINSIC PARAMETERS

The parameters of plant and animal tissues that are inherent part of the tissues are referred to as intrinsic parameter. These parameters are as follows,

1. pH

pH: It is the negative logarithm of the hydrogen ion activity.

$$PH = -\log(a_H) = \log\frac{1}{(a_H)}$$

$$= \log\frac{1}{[H^{-1}]}$$

pH = Hydrogen ion activity

+ H = Hydrogen ion concentration.

Every micro organism has a minimal, a maximal and an optimal pH for growth. Bacteria grow fastest in the pH range 6.0 – 8.0, yeasts 4.5 – 6.0 and filamentous fungi 3.5-4.0. Usually between pH 5.0 & 6.0.

Inherent acidity: Some foods have a low pH because of inherent property of the food.

Ex: Fruits & vegetables.

Biological acidity: Some foods develop acidity from the accumulation of acid daring fermentation. Ex: curd, sauerkraut, pickles etc. Molds can grow over a wide range of pH vales than the yeast and bacteria. Film yeasts grow well on acid foods such as sauerkraut and pickles. Most yeast does not grow well in alkaline substrates. Bacteria which are acid formers are favored by moderate acidity. Active proteolytic bacteria can grow in media with a high pH (alkaline.) Ex: Egg white. The compounds that resist changes in pH are important not only for their buffering capacity but also for their ability to be especially effective within a certain pH range.

Vegetable juices have low buffering power, permitting an appreciable decrease in pH with the production of small amount of acid by lactic acid bacteria during the early part of sauerkraut and pickle fermentations. This enables the lactic to suppress the undesirable pectin –hydrolyzing and proteolytic competing organisms. Low buffering power makes for a more rapidly appearing succession of micro-organisms during fermentation than high buffering power. Ex: Milk – High in protein content, act as good buffer. Lactic acid converted to pyruvic acid by glycolytic pathway. Acid again converts to lactic acid by lactic dehydrogenase enzyme. After 5- 10 minutes, there will be decreased in pH. Hence the lactic acid bacteria survive and activity slows dawn. Once the acidity increase, yeasts and molds will take upper hand and all the products used by these organisms. The quantity of acid decreases and pH increases to neutral.

Proteolytic bacteria acts on caesin and these proteins are broken down and give bad smell accompanied by removal of NH_3. pH increases and neutral due to deamination. Then lipolytic organisms which utilize the fat present and utilize the short chain fatty acids through hydrolysis which gives still bad smell. Egg white where the pH increases to around 9.2 as CO_2 is lost from the egg after laying. Fish spoil more rapidly than meat under chill conditions. The pH of post – rigor mammalian muscle, round 5.6 and it is lower than that of fish (6.2-6.5) and this contributes to the longer storage life of meat.

The ability of low pH to restrict microbial growth has been employed since the earliest times in the presentation of foods with acetic and lactic acids. Fruits are acidic than vegetables. pH of milk – neutral. Fruits generally undergo mold and yeast spoilage than vegetables.

Redox potential (Eh): - Oxidation – reduction potential

Oxygen tension or partial pressure of oxygen about a food and the O-R potential or reducing and oxidizing power of the food itself, influence the type of organisms which will grow and hence the changes produced in the food. The O- R potential of the food is determined by

1. Characteristic O-R potential of the original food.
2. The poising capacity i.e., the resistance to change in potential of the food.
3. The oxygen tension of the atmosphere about the food.
4. The access which the atmosphere has to the food.

Head space in an "evacuated" can of food contains low oxygen tension compared to air.

Micro organisms are classified as aerobic, anaerobic, and facultative based on the requirement of O2.

- Molds – aerobic, yeasts – Aerobic and facultative.
- Bacteria – Aerobic, anaerobic and facultative.
- High O - R potential favors aerobes and facultative organisms.
- Low O-R potential favors anaerobic and facultative organisms. However some aerobes grow at low O-R potential O-R potential of a system is usually written
- Eh are measured and expressed in terms of millivolts (mv).

Highly oxidised substrate would have a positive Eh and a reduced substrate have a negative Eh. Aerobic microorganisms require positive Eh. Ex: *Bacillus, Micrococcus, Pseudomonads, Acinetobacters*. Anaerobic micro organisms required negative Eh. Ex: *Clostridium.* Most fresh plant and animal foods have a low and well poised O – R potential in their interior because plants contain reducing substances like ascorbic acid and reducing sugars where as animal tissues contain –SH (Sulf hydryl) and other reducing groups. As long as the plant or animal cells respire and remain active, they have low level of O-R potential.

Meat could support the aerobic growth of shine forming or souring bacteria at the same time that anaerobic putrefaction was proceeding in the interior. Heating and processing may alter the reducing and oxidizing substances of food. Ex: Fruit juices lost reducing substances by their removal during extraction and filtration by their removal during extraction and filtration and therefore have become more favorable for the growth of yeasts.

Nutrient content

Food is required for energy and growth of micro organisms. Carbohydrates especially the sugars are commonly used as an energy source. Complex carbohydrates such as cellulose can be utilized by few organisms and starch can be hydrolyzed by limited number of organisms. Many organisms cannot use the disaccharide lactose (Milk sugar) and therefore do not grow well in milk. Maltose is not attacked by some yeast. Some micro organisms hydrolyze pectin of the fruits and vegetables. Limited number of micro organisms can obtain their energy from fats by producing lipases. Fats are hydrolyzed to glycerol and fatty acids. Aerobic micro organisms are more commonly involved in the decomposition of fats than are anaerobic ones and the lipolytic organisms usually are also proteolytic. Hydrolysis products of proteins, peptides and amino acids serve as an energy source for many proteolytic organisms when a better energy source is lacking. Meats are decomposed by proteolytic sps Ex: *Pseudomonas* sps. Concentration of food in solution increases the osmotic effect and amount of available moisture. Molds & yeasts can grow in the highest concentrations of sugars. Bacteria can grow best in low concentration of sugars.

Microorganisms differ in their ability to use various nitrogenous compounds as a source of nitrogen for growth. Many organisms are unable to hydrolyze proteins and hence cannot get nitrogen from them. Peptides, amino acids, urea, ammonia and other simpler nitrogenous

compounds may be available to some organisms but not to others. These compounds may be used under some environmental conditions but not under other conditions. Ex: Some lactic acid bacteria grow best with polypeptides as nitrogen foods cannot attack casein. Some microorganisms use fermentable carbohydrates and results in acid production which suppresses the proteolytic bacteria and hence it is called sparing action on the nitrogen compounds.
Many kinds of molds are proteolytic but very few types of yeast are actively proteolytic. Proteolytic bacteria grow best at pH values near neutrality and are inhibited by acidity. Carbon for growth may come partly from CO_2 and also from organic compounds. Minerals required by microorganisms are always present in low level. Sometimes an essential mineral may be unavailable, lacking or present in insufficient amounts.
Ex: Milk contains insufficient iron for pigmentation of the spores of *Penicillium roqueforti*. Accessory food substances or vitamins needed by the organisms.

Antimicrobial barriers and constituents (or) Inhibitory substances and biological structure

Inhibitory substances: These originally present in the food or added purposely to prevent growth of micro organisms.

- Freshly drawn milk – Lactenins, anticoliform factors.
- Egg white – Lysozyme
- Cran berries – Benzoic acid
- Short chain fatly acids on animal skin cabbage and other brassicas, garlic, onions and leeks.
- Allicin – Garlic, onion, leeks.
- Phytoalexins are produced by many plants in respone to microbial invasion.
- Antifungal compound phaseolin produced in green beans
- Eugenol – Allspice (pimento), cloves, cinnamon
- Thymol – thyme and oregano
- Cinnamic aldehyde – cinnamon and Cassia
- Inclusion of cinnamon in raisin bread retards mould spoilage.
- Humulones contained in the hop resin and isomers produced during processing, impart the characteristic bitterness of beer.
- Oleuropein – The bitter principle of green olives have antimicrobial properties.
- Lysozyme present in milk, egg is most active against gram positive bacteria.
- Egg – Ovotransferrin, avidin ovolflaroprotein.
- Milk – Lactoferrin
- Ovoflavo protein and avidin in egg white which sequester biotin and riboflavin restricting the growth of those bacteria.

Biological structures of food on the protection of foods against spoilage have been observed.
Ex: 1) Inner parts of healthy tissues of living plants and animals are sterile or low in microbial content.
2) Protective covering on the food like shell on egg, skin on poultry, shell on nuts, rind or skin on fruits and vegetables, artificial coating like plastic or wax.
3) Layers of fat over meat may protect the part of the flesh or scales may protect the outer part of the fish.

Water activity

Micro organisms have an absolute demand for water. Without water, no growth can occur. The exact amount of water needed for growth of micro organisms varies. This water requirement is best expressed in terms of available water or water activity (aw).

Factors that may affect water activity (aw). Requirements of micro organisms include the following.

1. The kind of solute employed to reduce aw. Potassium chloride is usually less toxic than NaCl. And less inhibitory than sodium sulphate.
2. The nutritive valve of the culture medium. The better the medium for growth, the lower the limiting aw.
3. Temperature: Most organisms have the greatest tolerance to low aw at about optimal temperatures.
4. Oxygen supply: Growth of aerobes takes place at lower aw in the presence of air than in its absence.
5. pH: Most organisms are more tolerant of low aw at pH valves near neutrality than in acid or alkaline media.
6. Inhibitors: The presence of inhibitors narrows the range of aw for growth of micro organisms.

Methods for the control of aware

1. Equilibrium with controlling solutions
2. Determination of the water – sorption isotherm for the food.
3. Addition of solutes.

0.85 – 0.93 Dried beef, raw ham, aged cheddar cheese, sweetened condensed milk, dry or fermented sausage. 0.60 – 0.85. Dried fruit, flour, cereals, jams & jellies, nuts. Below 0.60 Chocolate, confectionary, Honey, Biscuits, Crackers, Potato chips, dried eggs, milk and vegetables.

Methods for measuring or establishing aw valves of food:

1. Freezing point determinations by Clausius – Clayperson equation.
2. Manometric techniques
3. Electrical devices.

Favorable aw for bacteria growth in foods is 0.995 to 0.998. They grow best in low concentration of sugar or salt. 3-4% sugar and 1-2% salt may inhibit some bacteria.

Molds have optimum aw of 0.98 – 0.99; Mold spores germinate at min aw of 0.62.

Some general conclusions related to water requirement of micro organisms are

1. Each organism has its own characteristic optimal aw.
2. Bacteria require more moisture than yeasts and yeasts more than molds.

Minimum aw required for bacteria – 0.91
Minimum aw required for yeasts – 0.88
Minimum aw required for molds – 0.80
Minimum aw required for Halophilic bacteria – 0.75
Minimum aw required for Xerophilic fungi – 0.65
Minimum aw required for Osmophilic yeasts – 0.60

3. Micro organisms that can grow in high concentrations of solutes e.g. sugar and salt have low water activity (aw). Osmophilic yeasts grow best in high concentrations of sugar.

EXTRINSIC PARAMETERS (ENVIRONMENTAL LIMITATIONS)

Relative humidity (RH)

Relative humidity and water activity are interrelated. When food commodities having low water activity are stored in an atmosphere of high RH, water will transfer from the gas phase to the food. It may take a very long time for the bulk of the commodity to increase in water activity. Once micro organisms have started to grow and become physiologically active they usually produce water as an end product of respiration. Ex: Grain silos or in tanks in which concentrates and syrups is stored. Storage of fresh fruits and vegetables requires very careful control of relative humidity. If RH is too low; many vegetables will lose water and become flaccid. If it is too high, then condensation may occur and microbial spoilage may be initiated.

Temperature

Microbial growth can occur over a temperature range from about -8°C up to 100°C at atmospheric pressure.

Thermophiles have optimum: 55-75°C

Mesophile have optimum: 30 -40°C

Psychrophiles (Obligate psychrophiles): 12 – 15 °C

Psychotroph (facultative): 25-30 °C

Micro organisms can be classified into several physiological groups based on their cardinal temperatures. Low temperature affects the uptake and supply of nutrients to enzyme systems within the cell. Many microorganisms responds to growth at lower temperature by increasing the amount of unsaturated fatty acids in their membrane lipids and that psychrotrophs generally have higher level of unsaturation in a fatty acid decreases its melting point so that membranes containing higher levels of unsaturated fatly acid will remain fluid and hence functional at lower temperatures. As the temperature increases above the optimum, the growth rate declines as a result of denaturation of proteins.

Gaseous atmosphere

Oxygen comprises 21% of the earth's atmosphere and is the most important gas in contact with food under normal circumstances. The inhibitory effect of CO_2 on microbial growth is applied in modified atmosphere packing of food and is an advantage in carbonated mineral waters and soft drinks. Moulds and bacteria are sensitive to CO_2 condensation. Some yeast such as Bettanomyces sp. has tolerance to high CO_2 levels. Growth inhibition is usually greater under aerobic conditions than anaerobic and the inhibitory effect increases with decrease of temperature, presumably due to the increased solubility of CO_2 at lower temperatures. CO_2 dissolves in water to produce carbonic acid which decreases pH and partially dissociates into bicarbonate anions and protons. CO_2 also affects solute transport, inhibition of key enzymes involving carboxylation, decarboxylation reactions in which CO_2 is a reactant and reaction with protein amino groups causing change in their properties and activity.

SOURCES OF CONTAMINATION OF FOODS

Micro organisms from various natural sources act as source of contamination.

- From green plants and fruits
- From animals
- From sewage
- From soil
- From water
- From air
- During handling and processing.

From green plants and fruits

Natural surface flora of plants varies with the plant. But usually includes species of *Pseudomonas, Alcaligenes, Flavobacterium, Micrococcus, coliforms* and lactic acid bacteria. The no. of bacteria will depend on the plant and its environment and may range from a few hundred or thousand per square centimeter of surface to millions.

Ex: Surface of well washed tomato contains 400-700 microorganisms per square centimeter. Outer tissue of unwashed cabbage contains 1 million to 2 million microorganisms. Inner tissues of cabbage contain fewer micro organisms. Exposed surface of plants become contaminated from soil, water, sewage, air and animals, so that microorganisms from these sources are added to the natural flora. Whenever conditions for growth of natural flora and contaminants are present, special kinds of micro organisms may increase. Some fruits have been found to contain viable microorganisms in their interior.

From animals

Sources of microorganisms from animals include the surface flora, the flora of the respiratory tract, and the flora of the gastro intestinal tract. Hides, hooves, and hair contain microorganisms from soil, manure, feed and water but contain spoilage organisms. Feathers, feet of poultry carry heavy contamination of microorganisms. Skin of many meat animals may contain *Micrococci*, *Staphylococci* and beta haemolytic *Streptococci*. Pig or beef carcasses may be contaminated with salmonellae. Salmonellosis associated with eggs has been reduced because of the pasteurization of egg products. Meat from slaughter houses is not frequently associated with human salmonellosis. Many of these diseases have been reduced or eliminated by improvement in animal husbandry, but animal disease causing infections from foods include *Mycobacterium*, *Coxiella*, *Listeria*, *Salmonella* and entropathogenic *E.coli* and viruses. Insects and birds cause mechanical damage to fruits and vegetables, introduce microorganisms and open the way for microbial spoilage.

From sewage

When untreated domestic sewage is used to fertilize plant crops, there is a chance that raw plant foods will be contaminated with human pathogens especially those causing gastrointestinal diseases. The use of “night soil” as a fertilizer still persists in some parts of the world. In addition to the pathogens, *coliform* bacteria, anaerobes, *enterococci*, other intestinal bacteria and viruses can contaminate the foods from this source. Natural water contaminated with sewage contributes their micro organisms to shell fish, fish, and other seafood.

From soil

Soil contains greatest variety of micro organisms. They are ready to contaminate the surfaces of plants growing on or in them and the surfaces of animals roaming over the land. Soil dust is whipped up by air currents and soil particles are carried by running water to get into or onto foods. Soil is an important source of heat resistant spore forming bacteria.

From water

Natural water contains not only their natural flora but also microorganisms from soil and possibly from animals or sewage. Surface waters in streams or pools and stored waters have low microbial content because self purification of quiet lakes and ponds or of running water. Ground waters from springs or wells have passed through layers of rock and soil to a definite level hence most of the bacteria, suspended material have been removed. Kinds of bacteria in natural waters are chiefly of in *Pseudomonas*, *Chromobacterium*, *Proteus, Micrococcus, Bacillus, Streptococcus, Enterobacter* and *Escherichia coli*. Two aspects of water bacteriology are Public health aspects and Economic aspects.

Public health aspects and Economic aspects

Public health aspects include safe to drink, free from pathogens, water should be tested for *coliforms, enterobacter* before consumption. Water is used for processing of fruits & vegetables. The water commonly is chlorinated but there have been presence of chlorine resistant flora. Efficient filtration greatly reduces the microbial content.

From Air

Air does not contain a natural flora of micro organisms, but accidentally they are present on suspended solid material or in moisture droplets. Micro organisms get into air on dust or lint, dry soil, spray from stream, lakes or oceans, droplets of moisture from coughing, sneezing or talking and growth of sporulating molds on floors, etc. Micro organisms in air have no opportunity for growth but merely persist there and the organisms resistant to desiccations will live longer. Mold spores because of their small size, resistance to drying and large numbers of per mold plant are usually present in air. *Cocci* are more numerous than rod shaped bacteria. Yeasts especially asporogenous chromogenic ones are found in most samples of air. Number of microorganisms in air at any given time depends on factors like amount of movement, sunshine, humidity, location and the amount of suspended dust or spray. No. of micro organisms vary from mountains to dusty air. Less on mountains and more in dusty air. Direct rays from the sun kill microorganisms suspended in air and hence reduce numbers. Dry air contains more organisms than moist air. Rain or snow removes organisms from the air. Number of micro organisms in air may be reduced under natural conditions by sedimentation, sunshine and washing by rain or snow. Filters in ventilating or air conditioning systems prevent the spread of organisms from one part of a plant to another.

During handling and processing

Additional contamination may come from equipment coming in contact with foods, from packaging materials and from personnel.

Lecture plan

FOOD AND AGRICULTURAL MICROBIOLOGY (15MBU503)

SEMESTER – V **5H – 5C**

UNIT II

S. No	Duration	Topic	Reference
1	1	Preservation of food – Introduction	T1: 628
2	1	Principles of food preservation	R1: 84-85
3	1	Methods of food preservation	R1: 84-85
4	1	Physical methods – Temperature (high)	T1: 629-651
5	1	Physical methods – low temperature	
6	1	Drying and Radiation	
7	1	Chemical methods of food preservation	T1: 146-153
8	1	Food preservation – additives	
9	1	Characteristics of psychrophiles	
10	1	Characteristics of thermophiles	
11	1	Limitation	R1: 168-169
12	1	Commercial applications	
13	1	Tutorial hour	
14	1	Unit Revision	
Total Hrs: 14			

T1: Pelczar - Microbiology

R1: Frazier, WC and DC. Westhoff, 1995. Food: Microbiology. Tata McGraw – Hill Publishing Company limited. New Delhi.

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FOOD AND AGRICULTURAL MICROBIOLOGY

Prepared by - Ms. P. Akilandeswari, Asst Professor, Dept of Microbiology, KAHE

UNIT-2

Introduction

Foods are mainly composed of biochemical compounds which are derived from plants and animals. Carbohydrates, proteins and fats are the major constituents of food. In addition, minor constituents such as minerals, vitamins, enzymes, acids, antioxidants, pigments, flavours are present. Foods are subject to physical, chemical, and biological deterioration. The major factors affecting food spoilage are

1) Growth and activities of microorganisms (bacteria, yeasts, and molds)
2) Activities of food enzymes and other chemical reactions within food itself
3) Infestation by insects, rodents
4) Inappropriate temperatures for a given food
5) Either the gain or loss of moisture
6) Reaction with oxygen
7) Light

The vast majority of instances of food spoilage can be attributed to one of two major causes: (1) the attack by microorganisms such as bacteria and molds, or (2) oxidation that causes the destruction of essential biochemical compounds and/or the destruction of plant and animal cells. Chemical and/or biochemical reactions results in decomposition of food due to microbial growth. There is an adverse effect on appearance, flavour, texture, colour, consistence and/or nutritional quality of food.

Food Preservation

Food preservation is the process of treating and handling food to stop or greatly slow down spoilage (loss of quality, edibility or nutritive value) caused or accelerated by micro-organisms. Preservation usually involves preventing the growth of bacteria, fungi, and other micro-organisms, as well as retarding the oxidation of fats which cause rancidity. It also includes processes to inhibit natural ageing and discolouration that can occur during food preparation such as the enzymatic browning reaction in apples after they are cut. Preservative for food may be defined as any chemical compound and/or process, when applied to food, retard alterations caused by the growth of microorganisms or enable the physical properties, chemical composition and nutritive value to remain unaffected by microbial growth.

Principles of Food Preservation

The principles of various methods for food preservation are as

1) Prevention or delay of microbial decomposition

By keeping out microorganisms (asepsis), By removal of microorganisms, By hindering the growth and activity of microorganisms (e.g. by low temperatures, drying, anaerobic conditions, or chemicals), By killing the microorganisms (e.g. by heat or radiation)

2) Prevention or delay of self decomposition of the food

By destruction or inactivation of food enzymes (by blanching)
By prevention or delay of chemical reactions (By using antioxidant).

Methods of Food Preservation

Preservation of food is achieved by application of physical, chemical and/or biological methods are as follows:

Physical methods

Thermal treatment

The term "thermal" refers to processes involving heat. Heating food is an effective way of preserving it because the great majority of harmful pathogens are killed at temperatures close to the boiling point of water. In this respect, heating foods is a form of food preservation comparable to that of freezing but much superior to it in its effectiveness. A preliminary step in many other forms of food preservation, especially forms that make use of packaging, is to heat the foods to temperatures sufficiently high to destroy pathogens.

In many cases, foods are actually cooked prior to their being packaged and stored. In other cases, cooking is neither appropriate nor necessary. The most familiar example of the latter situation is pasteurization. Conventional methods of pasteurization called for the heating of milk to a temperature between 145 and 149 °F (63 and 65 °C) for a period of about 30 minutes, and then cooling it to room temperature. In a more recent revision of that process, milk can also be "flash-pasteurized" by raising its temperature to about 160 °F (71 °C) for a minimum of 15 seconds, with equally successful results. A process known as ultra high pasteurization uses higher temperatures of the order of 194 to 266 °F (90 to 130 °C) for periods of a second or more.

Low temperature

The lower the temperature, the slower will be chemical reactions, enzyme action, and microbial growth. Each microorganism present has an optimal temperature for growth and a minimal temperature below which it cannot multiply. As the temperature drops from this optimal temperature toward the minimal, the rate of growth of the organism decreases and is slowest at the minimal temperature. Cooler temperatures will prevent growth, but slow metabolic activity may continue. Most bacteria, yeasts, and molds grow best in the temperature range 16-38^{o}C (except psychrotrophs). At temperatures below 10 ^{o}C, growth is slow and becomes slower the colder it gets. The slowing of microbial activity with decreased temperatures is the principal behind refrigeration and freezing preservation.

Drying

One of the oldest methods of food preservation is by drying, which reduces water activity sufficiently to prevent or delay microbial growth. The term water activity is related to relative humidity. Relative humidity refers to the atmosphere surrounding a material or solution. Water activity is the ratio of vapour pressure of the solution to the vapour pressure of pure water at the same temperature. Under equilibrium conditions water activity equals RH/100. At the usual temperatures permitting microbial growth, most bacteria require a water activity as low as 0.90-1.00. Some yeasts and molds grow slowly at a water activity as low as 0.65. Food is dried either partially or completely to preserve it against microbial spoilage.

Chemical preservation

Chemical preservatives are added to kill or inhibit microorganisms in food. The may be incorporated into the foods or only their surface or the wrappers used for them may be treated, or they may be used as gas or vapors around the food. Some chemicals may be effective on selected group of microorganisms while others on a wide variety of them. Chemical preservatives may be harmless if they are added during the storage period and are removed before the food is consumed. But if they are consumed as such, they may be poisonous to man or animal, as well as to microorganisms.

Organic acids and their salts:

Several organic acids and their salts are common preservatives as they have marked microbiostatic and microbicidal action.

Benzoic acid and benzoate are used for the preservation of vegetables. Sodium benzoate is used in the preservation of jellies, jams, fruit juice and other acid foods.

Salicylic acid and salicylates are used as preservatives of fruits and vegetables in place of benzoate. However, it is considered to be deleterious to health of consumer.

Sorbic acid is recommended for foods susceptible to spoilage fungi, e.g., it inhibits mold growth in bread. Wrapping material for cheese may be treated with it. It is also used in sweet pickles and for control of lactic fermentations of olives and cucumbers.

Foods prepared by fermentation processes, e.g. milk products etc. are preserved mainly by lactic, acetic and propionic acids.

Flavoring extracts of vanilla, lemons are preserved in 50-70% alcohol as it coagulates cell proteins.

Inorganic acids and their salts:

Most common among the inorganic acids and their salts are, sodium chloride, hypochlorites, sulphurous acids and sulphites, sulphurdioxide, nitrate and nitrite.

a. Sodium chloride

Sodium chloride produces high osmotic pressure and therefore causes destruction of many microorganisms by plasmolysis. It causes dehydration of food as well as microorganisms, releases disinfecting chlorine ion by ionization, reduces solubility of oxygen in the moisture, sensitizes microbial cells against carbon dioxide and interferes with the action of proteolytic enzymes. These are the reasons why this common salt is used widely for preservation either directly or curing solutions.

b. Hypochlorites

The hypochlorous acid liberated by these salts is an effective germicide. It is oxidative in its action. The commonly used forms are sodium and calcium hypochlorites. Drinking water or water used for washing foods may be dissolved with hypochlorites.

c. Sulphurous acids and Sulphites

Sulphurous acids and sulphites are added to wines as preservatives. Sulphurous acid is used especially in the preservation of dry fruits. It helps in retention of original colour of the preserve and inhibition of molds more than either yeasts or bacteria. Potassium metabisulphite is used in canning.

d. Sulphur dioxide

Sulphur dioxide has a bleaching effect desired in some fruits, and also suppresses the growth of yeast and molds. It is used as a gas to treat drying fruits and is also used in molasses.

e. Nitrates and Nitrites

Nitrates and nitrites produce an inhibitory effect on bacterial growth and are used usually together in meat and fish preservation and for retention of red-colour of the meat. Nitrate is changed to nitrous acid which reacts with myoglobin to give nitric oxide myoglobin. It is the latter which gives a bright red colour to the meat making it more attractive in appearance. However, both nitrite and nitrate are poisonous, if present in potable water or food products in more than minimal amounts. It is why the generous use of these chemicals as preservative in meat and fish products has been questioned.

Antibiotics:

Aureomycin (chlorotetracycline) is the most commonly used antibiotic for the preservation of animal products under chilling conditions. It is extensively used for the preservation of poultry, meat and fish. The antibiotic is applied to the surface of the fresh meat by dipping it in a solution of the antibiotic or it may be fed to the animal, by mixing it with feed or water, for one to several days before slaughter. Fish are treated by adding the antibiotic in the ice or water in which they are to be transported.

The indiscriminate use of antibiotics as preservatives, however, should be prevented or the antibiotics used should be such that it is demobilized on cooking so that the internal flora of man using such food is not constantly exposed to the effect of the antibiotic. It is important for otherwise the use would lead to the development of the antibiotic resistant strains of microorganisms in the body. Aside from this, some individuals sensitive to antibiotics become exposed constantly to allergy.

Biological method

Souring (fermentation) lactic and acetic acid e.g. cheese and cultured milk.

Radiations

Low-frequency, long-wavelength, low energy radiation ranges from radio waves to infrared. Conversely, the high-frequency, shorter-wavelength radiations have high quantum energies and actually excite or destroy organic compounds and microorganisms without heating the product. Microbial destruction without the generation of high temperatures suggested the term "cold sterilization." Radiations of higher frequencies have high energy contents and are capable of actually breaking individual molecules into ions, hence the term ionizing irradiation.

Gamma rays and high-energy electron beams

Gamma rays and high-energy electron beams have been used for the preservation of fresh perishable canned and packaged foods. They have good penetration and are effective to a depth of about 15 cm in most foods. Food preservation by such radiation dosage is called "cold-sterilization" as it produces only a few degrees rise in temperature of the product.

Ultraviolet rays

Ultraviolet rays are short waves and are used to sterilize the surface of foods. These rays have been successfully used for the treatment of water for beverages, aging meat's packaging, and treatment of knives for slicing bread, for sterilizing utensils, for prevention of spoilage by organisms on the surface of preserved pickles, cheese and prevention of air contamination. Cold-storage rooms of meat-processing plants are sometimes equipped with germicidal lamps which reduce the surface contamination and permit longer periods of spoilage-free storage.

Radiation pasteurization or sterilization

It represents a term which describes the killing of over 98% but not 100% of the microorganisms by intermediate dosage of radiation. This method increases the storage life of some meats, sea-foods, certain fruits and vegetables when stored at low temperature. Radiation pasteurization provides the possibility of an entirely new approach to food preservation and could bring about a radical change in industrial methods of food processing.

However, the effect of radiation on colour, flavor nutritional quality of food, odor and texture needs to be more carefully understood. Similarly, chemical changes in food products brought about by radiations may cause bad effects on animal and human subjects and need to be more adequately investigated.

Other methods

There are many different methods for drying, each with their own advantages for particular applications. These include,

- Convection drying
- Bed dryers
- Drum drying
- Freeze Drying
- Microwave-vacuum drying
- Shelf dryers
- Spray drying
- Infrared radiation drying
- Sunlight
- Commercial food dehydrators and Household oven.

Lecture plan

FOOD AND AGRICULTURAL MICROBIOLOGY (15MBU503)

SEMESTER – V **5H – 5C**

UNIT III

S. No	Duration	Topic	Reference
1	1	Food borne disease – Introduction	R1:401-432
2	1	Food poisoning – Clostridium botulism	R1: 441-455
3	1	Staphylococcus food intoxication	R1: 412-419
4	1	Food borne illness – Salmonellosis	R1: 419-425
5	1	Clostridium prefringens	R1: 425-427
6	1	Vibrio parahaemolyticus	R1: 427-430
7	1	Bacillus cereus	R1: 431
8	1	Food control agencies – International	R1: 495-498
9	1	Federal agencies, state	R1: 496-498
10	1	Commercial, Professional, private agencies	R1: 498-499
11	1	Microbiological criteria for food	R1: 499-500
12	1	Microbial quality for food	R1: 415-418
13	1	Tutorial hour	
14	1	Unit Revision	
Total Hrs: 14			

R1: Frazier, WC and DC. Westhoff, 1995. Food: Microbiology. Tata McGraw – Hill Publishing Company limited. New Delhi.

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FOOD AND AGRICULTURAL MICROBIOLOGY

Prepared by – Ms. P. Akilandeswari, Asst Professor, Dept of Microbiology, KAHE

UNIT – 3

Food poisoning is a term customarily applied to represent the illness caused both by the ingestion of toxins produced by the organisms in the food as well as resulting from the infection of the host by the organisms carried in by the food. But, more correctly, all food-borne diseases can be classified into two categories: 'food-poisoning' or 'food-intoxications' and 'food-infections'. Food-poisoning or food-intoxication diseases are those which are caused by the consumption of toxins produced by organisms in the food whereas food-infection diseases are those trial are caused by the organisms which enter into the body through ingestion of contaminated food.

Microbial 'Food-poisoning' or 'Food-intoxications'

Bacterial "Food-Poisoning" (Bacterial food-intoxications)

There are two major food-poisonings or food-intoxications caused by bacteria. These are: **Botulism** and **Staphylococoal poisoning**.

a. Botulism

Botulism is caused by the ingestion of food containing the neurotoxin (toxin that affects the nervous system) produced by Clostridium botulinum, an anaerobic spore forming bacterium. Sixty to seventy percent-cases of botulism die. There are 7 types (type A,B,C, D,E,F,G) of these neurotoxins recognized on the basis of serological specificity. The neurotoxin of C. botulinum is a protein. It has been purified and crystallized and is so powerful that only a does as low as 0.01 mg is said to be fatal to human being. The toxin is absorbed mostly in the small intestine and paralyzes the involuntary muscles of the body.

Source

The main sources of botulism are canned meat, fish, string beans, sweet corn, beets and other low medium acid foods. The foods implicated are generally those of a type that have undergone some treatment intended for the preservation of the product such as canning, pickling or smoking, but one which failed to destroy the spores of this bacterium. When the intended preservative treatment is inadequate and is followed by storage conditions which permit the germination and growth of the microorganisms, one of the most lethal toxins known to humanity is produced, The toxin has been known to persist in foods for long periods, especially when storage has been at low temperatures. It is unstable at pH value above 6.8.

Temperature is considered to be the most important factor in determining whether toxin production will take place and what the rate of production will be. Various strains of C. botulinum types A and B vary in their temperature requirements; a few strains grow at 10 to 11 °C. However, the lowest temperature for germination of spores of most of the strains is 15 °C and maximum of 48 °C.

Symptoms

Symptoms generally occur within 12 to 36 hours after consumption of the spoiled food. Early symptoms are digestive disturbances followed by nausea, vomiting, diarrhea together with dizziness and headache. Double vision may occur early and there may be difficulty in speaking. Mouth may become dry, throat constricted; tongue may get swollen, and coated. Involuntary Muscles become paralyzed and paralysis spreads to the respiratory system and to the heart. Death normally results from respiratory failure.

Prevention

Canned food should be properly processed by using approved heat processes.

Avoiding food that has been cooked but not well heated. Raw foods, frozen foods thawed and held at room temperature should be avoided. Gassy and spoiled canned foods should be rejected. Boiling of suspected food for at least 15 minutes.

Treatment:

Successful treatment is by the administration of polyvalent antitoxin in the early stages of infection. Once the symptoms appear the fails to prove useful.

b. Staphylococcal-poisoning:

This is the most common type of food-poisoning caused due to the food contaminated with a potent toxin namely, **enterotoxin.** This toxin is produced by certain strains of Staphylococcus aureus. A sudden onset of illness starts usually within 3 to 6 hours after ingestion of the contaminated food.

Source

These bacteria are commonly present on the skin, nose and other parts of human body. People who handle foods carelessly usually transfer them to the food. Foods most commonly contaminated involve those which are eaten cold, e.g., cold meat, poultry, salads, bakery products etc.

Symptoms

As said earlier, the disease starts within 3 to 6 hours after ingestion of the contaminated food and is manifested by nausea, vomiting, abdominal pain and diarrhoea within 24 to 48 hours. If the case becomes severe, dehydration and collapse may follow. However, in usual conditions death is rare.

Control

The disease can be controlled by preventing the entry of the bacteria to food. It is important that all susceptible foods are kept under refrigeration to restrict the growth of the bacteria; and also by the destruction of the bacteria.

Bacterial Food Infections

a. Salmonellosis

This disease is caused through the ingestion of Salmonella bacteria present in food. A large number of species and serotypes are involved. An inoculum of about 600,500 cells is required to become established and cause illness in the host. These bacteria are gram-negative, non-spore forming rods and motile by means of peritrichous flagella. Various species of Salmonella get ingested with improperly cooked eggs, puddings and meat that have been contaminated by the carriers. The carriers may be cats, dogs, chickens and others.

The disease appears through gastrointestinal infections as a result of the growth of the bacteria in the intestine. Typical symptoms of salmonellosis are nausea, vomiting, abdominal pain and diarrhoea. Generally the symptoms persist for 2 to 4 days. The incubation period ranges between 4 to 36 hours.

Salmonellosis can be prevented by avoiding consumption of contaminated food, by heat destruction of the bacteria, or by refrigeration to check the growth of bacteria.

b. Perfringens poisoning

The disease caused by the strains of Clostridium perfringenes is called 'perfringens poisoning' or more technically, 'Clostridium perfringens - gastroenteritis'. This bacterium is a gram-positive, anaerobic non-motile, spore former with an optimum growth temperature of 37-43°C.

This disease has been caused by the ingestion of prepared meat, meat products and poultry. Generally, the meat that has been cooked and allowed to cool slowly before consumption allows the growth of these microorganisms. What happens is that the cooking destroys only the vegetative cells not the spores. The latter survive the heating applied during cooking and germinate into vegetative cells. It could be avoided by adequate refrigeration of the food.

Symptoms

Symptoms appear in the form of diarrhoea, acute abdominal pain and, rarely, vomiting when the, growth of microorganisms takes place in the human intestine. Disease manifestation occurs between 8 to 22 hours after the contaminated food has been taken.

Prevention

Prevention of the disease includes rapid cooling of cooked meats and other foods and reheating of the remaining food before further consumption.

Bacillus cereus gastroenteritis

Bacillus cereus is a gram-positive, aerobic, rod-shaped, spore forming bacterium that causes food infections called 'gastroenteritis'. Its spores are heat resistant and remain viable even after considerable degree of cooling; germinate and produce vegetative cells. It is believed that the bacterial cells undergo lysis in the intestinal tract and release enterotoxin.

Escherichia coli gastroenteritis

Escherichia coli bacterium is generally regarded as a part of the natural flora of the human and animal intestinal tract. In recent years, however, various serotypes of this bacterium have been thought responsible for human and animal diarrhoeal diseases. These bacteria can be classified into two groups: one group representing enteropathogenic E.coli and the other representing enterotoxin producing E. coli.

The enteropathogenic E. coli are pathogenic within the intestinal tract. They have ability to penetrate epithelial cells of the intestinal mucosa, cause spithelial necrosis and ulceration resulting in the presence of red blood cells and large number of neutrophils in the stool during dysentery. This acute gastroenteritis (dysentery-like syndrom) is generally reported in the new-born and in infant up to two years of age.

The enterotoxin-producing E. coli fails to invade the intestinal mucosa but release an enterotoxin which causes diarrhea like syndrome. The latter refers to a profuse watery discharge generally from the small intestine. Since these bacteria do not penetrate and cause epithelial necrosis, red blood cells and neutrophils are not present in the diarrheal stool.

Foods which are highly contaminated or inadequately preserved allow the growth of such E. coli serotypes. The latter are heat sensitive and can be destroyed by pasteurization or by proper cooking methods.

Cholera

This disease, generally called 'asiatic cholera', is caused by Vibrio cholerae and has been the cause of untold suffering and death. The symptoms include vomiting and profuse diarrhoeal (rice-water) stools which result in mineral deficiency, dehydration and increased blood acidity of the body tissues leading, finally, to the death.

Vibrio cholerae is a gram-negative, uniflagellate bacterium and is transmitted through contaminated flies, water, raw and exposed foods etc. They find their way through mouth into the intestines and produce endotoxins which disintegrate the epithelial cells of the intestines. Death rate is rather high and the course of the disease may be as short as 12 hours after the onset of the first symptoms. Individuals recovering from infection are said to be effective in controlling the disease. Cholera patients should be kept in quarantine and all materials contaminated by faeces burnt for checking infection spread.

Differentiate between the major types of food borne diseases -- infection, intoxication, and toxin-mediated infection.

Microbiological hazards cause most foodborne diseases in the United States. The three microbiological hazards of concern are bacteria, viruses, and parasites. These microorganisms can cause one of three types of illness -- infection, intoxication, or toxin-mediated infection.

Infection

A foodborne disease is when a person eats food containing harmful microorganisms, which then grow in the intestinal tract and cause illness. Some bacteria, all viruses, and all parasites cause foodborne illness via infection. The foodborne bacteria that cause infection are: Salmonella spp., Listeria monocytogenes, Campylobacter jejuni, Vibrio parahaemolyticus, Vibrio vulnificus, and Yersinia enterocolitica. The most common viral agents that cause foodborne disease are: Hepatitis A, norovirus, and rotavirus. The most common foodborne parasites are: Trichinella spiralis, Anisakis simplex, Giaria duodenalis, Toxoplasma gondii, Cryptosporidium parvum, and Cyclospora cayetanensis.

Intoxication

An intoxication results when a person eats food containing toxins that cause illness. Toxins are produced by harmful microorganisms, the result of a chemical contamination, or are naturally part of a plant or seafood. Some bacteria cause intoxication. Viruses and parasites do not cause food borne intoxication. The foodborne bacteria that cause intoxication are: Clostridium botulinum, Staphylococcus aureus, Clostridium perfringens, and Bacillus cereus. Chemicals that can cause intoxication include cleaning products, sanitizers, pesticides and metals (lead, copper, brass, zinc, antimony, and cadmium). Seafood toxins include ciguatera toxin, scombroid toxin, shellfish toxins, and systemic fish toxins. Plants and mushrooms can also cause intoxication.

Food laws and Regulations

- To meet a country's sanitary and phytosanitary requirements, food must comply with the local laws and regulations to gain market access.
- These laws ensure the safety and suitability of food for consumers.
- The requirement of food regulation may be based on several factors such as
- whether a country adopts international norms developed by the Codex Alimentarius Commission of the Food and Agriculture Organization of the United Nations and the World Health Organization or a country may also has its own suite of food regulations.
- Each country regulates food differently and has its own food regulatory framework.

Food laws in our country

The Indian Parliament has recently passed the *Food Safety and Standards Act, 200*6 that overrides all other food related laws.

Such as;

Prevention of Food Adulteration Act, 1954

Fruit Products Order,1955

Meat Food Products Order ,1973;

Vegetable Oil Products (Control) Order, 1947

Edible Oils Packaging (Regulation) Order 1988

Solvent Extracted Oil, De- Oiled Meal and Edible Flour (Control) Order, 1967,

Milk and Milk Products Order, 1992 etc are repealed after commencement of FSS Act, 2006.

Food Safety and Standards Authority of India (FSSAI)

The Food Safety and Standards Authority of India (FSSAI) has been established under Food Safety and Standards Act, 2006 which consolidates various acts & orders that have hitherto handled food related issues in various Ministries and Departments.

FSSAI has been created for laying down science based standards for articles of food and to regulate their manufacture, storage, distribution, sale and import to ensure availability of safe and wholesome food for human consumption.

Functions performed by FSSAI

- Framing of Regulations to lay down the Standards and guidelines in relation to articles of food and specifying appropriate system of enforcing various standards.
- Laying down mechanisms and guidelines for accreditation of certification bodies engaged in certification of food safety management system for food businesses.
- Laying down procedure and guidelines for accreditation of laboratories and notification of the accredited laboratories.
- To provide scientific advice and technical support to Central Government and State Governments in the matters of framing the policy and rules in areas which have a direct or indirect bearing of food safety and nutrition .
- Collect and collate data regarding food consumption, incidence and prevalence of biological risk, contaminants in food, residues of various, contaminants in foods products, identification of emerging risks and introduction of rapid alert system.
- Creating an information network across the country so that the public, consumers, Panchayats etc receive rapid, reliable and objective information about food safety and issues of concern.
- Provide training programmes for persons who are involved or intend to get involved in food businesses.
- Contribute to the development of international technical standards for food, sanitary and phyto-sanitary standards.
- Promote general awareness about food safety and food standards

Bureau of Indian Standards (BIS)

- The Bureau of Indian Standards (BIS), the National Standards Body of India, resolves to be the leader in all matters concerning Standardization, Certification and Quality.

Main Activities

- Harmonious development of standardization, marking and quality certification
- To provide new thrust to standardization and quality control.
- To evolve a national strategy for according recognition to standards and integrating them with growth and development of production and exports.
- Certification of Product
- Hallmarking of Gold Jewellery.
- Quality Management System
- Environmental Management Systems
- Occupational Health and Safety Management System
- Food Safety Management System
- Hazard Analysis and Critical Control Points
- Imported Products
- Laboratory Management
- International Activities
- Training Services

AGMARK

- The Directorate of Marketing and Inspection enforces the Agricultural Produce (Grading and Marketing) Act, 1937. Under this Act Grade standards are prescribed for agricultural and allied.
- AGMARK is a Quality Certification Mark .
- It ensures quality and purity of a product.
- It acts as a Third Party Guarantee to Quality Certified.
- Quality standards for agricultural commodities are framed based on their intrinsic quality.
- Food safety factors are being incorporated in the standards to complete in World Trade.
- Standards are being harmonized with international standards keeping in view the WTO requirements. Certification of agricultural commodities is carried out for the benefit of producer/manufacturer and consumer.
- Products available under AGMARK are as follows:-
- ✓ Pulses
- ✓ Whole spices & ground spices
- ✓ Vegetable oils
- ✓ Wheat Products
- ✓ Milk products.
- ✓ Other products such as Honey, Compounded asafetida, Rice, Tapioca Sago, Seedless tamarind, Besan (Gram flour).

 HACCP Plan: A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration.

HACCP System: The hazard analysis critical control point system (HACCP) is a scientific and systematic way of enhancing the safety of foods from primary production to final consumption through the identification and evaluation of specific hazards and measures for their control to ensure the safety of food. HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing.

Some establishments may use **Good Manufacturing Practices (GMP)** to reduce the likelihood of certain hazards. GMPs are minimum sanitary and processing requirements. GMPs are fairly broad and general, for example, "*Training: All employees should receive training in personal hygiene.*" GMPs are usually not designed to control specific hazards, but are intended to provide guidelines to help establishments produce safe and wholesome products.

- ✓ **Standard Operating Procedures (SOP)** are step-by-step directions for completing important procedures and are usually very specific. SOP may be used to address a specific hazard, for instance, an establishment may have specific preventive maintenance procedures for its processing equipment, which prevent the hazard of metal fragments.
- ✓ **Sanitation SOP (SSOP)** may be considered by establishments to reduce the likelihood of occurrence of some food safety hazards. For example, the SSOP may address washing and sanitizing of knife and hands between carcasses to reduce potential contamination with pathogens.

Product specific GMPs

- ➢ thermally processed low-acid canned foods
- ➢ acidified foods
- ➢ bottled drinking water

GMPs Regulations

21CFR Part 110

- o *Subpart A - General Provisions*
- o *Subpart B - Building and Facilities*
- o *Subpart C - Equipment*
- o *Subpart D - [Reserved]*
- o *Subpart E - Production and Process Controls*
- o *Subpart F - [Reserved]*
- o *Subpart G - Defect Action Levels*

GMPs - General Provisions

- o provides definitions necessary for *important in understanding implications and applications*

- ✓ Buildings and Facilities. Buildings must be designed and constructed to facilitate *effective maintenance and sanitation.The* results specified rather than method for achieving detailed expectations in sanitation of operations**.**
- ✓ The equipment and utensils are *designed and constructed to be easily and properly cleaned*, temperature is measured and recorded by refrigerators and freezers. Also the critical parameters are measured.
- ✓ Production and Process Controls-
  - o The end results emphasizes *ensuring that no adulterated food enters marketplace.The terms used subject to variation in interpretation.*
  - o *The* raw materials and ingredients properly *inspected, analyzed, segregated, stored and handled.*
  - o manufacturing operations must be monitored
    - o *pH, water activity, temperatures*
    - o *elimination of metal from product*
  - o personnel should be trained and aware of GMP requirements
- ✓ Defect Action Levels
  - o natural or unavoidable defects may be in food
    - o *not harmful at levels present*
    - o *present even with GMPs*
  - o FDA establishes DALs when necessary and possible
  - o defect level may not be reduced by blending

Thus GMPs are Intended to prevent adulteration. Opportunity for considerable judgment in defining and interpreting regulations. *“spirit” of GMPs is to do what is reasonable and necessary to ensure safe and unadulterated food supply.*

Specific GMPs:

Low acid canned foods

- *Life threatening risk if improperly processed*
- Requires supervision of personnel who have been trained
- Regulations quite detailed for equipment design and operation
- Extensive record keeping requirements

Acidified foods:

- Defined as a low acid food with
  - A_w *greater than 0.85*
  - *acid added to lower pH to 4.6 or lower*
- Product examples
  - *includes beans, cucumbers, cabbage*
  - *excludes carbonated beverages*
- Personnel trained under approved program

Bottled Drinking Water:

- *All water sealed in bottles, packages for human consumption*
- Regulations are general and similar to umbrella GMPs
- Source of water must be approved
- Sanitation, equipment designed, personnel emphasized

Extensive record keeping

What is HACCP?

- The National Advisory Committee on Microbiological Criteria for Food (NACMCF) working group created guidelines and redefined the seven basic principles of HACCP as an effective and rational means of assuring food safety from harvest to consumption.
- The working group published the HACCP principles and application guideline document in August 1997.
- The hazard analysis critical control point system (HACCP) is a scientific and systematic way of enhancing the safety of foods from primary production to final consumption through the identification and evaluation of specific hazards and measures for their control to ensure the safety of food. HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing.
- Under the HACCP regulatory system, establishments assume full responsibility for producing products that are safe for consumers.

History of HACCP

- Developed by Pillsbury in 1959 as a nontesting approach to assure the safety level required by NASA for foods produced for the space program
- NASA’s major concerns • Food crumbs • Foodborne illness
- NASA’s Zero Defects program Testing materials
- National Research Council – 1985 • An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients
- Microbiological hazards not controlled by testing

- Recommended using HACCP for food safety assurance
- **National Advisory Committee on Microbiological Criteria for Food (NACMCF) 1988**
- NACMCF proposed 7 principles of HACCP application, Published in 1989;
- 1st. Revision in 1992; 2nd. Revision (latest) in 1997

PRINCIPLES OF THE HACCP SYSTEM

The seven principles of HACCP, which encompass a systematic approach to the identification, prevention, and control of food safety hazards include:

PRINCIPLE 1 Conduct a hazard analysis.

PRINCIPLE 2 Determine the Critical Control Points (CCPs).

PRINCIPLE 3 Establish critical limit(s).

PRINCIPLE 4 Establish a system to monitor control of the CCP.

PRINCIPLE 5 Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.

PRINCIPLE 6 Establish procedures for verification to confirm that the HACCP system is working effectively.

PRINCIPLE 7 Establish documentation concerning all procedures and records appropriate to these principles and their application.

APPLICATION

The application of HACCP principles consists of the following tasks as identified in the Logic Sequence
for Application of HACCP (Diagram 1).

1. Assemble HACCP team

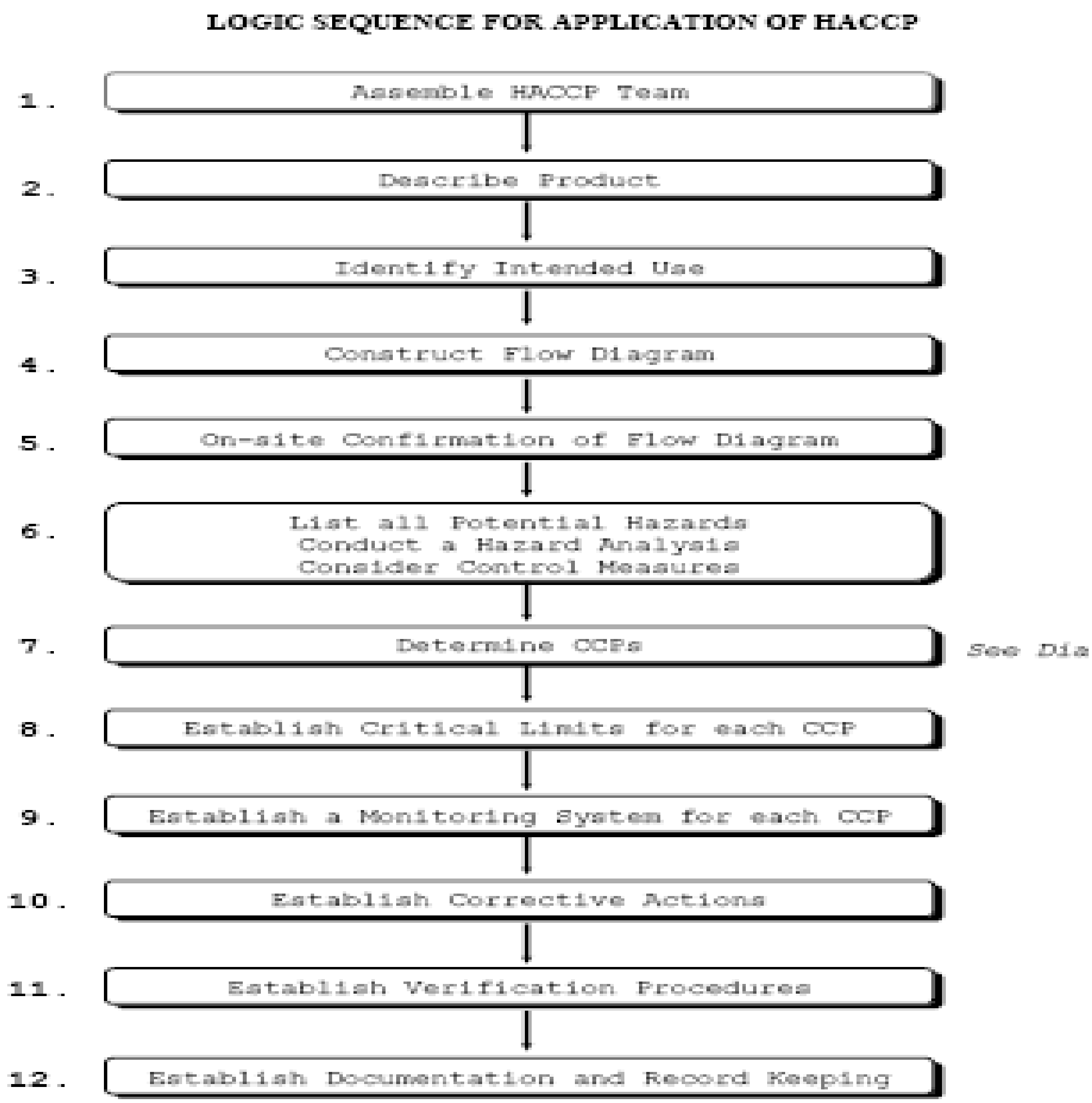
The food operation should assure that the appropriate product specific knowledge and expertise is
available for the development of an effective HACCP plan. Optimally, this may be accomplished by
assembling a multidisciplinary team. Where such expertise is not available on site, expert advice should
be obtained from other sources, such as, trade and industry associations, independent experts, regulatory
authorities, HACCP literature and HACCP guidance (including sector-specific HACCP guides). It may
be possible that a well-trained individual with access to such guidance is able to implement HACCP inhouse.

The scope of the HACCP plan should be identified. The scope should describe which segment
of the food chain is involved and the general classes of hazards to be addressed (e.g. does it cover all
classes of hazards or only selected classes).

2. Describe product

A full description of the product should be drawn up, including relevant safety information such as:
composition, physical/chemical structure (including Aw, pH, etc), microcidal/static treatments (heattreatment,
freezing, brining, smoking, etc), packaging, durability and storage conditions and method of

distribution. Within businesses with multiple products, for example, catering operations, it may be
effective to group products with similar characteristics or processing steps, for the purpose of development of the HACCP plan.



3. Identify intended use

The intended use should be based on the expected uses of the product by the end user or consumer. In
specific cases, vulnerable groups of the population, e.g. institutional feeding, may have to be considered.

4. Construct flow diagram

The flow diagram should be constructed by the HACCP team (see also paragraph 1 above). The flow
diagram should cover all steps in the operation for a specific product. The same flow diagram may be

used for a number of products that are manufactured using similar processing steps. When applying
HACCP to a given operation, consideration should be given to steps preceding and following the specified operation.

5. On-site confirmation of flow diagram

Steps must be taken to confirm the processing operation against the flow diagram during all stages and
hours of operation and amend the flow diagram where appropriate. The confirmation of the flow diagram should be performed by a person or persons with sufficient knowledge of the processing operation.

6. List all potential hazards associated with each step, conduct a hazard analysis, and consider any measures to control identified hazards

The HACCP team should list all of the hazards that may be reasonably expected to occur at each step according to the scope from primary production, processing, manufacture, and distribution until the point of consumption.

The HACCP team should next conduct a hazard analysis to identify for the HACCP plan, which hazards are of such a nature that their elimination or reduction to acceptable levels is essential to the production of a safe food.

In conducting the hazard analysis, wherever possible the following should be included:

• the likely occurrence of hazards and severity of their adverse health effects;
• the qualitative and/or quantitative evaluation of the presence of hazards;
• survival or multiplication of micro-organisms of concern;
• production or persistence in foods of toxins, chemicals or physical agents; and,
• conditions leading to the above.

Consideration should be given to what control measures, if any exist, can be applied to each hazard.

More than one control measure may be required to control a specific hazard(s) and more than one
hazard may be controlled by a specified control measure.

- ✓ A hazard is defined by NACMCF as a biological, chemical or physical agent that is **reasonably likely to occur**, and will **cause illness or injury in the absence of its control**. Establishments must consider all **three types of hazards – biological, chemical, and physical** – at each step of the production process.

7. Determine Critical Control Points

- ✓ A ***critical control point*** is defined as a point, step, or procedure in a food process at which control can be applied, and, as a result, a food safety hazard can be prevented, eliminated, or reduced to acceptable levels. Critical control points are locations in a process at which some aspect of control can be applied to control food safety hazards that have been determined reasonably likely to occur.
- ✓ Examples of CCPs include product temperature, certification of incoming product, microbiological testing, testing for foreign objects such as metal contamination, the chemical concentration of a carcass rinse or spray, and other such parameters.

There may be more than one CCP at which control is applied to address the same hazard. The determination of a CCP in the HACCP system can be facilitated by the application of a decision tree, which indicates a logic reasoning approach. Application of a decision tree should be flexible, given whether the operation is for production, slaughter, processing, storage, distribution or other. It should be used for guidance when determining CCPs. This example of a decision tree may not be applicable to all situations. Other approaches may be used. Training in the application of the decision tree is recommended.

If a hazard has been identified at a step where control is necessary for safety, and no control measure exists at that step, or any other, then the product or process should be modified at that step, or at any earlier or later stage, to include a control measure.

8. Establish critical limits for each CCP

Critical limits (CL) are the parameters that indicate whether the control measure at the CCP is in or out of control. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) states that a CL is **a maximum or minimum value** to which a biological, chemical, or physical parameter must be controlled at a CCP to prevent, eliminate, or reduce to an acceptable level the occurrence of a food safety hazard.

Critical limits must be specified and validated for each Critical Control Point. In some cases more than one critical limit will be elaborated at a particular step. Criteria often used include measurements of temperature, time, moisture level, pH, Aw, available chlorine, and sensory parameters such as visual appearance and texture.

Where HACCP guidance developed by experts has been used to establish the critical limits, care should be taken to ensure that these limits fully apply to the specific operation, product or groups of products under consideration. These critical limits should be measurable.

9. Establish a monitoring system for each CCP

Monitoring is the scheduled measurement or observation of a CCP relative to its critical limits. The monitoring procedures must be able to detect loss of control at the CCP. Further, monitoring should ideally provide this information in time to make adjustments to ensure control of the process to prevent violating the critical limits. Where possible, process adjustments should be made when monitoring results indicate a trend towards loss of control at a CCP. The adjustments should be taken before a deviation occurs. Data derived from monitoring must be evaluated by a designated person with knowledge and authority to carry out corrective actions when indicated. If monitoring is not continuous, then the amount or frequency of monitoring must be sufficient to guarantee the CCP is in control. Most monitoring procedures for CCPs will need to be done rapidly because they relate to online processes and there will not be time for lengthy analytical testing. Physical and chemical measurements are often preferred to microbiological testing because they may be done rapidly and can often indicate the microbiological control of the product.

All records and documents associated with monitoring CCPs must be signed by the person(s) doing the monitoring and by a responsible reviewing official(s) of the company.

10. Establish corrective actions

Specific corrective actions must be developed for each CCP in the HACCP system in order to deal with deviations when they occur.

The actions must ensure that the CCP has been brought under control. Actions taken must also include proper disposition of the affected product. Deviation and product disposition procedures must be documented in the HACCP record keeping.

The corrective actions consist of:

- ✓ Identifying and eliminating the cause of the deviation,
- ✓ Ensuring that the CCP is under control after the corrective action is taken,
- ✓ Ensuring that measures are established to prevent recurrence, and
- ✓ Ensuring that no product affected by the deviation is shipped.

11. Establish verification procedures

Establish procedures for verification. Verification and auditing methods, procedures and tests, including random sampling and analysis, can be used to determine if the HACCP system is working correctly. The frequency of verification should be sufficient to confirm that the HACCP system is working effectively.

Verification should be carried out by someone other than the person who is responsible for performing the monitoring and corrective actions. Where certain verification activities cannot be performed in house, verification should be performed on behalf of the business by external experts or qualified third parties.

Examples of verification activities include:

• Review of the HACCP system and plan and its records;
• Review of deviations and product dispositions;
• Confirmation that CCPs are kept under control.

Where possible, validation activities should include actions to confirm the efficacy of all elements of the HACCP system.

12. Establish Documentation and Record Keeping

Efficient and accurate record keeping is essential to the application of a HACCP system. HACCP

procedures should be documented. Documentation and record keeping should be appropriate to the
nature and size of the operation and sufficient to assist the business to verify that the HACCP controls
are in place and being maintained. Expertly developed HACCP guidance materials (e.g. sector-specific
HACCP guides) may be utilised as part of the documentation, provided that those materials reflect the
specific food operations of the business.

Documentation examples are:

Hazard analysis;

CCP determination;

Critical limit determination.

Record examples are:

• CCP monitoring activities;
• Deviations and associated corrective actions;
• Verification procedures performed;
• Modifications to the HACCP plan;

An example of a HACCP worksheet for the development of a HACCP plan is attached as Diagram 3.

A simple record-keeping system can be effective and easily communicated to employees. It may be
integrated into existing operations and may use existing paperwork, such as delivery invoices and
checklists to record, for example, product temperatures.

Benefits of HACCP

Although the adoption of HACCP systems worldwide is due primarily to the added food safety protection provided to consumers, there are other benefits to the food industry that can be realized by implementing a successful HACCP system.

a. Formally incorporates food safety principles as integral steps of production processes

HACCP recognition status cannot be completed without a firm commitment by senior management to formally support food safety control measures throughout the production process. The implementation and maintenance of those control measures play a critical role in raising awareness of front line production management and staff of the presence and importance of specific food safety procedures within their process.

b. Increased employees' ownership of the production of safe food

As a sign of this commitment, it is the responsibility of senior management to foster the idea within the facility that food safety is the responsibility of everyone. Through the process of developing and implementing a HACCP system, employees become more aware of food safety and their role in contributing to food safety. This increased knowledge leads to ownership of and pride in the production of a safe food product.

c. Increased buyer and consumer confidence

Establishments that have implemented a HACCP system provide buyers and consumers with a greater degree of confidence that the facility is producing a safe food product. Establishments can demonstrate by showing documents and records that food safety is under control.

d. Maintaining or increasing market access

Market forces continue to drive HACCP implementation throughout the food industry. In many cases, buyer demands and foreign governments require HACCP implementation to maintain market share and/or gain access to previously inaccessible markets. As HACCP systems are accepted worldwide, FSEP helps the Canadian industry to maintain and expand its international markets.

e. Reduced waste

The preventative nature of HACCP allows a company to control costs by minimizing the amount of product requiring rejection or recall, and by focusing resources on areas that have been identified as critical in the manufacture of a safe food product. With the regular monitoring inherent in a HACCP system, establishments become aware of problems earlier and the costs of waste are reduced.

Lecture plan

FOOD AND AGRICULTURAL MICROBIOLOGY (15MBU503)

SEMESTER – V **5H – 5C**

UNIT IV

S. No	Duration	Topic	Reference
1	1	Biological nitrogen fixation	R1: 29-32
2	1	Mechanism of nitrogen fixation	R1: 124-126
3	1	Symbiotic microorganism	R1: 166-173
4	1	Non-symbiotic microorganism	
5	1	Root nodule formation	
6	1	Nitrogen fixers	
7	1	Hydrogenase	R1: 196
8	1	Nitrogenase	
9	1	Nif genes	R1: 120-124
10	1	Regulation of nif genes	
11	1	Biochemistry of nitrogen fixation	
12	1	Interaction of microbes with plants	
13	1	Tutorial hour	
14	1	Unit Revision	
Total Hrs: 14			

R1: Subba Rao. NS. Soil Microbiology

2017

FOOD AND AGRICULTURAL MICROBIOLOGY

Prepared by P. Akilandeswari, Assistant Professor, Dept of Microbiology, Karpagam University

Unit – 4

Nitrogen fixation is a process in which nitrogen (N_2) in the atmosphere is converted into ammonia (NH_3). Atmospheric nitrogen or molecular dinitrogen (N_2) is relatively inert; it does not easily react with other chemicals to form new compounds.

Nitrogen fixation, natural and synthetic, is essential for all forms of life because nitrogen is required to biosynthesize basic building blocks of plants, animals and other life forms, e.g., nucleotides for DNA and RNA, the coenzyme nicotinamide adenine dinucleotide for its role in metabolism (transferring electrons between molecules), and amino acids for proteins. Therefore, as part of the nitrogen cycle, it is essential for agriculture and the manufacture of fertilizer. It is also an important process in the manufacture of explosives (e.g. gunpowder, dynamite, TNT, etc.).

Nitrogen fixation occurs naturally in the soil by nitrogen fixing bacteria affiliated with some plants (for example, *Azotobacter* and legumes). Some nitrogen-fixing bacteria have very close relationships with plants, referred to as symbiotic nitrogen fixation.

Biological nitrogen fixation

Biological nitrogen fixation was discovered by the German agronomist Hermann Hellriegel and Dutch Microbiologist Martinus Beijerinck. Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called a nitrogenase. All biological nitrogen fixation is done by the way of nitrogenase metallo enzymes which contain iron, molybdenum, or vanadium. Microorganisms that can fix nitrogen are prokaryotes (both bacteria and archaea, distributed throughout their respective kingdoms) called diazotrophs. Some higher plants, and some animals (termites), have formed associations (symbiosis) with diazotrophs.

The overall reaction for BNF is,

$$N_2 + 8\,H^+ + 8\,e^- \rightarrow 2\,NH_3 + H_2$$

The process is coupled to the hydrolysis of 16 equivalents of ATP and is accompanied by the co-formation of one molecule of H_2. The conversion of N_2 into ammonia occurs at a cluster called FeMoco, an abbreviation for the iron-molybdenum cofactor. The mechanism proceeds via a series of protonation and reduction steps wherein the FeMoco active site hydrogenates the N_2 substrate.

In free-living diazotrophs, the nitrogenase-generated ammonium is assimilated into glutamate through the glutamine synthetase/glutamate synthase pathway. The microbial genes required for nitrogen fixation are widely distributed in diverse environments. Enzymes responsible for nitrogenase action are very susceptible to destruction by oxygen. For this reason, many bacteria cease production of the enzyme in the presence of oxygen. Many nitrogen-fixing organisms exist only in anaerobic conditions, respiring to draw down oxygen levels, or binding the oxygen with a protein such as leghemoglobin.

Microorganisms that fix nitrogen

Diazotrophs are a diverse group of prokaryotes that includes cyanobacteria (e.g. the highly significant *Trichodesmium* and *Cyanothece*), green sulfur bacteria, and diazotrophs Azotobacteraceae, rhizobia and *Frankia*.

Cyanobacteria inhabit nearly all illuminated environments on Earth and play key roles in the carbon and nitrogen cycle of the biosphere. In general, cyanobacteria are able to utilize a variety of inorganic and organic sources of combined nitrogen, like nitrate, nitrite, ammonium, urea, or some amino acids. Several cyanobacterial strains are also capable of diazotrophic growth, an ability that may have been present in their last common ancestor in the Archean eon. Nitrogen fixation by cyanobacteria in coral reefs can fix twice the amount of nitrogen as on land—around 1.8 kg of nitrogen is fixed per hectare per day (around 660 kg/ha/year). The colonial marine cyanobacterium *Trichodesmium* is thought to fix nitrogen on such a scale that it accounts for almost half of the nitrogen fixation in marine systems on a global scale.

Symbiotic Nitrogen Fixation

Symbiosis is a close and often long-term interaction between two different biological species. Symbiotic nitrogen fixation occurs in plants that harbor nitrogen-fixing bacteria within their tissues. The best-studied example is the association between legumes and bacteria in the genus Rhizobium. Each of these is able to survive independently (soil nitrates must then be available to the legume), but life together is clearly beneficial to both. Only together can nitrogen fixation take place.

Rhizobium

Rhizobium is Gram-negative bacilli that live freely in the soil (especially where legumes have been grown). However, they cannot fix atmospheric nitrogen until they have invaded the roots of the appropriate legume.

Infection

The interaction between a particular strain of rhizobia and the "appropriate" legume is mediated by a Nod factor secreted by the rhizobia and transmembrane receptors on the cells of the root hairs of the legume. Different strains of rhizobia produce different Nod factors, and different legumes produce receptors of different specificity. The bacteria enter an epithelial cell of the root; then migrate into the cortex. Their path runs within an intracellular channel that grows through one cortex cell after another. This infection thread is constructed by the root cells, not the bacteria, and is formed only in response to the infection. When the infection thread reaches a cell deep in the cortex, it bursts and the rhizobia are engulfed by endocytosis into membrane-enclosed symbiosomes within the cytoplasm. The cortex cells then begin to divide rapidly forming a nodule. The rhizobia also go through a period of rapid multiplication within the nodule cells. Then they begin to change shape and lose their motility. The bacteroide almost fill the cell and nitrogen fixation begins. Thus the development of nodules, while dependent on rhizobia, is a well-coordinated developmental process of the plant.

Non symbiotic nitrogen fixation

Azotobacter

Azotobacter species are Gram-negative bacteria found in neutral and alkaline soils, in water, and in association with some plants. They are aerobic, free-living soil microbes which play an important role in the nitrogen cycle in nature, binding atmospheric nitrogen and releasing it in the form of ammonium ions into the soil (nitrogen fixation). *Azotobacter* species are free-living, nitrogen-fixing bacteria; in contrast to *Rhizobium* species, they normally fix molecular nitrogen from the atmosphere without symbiotic relations with plants, although some *Azotobacter* species are associated with plants. Nitrogen fixation is inhibited in the presence of available nitrogen sources, such as ammonium ions and nitrates.

Azotobacter species have a full range of enzymes needed to perform the nitrogen fixation are ferredoxin, hydrogenase, and an important enzyme nitrogenase. Nitrogenase is the most important enzyme involved in nitrogen fixation. *Azotobacter* species have several types of nitrogenase. The basic one is molybdenum-iron nitrogenase. Nitrogen fixation plays an important role in the nitrogen cycle. *Azotobacter* also synthesizes some biologically active substances, including some phytohormones such as auxins, thereby stimulating plant growth.

They also facilitate the mobility of heavy metals in the soil, thus enhancing bioremediation of soil from heavy metals, such as cadmium, mercury and lead.

Applications

Owing to their ability to fix molecular nitrogen and therefore increase the soil fertility and stimulate plant growth. *Azotobacter* species are widely used in agriculture, particularly in nitrogen biofertilizers such as azotobacterin.

Azospirillium

Azospirillum species are described as Gram negative, rod-shaped, very motile. The nitrogen sources used by Azospirillum for their growth are ammonium, nitrate, amino acids and elemental nitrogen. *Azospirillum* spp. is highly adaptable, being able to grow under anaerobic conditions (nitrate used as eletron acceptor), Microaerobic (elemental or ammonia used as N source), fully aerobic conditions (ammonia, nitrate, amino acid or combined N only).

Azospirillum as a "biofertilizer" is particularly important in agricultural systems where fertilizer inputs are either impractical (rangelands), undesirable (organic farming), or not possible (subsistence agriculture). Experiments on inoculation of crops with *Azospirillum* or other diazotrophs often resulted in enhanced plant growth or nitrogen content under environmental conditions, improve nutrient assimilation, alter root size and function.

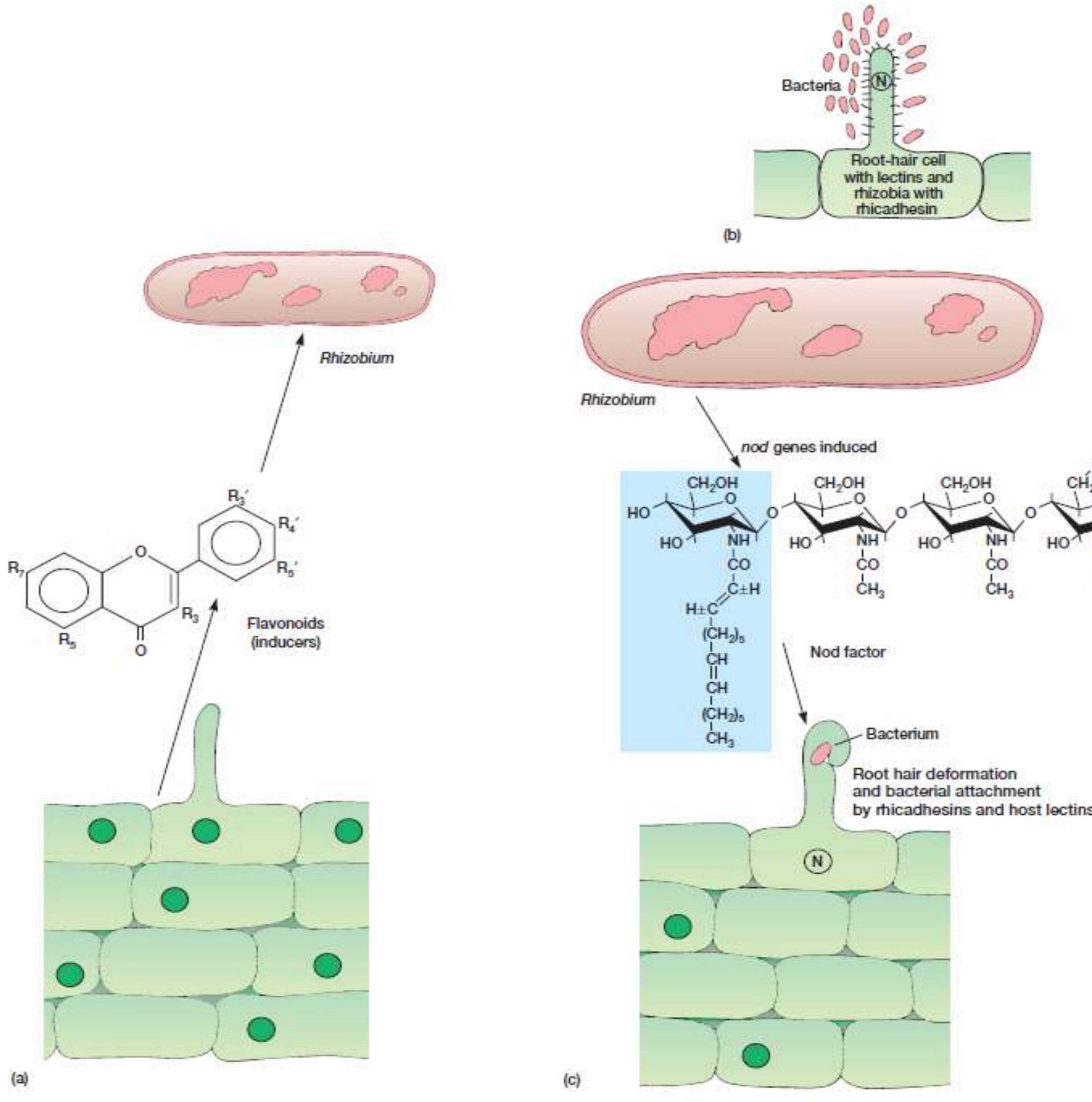
Azospirillum results in the following benefits,

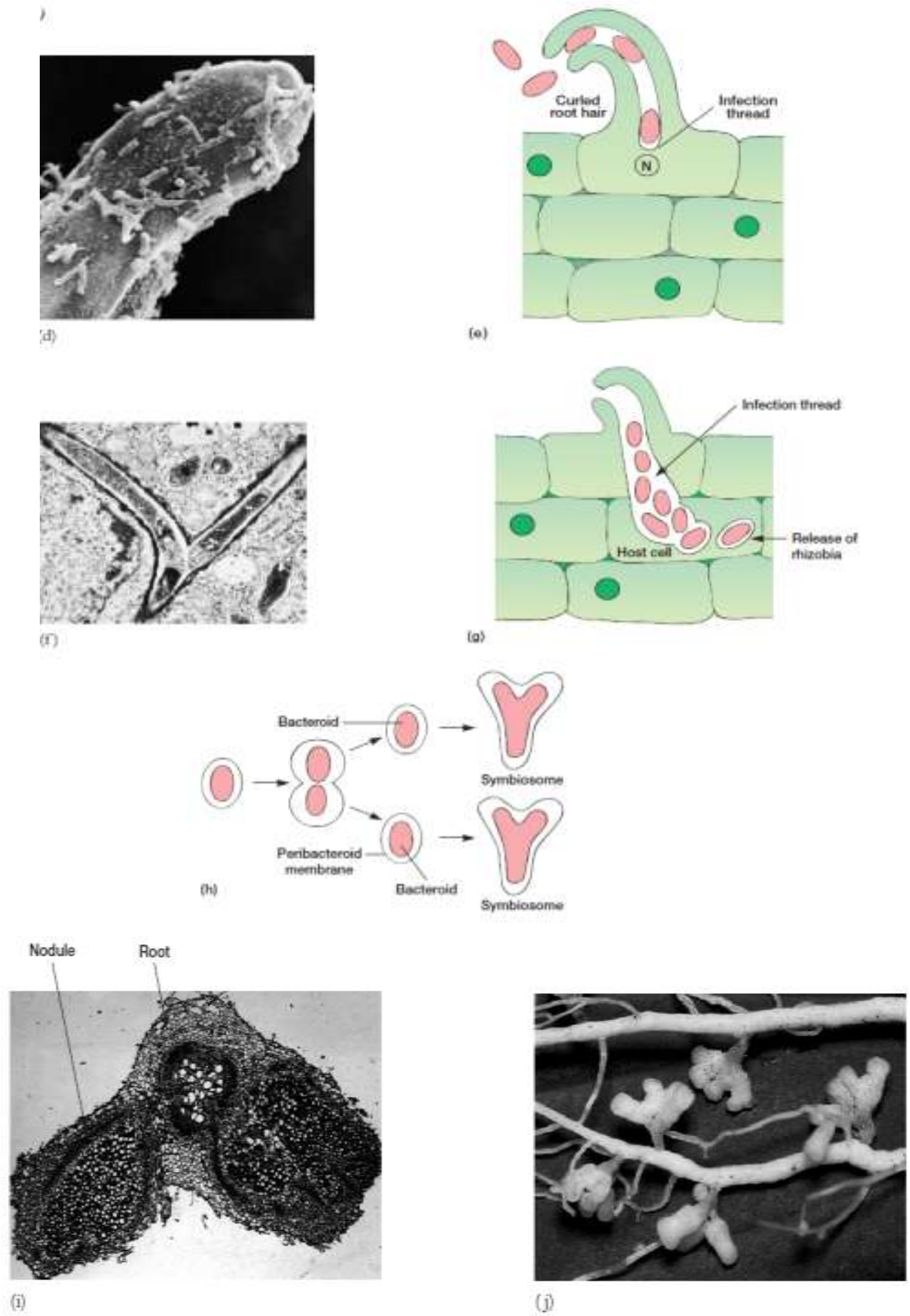
1. Promotion of root hair development and branching.
2. Increased uptake of N, P, K and microelements.
3. Improved water status of plants and
4. Increased dry matter accumulation and grain yield.

Root nodule formation

This complex infection process appears to involve a series of molecules produced by the host plant that lead to the exchange of recognition signals. Flavonoid inducers produced by the plant play a major role in this process by stimulating the *Rhizobium* to synthesize specific Nod factors that activate the host symbiotic processes necessary for root hair infection and nodule development. After bacterial attachment, the root hairs curl and the bacteria induce the plant to form an infection thread that grows down the root hair. The *Rhizobium* then spreads within the infection thread into the underlying root cells as noted in figure 30.8*e.* At no time does the *Rhizobium* actually enter the plant cytoplasm while it is in the infection thread! When the bacteria are released from the infection thread into the host cell, the *Rhizobium* is enclosed by a plant-derived membrane, called the peribacteroid membrane, to form a bacteroid. Further growth and differentiation lead to the development of a nitrogen-fixing form, a structure called a symbiosome. At this point, specific nodule components such as leghemoglobin, which protect the nitrogen fixation enzymes from oxygen, are produced to complete the nodulation process.

The symbiosomes within mature root nodules are the site of nitrogen fixation. Within these nodules, the differentiated bacteroids reduce atmospheric N2 and form ammonia (the primary product) and alanine; these compounds are released into the host plant cell, assimilated into various other nitrogen-containing organic compounds, and distributed throughout the plant. Because reduced nitrogen is the nutrient most commonly limiting plant growth, biological nitrogen fixation, as exemplified by the *Rhizobium*- legume symbiosis, is of major importance to agricultural productivity and the biogeochemical nitrogen cycle needed to sustain life on Earth.


Bacteria
N
Root-hair cell with lectins and rhizobia with rhicadhesin
(b)
Rhizobium
Flavonoids (inducers)
(a)
Rhizobium
nod genes induced
Nod factor
Bacterium
Root hair deformation and bacterial attachment by rhicadhesins and host lectins
N
(c)



Root nodule formation by Rhizobium

Nitrogenase

Nitrogenases are enzymes that are produced by certain bacteria, such as Cyanobacteria (blue-green algae). These enzymes are responsible for the reduction of nitrogen (N_2) to ammonia (NH_3). Nitrogenases are the only family of enzymes known to catalyze this reaction, which is a key step in the process of nitrogen fixation. Nitrogen fixation is required for all forms of life, with nitrogen being essential for the biosynthesis of molecules (nucleotides, amino acids) that create plants, animals and other organisms.

The nitrogenase complex consists of two proteins: The homodimeric Fe protein, a reductase which has a high reducing power and is responsible for the supply of electrons. The heterotetrameric MoFe protein, a nitrogenase which uses the electrons provided to reduce N_2 to NH_3.

Organisms that synthesize nitrogenase

There are two types of bacteria that synthesize nitrogenase and are required for nitrogen fixation. These are:

1. Free-living bacteria (non-symbiotic), examples include, Cyanobacteria (blue-green algae), Green sulfur bacteria, *Azotobacter*.
2. Mutualistic bacteria (symbiotic), examples include, *Rhizobium*, associated with leguminous plants, *Spirillum*, associated with cereal grasses and *Frankia*.

Hydrogenase

A hydrogenase is an enzyme that catalyses the reversible oxidation of molecular hydrogen. Most of these species are microbes and their ability to use H_2 as a metabolite arises from the expression of H_2 metalloenzymes known as hydrogenases. Hydrogenases are sub-classified into three different types based on the active site metal content: iron-iron hydrogenase, nickel-iron hydrogenase, and iron hydrogenase.

***Nif* gene**

The *nif* genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen into a form of nitrogen available to living organisms. The primary enzyme encoded by the *nif* genes is the nitrogenase complex which is in charge of converting atmospheric nitrogen (N_2) to other nitrogen forms such as ammonia which the organism can use for various purposes. Besides the nitrogenase enzyme, the *nif* genes also encode a number of regulatory proteins involved in nitrogen fixation. The *nif* genes are found in both free-living nitrogen-fixing bacteria and in symbiotic bacteria associated with various plants. The expression of the *nif* genes is induced as a response to low concentrations of fixed nitrogen and oxygen concentrations (the low oxygen concentrations are actively maintained in the root environment of host plants). The first Rhizobium genes for nitrogen fixation (nif) and for nodulation (nod) were cloned in the early 1980s by Gary Ruvkun and Sharon R. Long. In most bacteria, regulation of *nif* genes transcription is done by the nitrogen sensitive NifA protein. The *nif* genes can be found on bacterial chromosomes, but in symbiotic bacteria they are often found on plasmids. Example of *nif* gene is *Rhizobium* spp. Gram-negative, symbiotic nitrogen fixing bacteria that usually form a symbiotic relationship with legume species. In some rhizobia, the *nif* genes are located on plasmids called 'sym plasmids' (sym = symbiosis) which contain genes related to nitrogen fixation and metabolism, while the chromosomes contain most of the housekeeping genes of the bacteria. Regulation of the *nif* genes is at the transcriptional level and is dependent on colonization of the plant host.

Lecture plan

FOOD AND AGRICULTURAL MICROBIOLOGY (15MBU503)

SEMESTER – V **5H – 5C**

UNIT V

S. No	Duration	Topic	Reference
1	1	Biofertilizer – Introduction	R1: 166-172
2	1	Biofertilizer – Rhizobium	
3	1	Azospirillium	R1: 133-135
4	1	Azotobacter	R1: 116-128
5	1	Phosphobacteria	
6	1	Plant growth promoting rhizobacteria	R1: 103-105
7	1	Blue green algae	R1: 151-165
8	1	Azolla	R1:160-163
9	1	Production of biofertilizer	R1: 381
10	1	Quality control of biofertilizer	
11	1	Field application of biofertilizer	
12	1	Crop response	
13	1	Tutorial hour	
14	1	Unit Revision	
Total Hrs: 14			

R1: Subba Rao. NS. Soil Microbiology

2017

FOOD AND AGRICULTURAL MICROBIOLOGY

Prepared by - P. Akilandeswari, Asst Professor, Dept of Microbiology, KAHE

Unit – 5

Biofertilizer

Biofertilizer is defined as the microbial inoculation containing living cells of efficient strain of microorganisms such as cellulolytic N2-fixing or phosphate solubilizing microbes. Biofertilizers increases the fertility of the soil and thus enhances the growth of plants. Biofertilizers are used to reduce the use of chemical fertilizers in agriculture. Chemical fertilizers are much harmful to man, whereas the biofertilizers are harmless. The microbial conversion is of two type namely simple organic conversion and complex conversion. In simple conversion, the insoluble organic substances are directly converted into organic acids or nitrogenase compounds in the soil. In complex reactions, the conversion is carried out by a series of reactions catalyzed by a number of enzymes produced by microorganisms.

Rhizobium

Rhizobium inoculum:

Rhizobium inoculum contains the viable cells of Rhizobium which fixes the atmospheric nitrogen when the roots of higher leguminous plants are injected by Rhizobium.

Isolation of Rhizobium:

The leguminous plants are uprooted and tested of any nodule is present in the root. The root nodules which are brown to pink green in color are washed in water in order to eradicate the soil particles. Then a pinkish green nodule is selected and washed in distilled water. The washed root nodule is kept immersed in 0.1 acidified Kcl solution for 5 min. This Kcl is used as a disinfectant to sterilize the contaminants found on the surface of the nodule. Then again wash the nodule to remove the disinfectant. Finally the nodule is immersed in ethyl alcohol and later washed with sterile H_2O. The Rhizobium is isolated either by washing the nodule in pestle and morter or by cutting the nodule and streaking. The washed juice is collected by a sieve and serially diluted and plated. The nodule is streaked in a solid media to obtain proper growth of the bacteria. The media used for the growth of Rhizobium is yeast extract mannitol agar medium. The rhizobial cells from the culture are identified and mass cultured for the preparation of inoculum. The correct strain of Rhizobia is identified by nodule formation, cultural tests, microscopic observation and staining techniques.

Mass culture of Rhizobium:

The selected rhizobial strain is cultured in YEMA medium for about 7 days in order to establish better growth. The Rhizobium culture is tested. The tested Rhizobial culture is transferred to a large container containing the sterile YEMA medium are incubated at 30 ° C for 9 days. Sufficient nutrients should be supplied at regular intervals of 24hrs. The rhizobial culture is checked to detect the presence of contaminants in the culture. pH of the medium and the growth rate are used to determine the presence of contaminants in the culture.

Carrier- based inoculum for storage:

The cultured Rhizobial cells can be added to the carrier and used to preserve the inoculum in a viable condition

Field Application:

1. The cultured Rhizobium is diluted with H_2O and applied on seeds. The suspension is sprinkled over the seeds. Sucrose solution (10%) is used to enhance the surviving potential of Rhizobium on the seed coats.
2. Inoculum is diluted with H_2O and slurry is uniformly mixed with seeds. Then the inoculum is pellatized on the seed coats. The inoculum is protected from the agricultural chemicals and acids and alkaline reaction of the soil. Thus the inoculum is spread over the field along with the seeds during sowing.
3. Pelleting agents like dolomite, gypsum, charcoal rock phosphates are used along with the inoculum. They increase the sedimentation potential of the inoculum on the surface of seeds. It protects the seeds from winter season.

4. The inoculum is stored at 4 °C in a refrigerator. The stored inoculum is sprayed over the soil directly to increase the fertility of the soil.

Azotobacter

Azotobacter a soil habitant bacteria is a free living, nonsymbiotic Nitrogen fixing bacteria.

Inoculation:

The soil sample is collected and mixed with nitrogen free medium to prepare a paste of soil slurry. The slurry is diluted serially with the liquid medium to bring the growth of Azotobacter colonies. A mucoid colony on the agar medium was obtained after the incubation of culture for 3 days at 30 °C. The selected strain is mass-cultured in a large container Janson's medium.

Carrier based medium:

Powdered peat soil, lignite are used as carriers. The Azotobacter prefers 4 °C for its long term storage. Sometimes the powdered carriers are neutralized with CaCo3 and autoclaved for proper sterilization. This is mixed with culture and dried in air before storage.

Applications:

1. Seed treatment:

   The cultured inoculum is diluted with H2O and the seeds are kept dipped in the inoculum for one night. This seeds are sown in the main field. The slurry is directly poured over the nursery bed or in agricultural field.

   The seeds are spread on a polythene bag and the inoculum is sprinkled over the seeds for the mixing of the inoculum with the seeds. The inoculum-coated seeds are then dried in the air before sowing

2. Seedling treatment:

   In this method, the inoculum in diluted with the H2O and the roots of the seedlings are kept dipped in the inoculum for about 10-15 min. Paddy field gets benefited by this process.

3. In paddy field:

   A required amount of inoculum is mixed with farmyard manure. Then this mixture is properly mixed with soil. The resulting carrier based inoculum is directly used in the cultivation of rice.

   Azotobacter synthesis biologically active substances such as nicotinic acid, panthothenic acid, pyridoxine, biotin, giberellic acid. These are plant growth promoting substances (PGPS). Azotobacter provides a favorable micro environment to the root system of higher plants and induces the better growth of the roots which participates in the growth of root systems in higher plants.

Az*ospirillum*

Azospirillum, a free-living nitrogen-fixing bacteria closely associated with grasses. *Azospirillum* Bacteria is a Gram negative motile bacteria belonging to the order Rhodospirillales, associated with roots of monocots, including important crops, such as wheat, corn and rice. *Azospirillum* contains 109/gm spores of *Azospirillum* species. This *Azospirillum* bacterium fixes the atmospheric nitrogen and makes it available to plants in non-symbiotic manner that can replace 50-90% of the nitrogen fertilizer required by plants. *Azospirillum* biofertilizer also secretes some fungicides, enzymes but in minute amount. Use of *Azospirillum* biofertilizer increases the crop production in large scale. *Azospirillum* is mainly useful for monocot vegetables. *Azospirillum* is an eco-friendly liquid biological fertilizer formulation containing bacteria, *Azospirillum* which contain large amount of lipid granules, which enters the cortical cells of the root and fix up atmospheric nitrogen and also produces biologically active substances like vitamins, nicotinic acid, in dole acetic acid, gibberellins etc and helps in better retention of flowers and enhances the plant growth.

Inoculation Techniques

Azospirillum spp. can be inoculated directly on the seed surface or in the soil. Seed applications greatly outnumber soil applications. This happens because it is easy to use and requires a relatively small amount of inoculants because *Azospirillum* do not survive well in soils.

Application:

- Use for the non-leguminous crops before the mentioned expiry date.
- Mix the inoculants uniformly with the seeds gently with the minimum amount of water taking care to avoid damage to seed coat. Dry the inoculated seeds under shade over clean paper or gunny bag and sow immediately.
- For transplanted crops: Mix the inoculants in bucket of water stir the mixture vigorously. Dip the roots of seedlings in this mixture before transplanting. Transplant as usual.
- It can be mixed with pit mixture before planting of vegetables / fruit crop.
- If the seed is to be treated with pesticides; first follow the pesticide treatments and finally treat seeds with *Azospirillum* inoculant.

Dosage:

Use 15 to 20 gm / kg of seed, 1 to 2 kg for soil application per acre of land, 1 kg for root application (root dipping) of one acre of crop.

Blue green algae (BGA)

BGA fixes nitrogen in the soil. BGA such as *Anabena*, *Polypothium*, *Oscillotrian* actively fixes the nitrogen in soil. The BGA induces the growth of higher plants with the help vitamin B12, auxins etc. Thus they form an effective biofertilizer in agriculture. The blue green algal inoculum may be produced by several methods viz., in tubs, galvanized trays, and small pits and also in field conditions. However the large-scale production is advisable under field condition which is easily adopted by farmers.

Multiplication in trays

- Big metallic trays (6'x 3'x 6"lbh) can be used for small scale production
- Take 10 kg of paddy field soil, dry powder well and spread
- Fill water to a height of 3"
- Add 250 g of dried algal flakes (soil based) as inoculum
- Add 150 g of super phosphate and 30 g of lime and mix well with the soil
- Sprinkle 25 g carbofuran to control the insects
- Maintain water level in trays
- After 10 to 15 days, the blooms of BGA will start floating on the water sources
- At this stage stop watering and drain. Let the soil to dry completely
- Collect the dry soil based inoculum as flakes
- Store in a dry place. By this method 5 to 7 kg of soil based inoculum can be obtained.

Multiplication under field condition

Materials

- Rice field
- Super phosphate
- Carbofuran
- Composite BGA starter culture

Procedure

Select an area of 40 m2 (20m x 2m) near a water source which is directly exposed to sunlight. Make a bund all around the plot to a height of 15 cm and give it a coating with mud to prevent loss of water due to percolation.

- Plot is well prepared and levelled uniformly and water is allowed to a depth of 5-7.5 cm and left to settle for 12 hrs.

- Apply 2 kg of super phosphate and 200 g lime to each plot uniformly over the area.
- The soil based composite starter culture of BGA containing 8-10 species @ 5 kg / plot is powdered well and broadcasted.
- Carbofuran at 200 g is also applied to control soil insects occurring in BGA.
- Water is let in at periodic intervals so that the height of water level is always maintained at 5 cm.
- After 15 days of inoculation, the plots are allowed to dry up in the sun and the algal flakes are collected and stored.

Observations

The floating algal flasks are green or blue green in colour. From each harvest, 30 to 40 kg of dry algal flakes is obtained from the plot.

Method of inoculation of BGA in rice field

Blue green algae may be applied as soil based inoculum to the rice field following the method described below.

- Powder the soil based algal flakes very well.
- Mix it with 10 kg soil or sand (10kg powdered algal flakes with 10 kg soil / sand).
- BGA is to be inoculated on 7-10 days after rice transplanting.
- Water level at 3-4 is to be maintained at the time of BGA inoculation and then for a month so as to have maximum BGA development.

Observation

A week after BGA inoculation, algal growth can be seen and algal mat will float on the water after 2-3 weeks. The algal mat colour will be green or brown or yellowish green.

Azolla

Azolla is a free-floating water fern that floats in water and fixes atmospheric nitrogen in association with nitrogen fixing blue green alga *Anabaena azollae*. *Azolla* fronds consist of sporophyte with a floating rhizome and small overlapping bi-lobed leaves and roots. Rice growing areas in South East Asia and other third World countries have recently been evincing increased interest in the use of the symbiotic N2 fixing water fern. *Azolla* either as an alternate nitrogen sources or as a supplement to commercial nitrogen fertilizers. *Azolla* is used as biofertilizer for wetland rice and it is known to contribute 40 - 60 kg N ha-1 per rice crop. The agronomic potential of *Azolla* is quite significant particularly for rice crop and it is widely used as biofertilizer for increasing rice yields. Rice crop response studies with *Azolla* biofertilizer in the People's Republic in China and in Vietnam have provided good evidence that *Azolla* incorporation into the soil as a green manure crop is one of the most effective ways of providing nitrogen source for rice.

The utilization of *Azolla* as dual crop with wetland rice is gaining importance in Philippines, Thailand, Srilanka and India. The important factor in using *Azolla* as a biofertilizer for rice crop is its quick decomposition in soil and efficient availability of its nitrogen to rice. In tropical rice soils the applied *Azolla* mineralizes rapidly and its nitrogen is available to the rice crop in very short period. The common species of *Azolla* are *A. microphylla, A. filiculoides, A. pinnata, A. caroliniana, A. nilotica, A. rubra* and *A. mexicana*.

Mass multiplication of *Azolla* under field conditions

A simple *Azolla* nursery method for large scale multiplication of *Azolla* in the field has been evolved for easy adoption by the farmers.

Materials

- One cent (40 sq. m) area plot
- Cattle dung
- Super phosphate
- Furadan
- Fresh *Azolla* inoculum

Procedure

- Select a wetland field and prepare thoroughly and level uniformly.
- Mark the field into one cent plots (20 x 2m) by providing suitable bunds and irrigation channels.
- Maintain water level to a height of 10 cm.
- Mix 10 kg of cattle dung in 20 litres of water and sprinkle in the field.
- Apply 100 g super phosphate as basal dose.
- Inoculate fresh *Azolla* biomass @ 8 kg to each pot.
- Apply super phosphate @ 100 g as top dressing fertilizer on 4th and 8th day after *Azolla* inoculation.
- Apply carbofuran (furadan) granules @ 100 g/plot on 7th day after *Azolla* inoculation.
- Maintain the water level at 10 cm height throughout the growth period of two or three weeks.
- Observations
- Note the *Azolla* mat floating on the plot. Harvest the *Azolla*, drain the water and record the biomass.

Method of inoculation of *Azolla* to rice crop

The *Azolla* biofertilizer may be applied in two ways for the wetland paddy. In the first method, fresh *Azolla* biomass is inoculated in the paddy field before transplanting and incorporated as green manure. This method requires huge quantity of fresh *Azolla*. In the other method, *Azolla* may be inoculated after transplanting rice and grown as dual culture with rice and incorporated subsequently.

***Azolla* biomass incorporation as green manure for rice crop**

- Collect the fresh *Azolla* biomass from the *Azolla* nursery plot.
- Prepare the wetland well and maintain water just enough for easy incorporation.
- Apply fresh *Azolla* biomass (15 t ha-1) to the main field and incorporate the *Azolla* by using implements or tractor.

***Azolla* inoculation as dual crop for rice**

- Select a transplanted rice field.
- Collect fresh *Azolla* inoculum from *Azolla* nursery.
- Broadcast the fresh *Azolla* in the transplanted rice field on 7th day after planting (500 kg / ha).
- Maintain water level at 5 -7.5cm.
- Note the growth of *Azolla* mat four weeks after transplanting and incorporate the *Azolla* biomass by using implements or tranctor or during inter-cultivation practices.
- A second bloom of *Azolla* will develop 8 weeks after transplanting which may be incorporated again.

By the two incorporations, 20 - 25 tonnes of *Azolla* can be incorporated in one hectare rice field.

Plant Growth Promoting Rhizobacteria (PGPR)

Plant Growth Promoting Rhizobacterial Forms

Plant growth promoting rhizobacteria can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPR). The ePGPRs may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex while iPGPRs locates generally inside the specialized nodular structures of root cells. The bacterial genera such as *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcous*, *Pseudomonas* and *Serratia* belongs to ePGPR. The iPGPR belongs to the family of Rhizobiaceae includes *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium*, endophytes and *Frankia* species both of which can symbiotically fix atmospheric nitrogen with the higher plants.

Plant Growth Promotion: Mechanism of Action

Plant growth promotion by plant growth promoting rhizobacteria is a well-known phenomenon and this growth enhancement is due to certain traits of rhizobacteria. There are a number of mechanisms used by PGPR for enhancing plant growth and development in diverse environmental conditions. According to Kloepper and Schroth plant growth promoting rhizobacteria mediated plant growth promotion occurs by the alteration of the whole microbial community in rhizosphere niche through the production of various substances. Generally, plant growth promoting rhizobacteria promote plant growth directly by either often due to their ability for nutrient supply (nitrogen, phosphorus, potassium and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents, root colonizers, and environmental protectors.

Direct mechanisms

Plant growth promoting rhizobacteria having direct mechanisms that facilitate nutrient uptake or increase nutrient availability by nitrogen fixation, solubilization of mineral nutrients, mineralize organic compounds and production of phytohormones.

Nitrogen fixation: Nitrogen is an essential element for all forms of life and it is the most vital nutrient for plant growth and productivity. Although the nitrogen presents 78 % of the atmosphere, it remains unavailable to the plants. Regrettably no plant species is capable for fixing atmospheric dinitrogen into ammonia and expend it directly for its growth. Thus the atmospheric nitrogen is converted into plantutilizable forms by biological nitrogen fixation (BNF) which changes nitrogen to ammonia by nitrogen fixing microorganisms using a complex enzyme system known as nitrogenase. Plant growth promoting rhizobacteria have the ability to fix atmospheric nitrogen and provide it to plants by two mechanisms: symbiotic and non-symbiotic. Symbiotic nitrogen fixation is a mutualistic relationship between a microbe and the plant. The microbe first enters the root and later on form nodules in which nitrogen fixation occurs. Rhizobia are a vast group of rhizobacteria that have the ability to lay symbiotic interactions by the colonization and formation of root nodules with leguminous plants, where nitrogen is fixed to ammonia and make it available for the plant. The plant growth promoting rhizobacteria widely presented as symbionts are *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, and *Mesorhizobium* with leguminous plants, *Frankia* with non-leguminous trees and shrubs. On the other hand, non-symbiotic nitrogen fixation is carried out by free living diazotrophs and this can stimulate non-legume plants growth such as radish and rice. Non-symbiotic Nitrogen fixing rhizospheric bacteria belonging to genera including *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas* and cyanobacteria (*Anabaena*, *Nostoc*). The genes for nitrogen fixation, called *nif* genes are found in both symbiotic and free living systems. Nitrogenase (*nif*) genes include structural genes, involved in activation of the Fe protein, iron molybdenum cofactor biosynthesis, electron donation, and regulatory genes required for the synthesis and function of the enzyme. Inoculation by biological nitrogen fixing plant growth promoting rhizobacteria on crop provide an integrated approach for disease management, growth promotion activity, maintain the nitrogen level in agricultural soil.

Phosphate solubilization: Phosphorus is the most important key element in the nutrition of plants, next to nitrogen (N). It plays an important role in virtually all major metabolic processes in plant including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration. It is abundantly available in soils in both organic and inorganic forms. Plants are unable to utilized phosphate because 95-99%

phosphate present in the insoluble, immobilized, and precipitated form. Plants absorb phosphate only in two soluble forms, the monobasic (H_2PO_4) and the diabasic ($HPO_4{}^{2-}$) ions. Plant growth promoting rhizobacteria present in the soil employ different strategies to make use of unavailable forms of phosphorus and in turn also help in making phosphorus available for plants to absorb. The main phosphate solubilization mechanisms employed by plant growth promoting rhizobacteria include: (1) release of complexing or mineral dissolving compounds e.g. organic acid anions, protons, hydroxyl ions, CO_2, (2) liberation of extracellular enzymes (biochemical phosphate mineralization) and (3) the release of phosphate during substrate degradation (biological phosphate mineralization). Phosphate solubilizing PGPR included in the genera *Arthrobacter*, *Bacillus*, *Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium, Rhodococcus*, and *Serratia* have attracted the attention of agriculturists as soil inoculums to improve plant growth and yield.

Application of Biofertilizers

- Seed treatment or seed inoculation
- Seedling or seed inoculation
- Main field application.

Seed treatment

One packet of the inoculant is mixed with 200 ml of rice kanji to make slurry. The seeds required for an acre are mixed in the slurry so as to have a uniform coating of the inoculant over the seeds and then shade dried for 30 minutes. The shade dried seeds should be sown within 24 hours. One packet of the inoculant (200 g) is sufficient to treat 10 kg of seeds.

Seedling root dip

This method is used for transplanted crops. Two packets of the inoculant are mixed in 40 litres of water. The root portion of the seedlings required for an acre is dipped in the mixture for 5 to 10 minutes and then transplanted.

Main field application

Four packets of the inoculants is mixed with 20 kg of dried and powdered farm yard manure and then broadcasted in one acre of main field just before transplanting.

Types of biofertilizers

Nitrogen biofertilizers:

This type of biofertilizers helps the agriculturists to determine the nitrogen level in the soil. Nitrogen is a necessary component which is used for the growth of the plant. Plants need a limited amount of nitrogen for their growth. The type of the crops also determines the level of nitrogen. Some crops need more nitrogen for their growth while some cops need fewer amounts. The type of the soil also determines that which type of biofertilizer is need for this crop. For example, Azotobacteria is used for the non legume crops; Rhizobium is needed for the legume crops. Similarly blue green algae are needed to grow rice while Acetobacter is used to grow sugarcane.

Phosphorus biofertilizers:

Phosphorus biofertilizers are used to determine the phosphorus level in the soil. The need of phosphorus for the plant growth is also limited. Phosphorus biofertilizers make the soil get the required amount of phosphorus. It is not necessary that a particular phosphorus biofertilizers is used for a particular type of crop. They can be used for any types of the crops for example; Acetobacter, Rhizobium and other biofertilizers can use phosphorus for any crop type.

Compost biofertilizers: Compost biofertilizers are those which make use of the animal dung to enrich the soil with useful microorganisms and nutrients. To convert the animal waste into a biofertilizers, the microorganisms like bacteria undergo biological processes and help in breaking down the waste. Cellulolytic fungal culture and Azotobacter cultures can be used for the compost biofertilizers.

Advantages of biofertilizers:

- They help to get high yield of crops by making the soil rich with nutrients and useful microorganisms necessary for the growth of the plants.
- Biofertilizers have replaced the chemical fertilizers as chemical fertilizers are not beneficial for the plants. They decrease the growth of the plants and make the environment polluted by releasing harmful chemicals.
- Plant growth can be increased if biofertilizers are used, because they contain natural components which do not harm the plants but do the vice versa.
- If the soil will be free of chemicals, it will retain its fertility which will be beneficial for the plants as well as the environment, because plants will be protected from getting any diseases and environment will be free of pollutant.
- Biofertilizers destroy those harmful components from the soil which cause diseases in the plants. Plants can also be protected against drought and other strict conditions by using biofertilizers.
- Biofertilizers are not costly and even poor farmers can make use of them.
- They are environment friendly and protect the environment against pollutants.

Reg. No.___________________

[15MBU503]

KARPAGAM UNIVERSITY

(Established Under Section 3 of UGC Act, 1956)

Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

(For candidates admitted from 2009, onwards)

B. Sc., DEGREE INTERNAL EXAMINATION July – 2017

MICROBIOLOGY

FOOD AND AGRICULTURAL MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**

Date:

Part - A (20 X 1 = 20 Marks)

1. The undesirable change in a food that makes it or human consumption is referred as _________

a) Food decay b) Food spoilage

c) Food loss d) Food contamination

2. The time temperature combination for HTST pasteurization of 71.1 C for 15 sec is selected on the basis of________

a) Coxiella burnetii b) E. coli

c) Bacillus subtilis d) Clostridium botulinum

3. Contamination of foods from _______ may be important for sanitary as well as economic reasons

a) Air b) Soil

c) Water d) Sewage

4. _____ bacteria produce lipase enzyme that hydrolysis fat to fatty acids and glycerol

a) Saccharolytic b) Pectinolytic

c) Lipolytic d) Proteolytic

5. _________organic acid is used in syrups, drinks, jam and jellies

a) Lactic b) Acetic

c) Propionic d) Citric

6. The O-R potential of a system is measured by _______

a) Mm b) mV

c) a_w d) Eh

7. When microbes can use fat as an energy source _______

a) Absence of sugar molecule b) Presence of glucose

c) Presence of fructose d) Presence of high sugar

8. Most spoilage bacteria grow at _______

a) Acidic pH b) Alkaline pH

c) Neutral pH d) Any of the pH

9. The undesirable change in a food that makes it or human consumption is referred as _________

a) Food decay b) Food spoilage

c) Food loss d) Food contamination

10. In fruit juices the growth of the fermentative yeast are favored by _______ pH

a) 4.0-4.5 b) 6.0-6.5

c) 2.0-2.5 d) 3.0-3.5

11. Preservation affects the growth of microorganism by________

a) Inhibition b) Retardation

c) Arresting d) Prevention

12. Pasteurization is a _________

a) Low temperature treatment b) Steaming treatment

c) High temperature treatment d) Low and high temperature treatment

13. The sodium salt of ______ acid has been used extensively as an antimicrobial agent in foods

a) Propionic b) Benzoic

c) Acetic d) Sorbic

14. Radiation dose in kilograms of _______ inhibits sprouting in potatoes, onions and garlic

a) 0.05-0.15 b) 0.01-0.14

c) 0.05-0.07 d) 0.05-0.11

15. Bacteria which is present in raw or undercooked meat, eggs, sea food and unpasteurized milk is________

a) E. coli b) Salmonella

c) Staphylococcus d) Cyanobacteria

16. Food should be cooked to which temperature?

a) 5 °C b) 75 °C

c) 100 °C d) 60 °C

17. Disease organism that cause respiratory infections may be spread among employees by ______

a) Water b) Air

c) Sewage d) Inanimate objects

18. Most fermentative yeast are favored by a pH of about ______

a) 4 - 4.5 b) 7

c) 3 d) 6

19. Freeze drying is otherwise called as _________

a) Sterilization b) Tyndallization

c) Refrigeration d) Lyophilization

20. Food acts as a _______ for microorganism

a) Substrate b) Enzymes

c) Collagen d) pathogen

Part – B (3 X 10 = 30 Marks)

21. a) Explain briefly about the sources of contamination of food

(or)

b) What are the factors that influence the growth of microorganisms in food?

22. a) Briefly explain the physical methods of food preservation.

(or)

b) Write a short note on the importance of molds in food.

23. a) Write in detail the chemical method of food preservation

(or)

b) List out the microorganisms important in food microbiology.

FOOD AND AGRICULTURAL MICROBIOLOGY

CIA – I

Key Answer

Part - A (20 X 1 = 20 Marks)
1 – b, 2 – a, 3 – a, 4 – c. 5 – d, 6 – a, 7 – a, 8 – c, 9 – b, 10 – a, 11 – b, 12 – c, 13 – b, 14 – a, 15 – b, 16 – b, 17 – b, 18 – a, 19 – d, 20 – a.

Part – B (3 X 10 = 30 Marks)
21. a) **Sources of contamination of foods**
From green plants and fruits
Natural surface flora of plants varies with the plant.
Pseudomonas, Alcaligenes, Flavobacterium, Micrococcus, coliforms and lactic acid bacteria. Depend on the plant and its environment and may range from a few hundred or thousand per square centimeter of surface to millions.
Ex: Surface of well washed tomato contains 400-700 microorganisms per square centimeter. Outer tissue of unwashed cabbage contains 1 million to 2 million microorganisms. Inner tissues of cabbage contain fewer micro organisms.
From animals
Sources of microorganisms from animals include the surface flora, the flora of the respiratory tract, and the flora of the gastro intestinal tract.
Hides, hooves, and hair contain microorganisms from soil, manure, feed and water but contain spoilage organisms.
Feathers, feet of poultry carry heavy contamination of microorganisms. Skin of many meat animals may contain *Micrococci*, *Staphylococci* and beta haemolytic *Streptococci*.
Meat from slaughter houses is not frequently associated with human salmonellosis.
From sewage
When untreated domestic sewage is used to fertilize plant crops, there is a chance that raw plant foods will be contaminated with human pathogens especially those causing gastrointestinal diseases.
The use of “night soil” as a fertilizer still persists in some parts of the world. In addition to the pathogens, *coliform* bacteria, anaerobes, *enterococci*, other intestinal bacteria and viruses can contaminate the foods from this source.
Natural water contaminated with sewage contributes their micro organisms to shell fish, fish, and other seafood.
From soil
Soil contains greatest variety of micro organisms. They are ready to contaminate the surfaces of plants growing on or in them and the surfaces of animals roaming over the land.
Soil dust is whipped up by air currents and soil particles are carried by running water to get into or onto foods. Soil is an important source of heat resistant spore forming bacteria.
From water
Natural water contains not only their natural flora but also microorganisms from soil and possibly from animals or sewage.
Surface waters in streams or pools and stored waters have low microbial content because self purification of quiet lakes and ponds or of running water.

Ground waters from springs or wells have passed through layers of rock and soil to a definite level hence most of the bacteria, suspended material have been removed.

Pseudomonas, *Chromobacterium*, *Proteus, Micrococcus, Bacillus, Streptococcus, Enterobacter* and *Escherichia coli*.

From Air

Air does not contain a natural flora of micro organisms, but accidentally they are present on suspended solid material or in moisture droplets.

Yeasts especially asporogenous chromogenic ones are found in most samples of air. Number of microorganisms in air at any given time depends on factors like amount of movement, sunshine, humidity, location and the amount of suspended dust or spray.

No. of micro organisms vary from mountains to dusty air.

During handling and processing

Additional contamination may come from equipment coming in contact with foods, from packaging materials and from personnel.

21. b) **Factors that influence the growth of microorganisms in food**

Intrinsic parameters

The parameters of plant and animal tissues that are inherent part of the tissues are referred to as intrinsic parameter. These parameters are as follows,

pH

It is the negative logarithm of the hydrogen ion activity.

$$PH = -\log(a_H) = \log\frac{1}{(a_H)}$$

$$= \log\frac{1}{[H^{-1}]}$$

Every micro organism has a minimal, a maximal and an optimal pH for growth.

Bacteria grow fastest in the pH range 6.0 – 8.0, yeasts 4.5 – 6.0 and filamentous fungi 3.5-4.0.

Inherent acidity: Some foods have a low pH because of inherent property of the food.

Ex: Fruits & vegetables.

Biological acidity: Some foods develop acidity from the accumulation of acid daring fermentation. Ex: curd, sauerkraut, pickles etc.

Redox potential (Eh): - Oxidation – reduction potential

Oxygen tension or partial pressure of oxygen about a food and the O-R potential or reducing and oxidizing power of the food itself, influence the type of organisms which will grow and hence the changes produced in the food. The O- R potential of the food is determined by

1. Characteristic O-R potential of the original food.
2. The poising capacity i.e., the resistance to change in potential of the food.
3. The oxygen tension of the atmosphere about the food.
4. The access which the atmosphere has to the food.

Highly oxidised substrate would have a positive Eh and a reduced substrate have a negative Eh.

Aerobic microorganisms require positive Eh. Ex: *Bacillus, Micrococcus, Pseudomonads, Acinetobacters*.

Nutrient content

Food is required for energy and growth of micro organisms.

Carbohydrates especially the sugars are commonly used as an energy source.
Complex carbohydrates such as cellulose can be utilized by few organisms and starch can be hydrolyzed by limited number of organisms.
Microorganisms differ in their ability to use various nitrogenous compounds as a source of nitrogen for growth. Many organisms are unable to hydrolyze proteins and hence cannot get nitrogen from them.
Peptides, amino acids, urea, ammonia and other simpler nitrogenous compounds may be available to some organisms but not to others.
Water activity
Micro organisms have an absolute demand for water. Without water, no growth can occur. The exact amount of water needed for growth of micro organisms varies. This water requirement is best expressed in terms of available water or water activity (aw).
Extrinsic parameters
Relative humidity (RH)
Relative humidity and water activity are interrelated.
When food commodities having low water activity are stored in an atmosphere of high RH, water will transfer from the gas phase to the food.
Ex: Grain silos or in tanks in which concentrates and syrups is stored.
Storage of fresh fruits and vegetables requires very careful control of relative humidity.
Temperature
Microbial growth can occur over a temperature range from about -8°C up to 100°C at atmospheric pressure.
Thermophiles have optimum: 55-75°C
Mesophile have optimum: 30 -40°C
Psychrophiles (Obligate psychrophiles): 12 – 15 °C
Psychotroph (facultative): 25-30 °C
Gaseous atmosphere
Oxygen comprises 21% of the earth's atmosphere and is the most important gas in contact with food under normal circumstances.
The inhibitory effect of CO_2 on microbial growth is applied in modified atmosphere packing of food and is an advantage in carbonated mineral waters and soft drinks.
Moulds and bacteria are sensitive to CO_2 condensation.
Some yeast such as Bettanomyces sp. has tolerance to high CO_2 levels.

22. a) **Physical methods of food preservation**
Thermal treatment
The term "thermal" refers to processes involving heat.
Heating food is an effective way of preserving it because the great majority of harmful pathogens are killed at temperatures close to the boiling point of water.
A preliminary step in many other forms of food preservation, especially forms that make use of packaging, is to heat the foods to temperatures sufficiently high to destroy pathogens.
In many cases, foods are actually cooked prior to their being packaged and stored. In other cases, cooking is neither appropriate nor necessary.
The most familiar example of the latter situation is pasteurization. Conventional methods of pasteurization called for the heating of milk to a temperature between 145 and 149 °F (63 and 65 °C) for a period of about 30 minutes, and then cooling it to room temperature. In a more recent

revision of that process, milk can also be "flash-pasteurized" by raising its temperature to about 160 °F (71 °C) for a minimum of 15 seconds, with equally successful results.
A process known as ultra high pasteurization uses higher temperatures of the order of 194 to 266 °F (90 to 130 °C) for periods of a second or more.
Low temperature
The lower the temperature, the slower will be chemical reactions, enzyme action, and microbial growth.
Each microorganism present has an optimal temperature for growth and a minimal temperature below which it cannot multiply. As the temperature drops from this optimal temperature toward the minimal, the rate of growth of the organism decreases and is slowest at the minimal temperature.
Cooler temperatures will prevent growth, but slow metabolic activity may continue. Most bacteria, yeasts, and molds grow best in the temperature range 16-38ºC (except psychrotrophs). At temperatures below 10 ºC, growth is slow and becomes slower the colder it gets. The slowing of microbial activity with decreased temperatures is the principal behind refrigeration and freezing preservation.
Drying
One of the oldest methods of food preservation is by drying, which reduces water activity sufficiently to prevent or delay microbial growth.
The term water activity is related to relative humidity. Relative humidity refers to the atmosphere surrounding a material or solution. Water activity is the ratio of vapour pressure of the solution to the vapour pressure of pure water at the same temperature.

22. b) **Importance of molds in food**
Molds of Industrial Importance
Mucor: *Mucor* are involved in the spoilage of some foods and the manufacture of others. Widely distributed species is *M. racemosus*; *M. rouxii* is used in the "Amylo" process for the saccharification of starch, and mucors help ripen some cheese, (e.g., Gammelost) and are used in making certain Oriental foods.
Zygorrhynchus: These soil molds are similar to *Mucor* except that the zygo-spore suspensors are markedly unequal in size.
Rhizopus: *Rhizopus stolonifer*, the so-called bread mold, is very common and is involved in the spoilage of many foods: berries, fruits, vegetables, bread, etc.
Absidia: Similar to *Rhizopus*, except that sporangia are small and pear-shaped.
Thamnidium: *Thamnidium elegans* is found on meat in chilling storage, causing "whiskers" on the meat.
Aspergillus: The aspergillus is very widespread. Many are involved in the spoilage of foods, and some are useful in the preparation of certain foods.
Penicillium: P. expansum, the blue-green-spored mold, causes soft rots of fruits. Other important species are *P. digitatum*, with olive, or yellowish-green conidia, causing a soft rot of citrus fruits; *P. italicum*, called the “blue contact mold” with blue green conidia, also rotting citrus fruit; *P.camemberti*, with grayish conidia, useful in the ripening of Camembert cheese; and *P. roqueforti*, with bluish-green conidia, aiding in the ripening of blue cheeses, e.g., Roquefort.
Trichothecium: The common species, *T. roseum*, is a pink mold which grows on wood, paper, fruits such as apples and peaches, and vegetables such as cucumbers and cantaloupes.
Yeasts and yeast like fungi

Like mold, the term "yeast" is commonly used but hard to define. It refers to those fungi which are generally not filamentous but unicellular and ovoid or spheroid and which reproduce by budding or fission.

Yeasts may be useful or harmful in foods. Yeast fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar, and surface ripened cheese, and yeasts are grown for enzymes and for food. Yeasts are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups; molasses, honey, jellies, meats, wine, beer, and other foods.

23. a) **Chemical method of food preservation**

Chemical preservatives are added to kill or inhibit microorganisms in food. The may be incorporated into the foods or only their surface or the wrappers used for them may be treated, or they may be used as gas or vapors around the food.

Chemical preservatives may be harmless if they are added during the storage period and are removed before the food is consumed. But if they are consumed as such, they may be poisonous to man or animal, as well as to microorganisms.

Organic acids and their salts:

Several organic acids and their salts are common preservatives as they have marked microbiostatic and microbicidal action.

Benzoic acid and benzoate are used for the preservation of vegetables. Sodium benzoate is used in the preservation of jellies, jams, fruit juice and other acid foods.

Salicylic acid and salicylates are used as preservatives of fruits and vegetables in place of benzoate. However, it is considered to be deleterious to health of consumer.

Sorbic acid is recommended for foods susceptible to spoilage fungi,

e.g., it inhibits mold growth in bread. Foods prepared by fermentation processes, e.g. milk products etc. are preserved mainly by lactic, acetic and propionic acids.

Flavoring extracts of vanilla, lemons are preserved in 50-70% alcohol as it coagulates cell proteins.

Inorganic acids and their salts:

Most common among the inorganic acids and their salts are, sodium chloride, hypochlorites, sulphurous acids and sulphites, sulphurdioxide, nitrate and nitrite.

Sodium chloride

Sodium chloride produces high osmotic pressure and therefore causes destruction of many microorganisms by plasmolysis. It causes dehydration of food as well as microorganisms, releases disinfecting chlorine ion by ionization, reduces solubility of oxygen in the moisture, sensitizes microbial cells against carbon dioxide and interferes with the action of proteolytic enzymes.

Hypochlorites

The hypochlorous acid liberated by these salts is an effective germicide. It is oxidative in its action. The commonly used forms are sodium and calcium hypochlorites. Drinking water or water used for washing foods may be dissolved with hypochlorites.

Sulphurous acids and Sulphites

Sulphurous acids and sulphites are added to wines as preservatives. Sulphurous acid is used especially in the preservation of dry fruits. It helps in retention of original colour of the preserve and inhibition of molds more than either yeasts or bacteria.

Sulphur dioxide
Sulphur dioxide has a bleaching effect desired in some fruits, and also suppresses the growth of yeast and molds. It is used as a gas to treat drying fruits and is also used in molasses.
Nitrates and Nitrites
Nitrates and nitrites produce an inhibitory effect on bacterial growth and are used usually together in meat and fish preservation and for retention of red-colour of the meat.
Nitrate is changed to nitrous acid which reacts with myoglobin to give nitric oxide myoglobin.
It is the latter which gives a bright red colour to the meat making it more attractive in appearance.

23. b) **Microorganisms important in food microbiology**
Molds
Mold growth on foods, with its fuzzy or cottony appearance, sometimes colored, is familiar to everyone, and usually food with a moldy or "mildewed" food is considered unfit to eat.
Special molds are useful in the manufacture of certain foods or ingredients of foods. Thus, some kinds of cheese are mold-ripened, e.g., blue, Roquefort, Camembert, Brie, Gammelost, etc., and molds are used in making Oriental foods, e.g., soy sauce, miso, sonti, and other discussed later. Molds have been grown as food or feed and are employed to produce products used in foods, such as amylase for bread making or citric acid used in soft drinks.
Bacteria
Genus *Acetobacter*
These bacteria oxidize ethyl alcohol to acetic acid. They are rod-shaped and motile and are found on fruits, vegetables, souring fruits, and alcoholic beverages. They are a definite spoilage problem in alcoholic beverages.
Genus *Aeromonas*
These are gram-negative rods with an optimum temperature for growth of 22 to 28 °C. They are facultative anaerobes and can be psychrophilic. They are frequently isolated from aquatic environments. A. hydrophila can be a human pathogen; it is also pathogenic to fish, frogs, and other mammals.
Genus *Alcaligenes*
As the name suggests, an alkaline reaction usually is produced in the medium of growth. *A. viscolactis* causes ropiness in milk, and *A. metalcaligenes* gives a slimy growth on cottage cheese. These organisms come from manure, feeds, soil, water, and dust. This genus also contains organisms which were formerly classified in the genus *Achromobacter.*
Genus *Alteromonas*
Several former species of *Pseudomonas* are now classified as *Alteromonas*. They are marine organisms that are potentially important in sea foods.
Genus *Bacillus*
The endospores of species of this aerobic to facultative genus usually do not swell the rods in which they are formed. Different species may be mesophilic or thermophilic, actively proteolytic, moderately proteolytic, or non proteolytic, gas-forming or not, and lipolytic or not. In general the spores of the mesophiles, e.g., *B. subtilis*, are less heat-resistant than spores of the thermophiles. Spores of the obligate thermophiles.
Genus *Brevibacterium*
B. linens are related to *Arthrobacter globiformis* and may be synonymous.

Genus *Brochotrix*

These are gram-positive rods which can form long filamentous like chains that may fold into knotted masses. The optimum temperature for growth is 20 to 25 °C, but growth can occur over a temperature range of 0 to 45 °C depending on the strain.

Genus *Campylobacter*

These bacteria were originally classified in the genus *Vibrio*. Several strains of *C. fetus* subsp. *jejuni* have been associated with gastroenteritis in humans.

Genus *Clostridium*

The endospores of species of this genus of anaerobic to microaerophilic bacteria usually swell the end or middle of the rods in which they are formed. Different species may be mesophilic or thermophilic and proteolytic or non-proteolytic. *Clostridium thermosaccharoolyticum* is an example of a saccharolytic obligate thermophile; this organism causes gaseous spoilage of canned vegetables. Putrefaction of foods often is caused by mesophilic, proteolytic species, such as *C. lentoputrescens* and *C. putrefaciens*.

Genus *Corynebacterium*

The diphtheria organism, *C. diptheriae*, may be transported by foods. *C. bovis*, with the slender, barred, or clubbed rods characteristic of the genus, is commensally on the cow's udder, can be found in aseptically drawn milk, and may be a cause of bovine mastitis.

Genus *Erwinia*

The species of this genus are plant pathogens that cause necrosis, galls, wilts, or soft rots in plants and therefore damage the plants and vegetable and fruit products from them. *E.carotovora* is associated with the market disease called "bacterial soft rot." *E. carotovora* subsp. *carotovora* causes rotting in a large number of plants. *E. carotovora subsp. atroseptica* produces a black rot in potatoes. *E. carotovora* subsp. *betavasculorum* causes soft rot in sugar beets.

Genus *Escherichia*

Found in feces, a predominant gram-negative rod isolated from the intestinal tract of warm-blooded animals and widely distributed in nature. One of the "*coliform* groups," the genus is divided into many biotypes and serotypes, some of which can be pathogenic to humans.

Genus *Flavobacterium*

The yellow to orange-pigmented species of this genus may cause discolorations on the surface of meats and be involved in the spoilage of shellfish, poultry, eggs, butter, and milk. Some of the organisms are psychrotrophic and have been found growing on thawing vegetables.

Genus *Klebsiella*

Many are capsulated. Commonly associated with the respiratory and intestinal tracts of humans. *K. pneumoniae* is the causative organism for a bacterial pneumonia in humans.

Genus *Lactobacillus*

The lactobacilli are rods, usually long and slender, that form chains in most species. They are microaerophilic, (some strict anaerobes are known), are catalase-negative and gram-positive, and ferment sugars to yield lactic acid as the main product.

Reg. No.__________________

[15MBU503]

KARPAGAM UNIVERSITY

Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

(For candidates admitted from 2009, onwards)

MICROBIOLOGY

B. Sc., DEGREE INTERNAL EXAMINATION, August – 2017

FOOD AND AGRICULTURAL MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**

Date:

Part - A (20 X 1 = 20 Marks)

1. Gazing at ultraviolet lamps produces irritation of the ____ within few seconds
   a) Eye b) Ear
   c) Nose d) Throat
2. Flavoring extracts such as vanilla and lemon extracts are preserved by their content of ____
   a) Sugar b) Salt
   c) Alcohol d) Ethylene
3. The incubation period of Vibrio parahaemolyticus infection is ______
   a) 2-48 hrs b) 5-24 hrs
   c) 40 hrs d) 37 hrs
4. _____ involves the identification of ingredients and products that have effect on food safety
   a) Hazard analysis b) Critical control points
   c) Fishery service d) Research and development service
5. The FDA and USDA cooperative is a _____ surveillance program for dry milk products
   a) Pseudomonas b) E. coli
   c) Salmonella d) Vibrio
6. _____ rays are streams of electrons emitted from radioactive materials
   a) Beta b) Cathode
   c) Gamma d) X-rays
7. About ____ percent of the suspected samples contained viable spores
   a) 20 b) 10
   c) 50 d) 30
8. _____ is a term used to label foods treated with low level ionizing radiation
   a) Radicidation b) Radurization
   c) Picowaved d) Radappertization
9. Radiation dose in kilograys of ____ inhibits sprouting in potatoes, onions and garlic
   a) 0.05-0.15 b) 0.01-0.14
   c) 0.05-0.07 d) 0.05-0.11
10. The staphylococcal intoxication refers to presence of
    a) An enterotoxin b) Neurotoxin
    c) Mycotoxin d) Exotoxin
11. Common food poisoning microbes are_______
    a) Clostridium and Salmonella b) Clostridium and E. coli
    c) E. coli and Salmonella d) Clostridium and Streptococcus

12. Clostridium perfingens poison is an __________

a) Exotoxin
b) Enterotoxin produced during sporulation
b) Endotoxin
d) Enterotoxin produced during vegetative phase

13. Aflatoxin is produced by _____________

a) Aspergillus sp.
b) Salmonella sp.
c) Fusarium sp.
d) Streptococcal sp

14. ________ is caused by strains of *C. botulinum*

a) Botulism
b) Listeriosis
c) Salmonellosis
d) Shigellosis

15. The control measure of foods that cause disease by Vibrio parahaemolyticus infection is to ______

a) Reheat left over
b) Sanitize equipment
c) Control files
d) Pasteurization

16. _____ involves the identification of ingredients and products that have effect on food safety

a) Hazard analysis
b) Critical control points
c) Fishery service
d) Research and development service

17. Which of the following statements are true regarding Staphylococcus food poisoning __________

a) Is an enterotoxin
b) Causes gastroenteritis
c) Produced by S. aureus
d) Exotoxin

18. Botulism is caused by ________

a) Clostridium botulism
b) All Clostridium species
c) Clostridium tetanai
d) Clostridium subtilis

19. The symptoms such as nausea and dehydration is caused by _____

a) Shigella sonnei
b) Yersinia
c) Arizona
d) E.coli

20. The food and Drug Administration act was amende in the year ____

a) 1983
b) 1980
c) 1989
d) 1988

Part – B (3 X 10 = 30 Marks)

21. a) Give short notes on limitation and application of radiation used for food preservation.

(or)

b) Write in brief about preservation of food using radiation.

22. a) What is food intoxication? Explain with an example.

(or)

b) What is Salmonellosis? Explain in brief.

23. a) What is HACCP? Write brief notes on the history of HACCP.

(or)

b) Write a detailed note on food borne infection caused by *Clostridium botulism*

FOOD AND AGRICULTURAL MICROBIOLOGY
CIA – II

Key Answer
Part - A (20 X 1 = 20 Marks)
1 – a, 2 – c, 3 – a, 4 – a, 5 – c, 6 – a, 7 – b, 8 – c, 9 – a, 10 – a, 11 – a, 12 – b, 13 – a, 14 – a, 15 – b. 16 – a, 17 – b, 18 – a, 19 – a, 20 – b.

Part – B (3 X 10 = 30 Marks)
21. a) **Limitation and application of radiation used for food preservation.**
Low-frequency, long-wavelength, low energy radiation ranges from radio waves to infrared. Conversely, the high-frequency, shorter-wavelength radiations have high quantum energies and actually excite or destroy organic compounds and microorganisms without heating the product. Microbial destruction without the generation of high temperatures suggested the term "cold sterilization.
Radiations of higher frequencies have high energy contents and are capable of actually breaking individual molecules into ions, hence the term ionizing irradiation.
Gamma rays and high-energy electron beams
Gamma rays and high-energy electron beams have been used for the preservation of fresh perishable canned and packaged foods.
They have good penetration and are effective to a depth of about 15 cm in most foods.
Food preservation by such radiation dosage is called "cold-sterilization” as it produces only a few degrees rise in temperature of the product.
Ultraviolet rays
Ultraviolet rays are short waves and are used to sterilize the surface of foods.
These rays have been successfully used for the treatment of water for beverages, aging meat's packaging, and treatment of knives for slicing bread, for sterilizing utensils, for prevention of spoilage by organisms on the surface of preserved pickles, cheese and prevention of air contamination.
Cold-storage rooms of meat-processing plants are sometimes equipped with germicidal lamps which reduce the surface contamination and permit longer periods of spoilage-free storage.

21. b) **Preservation of food using radiation.**
It represents a term which describes the killing of over 98% but not 100% of the microorganisms by intermediate dosage of radiation.
This method increases the storage life of some meats, sea-foods, certain fruits and vegetables when stored at low temperature.
Radiation pasteurization provides the possibility of an entirely new approach to food preservation and could bring about a radical change in industrial methods of food processing.
However, the effect of radiation on colour, flavor nutritional quality of food, odor and texture needs to be more carefully understood.
Similarly, chemical changes in food products brought about by radiations may cause bad effects on animal and human subjects and need to be more adequately investigated.

22. a) **Food intoxication**

There are two major food-poisonings or food-intoxications caused by bacteria. These are: Botulism and Staphylococoal poisoning.

Staphylococcal-poisoning: This is the most common type of food-poisoning caused due to the food contaminated with a potent toxin namely, enterotoxin.

This toxin is produced by certain strains of Staphylococcus aureus.

A sudden onset of illness starts usually within 3 to 6 hours after ingestion of the contaminated food.

Source

These bacteria are commonly present on the skin, nose and other parts of human body. People who handle foods carelessly usually transfer them to the food. Foods most commonly contaminated involve those which are eaten cold, e.g., cold meat, poultry, salads, bakery products etc.

Symptoms

As said earlier, the disease starts within 3 to 6 hours after ingestion of the contaminated food and is manifested by nausea, vomiting, abdominal pain and diarrhoea within 24 to 48 hours.

If the case becomes severe, dehydration and collapse may follow. However, in usual conditions death is rare.

Control

The disease can be controlled by preventing the entry of the bacteria to food.

It is important that all susceptible foods are kept under refrigeration to restrict the growth of the bacteria; and also by the destruction of the bacteria.

22. b) **Salmonellosis**

This disease is caused through the ingestion of Salmonella bacteria present in food. A large number of species and serotypes are involved. An inoculum of about 600,500 cells is required to become established and cause illness in the host. These bacteria are gram-negative, non-spore forming rods and motile by means of peritrichous flagella. Various species of Salmonella get ingested with improperly cooked eggs, puddings and meat that have been contaminated by the carriers. The carriers may be cats, dogs, chickens and others.

The disease appears through gastrointestinal infections as a result of the growth of the bacteria in the intestine. Typical symptoms of salmonellosis are nausea, vomiting, abdominal pain and diarrhoea. Generally the symptoms persist for 2 to 4 days. The incubation period ranges between 4 to 36 hours.

Salmonellosis can be prevented by avoiding consumption of contaminated food, by heat destruction of the bacteria, or by refrigeration to check the growth of bacteria.

23. a) **HACCP and history of HACCP**

The hazard analysis critical control point system (HACCP) is a scientific and systematic way of enhancing the safety of foods from primary production to final consumption through the identification and evaluation of specific hazards and measures for their control to ensure the safety of food.

History of HACCP

Developed by Pillsbury in 1959 as a nontesting approach to assure the safety level required by NASA for foods produced for the space program

NASA’s major concerns • Food crumbs • Food borne illness

NASA's Zero Defects program - Testing materials
National Research Council – 1985 • An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients
Microbiological hazards not controlled by testing
Recommended using HACCP for food safety assurance
National Advisory Committee on Microbiological Criteria for Food (NACMCF) 1988
NACMCF proposed 7 principles of HACCP application, Published in 1989;
1st. Revision in 1992; 2nd. Revision (latest) in 1997.

23. b) **Food borne infection caused by *Clostridium botulism***

Botulism - The main sources of botulism are canned meat, fish, string beans, sweet corn, beets and other low medium acid foods.

The foods implicated are generally those of a type that have undergone some treatment intended for the preservation of the product such as canning, pickling or smoking, but one which failed to destroy the spores of this bacterium.

When the intended preservative treatment is inadequate and is followed by storage conditions which permit the germination and growth of the microorganisms, one of the most lethal toxins known to humanity is produced.

The toxin has been known to persist in foods for long periods, especially when storage has been at low temperatures. It is unstable at pH value above 6.8.

Temperature is considered to be the most important factor in determining whether toxin production will take place and what the rate of production will be.

Various strains of C. botulinum types A and B vary in their temperature requirements; a few strains grow at 10 to 11 °C.

The lowest temperature for germination of spores of most of the strains is 15 °C and maximum of 48 °C.

Symptoms

Symptoms generally occur within 12 to 36 hours after consumption of the spoiled food. Early symptoms are digestive disturbances followed by nausea, vomiting, diarrhea together with dizziness and headache. Double vision may occur early and there may be difficulty in speaking. Mouth may become dry, throat constricted; tongue may get swollen, and coated. Involuntary Muscles become paralyzed and paralysis spreads to the respiratory system and to the heart. Death normally results from respiratory failure.

Prevention

Canned food should be properly processed by using approved heat processes.

Avoiding food that has been cooked but not well heated.

Raw foods, frozen foods thawed and held at room temperature should be avoided. Gassy and spoiled canned foods should be rejected.

Boiling of suspected food for at least 15 minutes.

Reg. No.__________________

[15MBU503]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)

Coimbatore – 641 021

B.Sc., MODEL EXAMINATION, SEPTEMBER, 2017

FIFTH SEMESTER

MICROBIOLOGY

FOOD AND AGRICULTURAL MICROBIOLOGY

TIME: 3 HOURS **Max Marks: 60**

PART –A (Multiple choice questions) **20 x 1=20**

1. The different ACC's between food categories reflect the_________
   a) Level of contamination of the raw material b) Potential for microbial growth
   c) Potential shelf life d) Potential source
2. The undesirable change in a food that makes it or human consumption is _________
   a) Food decay b) Food spoilage
   c) Food loss d) Food contamination
3. _____ bacteria produce lipase enzyme that hydrolysis fat to fatty acids and glycerol
   a) Saccharolytic b) Pectinolytic
   c) Lipolytic d) Proteolytic
4. The microorganism which apparently have no mechanism to tolerate acidic pH _______
   a) Bacteria b) Fungi
   c) Viruses d) Protozoa
5. Gazing at ultraviolet lamps produces irritation of the ____ within few seconds
   a) Eye b) Ear
   c) Nose d) Throat
6. ______ can be used to control bacterial and fungal growth in tapholes of maple tree
   a) Paraformaldehyde b) Benzaldehyde
   c) Formaldehyde d) Acetaldehyde
7. During _____ the internal temperature of bread, cake or other bakery products approaches but never reaches 100 °C
   a) Heating b) Boiling
   c) Baking d) Cooling
8. Food should be cooked to which temperature?
   a) 5 °C b) 75 °C
   c) 100 °C d) 60 °C
9. ________ is caused by strains of *C. botulinum*
   a) Botulism b) Listeriosis
   c) Salmonellosis d) Shigellosis
10. Which of the following is a food infection?
    a) Salmonellois b) Botulism
    c) Staphylococcal intoxication d) Bacillus anthraxis
11. The optimal pH for enteropathogenic E. coli is _______
    a) 4.0 to 5.0 b) 7.0 to 7.5
    c) 3.0 to 4.0 d) 8.0 to 9.0

12. _____ involves the identification of ingredients and products that have effect on food safety

a) Hazard analysis b) Critical control points
c) Fishery service d) Research and development service

13. The majority of hydrogenases in prokaryotes are ________ containing enzymes

a) Nickel b) Copper
c) Molybdenum d) Sulfur

14. The enzyme nitrogenase is inhibited by______

a) CO_2 b) Sulfur
c) Hydrogen d) Oxygen

15. Most abundant gas in atmosphere________

a) Nitrogen b) Oxygen
c) Carbon dioxide d) Hydrogen

16. Example of associative nitrogen fixation_________

a) Legume-Rhizobium b) Rice-Azospirillum
c) Higher plants-Mycorrhizae d) Azolla-Anabaena

17. Majority of atmospheric nitrogen is obtained from________

a) Fossil fuel b) Hospital waste
c) Domestic waste d) Industrial waste

18. Cyanobacteria are_______

a) Photoheterotrophs b) Chemotrophs
c) Prototrophs d) Photoautotrophs

19. _____ is organic matter, mostly derived from animal waste/feces

a) Biomanure b) Fertilizer
c) Potash d) NPK

20. ________ is phosphte solubilizing bacteria

a) Bacillus megaterium b) Bacillus anthrax
c) Bacillus cereus d) Bacillus phosphatae

PART –B

(Answer all the questions) **5 x 8 = 40 marks**

21. a) List out and explain the genera of bacteria important in food.

(or)

b) Explain briefly about the extrinsic factors of food.

22. a) Write in brief about chemical preservation process.

(or)

b) Give short notes on the advantages of temperature as a preservation method.

23. a) What is food intoxication? Explain with an example.

(or)

b) What is Salmonellosis? Explain in brief.

24. a) Write an essay on *Rhizobium* as symbiotic nitrogen fixers.

(or)

b) Describe the role of nitrogenase activity in nitrogen fixation.

25. a) Describe the mass multiplication, field application of *Cyanobacteria.*

(or)

b) Give an elaborate note on the mass multiplication, field application of *Rhizobium*.

FOOD AND AGRICULTURAL MICROBIOLOGY

Model Examination Answer key

PART –A (Multiple choice questions
1 – a, 2 – b, 3 – c, 4 – b, 5 – a, 6 – a, 7 – c, 8 – b, 9 – a, 10 – a, 11 – b, 12 – a, 13 – a, 14 – d, 15 – a, 16 – b, 17 – a, 18 – d, 19 – a, 20 – a

PART –B
21. a) **Genera of bacteria important in food.**
Genus *Acetobacter*
These bacteria oxidize ethyl alcohol to acetic acid. They are rod-shaped and motile and are found on fruits, vegetables, souring fruits, and alcoholic beverages. They are a definite spoilage problem in alcoholic beverages.
Genus *Aeromonas*
These are gram-negative rods with an optimum temperature for growth of 22 to 28 °C. They are facultative anaerobes and can be psychrophilic. They are frequently isolated from aquatic environments. A. hydrophila can be a human pathogen; it is also pathogenic to fish, frogs, and other mammals.
Genus *Alcaligenes*
As the name suggests, an alkaline reaction usually is produced in the medium of growth. *A. viscolactis* causes ropiness in milk, and *A. metalcaligenes* gives a slimy growth on cottage cheese. These organisms come from manure, feeds, soil, water, and dust. This genus also contains organisms which were formerly classified in the genus *Achromobacter.*
Genus *Alteromonas*
Several former species of *Pseudomonas* are now classified as *Alteromonas*. They are marine organisms that are potentially important in sea foods.
Genus *Bacillus*
The endospores of species of this aerobic to facultative genus usually do not swell the rods in which they are formed. Different species may be mesophilic or thermophilic, actively proteolytic, moderately proteolytic, or non proteolytic, gas-forming or not, and lipolytic or not. In general the spores of the mesophiles, e.g., *B. subtilis*, are less heat-resistant than spores of the thermophiles. Spores of the obligate thermophiles.
Genus *Brevibacterium*
B. linens are related to *Arthrobacter globiformis* and may be synonymous.
Genus *Brochotrix*
These are gram-positive rods which can form long filamentous like chains that may fold into knotted masses. The optimum temperature for growth is 20 to 25 °C, but growth can occur over a temperature range of 0 to 45 °C depending on the strain.
Genus *Campylobacter*
These bacteria were originally classified in the genus *Vibrio*. Several strains of *C. fetus* subsp. *jejuni* have been associated with gastroenteritis in humans.
Genus *Clostridium*
The endospores of species of this genus of anaerobic to microaerophilic bacteria usually swell the end or middle of the rods in which they are formed. Different species may be mesophilic or thermophilic and proteolytic or non-proteolytic. *Clostridium thermosaccharoolyticum* is an example of a saccharolytic obligate thermophile; this organism causes gaseous spoilage of

canned vegetables. Putrefaction of foods often is caused by mesophilic, proteolytic species, such as *C. lentoputrescens* and *C. putrefaciens*.

Genus *Corynebacterium*

The diphtheria organism, *C. diptheriae*, may be transported by foods. *C. bovis*, with the slender, barred, or clubbed rods characteristic of the genus, is commensally on the cow's udder, can be found in aseptically drawn milk, and may be a cause of bovine mastitis.

Genus *Erwinia*

The species of this genus are plant pathogens that cause necrosis, galls, wilts, or soft rots in plants and therefore damage the plants and vegetable and fruit products from them. *E.carotovora* is associated with the market disease called "bacterial soft rot." *E. carotovora* subsp. *carotovora* causes rotting in a large number of plants. *E. carotovora subsp. atroseptica* produces a black rot in potatoes. *E. carotovora* subsp. *betavasculorum* causes soft rot in sugar beets.

Genus *Escherichia*

Found in feces, a predominant gram-negative rod isolated from the intestinal tract of warm-blooded animals and widely distributed in nature. One of the "*coliform* groups," the genus is divided into many biotypes and serotypes, some of which can be pathogenic to humans.

Genus *Flavobacterium*

The yellow to orange-pigmented species of this genus may cause discolorations on the surface of meats and be involved in the spoilage of shellfish, poultry, eggs, butter, and milk. Some of the organisms are psychrotrophic and have been found growing on thawing vegetables.

Genus *Klebsiella*

Many are capsulated. Commonly associated with the respiratory and intestinal tracts of humans. *K. pneumoniae* is the causative organism for a bacterial pneumonia in humans.

Genus *Lactobacillus*

The lactobacilli are rods, usually long and slender, that form chains in most species. They are microaerophilic, (some strict anaerobes are known), are catalase-negative and gram-positive, and ferment sugars to yield lactic acid as the main product.

21. b) **Extrinsic factors of food**

Relative humidity (RH)

Relative humidity and water activity are interrelated.

When food commodities having low water activity are stored in an atmosphere of high RH, water will transfer from the gas phase to the food. Ex: Grain silos or in tanks in which concentrates and syrups is stored. Storage of fresh fruits and vegetables requires very careful control of relative humidity.

Temperature

Microbial growth can occur over a temperature range from about -8°C up to 100°C at atmospheric pressure.

Thermophiles have optimum: 55-75°C

Mesophile have optimum: 30 -40°C

Psychrophiles (Obligate psychrophiles): 12 – 15 °C

Psychotroph (facultative): 25-30 °C

Gaseous atmosphere

Oxygen comprises 21% of the earth's atmosphere and is the most important gas in contact with food under normal circumstances.

The inhibitory effect of CO_2 on microbial growth is applied in modified atmosphere packing of food and is an advantage in carbonated mineral waters and soft drinks.
Moulds and bacteria are sensitive to CO_2 condensation. Some yeast such as Bettanomyces sp. has tolerance to high CO_2 levels.

22. a) **Chemical preservation**
Chemical preservatives are added to kill or inhibit microorganisms in food. The may be incorporated into the foods or only their surface or the wrappers used for them may be treated, or they may be used as gas or vapors around the food. Some chemicals may be effective on selected group of microorganisms while others on a wide variety of them. Chemical preservatives may be harmless if they are added during the storage period and are removed before the food is consumed. But if they are consumed as such, they may be poisonous to man or animal, as well as to microorganisms.
Organic acids and their salts:
Several organic acids and their salts are common preservatives as they have marked microbiostatic and microbicidal action.
Benzoic acid and benzoate are used for the preservation of vegetables. Sodium benzoate is used in the preservation of jellies, jams, fruit juice and other acid foods.
Salicylic acid and salicylates are used as preservatives of fruits and vegetables in place of benzoate. However, it is considered to be deleterious to health of consumer.
Sorbic acid is recommended for foods susceptible to spoilage fungi, e.g., it inhibits mold growth in bread.
Foods prepared by fermentation processes, e.g. milk products etc. are preserved mainly by lactic, acetic and propionic acids.
Flavoring extracts of vanilla, lemons are preserved in 50-70% alcohol as it coagulates cell proteins.
Inorganic acids and their salts:
Most common among the inorganic acids and their salts are, sodium chloride, hypochlorites, sulphurous acids and sulphites, sulphurdioxide, nitrate and nitrite.
Sodium chloride
Sodium chloride produces high osmotic pressure and therefore causes destruction of many microorganisms by plasmolysis. It causes dehydration of food as well as microorganisms, releases disinfecting chlorine ion by ionization, reduces solubility of oxygen in the moisture, sensitizes microbial cells against carbon dioxide and interferes with the action of proteolytic enzymes. These are the reasons why this common salt is used widely for preservation either directly or curing solutions.
Hypochlorites
The hypochlorous acid liberated by these salts is an effective germicide. It is oxidative in its action. The commonly used forms are sodium and calcium hypochlorites. Drinking water or water used for washing foods may be dissolved with hypochlorites.
Sulphurous acids and Sulphites
Sulphurous acids and sulphites are added to wines as preservatives. Sulphurous acid is used especially in the preservation of dry fruits. It helps in retention of original colour of the preserve and inhibition of molds more than either yeasts or bacteria. Potassium metabisulphite is used in canning.

Sulphur dioxide has a bleaching effect desired in some fruits, and also suppresses the growth of yeast and molds. It is used as a gas to treat drying fruits and is also used in molasses.
Nitrates and Nitrites
Nitrates and nitrites produce an inhibitory effect on bacterial growth and are used usually together in meat and fish preservation and for retention of red-colour of the meat. Nitrate is changed to nitrous acid which reacts with myoglobin to give nitric oxide myoglobin. It is the latter which gives a bright red colour to the meat making it more attractive in appearance.

22. b) **Advantages of temperature as a preservation method.**
The term "thermal" refers to processes involving heat. Heating food is an effective way of preserving it because the great majority of harmful pathogens are killed at temperatures close to the boiling point of water.
In this respect, heating foods is a form of food preservation comparable to that of freezing but much superior to it in its effectiveness.
A preliminary step in many other forms of food preservation, especially forms that make use of packaging, is to heat the foods to temperatures sufficiently high to destroy pathogens.
In many cases, foods are actually cooked prior to their being packaged and stored. In other cases, cooking is neither appropriate nor necessary.
The most familiar example of the latter situation is pasteurization. Conventional methods of pasteurization called for the heating of milk to a temperature between 145 and 149 °F (63 and 65 °C) for a period of about 30 minutes, and then cooling it to room temperature.
The lower the temperature, the slower will be chemical reactions, enzyme action, and microbial growth.
Each microorganism present has an optimal temperature for growth and a minimal temperature below which it cannot multiply.
As the temperature drops from this optimal temperature toward the minimal, the rate of growth of the organism decreases and is slowest at the minimal temperature. Cooler temperatures will prevent growth, but slow metabolic activity may continue.
Most bacteria, yeasts, and molds grow best in the temperature range 16-38ºC (except psychrotrophs). At temperatures below 10 ºC, growth is slow and becomes slower the colder it gets.
The slowing of microbial activity with decreased temperatures is the principal behind refrigeration and freezing preservation.

23. a) **Food intoxication**
There are two major food-poisonings or food-intoxications caused by bacteria. These are: Botulism and Staphylococoal poisoning.
Botulism
Botulism is caused by the ingestion of food containing the neurotoxin (toxin that affects the nervous system) produced by Clostridium botulinum, an anaerobic spore forming bacterium. Sixty to seventy percent-cases of botulism die. There are 7 types (type A,B,C, D,E,F,G) of these neurotoxins recognized on the basis of serological specificity. The neurotoxin of C. botulinum is a protein. It has been purified and crystallized and is so powerful that only a does as low as 0.01 mg is said to be fatal to human being. The toxin is absorbed mostly in the small intestine and paralyzes the involuntary muscles of the body.
Source

The main sources of botulism are canned meat, fish, string beans, sweet corn, beets and other low medium acid foods. The foods implicated are generally those of a type that have undergone some treatment intended for the preservation of the product such as canning, pickling or smoking, but one which failed to destroy the spores of this bacterium. When the intended preservative treatment is inadequate and is followed by storage conditions which permit the germination and growth of the microorganisms, one of the most lethal toxins known to humanity is produced, The toxin has been known to persist in foods for long periods, especially when storage has been at low temperatures. It is unstable at pH value above 6.8.

Temperature is considered to be the most important factor in determining whether toxin production will take place and what the rate of production will be. Various strains of C. botulinum types A and B vary in their temperature requirements; a few strains grow at 10 to 11 °C. However, the lowest temperature for germination of spores of most of the strains is 15 °C and maximum of 48 °C.

Symptoms

Symptoms generally occur within 12 to 36 hours after consumption of the spoiled food. Early symptoms are digestive disturbances followed by nausea, vomiting, diarrhea together with dizziness and headache. Double vision may occur early and there may be difficulty in speaking. Mouth may become dry, throat constricted; tongue may get swollen, and coated. Involuntary Muscles become paralyzed and paralysis spreads to the respiratory system and to the heart. Death normally results from respiratory failure.

Prevention

Canned food should be properly processed by using approved heat processes.

Avoiding food that has been cooked but not well heated. Raw foods, frozen foods thawed and held at room temperature should be avoided. Gassy and spoiled canned foods should be rejected. Boiling of suspected food for at least 15 minutes.

Treatment:

Successful treatment is by the administration of polyvalent antitoxin in the early stages of infection. Once the symptoms appear the fails to prove useful.

23. b) **Salmonellosis**

This disease is caused through the ingestion of Salmonella bacteria present in food. A large number of species and serotypes are involved. An inoculum of about 600,500 cells is required to become established and cause illness in the host. These bacteria are gram-negative, non-spore forming rods and motile by means of peritrichous flagella. Various species of Salmonella get ingested with improperly cooked eggs, puddings and meat that have been contaminated by the carriers. The carriers may be cats, dogs, chickens and others.

The disease appears through gastrointestinal infections as a result of the growth of the bacteria in the intestine. Typical symptoms of salmonellosis are nausea, vomiting, abdominal pain and diarrhoea. Generally the symptoms persist for 2 to 4 days. The incubation period ranges between 4 to 36 hours.

Salmonellosis can be prevented by avoiding consumption of contaminated food, by heat destruction of the bacteria, or by refrigeration to check the growth of bacteria.

24. a) ***Rhizobium* as symbiotic nitrogen fixers.**

Rhizobium is Gram-negative bacilli that live freely in the soil (especially where legumes have been grown). However, they cannot fix atmospheric nitrogen until they have invaded the roots of the appropriate legume.

Infection

The interaction between a particular strain of rhizobia and the "appropriate" legume is mediated by a Nod factor secreted by the rhizobia and transmembrane receptors on the cells of the root hairs of the legume. Different strains of rhizobia produce different Nod factors, and different legumes produce receptors of different specificity. The bacteria enter an epithelial cell of the root; then migrate into the cortex. Their path runs within an intracellular channel that grows through one cortex cell after another. This infection thread is constructed by the root cells, not the bacteria, and is formed only in response to the infection. When the infection thread reaches a cell deep in the cortex, it bursts and the rhizobia are engulfed by endocytosis into membrane-enclosed symbiosomes within the cytoplasm. The cortex cells then begin to divide rapidly forming a nodule. The rhizobia also go through a period of rapid multiplication within the nodule cells. Then they begin to change shape and lose their motility. The bacteroide almost fill the cell and nitrogen fixation begins. Thus the development of nodules, while dependent on rhizobia, is a well-coordinated developmental process of the plant.

24. b) **Nitrogenase activity in nitrogen fixation.**

Nitrogenases are enzymes that are produced by certain bacteria, such as Cyanobacteria (blue-green algae). These enzymes are responsible for the reduction of nitrogen (N_2) to ammonia (NH_3). Nitrogenases are the only family of enzymes known to catalyze this reaction, which is a key step in the process of nitrogen fixation. Nitrogen fixation is required for all forms of life, with nitrogen being essential for the biosynthesis of molecules (nucleotides, amino acids) that create plants, animals and other organisms.

The nitrogenase complex consists of two proteins: The homodimeric Fe protein, a reductase which has a high reducing power and is responsible for the supply of electrons. The heterotetrameric MoFe protein, a nitrogenase which uses the electrons provided to reduce N_2 to NH_3.

Organisms that synthesize nitrogenase

There are two types of bacteria that synthesize nitrogenase and are required for nitrogen fixation. These are:

Free-living bacteria (non-symbiotic), examples include, Cyanobacteria (blue-green algae), Green sulfur bacteria, *Azotobacter*.

Mutualistic bacteria (symbiotic), examples include, *Rhizobium*, associated with leguminous plants, *Spirillum*, associated with cereal grasses and *Frankia*.

25. a) **Mass multiplication, field application of *Cyanobacteria*.**

BGA fixes nitrogen in the soil. BGA such as *Anabena*, *Polypothium*, *Oscillotrian* actively fixes the nitrogen in soil. The BGA induces the growth of higher plants with the help vitamin B12, auxins etc. Thus they form an effective biofertilizer in agriculture. The blue green algal inoculum may be produced by several methods viz., in tubs, galvanized trays, and small pits and also in field conditions. However the large-scale production is advisable under field condition which is easily adopted by farmers.

Multiplication in trays

- Big metallic trays (6'x 3'x 6"lbh) can be used for small scale production
- Take 10 kg of paddy field soil, dry powder well and spread
- Fill water to a height of 3"
- Add 250 g of dried algal flakes (soil based) as inoculum
- Add 150 g of super phosphate and 30 g of lime and mix well with the soil
- Sprinkle 25 g carbofuran to control the insects
- Maintain water level in trays
- After 10 to 15 days, the blooms of BGA will start floating on the water sources
- At this stage stop watering and drain. Let the soil to dry completely
- Collect the dry soil based inoculum as flakes
- Store in a dry place. By this method 5 to 7 kg of soil based inoculum can be obtained.

Multiplication under field condition

Materials

- Rice field
- Super phosphate
- Carbofuran
- Composite BGA starter culture

Procedure

Select an area of 40 m2 (20m x 2m) near a water source which is directly exposed to sunlight. Make a bund all around the plot to a height of 15 cm and give it a coating with mud to prevent loss of water due to percolation.

- Plot is well prepared and levelled uniformly and water is allowed to a depth of 5-7.5 cm and left to settle for 12 hrs.
- Apply 2 kg of super phosphate and 200 g lime to each plot uniformly over the area.
- The soil based composite starter culture of BGA containing 8-10 species @ 5 kg / plot is powdered well and broadcasted.
- Carbofuran at 200 g is also applied to control soil insects occurring in BGA.
- Water is let in at periodic intervals so that the height of water level is always maintained at 5 cm.
- After 15 days of inoculation, the plots are allowed to dry up in the sun and the algal flakes are collected and stored.

Observations

The floating algal flasks are green or blue green in colour. From each harvest, 30 to 40 kg of dry algal flakes is obtained from the plot.

Method of inoculation of BGA in rice field

Blue green algae may be applied as soil based inoculum to the rice field following the method described below.

- Powder the soil based algal flakes very well.
- Mix it with 10 kg soil or sand (10kg powdered algal flakes with 10 kg soil / sand).
- BGA is to be inoculated on 7-10 days after rice transplanting.
- Water level at 3-4 is to be maintained at the time of BGA inoculation and then for a month so as to have maximum BGA development.

Observation

A week after BGA inoculation, algal growth can be seen and algal mat will float on the water after 2-3 weeks. The algal mat colour will be green or brown or yellowish green.

25. b) **Mass multiplication, field application of *Rhizobium***

The selected rhizobial strain is cultured in YEMA medium for about 7 days in order to establish better growth. The Rhizobium culture is tested. The tested Rhizobial culture is transferred to a large container containing the sterile YEMA medium are incubated at 30 ° C for 9 days. Sufficient nutrients should be supplied at regular intervals of 24hrs. The rhizobial culture is checked to detect the presence of contaminants in the culture. pH of the medium and the growth rate are used to determine the presence of contaminants in the culture.

Carrier- based inoculum for storage:

The cultured Rhizobial cells can be added to the carrier and used to preserve the inoculum in a viable condition

Field Application:

The cultured Rhizobium is diluted with H_2O and applied on seeds. The suspension is sprinkled over the seeds. Sucrose solution (10%) is used to enhance the surviving potential of Rhizobium on the seed coats.

Inoculum is diluted with H_2O and slurry is uniformly mixed with seeds. Then the inoculum is pellatized on the seed coats. The inoculum is protected from the agricultural chemicals and acids and alkaline reaction of the soil. Thus the inoculum is spread over the field along with the seeds during sowing.

Pelleting agents like dolomite, gypsum, charcoal rock phosphates are used along with the inoculum. They increase the sedimentation potential of the inoculum on the surface of seeds. It protects the seeds from winter season.

The inoculum is stored at 4 °C in a refrigerator. The stored inoculum is sprayed over the soil directly to increase the fertility of the soil.

UNIT- I

Most spoilage bacteria grow at
The microbiological examination of coliform bacteria in foods preferably use
Which of the following acid will have higher bacteriostatic effect at a given pH?
Water activity can act as
The different ACC's between food categories reflect the
Yeast and mould count determination requires
A psychrophilic halophile would be a microbe that prefers

NaCl can act as
Which of the bacteria can grow in alkaline pH?
The water activity range of fresh meat and fresh fish was ________
The O-R potential of a system is measured by
When microbes can use fat as an energy source _________.
The approximate range of bacteria present in fresh vegetable is _________
In fruit juices the growth of the fermentative yeast are favored by _______ pH
The water requirement of a microorganism is expressed in terms of ______
The microorganism which apparently have no mechanism to tolerate acidic pH _______
---------------------------- is the thermoduric bacteria
To retard the contamination and other microbial growth in meat is obtained by storing at _____
The percentage of relative humidity is obtained by multiplying by_______.
Which of the following can cause food to be contaminated because of physical hazards from fo
Which of the following can cause food to be contaminated because of chemical hazards from fo
Cross-contamination of food occurs when
Which of the following are allergens?
The undesirable change in a food that makes it or human consumption is referred as _________
______ microorganisms reqire positive Eh values or positive mV O-R potentials
_______ acid produced by the propionibacteria in swiss cheese is inhibitory to molds
______ bacteria oxidize ethylalcohol to acetic acid
The endospores of ______ do not swell the rods in which they are formed
_______ is associated with the market disease called bacterial soft rot
________ is the causative organism for a bacterial pneumonia in human.
________ bacteria grow and cause discoloration on foods high in salt
Aeromonas grows at an optimum temperature of _________
The culture of Brevibacterium produces ______ pigmentation and helps ripening
________ bacteria is found aseptically in drawn milk and cause bovine mastitis

Pectins are complex __________ that are responsible for cell wall rigidity in vegetables and fruits

________ bacteria are those which grow in high concentration of sugars

_______ bacteria are able to grow at commercial refrigeration temperatures

Truly halophilic bacteria require minimal concentration of dissolved ______ for growth

______ causes ropiness in milk

______ are short rods that are defined as aerobic and facultative anaerobic

The use of indicator microorganisms began with use of E. coli testing in ______

Many infectious disease agents of animals can be transmitted to people through _____

The _____ of many meat animals may contain micrococci, Staphylococci, and beta-hemolytic Streptococci

______ used to fertilize plant crops will be contaminated with human pathogens

________ contain the greatest variety of microorganisms of any source of contamination of food

Contamination of foods from _______ may be important for sanitary as well as economic reasons

______ does not contain a antural flora of microorganisms

There are ___ aspects of water bacteriology that are interested by food microbiologist

The surface of a well washed tomato show _____ microorganisms per square centimeter

Pig and beef carcasses may be contaminated with _____

Natural water contaminated with sewage contribute their microorganism to ____

Chlorination of _____ water is practised when there is any doubt about the sanitory quality of the water

Cannery cooling water often contain _____

Many microorganisms cannot use the disaccharide lactose and therefore do not grow well in ______

_____ yeast is grown with dairy starter cultures to maintain the activity and increase the longevity of the lactic acid bacteria

Saccharomyces are reclassified by Lodder in the year ____

______ has been used as starter culture in fermented sausages

______ bacteria produce lipase enzyme that hydrolysis fat to fatty acids and glycerol

UNIT -II

The time temperature combination for HTST paterurization of 71.1°C for 15 sec is selected on

The percentage fat constituent of double toned milk is

Which solvent is commonly used to determine fat content

Pasteurization is done to kill

Bacteria which is present in raw or undercooked meat, eggs, sea food and unpasteurized milk is

Milk and curry left over can be turned into sour and spoiled at

Preservation affects the growth of microorganism by
Souring of canned meat is caused by ______.
Sugars act as preservatives due to their ability to
The minimal pH for the growth of staphylococcus is about --------------------
The concentration of salt used in high protein containing vegetables is _____
Fruit juice is sterilized by _________.
The reddish liquid comes out from meat on thawing process is called as
------------- is a storage method uses bins or boxes for equalization of moisture
------------- is mostly used preservative to prevent mold growth
The spoilage organism bring about the spoilage of meat by
Significant numbers of *S. aureus* in a food can be determined by examining the food
To retard the contamination and other microbial growth in meat is obtained by storing at _____
------------- is a storage method uses bins or boxes for equalization of moist
To retard the contamination and other microbial growth in meat is obtained by storing at _____
Increase in the concentration of dissolved substances like sugar and salt helps in _______ of the
Sulfur stinker spoilage of canned food is caused by
The minimum growth temperature of Bifidobacteria range from
Food should be cooked to which temperature?

Sanitising is_______

Food preservation involves_______

Pasteurization is a _________

Which of the following statements are true about chemical preservatives _________
The sclerotia from a species of Penicillium can survive a heat treatment of ____
During _____ the internal temperature of bread, cake or other bakery products approaches but never reaches 100 °C
______ in 1765 preserved food by heating it in a sealed containers
Combination of ______ irradiation with chilling storage helps preserve foods
______ freezing usually refer to freezing in air with only natural air circulation
Christophersen classified microroganisms on the basis of sensitivity to freezing in the year_____
_____ temperature are more lethal
The simplest dryer is the _____

The sodium salt of ______ acid has been used extensively as an antimicrobial agent in foods
______ is used most extensively in the prevention of mold growth and rope development in baked goods
_____organic acid is used in syrups, drinks, jam and jellies
______ is used as treatment for wrappers used on butter
_____ alcohol is used as coagulant and enaturizer of cell proteins

The fumes of burning ____ are used to treat light colored dehydrated fruits
______ solvent is poisonous and should not be added to foods
______ can be used to control bacterial and fungal growth in tapholes of maple tree
_____ contains a large number of olatile compounds that may have bacteriostatic and bactericidal effect
_____ acid is used in soft drinks such as colas
_____ drying is limited to climates with a hot sun and dry atmosphere to fruits
_____ rays are streams of electrons emitted from radioactive materials
Gazing at ultraviolet lamps produces irritation of the ____ within few seconds
Radiation dose in kilograys of ____ inhibits sprouting in potatoes, onions and garlic
_____ can be dried by a process called explosive puffing
Jones and Loackhead found enterotoxin forming Staphylococci in _____food
_____ is a term used to label foods treated with low level ionizing radiation
97 to 99 % of E.coli in air were killed in ____ seconds with a 15 watts lamp
Flavoring etracts such as vanilla and lemon etracts are preserved by their content of ____
______ from retail market contain from 0 to2 million bacteria per piece
About ____ percent of the suspected samples contained viable spores

UNIT -III

Which of the following toxin causing botulism is less toxic to human beings?
Which of the following is a food infection?
The staphylococcal intoxication refers to presence of
A bacterial food intoxication refers to
The method of successful treatment of botulism prior to appearance of botulism symptoms invc
Botulism is caused by the presence of toxin developed by
Salmonellois is caused by the
Group I *C. botulinum* strains generally includes in
The application of Gamma rays destroys botulism toxin. The dose of gamma rays required for t
The *Bacillus* cereus causes gasteroenteritis by the production of an exoenterotoxin which is rel
Staphylococcal intoxication is caused by the toxin in the food from
What is the main type of micro-organism responsible for food poisoning?
Common food poisoning microbes are_______

Botulism prevention involves________
Clostridium perfingens poisoning is associated with_________

Clostridium perfingens poison is an __________

Which of the following statements are true regarding Staphylococcus food poisoning__________

Salmonellois involves _______
The major carrier of Salmonellosis are ___________
Aflatoxin is produced by _____________

Botulism is caused by ________

Which of the following statements are regarding botulinal toxin __________
Human beings and animals are directly or indirectly the source of the contamination of food with _____
The disease gastroenterities caused by C. perfringens was first reported in the year___-
The incubation period of Vibrio parahaemolyticus infection is ______
The etiologic agent of diarrheal syndrome is ____
The sore and throat symptom caused by ______ etiologic agent
The control measure of foods that cause disease by Vibrio parahaemolyticus infection is to ______
The symptoms such as nausea and dehydration is caused by _____
Entheropathogenic Escherischia coli infection is involved in ____ foods
The etiological agent of Arizona infection is ____
The optimal temperature for growth of Shigellosis is _____
Yersinia enterocolitica is a small _____ shaped bacteria
Nursery epidemics diarrheal disease in infants was implicated in the year _____
The term heat tolerant is a misnomer and refers to growth at ___ temperature
_____ is associated with warm blooded animals
Miller and Kolurger examined forty environmental isolates of P. shigelloides in the year _____
Aeroonas hydrophillia is a gram negative motile rods which are ubiquitous in ____
The toxin patulin is produced by ______fungi
The mold Penicillium islandicum produces _____ toxin

In the early _____ numerous surveys have been conducted on the detection aflatoxins in foods
The _____ virus enters a person through oral route in the fecal contamination of food
The mode of transmission of poliomyelitis is _______
The pH near ____ favors C. botulinum
The growth of Staphylococcus aureus on solid media is usually ____ in color
The term ___ is used to distinguish strains of different antigenetic complements
Depending on the food and the serotype the _____ values from 0.06 to 11.3 min
______ organism can be isolated from seafoods and sea water

Pathogenecity involves the release of a ______ endotoxin which affects the intestinal mucosa
The incubation period of Streptococcus faecalis is _____
The optimal pH for enteropathogenic E. coli is _______

A_____ refers to food borne illnesses caused by the entrance of bacteria into the body through ingestion of comtaminated food

Typhoid fever is caused by ________

______ agencies aprove the Good house keeping institute

The FDA and USDA cooperative is a _____ surveillance program for dry milk products

The food and Drug Administration act was amende in the year ____

_____ involves the identification of ingredients and products that have effect on food safety

UNIT -IV

______ are aerobic and free-living nitrogen nitrogen fixers

______ are genes encoding enzymes involved in the fixation of atmospheric nitrogen

________ catalyze conversion of atmospheric nitrogen to ammonia

________ is a typical example of symbiotic nitrogen fixation seen in paddy fields

_________ recycles the H_2 produced during N_2 fixation, thereby minimizing the loss of energy

A free-living anaerobic photosynthetic bacterium

A free-living soil bacteria that is involved in nitrogen fixation

Amount of ATP needed to form 2 moles of ammonia from 1 mole of nitrogen gas during biolog

Apart from biological nitrogen fixation by microbes, _________ can fix atmospheric nitrogen

Bacteria that forms root nodules in legume plants

Biological nitrogen fixation was discovered by

Chemicals produced by the Rhizobia called _________ that cause the colonized root hairs to cu

Example of associative nitrogen fixation

Frankia is a

Group of irregularly shaped bacteria in root nodules are called as

In biological nitrogen fixation, ____ moles of ammonia are produced from one mole of nitrogen

In Cyanobacteria, nitrogen fixation occurs in terminally differentiated cells known as

In root nodules, ___________ bind and regulate the levels of oxygen in the nodule

Legume plants belongs to

Most abundant gas in atmosphere

Nitrogenase enzyme consists of

Rhizobia are attracted to ___________ released by the host legume's roots

The enzyme nitrogenase is inhibited by

Which is not true about *Anabaena* and *Nostoc*

The majority of hydrogenases in prokaryotes are ________ containing enzymes

With associative nitrogen fixation, which one of the following genera is associated?

The conversion of nitrogen to ammonia or nitrogenous compounds is called as______
Symbiotic nitrogen cyanobacteria are present in all except______
All the following are free living nitrogen fixers except________
Anabena is a nitrogen fixer present in the root pockets of_______
Splitting of dinitrogen molecule into free nitrogen atom in biological nitrogen fixation is carried out by _________
Which of the following aid plants in the acquisition of nitrogen from nitrogen gas of the atmosphere?
A major plant macronutrient found in nucleic acids and proteins is _________
Organisms capable of converting nitrogen to nitrate are_______
The conversion of amino acids to ammonium by soil decomposers is called______
To fix one molecule of nitrogen _______

Conversion of nitrite to nitrate is carried out by _______
The nonsymbiotic bacteria which fix nitrogen live in the soil independently are
Which of the following is not the biofertilisers producing bacteria?
Which of the following is capable of oxidizing sulfur to sulfates?
Most soil protozoa are flagellates or amoebas, having their dominant mode of nitrogen as
Nitrifying bacteria can not be isolated directly by the usual techniques employed to isolate hetrc

The phenomenon of commensalism refers to a relationship between organisms in which
The population of algae in soil is __________ that of either bacteria or fungi.

Nitrogen fixation refers to the direct conversion of atmospheric nitrogen gas into
The diagnostic enzyme for denitrification is

Nitrogen oxidation (nitrification)refers to the
An example of a symbiotic nitrogen fixer is

In the process of nitrogen fixation, which of the following microorganism is involved?

The groups of bacteria which have the ability to fix nitrogen from air to soil are
The nitrogenase consists of
The conversion of molecular nitrogen into ammonia is known as
Some microorganisms have the ability to increase the nitrogen content of soils, are called as
Which are the main source of biofertilisers?

Syntrophism involves
The physical structure of soil is improved by the accumulation of ________
_______ play a key role in the transformatio nof rock in the transformation of rock to soil

Denitrification may be distinguished as

All of the following are examples of negative symbiosis________
The reservior for nitrogen is __________
Degree of compost maturity can be assesed by _______

UNIT- V

______ is organic matter, mostly derived from animal waste/feces
______ is the used for seed treatment of groundnut
_______ are best phosphate mobilizers
_______ is a biocontrol agent
________ are rich in beneficial microorganisms that enrich the nutrient quality of soil
________ is a best biofertilizer used in paddy fields
________ is a form of agriculture that relies on techniques such as crop rotation, green manure,
________ is phosphte solubilizing bacteria
________ is the biological oxidation of ammonia
_________ can be used with crops like wheat, maize,mustard, cotton, potato and other vegetabl
_________ is a plant growth promoting bacteria found naturally in soil
A carrier used in preparation of biofertilizers
A fertilizer consisting of growing plants that are ploughed back into the soil
Chemoautotrophic involved in nitrification
Cyanobacteria are
Denitrification is a microbially facilitated process of
Denitrifying bacteria
Enzyme involved in phosphate solubilization
Foliar spray is
Indole acetic acid and gibberelins are
Liquid extract of composting by earthworms
Majority of atmospheric nitrogen is obtained from
Microorganisms make soluble phosphate from insoluble phosphate by producing _______
PGPR is
Phyllosphere refers to
Rhizobacteria are bacteria growing in & around ______ of plants
VAM is
Which are important nutrients for plant growth in soil?
Which bacteria is used as biofertilizer in sugarcane crop?
Which forms symbiotic relation with higher plants?
Expect Rhizobium, which one of the following bacteria forms nitrogen fixing nodules in plants?

Rhizobium has symbiotic association with_______
Which of the following is not the biofertilisers producing bacteria?
Which of the following is capable of oxidising sulfur to sulfates?
Azolla is used as biofertilizer as it has _______

The most quickly available surce of nitrogen to plants are______

Most effective pesticide is _______
Which is true for DDT

Which is major component of bordeaux mixture?

Which one is correctly matched

IPM stands for

Which is major component of bordeaux mixture?

Insecticides generally attack
Organisms associated with sorghum and cotton which provide nutrition to them are

Azolla as biofertilizer, increase the yield of rice fields by ________

Denitrification is __________
Which of the following soil microorganism is involved in the reduction of sulfates to hydrogen sulphide
Which one of the following structure is formed in plant roots by mycorrhizae
Except Rhizobium, which one of the following bacteria forms nitrogen fixing nodules in plants
Which one of the following genes is responsible for nod factor in bacteria
In which one of the following relationship one partner benefits but the other is neither hurt nor helpless
The proteinaceous compounds ae converted to ammonia in the presence of which one of the following bacteria

In soil, which one of the following bacterial genera is responsible for degradation of cellulose
Which one of the following compound is known as the most resisant to microbial degradation during organic matter decomposition
Soil microorganisms influence above ground ecosystems by contributing to except which one of the following
Mycorrizha is a symbiotic association between a fungus and the roots of a vascular plant, was f
Denitrification is done only by microorganisms, usually by which one of the following
The plant disease control agents inclde to which one of the following microorganism, except?
In plants, the strains of which one of the following bacterium initiates to the formation of galls?
In 1888, a dutch microbiologist Beijerinck succeeded in isolating which one of the following ba

Ammonia produced in the bacteriod needs to be transported to the plants through which one of

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF MICROBIOLOGY
FOOD AND AGRICULTURAL MICROBIOLOGY

OPTION 1	OPTION 2
acidic pH	alkaline pH
MacConkey broth	violet Red Bile agar
Acetic acid	Tartaric acid
warm temperature	a processing factor
expected level of contamination of the raw material	potential for microbial growth durin
nutrient agar	acidified potato glucose agar
cold temperatures and increased amounts of salt	warm temperatures and increased ar
pH	Moisture
antagonist at optimal concentrations	synergistically if added in excess of
Lactobacilli	*Vibrio cholera*
0.93-0.98	0.98 and above
mV	mM
absence of sugar molecule	presence of glucose
10^9 -10^7/g	10^3 -10^9/g
4.0-4.5	6.0-6.5
water action	water adsorption
bacteria	fungi
Acenetobacteria	*Morexella*
10°C	0°C
aw*10	aw*1000
Jewellery	Dust
Hair	Dust
Cleaning and sanitising equipment and benches	Keeping food stored in food-grade
Sources of gluten and Red meat	Fruits and vegetables
food decay	food spoilage
Aerobic	anaerobic
sorbic	acetic
Aeromonas	Acetobacter
Streptococcus	Brochotrix
Erwinia	Enterobacter
Flavobacterium	Escherichia
Halobacterium	Enterobacter
27 to 37 °C	22 to 28 °C
orange-red	yellow
Corynebacterium	Clostridium

Proteins	lipids
Halophilic	thermophilic
Pschrotropic	halophilics
NaCl2	Hcl
Lactobacillus plantarum	Klebsiella pneumonia
Enterobacter	Coliforms
soil	plants
water	food
Hair	nail
sewage	distilled water
plants	sewage
air	soil
soil	air
2	5
100-200	400-700
Salmonellae	Klebsiella
meat	vegetables
sewage	drinking
Coliforms	Aeromonas
milk	water
Candida sp.	Trichosporon
1985	1978
Photobacterium	Pediococcus
Saccharolytic	Pectinolytic
Coxiella Burnetii	*E. coli*
0.5	1.5
Ethyl alcohol	Hexane
Selective microorganism	All the microorganism
E.coli	salmonella
high temperature	very low temperature

inhibition		retardation	
thermoduric cells		thermostatic cells	
make water unavailable to organism's		interfere with the action of proteoly	
	2.5		4.8
4.3-10.3		17.5-20.0	
filteration		freezing	
drying		wilting	
sweating		springer	
sodium propionate		springer	
purification		oxidation	
RNase		thermostable nuclease	
10°C		0°C	
Sweating		Springer	
10 °C		0°C	
drying		freezing	
E.coli		*D. nigrificans*	
43 to 45		25 to 28	
5°C		75°C	
Applying detergent to a clean surface		Done before washing	
increasing shelf life of food		ensuring safety for human consumption	
low temperature treatment		steaming treatment	
microbicidal or microstatic		chemical preservatives often hazardous to humans	
70 °C		90 to100 °C	
Heating		boiling	
Spallanzani		Ruiz-Argueso	
Ultraviolet		infra red	
Sharp		slow	
1984		1989	
high freezing		frozen storage	
sun		air	
propionic		benzoic	
calcium propionate		calcium sorbate	
lactic		acetic	
sodium diacetate		calcium carbonate	
methanol		ethanol	

sulfur	ethylene
propylene	ethanol
paraformaldehyde	benzaldehyde
spices	woodsmoke
phosphoric	benzoic
mechanical	solar
beta	cathode
eye	ear
0.05-0.15	0.01-0.14
meat	vegetables
frozen corn	cheese
Radicidation	radurization
40	10
sugar	salt
caramels	jellies
20	10

Type A	Type B
Salmonellois	Botulism
an enterotoxin	neurotoxin
illness caused by presence of pathogens	food borne illness caused by the pre
antibiotic	analgesic
Clostridium tyrobutyricum	*Clostridium sporogenes*
enterotoxin of *Salmonella* spp	endotoxin of *Salmonella* spp
all types of strains (proteolytic)A, B and F	all types of strains (non-proteolytic)
73 Gy	73 Rad
cell growth	cell autolysis
Staphylococcus aureus	*S. cerevisiae*
Bacteria	Mould
Clostridium and Salmonella	Clostridium and E. coli
Proper heat sterilization before food canning	addition of chemical preservatives
meat products	vegetables
exotoxin	enterotoxin produced during sporulation

is an enterotoxin	causes gastroenteritis
an enterotoxin and exotoxin	an enterotoxin and cytotoxin
meat and eggs	meat and fish
Aspergillus sp.	Salmonella sp.
Clostridium botulism	All Clostridium species
is a neurotoxin	water soluble exotoxin
Salmonella	Staphylococcus
1952	1961
2-48 hrs	5-24 hrs
Shigellosis	Yersiniosis
Streptococcus pyogenes	Staphylococcus aureus
reheat left over	sanitize equipment
Shigella sonnei	Yersinia
vegetables	apple cider
Vibrio	E. coli
27 °C	37 °C
cocci	chain
1950	1940
37 °C	40 °C
C. jejuni	C. botulinum
1987	1982
air	soil
Penicillium expansum	Fusarium
Luteoskyrin	aflatoxin
1980s	1940s
Poliomyelitis	Hepatitis
food	air
neutrality	alkalinity
red	brown
biovars	serovar
D50 C	D40 c
Vibrio cholerae	Vibrio vulnificus
lipopolysaccharides	monosaccharides
5 to 10	2 to 10
4.0 to 5.0	7.0 to 7.5

Food infection	food poisoning
Salmonell enteritidis	Salmonella infantis
Commercial	State
Pseudomonas	E. coli
1983	1980
Hazard analysis	critical control points
Frankia & *Azospirillum*	*Clostridium* & *Desulfovibrio*
mif	*nif*
Kinase	Hydrogenase
Azolla-*Anabaena*	Alder-*Frankia*
Reductase	Catalase
Anabaena azollae	*Clostridium thermocellum*
Alcaligenes	*Acetobacter*
8	16
Cyclone	Thunder
Rhizobium	*Azotobacter*
Winogradsky	Beijerinck
Pod factors	Nod factors
Legume-*Rhizobium*	Rice-*Azospirillum*
Bacteria	Actinomycete
Bacteroids	Asteroids
2	4
Cyanocysts	Nitrocycts
Teghemoglobin	Peghemoglobin
Solanaceae	*Rosaceae*
Nitrogen	Oxygen
Iron protein	Molybdenum-iron protein
Flavonoids	Enzymes
CO_2	Sulfur
Filamentous	Nitrogen fixing
Nickel	Copper
Azotobacter	Escherichia

Nitrogen assimilation	Nitrogen fixation
Anthoceros	Azolla
Rhizobium	Azotobacter
Marselia	Salvinia
hydrogenase	nitrogenase
Bacteria	Algae
calcium	nitrogen
yeast	bacteria
ammonification	mineralization
6 ATP molecules are required	12 Atp molecules are required
Nitrosomonas	Nitrosococcus
Azotobacter	Anabena
Nostoc	Anabaena
Thiobacillus thiooxidans	*Desulfotomaculum*
ingestion of bacteria	ingestion of mold
slow growth	medium growth
one species of a pair benefits	both the species of a pair benefit
generally smaller than	generally greater than
ammonia	glucose
nitrate reductase	nitrate oxidase
conversion of ammonium ions into nitrates through the activities of certain bacteria.	changing of atmospheric nitrogen (
Azotobacter	*Beijerinckia*
symbiotic and non symbiotic microroganisms	Non symbiotic microorganisms only
symbiotic	nonsymbiotic
dinitrogenase	dinitrogenase reductase
nitrification	denitrification
nitrogen fixation	denitrification
Cyanobacteria	Bacillus
exchange of nutrients between two species	exchange of nutrients among species
Mold mycelium	minerals
Cyanobacterium	pectin decomposing bacteria

dissimilative	assimilative
amensalism	competition
the atmosphere	rocks
infrared tehnique	germination test
Biomanure	Fertilizer
Azospirillum	*Azotobacter*
Mycorrhizae	*Bacillus*
Bacillus polymyxa	*Azospirillum*
Biofertilizers	Humus
Bradyrhizobium	*Azospirillum*
Terrestial farming	Hill farming
Bacillus megaterium	*Bacillus anthrax*
Oxidation	Nitrification
Anabaena	*Azotobacter*
Pseudomonas aeruginosa	*Staphylococcus aureus*
Rubber	Peat
Green manure	Vermicompost
Alcaligenes	*Fusarium*
Photoheterotrophs	Chemotrophs
Nitrate degradation	Nitrate assimilation
Thiobacillusdenitrificans	*Bacillus*
Oxidases	Reductases
Spraying on roots	Spraying on Stem
Hormones of bacteria	Hormones that retard plant growth
Vermiwash	Germiwash
Fossil fuel	Hospital waste
Hydrochloric acid	Sulphuric acid
Phosphorous growth promoting bacteria	Plant gibberellin promoting bacteria
Surface of roots	Surface of leaves
Leaf	Root
Ventricular arbuscular mycorrhizae	Vesicular augumenting mycorrhizae
Nitrogen	Phosphorous
Beijerinckia	*Acetobacter diazotrophicus*
Aspergillus fumigatus	*Bradyrhizobium*
Actinorhiza	Burholderia

Legumes	non-legume crop
Nostoc	Anabena
Thiobacillus thioxidans	Desulfotomaculum
Rizobium	Cyanobacteria
amide fertilizers	ammonia fertilizers
carbamates	organophosphates
not a pollutant	an antibiotic
copper sulphate	sodium chloride
carbamates-malathion	organophosphates-cabofuran
integrated plant manufacture	integrated plant management
copper sulphate	sodium chloride
respiratory system	muscular system
	Azotobacter, Azospirillum
Azospirillum, Azotobacter	
10%	20%
reduction of nitrate to nitrogen gas	reduction of nitrate to organic nitrogen compounds
Thiobacillus thiooxidans	Desulfotomaculum
Arbuscles	Hartig net
Actinorhiza	Burholderia
fix gene	gag gene
Amensalism	Commensalism
Ammonifying bacteria	Denitrifying bacteria
Escherichia	Pseudomonas
cellulose	chitin
plant nutrition and health	soil fertility
Crick	Fisher
Facultative anaerobes	obligate aerobe
Ampelomyces quisqualis	Bacillus subtilis
Agrobacterium	Rhizobium
Bradyrhizobium japonicum	Rhizobium leguminosarum

lipid membrane

periplasmic membrane

15MBU503

OPTION 3	OPTION 4
neutral pH	any of the pH
Mac conkey agar	nutrient broth
Citric acid	Maleic acid
an extrinsic factor	both b and c
potential shelf life	all of the above
MacConkey agar	violet Red Bile agar
cold temperatures and the absei	warm temperatures and increased amoun
Oxidation-Reduction Potential	All of these
Both (a) and (b)	None of the above
Salmonella	*Staphylococcus*
0.60-0.76	below 0.98
aw	Eh
presence of fructose	Presence of high sugar
10^3 -10^7/g	10^1 -10^7/g
2.0-2.5	3.0-3.5
water affinity	water activity
viruses	both a and b
Bacillus	*Flavobacterium*
100°C	-10°C
aw*100	aw*0.1
Rodent droppings	Incorrectly diluted chemicals
Live insects	Perfume
Washing hands before handling	Using food handling gloves for handling
Fish and fish products	None of the above
food loss	all of the above
facultative	none of these
propionic	acetic
Alcaligens	Alteromonas
Brevibacterium	Bacillus
Corynebacterium	Klebsiella
Klebsiella	Gluconobacter
Erwinia	Corynebacterium
35 to 37 °C	40 °C
black	red
Campylobacter	Enterobacter

carbohydrates	vitamins
osmophilic	none of these
auttrophic	heterotrophic
NaNo2	Cacl2
Klebsiella oxytoca	Flavobacterium
Proteus	Clostridium
water	all of these
soil	juices
skin	foot
mineralized water	drinking water
water	soil
water	sewage
water	sewage
6	4
100-300	200-400
E. coli	Enterobacter
fruits	seafoods
distilled water	surface water
Klebsiella	Clostridium
food	sewage
Rhodotorula	Torulopsis
1982	1984
Propionibacterium	Proteus
lipolytic	proteolytic
B. subtilis	*C. botulinum*
3	4.5
Acetone	Benzene
Yeast	Yeast and its spores
staphylococcus	cyano bacteria
room temperature	constant temperature

arresting	degradation
thermo liable cells	thermostable
osmotic effect	chemical changes
2	3.5
18.6-26.5	19.2-22.2
cooling	heating
bleeding	leakage
cooling	freezing
sorbates	acetate
decomposition	hydrolysis
protease	thermostable DNase
100°C	-10°C
Cooling	Freezing
100°C	-10°C
moistening	thawing
Bacillus	*Clostridium*
29 to 32	30 to 35
100°C	60°C
Reducing bacteria by applicatic	Wiping all surfaces with a clean cloth
both a and b	boiling
high temperature treatment	low and high temperature treatment
sodium benzoate is a widely used preservative	all these
50-60 °C	37 °C
baking	all of these
Rodrigeuz-Navarro	Christophersen
gamma	none of the above
quick	speed
1973	1981
freezing rate	thawing
heat	evaporator
sorbic	acetic
monocholroacetic acid	nitrates
propionic	citric
sodium nitrate	potassium nitrite
butanol	none of these

potassium	sodium
methanol	glycerol
formaldehyde	all of these

formaldehyde	alcohol
acetic	sorbic
freeze	chemical
gamma	X-rays
nose	throat
0.05-0.07	0.05-0.11
fruits	juices
bread	jam
picowaved	radappertization
50	30
alcohol	ethylene
fudges	candies
30	50

Type C	Type D
Staphylococcal intoxication	Tetanus
mycotoxin	exotoxin
both (a) and (b)	food poisoning
antitoxin	antipyretic
Clostridium botulinum	*Bacillus*
neurotoxin of *Salmonella* spp	exoenterotoxin of *Salmonella* spp
all types of strains (proteolytic)	none of the above
7.3 Mrad	173 Rad
cell permeation	cell damage
S. thermophillus	none of these
Virus	Parasite
E. coli and Salmonella	Clostridium and
Proper low temperature treatment before food canning	freezing
canned foods	fish products
endotoxin	enterotoxin produced during vegetative phase

is produced by Clostridium botulinum	Both a and b
is produced by Staphylococcus aureus	endotoxin
eggs and fish	eggs and fruits
Fusarium sp.	Streptococcal sp.
Clostridium tetanai	Clostridium subtilis
is produced by Clostridium botulinum	caused by Staphylococcus
Bacillus	E. coli
1978	1945
40 hrs	37 hrs
Bacillus cereus	Vibrio
Bacillus anthrax	E.coli
control files	pastuerization
Arizona	E.coli
ice creams	cheese
Arizona	Streptococcus
40 °C	50 **°C**
rod	bacilli
1962	1980
42 °C	25 °C
C. perferigens	E. coli
1980	1986
water	land
Aspergillus flavus	Mucor
penicillic acid	roquefortine
1950s	1960s
Adeno	Herpes
contaminated water	all of these
acidic	both b and c
pink	yellow
herbivore	none of these
D60 c	D30 c
Vibrio parahaemolyticus	All of these
polysaccharides	peptidoglycon
2 to 18	8 to 12
3.0 to 4.0	8.0 to 9.0

food intoxication	contamination
Salmonella typhi	Salmonella typhimurium
Federal	Private
Salmonella	Vibrio
1989	1988
fishery service	research and development service

Beijerinckia & *Klebsiella*	*Rhizobium* & *Anabaena*
sif	*nod*
Nitrogenase	Phosphatase
Legume-*Rhizobium*	Higher plants-*Mycorrhizae*
Nitrogenase	Hydrogenase
Rhodospirillum rubrum	*Klebsiella pneumoniae*
Pseudomonas	*Azotobacter*
32	64
Raining	Lightning
Azospirillum	Cyanobacteria
Pasteur	Koch
Sod factors	Mod factors
Higher plants-Mycorrhizae	Azolla-*Anabaena*
Fungi	Algae
Mesteroids	Histeroids
6	8
Heterocysts	Homocysts
Leghemoglobin	Hemoglobin
Astraceae	*Fabaceae*
Carbon dioxide	Hydrogen
Iron protein and a molybdenum	Hemoglobin
Toxins	Chemicals
Hydrogen	Oxygen
Cyanobacteria	Symbiotic
Molybdenum	Sulfur
Rhizobium	Anabena

Denitrification	Nitrification
Cycas	Gnetum
Rhodospirillum	Clostridium
Pistia	Azolla
dinitrogenase	nitrate reductase
Nematodes	Moulds
sulphur	iron
roundworms	moulds
deamination	both a and b
16 ATP molecules are required	20 ATP molecules are required
Nitrobacter	Clostridium
Rhizobium	Azolla
Both (a) and (b)	Clostridium
Rhodospirillum	*Rhodomicrobium*
ingestion of fungi	ingestion of virus
fast growth	no growth
one species of a pair is more b	two species are benefitef
equal to	not greater or smaller
ATP	nitrate
nitro oxidoreductase	nitrate
sulfur is oxidized to the sulfate form through Thiobacillus bacteria	conversion of nitrate to nitrites
Clostridium	*Rhizobium*
Symbiotic microorganisms only	Non symbiotic and symbiotic microorganisms only
both (a) and (b)	synergistics
both (a) and (b)	nitrogenase
nitrogen fixation	ammonification
nitrification	nitrogenase
Streptococcus	Azolla
no exchange of nutrients between two species	no exchange of nutrients among species
water	yeast
denitrifying bacteria	

both a and b	blue baby syndrome
commensalism	parasitism
ammonia	nitrates
both a and b	MPN test

Potash	NPK
Rhizobium	*Nostoc*
Citrobacter	*Candida*
Trichoderma viridae	*Aspergillus flavus*
NPK	Vermicompost
Anabaena	*Frankia*
Inorganic farming	Organic farming
Bacillus cereus	*Bacillus phosphatae*
Denitrification	Reduction
Rhizobium	*Mycorrhizae*
Pseudomonas fluorescens	*Aspergillus fumigatus*
Plastic	Soil
Biomanure	Organic fertilizer
Nitrosomonas	*Arthrobacter*
Prototrophs	Photoautotrophs
Nitrate oxidation	Nitrate reduction
Aspergillus	*Micrococcus denitrificans*
Kinases	Phytases
Spraying on leaves	Spraying on Flowers
Plant growth hormones	Weedicides
Wormiwash	Liquiwash
Domestic waste	Industrial waste
Nitric acid	Organic acids
Plant growth promoting biomas	Plant growth promoting bacteria
Surface of Stem	Surface of flowers
Stem	Fruit
Vesicular arbuscular mycorrhiz	Vesicular arbuscular mycobacterium
NPK	Potassium
Bacillus	*Pseudomonas*
Pseudomonas fluorescens	*Mycorrhizae*
Micrococcus	Pseudomonas

sugarcane	paddy
Both a and	Clostridium
Rhodospirillium	Rhodomicrobium
Mycorrhiza	Large quantity of humus
nitrate fertilizers	ammonia nitrate fertilizer
organochlorines	phosphates
an antiseptic agent	a non degradable pollutant
calcium chloride	magnesium sulphate
carbamates-malathion	organochloride-endosulphan
integrated plant management	integrated pest management
calcium chloride	magnesium sulphate
nervus system	circulatory system
Anabena, Rhizobium	Rhizobium, Azotobacter
30%	50%
both a and b	reduction of ammonia
Rhodospirillium	Rhodomicrobium
Haustoria	Rhizomorph
Micrococcus	Pseudomonas
nif gene	nol gene
Parasitization	Predation
Nitrifying bacteria	Putrefying bacteria
Salmonella	Staphylococcus
hemicellulose	lignin
soil structure	soil texture
Frank	Funk
phototrophic aerobe	Microaerophilic
Trichoderme sp.	Bacillus anthrax
Pseudomonas	Ralstonia
Sinorhizobium meliloti	Both a and b

symbiosome membrane plasma membrane

ANSWER KEY

neutral pH
MacConkey broth
Acetic acid
both b and c
all of the above
acidified potato glucose agar
cold temperatures and increased amounts of salt
All of these
Both (a) and (b)
Vibrio cholera
0.93-0.98
mM
absence of sugar molecule
10^3 -10^4/g
4.0-4.5
water activity
fungi
Morexella
0°C
aw*100
Jewellery
Perfume
Using food handling gloves for handling money
Sources of gluten and Red meat
food spoilage
Aerobic
propionic
Acetobacter
Bacillus
Erwinia
Klebsiella
Halobacterium
22 to 28 °C
orange - red
Corynebacterium

Carbohydrate
osmophilic
Pschrotropic
Nacl2
Klebsiella oxytoca
Coliforms
water
food

skin

sewage

soil

air
air
2
400-700
Salmonellae
sea foods

drinking
Coliforms

milk

Candida sp.
1984
Pediococcus
lipolytic

Coxiella Burnetii

1.5

Hexane
Selective microorganism
salmonella

room temperature

retardation
thermoduric cells
interfere with the action of proteolytic enzyme
4.8
18.6-26.5
filteration
bleeding
springer
sodium propionate
decomposition
protease
0°C
Springer
0°C
drying
D. nigrificans
43 to 45
75°C
Reducing bacteria by application of heat or chemical

both a and b

high temperature treatment

All of these
90 to100 °C

baking
spallanzani
ultraviolet
sharp

1973
high freezing
evaporator

benzoic

calcium propionate
citric
sodium diacetate
ethanol

sulfur
methanol
paraformaldehyde

woodsmoke
phosphoric
solar
beta
Eye
0.05-0.15
vegetables
frozen corn
Picowaved
10
alcohol
candies
10

Type B
Salmonellois
an enterotoxin
food borne illness caused by the presence of a bacterial toxin formed in food
antitoxin
Clostridium botulinum
endotoxin of *Salmonella* spp
all types of strains (proteolytic)A, B and F
7.3 Mrad
cell autolysis
Staphylococcus aureus
Bacteria
Clostridium and Salmonella

Proper heat sterilization before food canning
meat products

enterotoxin produced during sporulation

Both a and b

an enterotoxin and cytotoxin
meat and egs
Aspergillus sp.

Clostridium botulinum

is produced by Clostridium botulinum

Salmonella
1945
2-48 hrs
Bacillus cereus
Streptococcus pyogenes

sanitize equipment
Shigella sonnei
cheese
Arizona
37 °C
rod
1940
42 °C
C. jejuni

1986
water
Penicillium expansum
Luteoskyrin

1960s
Hepatitis
contaminated water
neutrality
yellow
serovar
D60 c
Vibrio vulnificus

lipopolysaccharides
2 to 18
7.0 to 7.5

food infection

Salmonella typhi
Private
Salmonella

1980

Hazard analysis

Beijerinckia & *Klebsiella*
nif
Nitrogenase
Azolla-*Anabaena*
Hydrogenase
Rhodospirillum rubrum
Azotobacter
16
Lightning
Rhizobium
Beijerinck
Nod factors
Rice-*Azospirillum*
Actinomycete
Bacteroids
2
Heterocysts
Leghemoglobin
Fabaceae
Nitrogen
Iron protein and a molybdenum-iron protein
Flavonoids
Oxygen
Symbiotic
Nickel
Azotobacter

Nitrogen fixation
Gentum
Rhizobium
Azolla
nitrogenase

Bacteria

nitrogen
bacteria
both a and b
16 ATP molecules are required

Nitrobacter
Azotobacter
Clostridium
Thiobacillus thiooxidans
ingestion of bacteria
slow growth
one species of a pair benefits

generally smaller than

ammonia
nitrate reductase

conversion of ammonium ions into nitrates through the activities of certain bacteria.
Rhizobium
Non symbiotic and symbiotic microorganisms only

both (a) and (b)
both (a) and (b)
nitrogen fixation
nitrogen fixation
Cyanobacteria
exchange of nutrients between two species

mold mycelium
Cyanobacteria

both a and b

competition
the atmosphere
both a and b

Biomanure
Rhizobium
Mycorrhizae
Trichoderma viridae
Biofertilizers
Both b and c
Organic farming
Bacillus megaterium
Nitrification
Azotobacter
Pseudomonas fluorescens
Peat
Green manure
Nitrosomonas
Photoautotrophs
Nitrate reduction
Both I & IV
Phytases
Spraying on leaves
Plant growth hormones
Vermiwash
Both a and c
Organic acids
Plant growth promoting bacteria
Surface of leaves
Root
Vesicular arbuscular mycorrhizae
NPK
Acetobacter diazotrophicus
Mycorrhizae
Burholderia

legumes
Clostridium
Thiobacillus thiooxidans
Cyanobacterium

amide fertilizers

carbamates
a non degradable pollutant

sodium chloride

organochloride-endosulphan

integrated plant management

sodium chloride

muscular system

Azotobacter-Azospirillum
10%

Both a and b

Desulfotomaculum
Hartig net
Burholderia
nol gene

Commensalism

Ammonifying bacteria

Pseudomonas

lignin

soil texture
Frank
Facultative anaerobes
Trichoderma sp.
Agrobacterium
Rhizobium leguminosarum

symbiosome membrane