Food and Dairy Microbiology

Unit -1

GENERAL

Food and Dairy microbiology is the study of the microorganisms that inhibit, create, or contaminate food and Dairy products.

This includes the study of microorganisms causing…….

- **Food spoilage**
- **That may cause disease especially if food is improperly cooked or stored**
- **Those used to produce fermented foods such as cheese, yogurt, bread, beer, and wine**
- **Then those researchers with other useful roles such as producing probiotics**

- **In addition to natural microflora every food may be contaminated from outside sources on the way from the field to the processing plant, or during storage, transport and distribution**
- **There are thousands of different types of micro-organisms everywhere in air, soil and water, and consequently on foods, and in the digestive tract of animals and human**
- **The majority of micro-organisms perform useful functions in the environment and also in some branches of food industry**
- **On the other hand unwanted spoilage of foods is generally caused by micro-organisms and contamination of food with pathogens causes food safety problems.**

Points to be considered in Food Microbiology

- **Food safety is a major focus of food microbiology**
- **Numerous agents of disease and pathogens are readily transmitted via food which includes bacteria and viruses**
- **Microbial toxins are also possible contaminants of food**
- **Probiotic bacteria, including those that produce bacteriocins can kill and inhibit pathogens**
- **Purified bacteriocins such as nisin can be added directly to food products**
- **Bacteriophages, viruses that only infect bacteria can be used to kill bacterial pathogens**
- **Thorough preparation of food, including proper cooking, eliminates most bacteria and viruses**
- **However, *toxins produced* by contaminants may not be liable to change to non-toxic forms by heating or cooking the contaminated food due to other safety conditions**

Food As a Substrate for Microorganisms

- The nutritional requirements of various microorganisms may differ appreciably but all of them require carbon, nitrogen (ammonia, ammonium salts, and nitrates) and phosphorus sources, as well as other minerals and frequently vitamins
- Other minerals such as calcium, magnesium, potassium and trace elements are infrequently mentioned as substrates for microbial growth
- In some instances, vitamins such as biotin, thiamin, or others have to be supplied to the growth medium under artificial conditions

Important micro organisms in food microbiology Molds, Yeasts and Bacteria (General characteristics and importance)

- The micro-organisms occurring on and/or in foods are divided into three groups: molds, yeast and bacteria
- Molds are generally concerned in the spoilage of foods; their use in the food industry is limited (e.g. mold ripened cheese)
- Yeasts are the most widely used micro-organisms in the food industry due to their ability to ferment sugars to ethanol and carbon-dioxide. Some types of yeast, such as baker's yeasts are grown industrially, and some may be used as protein sources, mainly in animal feed
- Bacteria are generally concerned in the fermentation of food and may be divided into groups according to the product of fermentation, e.g. lactic acid bacteria, acetic acid bacteria, propionic acid bacteria, proteolytic, saccharolytic and lipolytic bacteria Many bacteria are known as micro-organisms that cause spoilage and some are pathogens (e.g.salmonellae, staphylococci etc.)

Micro-organisms, in relation to food, can have one of these 3 roles:

1.Pathogenic micro-organisms can cause infections or intoxications

2.Saprophytic micro-organism play a role in biodegradation and cause food spoilage

3.Cultured micro-organisms like probiotic bacteria are used in food processing

Sources of food Microflora

- The fruit or vegetable is harvested, milk is drawn, fish and other products are obtained from natural waters, and animals are collected and slaughtered
- After initial handling, further contamination begins and it continues while the product is being processed and prepared.
- Foods may be contaminated by each other and by pieces of equipment with which they come in contact.
- Air, dust, water, and ingredients may add their quota of contaminants
- Whenever food is handled personally by human beings, there is always the possibility of addition of human pathogens

Bacteria

- These are unicellular microorganisms with about 1µm in length and somewhat smaller in diameter.
- Bacteria are classified according to their shape.
- Bacterial spores are more heat resistant than yeast or mold spores and with a few exceptions cannot grow in acid media in which yeasts and molds thrive.
- They multiply by ‘binary fission’. When a bacterium becomes mature it divides into two, these two become four and so on.
- Bacteria can be found virtually everywhere. They are in the air, the soil, and water, and in and on plants and animals, including us.
- Some bacteria (along with archaea) thrive in the most forbidding, uninviting places on Earth, from nearly-boiling hot springs to super-chilled Antarctic lakes buried under sheets of ice. Microbes that dwell in these extreme habitats are aptly called extremophiles.

Cultural Characteristics:

- Bacterial growth in and on foods often is extensive enough to make the food unattractive in appearance or otherwise objectionable.
- Pigmented bacteria cause discolouration on the surfaces of foods; films which may cover the surfaces of liquids; growth may make surfaces slimy; or growth throughout the liquids may result in undesirable cloudiness or sediment.
- Some bacteria have natural colours. Certain species contain pigments, such as various chlorophylls, that make them naturally green, yellow, orange, or brown. Colonies of millions of bacteria may appear pink, yellowish, or white.
- The growth of bacteria is very rapid and depends upon the nature of the food material, moisture, temperature and air. Some bacteria do not grow in air but temperature plays a major role in their growth, the optimum being generally 37°C for bacteria pathogenic to humans.
- Bacteria are very sensitive to acids and are destroyed in their presence even at temperature of boiling water. Hence, most fruits being acidic can be easily sterilized at 100°C whereas vegetables being non-acidic require a higher temperature of 116°C.
- A bacterium's genetic information is contained in a single DNA molecule suspended in a jelly-like substance called cytoplasm. In most cases, this and other cell parts are surrounded by a flexible cytoplasmic membrane that is itself surrounded by a tough, rigid cell wall.

The important groups of bacteria are:

a) Bacillus: rod-shaped.

b) Coccus: spherical.

c) Coccobacillus: oval-shaped.

d) Aerobes: require atmospheric oxygen for growth, e.g., Acetobacter aceti.

e) Facultative anaerobes: can grow with or without atmospheric oxygen.

f) Obligate anaerobes: do not grow in atmospheric oxygen.

g) Mesophiles: require a temperature below 38°C for growth.

h) Obligate thermophiles: grow between 38°C and 82°C.

i) Facultative thermophiles: grow over a wide range of temperatures covered by mesophiles and obligate thermophiles and below.

j) Psychrotrophs: grow fairly well at refrigeration temperatures and some can even grow slowly at temperatures below freezing.

Special significance:

- **Encapsulation: The presence of capsules or slime may account for sliminess or ropiness of a food. Most capsules are polysaccharides of dextrin, dextran or levan and they serve as a source of reserve nutrients and increase the resistance of bacteria under adverse conditions.**
- **Formation of Endospores: Bacteria of genera *Bacillus, Clostridium, Sporosarcina* etc have the ability to form endospores. Endospores are formed at an intracellular site and are resistant to heat, ultraviolet light and dessication. Lysis of the vegetative cell releases the free endospore, which may remain dormant with no detectable metabolism for years. Sporulation usually appears in the late logarithmic phase of growth, possibly because of nutrient depletion or product accumulation. The acquisition of heat resistance is closely related to the formation of dipicolinic acid and the Ca2+ uptake. Germination is favoured by conditions that are favourable for growth.**
- **Formation of Cell Aggregates: It is characteristic of some bacteria to form long chains or 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.**

Some useful bacteria:

- **Acetobacter sp. These bacteria, also known as “vinegar bacteria”, cause significant spoilage in the wine industry but are necessary for vinegar production. The important species are Acetobacter aceti, A. orleansis and A.schutzenbachi. They are very small, usually non-motile and generally do not form spores. These bacteria are aerobes and in the presence of oxygen convert ethyl alcohol to acetic acid. These bacteria can be easily destroyed by heating to 65°C.**
- **Lactobacillus sp. Different organisms of this group, also known as “lactic acid bacteria”, have different properties but all of them produce lactic acid from carbohydrates. The important species include Lactobacillus plantarum, Pediococcus cerevisiae, Leuconostoc mesenteroides, Streptococcus faecalis and Lactobacillus brevis. These bacteria cause “lactic souring” and spoil wines, which can be easily prevented by maintaining a sulphur dioxide concentration of 0.007 per cent in wine.**

- **Important Food Spoilage Bacteria**

Group	Genus
Acetics	**Acetobacter and Gluconobacter**
Lactics	**Lactobacillus, Leuconostoc, Pediococuus, Sreptococcus**
Butyrics	**Clostridium**
Propionics	**Propionibacterium**
Proteolytics	**Bacillus, Pseudomonas, Closridium, Proteus**

Fungi (Molds)

The term mold is applied to certain multicellular, filamentous fungi whose growth on foods is usually readily recognized by its fuzzy or cottony appearance. Fungi are eukaryotic organisms their DNA-containing chromosomes are enclosed within a nucleus inside their cells

Morphological Characteristics:

- **The gross appearance of a mold growing on a food is often enough to indicate its genus**
- **Some molds are fluffy, others are compact**
- **Some look velvety on the upper surface, some dry and powdery, and others wet or gelatinous**
- **Pigments in the mycelium—red, purple, gray, black, etc.—are also characteristic.**
- **Some molds are restricted in size, but others seem limited only by the food or container**
- **Macroscopically the mold consists of a mass of branching, interwined filaments called hyphae (singular hypha), and the whole mass of these hyphae is known as a mycelium.**

- **Hyphae may be classed as vegetative or fertile based on their biological function. The vegetative hyphae or growing hyphae are concerned with the nutrition of the mold and the fertile ones with the production of reproductive parts.**
- **The hyphae of some molds are full and smooth, but the hyphae of others are characteristically thin and ragged**
- **A few kinds of molds produce sclerotia (singular sclerotium) which are tightly packed masses of hyphae, often thick-walled, within the mycelium. These sclerotia are considerably more resistant to heat and other adverse conditions than the rest of the mycelium and for this reason may be important in some processed food products**
- **In the group of molds called septate the hyphae are divided by cross-walls into cells. The hyphae of the nonseptate group consist apparently of cylinders without cross walls.**
- **The reproductive parts or structures of molds are the spores, which are mainly asexual.**
- **Such spores are produced in large numbers and are readily spread by air. Spores that settle on favorable substrates can initiate a new phase of growth and develop into a new mycelium.**

Physiological Characteristics :

- **Moisture Requirements: In general most molds require less available moisture than do most yeasts and bacteria. 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.**
- **Temperature Requirements: Most molds would be considered mesophilic i.e. able to grow well at ordinary temperature. The optimal temperature for most molds is around 25 to 30°C, but some grow well at 35 to 37°C or above, e.g. Aspergillus spp. And some at still higher temperatures. A number of molds are psychrotophic or psychroduric i.e. they grow fairly well at temperatures of refrigeration, and some can grow slowly at temperatures below freezing. Growth has been reported at as low as – 5 to 10°C. A few are thermophilic; i.e. they have a high optimal temperature.**

- **Oxygen and pH Requirements Molds are aerobic; i.e. they require oxygen for growth; this is true at least for the molds growing on foods. Most molds can grow over a wide range of hydrogen-ion concentration (pH 2 to 8.5), but the majority are favoured by an acid pH.**
- **Food Requirements: Molds in general can utilize many kinds of foods, ranging from simple to complex. Most of the common molds possess a variety of hydrolytic enzymes, and some are grown for their amylases, pectinases, proteinases, and lipases.**
- **Inhibitors: Compounds inhibitory to other organisms are produced by some molds, such as penicillin from Penicillium chrysogenum and clavacin from Aspergillus clavatus. Certain chemical compounds are mycostatic, inhibiting the growth of molds (sorbic acid, propionates, and acetates are examples), or are specifically fungicidal, killing molds.**

Classification of Molds:

- **Genus *Mucor* (Mucor racemosus, Mucor rouxii): Mucors are involved in the spoilage of some foods and in the manufacture of others e.g. oriental fermented foods.**
- **Genus *Rhizopus*: *Rhizopus nigricans*, sometimes called „bread mold", is very common and is involved in the spoilage of many foods such as berries, fruits, vegetables, bread, etc.**
- **Genus *Aspergillus*: The members of this genus are very widespread. Many are involved in the spoilage of foods and some are useful in preparation of fermented foods. Many groups and hundreds of aspergillus species are known. *Aspergillus niger* is the leading species important for food microbiologists. Selected strains are used for commercial production of citric and gluconic acids.**
- **Genus *Penicillium*: This is another widespread genus important in foods. *Penicillium expansum*, a green spored species, causes soft rot of fruits. *Penicillium camemberti* with grayish conidia, useful in the ripening of Camembert cheese, and Penicillium roqueforti, used in ripening of blue cheeses, are also weel known members of this genus.**
- **Genus *Bothrytis*: The species Bothrytis cinerea causes the noble rot of grape in some wine producing areas such as Tokay (Hungary).**
- **Genus *Alternaria*: Molds of this genus are common causes of the spoilage of foods. *Alternaria citri, Alternaria tenuis and Alternaria brassicae* are the common species.**
- **Genus *Neurospora* (Monilia): The species of this genus grow on various foods.**

- **Generally molds are concerned in the spoilage of foods; moldy or mildewed food is considered unfit to eat.**
- **On the other hand some of molds are used in manufacture of different foods and are ingredients of some foods**
- **Some kinds of cheese are mold-ripened (e.g. Roquefort, Camembert).**
- **Molds are grown as feed and food and are employed to produce products used in foods, such as amylases and other enzymes for bread making or citric acid used in soft drinks.**
- **Molds are major contributors in the ripening of many oriental foods.**
- **A species of *Bothrytis cinerea*, is responsible for the noble rot of grape.**
- **Molds are used for production of several antibiotics.**

Yeasts

- Yeasts are unicellular fungi which are widely distributed in nature. They are somewhat larger than bacteria.
- The cell length is about 10µm and the diameter is about a third of this. Most yeasts are spherical or ellipsoidal.
- Yeasts that multiply by means of ‘budding’ are known as ‘true yeasts’.
- Yeasts grow luxuriously at a moderate temperature in a solution of sugar in plenty of water.
- Under suitable conditions the sugar is converted into alcohol and carbon dioxide is evolved. This is the reason that carbon dioxide is evolved from food materials spoiled by yeasts and pushes out corks from bottles with great force.
- Boiling destroys the yeast cells and spores completely.
- Some of the yeasts which grow on fruits are Saccharomyces, Candida and Brettanomyces.

Morphological Characteristics Form and structure:

- The form of yeasts may be spherical to ovoid, lemonshaped, pear-shaped, cylindrical, triangular, or even elongated into a false or true mycelium. They also differ in size.
- Most yeasts reproduce asexually by multilateral or polar budding, a process in which some of the protoplasm bulges out the cell wall; the bulge grows in size and finally walls off as a new yeast cell.
- A new species or yeasts reproduce by fission, and one reproduces by combination of fission and budding.
- Sexual reproduction of “true” yeasts (Ascomycotina) results in the production of ascospores, the yeast cell serving as the ascus.
- The ascospores may differ in colour, in smoothness or roughness of their walls, and in their shape (round, oval, reniform, bean or sickle-shaped, hemispherical, angular, fusiform, or needle-shaped).

Cultural Characteristics:

- **The appearance of massed yeast growth is not useful in the identification of yeasts, although growth as a film on the surface of liquid media suggests an oxidative or film yeasts, and production of a carotenoids pigment indicates the genus Rhodotorula.**
- **Yeasts are oxidative, fermentative, or both. The oxidative yeasts may grow as a film, pellicle, or scum on the surface of liquid and then are termed film yeasts.**
- **Fermentative yeasts usually grow throughout the liquid and produce carbon dioxide.**

Physiological Characteristics:

- Most common yeasts grow best with a plentiful supply of available moisture. But since many yeasts grow in the presence of greater concentration of solutes (such as sugar or salt) than most bacteria it can be concluded that these yeasts require less moisture than the majority of bacteria.
- Most yeast require more moisture than molds, however, on the basis of water activity or aw yeasts may be classified as ordinary if they do not grow in high concentrations of solutes, i.e. in a low aw, and as osmophilic if they do. However limits of aw for ordinary yeasts tested thus far ranges from 0.88 to 0.94.
- The range of temperature for growth of most yeasts is, in general, similar to that for molds, with the optimum around 25°C to 30°C and the maximum about 35°C to 47°C. Some kinds can grow at 0°C or less.

- **The growth of most yeasts if favoured by an acid reaction in the vicinity of pH 4 to 4.5, and they will not grow well in an alkaline medium unless adapted to it.**
- **Yeasts grow best under aerobic conditions, but the fermentative types can grow anaerobically, although slowly.**
- **Sugars are the best source of energy for yeasts, although oxidative yeasts, e.g., the film yeasts, oxidize organic acids and alcohol. Carbon dioxide produced by bread yeasts accomplishes the leavening of bread, and alcohol made by the fermentative yeasts is the main product in the manufacture of wines, beer, industrial alcohol, and other products.**
- **The yeasts also aid in the production of flavors or "bouquet" in wines.**
- **Nitrogenous foods utilized vary from simple compounds such as ammonia and urea to amino acids and polypeptides.**
- **Yeasts require accessory growth factors.**

- **Yeasts causing food spoilage**

Yeast	Product Spoilage
Saccharomyces	Low sugar products
Candida	High-acid foods, salty foods, butter Brettanomyces Beers, wines
Zygosaccharomyces (osmophillic)	Honey, syrups, molasses, wines, soy sauce
Pichia	Wines
Hansenula	Beers
Torulopsis	Milk products, fruit juices, acid foods
Rhodotorula	Meat, sauerkraut