**ADVANCED FOOD MICROBIOLOGY MCB 402**

Man’s sources of food are of plant and animal origin. Therefore, it is important to understand the biological principles of the microbial flora associated with plants and animals in their natural habitats and their respective roles.

While it sometimes appears that microorganisms are trying to ruin our food sources by infecting and destroying plants and animals, including man, this is by no means their primary role in nature. From our present concepts of life on this planet, the primary role of microorganism in nature is self-perpetuation. During this process, the heterotrophs carry out the following general reaction:-

**All organic matter (CH2 O, proteins, lipids etc.)**

Energy + Inorganic compacts (nitrates, SO4 etc). This of course is nothing more than the operation of the nitrogen cycle and the cycle of other elements. In spite of their simplicity when compared to higher forms, microorganisms are capable of carrying out many complex chemical reactions essential to their perpetuation. To do this, they must obtain nutrients from organic matter, some of which constitutes our food supply.

If one considers the types of microorganism associated with plant and animal foods in their natural states one can then predict the general types of microorganisms to be expected on this particular food product at some later stage in its history. Results from many laboratories show that untreated foods may be expected to contain varying numbers of bacteria molds or yeasts and the question often arises as to the safety of a given food product based upon total microbial numbers. The question should be two fold

1. What are the types of microorganism present by gram or per ml?
2. What are the types of microorganism that are represented in this number

It is necessary to know which organisms are associated with a particular food in its natural state and which of the organisms present are not normal for that particular food in its natural state and which of the organism present are not normal for that particular food. It is therefore, of value to know the general distribution of bacteria in nature and the general types of organisms normally present under given conditions where foods are grown and handled.

Microorganism are quite ubiquetos having been found everywhere in the earths atmosphere except in volcanic land and on the inside of healthy plant and animal tissues. Their primary role in nature as earlier mentioned is self-perpetuation, the result of which is the conversion of organic matter to in organic compounds through which process they obtain the necessary energy for growth. All microorganisms such as bacteria, molds, and yeasts, are basically soil and water creatures with the parasitic state being relatively recent in the existence of these forms. From soil and water, these organisms find their way onto plants, into the air, dust, the intestinal tract of man and other animals, and back onto soil and into water where the cycle continues. Each genus appears to occupy a more-or-less specific niche in nature, and knowledge of this fact will allow one to make certain general predictions about the existence of these organisms in and on foods.

**INTRINSIC AND EXTRINSIC PARAMETERS OF FOODS THAT AFFECT MICROBIAL GROWTH**

Since our foods are of plant and animal origin, it is worthwhile to consider those characteristics of plant and animal tissues that affect the growth of microorganism therein. The plants and animals that serve as food sources have all evolved mechanisms of combating contamination against the invasion and proliferation and some of these remain in effect in fresh foods. By taking these natural phenomena into account, one can make effective use of each or all in preventing or retarding the microbial spoilage of the products which are derived from them.

**Intrinsic parameters**

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

1. pH
2. moisture content
3. oxidation – reduction potential (Eh)
4. nutrient content
5. anti microbial constituents
6. biological structures

**Extrinsic parameters**

The extrinsic parameters of foods are those properties of the storage environment that affect both the foods and their microorganisms. Those of greatest importance to the welfare of food-borne organism are (1) Temperature of storage (2) relative humidity of environment and (3) presence and concentration of gases in the environment.

Each food product is characterized by a set of intrinsic parameters enumerated above which is capable of preventing the entry of many microorganisms to certain foods. When a given food is characterized with respect to these parameters, it then becomes possible to make predictions about the perishability of that food by taking into consideration the overall capacity of certain flora to grow within these parameters.

The microorganisms of importance in foods all have limiting relative to these parameters and may be inhabited by one or more of them. the extrinsic parameters of temperature of storage, relative humidity of the storage environment and the presence or absence of gases such as Co2 and O2 may also be employed to inhabit the growth of some organisms. The storage of dried foods in areas of high Relative Humidity may offset the intrinsic parameter of water content and allow for growth in an otherwise stable product. The operation of these as well as other less definable parameters lead to the establishment of certain organisms as the dominant types in given foods.

For example, citrus fruits are largely spoiled by certain molds, refrigerated ground beef is spoiled mainly by certain gram negative bacteria, bread is spoiled mainly by certain molds etc.

There are a number of aspects to the role of the food microbiologist in the food industry, these include quality control on incoming raw materials, quality control of production, hygiene training for production staff, the development of suitable codes of practice for hygienic food production, assessment of detergent – disinfectants and the establishment of efficient cleaning regimes, the examination of samples of finished products and the investigation of customer complaints of a microbiological nature. Often, undue emphasis is placed on the microbiological examination of many and frequent samples of the finished products only with over simple tests being performed which provide very little real information about the microbiological status of the food.

It may be possible for a manufacture, or caterer to require that the suppliers of the materials meet certain specifications for the supplies to be accepted. For example in the production of many low-acid canned foods, the heat treatment given needs to be far in excess of any legally required “botulium cook” in order to achieve an acceptable low level of spoilage in the finished product. This is because the spore forming spoilage bacteria will be present in the foodstuff before heat treatment in much greater numbers than will *C.botulinum* spores, and very many of such spores will have much higher. D-values than will the *Cl*. *botulinum spores. If the raw materials e.g. rice which are used in the manufacture of the canned foods and which are known to contribute the majority of the bacterial spores are required to meet a specification relating to a low spore count, a less severe heat treatment will be needed to obtain an acceptable low level of spoilage, with* a consequent improvement in organoleptic quality.

Quality control on production is likely to operate at three levels, hygiene control on the processing and this includes cleaning regimes for equipment etc, detection of possible hazards from pathogenic organisms in the product and the assessment of the potential shelf – life (or storage life) of the product. The application of a repressive system of QC based only on the sampling of finished products and rejection of batches which fail the standard is unlikely to succeed in its aim since microbiological testing is destructive and in consequence only a relatively small sample of the entire batch can be taken. There is no way in which a food company can protect itself against a statutory zero tolerance on pathogens by microbiological testing of samples of the finished product. The only possibility of protection would come from extremely rigorous on line control of the hygienic precautions taken. For this to be successful the co-operation of the food handlers and production staff would be necessary.

A more positive approach to QC is one based on the provision of training courses in hygiene for production staff and the application of agreed codes of practice which, although they may be based on officially published Codes of Practice will be more helpful and easier to understand if they are written for the specific production situation.

Detailed microbiological examinations of samples taken during and after the production and processing of food products can provide useful information, allowing an assessment of the probable shelf life, a check of the process in order to correct or to anticipate any deterioration in the production methods, and some indication of potential public health hazard.

However, the choice of the methods of exam in action of a particular food product requires amongst other things an intimate knowledge of the preparation storage and distribution of the raw constituents.

**PRIMARY SOURCES OF MICROORGANISMS TO FOOD**

There are about 25 of the most important general of bacteria known to cause food spoilage and food poisoning. Some of them are ***Acetobacter, Acinetobacter, Alkaligenes, Bacillus, Citrobacter, Clostridium, Corgnebaterium, Enterobacter, Erwinia, Escherichia, Flavobacterium, Lactobacillus Leuconostoc, Micrococcus, Proteus, Pseudomonas, Salmonella, Serratia, Shigella, Staphyloccus, Streptococcus and Streptomyces.***

These are the most important bacteria normaly found in food. Each general has its own particular functions of nutrition and consequent degradative processes. These organisms may be generally associated with the particular aspects of the food environ presented below:-

1. **Soil and Water:-** It may be assumed that at one time, all microorganism existed in water. The drying of surface soils give rise to dust which, when disseminated by winds, carries adhering microorganisms to many places; including other areas of the soil, rivers, oceans etc. The formation of clouds over large bodies of water and the subsequent rainfall over land as well as other waters has the same effect. It is not surprising, then that soil and water microorganisms are often one and the same.

The following general of food-borne bacteria generally found in soils and water may be expected in foods: ***Alcaligenes, Bacillus, Citrobater, Clostridium, Corynebacterium, Enterobacter, Micrococcus, Proteus, Pseudomonas, Serratia, Streptomyces etc.***

Molds are in general, very widespread in nature, where they participate in the degradation of both plants and animal matter as well as cause many diseases of plants and animals.

Among most microorganisms that are nearly always present in soils are ***Aspergillus, Rhizopus, Penicillium, Botrytis, Fusarium*** and others.

1. ***Plants and Plants Products****:-* Most of the organisms discussed above for soil and water are also found on plants, since soil and water constitute the primary sources of microorganism to plants. On the other hand, there are some bacteria that are associated more with plants than with soil. Among these genera are*:* ***Acetobacter, Erwinia, Lactobacillus, Leuconostoc*** *and* ***Streptococcus****.*

Among the molds, the most important plant-borne genera are those that cause the spoilage of vegetables and fruits which are referred to as market diseases.

Genus **saccharomyces**, is the most notable yeast that may be found in many plants products especially fruits. Others are genera ***Rhodotorula*** and ***Torula***

1. ***Food Utensils***:-The genera of microorganisms to be found on food utensils depends upon the types of food handled; the care of the utensils, their storage, and other factors. If vegetables are handled in a given set of utensils, one would, of course, expect to find some or all of the organisms associated with vegetables. When utensils are cleaned with hot or boiling water the remaining Flora would normally be those best able to withstand effects of this treatment. Utensils that are stored in the open where dust might collect should be expected to have air-borne bacteria, yeasts and malds.
2. **Intestinal tract of Man and Animals:-** There are several genera of bacteria that are more commonly found in this environ, than in soils, water, or other places. Among these are:- ***Bacteroides, Eschericlia, Lactobaccillus, Proteus, Salmonella, Shigella, Staphylococcus*** and ***Streptococcus***. The most notable of these is ***Escherichea***, which has as its natural habitat the intestinal tract of man and other animals.

Species of other genera common to the intestinal tract include, ***Clostridium***, ***Citrobacter***, ***Enterobacter*** and ***Pseudomonas***.

From the intestinal tract of animals, intestinal microorganism find their way directly to the soil and water. And from the soil, they may find their way onto plants, in dust, to intensils etc.

Molds are not thought to be transmitted by feacal sources though the yeast genus ***Candida*** is very often found in the intestinal tract of man.

1. **Food Handlers:**- The microflora on the hands and other outer garments of food handlers generally reflects the environ and habits of individuals. This flora would normally consist of oras found on any object handled by the individual as well as some of those picked up from dust, water, soil, and the like. In addition there are several genera of bacteria that are specifically associated with hands, nasal cavities, and mouth. Among these are the genera ***Micrococcus*** and ***Staphylococcus***, the most notable of which are ***staphylococci*** which are found on hands, arms, in nasal cavities, the mouth, and other parts of the body.

While the genera ***Salmonella*** and ***Shigella*** are basically intestinal forms, they may be deposited onto food and utensils by food handlers if sanitary practices are not followed by each individual. Any number of molds and yeasts may be found on the hands and garments of food handlers depending upon the immediate history of each individual.

1. **Air and Dust:**- The types of organisms to be found in air and dust, with the exception of some of the pathogens, include the 25 years of bacteria, the 16 genera of molds and many of the yeasts. Although ***Staphylococcus*** and ***Salmonella*** spp may at times be found in air and dust, these are not the major sources of these organisms to foods. Notable among the bacteria genera in air and dust are ***Bacillus*** and ***Micrococcus*** species all of which are able to endure dryness to varying degrees. Notable among the yeasts is the genus ***Torulopsis***, and many mold genera may be found from time to time.

**MOLDS COMMON IN AND ON FOODS**

Unlike the true bacteria and most yeasts, molds grow in the form of a tangled mass which spreads rapidly and may cover several inches of area in 2 – 3 days. The total of the mass or any large single portion of it is referred to as ***Mycelium***. At the time of axesual reproduction, ***sporangiophores*** or ***conidiophores*** are sent up which bear, usually, at their tips, sporangia or conidia.

In those molds that produce sporangia, the spores are borne by these structures and are responsible for the various colours displayed by molds. Comidia, represent unprotected spores. In addition to these characteristics, and common asexual spores, some molds produce asexual spores.

***Chlamydospores*** results when a thick wall develops around any cell of the mycelium.

These structures are somewhat resistant to adverse environmental conditions. Arthrospores or oidia result from fragmentation by some molds that produce a separate mycelium.

Chlamydospores and arthrospores are a bit more difficult to destroy than other parts of the mold mycelium, and they sometimes cause concern in the food industry.

Most molds of importance in foods are now placed in the ***Fungi Imperfect*** group. These are molds whose sex, cycles are not known. Most or all are said to be related to the ***Ascomycetes*** group.

Some common molds and what they spoil are listed below:-

1. **Alternaria:-** Active in the spoilage of many plant products.
2. **Aspergillus:**- Molds appear yellow to green to black on a large number of foods some species of this genus produce carcinogenic aflatoxins while others are employed as commercial sources of proteases and citric acid. They are widespread and may be found on cakes, fruits, vegetables, meats, and other foods.
3. **Bofrytis:**- They cause a “grey mold” condition on many plants and plant foods. They are important causes of market diseases of fruits and vegetables.
4. **Cladosporuim:**- ***C***. ***herbarum*** produces black spots on beef
5. **Fusarium:**- These fungi are important in the spoilage of many fruits and vegetables. They have been implicated in “neck rot” of bananas.
6. **Geotrichum (oidium):** They are yeastlike fungi that produce various colors but generally white. These organisms are sometimes referred to as the “dairy mold” since they impart flavor and aroma to many types of cheese. They are referred to also as “machinery molds” since they build up on food contact equipment in food processing plants, especially tomato canning plants.
7. **Mucor:**- May be found growing on a large number of foods e.g. bread
8. **Penicillium:**- Typical colours on foods are blue to blue green. These molds are important in the making of some cheeses. Some are important in the production of antibiotics (penicillin e.g.). They are widespread in the soil, air, dust, and in many other places and may be found on foods such as breads, cakes, fruits and preserves. Some cause soft rots of fruits.
9. ***Rhizopus***:- Like the penicillia, they are very widespread in nature and may be found growing on foods, such as fruits, cakes, preserves and bread. One species ***R***. ***stolonifer***, is often called the “bread mold”. Some are employed in the fermentation of starch to alcohol.

**YEARS ASSOCIATED WITH FOOD SPOILAGE**

Yeasts are microscopic organisms that may be differentiated from the common bacteria by their large cell size, their oval, enlongate, elliptical or spherical cell shapes; and by their production of buds during the process of division.

The sizes of yeast cells vary, some ranging from 5 – 8Nm in diameter, while others may be as large as 100Nm in length. In general older cells of yeast tend to be smaller in size than young growing cells. Yeasts can grow over rather wide ranges of pH, alcohol and sugar concentration. Some have been reported to grow over a pH as low as 1.5 and in up to 18% ethanol. Many grow in the presence of 55% or more sucrose.

Yeasts are often placed into groupings based upon some particular function or activity. For example, film yeasts are those that grow at the surface of certain products such as *saver kraut* and pickles.

Some film yeasts are species and strains of the genera ***candida*** and ***Hansenula****.* These organisms are capable of oxidizing acids and alcohols as sources of energy. Top yeasts are those that carry out the conversion of sugar to alcohols at top of a vessel, while bottom yeasts are those that can do the same but from the bottom of the vessel. ***Apiculate*** or lemon-shaped yeasts are undersirable in wine fermentations where they produce off-flavours.