Food Microbiology

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Book Contents

Food microbiology

INTRODUCTION

Microorganisms are living entities of microscopic size and include bacteria, viruses, yeasts and molds (together designated as fungi), algae, and protozoa. While bacteria are classified as prokaryotes (cells without definite nuclei), the fungi, algae, and protozoa are eukaryotes (cells with nuclei); viruses do not have regular cell structures and are classified separately. Micro- organisms are present everywhere on earth, which includes humans, animals, plants and other living creatures, soil, water, and atmosphere, and they can multiply everywhere except in the atmosphere. Together, their numbers far exceed all other living cells on this planet. They were the first living cells to inhabit the earth over 3 billion years ago; and since then they have played important roles, many of which are beneficial to the other living systems.

Among the micro-organisms, some molds, yeasts, bacteria, and viruses have both desirable and

undesirable roles in our food. In this unit, the scope of food microbiology, importance of microbes in food and predominant microorganisms associated with food have been discussed.

The science of microbiology

Microbiology is the branch of the biological sciences that deals with micro- organisms, i.e. bacteria, fungi, some algae, protozoa, viruses, viroids and prions. Most micro-organisms have the following characteristics: -

1) They are generally too small to be seen with the unaided human eye, and some form of microscopy is required for the study of their structure.

2) Cells or other structures are relatively simple and less specialized than those of higher plants and animals.

3) They are handled and cultured in the laboratory in ways that are generally quite similar.

Microbiology has developed into a science that can be studied from a number of perspectives. A specialist study can be made of each of the individual groups giving rise to the following disciplines:

• Bacteriology - the study of bacteria

• Mycology - the study of fungi

• Protozoology - the study of protozoa

• Phycology (algology) - the study of algae

• Virology - the study of viruses.

Micro-organisms can also be studied from the applied viewpoint, i.e. the relationship between micro-organisms, the environment and human activity.

This again gives rise to a number of areas of specialist study:

• Medical microbiology includes some aspects of pathology (the study of diseases), immunology (how the immune system operates to prevent invasion by

micro-organisms) and epidemiology (how diseases are distributed and spread).

• Agricultural microbiology: The study of micro-organisms for crop/ plant health and related areas.

• Industrial microbiology / biotechnology: The study of the use of Microorganisms in large scale industrial processes.

• Food microbiology: The study of the role that micro-organisms play in food spoilage, food production, food preservation and food-borne disease.

None of these areas of specialist study can operate in isolation, e.g. food microbiology encompasses various aspects of industrial microbiology and biotechnology in the manufacture of fermented food and the production of single-cell protein. A study of food-borne disease involves aspects of medical microbiology and agricultural microbiology.

Specialist knowledge needs to be underpinned by an understanding of fundamental principles. The food

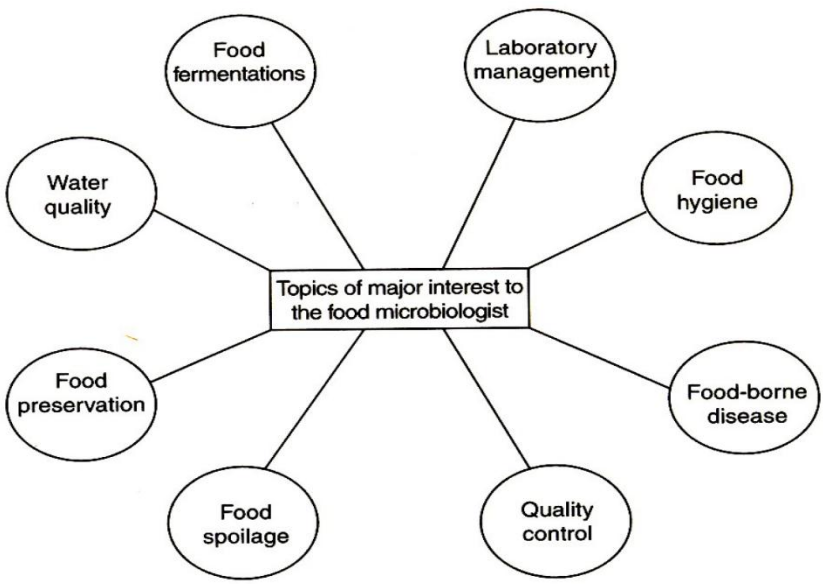
microbiologist, for example, needs to have an understanding of microbial structure; the classification and identification of micro-organisms; how micro-organisms grow; the factors that influence growth and how growth can be controlled; death of microorganisms; nutrition of microorganisms and how they are cultured in the laboratory

Food microbiology - its origin and scope

Although processes of food spoilage and methods of food preservation and food fermentation have been recognized since ancient times, it was not until the 1800s that the relationship between foods and micro-organisms was established. In 1837 Schwann proposed that the yeast which appeared during alcoholic fermentation was a microscopic plant, and between 1857 and 1876 Pasteur showed that micro-organisms were responsible for the chemical changes that take place in foods and beverages. Their observations laid the foundation for the development

of food microbiology as we know it today. Soon after these early discoveries were made, knowledge about the role that micro-organisms play in food preservation, food spoilage and food preservation, food spoilage and food poisoning accelerated rapidly until food microbiology gradually emerged as a discipline in its own right.

Food microbiology is now a highly developed area of knowledge with the main areas of interest highlighted in Fig. 1.1



Not all groups of micro-organisms are of equal interest to the food microbiologist. Bacteria come very much on top of the list with molds and yeasts also of considerable importance and viruses less so. The associations that these organisms have with the manufacture and consumption of foods are summarized in Fig. 1.2.

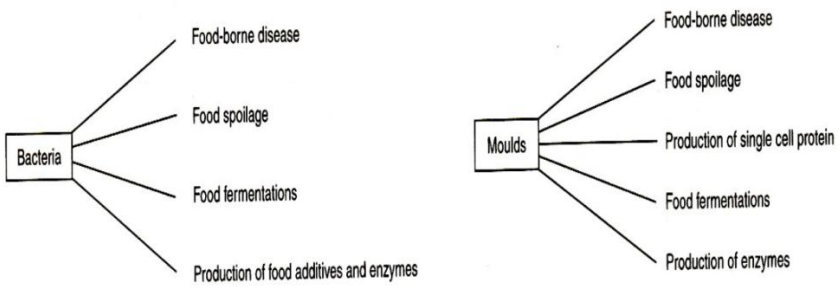


Fig.2 a. Various groups of microorganisms and their associations with food.

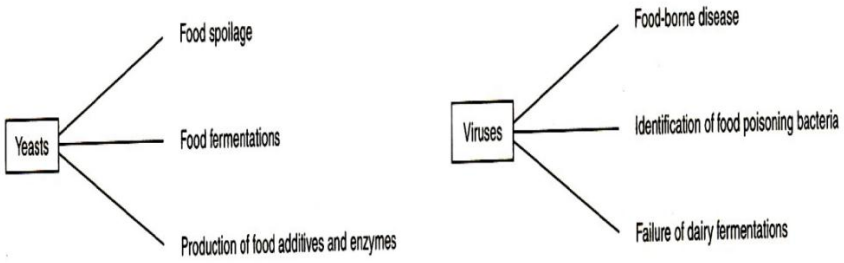


Fig. 2b. Various groups of microorganisms and their associations with food.

Protozoa and algae have minimum direct impact on the production, processing and consumption of food. Food-borne disease can be caused by some protozoa and others belonging to this group are important in the treatment of wastes. Algae are used to produce alginates; some have the potential for use in the production of single-cell protein and some marine species produce toxins that might enter our food along with sea foods.

Importance of microorganisms in foods

Since 1900 A.D. our understanding of the importance of micro-organisms in food has increased greatly. Their role in food can be either desirable (food bioprocessing) or undesirable (food borne diseases and food spoilage), which is briefly discussed here.

Food-borne diseases

Many pathogenic micro-organisms (bacteria, molds and viruses) can contaminate foods during various stages of their handling, between production and consumption. Consumption of these foods can cause

food borne diseases. Food borne diseases can be fatal and may also cause large economic losses. Foods of animal origin are associated, more with food borne diseases than foods of plant origin. Mass production of food, introduction of new technologies in the processing and storage of food, changes in food consumption patterns, and increased import of food from other countries have increased the chances of large outbreaks as well as the introduction of new pathogens. Effective intervention technologies are being developed and implemented to ensure the safety of consumers against food borne diseases. New methods are also being developed to effectively and rapidly identify the pathogens in contaminated foods.

Food spoilage

Except for sterile foods, all foods harbor micro-organisms. Food spoilage stems from the growth of these micro-organisms in food or is due to the action of microbial enzymes. New marketing trends, consumers' desire for foods that are not overly

processed and preserved, extended shelf life, and chances of temperature abuse between production and consumption of foods have greatly increased the chances of food spoilage and, in some instances, with new types of micro-organisms. The major concerns are the economic loss and wastage of food. New concepts are being studied to reduce contamination as well as control the growth of spoilage microbes in foods.

Food bioprocessing

Many food microorganisms are used to produce different types of fermented foods using raw materials from animal and plant sources. Consumption of these foods has increased greatly over the last 15-20 years and is expected to increase further in the future. There have been great changes in the production and availability of these micro-organisms (starter cultures) to meet the large demand. In addition, novel and better strains are being developed by using genetic engineering

techniques.

Food additives

Microbial enzymes are also being used to produce food and food additives. By employing genetic recombination techniques, and using diverse microbial sources enzymes of higher purity & activity are obtained. Many types of additives from microbial sources are being developed and used in food. Some of these include single-cell proteins, essential amino acids, colour compounds, flavour compounds, stabilizers and organic acids.

Food biopreservation

Antimicrobial metabolites (e.g. bacteriocins and organic acids like acetic, propionic and lactic acids) of desirable Micro-organisms are being developed and used in foods in place of preservatives of non-food (chemical) origin to control pathogenic and spoilage micro-organisms in food. Economic production of these antimicrobial compounds and their effectiveness in food systems have generated wide interest.

Probiotics

Consumption of foods containing live cells of bacteria and that have apparent health benefits has generated interest among consumers. The role of these bacteria for health and bacterial efficacy benefits is being critically investigated.

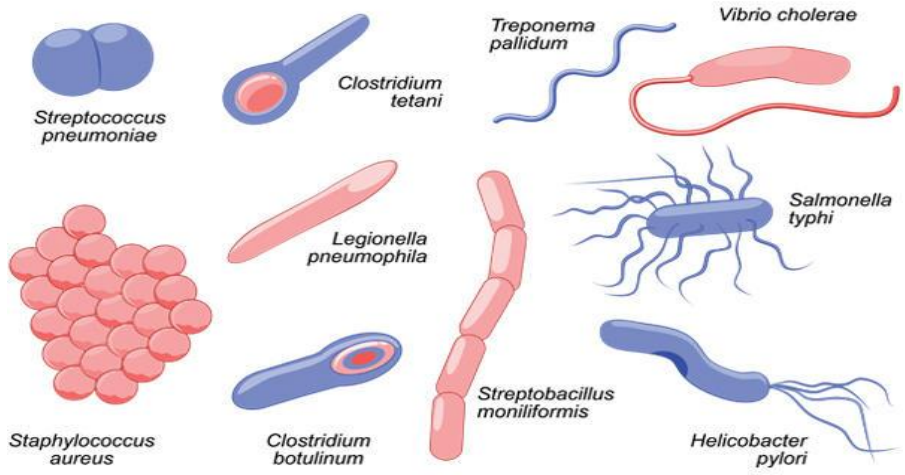
Microorganisms in food

The microorganisms most common to food are bacteria and fungi. The fungi, which are less common than bacteria, consist of two major types of Micro- organisms, viz. molds and yeasts. Apart from these, food may contain viruses and other parasites such as protozoans, worms etc.

Bacteria

Bacteria are unicellular microorganisms that are approximately one micro meter (10^{-3} mm) in diameter with variations in morphology from short and elongated rods (bacilli), spherical or ovoid forms(cocci), vibrio (comma shaped) and even spiral in shape (Refer Fig. 1.3). Cocci (meaning “berry”) are sphere shaped bacteria. Individual bacteria

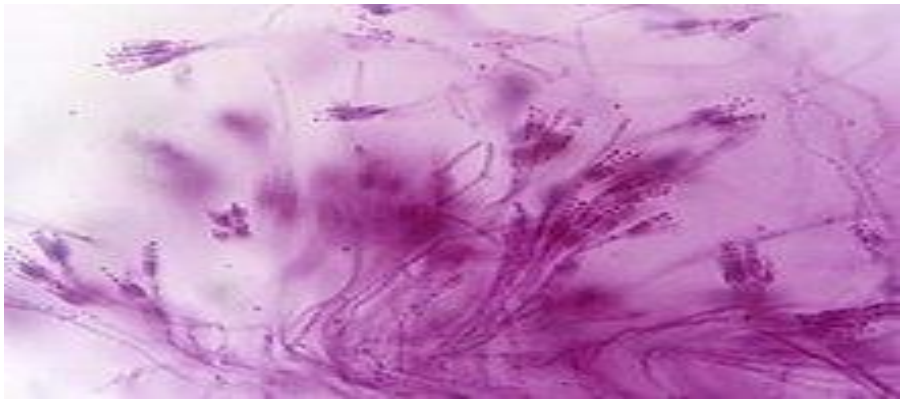
closely combine in various forms according to genera. Some sphere-shaped bacteria occur in clusters similar to a bunch of grapes (i.e. *staphylococci*). Other bacteria (rod shaped or sphere shaped) are linked together to form chains (i.e. streptococci in case of cocci chain). Certain genera of sphere-shaped bacteria are found together in pairs (*diplococci i.e. Pneumococci*) or as a group of four (Square or cubical packets formation; i.e. *Sarcinia*), while other genera appear as an individual bacterium. Other bacteria (in majority) are rod shaped and possess flagella and are motile. Bacteria produce various pigments which range from shades of yellow to dark pigments such as brown or black. Certain bacteria have pigmentation of intermediate colors such as red, pink, orange, blue, green, or purple. These bacteria cause food discoloration, especially, among foods with unstable color pigments such as meat. Some bacteria also cause discoloration by slime formation.



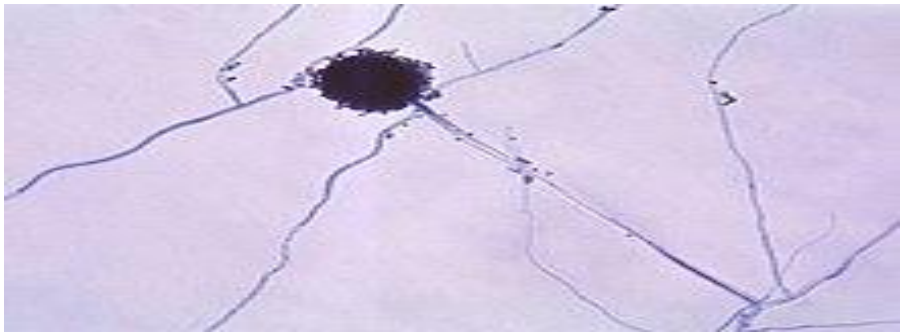
Molds

Molds are multicellular micro-organisms with mycelial (filamentous) morphology. These microbes are also characterized by their display of a variety of colors and are generally recognized by their mildewy or fuzzy, cotton like appearance. Molds can develop numerous tiny spores that are found in the air and can be spread by air currents. These spores can produce new mold growth if they are transferred to a location that has conditions conducive to germination. Molds generally withstand greater fluctuation in pH than bacteria and yeasts and can frequently tolerate more temperature fluctuation. Although molds thrive best

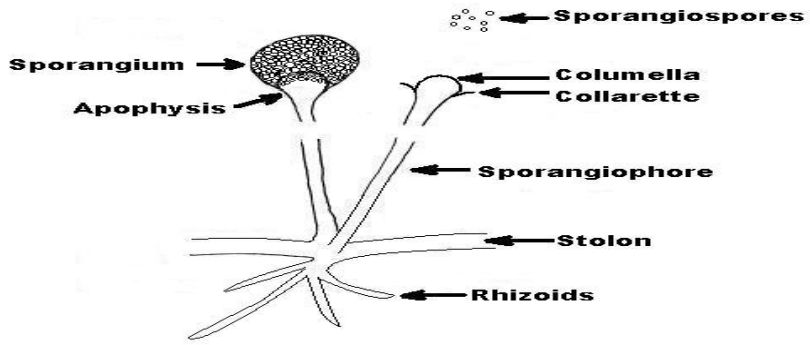
at or near a pH of 7.0, a pH range of 2.0 to 8.0 can be tolerated, even though an acid to neutral pH is preferred. Molds thrive better at ambient temperature than in a colder environment, even though growth can occur below 0°C. Although mold growth is optimal at a water activity (Aw) of approximately 0.85, growth can and does occur below 0.80. At an A w of 0.90 or higher, bacteria and yeasts grow more effectively and normally utilize available nutrients for growth at the expense of molds. When the A w goes below 0.90, molds grow more effectively. That is why foodstuffs, such as pastries, cheeses, and nuts, that are low in moisture content are more likely to spoil from mold growth.



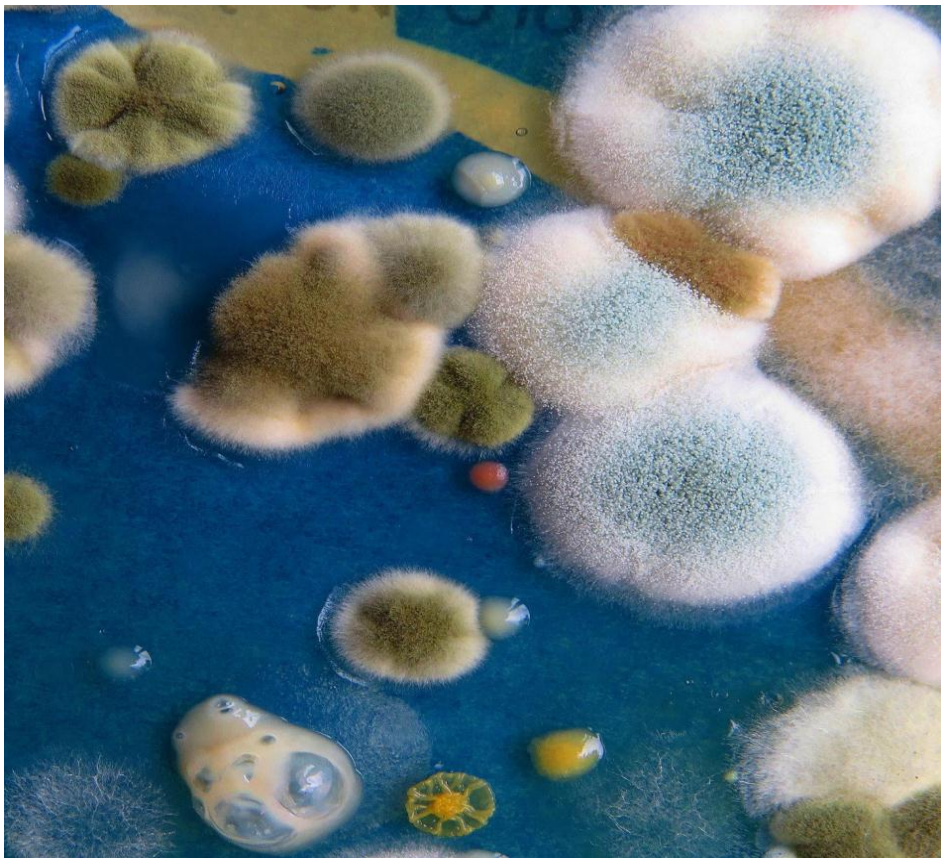
Pencillium sp.



Aspergillus sp.



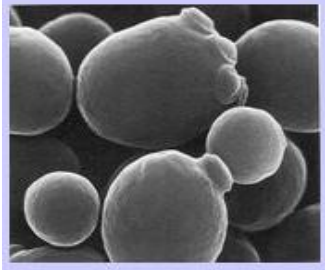
Rhizopus stolonfier

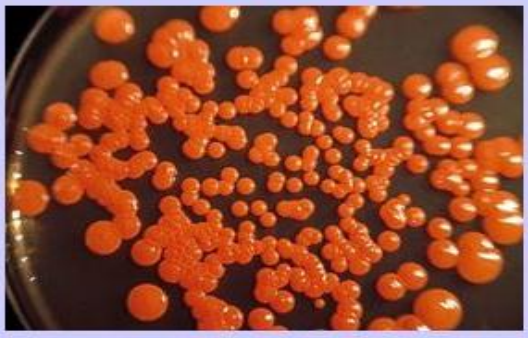


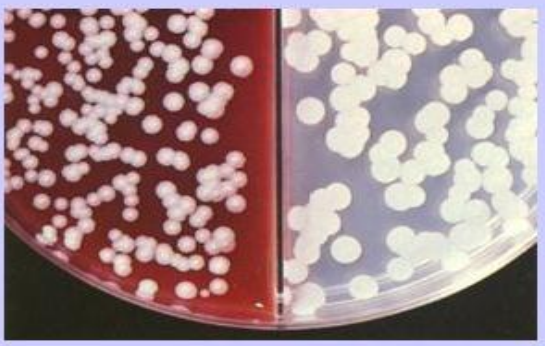
Yeasts

Yeasts are generally unicellular and differ from bacteria in their large cell size and morphology, and because they produce buds during the process of reproduction by division. Like molds, yeasts can be spread through the air, or other means, and alight on the surface of foodstuffs. Yeast colonies are

generally moist or slimy in appearance and creamy white colored. Yeasts prefer an A w of 0.90 - 0.94, but can grow below 0.90. These micro-organisms grow best in the intermediate acid range, pH from 4.0 to 4.5. Food that is highly contaminated with yeasts will frequently have a slightly fruity odour.





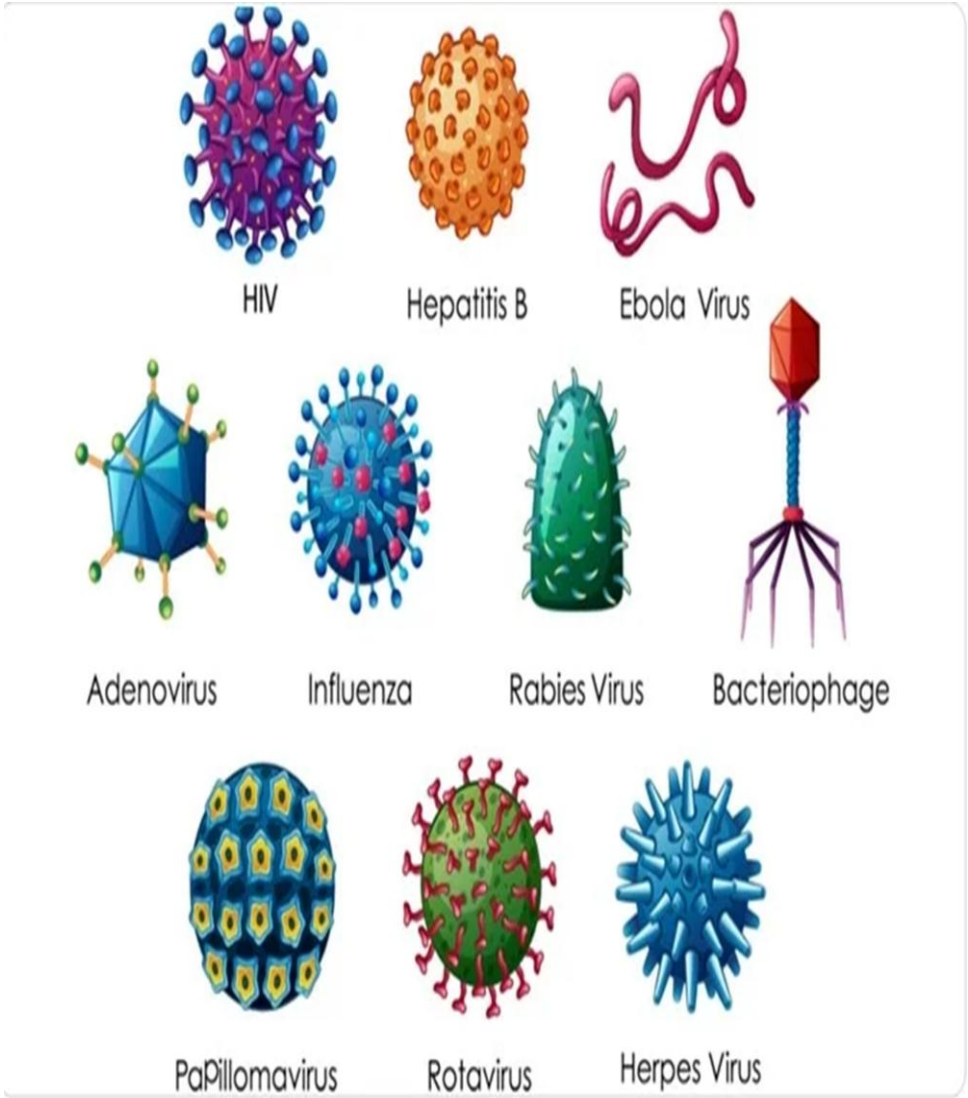


Viruses

Viruses are 10- 450 nm in size; cannot reproduce without a living host; attack only susceptible host cell lines; infect plants, animals, and bacteria; and have the capacity to produce specific diseases in specific hosts. Transmission occurs in foods, water and air. Viruses that infect bacteria are called bacteriophages.

Viruses are included in the order virales. Viruses are too small to be visualized with an ordinary compound microscope.

Only after the electron microscope was developed, the direct observation of viruses was possible. Viruses consist of a DNA or RNA core surrounded by a protein coat. Because they lack all the apparatus for normal cellular metabolism, they must utilize the cellular machinery of the host cell in order to grow and divide. Once they invade a host cell, however, viruses can multiply very rapidly.


HIV
Hepatitis B
Ebola Virus
Adenovirus
Influenza
Rabies Virus
Bacteriophage
PaPillomavirus
Rotavirus
Herpes Virus

Important micro-organisms in food

Important mold genera

Molds are important in food because they can grow in conditions in which many bacteria cannot, such as low pH, low water activity (a w), and high osmotic pressure. They are important spoilage micro-organisms. Many strains also produce mycotoxins and have been implicated in food borne intoxication. Many are used in food bioprocessing. Finally, many are used to produce food additives and enzymes. Some of the most common genera of molds found in food are listed here.

1) *Aspergillus*: They are widely distributed and contain many species that are important in food. They have septate hyphae and produce a sexual spore (black color) or conidia. Many are xerophilic (able to grow in low Aw) and can grow in grains, causing spoilage. They are also involved in spoilage of foods such as jams, cured ham, nuts, and fruits and vegetables (rot). Some species/strains produce mycotoxin (e.g., *Aspergillus flavus* produces

aflatoxin). Many species/strains are also used in food and food additive processing. *Aspergillus oryzae* is used to hydrolyze starch by alpha-amylase in the production of sake. *Aspergillus niger* is used to process citric acid from sucrose and to produce enzymes like-galactosidase.

2) *Alternaria*: They are also septate and form dark-brown colored many celled conidia on the conidiophere. They cause rot in tomatoes and rancid flavor in dairy products. Species: *Alternaria tenuis*.

3) Geotrichum: The hyphae are septate and form rectangular asexual arthrospores. They grow forming a yeast like, cottony, creamy colony. They establish easily in equipment and often grow on dairy products (also known as dairy mold). Species: *Geotrichum candidum*.

4) *Mucor*: They are widely distributed. They have nonseptate hyphae and produce sporangiophores. They produce cottony colonies. Some species are used in food fermentation and production of

enzymes. They cause spoilage of vegetables. Species: *Mucor rouxii*.

5) *Penicillium*: They are widely distributed and contain many species. They have septate hyphae and form conidiophore on a blue-green, brush- like conidia head. Some species are used in food production, such as *Penicillium roquefortii* and *Penicillium camembertii* in cheese. Many species cause fungal rot in fruits and vegetables.

6) *Rhizopus*: The hyphae are aseptate and form sporangiophores in sporangium. They are involved in the spoilage of many fruits and vegetables. *Rhizopus stolonifer* is the common black bread mold.

Important yeast genera

Yeasts are important in food due to their ability to cause spoilage. Many are also used in food bioprocessing. Some are used to produce food additives. Several important genera are briefly described below.

1) *Saccharomyces*: Cells are round, oval, or elongated. It is the most important genus and

contains heterogeneous groups. *Saccharomyces cerevisiae* variants are used in baking for leavening of bread and in alcoholic fermentation. They are also involved in spoilage of food with the production of alcohol and CO_2.

2) *Pichia*: They are oval to cylindrical cells and form pellicle in beer, wine, and brine to cause spoilage. Some are also used in oriental food fermentation. Species: *Pichia membranaefaciens*.

3) *Rhodotorula*: They are pigment (red, pink or yellow) forming yeasts and can cause discoloration of foods, such as in meat, fish, and sauerkraut. Species *Rhodotorula glutinis*.

4) *Torulopsis*: They have spherical to oval structure. They cause spoilage of milk due to the ability to ferment lactose (*Torulopsis sphaerica*). They also spoil fruit juice concentrates and acid foods.

5) *Candida*: Many spoil foods with high acid, salt, and sugar and form pellicle on the surface of liquids. Some can cause rancidity in butter and dairy products (*Candida lipolytica*).

6) Zygosaccharomyces: Involved in spoilage of foods, containing high sugar/ salt levels ex. honey, sirups, molasses, soy sauce. (*Zygosaccharomyces nussbaumeri*). These yeasts are termed osmophilic, because they can grow in high concentrations of solutes.

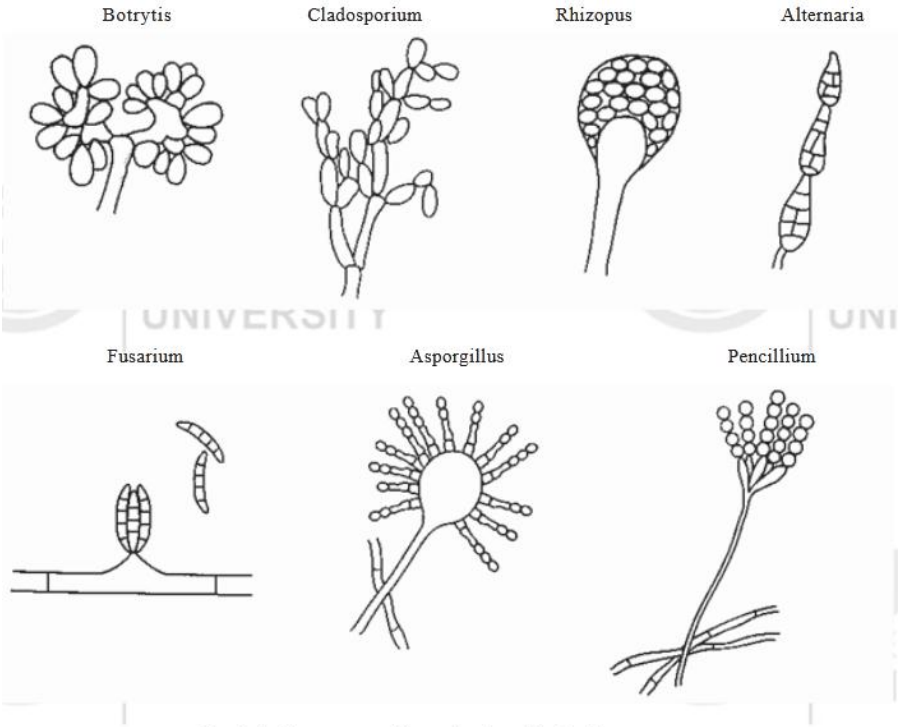


Fig. 1.4: Common mold species found in foods

Important Viruses

Viruses are important in food for three reasons. Some are able to cause enteric disease and thus, if present in a food, can cause food borne diseases. Hepatitis A and Norwalk viruses have been implicated in food borne outbreaks. Several other enteric viruses, such as Poliovirus, Echovirus, and Coxsackievirus, have the potential of causing food borne diseases. In some countries where the level of sanitation is not very high, they can contaminate foods and cause disease. Some bacterial viruses (bacteriophages) are used in the identification of species/ strains by a process called tranduction (e.g., in *Escherichia coli*, *Lactococcus lactis*).

Finally, some bacteriophages can be very important due to their ability to cause fermentation failure. Many lactic acid bacteria, used as starter cultures in food fermentation, are sensitive to different bacteriophages. These phages can infect and destroy starter culture bacteria, causing product failure. Among the lactic acid bacteria, bacteriophages have

been isolated for many species in genera *Lactococcus, Streptococcus, Leuconostoc, and Lactobacills*. Methods are being studied to genetically engineer lactic acid start cultures so that they become resistant to multiple bacteriophages.

Human Intestinal Viruses with High Potential as Food Contaminants

Types of Viruses	**Example**
Picornaviruses	Polioviruses Coxsackie virus A Coxsackie virus B Echovirus Enterovirus
Reoviruses	Reovirus Rotavirus
Parvoviruses	Human gastrointestional viruses
Papovaviruses	Human BK and JC viruses
Adenoviruses	Human adenoviruses

Common Bacterial Groups in Foods

Among the microorganisms found in foods, bacteria constitute a major important group. This is not only because many different species can be present in foods, but is also due to their rapid growth rate, ability to utilize food nutrients, and their ability to grow under a wide range of temperatures, aerobiosis, pH, and water activity, as well as to survive under adverse situations, such as survival of spores at high temperature. For convenience, bacteria important in foods have been arbitrarily divided into several groups on the basis of similarities in certain characteristics. This grouping does not have any taxonomic significance. Some of these groups and their importance in foods are listed here.

1) Lactic acid bacteria

Those bacteria that produce relatively large quantities of lactic acid from carbohydrates. Include species mainly from genera *Lactococcus, Leuconostoc, Pediococcus, Lactobacillus* and *Streptococcus thermophilus*.

2) Acetic acid bacteria

Those bacteria that produce acetic acid, such as *Acetobacter aceti*.

3) Propionic acid bacteria

Those bacteria that produce propionic acid and are used in dairy fermentation. Include species such as *Propionibacterium freudenreichii*.

4) Butyric acid bacteria

Those bacteria that produce butyric acid in relatively large amounts. Some *Clostridium* spp., such as *Clostridium butyricum*.

5) Proteolytic bacteria

Those bacteria that are capable of hydrolyzing proteins due to production of extracellular proteinases. Species in genera *Micrococcus, taphylocccus, Bacillus, Clostridium, Pseudomonas, Alteromonas, Flavobacerium,* and *Alcaligenes*; some in *Enterobacteriaceae* and *Brevibacterium* are also included in this group.

6) Lipolytic bacteria

Able to hydrolyze triglycerides due to production of extracellular lipases. Species in genera *Micrococcus, Staphylococcus, Serration, Pseudomonas, Alteromonas, Alcaligenes* and *Flavobacterium* are included in this group.

7) Saccharolytic bacteria

Able to hydrolyze complex carbohydrates. Include some species in genera *Bacillus, Clostridium, Aeromonas, Pseudomonas,* and *Enterobacter*.

8) Thermophillic bacteria

Able to grow at 50 ^{0}C and above. Include some species from genera *Bacillus, Clostridium, Pediococcus, Streptococcus,* and *Lactobacillus*.

9) Psychrotrophic bacteria

Able to grow at refrigerated temperature (<5 ^{0}C). Include some species of *Pseudomonas, Alteromonas, Alcaligenes, Flavobacterium, Serratia, Bacillus, Clostridium, Lactobacillus, Leuconostoc, Listeria, Yersinia* and *Aeromonas.*

10) Thermoduric bacteria

Able to survive pasteurization temperature. Include some species of *Micrococcus, Enterococcus, Lactobacillus, Pediococcus, Bacillus* (spores) and *Clostridium* (spores).

11) Halotolerant bacteria

Able to survive high salt concentrations (>10%). Include some species of *Bacillus, Micrococcus, Staphylococcus, Pediococcus, Vibrio Streptococcus, Clostridium* and *Corynebacterium.*

12) Aciduric bacteria

Able to survive at low pH (below 4.0). Include some species of *Lactobacillus, Pediococcus, Lactococcus, Enterococcus* and *Streptococcus*.

13) Osmophilic bacteria

Can grow at a relatively higher osmotic pressure (environment) than other bacteria. Some species from genera *Staphylococcus, Leuconostoc*, and *Lactobacillus* are included in this group. They are much less osmophilic than yeasts and molds.

14) Gas-producing bacteria

Produce gas (CO_2, H_2, H_2S) during metabolism of nutrients. Include spices from genera *Leuconostoc*, *Lactobacillus*, *Brevibacterium* and *Escherichia*.

15) Slime producers

Produce slime due to synthesis of polysaccharides. Include some species or strains of *Xanthomonas, Leuconostoc, Alcaligenes, Enterobacter, Lactococcus,* and *Lactobacillus.*

16) Spore formers

Ability to produce spore. Include *Bacillus*, *Clostridium* and *Desulfotomaculum* spp. They are again divided into aerobic, anaerobic, flat sour thermophilic and sulfide-producing spore formers.

17) Aerobes

Require oxygen for growth and multiplication. Species of *Pseudomonas, Bacillus*, and *Flavobacterium* are included in this group.

18) Anaerobes

Cannot grow in the presence of oxygen. Include species of *Clostridium*.

19) Facultative anaerobes

Able to grow both in the presence and absence of oxygen. *Lactobacillus, Pediococcus, Leuconostoc, enteric pathogens,* some *species* of *Bacillus, Serratia*, and coliforms are included in this group. Coliforms Include mainly species from *Escherichia, Enterobacter, Citrobacter,* and *Klebsiella*, and used as index of sanitation.

21) Fecal Coliforms

Include mainly Escherichia coli. Also used as index of sanitation.

22) Enteric Pathogens

Include pathogenic *Salmonella, Shigella, Campylobacter, Yersinia, Escherichia, Vibrio, Listeria*, Hepatitis A, and others that can cause gastrointestinal infection.

Chapter (ll)

Food contamination and spoilage

Introduction

Most foods are excellent media for rapid growth of micro-organisms. There is abundant organic matter in foods, their water content usually sufficient, and the pH is either neutral or slightly acidic. Foods consumed by man and animals are ideal ecosystems in which bacteria and fungi can multiply. The mere presence of micro-organisms in foods in small numbers however, need not be harmful, but their unrestricted growth may render the food unfit for consumption and can result in spoilage or deterioration. There are many opportunities for food to become contaminated as it is produced and prepared. Many food borne microbes are present in healthy animals (usually in their intestines) raised for food. Meat and poultry carcasses can become contaminated during slaughter by contact with small amounts of intestinal contents. Similarly, fresh fruits

and vegetables can be contaminated if they are washed or irrigated with water that is contaminated with animal manure or human sewage. Some types of Salmonella can infect a hen's ovary so that the internal contents of a normal looking egg can be contaminated with Salmonella even before the shell in formed. Oysters and other filter feeding shellfish can concentrate Vibrio bacteria that are naturally present in sea water, or other microbes that are present in human sewage dumped into the sea. Later in food processing, other food borne microbes can be introduced from infected humans who handle the food, or by cross contamination from some other raw agricultural products. For example, *Shigella* bacteria, hepatitis A virus and Norwalk virus can be introduced by the unwashed hands of food handlers who are themselves infected. In the kitchen, microbes can be transferred from one food to another food by using the same knife, cutting board or other utensil to prepare both without washing the surface or utensil in between. A food that is fully cooked can

become recontaminated if it touches other raw foods or drippings from raw foods that might contain pathogens microbes responsible for spoilage. The way in which food is handled after it is contaminated can also make a difference in whether or not an outbreak occurs. Many bacteria need to multiply to a large number before enough are present in food to cause disease. Given warm moist conditions and an ample supply of nutrients, a bacterium that reproduces by dividing itself every half hour can produce 17 million progenies in 12 hours. As a result, lightly contaminated food left out overnight can be highly infectious by the next day. If the food is refrigerated promptly, the bacteria multiply at a slower rate. In general, refrigeration or freezing prevents virtually all bacteria from growing and multiplying but generally preserves them in a state of suspended animation. This general rule has a few surprising exceptions. Two food borne bacteria, *Listeria monocytogenes* and *Yersinia enterocolitica* can actually grow at refrigerator temperatures.

Food contamination

Food contamination is the introduction or occurrence of a contaminant in food. A contaminant is any biological or chemical agent, foreign matter, or other substance unintentionally added to food that may compromise food safety or suitability. Among these contaminants are biological, chemical or physical agents in, or condition of, food with the potential to cause an adverse health effect. The contamination of food by chemicals is a worldwide public health concern and is a leading cause of trade problems internationally. Contamination may occur through environmental pollution, as in the case of toxic heavy metals, Poly Chlorinated Biphenyl (PCBs) and dioxins, or through the intentional use of chemicals, such as pesticides, animal drugs and other agrochemicals. Food additives and contaminants resulting from food manufacturing and processing can also adversely affect health. When foods are contaminated with unsafe levels of pathogens, chemical contaminants, or metals, they can pose

substantial health risk to consumers and place severe economic burden on individual communities or nation. Cross-contamination of food is a common factor in the cause of food borne diseases. Cross-contamination is the contamination of a food product from another contaminated source. Foods can become contaminated by micro- organisms (bacteria and viruses) from many different sources during the food preparation and storage process. There are three main ways cross- contamination can occur:

• Food to food

• People to food

• Equipment to food

Contamination of living plants and animals

The internal tissues of healthy plants and animals are essentially sterile including in the case of animals body fluids such as blood. Plants have a natural micro flora associated with the surfaces of root, stem, leaves, flowers and fruits. Invasion of healthy tissues

and subsequent growth of microorganisms is prevented by:

• Outer mechanical barriers, e.g. epidermis with an outer waxy layer, and outer corky layers

• Internal chemical constituents that are anti-microbial, e.g. tannins, organic acids and essential oils;

• Inert cell walls welded into tissues that are difficult to penetrate

• Active cells with intact membranes.

Plant materials are harvested in the living state and, as long as the mechanical barriers remain intact, can remain in storage at low temperature for several months without spoilage.

Animals have a natural micro flora associated with the skin, the gut content and external openings, e.g. the mouth. Lymph nodes and liver may also be contaminated with invading microorganisms.

Invasion of healthy tissues and subsequent growth of micro-organisms is prevented by:

• Epithelial barriers e.g. stratified skin epithelium (epidermis) and intestinal mucosa

• The immune system consisting of the lymphatic system, white blood corpuscles and antibodies;

Active cells with intact membranes

• Presence of natural antimicrobials, e.g. lysozyme in tears, saliva and egg white

• Voiding mechanisms such as vomiting.

Once an animal or plant is dead the activity of the majority of factors (defense mechanisms) that prevent microbial invasion of tissues by micro-organisms ceases and invasion is only temporarily hindered by mechanical barriers such as stratified epithelium or plant epidermis. Cell membranes are no longer active and leak cell contents, providing nutrients for microbial growth.

Sources of food contamination

It helps to understand at which point our food might become contaminated, as this will provide us with a better impetus for taking personal responsibility to reduce the potential for further contamination. There are five main events that can cause food contamination:

i) Food production: The use of chemicals, fertilizers, manures etc. all have the potential to contaminate food as it is being grown.

ii) Environmental factors: Bacteria, parasites, fungal sporus etc. travel in the wind, float in the water, hitch lifts with dust and reside snugly in the soil. They are a part of nature's web of life and will always be a possible source of contamination if not dealt with appropriately as part of a consistent and dedicated approach to food hygiene.

iii) Food processing: Whether in a large factory or in your own kitchen, food processing can be a major source of contamination. Areas used for processing

need to be kept scrupulously clean or cross-contamination can easily occur, especially with meat products (natural bacteria residing in the intestines of animals are a major source of cross-contamination when mishandled).

iv) Food storage: Food that is stored incorrectly, for instance an uncooked chicken thigh resting next to a bunch of grapes, can be a source of transferring bacteria and other contaminants from one food to another.

v) Food preparation: A great deal of food contamination occurs during the preparation stage. A sick person can pass on germs, ranging from flu to gastroenteritis. A chopping board used for meat that is not washed and then used for vegetables is another source of possible contamination. Unwashed hands, dirty kitchen spaces, insects and rodents in the kitchen etc. are all possible sources of food contamination.

Food spoilage

Spoilage of food may be due to chemical or biological causes; the latter include action of inherent enzymes, growth of micro-organisms, invasion by insects, and contamination with trichinae and worms. About one-fourth of the world's food supply is lost through the action of micro-organisms alone. The "spoilage" concept includes concepts about edibility, means the food is unfit to eat or fit to eat. Spoilage is decomposition. Many foods may not be decomposed, but harbor certain kinds of bacteria, or their toxins, in number or amounts which make the food poisonous and thus unfit for human consumption.

The criteria for assurance in foods suitable for consumption are:

• The desired stage of development or maturity of the food.

• Freedom from pollution at any stage in the production and subsequent handling of the food.

• Freedom from objectionable, chemical and physical changes resulting from action of food enzymes; activity of microbes, insects, rodents, invasion of parasites; and damage from pressure, freezing, heating, drying, and the like.

• Freedom from micro-organisms and parasites causing food borne illnesses. Enzymatic and microbial activities are undesirable when they are unwanted or uncontrolled. An example is the souring of milk; if unwanted, it is spoilage, yet the same process is purposely used in the production of certain cheeses and other fermented products made from milk.

Types of Spoilage

The food may become unacceptable due to the following factors:

1) Growth and activities of micro-organisms principally bacteria, yeasts and moulds (This is by far the most important and common cause of food spoilage).

2) Activities of food enzymes (enzymatic browning is a common example).

3) Infestation by insects, parasites and rodents.

4) Chemical changes in a food (i.e. not catalyzed by enzymes of the tissues or of microorganisms). For example the chemical oxidation of fats producing rancidity as well as non-enzymatic browning reactions in foods like Maillard Browning.

5) Physical changes or damages such as those caused by freezing (freezer burn), by drying (caking) etc.

6) Presence of foreign bodies.

Classification of foods on the basis of stability

Foods are frequently classified on the basis of their stability as nonperishable, semi perishable, and perishable.

An example of the first group is sugar. Few foods are truly nonperishable. Hermetically sealed, heat-processed, and sterilized (canned) foods are usually listed among the nonperishable items.

For all intents and purposes, they belong there. However, canned food may become perishable under certain circumstances, when, by accident, there is a chance for recontamination following processing because of faulty seams of the cans, or through rusting or other such damage so that the can is no longer hermetically sealed.

Classified as semi perishables are usually the dry goods, such as flour, dry legumes, baked goods, hard cheeses, dried fruits and vegetables, and even waxed vegetables. Frozen foods, though basically perishable, may be classified as semi perishables provided they are freezer-stored properly. The majority of our food materials must be classified as perishables.

This group includes meat, poultry, fish, milk, eggs, many fruits and vegetables, and all cooked or "made" food items, except the dry and very acid ones.

Role of microorganisms

Microbial spoilage of foods is the beginning of the complex natural process of decay that under natural circumstances leads to recycling of the elements present in the animal or plant tissues in the natural environment.

Microorganisms involved in spoilage

Microorganisms which may cause food to spoil include molds, yeasts, and bacteria. The contamination with molds, as a rule, is easily detected because of the presence of furry hyphae or threadlike structures which, in many instances, are colored. They often contribute a musty odor and flavor to the food they invade. Some molds, because of toxins they produce, are not altogether harmless. Semi moist foods or foods with low water activity having been partially dehydrated, and where the remaining water is sufficiently bound to hold the growth of bacteria are ideal for contamination by

molds and yeasts. Yeasts are unicellular organisms of small sizes which multiply by budding.

In general, sugars are the best food source for energy for yeast; Carbon dioxide and alcohol are the end products of the fermentation mediated by yeast.

Spoilage due to yeast may usually be recognized by the presence of bubbles and an alcoholic smell and taste.

Bacteria spoil food in many ways and it is not always possible to recognize the spoilage by sight, smell, or taste. Unfortunately, some of the bacteria that are important from a public health point of view may multiply to dangerously high numbers in food without changing the appearance, odor, or taste of the food. Disease-producing food has usually no decomposed appearance, but is certainly unfit for human consumption, and must be considered to be spoiled.

It is an important fact that almost any food will spoil if it is moist and not kept frozen. Spoilage must be

expected within a wide temperature range. The various types of micro-organisms as well as the genera, species, and strains vary in their temperature and food requirements. Thus the bacterial flora of a spoiled food item will vary greatly.

The origin of micro-organisms also varies. The micro-organisms may include the original flora of the particular food, as well as contaminants added during handling, processing, transporting, storing, preparing, and serving.

Growth of microorganisms

The multiplication of spoilage organisms on or in the food materials depends on many factors – the type of organism involved, its ability to gain nourishment from the food, competition from other micro-organisms, initial load, and environmental conditions. Micro-organisms grow rapidly; we call it logarithmic growth. The time a bacterium takes to multiply (double its number) is known as its generation time.

Four distinct phases occur in the growth curve: lag; log or growth phase; stationary phase and death phase. Bacteria need about four hours to adapt to a new environment before they begin rapid growth. In handling food, this means we have less than four hours to make a decision to either cool the food, heat it or eat it. As micro-organisms grow, they tend to form colonies. These colonies are made up of millions of individual cells. Once a colony forms, the food available to each cell is limited and excretions from these millions of cells become toxic to a microbe. This is the stationary phase. Some of the cells now begin to die.

If we can control bacterial growth, we can control the major cause of food spoilage.

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Factors affecting spoilage

Food is a chemically complex matrix, and predicting whether, or how fast, micro-organisms will grow in any given food is difficult. Most foods contain sufficient nutrients to support microbial growth. Several factors encourage, prevent or limit the growth of microorganisms in foods; the most important are water activity a w, pH and temperature. Factors affecting microbial growth are divided into two groups -intrinsic and extrinsic parameters (Table 2.1). These factors affect the growth of micro-organisms on foods. When spoilage of a food occurs under a given set of circumstances, not all of the different types of organisms contaminating a food are associated with the spoilage process. In fact, the spoilage flora always is dominated by just a few and sometimes only one organism. Components of the micro flora compete with one another for the

available nutrients and the organism(s) with fastest growth under a particular set of circumstances will become dominant and give rise to the spoilage symptoms.

Table 2.1: Intrinsic and Extrinsic Parameters Affecting Microbial Growth

Intrinsic parameters	Extrinsic parameters
Water activity, humectant identity	Temperature
Oxygen availability	Relative humidity
pH, acidity, acidulant identity	Atmospheric composition
Buffering capacity	Packaging
Available nutrients	
Natural antimicrobial substances	
Presence and identity of natural microbial flora	
Colloidal form	

The component of the micro flora which becomes dominant is determined by a complex interaction between the components of the contaminating micro flora (implicit factors), the storage environment (extrinsic factors) and the physico-chemical properties of the food (intrinsic factors).

All of the factors that influence the growth of micro-organisms have been dealt with as under. Time is included because, under any given set of circumstances, spoilage takes a finite period to occur and equates with the storage life of a product. A knowledge of the intrinsic and extrinsic parameters should enable you to decide which broad group of organisms is likely to spoil a particular type of food, i.e. whether the food is likely to be spoiled by bacteria, yeasts or moulds. For example, foods that have a high water activity and a pH above 5.0 are likely to be spoiled by bacteria simply because under these conditions bacteria grow the fastest. Foods with pH below 4.2 are likely to be spoiled by yeasts and moulds even when the water activity is high.

Extrinsic Factors

Extrinsic factors relate to the environmental factors that affect the growth rate of micro-organisms. They are as follows:

A. Temperature

Microbes have an optimum temperature as well as minimum and maximum temperatures for growth. Therefore, the environmental temperature determines not only the proliferation rate but also the genera of micro-organisms that will thrive and the extent of microbial activity that occurs. For example, a change of only a few degrees in temperature may favor the growth of entirely different organisms and result in a different type of food spoilage and food poisoning.

These characteristics have been responsible for the use of temperature as a method of controlling microbial activity.

The optimal temperature for the proliferation of most micro-organisms is from 15 to 40 ^{0}C. However, many genera of microbes are capable of growth from 0 to 15 ^{0}C and other even micro-organisms will grow at subzero temperatures. Still other genera will grow at temperatures up to and exceeding 100 ^{0}C.

Microbes classified according to temperature of optimal growth include:

• **Thermophiles** (high-temperature-loving micro-organisms), with growth optima at temperatures above 45 ^{0}C (e.g., *Bacillus stearothermophilus*, *Bacillus coagulans*, and *Lactobacillus thermophilus*).

• **Mesophiles** (medium-temperature-loving micro-organisms), with growth optima between 20 and 45 ^{0}C (e.g., most *Bactobacilli* and *Staphylococci*).

• **Psychrotrophs** (cold-temperature-tolerant micro-organisms), which tolerate and thrive at temperatures below 20 ^{0}C (e.g., *Pseudomonas* and *Acinetobacter*).

Bacteria, molds and yeasts each have some genera with temperature optima in the range characteristic of thermophiles, mesophiles, and psychrotrophs.

Molds and yeasts tend to be less thermophilic than bacteria. As temperature approaches 0 ^{0}C, fewer micro-organisms can thrive and their proliferation is slower. As temperature falls below approximately 50

C, proliferation of spoilage micro-organisms is regarded as the growth of nearly all pathogens ceases.

B. Oxygen availability

As with temperature, the availability of oxygen determines which micro- organisms will be active. Some micro-organisms have an absolute requirement for oxygen, whereas others grow in total absence of oxygen. Yet other micro- organisms can grow either with or without available oxygen. Micro-organisms that require free oxygen are called aerobic micro-organisms (e.g., *Pseudomonas spp*.) and those that thrive in the absence of oxygen are called anaerobic micro-organisms (e.g., *Clostridium spp*.).

Microorganisms that can grow with or without the presence of free oxygen are called facultative micro-organisms (e.g., *Lactobacillus spp*.).

C. Relative humidity

This extrinsic factor affects microbial growth and can be affected by temperature. All micro-organisms

have high requirements for water to support their growth and activity. A high relative humidity can cause moisture condensation on food, equipment, walls, and ceilings. Condensation causes moist surfaces, which are conducive to microbial growth and spoilage. Also, microbial growth is inhibited by a low relative humidity. Microorganisms bacteria require the highest relative humidity of the various. optimal relative humidity for bacteria is 92% or higher, whereas yeasts need 90% or higher and for molds, the value of relative humidity is 85-90%.

Intrinsic Factors

Intrinsic factors that affect the rate of proliferation relate more to the characteristics of the substrates (foodstuff or debris) that support or affect growth of micro-organisms. These major intrinsic factors are:

A. Water activity (a w)

Water is required by micro-organisms, and a reduction of water availability constitutes a method

of food preservation through reduction of microbial proliferation. It is important to recognize that it is not the total amount of moisture present that determines the limit of microbial growth, but the amount of moisture which is readily available for metabolic activity of microbes. The unit of measurement for water requirement of microorganism is usually expressed as water activity (aw). Water activity is defined as the vapor pressure of the subject solution divided by the vapor pressure of the pure solvent: a w = p/p0, where p is the vapor pressure of the solution and po is the vapor pressure of pure water. The approximate optimal a w for the growth of many micro-organisms is 0.99, and most microbes require a w higher than 0.91 for growth. The relationship between relative humidity (RH) and aw is RH = a w × 100. Therefore a w of 0.95 is equivalent to an RH of 95%. Generally, bacteria have the highest water activity requirements of the micro- organisms. Molds normally have the lowest aw requirements, with yeasts being intermediate. Most spoilage bacteria do

not grow at a w below 0.91, but molds and yeasts can grow at a w of 0.80 or lower. Molds and yeasts are more likely to grow in partially dehydrated surfaces (including food), whereas bacterial growth is retarded.

Approximate Minimum (a_w) Values for Growth

ORGANISMS GROUPS	WATER ACTIVITY
MOST SPOILAGE BACTERIA	0.90
MOST SPOILAGE YEASTS	0.88
MOST SPOILAGE MOLDS	0.80

B. pH

The pH for optimal growth of most micro-organisms is near neutrality (7.0). yeasts can grow in an acid environment, but grow best in an intermediate acid (4.0-4.5) range. Molds tolerate a wider range of pH (2.0-8.0), although their growth is generally greater with an acid pH. Molds can thrive in a medium that is too acid for either bacteria or yeasts. Bacterial

growth is usually favoured by near-neutral pH values. However, acidophilic (acid-loving) bacteria will grow on food or debris down to a pH value of approximately 5.2. Below pH 5.2, microbial growth is dramatically reduced when compared from growth in the normal pH range.

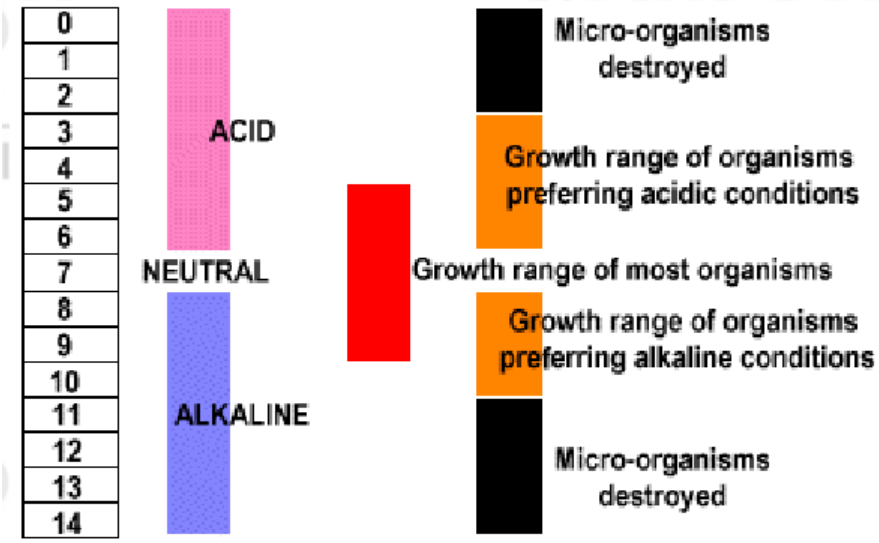

Fig. 2.2: pH and growth of micro-organisms

C. Oxidation – Reduction Potential

The oxidation – reduction (redox) potential is an indication of the oxidizing and reducing power of the substrate. To attain optimal growth, some micro-organisms require reduced conditions while others

need oxidized conditions. Thus, the importance of the oxidation-reduction potential is apparent. Aerobic micro-organisms grow more readily under a high oxidation – reduction potential (oxidizing reactivity). A low potential (reducing reactivity) favors the growth of anaerobes. Facultative micro-organisms are capable of growth under either condition. Micro-organisms can alter the oxidation-reduction potential of food to the extent that the activity of other micro-organisms is restricted.

For example, anaerobes can decrease the oxidation – reduction potential to such a low level that the growth of aerobes can be inhibited.

D. Nutrient requirements

In addition to water and oxygen (except for anaerobes), micro-organisms have other nutrient requirements. Most microbes need external sources of nitrogen, energy (i.e., carbohydrates, proteins, or lipids), minerals, and vitamins to support their growth. Nitrogen is normally obtained from amino

acids and other non-protein nitrogen sources; however, some micro-organisms utilize peptides and proteins. Molds are the most effective in the utilization of proteins, complex carbohydrates, and lipids because they contain enzymes capable of hydrolyzing these molecules into less complex components. Many bacteria have a similar capability, but most yeasts require the simple forms of these compounds. Minerals (micronutrients) are needed by all micro-organisms, but requirements for vitamins vary. Molds and some bacteria can synthesize enough B vitamins to fulfill their needs, while other micro-organisms require a ready-made supply.

E. Inhibitory substances

Microbial proliferation can be affected by the presence or absence of inhibitory substances. Substances or agents that inhibit microbial activity are called bacteriostatic, and those which destroy micro-organisms are called bactericides.

Some bacteriostatic substances are added during food processing (i.e., nitrites). Most bactericides are utilized as a method of decontaminating foodstuffs or as a sanitizer for cleaned equipment, utensils, and rooms.

Interaction between growth factors

The effects that factors such as temperature, oxygen, pH, and a w have on microbial activity may be dependent on each other. Microorganisms generally become more sensitive to oxygen availability, pH, and aw at temperatures near growth minima or maxima. For example, bacteria may require a higher pH, a w, and minimum temperature for growth under anaerobic conditions than when aerobic conditions prevail. Microorganisms that grow at lower temperatures are usually aerobic and generally have a high a w requirement.

Lowering a w by adding salt or excluding oxygen from foods such as meat which have been held at a refrigerated temperature dramatically reduces the

rate of microbial spoilage. Normally, some microbial growth occurs when any one of the factors that control the growth rate is at a limiting level. Yet, if more than one factor becomes limiting, microbial growth is drastically curtailed or completely stopped.

Deteriorative effects of microorganisms

Food is considered spoiled when it becomes unfit for human consumption. Spoilage is usually equated with the decomposition and putrefaction that result from activity of micro-organisms. Some of the more common physical and chemical changes due to the micro-organisms have been described under:

Physical changes

The physical changes caused by micro-organisms usually are more apparent than the chemical changes. Microbial spoilage usually results in an obvious change in physical characteristics such as color, body, thickening, odor, and flavor degradation. Food spoilage is normally classified as being either aerobic or anaerobic, depending upon the spoilage

conditions, including whether the principal microorganism causing the spoilage were bacteria, molds, or yeasts. Aerobic spoilage by bacteria and yeasts usually results in slime formation; undesirable odors and flavors (taints); color changes; and rancid, tallowy, or chalky flavors from the breakdown of lipids. Slime formation by certain species of bacteria or yeasts depends upon environmental conditions, especially those of temperature and aw. Colour changes can be pigment oxidation resulting in a gray, brown, or green color. Physical deterioration through aerobic spoilage by molds results in sticky surface of many foods. A filamentous appearance frequently referred to as "whiskers" can also occur as a result of physical deterioration. Discoloration from molds can give surface colorations, such as creamy, black, or green. Deterioration from molds can affect appearance of lipids of foods in a way similar to that of bacteria and yeasts and will produce musty odors and alcohol flavors Aerobic spoilage of foods from molds is

normally limited to the food surface, where oxygen is available. Therefore, molded surfaces of foods such as meats and cheeses can be trimmed off and the remainder is generally acceptable for consumption. This is especially true for aged meats and cheeses. When these surface molds are trimmed, surfaces underneath usually have limited microbial growth. However, if extensive bacterial growth occurs on the surface, penetration inside the food surface usually follows and toxins may be present. Anaerobic spoilage occurs within the interior of food products or in sealed containers, where oxygen is either absent or present in limited quantities. Spoilage is caused by facultative and anaerobic bacteria and is expressed through souring, putrefaction, or taint. Souring occurs from the accumulation of organic acids during the bacterial enzymatic degradation of complex molecules (carbohydrate). Also, proteolysis without putrefaction may contribute to souring. Souring can be accompanied by the production of various gases.

Examples of souring are milk, round sour or ham sour, and bone sour in meat. These meat sours, or taints, are caused by anaerobic bacteria that may originally have been present in lymph nodes or bone joints, or which might have gained entrance along the bones during storage and processing.

Chemical changes

Through the activity of endogenous hydrolytic enzymes that are present in foodstuffs (and the action of enzymes that micro-organisms produce), proteins, lipids, carbohydrates, and other complex molecules are degraded to smaller and simpler compounds. Initially, the endogenous enzymes are responsible for the degradation of complex molecules. As microbial load and activity increase, degradation subsequently occurs. These enzymes hydrolyze the complex molecules into simpler compounds that are subsequently utilized as nutrient sources for supporting microbial growth and activity. Oxygen availability determines the end products of microbial

action. Availability of oxygen permits hydrolysis of proteins into end products such as simple peptides and amino acids. Under anaerobic conditions, proteins may be degraded to a variety of sulfur-containing compounds, which are odorous and generally obnoxious. The end products of non-protein nitrogenous compounds usually include ammonia.

Other chemical changes include action of lipases secreted by micro-organisms which hydrolyze triglycerides and phospholipids into glycerol and fatty acids. Phospholipids are also hydrolyzed into nitrogenous bases and phosphorus.

Lipid oxidation is also accelerated by extensive lipolysis. Most micro-organisms prefer carbohydrates to other compounds as a source of energy. When available, carbohydrates are more readily utilized for energy.

Utilization of carbohydrates by micro-organisms results in a variety of end products such as alcohols

and organic acids. In many foods such as sausage products and cultured dairy products, microbial fermentation of sugar that has been added yields organic acid (e.g., lactic acid) which contribute to their distinct and unique flavors.

Different types of food spoilage

Food undergoes different types of spoilage depending on its composition.

Common methods of food preservation

Principles of Food Preservation include the following:

i) Prevention or delay of microbial decomposition.

By keeping microbes out (asepsis).

By removal of microbes (e.g. filtration).

By reducing the rate of microbial growth (e.g. by low temperature, drying, anaerobic conditions and chemical inhibitors).

By killing microbes (e.g. by heat or radiation).

ii) Prevention or delay of self-decomposition of food By inactivation of food enzymes (e.g. blanching). By prevention of chemical reactions (e.g. by using antioxidants)

Food preservation aims at preventing the microbial spoilage of food products and the growth of the food borne pathogens. Thus, the two principal goals of food preservation methods are:

(i) increasing the shelf life of the food and (ii) ensuring the safety for human consumption. There are a variety of food preservation methods. Table 2.4 consolidates the methods and details are given here.

Heat

Heat kills micro-organisms by inactivating their proteins and by changing the physical and chemical properties of their proteins. The most common use of heat is in the process of canning. The food product is washed, sorted, and graded and then subjected to steam heat for three to five minutes. This last

process called blanching, destroys many enzymes in the food product and prevents further cellular metabolism.

The food is then peeled and cored, and diseased portions are removed. For canning, containers are evacuated and placed in a pressurized steam sterilizer, similar to an autoclave at 121°C. This removes especially Bacillus and Clostridium spores. If canning is defective, foods may become contaminated by anaerobic; bacteria which produce gas. These are species of *Clostridium*, and coliform bacteria (a group of Gram-negative nonspore-forming rods which ferment lactose to acid and gas at 32°C in 48 hours).

Low temperature

Exposure of micro-organisms to low temperatures reduces their rates of growth and reproduction. This principle is used in refrigeration and freezing. Microbes are however not killed. In refrigerators at 5°C, food remains unspoiled. In a freezer at -5°C the

crystals formed tear, shred and thereby may kill micro- organisms.

However, some are able to survive. *Salmonella* spp. and *Streptococci* survive freezing. For these types rapid thawing and cooking is necessary. Deep freezing at -60°C forms smaller crystals. It reduces biochemical activities of microbes.

Blanching of fruits and vegetables, by scalding with hot water or steam prior to deep freezing, inactivate plant enzymes that may produce toughness, change in colour etc. A brief scalding prior to freezing also reduces the number of micro-organisms on the food surface by up to 99% enhances the colour of green vegetables.

Drying or desiccation

Water from foods is removed in different ways. It may be done by spray dryer which expels a fine mist of liquid such as coffee into a barrel cylinder containing hot air. A heated drum may be used onto which liquids like soup may be poured. Another

example is a belt heater that exposes liquids like milk to a steam of hot air that evaporates water and produces dried milk solids. A common process of freeze drying or lyophilization is used these days. The food is deep frozen, after which the water is drawn off by a vacuum pump in a machine. The dry product is then sealed in foil and is reconstituted with water. This method is very useful for storing, transporting and preserving bacterial cultures.

Osmotic Pressure

The principle of osmosis is applied in the age old traditional method of adding Salt or Sugar to pickles, preserves and jams. Foods are preserved by adding salts and sugars to them. These solutes remove the water out of microbial cells causing them to shrink, thus stopping their metabolism. Jams, jellies, fruit syrups, honey etc. are preserved by using high sugar concentration. Fish, meat, beef and vegetable products are preserved with salt mediated

process.

Chemical Preservatives

The most commonly used preservatives are the acids, such as sorbic acid, benzoic acid and propionic acid. These check mainly the growth of yeasts and molds. Sorbic acid is used for preservation of syrups, salads, jellies and some cakes. Benzoic acid is used for beverages, margarine, apple cider etc. Propionic acid is an ingredient of bread and bakery products. Sulphur dioxide, as gas or liquid is also used for dried fruits, molasses and juice concentrates. Ethylene oxide is used for spices, nuts and dried fruits.

Radiation

Ultra Violet (UV) radiation is used in meat storage facilities which reduce surface contamination, on meat products. Gamma rays are also used for some meat products.

Anaerobiosis

Packaging of food products under anaerobic

conditions - anaerobiosis is effective in preventing aerobic spoilage process. Vacuum packing in an airtight container is used to eliminate air.

Controlled atmosphere

Atmospheres containing 10% CO_2 are used to preserve stored food products such as apples and pears. This checks fungal growth. Ozone can also be added.

Other Methods

These are asepsis i.e. washing utensils that come in contact with food and filtration and centrifugation to remove microbes. Filtration is used for fruit juices and other drinks. Bacteriological filters are used in industries.

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Practical Part

Food microbiology

It is a study of microorganism that contaminate food and also that enter in production of food products.

Why microorganism found in food?

Because food is an ideal culture media for microbial growth.

Microbial types in food

Bacteria (Gram positive and Gram negative) e.g. *Campylobacter*, *Salmonella*, *Shigella*, *E. coli*

Viruses such as virus A

Animal parasite

Yeast

Molds (filamentous fungi such as *Rhizopus* and *Pencillium*

Food spoilage

It is a process where food product becomes unsuitable to ingest by consumer (human or animal)

Signs of food spoilage

- Odour (breakdown of protein such as rotten egg)
- Sliminess
- Discolouration such as mould on bread
- Souring as sour milk
- Gas formation such as swollen of canned food

Spoilage of fruit

Usually fruits inhibit growth of microbes until harvesting, ripening weakens cell wall so microbes can attack cell easily, fruit have low pH that inhibit most bacteria, but there is moulds tolerant to acidic condition can grow.

Spoilage of vegetables

Vegetables is an ideal media for microbial growth due to neutral pH, high water activity. The most common in vegetables is *Pseudomonas sp.*

Spoilage of meat

Edible meat has muscular tissue; animal protect its cells by immune system

Types of meat spoilage

1-Spoilage under aerobic condition

2- Spoilage under anaerobic condition

Spoilage of canned food

Causes

1-Chemical

2- Biological that occur due to:

- Action of *Clostridium* and *Bacillus*
- Survival of microbes after heat treatment
- Leakage of container after heat treatment

Microorganisms as food

- Some fungi such as mushroom
- ScP (single cell protein)

Refer to total protein extracted from the pure culture of microorganism that can be used as protein rich food supplements by human or animal

Examples of SCP

1-Algae such as *Scendesmus* and *Chorella*

2-Yeast such as *Saccharomyces cerevesia*

3-Bacteria such as Methylophilus methylotrophus

4- Filamentous fungi such as *Fusarium graminearum*

Advantages of SCP

1- Microorganism have very fast growth rate

2- Protein content is high

3- Can grow in varieties of raw substance such as waste water

Yeast	45-55%
Bacteria	50-85%
Fungi	30-34%

Algae 45-65%

Application of SCP

1-Cholestrol lowering effect

2-Lower blood sugar in diabetes

3-Contain p-carotein which good for eyes, skin

4-Used in some cosmetics

5-Used to feed cattle and fish

Isolation of fungi from different food

Types of fungi

1-Exophytic fungi: fungi that grow upon or attached to living plant

2-Endophytic fungi: fungi that can internally infect plant tissue.

Method of isolation fungi

A) **Dilution method:** By making a spore suspension of sample

Spore suspension is made by putting a weight of sample in sterile distilled water with shaking.

B) <u>Patting method</u>: In this method, we put parts of sample after surface sterilization directly on sterile petri dishes containing sterile solid media.

In our section, we will isolate fungi from different sources of food by using dilution and patting method.

Materials

1-Sterile czapeks media (10 gm glucose, 3gm $NaNO_3$, 1gm KH_2PO_4, 0.5gm KCl, 0.5 gm $MgSO_4.7H_2O$,1gm Chloramphenicol, 20 gm agar) in 1000 ml distilled H_2O)

2- Conical flask 250 ml

3-Conical flask 500ml

4- Petri dishes

5-Cotton, Rose Bengal.

1-Isolation of fungi from Nescafé by dilution method:

1-1 1g of sample in sterile conical flask contain 100 ml distilled water and shaking very well

1-2 then take 10 ml from this conical by using pipette and put them in sterile conical flask contain 90 ml dist. water and so on.

1-3 Take 1 ml of best dilution and put it in sterile Petri-dish

1-4 Pour sterile culture medium in Petri-dish

1-5 The plates were gently rotated clockwise and anticlockwise to ensure uniform distribution of homogenates and then incubated at 27°C for 7 days and the numbers of fungi were calculated.

2- Isolation of fungi from different food by using patting method:

1) The samples were subjected to a series of washing with sterile distilled water.

2)Dried them by using sterile filter papers.

3)Cut them into equal segments (1cm each).

4) Four particles were placed into each of 3 plates containing media.

5) then incubated them in incubator at 27°C for 7 days.

6) Developing fungi were counted, identified and calculated.

Observation

1-Growth of fungi.

2- No growth of fungi.

Comment

1- Food is invalid for using

2- Food is valid for using

Isolation of Bacteria from chicken

1-Put 1gm of sample in test tube containing 5 ml of sterile neutral broth media.

2-Incubate in incubator for 24h at 37 ^{o}C (for enrichment).

3-Then take 1 ml from this tube by using pipette and put them in sterile test tube contain 9 ml N. B media and so on (Serial dilution).

4- Pour sterile N.A medium in Petri-dish and let them for solidification.

5- Take 100 M of each dilution and spread on the surface of solid N.A media in Petri-dish.

6- Incubate in incubator for 24 h at 37 ^{o}C.

7- Count colonies of bacteria.

Observation

1- Number of colonies >300

Sample should be diluted again

2- Number of colonies <30

Sample is not contaminated

……..

Calculation of CFU= No. of colonies *(1/Dilution)

Determination of CFU per ml of bacteria

Serial dilutions are used to calculate the concentration of microorganisms. As it would usually be impossible to actually count the number of microorganisms in a sample, the sample is diluted and plated to get a reasonable number of colonies to count. Since each colony on an agar plate

theoretically grew from a single microorganism, the number of colonies or Colony Forming Units is representative of the number of viable microorganisms. Since the dilution factor is known, the number of microorganisms per ml in the original sample can be calculated.

A dilution problem such as the one shown above is relatively easy to solve if taken step by step. Follow the steps below.

1. First determine which the countable plate is.

- Count the number of colonies on each plate. If there are too many colonies on the plate, the colonies can run together and become indistinguishable as individual colonies. In this case the plate is called confluent or Too Numerous to Count (TNTC). The countable plate has between 30 and 300 colonies. More than 300 colonies would be difficult to count, and less than 30 colonies are too small a sample size to present an accurate representation of the original sample. As stated above, the number of colonies is

the number of Colony Forming Units which represents the number of microorganisms per ml.

2. **Sample Dilution Factor (SDF)**
   - A sample is often diluted prior to doing the serial dilutions. If it is, the sample dilution factor will be shown in the diagram as above (the 1/2 in the Erlenmeyer flask is the sample dilution factor). If the sample remains undiluted, use 1/1 as the Sample Dilution Factor.
3. **Individual Tube Dilution Factor (ITDF)**
   - The individual tube dilution factors are a calculation of how much the sample was diluted in each individual tube. This is just the amount of sample added to the tube divided by the total volume in the tube after adding the sample. In tube I above, 1 ml of sample was added to 9 ml of water, so the ITDF for tube I is: 1ml/1ml + 9 ml = 1/10
4. **Total Series Dilution Factor (TSDF)**
   - The total series dilution factor is a calculation of how much the sample was diluted in all of the tubes combined. This is accomplished by multiplying each

of the appropriate ISDF. This series does not include any dilutions after the countable plate. In the example above, since the countable plate was plate C, tube IV is not included in the TSDF. The TSDF for the example above is 1/10 (ITDF for tube I) x 1/10 (ITDF for tube II) x 1/6 (ITDF for tube III) = 1/600.

5. **Plating Dilution Factor (PDF)**

- When the sample is plated, a dilution factor must also be calculated for this transfer. Since the object of these calculations is to determine CFU/ml, the amount plated for the countable plate is divided by 1 ml to get the PDF. In the example above, 0.3 ml from tube III was plated onto plate C, so the PDF is 0.3ml/1.0 ml = 0.3ml/1.0ml x 10/10 = 3/10.

6. **Final Dilution Factor (FDF)**

- The FDF takes into account all of the above dilution factors, giving you the total dilution from the original sample to the countable plate. The FDF = SDF x TSDF x PDF, so in this example, the FDF = 1/2 x 1/600 x 3/10 = 3/12000 = 1/4000. This means that

the original sample was 4000 times as concentrated as the plated sample from tube III. In other words, it would take 4 L of the sample in tube III to contain the same number of bacteria as 1 ml of the original sample.

7. **Colony Forming Units/ml (CFU/ml)** in original sample

- To find out the number of CFU/ ml in the original sample, the number of colony forming units on the countable plate is multiplied by 1/FDF. This takes into account all of the dilution of the original sample. For the example above, the countable plate had 200 colonies, so there were 200 CFU, and the FDF was 1/4000.
  - 200 CFU x 1/1/4000 = 200 CFU x 4000 = 800000

  CFU/ml = 8 x 10
  - CFU/ml in the original sample.

Citrate Utilization Test- Principle, Media, Procedure and Result

This test is among a suite of IMViC Tests (Indole, Methyl-Red, Vogues-Proskauer, and Citrate) that are used to differentiate among the Gram-Negative bacilli in the family Enterobacteriaceae.

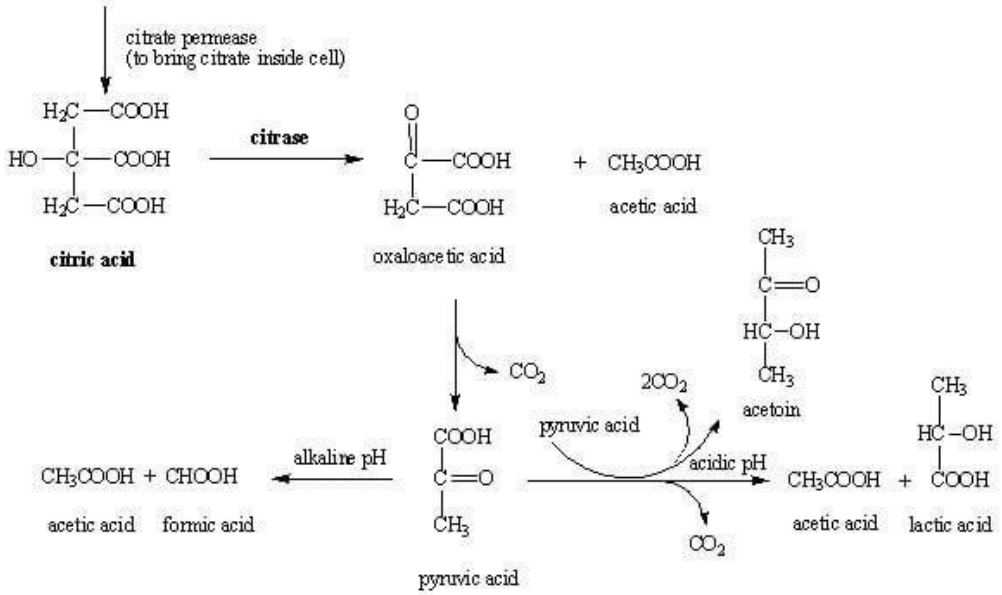
Principle of Citrate Utilization Test

Citrate agar is used to test an organism's ability to utilize citrate as a source of energy. The medium contains **citrate** as the sole **carbon source** and **inorganic ammonium salts ($NH_4H_2PO_4$)** as the sole source of **nitrogen**.

Bacteria that can grow on this medium produce an enzyme, **citrate-permease**, capable of converting **citrate** to **pyruvate**. **Pyruvate** can then enter the organism's metabolic cycle for the production of **energy**. Growth is indicative of utilization of citrate, an intermediate metabolite in the **Krebs cycle**.

When the bacteria metabolize **citrate**, the **ammonium salts** are broken down to **ammonia**, which increases **alkalinity**. The shift in pH turns the **bromthymol blue** indicator in the medium from **green to blue** above pH 7.6.

Christensen developed an alternative citrate test medium that does not require the organism to use citrate as a sole carbon source. **Christensen's medium** contains both **peptone** and **cysteine**. Thus citrate-negative bacteria can also grow on this medium. A positive reaction shows that the organism can use citrate but not necessarily as the sole carbon source.



Media used in Citrate Utilization Test

Simmon's Citrate Agar

Composition	
Sodium Chloride	5.0 gm
Sodium Citrate (dehydrate)	2.0 gm
Ammonium Dihydrogen Phosphate	1.0 gm
Dipotassium Phosphate	1.0 gm
Magnesium Sulfate (heptahydrate)	0.2 gm
Bromothymol Blue	0.08 gm
Agar	15.0 gm

Deionized water = 1,000 ml, Final pH 6.9 +/- 0.2 at 25 degrees C.

Preparation

1. Dissolve above salts in deionized water.
2. Adjust pH to 6.9.
3. Add agar and bromothymol blue.
4. Gently heat, with mixing, to boiling until agar is dissolved.
5. Dispense 4.0 to 5.0 ml into 16-mm tubes.
6. Autoclave at 121-degree C under 15 psi pressures for 15 minutes.
7. Cool in slanted position (long slant, shallow butt).
8. Tubes should be stored in a refrigerator to ensure a shelf life of 6 to 8 weeks.
9. The uninoculated medium will be a deep forest green due to the pH of the sample and the bromothymol blue.

Procedure of Citrate Utilization Test

1. Streak the slant back and forth with a light inoculum picked from the center of a well-isolated colony.
2. Incubate aerobically at 35 to 37C for up to 4-7 days.
3. Observe a color change from green to blue along the slant.

Result Interpretation of Citrate Utilization Test

Positive Reaction: Growth with color change from green to intense blue along the slant.

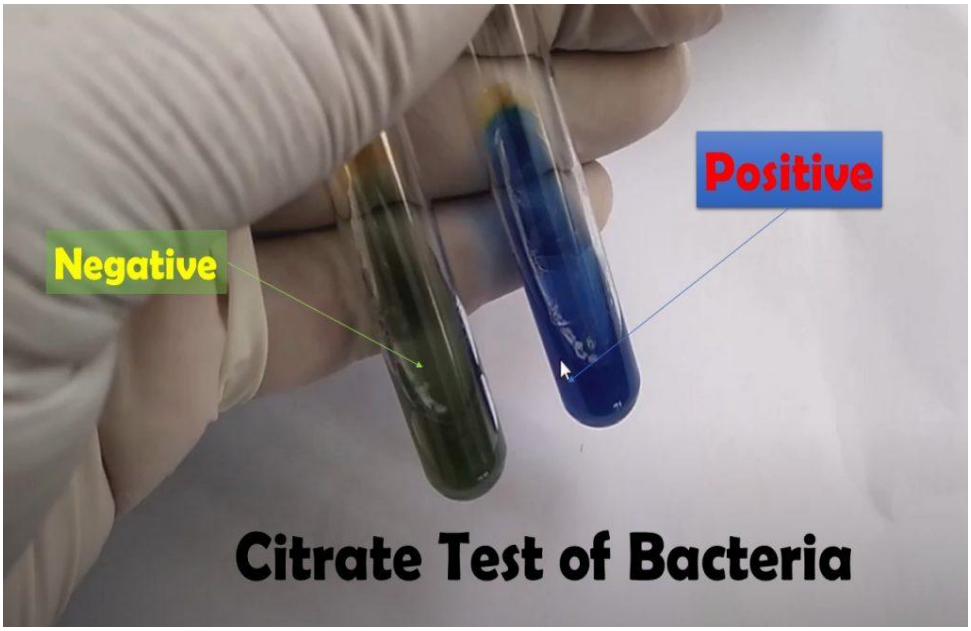
Examples:

Salmonella, Edwardsiella, Citrobacter, Klebsiella, Enterobacter, Serratia, Providencia, etc.

Negative Reaction: No growth and No color change; Slant remains green.

Examples:

Escherichia, Shigella, Morganella, Yersinia etc.



Urease Test- Principle, Media, Procedure and Result

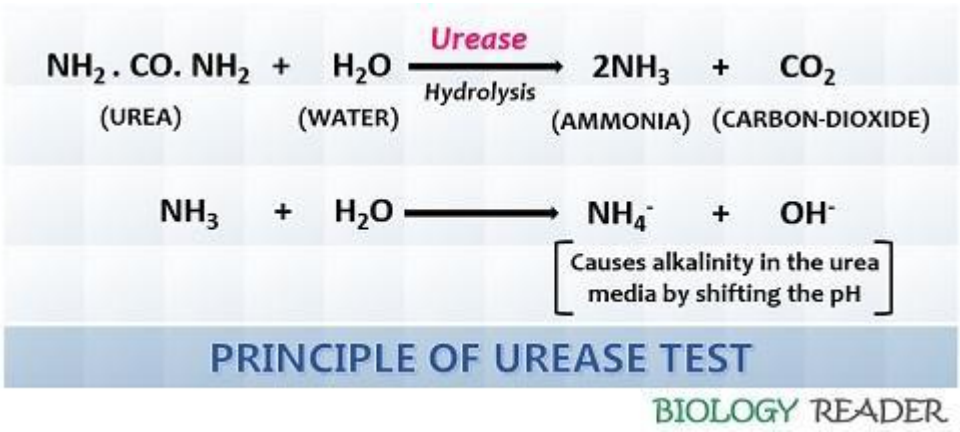
Urea Agar was developed by Christensen in 1946 for the differentiation of enteric bacilli. The urease test is used to determine the ability of an organism to split urea, through the production of the enzyme urease.

Principle of Urease Test

Urea is the product of decarboxylation of **amino acids**. Hydrolysis of **urea** produces **ammonia** and **CO2**. The formation of **ammonia**

alkalinizes the medium, and the pH shift is detected by the color change of **phenol red** from **light orange** at pH 6.8 to **magenta (pink)** at pH 8.1. Rapid urease-positive organisms turn the entire medium **pink** within 24 hours.

Weakly positive organisms may take several days, and negative organisms produce **no color change** or **yellow** as a result of **acid production**.



Uses of Urease Test

1. This test is used to differentiate organisms based on their ability to hydrolyze urea with the enzyme urease.

2. This test can be used as part of the identification of several genera and species of Enterobacteriaceae, including *Proteus*, *Klebsiella*, and some *Yersinia* and *Citrobacter* species, as well as some *Corynebacterium* species.
3. It is also useful to identify *Cryptococcus* spp., *Brucella*, *Helicobacter pylori*, and many other bacteria that produce the urease enzyme.

Media used in Urease Test (Christensen's Urea Agar)

Composition (Ingredients per liter of deionized water)	
Urea	20.0 gm
Sodium Chloride	5.0 gm
Monopotassium Phosphate	2.0 gm
Peptone	1.0 gm
Dextrose	1.0 gm
Phenol Red	0.012 gm
Agar	15.0 gm

Final pH 6.7 +/- 0.2 at 25 degrees C.

Preparation

1. Dissolve the ingredients in 100 ml of distilled water and filter sterilize (0.45-mm pore size).
2. Suspend the agar in 900 ml of distilled water, boil to dissolve completely.
3. Autoclave at 121-degree C and 15 psi for 15 minutes.
4. Cool the agar to 50 to 55-degree C.
5. Aseptically add 100 ml of filter-sterilized urea base to the cooled agar solution and mix thoroughly.
6. Distribute 4 to 5 ml per sterile tube (13 x 100 mm) and slant the tubes during cooling until solidified.

Procedure of Urease Test

1. Streak the surface of a urea agar slant with a portion of a well-isolated colony or inoculate slant with 1 to 2 drops from an overnight brain-heart infusion broth culture.
2. Leave the cap on loosely and incubate the tube at 35°-37°C in ambient air for 48 hours to 7 days.

3. Examine for the development of a pink color for as long as 7 days.

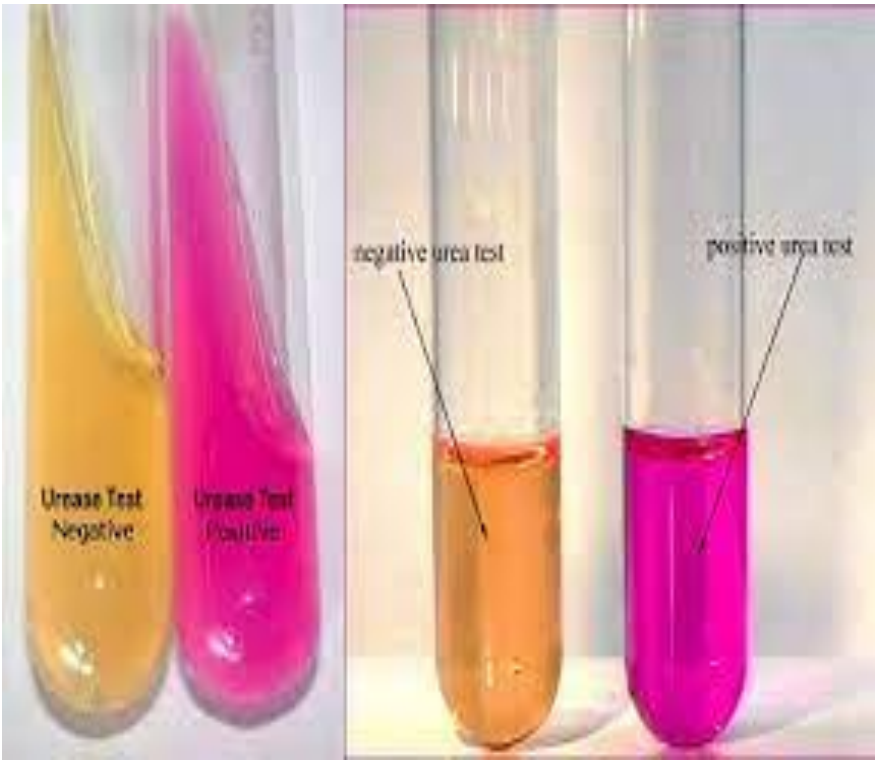
   Positive Reaction: Development of an intense magenta to bright pink color in 15 min to 24 h.

   Examples:

   Proteus spp, *Cryptococcus* spp, *Corynebacterium* spp, *Helicobacter pylori*, *Yersinia* spp, *Brucella* spp, etc.

   Negative Reaction: No color change.

   Examples: *Escherichia, Shigella, Salmonella*, etc.



The Triple Sugar Iron (TSI) Test – Principle, Procedure, Uses and Interpretation

Most bacteria have the ability to ferment carbohydrates, particularly sugars. Among them, each bacterium can ferment only some of the sugars, while it cannot ferment the others. Thus, the sugars, which a bacterium can ferment and the sugars, which

it cannot is the characteristic of the bacteria and thus an important criterion for its identification.

The Triple Sugar Iron (TSI) test is microbiological test named for its ability to test a microorganism's ability to ferment sugars and to produce hydrogen sulfide.

An agar slant of a special medium with multiple sugars constituting a pH-sensitive dye (phenol red), 1% lactose, 1% sucrose, 0.1% glucose, as well as sodium thiosulfate and ferrous sulfate or ferrous ammonium sulfate is used for carrying out the test.

All of these ingredients when mixed together and allowed solidification at an angle result in a agar test tube at a slanted angle. The slanted shape of this medium provides an array of surfaces that are either exposed to oxygen-containing air in varying degrees (an aerobic environment) or not exposed to air (an anaerobic environment) under which fermentation patterns of organisms are determined.

Objective

To determine the ability of an organism to ferment glucose, lactose, and sucrose, and their ability to produce hydrogen sulfide.

Principle

The triple sugar- iron agar test employing Triple Sugar Iron Agar is designed to differentiate among organisms based on the differences in carbohydrate fermentation patterns and hydrogen sulfide production. Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow.

To facilitate the observation of carbohydrate utilization patterns, TSI Agar contains three fermentative sugars, lactose and sucrose in 1% concentrations and glucose in 0.1% concentration. Due to the building of acid during fermentation, the pH falls. The acid base indicator Phenol red is incorporated for detecting carbohydrate fermentation

that is indicated by the change in color of the carbohydrate medium from orange red to yellow in the presence of acids. In case of oxidative decarboxylation of peptone, alkaline products are built and the pH rises. This is indicated by the change in color of the medium from orange red to deep red. Sodium thiosulfate and ferrous ammonium sulfate present in the medium detects the production of hydrogen sulfide and is indicated by the black color in the butt of the tube.

To facilitate the detection of organisms that only ferment glucose, the glucose concentration is one-tenth the concentration of lactose or sucrose. The meager amount of acid production in the slant of the tube during glucose fermentation oxidizes rapidly, causing the medium to remain orange red or revert to an alkaline pH. In contrast, the acid reaction (yellow) is maintained in the butt of the tube since it is under lower oxygen tension.

After depletion of the limited glucose, organisms able to do so will begin to utilize the lactose or sucrose. To enhance the alkaline condition of the slant, free exchange of air must be permitted by closing the tube cap loosely.

Media:

TSI Agar

Enzymatic digest of casein (5 g), enzymatic digest of animal tissue (5 g), yeast enriched peptone (10 g), dextrose (1 g), lactose (10 g) sucrose (10 g), ferric ammonium citrate (0.2 g), NaCl (5 g), sodium thiosulfate (0.3 g), phenol red (0.025 g), agar (13.5 g), per 1000 mL, pH 7.3.

Method

1. With a straight inoculation needle, touch the top of a well-isolated colony.

2. Inoculate TSI by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant.
3. Leave the cap on loosely and incubate the tube at 35°-37°C in ambient air for 18 to 24 hours.
4. Examine the reaction of medium.

Expected Results

- **An alkaline/acid (red slant/yellow butt) reaction:** It is indicative of dextrose fermentation only.
- **An acid/acid (yellow slant/yellow butt) reaction:** It indicates the fermentation of dextrose, lactose and/or sucrose.
- **An alkaline/alkaline (red slant, red butt) reaction:** Absence of carbohydrate fermentation results.
- **Blackening of the medium:** Occurs in the presence of H_2
- **Gas production**: Bubbles or cracks in the agar indicate the production of gas (formation of CO_2 and H_2)

Triple sugar iron agar. A, Acid slant/acid butt with gas, no H2S (A/A). **B,** Alkaline slant/acid butt, no gas, H2S-positive (K/A H2S+). **C,** Alkaline slant/alkaline butt, no gas, no H2S (K/K). **D,** Uninoculated tube.

Uses

- The test is used primarily to differentiate members of the Enterobacteriaceae family from other gram-negative rods.
- It is also used in the differentiation among Enterobacteriaceae on the basis of their sugar fermentation patterns.


TSI
LOT 5536
EXP 9-30-97
TSI
Microbiology Info.com

Detection of endotoxins in food samples by Limulus Amoebocyte Lysate test.

Theory:

Limulus Amoebocyte Lysate Test

The limulus amoebocyte lysate (LAL) test is a simple method for the detection of viable and non-viable Gram-negative bacteria. Certain cell-wall lipopolysaccharides (i.e. endotoxins) of this bacterial group lead to gelation of blood cell (amoebocytes) lysates of the *Limulus_polyphemus* crab. Using a dilution row and determining the limit at which no more gel formation occurs, a semi-quantitative estimation of the Gram-negative content is possible.

Several test-kits are available. Mostly used for pyrogen control of pharmaceutical products the LAL-test is applicable for predominantly Gram-negative containing foods such as fresh meat, milk and eggs. Another field of application may be the retrospective assessment of the microbiological quality of heated products.

Microbial toxins are organic poisons produced by microorganisms. When such poisons are ingested, absorbed, or otherwise introduced into the body of different living organisms, they cause damage to tissues and/or interfere with normal physiological functions. Microbial toxin s are complex in terms of structure and chemical composition, and they possess antigenic properties. Numerous types of microbial toxins have been described; these are bacterial toxins, fungal toxins (mycotoxins), algal toxins (phycotoxins), etc. Various types of toxins are produced by bacteria and potent toxins producers are *Corynebacterium diphtheria. Clostridium tetani, Clostridium botuilinum, E. coli, Staphylococcus aureus* etc.

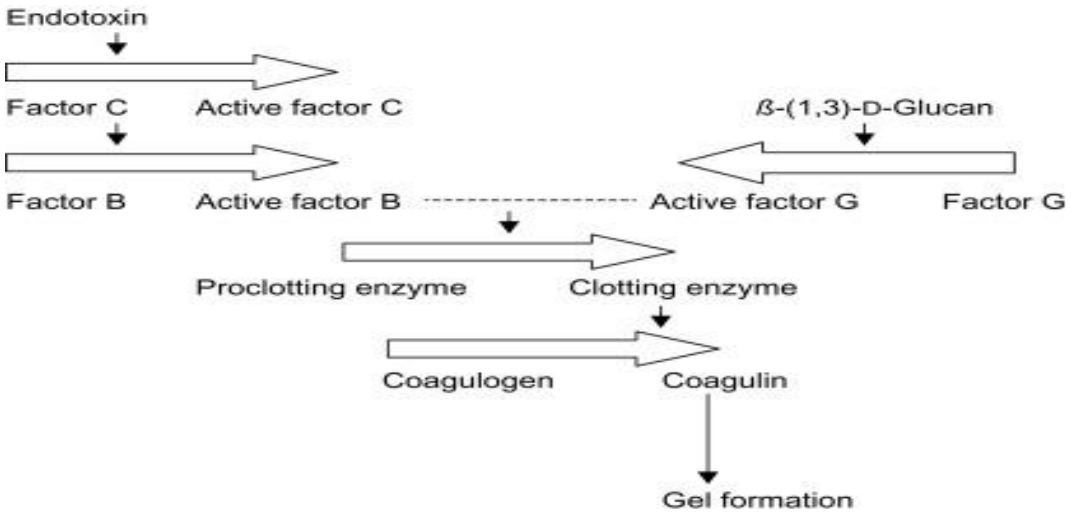
Exotoxins are soluble substances produced with in cells but secreted to the cell's exterior environment during periods of active growth. Endotoxins are bound to the bacterial cell wall as a structural component - or, in certain types of microbes, contained within the cell's cytoplasm.

Exotoxins are proteinaceous substances, specially characterized by their lack of chemical association with other macromolecules. These are sensitive to denaturation by heat and other chemical substances. Exotoxins can be neutralized by their homologous antibodies.

Endotoxin is complex lipo-polysaccharide-protein component of the bacterial cells wall. These are relatively heat-stable and cannot be neutralized by homologous antibodies.

Limulus polyphemus present in the blue blood of the horseshoe crab is a nucleated cell, called amoebocyte. The cytoplasm of amoebocyte is densely packed with granules. Limulus lysate, extract of amoebocyte granules, contains all the necessary clotting factors. Limulus lysate clots in the presence of bacterial lipopolysaccharides (endotoxins). Hence this test can be used to rapidly detect the endotoxins present in food samples. This

test is very specific and sensitive and can detect up to 10^{-15} g endotoxins per milliliter of sample.



Material required:

Food samples, clean and dry test tubes, Limulus lysate reagent, Water bath incubator (37 °C), wax marking pencils, sterile 0. 1 and 1 ml tips, auto-pipettes.

Procedure:

• Prepare the serial decimal dilution of samples using normal saline.

• Take equal volume of diluted sample and limulus lysate reagent in clean and dry test tubes and mix the content gently.

• Incubate the test tube in a water bath incubator at 37°C for 4 h

• After incubation is over, invert the test tube and see the flow behaviour of the fluid in the test tube.
• If the mixture remain s unchanged and runs down the test tube wall, indicate the negative LAL test and thus endotoxins are absent in the sample dilution.

• If a firm and opaque gel is formed and sticks to the bottom of the tube, indicates the positive LAL test and thus endotoxins are present in the sample dilution.

Checking the presence of antibiotics in milk samples through DSM DELVO test

Theory:

Antibiotic contamination in milk can seriously affect consumers' health by causing allergic reactions to residues or by the development of resistant strains of microorganisms. Therefore, subsequently antibiotic contamination in milk can also cause significant economic losses for producers and manufacturers of milk and milk products.

For determination of antibiotic residues in milk, use commercially available e Antibiotic

Detection Kit: Delvo-X-Press bL-II, COPAN TEST, CHARM FARM TEST or CHARM AIM-96, BITA STAR KIT

The growth of the *Bacillus stearothermophilus* spores at 64°C initiates an acidification process which causes the turning of a pH indicator from purple to yellow; the presence of antibacterial substances will cause delay or inhibition of the

spores, depending on the concentration of the residues, In the presence of residues the spores will not multiply and the pH indicator will remain purple.

Material Required:

DSM DEL YO test kit, raw milk samples, Water bath, agar well strips

Procedure:

• Add I nutrient tablet to each of the agar wells in the strip,

• Inoculate 100 ml of milk into the agar well plus nutrient tablet.

• Seal the wells for incubation

Incubate the strip or wells in a water bath at 64°C ± 0.5°C for 2 h 30 min* (at the time the negative control has been changed to yellow)

• Examine the strip for color change from purple to yellow. a yellow reading indicates that no inhibitory substances are present; a purple reading indicates

that antibiotic residues are present and a yellow/purple reading indicates a doubtful result.

*For best sensitivity a control time reading is advised using a negative control sample.

The sample incubation period is crucial to the accuracy of the Delvo® SP test method. This particularly applies to the detection of sulphonamides as the sensitivity to sulphonamides is greatly reduced by an increased incubation time. An incubation time of 2 hr 30 min (at the time the negative control has been changed to yellow) is recommended with a 15-minute extension time of the test in the case of a suspect sample. For reading times of 2 h 45 min or 3 hours the sensitivity of the test will diminish. The use of antibiotic free skim milk powder as a control is also advised. It is essential that the correct temperature is maintained in the water bath (64°C ±0.5°C). The use of a proper water bath lid, a sloping lid is advised, the temperature control in the water· bath should be digital temperature

readout. Good circulation should be maintained in the water-bath

Results:

Yellow colours: no antibiotic; Purple colour: antibiotic present