Bule Hora University

College of Medicine and Health Sciences

Department of Environmental Health Science

Food Microbiology for 3rd Year Environmental Health Students

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Course objectives

- To give brief highlight on Important Microorganisms in Food
- To know sources of microorganisms in food
- To know growth requirements of microorganisms in food
- To know the public health importance of some microorganisms in food

Chapter One

Important Microorganisms in Food

- Bacteria, yeasts, molds, and viruses are important in food for their ability to cause food borne diseases and food spoilage and to produce food and food ingredients.
- Many **bacterial species and some molds and viruses, but not yeasts**, are able to cause **foodborne diseases**.
- **Most bacteria, molds, and yeasts, because of their ability to grow in foods (viruses cannot grow in foods), can potentially cause food spoilage**.
- Several species of bacteria, molds, and yeasts are considered safe or food grade, or both, and are used to produce fermented foods and food ingredients.
- Among the four major groups, bacteria constitute the largest group.

Taxonomy

- kingdom: divisions,
- classes,
- orders,
- families,
- genera (singular genus), and
- species

- a **species** is regarded as a collection of strains having many common features

NOMENCLATURE

Morphology and structure of microorganisms

- **Eucaryotic cells**

- are generally much larger (20 to 100 mm) than procaryotic cells (1 to 10 mm)
- have rigid cell walls and thin plasma membranes.
- The cell wall does not have mucopeptide, is rigid, and is composed of carbohydrates.
- The plasma membrane contains sterol.
- The cytoplasm is mobile (streaming) and contains organelles (mitochondria, vacuoles) that are membrane bound.
- Ribosomes are 80S type and attached to the endoplasmic reticulum.
- The DNA is linear (chromosomes), contains histones, and is enclosed in a nuclear membrane.
- Cell division is by mitosis (i.e., asexual

Yeasts and Molds

- Both yeasts and molds are eucaryotic, but **yeasts are unicellular** whereas **molds are multicellular**

❖ Molds are

- ✓ nonmotile, filamentous, and branched
- ✓ The cell wall is composed of cellulose, chitin, or both.
- ✓ A mold (thallus) is composed of large numbers of filaments called hyphae.
- ✓ An aggregate of hyphae is called mycelium.
- ✓ A hypha can be nonseptate, septate-uninucleate, or septate-multinucleate.
- ✓ A hypha can be vegetative or reproductive.
- ✓ The reproductive hypha usually extends in the air and form exospores, either free (conidia) or in a sack (sporangium).
- ✓ Shape, size, and color of spores are used for taxonomic classification.

- Yeasts are
  - ✓ widely distributed in nature.
  - ✓ The cells are oval, spherical, or elongated
  - ✓ They are non motile.
  - ✓ The cell wall contains polysaccharides (glycans), **proteins**, and lipids.
  - ✓ The wall can have scars, indicating the sites of budding.
  - ✓ The membrane is beneath the wall.
  - ✓ The cytoplasm has a finely granular appearance for ribosomes and organelles.
  - ✓ The nucleus is well defined with a nuclear membrane.

1. Important Mold Genera

- **Molds** are important in food because they can grow even in conditions in which many bacteria cannot grow, such as low pH, low water activity (Aw), and high osmotic pressure.
- Many types of molds are found in foods.
- They are important **spoilage microbes**
- Many strains also produce **mycotoxins** and have been implicated in **foodborne intoxication**.
- Many are used in food bioprocessing.
- Finally, many are used to produce food additives and enzymes.

I. Aspergillus

- It is widely distributed and contains many species important in food.
- Members have septate hyphae and produce **black-colored asexual spores on 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 (food made of fruit and sugar), cured ham, nuts, and fruits and vegetables (rot)
- Some species or strains produce mycotoxins (e.g., *Aspergillus flavus* produces aflatoxin)

II. Alternaria

- Members are septate and form **dark-colored spores on conidia**.
- They cause rot in tomatoes and rancid flavor in dairy products.
- Some species or strains produce mycotoxins. Example the species: *Alternaria tenuis.*

III. Fusarium

- Many types are associated with rot in citrus fruits, potatoes, and grains.
- They form **cottony growth** and produce septate, sickle-shaped conidia. Example species: *Fusarium solani.*

IV. Geotrichum

- Members are septate and form rectangular arthrospores.
- They grow, forming a yeast-like cottony, creamy colony.
- They establish easily in equipment and often grow on **dairy products** (dairy mold). Example species: *Geotrichum candidum.*

V. Mucor: It is widely distributed.

- Members have nonseptate hyphae and produce sporangiophores(spore sack).
- They produce cottony colonies.
- Some species are used in **food fermentation** and as a source of enzymes.
- They cause **spoilage of vegetables**. Example species: *Mucor rouxii.*

VI. Rhizopus

- Hyphae are aseptate and form **sporangiophores** in sporangium.
- They cause **spoilage of many fruits and vegetables**.
- ***Rhizopus stolonifer* is the common black bread mold**.

VII. Penicillium

- It is widely distributed and contains many species.
- Members have septate hyphae and **form conidiophores** on a blue-green, brush-like conidia head.
- Some species are used in **food production**, such as *Penicillium roquefortii* and *Pen. camembertii* in cheese.
- Many species cause fungal **rot in fruits and vegetables.**
- They also cause spoilage of grains, breads, and meat.
- Some strains produce mycotoxins (e.g., Ochratoxin A).

2. Important Yeast Genera

- Yeasts are important in food because of their ability to cause spoilage.
- Many are also used in **food bioprocessing**.
- Some are used to produce **food additives**.

I. Saccharomyces

- Cells are round, oval, or elongated.
- It is the most important genus and contains heterogenous groups.
- *Saccharomyces cerevisiae* variants are used in **baking for leavening bread** and in **alcoholic fermentation**.
- They also cause spoilage of food, producing alcohol and CO2.

Cont…

II. Pichia

- Cells are oval to cylindrical and form pellicles(thin skin /film) in beer, wine, and brine to cause spoilage.
- Species: *Pichia membranaefaciens.*

III. Rhodotorula

- They are pigment-forming yeasts and can cause **discoloration of foods** such as meat, fish, and sauerkraut.
- Species: *Rhodotorula glutinis.*

IV. *Torulopsis*

- Cells are spherical to oval.
- They cause **spoilage of milk** because they can ferment lactose (e.g., *Torulopsis versatilis).*
- They also spoil fruit juice concentrates and acid foods.

V. *Candida*

- Many species spoil foods with high acid, salt, and sugar and form pellicles on the surface of liquids.
- Some can cause rancidity in butter and dairy products (e.g., ***Candida lipolyticum).***

VI. *Zygosaccharomyces*

- Cause spoilage of **high-acid foods**, such as sauces, ketchups, pickles, mustards, mayonnaise and **salad dressings especially those with less acid and less salt and sugar** (e.g., *Zygosaccharomyces bailii).*

3. 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 foodborne diseases.
- **Hepatitis A** and **Norwalk-like viruses** have been implicated in foodborne outbreaks.
- Several other enteric viruses, such as **poliovirus, echo virus, and Coxsackie virus**, can cause foodborne diseases.
- In some countries where the level of sanitation is not very high, they can contaminate foods and cause disease.

- **Bacteriophages** are used to transfer genetic traits in some bacterial species or strains by a process called transduction (e.g., in *Escherichia coli or Lactococcus lactis).*
- Finally, some bacteriophages can be very important because they can cause fermentation failure.
- Many lactic acid bacteria, used as starter cultures in food fermentation, are sensitive to different bacteriophages.

- They can infect and destroy starter-culture bacteria, causing product failure.
- Among the lactic acid bacteria, bacteriophages have been isolated for many species in the genera *Lactococcus, Streptococcus, Leuconostoc, and Lactobacillus*
- Methods are being devised to genetically engineer lactic starter cultures so that they become resistant to multiple bacteriophages

4. Important Bacterial Genera

- **Among the microorganisms found in foods, bacteria constitute major important groups.**
- **This is not only because many different species can be present in foods, but also because of their rapid growth rate, ability to utilize food nutrients, and ability to grow under a wide range of temperatures, aerobiosis, pH, and water activity, as well as to better survive 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.**

A. Lactic Acid Bacteria(CH3.CHOH.CO2H)

- They are bacteria that produce relatively large quantities of lactic acid from carbohydrates.
- Species mainly from genera *Lactococcus, Leuconostoc, Pediococcus, Lactobacillus,* and *Streptococcus thermophilus* are included in this group.

B. Acetic Acid Bacteria(CH3COOH)

- They are bacteria that produce acetic acid, such as *Acetobacter aceti.*

C. Propionic Acid Bacteria(CH3CH2COOH)

- They are bacteria that produce propionic acid and are used in dairy fermentation.
- Species such as *Propionibacterium freudenreichii* are included in this group.

D. Butyric Acid Bacteria(C3H7COOH) OR CH3(CH2)2COOH

- They are bacteria that produce butyric acid in relatively large amounts.
- Some *Clostridium spp.* such as *Clostridium butyricum* are included in this group.

E. Proteolytic Bacteria

- They are bacteria that can hydrolyze proteins because they produce extracellular proteinases.
- Species in genera *Micrococcus, Staphylococcus, Bacillus, Clostridium, Pseudomonas, Alteromonas, Flavobacterium, Alcaligenes,* some in *Enterobacteriaceae,* and *Brevibacterium* are included in this group.

F. Lipolytic Bacteria

- They are bacteria that are able to hydrolyze triglycerides because they produce extracellular lipases.
- Species in genera *Micrococcus, Staphylococcus, Pseudomonas, Alteromonas, and Flavobacterium* are included in this group.

G. Saccharolytic Bacteria

- They are bacteria that are able to hydrolyze complex carbohydrates.
- Species in genera *Bacillus*, *Clostridium*, *Aeromonas*, *Pseudomonas*, and *Enterobacter* are included in this group.

H. Thermophilic Bacteria

- They are bacteria that are able to grow at **50 oC and above**.
- Species from genera *Bacillus*, *Clostridium*, *Pediococcus*, *Streptococcus*, and *Lactobacillus* are included in this group.

I. Psychrotrophic Bacteria

- They are bacteria that are able to grow at **refrigerated temperature (<5 oC).**
- Some species included are *Pseudomonas, Alteromonas, Alcaligenes, Flavobacterium, Serratia, Bacillus, Clostridium, Lactobacillus, Leuconostoc, Carnobacterium, Brochothrix, Listeria, Yersinia,* and *Aeromonas*

J. Thermoduric Bacteria

- They are bacteria that are able to survive **pasteurization temperature** treatment.
- Some species from *Micrococcus, Enterococcus, Lactobacillus, Pediococcus, Bacillus* (spores), and *Clostridium* (spores) are included in this group.

K. Halotolerant Bacteria

- They are bacteria that are able to survive high salt concentrations (≥10%).
- Some species from *Bacillus, Micrococcus, Staphylococcus, Pediococcus, Vibrio,* and *Corynebacterium* are included in this group.

L. Aciduric Bacteria

- They are bacteria that are able to survive at low pH (<4.0).
- Some species from *Lactobacillus, Pediococcus, Lactococcus, Enterococcus,* and *Streptococcus* are included in this group.

M. Osmophilic Bacteria

- They are bacteria that can grow at a relatively higher osmotic environment than that needed for other bacteria.
- Some species from genera *Staphylococcus, Leuconostoc,* and *Lactobacillus* are included
- They are much less osmophilic than **yeasts and molds.**

N. Gas-Producing Bacteria

- They are bacteria that produce gas (CO2, H2, H2S) during metabolism of nutrients.
- Species from genera *Leuconostoc, Lactobacillus, Propionibacterium, Escherichia, Enterobacter, Clostridium,* and *Desulfotomaculum* are included

O. Slime Producers

- They are bacteria that produce **slime(cream)** because they synthesise polysaccharides.
- Some species or strains from *Xanthomonas*, *Leuconostoc*, *Alcaligenes*, *Enterobacter*, *Lactococcus*, and *Lactobacillus* are included

P. Spore Formers

- They are bacteria having the ability to produce spores.
- Species from *Bacillus*, *Clostridium*, and *Desulfotomaculum* are included

Q. Aerobes

- They are bacteria that require oxygen for growth and multiplication.
- Species from *Pseudomonas,* *Bacillus,* and *Flavobacterium* are included in this group.

R. Anaerobes

- They are bacteria that cannot grow in the presence of oxygen.
- Species from *Clostridium* are included in this group.

S. Facultative Anaerobes

- They are bacteria that are able to grow in both the presence and absence of oxygen.
- *Lactobacillus, Pediococcus, Leuconostoc, enteric pathogens,* and some species of *Bacillus, Serratia,* and coliforms are included in this group.

T. Coliforms

- Species from *Escherichia, Enterobacter, Citrobacter,* and *Klebsiella* are included in this group.
- They are used as an index of sanitation.

U. Fecal Coliforms

- Mainly *Escherichia coli* is included in this group.
- They are also used as an index of sanitation.

V. Enteric Pathogens

- Pathogenic *Salmonella, Shigella, Campylobacter, Yersinia, Escherichia, Vibrio, Listeria,* hepatitis A, and others that can cause gastrointestinal infection are included in this group.

Chapter Two

Sources of Microorganisms in Foods

Introduction

- The internal tissues of healthy plants (fruits and vegetables) and animals (meat) are essentially sterile.
- Yet raw and processed (except sterile) foods contain different types of molds, yeasts, bacteria, and viruses.
- Microbes get into foods from both **natural** (including internal) and **external sources** to which a food comes into contact from the time of **production** until the time of **consumption**.
- **Natural sources for foods of plant origin** include the surfaces of fruits, vegetables, and grains, and the pores in some tubers (e.g., radish and onion).

- **Natural sources for foods of animal origin** include skin, hair, feathers, gastrointestinal tract, urinogenital tract, respiratory tract, and milk ducts (teat canal) in udders of milk animals.
- **External source of microbes** includes: air, soil, sewage, water, feeds, humans, food ingredients, equipment, packages, and insects.
- **Microbial types and their levels** from these sources getting into foods vary widely and depend on the degree of sanitation used during the handling of foods.

- An understanding of the **sources of microorganisms in food is important to;**
  - Develop methods to control access of some microorganisms in the food,
  - Develop processing methods to kill them in food,
  - Determine the microbiological quality of food, as well as
  - Set up microbiological standards and specifications of foods and food ingredients.

I. Plants (Fruits and Vegetables)

- The inside tissue of foods from plant sources are essentially sterile, except for a few porous vegetables (e.g., radishes and onions) and leafy vegetables (e.g., cabbage and Brussels sprouts)
- Some plants produce natural antimicrobial metabolites that can limit the presence of microbes
- Fruits and vegetables harbor microbes on the surface; their type and level vary with soil condition, type of fertilizers and water used, and air quality.
- Molds, yeasts, lactic acid bacteria, and bacteria from genera *Pseudomonas, Alcaligenes, Micrococcus, Erwinia, Bacillus, Clostridium,* and *Enterobacter* can be expected from this source.

- **Pathogens**, especially of enteric types, can be present if the soil is contaminated with **untreated sewage.**
- Diseases of the plants, damage of the surface (before, during, and after harvest), long delay between harvesting and washing, and unfavorable storage and transport conditions after harvesting and before processing can greatly increase microbial numbers as well as predominant types.
- Improper storage conditions following processing can also increase their numbers.

The following activities can be used to reduce microbial load in foods of plant origin.

- Proper methods used during growing (such as use of treated sewage or other types of fertilizers),
- Damage reduction during harvesting,
- Quick washing with good quality water to remove soil and dirt,
- Storage at low temperature before and after processing

II. Animals, Birds, Fish, and Shellfish

- **Food animals and birds** normally carry many types of **indigenous microbes** in the digestive, respiratory, and urinogenital tracts, the teat canal in the udder, as well as in the skin, hooves, hair, and feathers

- Many, as carriers, can harbor pathogens such as *Salmonella serovars,* pathogenic *Escherichia coli, Campylobacter jejuni, Yersinia enterocolitica,* and *Listeria monocytogenes* without showing symptoms

- Laying birds have been suspected of asymptomatically carrying ***Salmonella Enteritidis*** **in the ovaries and contaminating the yolk during ovulation**

- Disease situations, such as **mastitis in cows** and intestinal, respiratory, and uterine infections, as well as injury can change the ecology of normal microflora.
- Similarly, poor husbandry resulting in fecal contamination on the body surface (skin, hair, feathers, and udder) and supplying contaminated water and feed (e.g., contaminated with salmonellae) can also change their normal microbial flora.
- Fish and shellfish also carry normal microflora in the scales, skin, and digestive tracts.
- Water quality, feeding habits, and diseases can change the normal microbial types and level.

- Pathogens such as *Vibrio parahaemolyticus, Vib. vulnificus, and Vib. cholerae* are of major concern from these sources.
- Many **spoilage and pathogenic microbes** can get into foods of animal origin (milk, egg, meat, and fishery products) during production and processing
- **Milk** can be contaminated with fecal materials on the udder surface, egg shells with fecal material during laying, meat with the intestinal contents during slaughtering, and fish with intestinal contents during processing

Prevention of food contamination from these sources

- Use of effective husbandry of live animals and birds, which includes good housing and supply of uncontaminated feed and water
- Testing animals and birds for pathogens and culling (collecting) the carriers
- Thorough cleansing using good-quality water for washing carcasses (preferably with acceptable antimicrobial agents); hair removal; feather removal; careful removal of digestive, urinogenital, and respiratory organs without contaminating tissues; removal of contaminated parts; and proper

- Proper cleaning of the udder before milking, cooling milk immediately after milking and sanitization at all stages are important reduce microbial levels in milk
- Eggs should be collected soon after laying and washed and stored as per recommended procedures
- Fish and marine products should be harvested from unpolluted and recommended water
- Proper sanitation should be used during processing
- They should be stored properly to prevent further contamination and microbial growth
- Ice to be used for storage should be produced from potable water

III. Air

- Microbes are present in dust and moisture droplets in the air.
- They do not grow in dust, but are transient and variable, depending on the environment.
- **Their level is controlled by the degree of humidity, size and level of dust particles, temperature and air velocity, and resistance of microbes to drying.**
- Generally, dry air with low dust content and higher temperature has a low microbial level.
- Spores of *Bacillus spp., Clostridium spp.,* and molds, and cells of some *Grampositive* bacteria (e.g., *Micrococcus spp.* and *Sarcina spp.),* as well as yeasts, can be predominantly present in air.

- If the surroundings contain a source of pathogens (e.g., animal and poultry farms or a sewage-treatment plant), different types of bacteria, including pathogens and viruses (including bacteriophages), can be transmitted via the air
- Microbial contamination of food from the air can be reduced by removing the potential sources, controlling dust particles in the air (using filtered air), reducing humidity level, and installing UV light

IV. Soil

- Many types of molds, yeasts, and bacterial genera (e.g., *Enterobacter, Pseudomonas, Proteus, Micrococcus, Enterococcus, Bacillus,* and *Clostridium)* can enter foods from the soil.
- Soil contaminated with fecal materials can be the source of enteric pathogenic bacteria and viruses in food.
- Sediments where fish and marine foods are harvested can also be a source of microbes
- Different types of parasites can also get in food from soil.
- Removal of soil (and sediments) by washing and avoiding soil contamination can reduce microorganisms in foods

V. Sewage

- **Sewage, especially when used as fertilizer in crops**, can contaminate food with microbes, the most significant of which are different enteropathogenic bacteria and viruses.
- This can be a major concern with organically grown food and many imported fruits and vegetables, in which untreated sewage and manure might be used as fertilizer.
- Pathogenic parasites can also get in food from sewage.
- **To reduce incidence of microbial contamination** of foods from sewage, it is better **not to use sewage as fertilizer.**
- If used, it should be efficiently treated to kill the pathogens.
- Also, effective washing of foods following harvesting is important.

VI. Water

- Water is used to **produce and process** foods.
- It is used for irrigation of crops, drinking by food animals and birds, raising fishery and marine products, washing foods, processing (pasteurization, canning, and cooling of heated foods) and storage of foods (e.g., fish on ice), washing and sanitation of equipment, and processing and transportation facilities.
- Water is also used as an **ingredient** in many processed foods.
- Thus, water quality can greatly **influence**

- Wastewater can be recycled for irrigation.
- However, **chlorine-treated potable water** (drinking water) **should be used** in processing, washing, sanitation, and as an ingredient.
- Although **potable water does not contain coliforms and pathogens** (mainly enteric types), it can contain other bacteria capable of causing **food spoilage**, such as *Pseudomonas, Alcaligenes, and Flavobacterium*.
- Improperly treated water can contain pathogenic and spoilage microbes

VII. Humans

- Between production and consumption, foods come in contact with different **people handling the foods**.
- They include not only people working in farms and food processing plants, but also those handling foods at restaurants, catering services, retail stores, and at home.
- **Human carriers** have been the source of pathogenic microbes in foods that later caused **foodborne diseases**, especially with **ready-to-eat foods.**
- Improperly cleaned hands, personal hygiene, and dirty clothes and hair can be major sources of microbial contamination in foods.

- The presence of minor cuts and infection in hands and face and mild generalized diseases (e.g., flu, strep throat, or hepatitis A in an early stage) **can amplify the situation**.
- In addition to spoilage bacteria, pathogens such as *Sta. aureus, Salmonella* serovars, *Shigella spp., pathogenic Esc. coli, and hepatitis A* can be introduced into foods from human sources.
- Proper **training of personnel in personal hygiene, regular checking of health, and maintaining efficient sanitary and aesthetic standards** are necessary to reduce contamination from this source.

VIII. Food Ingredients

- In prepared or fabricated foods, many ingredients/additives are included in different quantities.
- Many of these ingredients can be the source of both **spoilage and pathogenic microbes.**
- **Various spices** generally have very high populations of mold and bacterial spores.
- Starch, sugar, and flour might have spores of thermophilic bacteria.
- The ingredients should be produced under sanitary conditions and given antimicrobial treatments.
- In addition, **setting up acceptable microbial**

IX. Equipment

- A wide variety of equipment is used in harvesting, slaughtering, transporting, processing, and storing foods.
- Many types of microbes from air, raw foods, water, and personnel can get into the equipment and contaminate foods.
- Depending on the environment and time, microbes can multiply and, even from a low initial population, reach a high level and contaminate large volumes of foods.
- Also, when processing equipment is used

- In some equipment, small parts, inaccessible sections, and certain materials might not be efficiently cleaned and sanitized.
- These dead spots can serve as sources of both pathogenic and spoilage microbes in food.
- Small equipment, such as cutting boards, knives, spoons, and similar articles, because of improper cleaning, can be sources of cross contamination.
- Proper cleaning and sanitation of equipment at **prescribed intervals** are important to reduce microbial levels in food.
- In addition, developing means to prevent or reduce contamination from air, water, personnel, and insects is important.

X. Miscellaneous Sources

- Other sources include **packaging and wrapping materials**, containers, flies, vermins, birds, house pets, and rodents.
- Because they are used in products ready for consumption and in some cases without further heating, proper microbiological standards (or specifications) for packaging materials are necessary.
- Flies, vermins, birds, and rodents in food processing and food preparation and storage facilities should be viewed with concern as they can carry pathogenic microorganisms.
- House pets can also harbor pathogens; proper care should be taken not to contaminate food from this source.

Chapter Three

Microbial Growth in Food Environment

Introduction

- **Microbes grow or multiply** in numbers when exposed to a **favorable environment** such as food
- Their growth is associated with **food spoilage, foodborne diseases**, and **food bioprocessing**
- Growth is also important **to isolate an unknown microbial strain** involved in food spoilage, foodborne diseases, or food bioprocessing, in pure form, and study its morphological, physiological, biochemical, and genetic characteristics
- This help to design methods to control or stimulate its growth in food, destroy it, or improve its genetic makeup for better use

Microbial Reproduction or Growth

A. Binary Fission

- An increase in number or mass of vegetative cells of bacteria, yeasts, and molds is customarily used to reflect growth for microbes
- Bacteria reproduce by a process called binary fission.
- A bacterial cell has a specific **surface area-to-volume** (s/v) ratio.
- **A newly divided cell has a higher s/v ratio, which helps in the rapid transport of nutrients from the environment**
- A young cell predominantly uses nutrients to synthesize energy and cellular components, leading to an increase in cell size
- As the cell size increases, the s/v ratio decreases, which adversely affects the transport of nutrients into and by-products out of the cell.

- **Yeasts and molds** can also **reproduce asexually**
- A yeast cell produces a bud that initially is much smaller in size and remains attached to the surface of the original cell
- As it grows in size, it also can produce a bud, giving an appearance of a chain of buds growing on the surface of the original yeast cell
- A yeast cell can have several buds forming on its surface
- As the buds mature, they separate from the original cell

- Both yeasts and molds can also reproduce sexually
- Viruses do not reproduce by themselves
- Instead, they attach on the surface and inject their nucleic acid inside specific host cells, which then replicate the viral nucleic acid and produce viral particles
- The viral particles are released into the environment following lysis of the host cells
- **Viruses associated with foodborne diseases do not increase in numbers by replication in food**
- **However, if the specific host bacterial cells and the bacteriophages are present in a food,**

B. Generation Time (or Doubling Time)

- The time that a single cell takes to divide into two is **called generation time**
- However, in practice, generation time is referred to as the **doubling time** for the entire population
- In a population of a microbial species, not all cells divide at the same time or at the same rate
- The generation time of a microbial species under different conditions provides valuable information for developing methods to preserve foods
- In general, **under optimum conditions of growth, bacteria have the shortest generation time, followed by yeasts and molds.**

- The generation time of a microbial population can be calculated mathematically from the differences in population during a given time period.
- Because of large numbers, the calculation is done in logarithmatics (log10) by using the formula:

$$G = \frac{0.3t}{log10z - log10x}$$

where

- G is generation time (with time unit in minutes; also expressed as doubling time(td) with time unit usually in hours
- 0.3 is a constant (value of log102 and indicates doubling),
- t is the duration of study (min),
- log10x is initial and
- log10z is final cell numbers per milliliter or colony-forming units, (CFUs), per milliliter

example

- **Define generation time as related to bacterial growth. If a pure culture of a bacterial population during incubation at 35oC in a nutritionally rich broth increases to 5 X 106/ml from an initial population of 2.5 X 102 in 300 min, what is the generation time of the strain??????????**

D. Growth Curve

- The growth rate and growth characteristics of a microbial population under a given condition can be graphically represented by counting cell numbers, enumerating as CFUs
- The plot has several features that represent the conditions of the cells at different times.

A. lag phase

- **At the population does not change**
- the **cells assimilate nutrients and increase in size**
- Although the **population remains unchanged** because of **change in size like cell mass** and show some increase.

B. exponential phase (also called logarithmic phase)

- the cell number starts increasing, first slowly and then very rapidly
- The cells in the population differ initially in metabolic rate and only some multiply, and then almost all cells multiply.
- Growth rate at the exponential phase used to determine **generation time**

C. **stationary phase**

- the growth rate slows down
- **because of nutrient shortage and accumulation of waste products**, **a few**

D. Decline (death) phase

- the rate of cell death is higher than the rate of cell multiplication
- Depending on the strain and conditions of the environment, after a long period of time (may even be a few years) some cells may still remain viable
- **Dead cells, by the action of autolytic enzymes, may lyse and release the cellular enzymes in a food, which then can act on food components.**

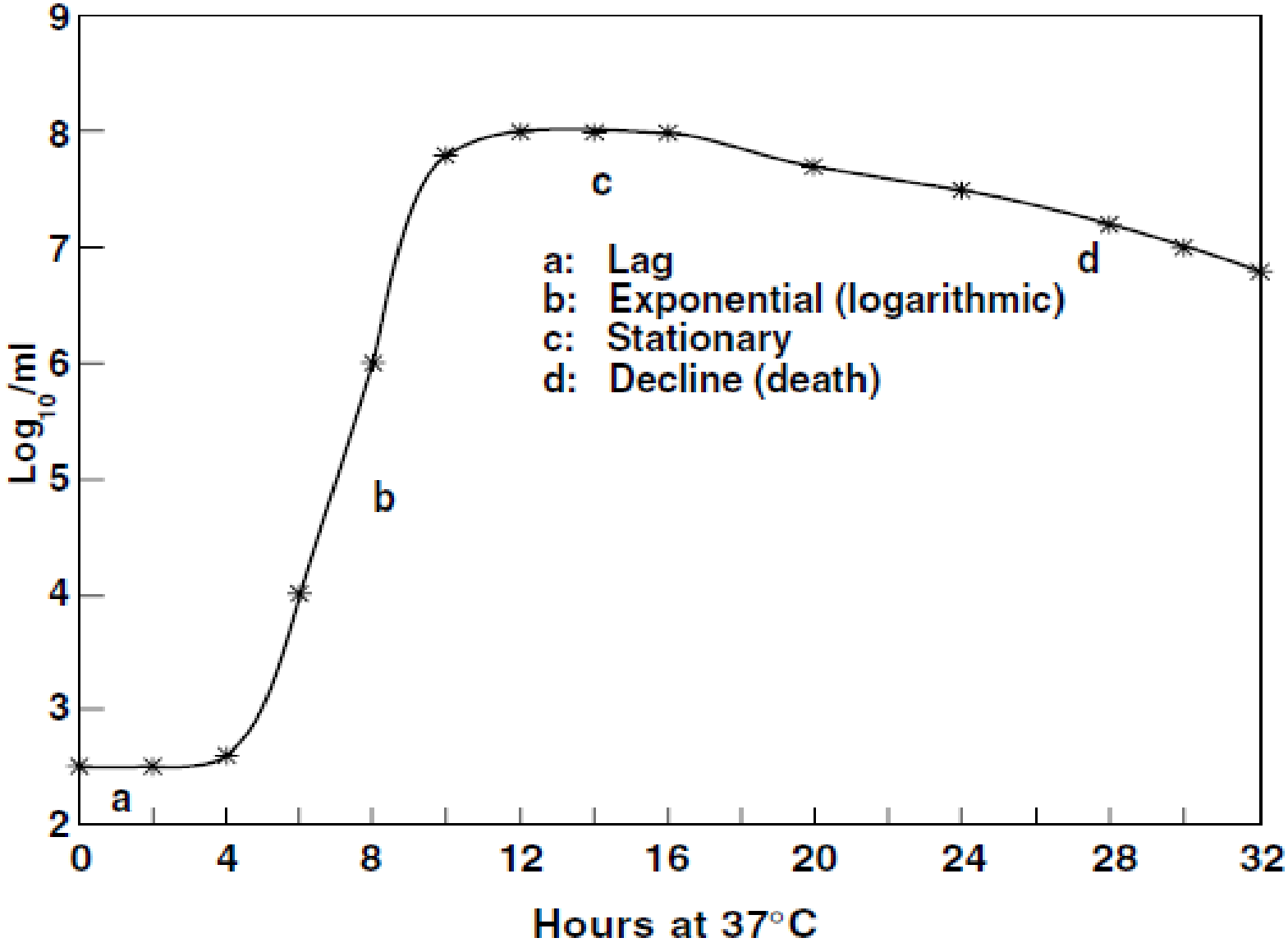


Fig. Bacterial Growth Curve

Relationship of microbial growth

A. Symbiotic Growth

- Symbiosis, or helping one another, during growth often occurs in food containing two or more types of microorganisms.
- One type may produce metabolic products that the second type needs for proper growth, but cannot produce by itself.
- In turn, the second species produces a nutrient that stimulates the first one to grow better.
- This is found in the production of some fermented foods such as yogurt.
- Initially, ***Streptococcus thermophilus*** *hydrolyzes milk proteins by its extracellular proteinases* and generates amino acids, which are necessary for good growth of *Lactobacillus delbrueckii subsp. bulgaricus.*

B. Synergistic Growth

- This is observed during symbiotic growth of two or more microbial types in a food.
- In synergistic growth, each type is capable of growing independently and producing some metabolites at lower rates.
- However, when the types are allowed to grow in a **mixed population**, both the growth rate and the level of by-product formation greatly increase.
- The **increase is more than the additive of the amounts produced by growing the two separately**.
- For example, both *Str. thermophilus and Lab. delbrueckii subsp. bulgaricus, when growing in milk independently, produce 8 to 10 ppm* ***acetaldehyde***, the **desirable flavor component of yogurt**.
- However, when **growing together in milk, 30 ppm or more of acetaldehyde is produced, which is**

C. Antagonistic Growth

- Two or more types of microorganisms present in a food can adversely affect the growth of each other, or one can interfere with the growth of one or more types; sometimes one can kill the other.
- This has been found **among many bacterial strains**, **between bacteria and yeasts**, **between yeasts and molds**, and **between bacteria and molds**.
- This occasionally occurs due to the production of one or more **antimicrobial compounds** by one or more strains in the mixed population.
- **Some** Gram-positive bacteria produce antibacterial proteins or bacteriocins that can kill many other types of Gram-positive bacteria.

Factors Influencing Microbial Growth in Food

- The ability of microorganisms (except viruses) **to grow or multiply in a food** is determined by the **food environment** as well as the **environment in which the food is stored**, designated as the **intrinsic and extrinsic environment of food**, respectively.
- It is not possible to study the influence of any one factor on growth independently as the factors are interrelated.
- Instead, the influence of any one factor at different levels on growth is compared keeping other factors unchanged.

Intrinsic factors or food environment

A. Nutrients and Growth

- Microbial growth is accomplished through the synthesis of cellular components and energy
- Microbes can find the nutrients in the food they are growing
- Microorganisms normally present in food **vary greatly** in nutrient requirements, with bacteria requiring the most, followed by yeasts and molds.
- Microorganisms also differ greatly in their ability to utilize large and complex carbohydrates (e.g., starch and cellulose), large proteins (e.g., casein in milk), and lipids.

- Microorganisms capable of using these molecules do so by producing specific **extracellular enzymes** (exoenzymes) and **hydrolyzing the complex molecules** to simpler forms outside before transporting them inside the cell
- Molds are the most capable of doing this.
- Microbial cells, following **death and lysis**, release **intracellular enzyme**s that can also catalyze breakdown of complex food nutrients to simpler forms, which can then be **utilized by other microorganisms.**

1. Carbohydrates in Foods

- All microbes normally found in food metabolize glucose, but their ability to utilize other carbohydrates differs considerably
- This is because of the **inability** of some microbes to transport the specific monosaccharides and disaccharides inside the cells and the inability to hydrolyze polysaccharides outside the cells
- Molds are the most capable of using polysaccharides
- **carbohydrates** are metabolized by microbes principally to **supply energy** through several metabolic pathways
- **Some of the metabolic products can be used to synthesize cellular components of microbes (e.g., to produce amino acids by amination of some keto acids)**

- Microbes also **produce metabolic byproducts** associated with **food spoilage** (CO_2 to cause gas defect) or **food bioprocessing** (lactic acid in fermented foods).
- Some are also metabolized to **produce organic acids**, such as lactic, acetic, propionic, and butyric acids, which have an antagonistic effect on the growth and survival of many bacteria.
- Microorganisms can also polymerize some monosaccharides to produce complex carbohydrates such as dextrans, capsular materials, and cell wall (or outer membrane and middle membrane in Gram-negative bacteria).
- Some of these carbohydrates from pathogens may cause health hazards (forming complexes with proteins), some may cause food spoilage (such as slime defect), and some can be used in food production (such as dextrans as stabilizers)

2. Proteins in Foods

- Proteins are present in higher quantities in foods of animal origin than in foods of plant origin
- But plant foods, such as **nuts and legumes**, are rich in proteins.
- Proteins as ingredients can also be added to foods
- Many microorganisms can hydrolyze albumin, which is soluble in water.
- In contrast, **collagens, which are insoluble in water or weak salt and acid solutions**, are hydrolyzed **only by a few microorganisms**.

- Microorganisms differ greatly in their ability to metabolize food proteins.
- Most transport amino acids and small peptides **in the cells**; small peptides are then hydrolyzed to amino acids inside the cells, such as in some *Lactococcus spp.*
- Microorganisms also produce **extracellular proteinases and peptidases** to hydrolyze large proteins and peptides to small peptides and amino acids before they can be transported inside the cells.
- Soluble proteins are more susceptible to this hydrolytic action than are the insoluble proteins.
- Hydrolysis of food proteins can be either undesirable

- Amino acids inside microbial cells are metabolized via different pathways to synthesize **cellular components, energy, and various by-products.**
- Many of these byproducts can be undesirable (e.g., NH3 and H2S production causes spoilage of food, and toxins and biological amines cause health hazards) or **desirable** (e.g., some sulfur compounds give **cheese flavor**)
- Production of **specific metabolic products** is **used for the laboratory identification** of microbial isolates from food.
- An example of this is the ability of *Escherichia coli* to produce indole from tryptophan, which is used to differentiate this species from non-indole-producing related species (e.g., *Enterobacter spp.).*

3. Lipids in Foods

- Lipids are relatively higher in foods of animal origin than in foods of plant origin, although nuts, oil seeds, coconuts, and olives have high amounts of lipids.
- Lipids are, in general, less preferred substrates for the microbial synthesis of energy and cellular materials.
- Many microorganisms can produce extracellular lipases, which can hydrolyze glycerides to fatty acids and glycerol.
- Fatty acids can be transported in cells and used for energy synthesis, whereas glycerol can be metabolized separately.

- In general, **molds** are more capable of producing lipid oxidase enzymes.
- However, certain bacterial groups such as *Pseudomonas, Achromobacter, and Alcaligenes* can produce lipid oxidase enzymes.
- Lysis of dead microbial cells in foods causes release of intracellular lipases and oxidases, which then can carry out these reactions.
- In many foods, the action of these enzymes is associated with **spoilage (such as rancidity),** whereas in other foods the enzymes are credited for **desirable flavors** (such as in mold-ripened cheeses).
- Some beneficial intestinal microorganisms, such as ***Lactobacillus acidophilus* strains**, can **metabolize cholesterol and are believed to be capable of reducing serum cholesterol levels in humans**.

4. Minerals and Vitamins in Foods

- Microorganisms need several elements in small amounts, such as phosphorous, calcium, magnesium, iron, sulfur, manganese, and potassium.
- Most foods have these elements in sufficient amounts.
- Many microorganisms can synthesize B vitamins, and foods also contain most B vitamins.

B. Growth Factors and Inhibitors in Food

- The exact nature of **growth factors** is not known, but they are naturally present in some foods



- An example is the **growth factors** in **tomatoes** that stimulate growth of some ***Lactobacillus* species**
- These factors can be added to raw materials during food bioprocessing or to media to isolate some **fastidious** bacteria from foods
- Foods also contain many chemicals, either naturally or added, that adversely affect microbial growth.
- Some of the **natural inhibitors** are **lysozyme in egg, agglutinin in milk, and eugenol in cloves.**
- The inhibitors, depending on their mode of action, can prevent or reduce growth of and kill microorganisms.

C. Water Activity and Growth

- ✓ **Water activity (Aw) is a measure of the availability of water for biological functions and relates to water present in a food in free form**.
- ✓ In a food system, total water or moisture is present in free and bound forms.
- ✓ **Bound water** is the fraction used to hydrate hydrophilic molecules and to dissolve solutes, and is not available for biological functions; thus, it does not contribute to *Aw.*
- ✓ The Aw of food ranges from 0.1 to 0.99.
- ✓ Pure water has Aw of 1.0

- ✓ The Aw of a food can be reduced by several means, such as adding solutes, ions, hydrophilic colloids, and freezing and drying
- ✓ The free water in a food is necessary for microbial growth.
- ✓ It is necessary to transport nutrients and remove waste materials, carry out enzymatic reactions, synthesize cellular materials, and take part in other biochemical reactions, such as hydrolysis of a polymer to monomers (proteins to amino acids)
- ✓ Each microbial species (or group) has an optimum, maximum, and minimum Aw level for growth.

- ✓ In general, the minimum Aw values for growth of microbial groups are as follows:
- ✓ **Most molds, 0.8, with xerophilic molds as low as 0.6;**
- ✓ **Most yeasts, 0.85, with osmophilic yeasts, 0.6 to 0.7;**
- ✓ **Most Gram-positive bacteria, 0.90; and Gram-negative bacteria, 0.93.**
- ✓ **Some exceptions are growth of *Staphlococcus aureus* at 0.85 and halophilic bacteria at 0.75.**
- ✓ The Aw need for spore-forming bacteria to sporulate and the spores to germinate and the toxin-producing microorganisms to produce toxins is generally higher than the minimum Aw for their growth.

- **Also, the minimum Aw for growth in an ideal condition is lower than that in a nonideal condition.**
- **As an example, if minimum Aw for growth of a bacterial strain at pH 6.8 is 0.91, then at pH 5.5, it can be 0.95 or more.**
- When the Aw is reduced below the minimum level required for growth of a microorganism, the cells remain viable for a while.
- But if the Aw is reduced drastically, microbial cells in a population lose viability, generally rapidly at first and then more slowly.
- This **information is used to control spoilage and pathogenic microorganisms in food as well as enhance the growth of desirable types in food bioprocessing**

D. pH and Growth

- On the basis of pH, foods can be grouped as **high-acid foods (pH below 4.6) and low-acid foods (pH 4.6 and above).**
- Most fruits, fruit juices, fermented foods (from fruits, vegetables, meat, and milk), and salad dressings are high-acid (low-pH) foods, whereas most vegetables, meat, fish, milk, and soups are low-acid (high-pH) foods.
- **Tomato, however, is a high-acid vegetable (pH 4.1 to 4.4).**
- **The higher pH limit of most low-acid foods remains below 7.0**; only in a few foods, such as clams (pH 7.1) and egg albumen (pH 8.5), does the pH exceed 7.0.

- Similarly, **the low pH limit of most high-acid foods remains above 3.0**, except in some citrus fruits (lemon, lime, grape fruit) and cranberry juice, in which the pH can be as low as 2.2.
- The acid in the foods can either be present **naturally** (as in fruits), **produced** during **fermentation** (as in fermented foods), or **added during processing** (as in salad dressings).
- Foods can also have compounds that have a buffering capacity.
- A food such as **milk or meat, because of good buffering capacity, does not show pH reduction when compared with a vegetable** product in the presence of the same amount of acid.

- pH of a food has a profound effect on the growth and viability of microbial cells.
- Each species has an optimum and a range of pH for growth.
- In general**, molds and yeasts are able to grow at lower pH than do bacteria, and Gram-negative bacteria are more sensitive to low pH than are Gram-positive bacteria.**
- The pH range of growth for
  - ***molds is 1.5 to 9.0***
  - ***yeasts, 2.0 to 8.5***
  - ***Gram-positive bacteria, 4.0 to 8.5 and***
  - ***Gram-negative bacteria, 4.5 to 9.0***.

- When the pH in a food is reduced **below the lower limit** for growth of a microbial species, the **cells not only stop growing but also lose viability**, the rate of which depends on the extent of pH reduction.
- The information about pH is important to develop methods to prevent the growth of undesirable microorganisms in food, used to produce some fermented foods and to selectively isolate aciduric microorganisms from food

E. Redox Potential, Oxygen, and Growth

- It measures the potential difference in a system generated by a coupled reaction in which one substance is oxidized and a second substance is reduced simultaneously.
- The process involves the loss of electrons from a reduced substance (thus it is oxidized) and the gain of electrons by an oxidized substance (thus it is reduced).
- The electron donor, because it reduces an oxidized substance, is also called a reducing agent.
- Similarly, the electron recipient is called an oxidizing agent.

- **In biological systems, the oxidation and reduction of substances are the primary means of generating energy.**
- If free oxygen is present in the system, then it can act as an electron acceptor.
- In the absence of free oxygen, oxygen bound to some other compound, such as NO_3 and SO_4, can accept the electron.
- In a system where no oxygen is present, other compounds can accept the electrons.
- Thus, presence of oxygen is not a requirement of O–R reactions

- **On the basis of their growth in the presence and absence of free oxygen, microorganisms have been grouped as aerobes, anaerobes, facultative anaerobes, or microaerophiles**.
  - Aerobes need free oxygen for energy generation, as the **free oxygen** acts as the final electron acceptor through aerobic respiration.
  - Facultative anaerobes can generate energy if **free oxygen** is available, or they can use **bound oxygen** in compounds such as NO3 or SO4 as final electron acceptors through anaerobic

- **Anaerobic and facultative anaerobic microorganisms can only transfer electrons through fermentation**.
- Many anaerobes (obligate or strict anaerobes) cannot grow in the presence of even small amounts of free oxygen as they lack the **superoxide dismutase** necessary to scavenge the toxic oxygen **free radicals**.
- **Addition of scavengers, such as thiols (e.g., thiolglycolate), helps overcome the sensitivity to these free radicals.**
- **Microaerophiles grow better in the presence of less oxygen.**
- Growth of microorganisms and their ability to generate energy by the specific metabolic reactions depend on the redox potential of foods.

Extrinsic Factors

- Extrinsic factors important in microbial growth in a food include the environmental conditions in which it is stored.
- These are temperature, relative humidity, and gaseous environment.

A. Temperature

- Microbial growth is accomplished through enzymatic reactions.
- It is well known that within a certain range, with every 10oC rise in temperature, the catalytic rate of an enzyme doubles.
- Similarly, the enzymatic reaction rate is reduced to half by decreasing the temperature by 10oC.

- Microorganisms important in foods are divided into three groups on the basis of their temperature of growth, each group having an optimum temperature and a temperature range of growth:
- **thermophiles** (grow at relatively high temperature), with optimum ca. 55 oC and range 45 to 70 oC;
- **mesophiles** (grow at ambient temperature), with optimum at 35 oC and range 10 to 45 oC; and
- **psychrophiles** (grow at cold temperature), with optimum at 15 oC and range –5 to 20 oC.
- However, these divisions are not clear-cut and overlap each other.

- Psychrotrophs are microorganisms that grow at refrigerated temp. (0 to 5 oC), irrespective of their optimum range of growth temperature.
- They usually grow rapidly between 10 and 30 oC.
- **Molds; yeasts; many Gramnegative bacteria from genera *Pseudomonas, Achromobacter, Yersinia, Serratia, and Aeromonas;* and Gram-positive bacteria from genera *Leuconostoc, Lactobacillus, Bacillus, Clostridium,* and *Listeria* are included in this group**
- Microorganisms that survive pasteurization temperature are designated as thermodurics.

- When the foods are exposed to temperatures beyond the maximum and minimum temperatures of growth, microbial cells die rapidly at higher temperatures and relatively slowly at lower temperatures.
- Microbial growth and viability are important considerations in reducing food spoilage and enhancing safety against pathogens, as well as in food bioprocessing.

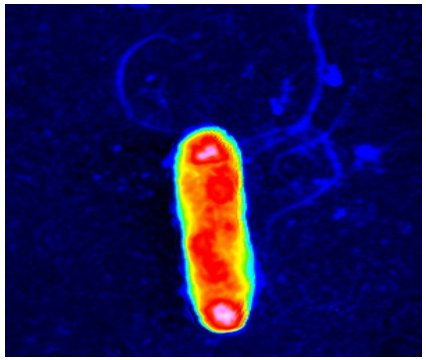
Chapter Four

Microbial Food Spoilage

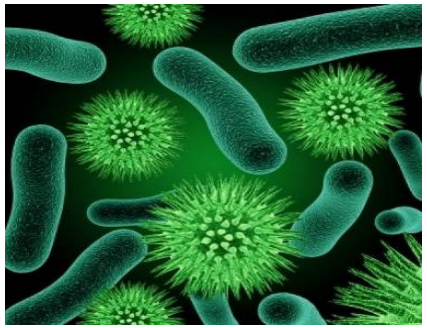
How do people get Food Poisoning?

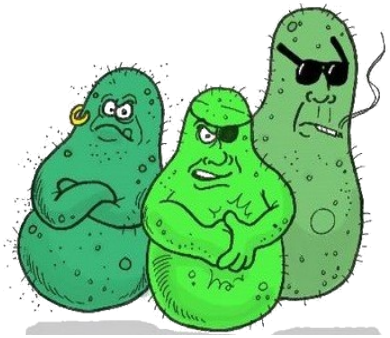
Because of Bad Bacteria

What is Bad Bacteria?









Where does bad bacteria come from?

Introduction

- A food is considered spoiled when it loses its **acceptance qualities**

- The factors considered in judging the acceptance qualities of a food include color, texture, flavor (smell and taste), shape, and absence of abnormalities.
- Loss of one or more normal characteristics in a food is considered to be due to **spoilage**

- The acceptance qualities of a food can be lost because of **infestation with insects and rodents, undesirable physical and chemical actions, and growth of**

- An example of **physical spoilage** is **dehydration of fresh vegetables** (wilting)
- **Chemical spoilage** includes oxidation of fat, browning of fruits and vegetables, and autolytic degradation of some vegetables (by pectinases) and fishes (by proteinases).
- Microbial spoilage results either as a consequence of microbial growth in a food or because of the action of some microbial enzymes present in a food.

Important Factors in Microbial Food Spoilage

- **Spoilage by microbial growth occurs much faster than spoilage by microbial extra or intracellular enzymes** in the absence of viable microbial cells

- Between initial production (such as harvesting of plant foods and slaughter of food animals) and final consumption, different methods are used to preserve the acceptance qualities of foods, which include the reduction of microbial numbers and growth

- Yet microbes grow and cause food spoilage, which for some foods could be relatively high

- It is important to understand the factors associated with microbial food spoilage, both for recognizing the

Sequence of Events

- Generally, **for microbial food spoilage to occur, several events need to take place in sequence**
- Microbes have to **get into** the food from one or more sources;
- The **food environment** (pH, Aw, nutrients, inhibitory agents) should favor growth of one or more types of these contaminating microorganisms;
- The food must be stored (or abused) at a **temperature** that enables one or more types to multiply; and
- Finally, the food must be stored under conditions of growth for sufficient **length of time** for the

- **In a heat-treated food**, the microbes associated with spoilage either survive the specific heat treatment (thermodurics) or get into the food following heating (as post-heat contaminants)
- **Spoilage of a heated food** by microbial enzymes, in the absence of viable microbial cells, can result from some **heat-stable enzymes** produced by microorganisms in the foods before heat treatment
- In addition, the foods need to be stored at a temperature for a sufficient length of time for the catalytic activities of the enzymes to occur to produce the detectable changes

Significance of Microorganisms

A. Microbial Types

- **Raw and most processed foods** normally contain many types of **molds, yeasts, and bacteria** capable of multiplying and causing spoilage.

- As multiplication is an important component in spoilage, **bacteria** (because of shorter generation time), followed by **yeasts, are in favorable positions over molds to cause rapid spoilage of foods.**

- However, in foods where bacteria or yeasts do not grow favorably and the foods are stored for a relatively longer period of time, such as breads, hard cheeses and acidic fruits and vegetables, spoilage due to mold growth is more prevalent.

B. Microbial Numbers

- To produce detectable changes in color, odor, and texture of a food, microbes need to reach a certain level called "**spoilage detection level**"
- **Although it varies with the type of foods and microorganisms, bacteria and yeasts need to grow and reach to 107 cells/g, /ml, or /cm2, of a food from the level present normally in a food.**
- Depending on the **specific nature of spoilage** and **microbial types**, the spoilage detection level can range from **106–8 cells/g, /ml, or /cm2.**

- Spoilage associated with H2S, some amines, and H2O2 formation can be detected at a **lower microbial load**,
- Whereas formation of lactic acid may be detected at a higher microbial load
- **Slime formation**, associated with accumulation of microbial cells, is generally detected at ≥108 cells/g, /ml, or /cm2 of a food
- It appears, then, that **a food with relatively higher initial loads of spoilage bacteria (or yeasts) and a storage condition that favors rapid growth (shorter generation time) will spoil more rapidly than a food with a low initial load of microbes with longer generation time**

C. Predominant Microorganisms

- The microbiological profile of a food is quite different from that of a pure culture growing in a laboratory medium
- An **unspoiled, nonsterile** food generally contains many types of microorganisms, such as bacteria, yeasts, and molds (also viruses) from different genera
- The population level of each type can vary greatly
- However, **when the same food is spoiled, it is found to contain predominantly one or two types, and they may not even be present initially in the highest numbers in the unspoiled or fresh product**

- Among the different species initially present and capable of growing in a particular food, **only those with the shortest generation time under the storage conditions attain the numbers rapidly and cause spoilage**
- In a study, a beef sample (pH 6.0) was found to initially contain 103 bacterial cells/g, with relative levels of *Pseudomonas spp.* 1%, *Acinetobacter* and *Morexella* 11%, *Brochothrix thermosphacta* 13%, and others 75%
- Following aerobic storage at 2 C for 12 day, the population reached 6*107 cells/g, with the relative levels of *Pseudomonas spp.* 99% and all others 1%

Some Important Food Spoilage Bacteria

- Theoretically, any microbe (including microbes used in food fermentation and pathogens) that can multiply in a food to reach a high level (spoilage detection level) is capable of causing it to spoil
- Yet, in reality, bacterial species from only several genera have been implicated more with spoilage of most foods
- This is dictated by the **bacterial characteristics, food characteristics, and the storage conditions**

A. Psychrotrophic Bacteria

- **Psychrotrophic bacteria** constitute the bacterial species capable of growing at 5 oC and below, but multiply quite rapidly at 10 to 25oC and even at higher temperatures
- Many foods are stored on ice (chilling) and in refrigerators, and some are expected to have a long shelf life (50 d or more).
- Between processing and consumption, they can be **temperature abused** to 10 C and higher.
- **Psychrotrophic bacteria** (also many yeasts and molds that are psychrotrophic) can **cause spoilage** in these foods.

- If the food is stored under aerobic conditions, **psychrotrophic aerobes** are the predominant spoilage bacteria.
- In foods stored under **anaerobic conditions** (also in the interior of a prepared food), anaerobic and facultative anaerobic bacteria predominate.
- If the food is given low-heat treatment and not exposed to post-heat contamination during storage at low temperature, **psychrotrophic thermoduric** bacteria can cause it to spoil.

B. Thermophilic Bacteria

- The bacteria in this group grow between **40 and 90 oC**, with optimum growth at **55 to 65 oC**.
- Some high-heat-processed foods are kept warm between 50 and 60C for a long period of time
- **Spores** of some thermophilic *Bacillus and Clostridium spp.* can be present in these heat-treated foods, which at warm temperature germinate and multiply to cause **spoilage**

- In addition, some thermoduric vegetative bacteria surviving low-heat processing (such as pasteurization) or thermophiles getting in food as post-heat contamination can also multiply in these warm foods, especially if the temperature is close to 50C.
- These include some **lactic acid bacteria**, such as *Pediococcus acidilactici* and *Streptococcus thermophilus,* as well as some *Bacillus* and *Clostridium* spp.
- They can also **survive and cause spoilage** of foods that are **cooked at low heat** (60 to 65C as for some processed meats) or kept warm for a long time.

C. Aciduric Bacteria

- Bacteria that can grow relatively rapidly in food at pH 4.6 or below are generally regarded as aciduric (or acidophilic).

- They are usually associated with **spoilage of acidic food products** such as fruit juices, pickles, salsa, salad dressings, mayonnaise, and fermented sausages.

- Heterofermentative lactic acid bacteria (such as *Lab. fructivorans, Lab. fermentum,* and *Leu. mesenteroides)* and homofermentative lactic acid bacteria (such as *Lab. plantarum* and *Ped. acidilactici)* have been associated with such spoilage.

- **Yeasts and molds** are aciduric and thus are also associated with spoilage of such foods

Significance of Foods

A. Food Types

- Foods differ greatly in their susceptibility to spoilage by microbes

- This is mainly because of their **differences in intrinsic factors** (Aw, pH, nutrient content, antimicrobial substances, and protective structures).

- **A food with a lower Aw (0.90) or a lower pH (5.3) is less susceptible to bacterial spoilage than one with Aw of 0.98 or pH of 6.4.**
- However, molds and yeasts will probably grow equally well under both conditions.

On the basis of **susceptibility of spoilage**, foods can be grouped as

- **Perishable**: spoil quickly, in days
- **Semi-perishable**: have a relatively long shelf life, few weeks or months, and
- **Non-perishable**: have a very long shelf life, many months or years.
  - In addition to intrinsic parameters, extrinsic parameters (storage conditions) play important roles in determining the ease of microbial spoilage of many foods

B. Food Nutrients

- Microbial growth in a food is associated with the metabolism of some food **carbohydrates, proteinaceous and non-protein nitrogenous (NPN) compounds, and lipids.**
- Microbes differ greatly in their abilities to metabolize different food nutrients such as ability or inability to utilize cellulose and lactose as carbon sources, casein as nitrogen source, and oxidation of oleic acid.
- Similarly, the same nutrient (substrate) can be utilized by different microbes by different metabolic pathways to produce different end products (e.g., glucose metabolized by homolactic and heterolactic acid bacteria).

- The same nutrient (substrate) can be degraded to produce different end products under aerobic and anaerobic metabolism (respiration and fermentation, respectively).
- Thus, ***glucose is metabolized (catabolized) by Micrococcus spp. aerobically to produce CO2 and H2O, and by Lab, acidophilus anaerobically to produce mainly lactic acid***
- *Saccharomyces cerevisiae* metabolizes glucose aerobically to CO2 and H2O, but anaerobically to ethanol and CO2.

- Under specific conditions, some microorganisms can also synthesize (anabolism) polymeric compounds as end products, such as dextran (polymer of glucose) production by ***Leu. mesenteroides*** while metabolizing sucrose.

- Some microorganisms can also secrete extracellular enzymes to break down large molecular nutrients (polymers) in a food (such as breakdown of starch by amylase produced by some molds).

- Finally, some microorganisms can **synthesize pigments** while growing in a food (such as ***Micrococcus luteus* producing a yellow pigment**).

- Thus, metabolism of food nutrients during growth of microbes in a food can adversely change its acceptance quality in several ways. Some of the changes are;

- **Odor**: due to production of volatile end products
- **Color**: pigment production or oxidation of natural color compounds, such as oxidation of **meat myoglobin**
- **Texture**: breakdown of pectin by pectinases in vegetables, softening of the tissues in meat by proteinases, or thickening of milk by proteolytic enzymes
- **Accumulation of gas**: due to production of CO_2, H_2, or H_2S
- **Formation of slime:** due to production of dextran and different types of exopolysaccharides or too many microbial cells

- Some of these changes also occur from the effect of microbial metabolites on food pH
- *Production of organic acids by microbes, causing lowering of food pH, can reduce the water-holding ability of food (such as growth of some lactic acid bacteria in low-fat, high-pH-processed meat products).*
- Similarly, production of **basic compounds** by microbes in a food can shift its pH to the alkaline side and reduce its acceptance quality (such as **decarboxylation of amino acids in some low heat- processed meat products with the production of amines**, shifting the pH to basic, and changing product color from **light brown to pink** in some processed meats

C. Utilization of Food Nutrients

- The characteristics of food spoilage differ greatly because of differences in the nature and the amount of a specific nutrient present in a food, the type of micrbes growing in the food, and the nature of metabolism (respiration or fermentation).

- In general, for energy production, microbes prefer to use metabolizable monosaccharides, disaccharides, and large carbohydrates first; followed by NPN, small peptides, and large proteinaceous compounds; and finally lipids

- However, again metabolic characteristics depend on whether a particular species can use a specific carbohydrate (such as the ability or inability to utilize lactose) and the concentration of it present (limiting

- Also, with any nutrient, small molecules are used first before large molecules (polymers)
- In a mixed microbial population, as present normally in a food, availability and amount of metabolizable carbohydrates greatly affect the spoilage pattern
- **Fresh meats, because of a low level of glucose, are susceptible to spoilage through microbial degradation of NPN and proteinaceous compounds**
- However, if a metabolizable carbohydrate (such as glucose, sucrose, or lactose) is added to meat, metabolism of carbohydrates will predominate

- If **lactic acid bacteria** are present as natural microflora and the growth environment is favorable, they will produce enough acids to arrest the growth of many normal microflora that preferentially metabolize NPN and proteinaceous compounds (e.g., Gram-negative pyschrotroph)

- **This is commonly known as the protein-sparing effect (proteins are not metabolized).**
- ***In the formulation of many processed meat products, metabolizable simple carbohydrates (usually glucose) are used to produce the protein-sparing effect***

Spoilage of Specific Food Groups

- Fresh and Ready-to-Eat Meat Products
- Eggs and Egg Products
- Fish, Crustaceans, and Mollusks
- Milk and Milk Products
- Vegetables and Fruits
- Soft Drinks, Fruit Juices and Preserves, and Vegetable Juices
- Cereals and Their Products
- Liquid Sweeteners and Confectioneries
- Mayonnaise, Salad Dressings, and Condiments
- Fermented Foods
- Canned Foods

I. Fresh and Ready-to-Eat Meat Products

A. Raw Meat

- Fresh meats from food animals and birds contain a large group of potential spoilage bacteria that include species of *Pseudomonas, Acinetobacter…..* as well as yeasts and molds
- The predominant spoilage flora in a meat is determined by;
  - **Nutrient availability**
  - **Oxygen availability**
  - **Storage temperature**
  - **pH**
  - **Storage time** of the product, and
  - **Generation time** of the microbes present in a given environment.

- To delay microbial spoilage, fresh meats are stored at refrigerated temperature (≤5°C)
- Thus, normally psychrotrophic bacteria are the most predominant types in raw meat spoilage
- Under aerobic storage at low temperature, growth of psychrotrophic aerobes and facultative anaerobes is favored
- In retail-cut meats, because of a shorter generation time, *Pseudomonas spp.* grows rapidly, using glucose first and then amino acids;
- **The metabolism of amino acids is accompanied by the production of malodorous methyl sulfides, esters, and acids**

- Psychrotrophic facultative anaerobes and anaerobes can grow in vacuum-packaged meat to produce different types of spoilage.
- *Lactobacillus curvatus* and *Lab. sake* metabolize glucose to produce lactic acid and the amino acids
- **Comminuted meats spoil more rapidly than retail cuts because they have more surface area**
- Under aerobic storage, growth of aerobic bacteria (predominantly *Pseudomonas spp.)* causes changes in odor, texture, color, and sliminess.

- To reduce spoilage of fresh meats, initial microbial level should be reduced
- In addition, storage at low temperatures (close to 0 to -1^{o}C), modified atmosphere packaging, and vacuum packaging should be done
- *Several other methods to reduce initial microbial load and slow growth rate of Gram-negative rods are being either used or tested*
- These include the addition of small amounts of organic acids to lower the pH of meat (slightly above pH 5.0), drying of meat surfaces (to reduce Aw), and a combination of the factors given previously, including lower storage temperature

B. Ready-to-Eat Meat Products

- This group includes high-heat-processed and low-heat-processed uncured and cured meat products
- High-heat-processed cured and uncured meats are given heat treatment to make them commercially sterile
- Thus, they may only have some thermophilic spores surviving, which will not germinate unless the products are temperature abused
- Low-heat-processed uncured meats, such as roasts, are given heat treatment at an internal temperature of 60 to 71°C
- Generally, the surface of the meats is exposed to the final temperature for 1h or more, depending on the size of the meat

- Under this condition, only the **spores** of *Bacillus* and *Clostridium spp.* and some extremely thermoduric vegetative species (*Lab. viridescens,* some *Enterococcus, Micrococcus* inside the product) can survive
- Many types of microbes can enter as post-heat contaminants into the products from equipment, personnel, water, and air
- In some situations, **spices** and other **ingredients** are added to the products after heating, which can be the source of microbial contamination of the products
- Some products are sliced before vacuum packaging, which increases the chances of contamination on the surface area of the product from the equipment and environment

- Psychrotrophic facultative anaerobic and anaerobic bacteria have been implicated in the spoilage of these products

- In vacuum-packaged roast beef, roast turkey, and roast chicken, hetero-fermentative *Lactobacillus spp.* and *Leuconostoc* spp., as post-heat contaminants, have been involved in spoilage, with the accumulation of large quantities of gas (CO_2) and liquid (due to acid production) inside the bag, without causing much flavor, color, or texture changes

- **Gas production** and **purge accumulation** in vacuum-packaged roast beef by psychrotrophic *Clostridium* spp., along with off-flavor and color changing from **brown to pink to red** (after 4 weeks), have been detected
- In spoiled sliced roast beef, the brown color of roast beef changes to pink (in 1 week) and the beef has a putrid odor (after 6 weeks)
- *Proteus* and *Hafnia spp.* are involved in spoilage of this product

- **Unpackaged cooked products** that do not have carbohydrates can become putrid from the growth of and protein degradation by proteolytic Gram-negative bacteria

- If the products are stored for long times, yeasts and molds can also grow, causing off-flavor, discoloration, and sliminess

- Because of growth of H_2O_2-producing lactic acid bacteria, they can have **green to gray discolorations**

II. Eggs and Egg Products

A. Shell Eggs

- The pores in the eggshell and inner membrane do not prevent entrance of bacteria and hyphae of molds, especially when the pore size increases during storage
- The presence of moisture enhances the entrance of motile bacteria
- The most predominant spoilage of shell eggs is caused by Gram-negative motile rods from several genera that include *Pseudomonas, Proteus, Alcaligenes, Aeromonas,* and the coliform group.

- The different types of spoilage are designated as rot. Some examples;
  - **Green rot**: causing greening of albumen because of growth of *Pseudomonas fluorescens;*
  - **Black rot:** causing muddy discoloration of yolk because of H_2S production by *Proteus vulgaris;*
  - **Red rot:** caused by production of red pigment by *Ser. mercescens*
- On some occasions, molds from genera *Penicillium, Alternaria, and Mucor* can grow inside eggs, especially when the eggs are oiled, and produce different types of fungal rots

B. Egg Products

- Liquid eggs consisting of whole, yolk, or white are generally pasteurized or frozen, or both, to prevent microbial growth

- If the liquid products are held at room temperature following breaking prior to pasteurization, spoilage bacteria can grow and cause off-flavor (putrid), sourness, or fish flavor (due to formation of trimethylamine)

- Pasteurized eggs at refrigerated temperature have a limited shelf life unless additional preservatives are added

- The predominant bacteria in pasteurized products are some Gram-positive bacteria that survive

III. Fish Spoilage

- Gram-negative **aerobic** rods, such as *Pseudomonas spp., Acinetobacter, Moraxella,* and *Flavobacterium,* and facultative **anaerobic** rods, such as *Shewanella, Alcaligenes, Vibrio*, and coliforms, are the major spoilage bacteria
- However, because of the relatively shorter generation time, spoilage by psychrotrophic *Pseudomonas spp.* predominates under aerobic storage at both refrigerated and slightly higher temperature
- In fish stored under vacuum or CO_2, lactic acid bacteria (including *Enterococcus)* can become predominant

- Proteolytic bacterial species also produce extracellular proteinases that hydrolyze fish proteins and supply peptides and amino acids for further metabolism by spoilage bacteria
- The volatile compounds produce different types of off-odors, namely, stale, fishy (due to trimethylamine), and putrid
- Bacterial growth is also associated with slime production, discoloration of gills and eyes (in whole fish), and loss of muscle texture (soft due to proteolysis).

- In fish stored by vacuum or by CO_2 packaging, growth of aerobic spoilage bacteria is prevented
- However, anaerobic and facultative anaerobic bacteria can grow, including lactic acid bacteria
- Under refrigeration, products have relatively long shelf life due to slower growth of spoilage bacteria
- Salted fish, especially lightly salted fish, are susceptible to spoilage by halophilic bacteria such as *Vibrio* (at lower temperature) and *Micrococcus* (at higher temperature)
- Smoked fish, especially with lower Aw, inhibit growth of most bacteria
- However, molds can grow on the surface

- Minced fish flesh, surimi, and seafood analogs prepared from fish tissues generally have high initial bacterial levels due to extensive processing 105–6/g).
- The types include those present in fish and those that get in during processing
- These products, such as fresh fish, can be spoiled rapidly by Gram-negative rods unless frozen quickly or used soon after thawing
- Canned fish (tuna, salmon, and sardines) are given heat treatment to produce commercially sterile products
- They can be spoiled by thermophilic sporeformers

IV. Milk and Milk Products

A. Raw Milk

- Microbial spoilage of raw milk can potentially occur from the metabolism of lactose, proteinaceous compounds, fatty acids (unsaturated), and the hydrolysis of triglycerides
- If the milk is refrigerated immediately following milking and stored for days, the spoilage will be predominantly caused by Gram-negative psychrotrophic rods, such as *Pseudomonas, Alcaligenes, Flavobacterium spp.,* and some coliforms

- They also produce heat-stable lipases (producing rancid flavor) and heat-stable proteinases

- The growth of lactose-positive coliforms produces lactic acid, acetic acid, formic acid, CO_2, and H_2 (by mixed-acid fermentation) and causes curdling, foaming, and souring of milk

- Some *Alcaligenes spp. (Alc. faecalis)* and coliforms can also cause ropiness (sliminess) by producing viscous exopolysaccharides.

- However, if the raw milk is not refrigerated soon, growth of mesophiles, such as species of *Lactococcus, Lactobacillus, Enterococcus, Micrococcus, Bacillus, Clostridium*, and coliforms,

- But lactose-hydrolyzing species, such as *Lactococcus spp.* and *Lactobacillus spp.*, generally predominate, producing enough acid to lower the pH considerably and prevent or reduce growth of others
- In such situations, curdling of milk and sour flavor is the predominant spoilage
- If other microorganisms also grow, gas formation, proteolysis, and lipolysis become evident
- Yeast and mold growth, under normal conditions, is generally not expected

B. Pasteurized Milk

- Thermoduric bacteria (*Micrococcus, Enterococcus,* some *Lactobacillus, Streptococcus, Corynebacterium,* and spores of *Bacillus* and *Clostridium)* survive the process
- In addition, coliforms, *Pseudomonas, Alcaligenes, Flavobacterium,* and similar types can enter as post-pasteurization contaminants
- Pasteurized milk, under refrigerated storage, has a limited shelf life, mainly due to growth of these psychrotrophic contaminants
- Their spoilage pattern is the same as described for raw milk spoilage
- Flavor defects from their growth are detectable when the population reaches 10^6cells/ml

- Growth of psychrotrophic *Bacillus spp.,* such as *Bacillus cereus,* cause spoilage of pasteurized refrigerated milk, especially when the levels of post-pasteurization contaminants are low
- Spores of psychrotrophic *Bacillus spp.,* surviving pasteurization, germinate and multiply to cause a defect known as bitty
- They produce the enzyme lecithinase, which hydrolyzes phospholipids of the fat globule membrane, causing aggregation of fat globules that adhere to the container surfaces.
- Production of rennin-like enzymes by psychrotrophic *Bacillus spp.* and others can cause sweet curdling of milk at a pH higher than that required for acid curdling

C. Butter

- The microbiological quality of butter depends on the quality of cream and the sanitary conditions used in the processing
- Growth of bacteria (*Pseudomonas spp.),* yeasts *(Candida spp.),* and molds (*Geotrichum candidum)* on the surface causes flavor defects (putrid, rancid, or fishy) and surface discoloration
- In unsalted butter, coliforms, *Enterococcus,* and *Pseudomonas* can grow favorably in the water phase (which has nutrients from milk) and produce flavor defects.

V. Vegetables and Fruits

- ✓ Fresh vegetables contain microbes coming from soil, water, air, and other environmental sources, and can include some plant pathogens
- ✓ Except tomatoes, they have high pH.
- ✓ Microbes grow more rapidly in damaged or cut vegetables.
- ✓ The presence of air, high humidity, and higher temperature during storage increases the chances of spoilage
- ✓ The most common spoilage is caused by different types of molds, some of those from genera *Penicillium*, *Phytopthora*, *Alternaria*, *Botrytis*, and

- ✓ Microbial vegetable spoilage is generally described by a common term rot, along with the changes in the appearance, such as black rot, gray rot, pink rot, soft rot, stem-end rot
- ✓ In addition to changes in color, microbial rot causes off-odor and loss of texture
- ✓ This is more apparent in ready-to-eat cut salads, and sliced vegetables and fruits that are refrigerated for a long time (3 to 4 weeks) and can be temperature abused
- ✓ Refrigeration, vacuum or modified atmosphere packaging, freezing, drying, heat treatment, and chemical preservatives are used to reduce microbial spoilage of vegetables

- ✓ Fresh fruits are high in carbohydrates (generally 10% or more), very low in proteins (≤1.0%), and have a pH 4.5 or below.
- ✓ Thus, microbial spoilage of fruits and fruit products is confined to **molds, yeasts, and aciduric bacteria** (lactic acid bacteria, *Acetobacter, Gluconobactor).*
- ✓ Like fresh vegetables, fresh fruits are susceptible to rot by different types of molds from genera *Penicillium, Aspergillus, Alternaria, Botrytis, Rhizopus,* and others.

- ✓ Yeasts from genera *Saccharomyces, Candida, Torulopsis,* and *Hansenula* are associated with fermentation of some fruits, such as apples, strawberries, citrus fruits, and dates.
- ✓ Bacterial spoilage associated with souring of berries and figs has been attributed to the growth of lactic acid and acetic acid bacteria.
- ✓ To reduce spoilage, fruits and fruit products are preserved by refrigeration, freezing, drying, reducing Aw, and heat treatment.

VI. Canned Foods

- Canned foods are heat treated to kill microorganisms present, and the extent of heat treatment is predominantly dependent on the pH of a food
- High-pH or low-acid (pH 4.6 and above) foods are heated to destroy most heat-resistant spores of the pathogenic bacteria, *Clo. botulinum*, to ensure that a product is free of any pathogen
- However, spores of some spoilage bacteria, which have greater heat resistance than do spores of *Clo. botulinum,* can survive
- Thus, these products are called commercially sterile (instead of sterile, which means free of any living organism) foods

- The spores that survive the heat treatment designed to destroy *Clo. botulinum* spores are thermophilic spores and can germinate at 43 ^{o}C and above
- The other food group, designated as low-pH or high-acid food with pH below 4.6, is heat treated to kill all vegetative cells and some spores

- Although low pH inhibits germination of spores and subsequent growth of *Clo. botulinum,* spores of some aciduric thermophilic spoilage bacteria can germinate and grow when the products are stored at higher temperatures, even for a short time, which induces germination

- Canned food spoilage is both due to non-microbial (chemical and enzymatic reactions) and microbial reasons.

Non-microbial spoilage includes;

- Production of hydrogen (hydrogen swell), CO2, browning, corrosion of cans due to chemical reactions,
- Liquification, gelation, and discoloration due to enzymatic reactions

Microbial spoilage is due to three main reasons:

A. Inadequate cooling after heating or high-temperature storage, allowing germination and growth of thermophilic sporeformers;

B. Inadequate heating, resulting in survival and growth of mesophilic microorganisms (vegetative cells and spores); and

C. Leakage (can be microscopic) in the cans, allowing microbial contamination from outside following heat treatment and their growth.

Chapter Five

Microbial Foodborne Diseases

- The incidence of foodborne illnesses in most of the developed countries is lower than in many developing countries
- The major reasons for the low incidence are the **implementation of necessary regulations** in production and handling of foods, **good sanitary practices**, and the **availability of necessary facilities** to reduce abuse
- Foodborne illnesses can be **fatal** as well as cause suffering, discomfort, and debilitation (make very weak) among the survivors

Types of Microbial Foodborne Diseases

- Foodborne diseases in humans result from the **consumption of either food and water** contaminated with viable pathogenic bacterial cells (or spores in the case of infant botulism) or food containing toxins produced by toxigenic bacteria and molds.
- On the basis of **mode of illnesses**, these can be arbitrarily divided into three groups:
  - Intoxication or poisoning,
  - Infection, and
  - Toxicoinfection

Intoxication

- **Illness occurs as a consequence of ingesting a preformed bacterial or mold toxin because of its growth in a food**
- A toxin has to be present in the contaminated food
- Once the microorganisms have grown and produced toxin in a food, there is no need of viable cells during consumption of the food for illness to occur
- Staph food poisoning is an example

Infection

- Illness occurs as a result of the consumption of food and water contaminated with enteropathogenic bacteria or viruses.
- It is necessary that the cells of **enteropathogenic bacteria and viruses remain alive** in the food or water during consumption.
- Viable cells, even if present in small numbers, have the potential to establish and multiply in the digestive tract to cause the illness
- Salmonellosis and hepatitis A are examples

Toxicoinfection/toxin mediated infection

- Illness occurs from **ingesting** a large number of viable cells of some pathogenic bacteria through contaminated food and water.
- Generally, the bacterial cells either sporulate or die and release toxins to produce the symptoms
- *Clo. Perfringens* gastroenteritis is an example

- In addition to the pathogenic microorganisms associated with foodborne illnesses, some bacterial species and strains normally considered nonpathogenic can cause gastroenteritis, especially in susceptible individuals
- They are designated as **opportunistic pathogens**
- They are normally required to be alive and present in large numbers when consumed through a contaminated food

I. Foodborne Intoxications

Some general characteristics of food poisoning are:

1. The toxin is produced by a pathogen while growing in a food
2. A toxin can be heat labile or heat stable.
3. Ingestion of a food containing active toxin, not viable microbial cells, is necessary for poisoning (except for infant botulism, in which viable spores need to be ingested)
4. Symptoms generally occur quickly, as early as 30min after ingestion
5. Symptoms differ with type of toxin; enterotoxins produce gastric symptoms and neurotoxins produce neurological symptoms
6. Febrile (fever) symptom is not present

A. Staphylococcal Intoxication

- **Staphylococcal food poisoning** (staphylococcal gastroenteritis) caused by toxins of *Staphylococcus aureus,* is considered one of the most frequently occurring foodborne diseases worldwide

Characteristics of *Staphylococcus aureus*

- *Sta. aureus* are Gram-positive cocci, occur generally in bunches, and are non-motile, non-capsular, and non-sporulating
- Most strains ferment mannitol and produce coagulase, thermonuclease, and hemolysin, but differ in their sensitivity to bacteriophages

- The cells are killed at 66 ^{o}C in 12min, and at 72 ^{o}C in 15s.
- *Sta. aureus* are facultative anaerobes, but grow rapidly under aerobic conditions
- They can ferment carbohydrates and also cause proteolysis by extracellular proteolytic enzymes
- They are mesophiles with a growth temperature range of 7 to 48 ^{o}C, with fairly rapid growth between 20 and 37 ^{o}C

- They can grow at relatively low Aw (0.86), low pH

- However, their growth can be reduced by combining two or more parameters.
- Because of their ability to grow under several adverse conditions, *Sta. aureus* can grow in many foods.
- Normally, they are poor competitors to many other microorganisms found in foods.
- But their ability to grow under adverse environments gives them an edge in growth in many foods in which others do not grow favorably
- Enterotoxin-producing *Sta. aureus* strains have generally been associated with staphylococcal food intoxication.

- Although strains of several other *Staphylococcus* species are known to be enterotoxin producers, their involvement in food poisoning is *not fully known.*
- *Sta. aureus,* along with many other *staphylococci,* are **naturally present** in the nose, throat, skin, and hair (feathers) of healthy humans, animals, and birds
- *Sta. aureus* can be present in infections, such as cuts in skin and abscesses in humans, animals, and birds, and cuts in hands and facial-erupted acne in humans.
- Food contamination generally occurs from these sources.

Toxins and Toxin Production

- Enterotoxigenic strains of *Sta. aureus* produce seven different enterotoxins: A, B, C1, C2, C3, D, and E
- They are serologically distinct **heat-stable** proteins and differ in toxicity
- The toxins vary in heat stability
- Normal temperature and time used to process or cook foods do not destroy the potency of the toxins
- Rate of toxin production by a strain is directly related to its rate of growth and cell concentrations
- Optimum growth occurs ca. 37 to 40^{o}C

- Under optimum conditions of growth, toxins can be detected when a population has reached over a few million per gram or milliliter of food and generally in 4h
- Some of the lowest environmental parameters of toxin production are $10^{o}C$, pH 5.0, or Aw 0.86

Disease and Symptoms

- Staphylococcal toxins are enteric toxins and cause **gastroenteritis**
- A healthy adult has to consume 30g or ml of a food containing **100 to 200ng** toxins produced by 10^{6-7} cells/g or /ml
- Infants, old and sick individuals need lesser amounts

- **The symptoms occur within 2 to 4h, with a range of 30min to 8h, and are directly related to the potency and amounts of toxin ingested and an individual's resistance**
- The disease lasts for 1 to 2d and is **rarely** fatal
- The **primary symptoms**, from stimulation of the autonomic nervous system by the toxins, are
  - ✓ salivation,
  - ✓ nausea and vomiting,
  - ✓ abdominal cramps, and
  - ✓ diarrhea
- Some **secondary symptoms** are
  - ✓ sweating,
  - ✓ chills, headache, and
  - ✓ dehydration.
- However, the symptoms and their severity vary

- Many foods have been implicated in staphylococcal foodborne outbreaks
- **In general, the bacterium grows in the food and produces toxins without adversely affecting the acceptance quality**
- Many **protein-rich foods**, **foods that are handled extensively**, foods in which **associated bacteria grow poorly**, and foods that have been **temperature abused** are associated with **staphylococcal gastroenteritis**
- Some of the foods that have been more frequently implicated are ham, corned beef, salami, bacon, barbecued meat, salads, baking products containing cream, sauces, and cheeses

Prevention (Reduction) of the Disease

- To reduce the incidence of staphylococcal food poisoning, the aim is to reduce initial load of *Sta. aureus* in a food by;
  - Proper selection of the quality of the raw materials and ingredients,
  - Sanitation of the food environments, and
  - Proper personal hygiene among food handlers
  - People with respiratory diseases, acute types of facial acne, skin rash, and cuts in hands should not handle the food.
  - **Where possible, products should be heat-treated to ensure killing of live cells.**

Identification Methods

- ✓ Enumeration technique in one or more selective differential agar media to determine the load of viable cells of *Sta. aureus,* followed by several biochemical tests
- ✓ Enterotoxins from the food or vomit samples are extracted and tested, either by biological means or by serological means
- ✓ In the **biological method**, animals (e.g., cats) are given the enterotoxin preparation orally or it is injected intraperitoneally or intravenously
- ✓ Vomiting symptoms by the test animals is a positive indication of the presence of staphylococcal enterotoxin

- ✓ In **the serological methods**, the enterotoxins are purified and examined by one of the several recommended immunological methods

- Following heating, recontamination of the products should be avoided
- The most important aim is to chill the processed products and ready-to-eat foods to ≤5^{o}C quickly
- Suitable preservatives can also be used to kill or arrest growth
- The inside of the food, and not only the surface, should reach the chilled temperature, preferably within 1h
- Finally, the food should not be subjected to temperature abuse and stored for a long period of time at growth temperature before eating
- **Once heat-stable toxins are formed, heating before eating does not ensure safety**

B. Botulism

- **Botulism** results following consumption of food containing the potent toxin **botulin** of *Clostridium botulinum.*
- It is a neurotoxin and produces neurological symptoms along with some gastric symptoms
- Unless prompt treatment is administered, it is quite **fatal.**
- **Infant botulism occurs when an infant ingests *Clo. botulinum* spores, which germinate, grow, and produce toxins in the GI tract and cause specific symptoms.**

Characteristics

- Cells of *Clo. botulinum* strains are **Gram-positive** rods, occur as single cells or in small chains;
- Many are **motile, obligate anaerobes, and form spores**.
- Cells are sensitive to low pH (<4.6), low Aw (0.93), and moderately high salt (5.5%).
- **Spores do not germinate in the presence of nitrite (250 ppm).**
- **Spores are highly heat resistant** (killed at 115 ◦C), but cells are killed at moderate heat (pasteurization).
- Strains can either be proteolytic or non-proteolytic

- *Clo. botulinum* strains, on the basis of the type of toxin production, have been divided into six types: **A, B, C, D, E, and F**
- Of these, A, B, E, and F are associated with human foodborne intoxications
- Type A strains are proteolytic, Type E strains are nonproteolytic, but Types B and F strains can be either proteolytic or nonproteolytic.
- **Proteolytic strains** can grow between 10 °C and ca. 48 °C, with the optimum at **35 °C**
- **Nonproteolytic strains** grow optimally at **30 °C**, with a range from 3.3 to 45 °C.

- Optimum growth facilitates optimum toxin production.
- **Anaerobic conditions** are necessary for growth
- Spore germination and outgrowth before cell multiplication are favored in the environment of cell growth.
- Similarly, conditions that prevent cell growth also adversely affect spore germination.
- **A pH of 4.6, Aw of 0.93, or 5.5% NaCl** can prevent cell growth, but by using two or more

Habitat

- Spores of *Clo. botulinum* are widely distributed in soil, sewage, mud, sediments of marshes, lakes and coastal waters, plants, and intestinal contents of animals and fishes.
- **Fruits and vegetables** can be contaminated with spores from soil, **fishes** from water and sediments, and various other foods from many of the given sources.
- Type **A and B spores** are more prevalent in soil, sewage, and fecal matters of animals, whereas **Type E** spores are generally found in marine environments.

Toxins and Toxin Production

- The toxins of *Clo. botulinum* are **neurotoxic proteins**
- In general, toxins associated with food intoxication in humans (Types A, B, E, and F) are extremely potent, and only a small amount of toxin is required to produce the symptoms and cause death
- Following ingestion, toxin molecules are absorbed from the upper portion of the intestine through the intestinal wall and spread via the blood to the peripheral nerves
- **The toxins block signal transfers, irreversibly** causing **paralysis** of all **involuntary muscles.**
- The toxins move slowly through the body.

- The toxins produced by nonproteolytic strains are **heat labile** and can be destroyed in a contaminated food by high and uniform heat, such as **90 °C for 15 min or boiling for 5 min**.
- Cell growth is necessary for toxin production.
- At optimum growth temperature, toxins are produced in large amounts.
- However, at both extreme growth ranges, enough toxin can be produced by a strain in a food to cause disease and death following ingestion.

Disease and Symptoms

- At the initial stage (generally 12 to 36 h, but can be 2 h), some **gastrointestinal disorders** (e.g., nausea, vomiting, diarrhea, and constipation) may be evident.

- **Neurological symptoms** develop within a short time, especially if the amount of botulin consumed is high.
- As they are **highly potent toxins**, only a very small amount (1ng/kg body weight) is necessary for severe symptoms and even death.

In general, neurological symptoms include

- ✓ **Blurred or double vision**
- ✓ **Difficulty in swallowing, breathing, and speaking**
- ✓ **Dryness of the mouth and**

- Death usually results from respiratory failure
- The toxins are antigenic; thus antitoxins are available.
- Soon after the onset of the symptoms and if the amount of **toxin consumed** is **fairly low, trivalent antitoxins** (against botulin A, B, and E) can be administered to treat the disease.
- But in some **advanced cases, especially if the diagnosis is delayed, antitoxin administration may not be successful**.

Food Association

- The **largest number of outbreaks** are associated with fruits and vegetables, mainly low-acid vegetables (e.g., green beans, corn, spinach, asparagus, pepper, and mushrooms) and fruits (e.g., figs and peaches).
- The **next highest incidence** is with finfish, which includes fermented, improperly cooked, and smoked fish and fish eggs.
- The **major cause** of outbreaks are **improper home canning** of the contaminated products.

- The occurrence of botulism from meat, poultry, and dairy products is low, probably because they are mostly heated and eaten quickly.
- Some condiments, such as chili peppers, relish, and sauce, have also been associated with outbreaks.
- As the toxins are heat labile, high uniform heating (90 ^{o}C for 15 min or boiling for 5 min) of a suspected food destroys the toxins and makes the food theoretically safe.
- But because only a small amount of toxin is enough to cause disease, it is advisable not to consume a suspected food

Prevention of Botulism

- The **single most important** control method is to use proper temperature and time in home canning of low-acid products
- Some foods (e.g., fish) should be properly and uniformly cooked at high temperatures.
- Foods cooked at temperatures in which spores survive should be stored at low temperatures (at 3 °C or below); at refrigerated temperature (4 to 5 °C), storage should not be prolonged unless some additional precautions are used, such as NO2, low pH, low Aw, or NaCl.
- Suspected foods should be properly heated before consumption, but it is better not to eat them.

Identification Methods

- In a suspected food, the presence of *Clo.* botulinum can be determined by enumeration techniques using selective agar media and anaerobic incubation, followed by biochemical and toxicological testing.
- The presence of toxins in the food is more often tested.
- This involves injecting a food extract intraperitoneally into mice.
- Development of characteristic neurological symptoms, followed by death in 92h, suggest the presence of toxin.
- The people engaged in testing for the organism or the toxins need to immunize themselves before handling the materials.

Infant Botulism

- *Clo. botulinum spores, ingested by infants through food and the environment, can germinate in the intestine and produce the toxin to cause infant botulism.*
- *The spores fail to produce the same disease in individuals above 1 year of age, probably because the* ***well-established normal population of gastrointestinal flora discourages spore germination and cell multiplication by Clo. botulinum.***
- *Symptoms include general*
  - ✓ *Weakness*
  - ✓ *inability to suck and control the head*
  - ✓ *loss of reflexes and*
  - ✓ *constipation.*
- *Foods such as honey and corn syrup and dirt have*

C. Mycotoxicosis

- Many strains of **molds**, while growing in a suitable environment (including in foods), produce metabolites that are toxic to humans, animals, and birds, and are grouped as mycotoxins
- Consumption of foods containing mycotoxins causes **mycotoxicosis**
- ***They are secondary metabolites and not proteins or enteric toxins***.
- Many are **carcinogens**
- Some cause toxicity of organs by unknown mechanisms.

Some well-known mycotoxicosis incidents in humans;

- **Ergotism**: from consumption of bread made from rye infected with *Claviceps purpurea*
- **Yellow rice disease**: from the consumption of rice infested with toxigenic strains of several *Penicillium* species
- **Alimentary toxic aleukia**: from the ingestion of grains infested with toxigenic strains of *Fusarium* species
- **Liver necrosis**: feeding peanut meal in which *Aspergillus flavus* grew and produced the toxin **aflatoxin.**

Characteristics

- Toxigenic strains from several species and genera and the toxins they produce include *Asp. flavus, Asp. parasiticus (both produce aflatoxins), Asp. nidulans, Asp. virsicolor (sterigmatocystin), Penicillium viridicatum (ochratoxin), Pen. patulum (patulin), Pen. roquefortii (roquefortin), and Cla. purpurea (ergotoxin).*
- Toxigenic strains cannot be differentiated from nontoxigenic strains by morphological characteristics only.
- It is necessary to grow a strain under suitable conditions and test the material for the presence or absence of a mycotoxin.

- In general, molds grow best in humid and warm environments.
- They are aerobic and thus need air for growth.
- They can grow, though slowly, at very low Aw (0.65), low temperature (refrigerated temperature), and low pH (pH 3.5).
- These conditions are often used to extend the shelf life of many foods.
- Unless other methods (such as vacuum packaging) are used, they can grow in these foods, and, if toxigenic, can produce toxins in the foods
- The spores are present in soil, dust, and the environment.
- Many foods can have viable spores or mycelia, especially before a heat treatment

Toxins and Toxin Production

- Mycotoxins are small-molecular-weight heterocyclic organic compounds and some have more than one chemical type. An example is aflatoxin
- Mycotoxins are produced by toxigenic mold strains as secondary metabolites.
- Toxin production, in general, is directly related with the growth rate of a mold strain.
- In microbiological media suitable for growth of molds, *Asp. flavus* strains can produce optimum concentrations of aflatoxin at 33 ^{o}C, pH 5.0, and Aw 0.99

Food Association

- The growth of toxigenic mold strains and the presence of specific mycotoxins have been detected in many foods, such as corn, wheat, barley, rye, rice, beans, peas, peanuts, bread, cheeses, dry sausages, spices, apple cider, grain meals, dough cassava, cotton seeds, and spaghetti
- Consumption of mycotoxin-contaminated food can cause mycotoxicosis in humans
- Feeding moldy products to food animals (including moldy silage) and birds can also produce foods of animal origin (milk, eggs) that are contaminated with mycotoxins
- Many mycotoxins are resistant to heat used in the normal preparation of foods
- Thus, their elimination by heating is not used to remove them from foods

Prevention of Mycotoxicosis

- Contamination of food with toxigenic mold strains should be reduced
- Proper packaging
- Heat treatment used for killing the molds and their spores.
- Preventing growth in food (and feeds) should be a major consideration in reducing the incidence of human mycotoxicosis.
- This can be achieved by using anaerobic packaging; reducing Aw, where possible, to 0.6 or below; freezing; and using specific preservatives against mold growth
- Some chemicals (e.g., ammonia, hydrogen peroxide, and sodium hypochlorite) were found to inactivate aflatoxins.

II. Foodborne Infections

Some characteristics of foodborne infections:

- Live cells of the enteric pathogens (bacteria and viruses) have to be consumed through food.
- The surviving cells (from gastric environment) penetrate through the membrane and establish in the epithelial cells of the intestines, **multiply**, and produce toxins (infection)
- Dose levels that cause infection vary greatly
- Symptoms generally occur after 24 h,
- Enteric symptoms are local
- Nonenteric symptoms (along with enteric

A. Salmonellosis By *Salmonella*

- *Salmonella* cells are Gram-negative, non-sporulating, facultative anaerobic, motile rods.
- They form gas while growing in media containing glucose.
- ***Salmonella Typhimurium*** and ***Salmonella* Enteritidis** are the causative agents
- Generally, they ferment dulcitol, but not lactose; utilize citrate as a carbon source; produce hydrogen sulfide, decarboxylate lysine, and ornithine; do not produce indole; and are negative for urease.
- They are **mesophilic**, with optimum growth temperature (35-37 ^{o}C), but generally have a growth range of 5 to 46 ^{o}C

- They are killed by pasteurization temperature and time, sensitive to low pH (4.5 or below), and do not multiply at an Aw of 0.94, especially in combination with a pH of 5.5 and below.
- The cells survive in frozen and dried states for a long time.
- They can multiply in many foods without affecting the acceptance qualities
- Salmonellae are natural inhabitants of the gastrointestinal tracts of domesticated and wild animals, birds, and pets (including turtles and frogs), and insects.
- In animals and birds, they can cause **salmonellosis** and then persist in a carrier state.

- Humans can also be carriers following an infection and shed (get rid of) the pathogens through feces for a long time.
- They have also been isolated from soil, water, and sewage contaminated with fecal matters

Toxins

- Following ingestion of Salmonella cells, the pathogens invade mucosa of the small intestine, proliferate in the epithelial cells, and produce a toxin, resulting in an **inflammatory reaction and fluid accumulation in the intestine**.
- ***The ability of the pathogens to invade and damage the cells is attributed to the production of a thermostable cytotoxic factor.***

Disease and Symptoms

- For salmonellosis, an individual generally has to consume 10^{5-6}cells
- **Strains sensitive to gastric acidity** generally need more cells to establish in the intestine and cause the disease;
- Conversely, **acid-resistant strains** may require fewer cells to cause the disease.
- Following ingestion of the pathogen, symptoms appear in 8 to 42 h, generally in 24 to 36 h.
- Symptoms last for 2 to 3 days, but in certain individuals can linger for a long time.
- An individual remains in a **carrier state** for several months following recovery.
- The general symptoms are **abdominal cramps, diarrhea, nausea, vomiting, chills, fever**, and prostration.

Food Association

- These include beef, chicken, turkey, pork, eggs, milk, and products made from them.
- These foods are contaminated directly or indirectly with **fecal matters** from carriers (animals, birds, and humans) and eaten either raw or improperly cooked, or contaminated following adequate heat treatment.
- Cross-contamination at home and at food services are the major sites of contamination of heated foods with *Salmonella.*
- Salmonellae have also been isolated from many foods of plant origin (use of sewage as fertilizer or washing products with polluted water), seafood, and finfish (harvested from polluted water).

Prevention and Control

- **Proper cooking of foods** (minimum to pasteurization temperature and time, such as 71.7 °C for 15 sec or equivalent) and prompt cooling (to 3 to 4 °C or freezing, if not used in 2 h);
- **Preventing cross-contamination** of ready-to-eat food with a raw food through cutting boards, equipment, utensils, and hands;
- Using **proper sanitation and personal hygiene**;
- Making sick people to do not handle a food; and
- **Properly reheating a food refrigerated** for a long time

B. Listeriosis by *Listeria Monocytogenes*

- **Human listeriosis** is considered as an **opportunistic disease ???**
- Individuals with normal health may not develop symptoms or show a very mild enteric form of the disease.
- However, it is highly fatal (30 to 40%) to fetuses, newborns, infants, the elderly, pregnant women, and immunocompromised people, such as those with cancer, renal diseases, heart diseases, and AIDS.
- In addition, its ability to **grow in many foods at refrigerated temperature** helps the organism reach from a low initial level to an infective dose level during storage of refrigerated foods

- The increase in consumption of many types of ready-to-eat foods stored for fairly long periods of time, and consumption of foods without properly reheating , has increased the likelihood for the pathogen to cause the disease.
- Any temperature abuse, even for a short time, can accelerate the growth rate.
- *Lis. monocytogenes* is a Gram-positive, psychrotrophic, facultative anaerobic, nonsporulating, motile, small rod.
- *Lis. monocytogenes* is a psychrotroph and grows between 1 and 44 ^{o}C, with optimum growth at 35 to 37 ^{o}C.

- At 7 to 10 °C, it multiplies relatively rapidly.
- It ferments glucose without producing gas.
- It can grow in many foods and environments.
- The cells are relatively **resistant to freezing, drying, high salt, and pH 5.0 and above.**
- They are **sensitive to pasteurization temperature** (71.7 °C for 15 s or 62.8 °C for 30 min), but when inside the white blood cells, a temperature of 76.4 to 77.8 °C for 15s is required to kill the cells

Habitat

- *Lis. monocytogenes* is isolated from many environmental samples, such as soil, sewage, water, dead vegetation and from the intestinal contents of domesticated animals and birds.
- Humans can also carry the organisms in the intestine without any symptoms.
- A large proportion of uncooked meat, milk, egg, seafoods, and fish, as well as leafy vegetables and tubers (potatoes and radishes, in particular), contains *Lis. monocytogenes.*
- Many heat-processed foods, such as pasteurized milk, stored foods and dairy products, and ready-to-eat meat preparations also contain the organism.

Toxin

- The virulence factor of *Lis. monocytogenes* is a specific type of hemolysin, listeriolysin O.
- It is produced during the exponential growth of the cells.
- The pathogens invade different body tissues and multiply inside the body cells, releasing the toxin.
- The toxin causes **death of the cells**

Disease and Symptoms

- People with normal health, following ingestion of a food contaminated with *Lis. monocytogenes,* may or may not produce symptoms.
- Most often, symptoms appear 1 to 7 d following

- The symptoms among sensitive groups are quite different.
- These groups include pregnant women, unborn fetuses, infants, elderly people with reduced immunity due to diseases, and people taking special medications, such as steroids and chemotherapeutic agents.

- Initial symptoms are enteric, with nausea, vomiting, abdominal cramps, and diarrhea, along with fever and headache.
- The pathogens then spread through the bloodstream and invade tissues in different vital organs, including the **central nervous system.**

- In pregnant women, the pathogen can invade organ tissues of the fetus through the placenta.
- Symptoms include
  - ✓ bacteremia (septicemia)
  - ✓ Meningitis
  - ✓ encephalitis and
  - ✓ endocarditis.
- The fatality rate in fetuses, infected newborn infants, and immuno-compromised individuals is very high.
- The infective dose is considered to be 100 to 1000 cells, particularly for the highly sensitive individuals.

Food Association

- Consumption of contaminated coleslaw, pasteurized milk, raw milk and dairy products, soft cheeses meat pâté, turkey franks, cold cut meats, improperly cooked chicken, and smoked mussels.
- As many raw foods of both animal and plant origin harbor *Lis. monocytogenes,* consumption of raw foods or recontaminated heat-processed foods has caused listeriosis.
- Growth during long refrigerated storage and temperature abuse before eating have been implicated in many cases.

Prevention and Control

- Thoroughly cooking raw foods of animal origin;
- Thoroughly washing raw vegetables before eating;
- Keeping uncooked meats separate from vegetables, cooked foods, and ready-to-eat foods;
- Not consuming raw milk or foods made with raw milk; and
- Washing hands, knives, and cutting boards after handling uncooked foods.

C. Pathogenic *Escherichia Coli*

- *Escherichia coli* was considered a harmless, Gram-negative, motile, non-sporulating, rod-shaped, facultative anaerobic bacterium,
- It is a normal inhabitant of the intestinal tract of humans and warm-blooded animals and birds
- Because it is normally present at a very high level for a long time it has been used as an index organism for possible fecal contamination and presence of enteric pathogens in food and water
- Current evidence indicates that pathogenic strains of *Esc. coli* subdivided into four groups

1. Enteropathogenic *E. coli* (EPEC)

- These strains are important in ***infant diarrhea*** worldwide, especially in places with poor sanitation.
- They are transmitted directly or indirectly through human carriers.
- The pathogenesis is produced by their ability to form a close physical contact with the intestinal epithelial cells and cause lesions.
- One needs to ingest high numbers of cells ($10^{6–9}$) to develop symptoms.
- The predominant symptom is gastroenteritis.

2. Enterotoxigenic *E. coli* (ETEC)

- These strains are the major cause ***of diarrhea in travelers, as well as in infants*** in many developing countries with poor sanitation.
- The pathogens produce an invasive factor and a heat-labile (HL), a heat-stable (HS), or both HL and HS, enterotoxins to cause the disease
- The symptom is gastroenteritis, like a mild form of cholera.
- The pathogen is spread directly or indirectly by human carriers.
- Both food and water have been implicated in outbreaks and sporadic cases in humans.
- Ingestion of large numbers of cells ($10^{8–9}$) is necessary for an individual to develop symptoms.

3. Enteroinvasive *E. coli* (EIEC)

- These strains are known to cause *dysentery* similar to that of shigellosis.
- The ability of the strains to produce an invasive factor is thought to be the cause of the disease.
- Human carriers, directly or indirectly, spread the disease.
- Ingestion of as many as 10^6cells may be necessary for an individual to develop symptoms

4. Enterohemorrhagic *E. coli* (EHEC)

- The strains in this group (a principal serogroup is O157:H7) have been recognized relatively recently as the cause of severe bloody diarrhea (hemorrhagic colitis) and hemorrhagic uremic syndrome (HUS) in humans.
- Animals, particularly dairy cattle, are thought to be the carriers.
- Ingestion of as few as 10 to 100 cells can produce the disease, especially in sensitive individuals.
- Three enterotoxins produced by the serotype cause the disease symptoms.

Gastroenteritis Due to EIEC

- Invade epithelial cells and cause infection in the colon.
- symptoms appear as abdominal cramps, profuse diarrhea, headache, chills, and fever.
- A large number of pathogens are excreted in the feces.
- Symptoms can last for 7 to 12d, but a person can remain a carrier and shed the pathogens in feces for a long time
- Only humans are known to be the host of the pathogen, and a food (cheese and potato salad) can get contaminated directly or indirectly though fecal contamination.

Prevention

- The pathogen is sensitive to pasteurization temperature.
- Thus, proper heat treatment, elimination of post-heat contamination for a ready-to-eat food, and refrigeration of a food soon after preparation are necessary to control the disease.
- In addition, proper sanitation at all stages of food processing and handling is an important factor.
- Individuals suspected of being carriers should not handle food, especially ready-to-eat food

Gastroenteritis due to EHEC

- Like other *Esc. coli,* it grows rapidly at 30 to 42°C, grows poorly at 44 to 45°C, and does not grow at 10°C or below.
- Strains resistant to pH 4.5 or below have been identified.
- The organism is destroyed by pasteurization temperature and time and killed at 64.3°C in 9.6 sec.
- The cells survive well in food at –20°C
- *Esc. coli* O157:H7 produces toxin

- The cells probably colonize in the intestine by adhering to the epithelial cells and produce toxins, which then act on the colon.
- Toxins are also absorbed into the bloodstream and damage the small blood vessels in the intestine, kidneys, and brain
- *Esc. coli O157:H7* causes hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP).
- Symptoms occur 3-9 d after ingestion and generally last for 4d

- The colitis symptoms include a sudden onset of abdominal cramps, watery diarrhea (which in 35 to 75% of cases turns to bloody diarrhea), and vomiting.
- Fever may or may not be an associated symptom.
- Damage to the lining of the large intestine causes bleeding.
- Toxins also cause breakdown of red blood cells, and clotting in small blood vessels of the kidney, causing kidney damage and occasional kidney failure, causing HUS.
- It can be fatal, particularly in children.
- TTP results from a blood clot in the brain, with seizures, coma, and often death

- The pathogen is probably present in the intestine of animals, particularly in dairy cattle, without producing symptoms.
- Proper sanitation, cooking or heating at appropriate temperatures, proper refrigeration, and prevention of cross-contamination should be practiced to control the disease

D. Shigellosis (Bacillary Dysentery) by *Shigella Spp.*

- The genus *Shigella* contains four species*: Shigella dysenteriae, Shi, flexneri, Shi. boydii* and *Shi. Sonnei*
- The organisms are either transmitted directly through **fecal–oral routes** or indirectly through fecal-contaminated food and water.
- It occurs more frequently in places with **poor sanitation.**
- In developing countries, there is a **high fatality rate** among children suffering from shigellosis.
- The cells of the species are Gram-negative, non-motile, facultative anaerobic rods.
- They are generally catalase positive and oxidase and lactose negative.
- They ferment sugars, usually without forming gas

- The strains grow between 7 and 46°C, with an optimum at 37°C.
- They survive for days under different physical and chemical stresses, such as refrigeration, freezing, 5% NaCl, and pH 4.5.
- They are killed by pasteurization.
- The strains can multiply in many types of food when stored at growth temperature range
- The intestine of humans and some primates is the only habitat known
- Following recovery from shigellosis, an individual can remain a carrier for months

Toxins

- Once engulfed by the epithelial cells, they can produce an **exotoxin** that has an enterotoxigenic property.
- The toxin is designated as Shiga toxin (ST).
- The engulfed shigellae cells kill the epithelial cells and then attack fresh cells, causing ulcers and lesions
- The symptoms are the consequence of both invasiveness of epithelial mucosa and the enterotoxin and include **abdominal pain, diarrhea often mixed with blood, mucus and pus, fever, chills, and headache.**
- Generally, children are more susceptible to the disease than adults

- **Direct contamination occurs from poor personal hygiene.**
- **Indirect contamination occurs from the use of fecal-contaminated water to wash foods that are not subsequently heat processed. Also, cross-contamination of ready-to-eat foods**
- In many developed countries, the most frequently involved foods are different types of salads (potato, tuna, shrimp, and chicken), with potato salads ranked at the top.
- Foods that are chopped or cut prior to eating, such as vegetables used in salads
- Shellfish harvested from sewage polluted water and eaten raw

Prevention

- Proper education of the food handlers about the importance of good personal hygiene and the need to not handle food if one suspects having a digestive disorder is important.
- Use of rigid sanitary standards to prevent cross-contamination of ready-to-eat food, use of properly chlorinated water to wash vegetables to be used for salads, and refrigeration of foods are necessary to reduce foodborne shigellosis

E. Campylobacteriosis by *Campylobacter Spp.*

- *Campylobacter* species can cause human gastroenteritis;
- However, ***Campylobacter jejuni* and *Cam. coli*** are considered the most common causative agents of human diarrheal disease
- ***Cam. jejuni*** is a Gram-negative, motile, non-sporulating, rod-shaped bacterium.
- The strains are microaerophilic and catalase and oxidase positive.
- The strains require a microaerophilic environment of 5% oxygen, 8% CO_2, and 87% N_2 for growth. Growth temperature ranges between 32 and 45^{o}C, with optimum 42^{o}C.

- They grow better in **amino acids** than in carbohydrates.
- They generally grow slowly and are not a good competitor while growing with other bacteria.
- They do not generally grow well in many foods.
- They are sensitive to many environmental parameters, including oxygen (in air), NaCl (above 2.5%), low pH (below pH 5.0), temperature (below 30C), heat (pasteurization), and drying.
- However, they survive well under refrigeration and for months in the frozen state

- *Cam. jejuni* is an enteric organism.
- It has been isolated in high frequency from feces of animals and birds.
- Human carriers were also found to shed the organisms in feces.
- Water, sewage, vegetables, and foods of animal origin are easily contaminated with *Cam. jejuni* excreted through feces
- *Cam. jejuni* has a thermolabile enterotoxin that is responsible for enteric disease symptoms.
- In addition, the strains produce an invasive factor that enables the cells to invade and establish in epithelial cells in both the small and large intestines in humans

Disease and Symptoms

- The infective dose for campylobacteriosis is considerably low, only 500 cells.
- Following ingestion, symptoms of the disease occur in 2 to 5d.
- Symptoms generally last for 2 to 3d, but can linger for 2 weeks or more.
- Persons with no visible symptoms can shed the cells in feces for a long time.
- The main symptoms are enteric and include abdominal cramps, profuse diarrhea, nausea, and vomiting.
- Other symptoms include fever, headache, and chills.

Food Association

- The foods can be contaminated directly with fecal material from animals and infected humans or indirectly from sewage and contaminated water.
- *Cam. Jejuni* has been isolated at a very high frequency from raw meats (beef, lamb, pork, chicken, and turkey), milk, eggs, vegetables, mushrooms, and clams.
- In heat-processed food, their presence has been related to cross-contamination following heat treatment or to improper heating.
- The use of animal feces as fertilizers was found to contaminate vegetables.

Prevention

- Proper sanitation can be used to reduce its load in raw foods during production, processing, and future handling.
- Preventing consumption of raw foods of animal origin, heat-treatment of a food, when possible, and preventing post-heat contamination are important to control campylobacteriosis in foods of animal origin.
- Contamination of vegetables can be controlled by not using animal feces as fertilizer and not using contaminated water to wash vegetables (especially ready-to-eat types).
- Contamination from humans can be reduced by establishing good personal hygiene and not allowing sick individuals to handle foods, especially ready-to-eat foods

F. Yersiniosis by *Yersinia Enterocolitica*

- *Yer. enterocolitica* cells are Gram-negative short rods, non-spore forming, motile below 37°C, and facultative anaerobic.
- The strains grow between 0 and 44°C, with an optimum growth at 25 to 29°C.
- Growth occurs in milk and raw meat at 1°C, but at a slower rate.
- Cells can grow in 5% NaCl and at a pH above 4.6.
- Cells are sensitive to pasteurization.
- *Yer. enterocolitica* is a normal inhabitant of intestines of food animals and birds, pets, wild animals, and humans.
- Human carriers do not show any disease symptoms.

Toxins

- Not all strains can produce yersiniosis.
- Most strains isolated from the environment are nonpathogenic.
- Pathogenic strains are predominant in pigs.
- Both **the pathogenic and nonpathogenic strains produce a heat-stable toxin; thus, toxin production is not directly related to the ability of a strain to cause yersiniosis.**
- Pathogenic strains also carry an invasive factor that enables the cells to colonize intestinal epithelial cells and lymph nodes.
- Only after colonization is the heat-stable toxin capable of causing the disease.

Disease and Symptoms

- Young children are more susceptible to foodborne yersiniosis.
- Symptoms are severe abdominal pain at the lower quadrant of the abdomen, diarrhea, nausea, vomiting, and fever.
- Symptoms generally appear 24 to 30 h following consumption of a contaminated food and last 2 to 3 d.
- The disease can be fatal in rare cases
- Foods implicated in yersiniosis include raw and pasteurized milk, ice cream, and improperly cooked meats.
- Good sanitation at all phases of handling and processing and proper heat treatment are important to control the occurrence of yersiniosis.
- Consumption of raw milk or meat cooked at low temperatures should be avoided

Other Foodborne Infections

A. Brucellosis

- Human brucellosis is caused by *Brucella spp.,* namely, *Brucella abortus, Bru. suis, and Bru. melitensis.*
- They are Gram-negative, nonmotile, nonsporeforming, aerobic small rods pathogenic to animals and humans.
- In infected animals, the organisms are located in the **uterus of pregnant animals and in the mammary glands of lactating females.**
- Thus, the pathogens can be excreted in milk.
- People working with animals can become infected with *Brucella spp.*
- *Also, people* working with meat can be infected.
- Consumption of raw milk and some products made from raw milk have been implicated in foodborne brucellosis.

- The cells survive for a long time in milk and milk products.
- Pasteurization of milk and milk products kills Brucella cells.
- Symptoms of brucellosis in humans include undulant fever with irregular rise and fall of temperature, profuse sweats, body aches, aching joints, chills, and weakness.
- Symptoms appear in 3 to 21 d following consumption of a contaminated food.
- Control measures for foodborne brucellosis include pasteurization of milk, manufacturing of dairy products from pasteurized milk, and proper sanitation to prevent recontamination of pasteurized products.

B. Streptococcal Infection

- ✓ *Streptococcus pyogenes* is a pathogen and has been isolated from lactating animals with mastitis.
- ✓ It is a Gram-positive coccus and has been associated with human pharyngitis with symptoms of sore throat, fever, chills, and weakness
- ✓ In some cases, nausea, vomiting, and diarrhea can be present.
- ✓ Some strains can cause scarlet fever.
- ✓ Foodborne infections have been recorded from the consumption of contaminated raw milk and milk products made with raw milk and different types of salads

- Control measures include pasteurization of dairy products and avoiding the consumption of raw milk and products made with raw milk.
- People who are suffering from streptococcal infections or are carriers should not handle ready-to-eat foods such as salads.
- Proper sanitation in all phases of processing and proper refrigeration will help reduce the incidence.

C. Q Fever

- ✓ Q fever in humans is caused by a rickettsia, *Coxiella burnetii.*
- ✓ Animals carry this organism without any symptom.
- ✓ People handling animals, raw milk, and meat can be infected by the rickettsia and develop symptoms of Q fever.
- ✓ Symptoms include fever, malaise, anorexia, muscular pain, and headache.
- ✓ Symptoms appear 2 to 4 weeks after infection and last for ca. 2 weeks.

- ✓ Foodborne infection occurs from the consumption of raw or improperly pasteurized milk and milk products.
- ✓ *Cox. Burnetii* is more resistant to heat than many pathogenic bacteria are.
- ✓ Because of this, the current temperature and time of pasteurization of milk have been set to either 145F (62.8^{o}C) for 30 min or 160F (71.1^{o}C) for 15 sec so that *Cox. burnetii* is killed.

Foodborne Toxicoinfections

Some of the characteristics of foodborne toxicoinfection:

1. For spore-formers, ingestion of large numbers of live vegetative cells is usually necessary.
2. Vegetative cells of spore-formers do not multiply in the digestive tract, but sporulate and release toxins.
3. For Gram-negative bacteria, live cells can be ingested in moderate numbers.
4. Gram-negative cells rapidly multiply in the digestive tract.
5. Many cells also die, releasing toxins.
6. Toxins of both groups produce the gastroenteritis symptoms

A.*Clostridium Perfringens* Gastroenteritis

- The cells are Gram-positive rods, motile, and sporeformers.
- *Clo. perfringens* is anaerobic but can tolerate some air (oxygen).
- The vegetative cells are sensitive to low-heat treatment (pasteurization), but the spores are extremely heat resistant, and some can survive even boiling for several hours.
- In the presence of suitable substrates, H2S is formed during growth.
- The cells need several amino acids for growth.
- Thus, they can grow very effectively in many protein foods

- The temperatures of growth of vegetative cells and germination of spores and outgrowth range between 10 and 52°C.
- The optimum growth occurs at 45°C.
- At optimum conditions, cell multiplication can be very rapid, in 9 min.
- The cells fail to grow well at pH <5.0, in NaCl concentration >5%, at Aw, <0.93, and in 500 ppm nitrite
- Spores and vegetative cells are found in soil; dust; intestinal contents of animals, birds, and humans; and sewage.

- The enterotoxin associated with the foodborne disease is a heat-labile protein.
- It is an intracellular protein produced by the cells during sporulation in the intestine and released.
- Unlike toxins of food poisoning microorganisms, the enterotoxin is produced in the digestive tract.
- The environmental parameters for the production of enterotoxin are directly related to the sporulation environment.
- There are some reports that, in addition to the intestine, sporulation and enterotoxin production to certain levels can also occur in some foods

- The main symptoms are diarrhea and abdominal pain.
- Nausea, vomiting, and fever also can occur but are less common.
- The toxin changes the permeability of intestinal cells.
- Fatality, although rare, can occur among the very young, elderly, and sick.
- Raw meat from animals and birds is most commonly contaminated with the spores and cells from the digestive tract content, whereas vegetables and spices commonly get them from soil and dust.

Prevention

- Proper sanitation in all phases of food preparation and handling.
- Food should be cooked to the highest temperature recommended to kill the cells and as many spores as possible.
- The food should be cooled quickly and uniformly (preferably within 1 h) to refrigerated temperature.
- If a food is stored for a long time, it is important to reheat it quickly and uniformly (to kill vegetative cells) and keep it hot (above 60°C) while being served.

B. *Bacillus Cereus* Gastroenteritis

- The cells are Gram-positive motile rods, which form endospores in the middle of the cells.
- Cells are sensitive to pasteurization.
- Spores can survive high heat treatment used in many cooking procedures.
- *Bac. cereus* is aerobic, but can also grow under some degree of anaerobic environment.
- The cells can multiply in a temperature range of 4 to 50^{o}C, with the optimum ca. 35 to 40^{o}C.
- Other parameters of growth are pH of 4.9 to 9.3, Aw of 0.95 and above, and NaCl concentration below 10%.

- Spores and cells of *Bac. cereus* are common in soil and dust and can be readily isolated in small numbers from many foods, which include both raw and finished products.
- Intestinal tracts of 10% of healthy adult humans have *Bac. cereus* under normal conditions.
- The strains produce at least two types (emetic and enteric) of enterotoxins, each probably associated with specific types of symptoms
- The toxins are produced during growth of cells at the growth temperature range and retained in the cells.
- The toxins released only when the cells are lysed
- They occur in the intestinal tract but can also occur in foods.
- Thus, the cases can also be regarded as food poisoning, as in staphylococcal food poisoning.

Disease and Symptoms

- The enterotoxin associated with the diarrheal form is a heat-labile protein, and that associated with the emetic form is a heat-stable protein
- In the diarrheal form, symptoms occur 6 to 12h following consumption of a food containing the viable cells
- Symptoms include abdominal pain, profuse watery diarrhea, and perhaps nausea, but no vomiting or fever
- Recovery is usually within 24h
- These symptoms in many respects are similar to those produced by *Clo. perfringens*

- **In the emetic form:** the symptoms occur 1 to 5h following ingestion of a food containing the viable cells.
- As the toxin is heat stable, once the toxin forms in cells, heating food containing a large number of cells before eating can produce the symptoms.
- Symptoms are nausea and vomiting; abdominal pain and diarrhea may also be present.
- Symptoms last for ca. 24 h.
- In some respects, the symptoms are similar to those of staphylococcal gastroenteritis.

- In the diarrheal outbreaks, a variety of foods, including vegetables, salads, meats, pudding, casseroles, sauces, and soups, has been implicated, mostly because of their improper cooling.
- **However, in the emetic form, outbreaks mostly involve rice and sometimes other starchy foods.**
- The predominating contributing factors associated with *Bac. cereus* gastroenteritis, in order of relative importance, are improper holding temperature, contaminated equipment, inadequate cooking, and poor personal hygiene.
- The heat treatment normally used in food preparation, except for pressure cooking, may not destroy *Bac. cereus* spores.

Prevention

- Keep food at a temperature at which the spores do not germinate and cells do not grow (4 to 5 ^{o}C or holding the food above 60^{o}C)
- Food should not be stored at low temperatures for long periods of time
- Because cells can get in a food through cross-contamination, proper sanitary measures should be adopted while handling a food
- There should be uniform reheating of a suspected food to above 75^{o}C before serving

C. Cholera

- Cholera, caused by *Vib. Cholerae* is a non-contagious disease but can cause large epidemics with high mortality.
- *Vib. Cholerae* is a Gram-negative motile, curved rod.
- Improper heating (at lower temperature for a shorter time) may not be able to kill all the cells present in a food.
- The optimum temperature of growth is between 30 and 35^{o}C.
- The growth rate is very rapid, even at room temperature.
- The cells do not multiply in contaminated live crabs,

Habitat

- Cholera is a human disease.
- The disease results from the ingestion of infective doses of *Vib. cholerae* cells through food and water contaminated with feces of humans suffering from the disease.
- **Marine environments may serve as long-term reservoirs.**
- Sea foods (crab, oysters, and finfish) harvested from these areas can carry contamination and provide the infective dose.
- Contaminated water can also be the source of the disease.

- **It produces cytotoxic protein causing massive secretion of water along with chloride, potassium, and bicarbonate in the lining of the intestine.**
- Following ingestion of *Vib. cholerae* cells in sufficient numbers, the cells colonize the small intestine and multiply rapidly and produce toxins.
- When the cells die and lyse, the toxins are released into the intestine.

Disease and Symptoms

- A person must consume a large number of viable cells through contaminated food or water to contract the disease
- Fecal–oral infection is also possible
- The infective dose for cholera is 10^6 viable cells per person, but it varies with age and health
- The incubation period ranges from 1 to 5 d, but is usually 2d
- The symptoms include the sudden onset of profuse watery diarrhea and vomiting.
- Loss of fluid results in dehydration.
- Other symptoms in severe cases are painful muscle

Food Association

- Food can serve as a source of *Vib. cholerae* if it is contaminated directly with human feces from the patient or previously contaminated water
- The handling of food by a person suffering from the disease can also contaminate food, because of poor personal hygiene.
- In addition, food originating from natural reservoirs of the causative bacteria can be contaminated and spread cholera.
- The natural reservoirs include marine and brackish water environments
- Consumption of raw seafood is associated with the disease

Prevention

- The disease can be prevented or reduced by adopting proper hygienic measures. These include;
- Provision for properly treated municipal water, decontamination of other water by boiling or chemical treatment, and proper disposal of sewage.
- Unexposed people can be vaccinated to protect them from the disease.
- Seafood should not be harvested from polluted water nor from water found to harbor *Vib. cholerae.*
- Finally, seafoods should not be eaten raw.
- The time- temperature of heat treatment of a suspected food should be enough to kill the pathogen.

Algal Toxins

- **Finfish and shellfish** feeding on several species of toxic algae can accumulate toxins
- Consumption of these fish and shellfish can produce disease symptoms

A. Ciguatera Poisoning

- **Finfish,** especially those feeding on reef and mud, while feeding on the **toxic algae *Gambierdiscus toxicus*** and several others, accumulate toxin in the muscle tissues
- Liver, intestines, and roe accumulate more toxin than muscle tissues.
- Consumption of these fish results in disease, with gastrointestinal and neurological symptoms developing within a few hours

- The gastrointestinal symptoms are nausea, vomiting, cramps, and diarrhea, and last for a short time.
- Neurological symptoms include tingling and numbness of lips and tongue, dryness of mouth, chills, sweating, blurred vision, and paralysis.
- The disease can be fatal under severe conditions.
- Incidence of the disease can be reduced by avoiding consumption of large fish from the reef, particularly their inner organs

B. Shellfish Poisoning

1. Paralytic Shellfish Poisoning

- **Scallops, clams, and mussels** feeding on ***Gonyaulax catenella*** and related toxic species of algae accumulate heat-stable toxin in the tissues
- Consumption of these shellfish produces paralytic poisoning
- The common neurological symptoms include tingling and numbness of lips and fingertips, drowsiness, poor coordination, incoherent speech, and dryness of the throat
- In extreme cases, respiratory failure and death can result.
- The symptoms appear within 1h and subside within a few days

- Occurrence of paralytic shellfish poisoning is sporadic and depends on the growth (bloom) of the toxic algae, as influenced by water temperature (8^{o}C), salinity, run-off, presence of nutrients, and others
- Shellfish growing in shallow water tend to accumulate more toxins

2. Neurotoxic Shellfish Poisoning

- Growth of the toxic algae ***Ptychodiscus brevis*** produces red tide, and feeding of the algae by shellfish causes them to accumulate toxin in the muscle
- Consumption of these shellfish produces neurological symptoms as described for paralytic shellfish poisoning, but less severe
- The symptoms appear very quickly and generally subside in a few hours

3. Diarrhetic Shellfish Poisoning

- They are Gastrointestinal disorders from the consumption of scallops, mussels, and clams that feed on the toxic **algae *Dinophysis fortii***
- The duration of the symptoms is short.
- The toxin is heat-stable, and thus cooking does not eliminate it.
- The incidence of the disease can be reduced by removing the digestive organs of the shellfish, because they tend to accumulate the majority of the toxins

Parasites

- ✓ Included in this group are several intestinal and tissue helminths (roundworms(nematod), flatworms(trematode), and tapeworms(cestode)) and protozoa that are known to cause human illness and where a food association has either been confirmed or suspected.
- ✓ They can stay in the GI tract and produce gastrointestinal symptoms.
- ✓ Some invade body tissues and produce specific problems.

A. Trichinosis by *Trichinella spiralis*

- ***Trichinella spiralis* is a roundworm** and can be present in high frequencies in **pigs feeding on garbage**.
- A person gets infected by consuming raw or insufficiently cooked meat of infected animals.
- The infected meat contains encysted larvae.
- Once the meat is consumed, the cysts dissolve in the GI tract, releasing the larvae, which then infect the GI tract epithelium.
- Here, the parasites mate and deposit larvae in the lymphatic system.
- Through lymphatic circulation, the larvae infect other body tissues.

- ✓ The symptoms of trichinosis appear in 2 to 28d following ingestion.
- ✓ Initial symptoms are nausea, vomiting, and diarrhea, followed by fever, swelling of eyes, muscular pain, and respiratory difficulties.
- ✓ In extreme cases of infection, toxemia (blood poisoning from by toxins) and death may result.
- ✓ The prevention methods consist of not raising pigs on garbage and cooking meat thoroughly [140F (60^{o}C) and above].
- ✓ Freezing meat for 20d at −30°C (home

B. Anisakiasis by *Anisakis simplex*

- ✓ The **nematode *Anisakiasis simplex*** is a parasite in many fish, particularly of marine origin.
- ✓ Human infection results from the consumption of raw infected fish.
- ✓ Inadequately cooked, brined, or smoked fish has also been implicated in some cases.
- ✓ Symptoms appear after a few days and generally include irritation of throat and digestive tract.
- ✓ Proper cooking, salting, or freezing at –20^{o}C for 3d can be used as a preventative measure

C. Taeniasis by *Taenia spp.*

- ✓ Taeniasis is a tapeworm disease caused by *Taenia solium* from pork and by *Tae. saginata* from beef.
- ✓ The illness results from the ingestion of raw or improperly cooked meat contaminated with larvae of the tapeworms.
- ✓ Symptoms appear within a few weeks and are digestive disorders.
- ✓ If an organ is infected by the larvae, especially a vital organ, the consequences can be severe

D. Toxoplasmosis by *Toxoplasma gondii*

- Toxoplasmosis is a tissue protozoan disease transmitted to humans from the consumption of undercooked and raw meat and raw milk contaminated with oocysts of *Toxoplasma gondii.*
- Contamination of food occurs with feces, especially from cats
- In individuals with low resistance, the symptoms are generally flu-like, with fever and headache.
- It can cause fatal deformation and be life-threatening in immunocompromised people.
- Preventative measures include cooking meat to a minimal internal temperature of 70^{o}C or freezing at –20^{o}C

E. Giardiasis by *Giardia lamblia*

- ✓ Giardiasis is produced by the intestinal protozan *Giardia lamblia.*
- ✓ *Giardiasis* has been considered as food- and waterborne disease
- ✓ Contaminated raw vegetables; foods such as salads and sandwiches, contaminated with water containing the causative agent; and poor personal hygiene are considered the major causes of the disease.
- ✓ The main symptoms are acute or chronic diarrhea and abdominal pain.
- ✓ The oocysts and cysts are excreted in the feces of the affected individuals
- ✓ Improved sanitary conditions and personal hygiene are important to reduce the incidence

F. Cryptosporidiosis by *Cryptosporidium parvum*

- ***Cryptosporidium parvum*** is an intestinal protozoan and causes cryptosporidiosis in humans through the consumption of contaminated food and water with the oocysts.
- It can also be present in the GI tract of domesticated and wild animals and contaminate food and water.
- The major symptoms of infection in humans are watery diarrhea, fever, muscle aches, dehydration, and, in some debilitated individuals, such as AIDS patients, death.
- The oocysts are resistant to disinfectants normally used to treat water

G. Cyclosporiasis by *Cyclospora cayetanensis*

- ***Cyclospora cayetanensis*** is a coccidian intestinal protozoan and is considered a newly emerging foodborne pathogen
- Humans and some primates are the only natural hosts.
- Consumption of food and water contaminated with the oocysts and subsequent infection results in the disease.
- The main symptoms are watery diarrhea, cramps, nausea, vomiting, fever, and fatigue.
- It comes from the consumption of contaminated raspberries, lettuce, and basil.

Chapter

Beneficial Uses of Microorganisms in Food

- The **major concern** of microbial presence in food is due to its undesirable properties
- Most are able to spoil foods, and several are associated with foodborne health hazards
- However, there are other microorganisms that have beneficial properties in food production, maintaining normal health of the gastrointestinal tract of humans and controlling the undesirable spoilage and pathogenic bacteria in food
- Beneficial microorganisms are used in foods in several ways
- These include actively growing microbial cells, nongrowing microbial cells, and metabolic by-products and cellular components of microbes

Microbiology of Fermented Foods Production

- **Food fermentation** involves a process in which raw materials are converted to fermented foods by the growth and metabolic activities of the desirable microbes.
- The microbes utilize some components present in the raw materials as substrates to generate energy and cellular components, to increase in population, and to produce many usable by-products that are excreted in the environment.
- **The unused components of the raw materials and the microbial by-products together constitute fermented foods.**
- The raw materials can be milk, meat, fish, vegetables, fruits, cereal grains, seeds, and beans, fermented individually or in combination.

- Microbial species, when used in **controlled fermentation**, are also referred to as **starter cultures.**
- Many of these microbial species are present in raw materials that are naturally fermented, along with other associated microorganisms, some of which may contribute to the desirable characteristics of the products.

General Method of Production

- **Fermentation** involves exposing the raw or starting food materials to conditions that favor growth and metabolism of specific and desirable microorganisms.
- As the desirable microorganisms grow, they utilize some nutrients and produce some end products.
- These end products, along with the un-metabolized components of the starting materials, constitute the fermented foods having desirable acceptance qualities, many of which are attributed to the metabolic end products.

A. Raw (or Starting) Materials

- A large number of raw materials from plant and animal sources are used to produce fermented foods.
- These include milk, meat, fish, eggs, vegetables and vegetable juices, many fruits and fruit juices, cereal grains, tubers, lentils, beans, and seeds. Some are used in combination.

B. Microorganisms Used

- Many desirable species and strains of bacteria, yeasts, and molds are associated with fermentation of foods.
- Depending on a product, fermentation may be achieved by a single predominating species and strain.
- However, in most fermentations, a mixed population of several bacterial species and strains, or even bacteria and yeasts or bacteria and molds, is involved.

- **When a fermentation process involves a mixed population, the members should not be antagonistic toward one another; rather, they should preferably be synergistic.**
- Maximum growth of a desirable microorganism and optimum fermentation rate are dependent on environmental parameters such as nutrients, temperature of incubation and pH
- In the fermentation process, if the different species in a mixed population need different environmental conditions (e.g., temperature of growth), a compromise is made to facilitate growth of all the species at a moderate rate

- Depending on a raw or starting material and a specific need, carbohydrates (dextrose in meat fermentation), salts, citrate, and other nutrients are supplemented
- In some **natural fermentations**, several species may be involved for the final desirable characteristics of the product
- However, instead of growing at the same time, they appear in sequence, with the consequence that a particular species predominates at a certain stage during fermentation

C. Fermentation Process

- Foods can be fermented in three different ways, based on the sources of the desirable microorganisms:

1. Natural Fermentation

- A product produced by natural fermentation can have some desirable aroma resulting from the metabolism of the associated flora.
- However, because the natural microbial flora in the raw materials may not always be the same, it is difficult to produce a product with consistent characteristics over a long period of time
- Also, chances of product failure because of growth of undesirable flora and foodborne diseases by the pathogens are high

2. Back Slopping

- In this method, some products from a successful fermentation are added to the starting materials, and conditions are set to facilitate the growth of the microorganisms coming from the previous product.
- Retention of product characteristics over a long period may be difficult because of changes in microbial types.
- Chances of product failure and foodborne diseases are also high

3. Controlled Fermentation

- The starting materials (may be heat treated) are inoculated with a high population (10^6 cells/ml or more) of a pure culture of single or mixed strains or species of microbes (starter culture).
- Incubation conditions are set for the optimum growth of the starter cultures.
- Large volumes of products can be produced with consistent and predictable characteristics each day.
- Generally, there is less chance of product failure and foodborne diseases.
- However, there may be no growth of desirable secondary flora.
- As a result, a product may not have some delicate flavor characteristics.

Fermented Dairy Products

- Fermented dairy products can be broadly divided into two groups: fermented milk products and cheeses.
- In fermented milk products, all the constituents of the milk are retained in the final products, with the exception of those partially metabolized by the bacteria.
- In cheeses, a large portion of milk constituents is removed in whey to obtain the final products.

Milk

- The growth of desirable microorganisms can be adversely affected by several components that are either naturally present or have entered in the milk as contaminants.
- The natural antimicrobials are agglutinins and the lactoperoxidase–isothiocynate system.
- The agglutinins can induce clumping of starter culture cells and slow their growth and metabolism.
- The lactoperoxidase–isothiocynate system can inhibit starter cultures.
- Antimicrobials can cause problems only when raw milk is used, because both are destroyed by heating milk.

- Milk can also contain antibiotics, either used in the feed or used to treat animals for some infections, such as mastitis.
- Their presence can also affect the growth of starter cultures.
- Some milk can contain heat-stable proteases and lipases produced by some psychrotrophic bacteria, such as *Pseudomonas* species, during refrigerated storage of raw milk before pasteurization
- These enzymes remain stable after heating and can cause product defects (low yield of cheese, proteolysis, rancidity).
- Before milk is used for fermentation, these aspects need to be considered.

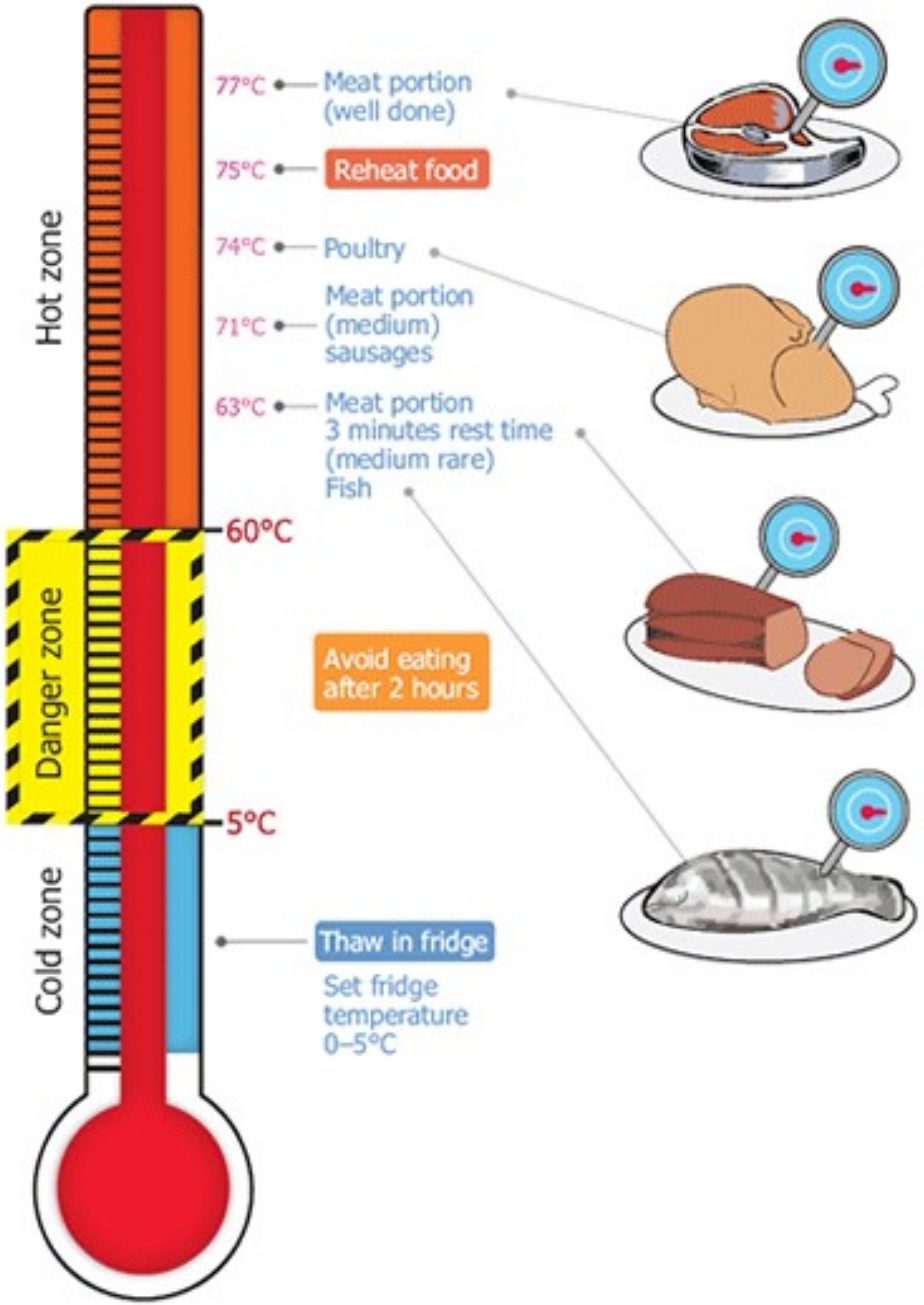
Microbiology of Cultured Buttermilk Fermentation

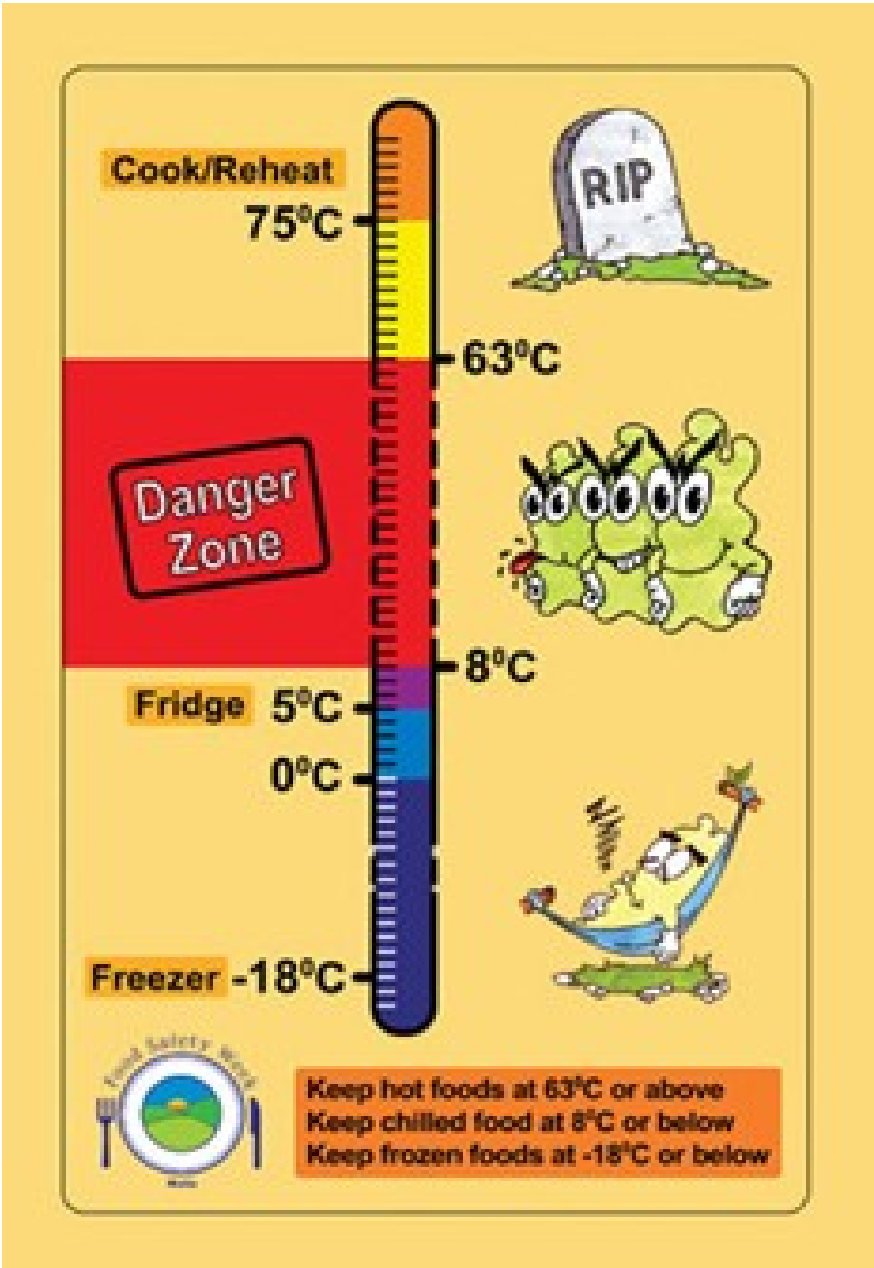
- It is produced from partially skim milk through controlled fermentation with starter cultures.

Product Characteristics

- It should have a pleasant acid taste (from lactic acid) and high degree of aroma (from diacetyl), with slight effervescence (from CO2).
- It should have white color with smooth, thick body and pour easily.

Temperature factor most concern for microbiology of food safetv







Thank you