CHAPTER 4:

DEMONSTRATE KNOWLEDGE OF FOOD MICROBIOLOGY TECHNIQUES

4.1 Introduction of the Unit of Learning / Unit of Competency

This unit specifies the competencies required to apply microbiological techniques. It involves demonstrating the knowledge of microorganisms in foods and food environments, physiology, genetics, biochemistry and behaviour of microorganisms, microbiology of food fermentation, microbiological aspects of food safety, methods of detection, identification and enumeration of food microorganisms.

4.2 Performance Standard

By the end of this unit of learning/competency, the trainee should demonstrate ability to carry out microbiological tests and analysis as per workplace guidelines; process different ferment food products based on resource materials; apply microbiological aspects of food safety based on ISO standards; and, identify and control microorganisms associated with food as per resource materials

4.3 Learning Outcomes (Elements in the OS)

4.3.1 List of the Learning Outcomes

i) Demonstrate the knowledge of microorganisms in food and food environment

ii) Demonstrate the knowledge of physiology, genetics, biochemistry and behaviour of food microorganisms

iii) Demonstrate the knowledge on microbiology of food fermentation

iv) Demonstrate the knowledge of microbiological aspects of food safety

v) Demonstrate the knowledge on methods of detection, identification and enumeration of food microorganism

4.3.2 Learning Outcome 1: Demonstrate the knowledge of microorganisms in food and food environment

4.3.2.1 Learning Activities

Learning activity	Special instructions
i) Identify and describe the terminologies in food microbiology • Define toxins & toxicants • Describe incubation period	
ii) Identify and describe basic types of food microorganism	Illustrate the shape of various microorganisms using very clear diagrams
iii) Identify and describe roles of microorganisms in food safety and spoilage	Consider both the beneficial and destructive roles of the microorganisms
iv) Apply the use of microscope	Mount and identify basic types of microorganisms on a microscope; o Bacteria o Virus o Yeast o Mold o Fungi o Protozoa

4.3.2.2 Information Sheet

Definitions

Incubation period is the period between exposure to an infection and the appearance of the first symptom

Toxins are natural products such as the ones found in poisonous mushrooms, or in a snakes' venom.

Toxicants are man-made products, artificial products introduced into the environment due to human activity; examples are industrial waste products and pesticides

Food safety: overall quality of food fit for consumption

Food spoilage: defined as damage or injury to food rendering in unsuitable for human consumption.

Microbiology: The study of microorganisms, which are microscopic, unicellular, and cell-cluster organisms

Food microbiology: is the study of the microorganisms that inhabit, create, or contaminate food. Of major importance is the study of microorganisms causing food spoilage

Basic types of food microorganisms

Bacteria

- Are single-celled prokaryotic organisms
- They have a rapid reproduction
- Are the major cause of foodborne diseases
- Are of many different types and there many ways of identifying them.

Protozoans

- Single celled eukaryotic organisms – larger than bacteria and are found in soil and water
- They cause diseases such as amoebic dysentery
- Fungi
- An eukaryotic organism with rigid cell walls
- Grow mainly as single-celled and reproduce by budding
- Examples include Yeast and molds
- They cause superficial infections such as athlete's foot, ringworm and thrush.

Parasites

- Organism that lives on or in another and uses that organism to provide nourishment
- Infections caused by parasites are called infestations. They include worms and parasites
- Viruses
- Smallest known infectious agents
- Cannot be seen by regular microscope
- Consist of only nucleic acid surrounded by a protein coat
- Viruses such as Norwalk virus are known to cause foodborne illnesses.

Roles of microorganisms in food safety and spoilage

Food borne infections are caused by the entrance of pathogenic microorganisms contaminating food into the body, and the reaction of the body tissues to their presence.

These can either be fungal, bacterial, viral or parasitic food borne infections tend to have long incubation periods and are usually characterized by fever.

Bacterial food borne infections include Cholera, salmonellosis, typhoid fever, shigellosis, Yersiniosis, Escherichia coli infection campylobacteriosis, Vibrio parahemolyticus and listeriosis

Mycotic food borne infections include Candida species, Sporothrix spp., and Wangiella species.

Viral food borne infections include hepatitis A, Norwak virus and poliomyelitis virus

Bacterial foodborne outbreaks occur in different forms;

a). Sporadic cases involving only one or two persons in a household

b). Family outbreaks in which several members of the family are affected

c). Large outbreaks caused by a widely distributed infective food item

d). Institutional outbreaks which may be caused by a contaminated single food item.

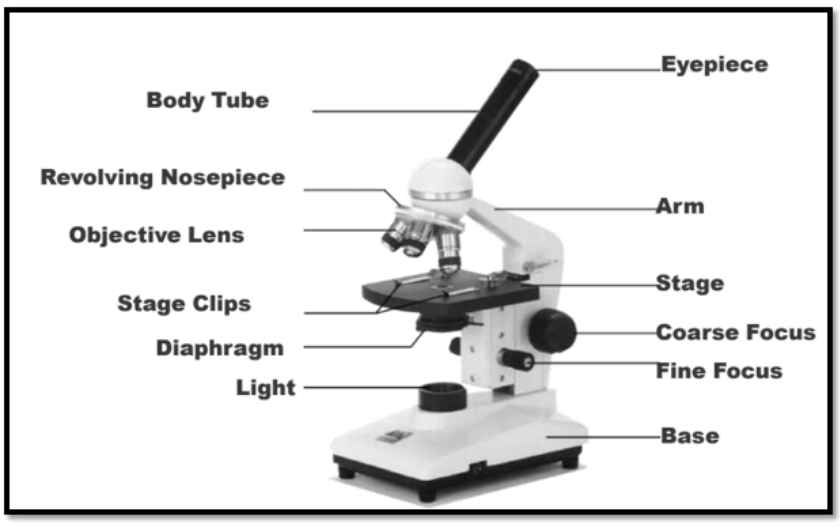
Viruses are common pathogens transmitted through food. Hepatitis A and Norwalk-like virus (Novovirus) are the most important viral food borne pathogens. These viruses are highly infectious and may lead to widespread outbreaks. Only a few viral particles are necessary for the disease to develop. High numbers of viral particles are further transmitted via feces of infected persons (up to 1011 particles per gram of feces. Specific lining cells are necessary for virus replication. Accordingly they cannot multiply in foods or water. Food borne virus are relatively stable and acid resistant outside host cells

Fungal intoxications are caused by consumption of metabolites produced by fungi, when growing in food. These metabolites are called mycotoxins. Grains, oilseeds, fruits and vegetables are mostly involved if they are stored at high humidity (≥ 0.75) or if they are not properly dried before storage. Poor dry storage practices of grains and other foods leads to mould growth and production of mycotoxins. Of significance to public health is aflatoxicosis.

Microscopy (structure, use, care and maintenance)

A microscope is an instrument that magnifies objects otherwise too small to be seen, producing an image in which the object appears larger. Most photographs of cells are taken using a microscope, and these pictures can also be called micrographs.

Parts of a microscope



Magnification

The microscope has three magnifications;

a. Scanning

b. Low

c. High

The ocular lens (eye piece) has a magnification hence total magnification is the ocular magnification multiplied by objective magnification as shown below.

	Magnification	Ocular lens	Total magnification
Scanning	4x	10x	40x
Low power	10x	10x	100x
High power	40x	10x	400x

General procedure for microscope use

- Make sure all backpacks and materials are out of the aisles and off the tops of desks.
- Plug your microscope into the outlet.
- Store with cord wrapped around microscope and the scanning objective clicked into place.
- Carry by the base and arm with both hands.

Focusing specimens

1. Always start with the scanning objective: Use the Coarse Knob to focus and then the fine adjustment knob until clear, image may be small at this magnification, but you won't be able to find it on the higher powers without this first step.
2. Once you have focused on scanning, switch to low power- use the Coarse Adjustment Knob to refocus. Then use the Fine Adjustment Knob to make the image crystal clear. Again, if you haven't focused on this level, you will not be able to move to the next level.
3. Now switch to High Power- (If you have a thick slide, or a slide without a cover, do NOT use the high power objective). At this point, ONLY use the Fine Adjustment Knob to focus specimens.

Drawing specimen

1. Use pencil - you can erase and shade areas

2. All drawings should include clear and proper labels (and be large enough to view details). Drawings should be labeled with the specimen name and magnification.

3. Labels should be written on the outside of the circle. The circle indicates the viewing field as seen through the eyepiece, specimens should be drawn to scale - ie.if your specimen takes up the whole viewing field, make sure your drawing reflects that.

Units for measurements of microorganisms include:

Micrometers - µm

Nanometers – nm

1mm = 1000µm

1µm = 1000nm

Simple microscopes have single magnifying lens (like a magnifying glass).Compound microscopes have two sets of lenses for magnification. Lens closer to the eye is called ocular lens (magnifying power of 10x) while the lenses closer to the object being viewed is called objective lens. (Most light microscopes used in biology have three or four objective lenses).

The Bright Field microscope

This is the commonest type and most used microscope.

Bright field light microscopes produce a dark image against brighter, backlit background.

They provide a 2-D image. It is commonly used to view stained cells.

The ocular lens magnifies the specimen 10x. You will always be looking through the ocular and objective lens simultaneously, so multiply ocular magnification x objective power to calculate the Total Magnification (xTM).

Rotary nosepiece of your microscope has four objective lenses attached. Shortest lens (red band) should have been pointing down when your scopes were last put away.

The quality of your image depends on the Numerical Aperture (NA) and resolution.

NA relates to the light gathering properties of the optical components of the microscope, whereas resolution is the ability to distinguish details within your specimen.

Using an immersion lens and oil can improve both your resolution and NA

Electron microscopes

Use an electron beam instead of light, which is focused using electromagnets.

The specimen has to be specially prepared and held inside a vacuum chamber from which the air has been pumped out (because electrons do not travel very far in air).

The image is formed as a photograph (called an electron micrograph) or as an image on a TV screen.

Are of two types

i. Scanning electron microscope (SEM)

ii. Transmission electron microscope (TEM)

Many microscopic images in textbook, journals and publications are obtained using electron microscopes. Electron beam wavelengths are shorter than light wavelengths, so better resolving power. Electrons go through very thin slice of specimen and detailed image is viewed on a screen.Beam of electrons across a whole specimen (sprayed with fine metal coating). Three dimensional views of surface features on a screen

Phase-Contrast microscope

This is used to study the behavior of living cells, observe the nuclear and cytoplasmic changes taking place during mitosis and the effect of different chemicals inside the living cells. One of the major advantages of phase contrast microscopy is that living cells can be examined in their natural state without previously being killed, fixed, and stained(usually kills cells) Offers more contrast than bright field microscopy. It is especially useful for examining living, unpigmented cells

Fluorescence Microscope

A fluorescence microscope is a light microscope used to study properties of organic or inorganic substances using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption. In most cases, a component of interest in the specimen is specifically labeled with a fluorescent molecule called a fluorophore such as Green fluorescent protein, fluorescein

The specimen is illuminated with light of a specific wavelength or wavelengths which is absorbed by the fluorophore, causing them to emit longer wavelengths of light of a different color than the absorbed light.

Dark Field Microscopy

Type of microscopy which is the exact opposite of a bright field microscope

Dark background/field with the specimen being the only one illuminated.

Used in observing unstained specimens. Most microscopes have the potential to do dark field microscopy such as compound or stereomicroscopes.

Microscopy techniques

Wet Mounts

Smears

Staining

Care and maintenance of microscopes

i. Handle with care

Most microscope problems occur as a result of improper handling. When carrying your microscope, hold it by the base and the metal support arm. Do not pick it up by the stage, as this can cause misalignment. When transporting it, use a microscope bag.

ii. Keep lenses clear of slides

When using your microscope and adjusting the focus you will need to lower the objective lens down as far as it will go. However, you should never allow the lens to touch the slide you are looking at. Dirty lenses can be difficult to clean.

iii. Clean after using immersion oil

If using immersion oil, always ensure the objectives are cleaned immediately after use. Objective, eyepieces and condenser may be removed for cleaning. Use only lens paper and lens cleaner. Do not use solvents.

iv. Cover when not in use

All microscopes are sold with dust covers. Always keep your microscope covered when not in use even if the microscope is stored in a cabinet. Eye tubes also need to be kept free of dust so do not store a microscope without the eyepieces. If the microscope eyepieces must be removed, cover the tubes with caps or a plastic bag with a rubber band around the eye tube.

v. Look after the bulb

After using the microscope, turn off the illuminator and wait for it to cool for several minutes before putting it away. By allowing the bulb to cool you will extend its life. When turning the microscope on and off, use the switch not the power point. Do not switch the microscope on while using full light intensity. Never touch the bulb with your fingers as the body oils can burn into the bulb and reduce its life. Use a tissue. Keep a store of replacement bulbs and always use the correct bulb.

vi. Store in a clean, dry place

Make sure you do not store your microscope in an area that has corrosive chemical fumes that can destroy lenses or metal parts or beside solutions that may leak. Salt air and pervasive damp can also cause damage over time. Make sure your cabinet is ventilated.

4.3.2.3. Self-assessment

1. Define the following terms as used in food microbiology
   A. Incubation period
   B. Microbiology
   C. Food spoilage
2. _____ are eukaryotic cells with rigid cell walls and they grow mainly as single-celled and reproduce by budding
   A. Protozoa
   B. Fungi
   C. Viruses
   D. Bacteria

3. The microscope which is used to study the behaviour of living cells, observe the nuclear and cytoplasmic changes taking place during mitosis is called___
   A. Phase-contrast microscope
   B. Electron microscope
   C. Fluorescence microscope
   D. Dark field microscopy
4. The metabolites of fungal intoxications are known as_____
   A. Aflatoxins
   B. Mycotoxins
   C. Toxins
   D. Aflatoxicosis
5. Describe the basic types of food microorganisms
6. Discuss the roles of microorganisms in food safety and spoilage
7. Discuss the different types of microscopes
8. Explain the care and maintenance of microscopes

4.3.2.4 Tools, Equipment, Supplies and Materials

Laboratories | Microscopes | Glass slides | Dye | Reagents | Specimen

Stationery

4.3.2.5 References

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4.3.3 Learning Outcome 2: Demonstrate the knowledge of physiology, genetics, biochemistry and behavior of food microorganisms

4.3.3.1 Learning Activities

Learning activity	Special instructions
Demonstrate understanding of physiology, genetics and biochemistry of microorganisms	Describe physiology, genetics an biochemistry of microorganisms
Describe bacterial anatomy	Illustrate the anatomical structure of a bacterial cells
Identify and describe factors that influence growth and activity of food microorganism	Consider how the food can be manipulated to eliminate factors that support growth of microorganisms
Describe the growth pattern of a typical bacterial colony	Identify the activities that may hinder the growth of a bacterial colony
Demonstrate and describe the gram stain method and AFB test	Carry out gram staining on various bacterial cells Mount the cells on a light microscope and Identify the cells Draw your observation and label the cells and AFB tests for various

4.3.3.2 Information Sheet

Introduction

Definitions

- Pathogens - are disease causing microorganisms (bacteria, viruses, parasite and fungi)
- Bacteria - single celled living microorganisms responsible for the decay of many plant and animal diseases.
- Virus - The smallest of the microbial food contaminants, viruses rely on a living host to reproduce.
- Parasite - An organism that needs a living host to survive.
- Fungi - can be single celled or multi cellular microorganisms can that can cause food spoilage and lives by absorbing nutrients from organic matter
- pH - – potential of Hydrogen. A measure of the acidity or alkalinity of a solution, numerically equal to 7 for neutral solutions, with increasing alkalinity and decreasing with increasing acidity. The pH scale commonly in use ranges from 0 to 14.
- Spore - The spore is formed by some bacteria, thickens walls to protect from adverse condition such as extreme acidity and temperature.

- Vegetative Stage - is a condition favorable for bacteria to grow and multiply rapidly.
- Budding Reproduction – a form of asexual reproduction where in new bud or bump is formed from the mother cell.
- Water Activity – The amount of moisture available in food for microorganisms to grow.

Characteristics of predominant microorganisms in food

Based on the organization of their cellular structures, all living cells can be divided into two groups: eukaryotic and prokaryotic

Eukaryotic cell types include animals, plants, fungi, protozoans, and algae

Prokaryotic cell types include bacteria & blue green algae

Bacteria

- Bacteria cells are prokaryotic
- Bacteria consist of only a single cell (unicellular)
- Bacteria reproduce through "binary fission" when one cell divides to form two new cells
- All bacteria exist in a vegetative stage
- Some bacteria has the ability to form a spore where they can survive in an adverse or extreme conditions "spore forming bacteria"
- Bacteria are "photosynthetic", they have the ability to make their own food through the use of the sunlight, thus bacteria also gives off oxygen.
    - An average bacterium measures 1 micrometer

Bacterial Shapes and Arrangement

Bacteria are majorly classified on the basis of;

i. Shape – coccus, bacillus, spirillum, vibrio

ii. Ability to retain certain dyes

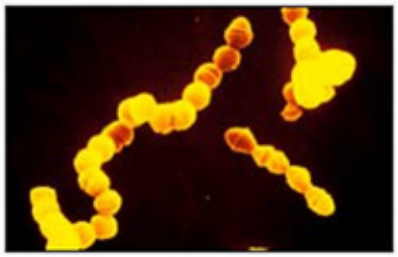
iii. Ability to grow in presence or absence of air

iv. Biochemical reactions

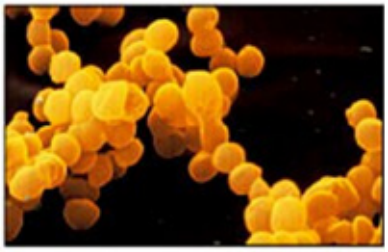
Shapes of bacteria

Coccus are spherical/round shaped

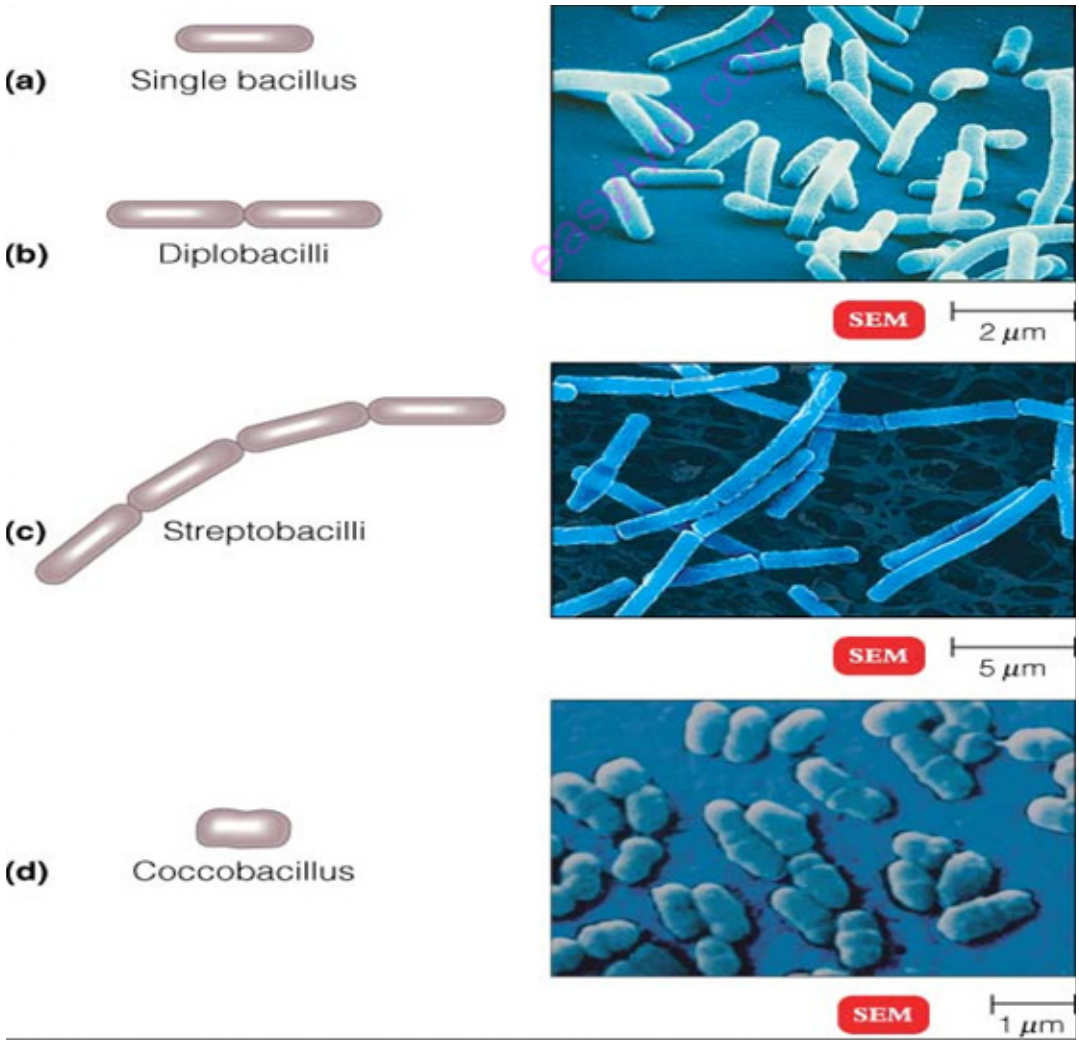
If they appear as a chain they are referred to as Streptococcus



If they appear in a Cluster form, they are referred to as Staphylococcus



- Bacillus – are rod shaped bacteria



- If a in a chain form, they are referred to as Streptobacillus
- Cocco bacillus is intermediate form of Coccus and bacillus
- Vibrio are curved
- Spirillums have a helical rigid shape
- Spirochetes have a helical flexible shape
- Actinomycetes are bacteria that have branching filamentous bacteria
- Mycoplasmas are bacteria that lack cell wall

BACTERIA CELL CYTOLOGY

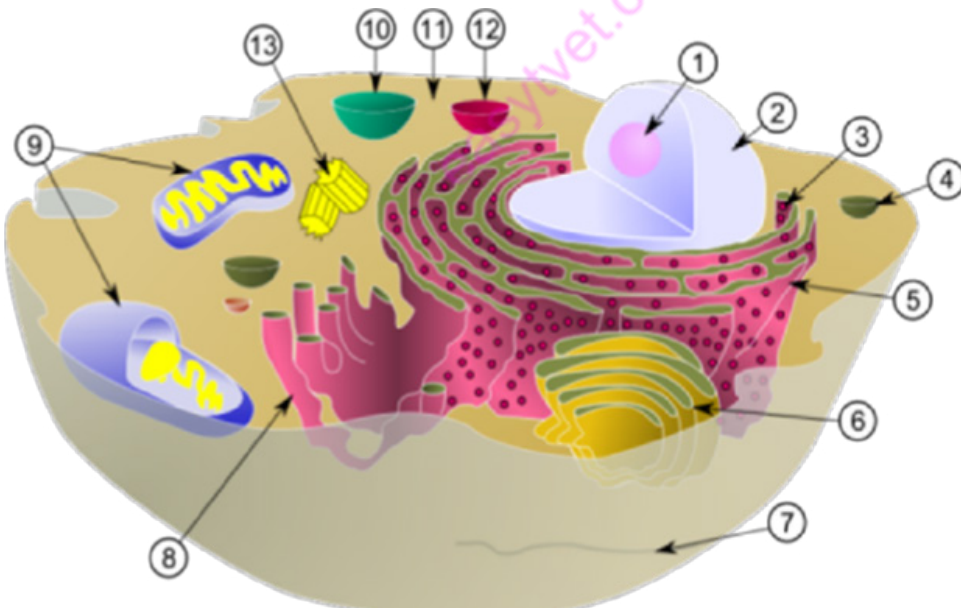
Prokaryotic Cells

Much smaller (microns) and more simple than eukaryotes

Prokaryotes are molecules surrounded by a membrane and cell wall.

They lack a true nucleus and don't have membrane bound organelles like mitochondria, etc.

Large surface-to-volume ratio: nutrients can easily and rapidly reach any part of the cells interior.



Schematic of typical animal (eukaryotic) cell, showing subcellular components

Organelles:

(1) Nucleolus (2) Nucleus

(3) Ribosome (4) Vesicle

(5) Rough endoplasmic reticulum (ER) (6) Golgi apparatus

(7) Cytoskeleton
(8) Smooth ER
(9) Mitochondria
(10) Vacuole
(11) Cytoplasm
(12) Lysosome
(13) Centrioles

Size of Bacteria

Unit of measurement in bacteriology is the micron (micrometer, µm)

Bacteria of medical importance range form 0.2 – 1.5 µm in diameter and 3 – 5 µm in length

CELL WALL

Outermost layer, encloses cytoplasm

- Confers shape and rigidity
- 10 - 25 nm thick
- Composed of complex polysaccharides (peptidoglycan/ mucopeptide) - formed by N acetyl glucosamine (NAG) & N acetyl muramic acid (NAM) alternating in chains, held by peptide chains.
- It carries bacterial antigens – important in virulence & immunity
- Chemical nature of the cell wall helps to divide bacteria into two broad groups – Gram positive & Gram negative

Gram negative: contain outer membrane which is composed of lipopolysaccharides (due to many enzymes, antibiotics, salts, etc

Gram positive: have thick wall composed of several layers of mucopeptide and teicnoic

- Gram +ve bacteria have simpler chemical nature than Gram –ve bacteria.
- Several antibiotics may interfere with cell wall synthesis e.g. Penicillin, Cephalosporin

There two types of cells walls Gram positive cell wall and Gram negative cell wall

CYTOPLASMIC ORGANELLES

Cytoplasmic (Plasma) membrane

- Thin layer 5-10 nm, separates cell wall from cytoplasm
- Acts as a semipermeable membrane: controls the inflow and outflow of metabolites
- Composed of lipoproteins with small amounts of carbohydrates

Cytoplasmic Components

Ribosomes; site for protein synthesis

Mesosomes – Multilaminated structures formed as invaginations of plamisc membrane

- Principal sites of respiratory enzymes
- Coordinate nuclear & cytoplasmic division during binary fission
- More prominent in Gram +ve bacteria

Intracytoplasmic inclusions – reserve of energy & phosphate for cell metabolism e.g. metachromatic granules in diphtheria bacilli

Nucleus

Has no nucleolus and does also not have a nuclear membrane

Its' genome is single, circular double stranded DNA.

It is a haploid and divides by binary fission

Additional Organelles

- Plasmid

Extranuclear genetic elements consisting of DNA. They are transmitted to daughter cells during binary fission. May be transferred from one bacterium to another but are not essential for life of the cell. They confer certain properties e.g. drug resistance, toxicity

- Capsule & Slime layer

Is a viscous layer secreted around the cell wall.

It is polysaccharide / polypeptide in nature, sharply defined structure, antigenic and Protects bacteria from lytic enzymes, it inhibits phagocytosis and is stained by negative staining using India ink.

Slime layer is a loose undemarcated secretion.

- Flagella

Long (3 to 12 μm), filamentous surface appendages

They are organs of locomotion.

Chemically, composed of proteins called flagellins

The number and distribution of flagella on the bacterial surface are characteristic for a given species - hence are useful in identifying and classifying bacteria

Flagella may serve as antigenic determinants (e.g. the H antigens of Gram-negative enteric bacteria)

Presence shown by motility e.g. hanging drop preparation.

Types of flagella arrangement

Polar/ Monotrichous – single flagellum at one pole

Lophotrichous – tuft of flagella at one pole

Amphitrichous – flagella at both poles

Peritrichous – flagella all over

Amphilophotrichous – tuft of flagella at both ends

Additional Organelles

Fimbriae/ Pili

Thin, hair like appendages on the surface of many Gram-negative bacteria 10-20µ long, acts as organs of adhesion (attachment) - allowing bacteria to colonize environmental surfaces or cells and resist flushing.

Made up of proteins called pilins.

Pili can be of two types

i. Common pili; short & abundant

ii. Sex pili; small number (one to six), very long pili, helps in conjugation (process of transfer of DNA)

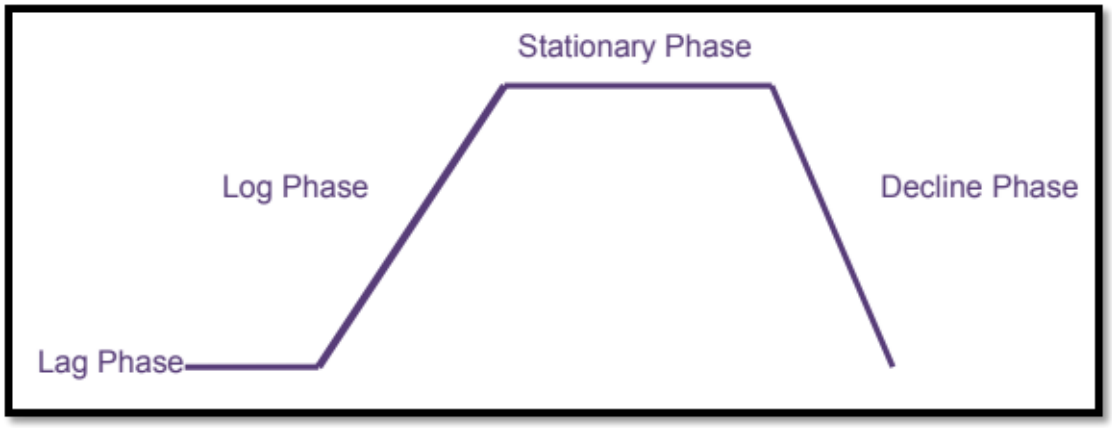
Additional Organelles

Highly resistant resting stages formed during adverse environment (depletion of nutrients).

They are formed inside the parent cell, hence called endospores. They are very resistant to heat, radiation and drying and can remain dormant for hundreds of years. Are formed by bacteria like Clostridia and bacillus

Phases of growth of bacteria

1. Lag Phase –bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide.
2. Log Phase or Logarithmic Phase –"exponential phase" growth is very rapid, doubling in numbers in every few minutes
3. Stationary Phase - the growth rate slows as a result of nutrient depletion and accumulation of toxic products. This phase is reached as the bacteria begin to exhaust t.he resources that are available to them.
4. Death or Decline Phase - bacteria run out of nutrients and die



Conditions bacteria needs to grow and multiply

1. Food
2. Acidity
3. Temperature
4. Time
5. Oxygen
6. Moisture

Food

Bacteria feed on Protein and Carbohydrates. Foods that contain these items can support the growth of microorganisms.

Potentially Hazardous Foods have the potential for contamination, they have the characteristics to allow microorganisms to grow and multiply.

How to Control the Growth of Bacteria in Food

1. Purchase from reputable suppliers
2. Avoid cross-contamination of food
3. Cook food to safe internal temperature and test with food thermometer

Acidity:

- Bacteria grows best at a slightly acidic and slightly neutral environment (pH 4.6 to 7.5)
- Some bacteria can develop a "spore" such as acidophilic bacteria, where it could grow and multiply in an acidic environment
- Bacteria such as E-Coli can grow in unpasteurized apple that has a pH value of 4.0

Growth of bacteria in different pH

	Total magnification
Below 4.6	Bacteria will not grow
Between 4.6 to 7.0	Bacteria will thrive
Between 7.0 to 9.0	Bacteria may survive

How to Control Acidity to Control the Growth of Bacteria:

1. Highly acidic foods such as vinegar and lemon inhibit the growth of microorganism.
2. Salad dressing made with vinegar, oil and garlic can make as a marinade for meat

Time

- Under ideal conditions, bacterial cells can double in number every 25 minutes to 30 minutes.
- Pathogens starts to multiply in four hours at the Temp. Danger Zone

How to Control Time to Control the Growth of Bacteria

1. Store received foods as quickly as possible to limit the time in Temp. Danger Zone
2. If the foods will not be cooked or served right away, store it inside the refrigerator or freezer
3. Check temperature on holding cabinets, make sure that it maintains the internal of 135ºF and above
4. Document food inside the storage room, practice First In First Out
5. Reheat foods at the internal temperature of 165ºF for 15 seconds

Temperature

- Temperature danger zone: temperature range 5°C to 60°C
- Food borne bacteria grow and reproduce.
- Temperature Abuse –foods that have not been to a safe temperature or kept at the proper temperature
- Psychrophilic bacteria – grow within the temperature range of 32ºF(0ºC) – 70ºF (21ºC) (spoilage organisms)
- Mesophilic bacteria – grow at temp. 70ºF(21ºC) – 110ºF(43ºC)
- Thermophilic bacteria – grows best above 110ºF (43º C)

How to control temperature to Control the Growth of Microorganism

1. Cold foods, must be stored at 5°C and below
2. Hot foods, must be held at 140°C (60°C) and above
3. Control the temperature of food during storing, preparing, cooking, holding, reheating and serving.
4. Check internal temperature regularly
5. Cook food at a required internal temperature with a food thermometer
6. Keep food out of Temperature danger zone

Oxygen

Bacteria differ in their oxygen requirement.

Anaerobic bacteria – cannot survive when oxygen is present because it is toxic to them.

Anaerobic bacteria grow well in vacuum packaged foods or canned foods where oxygen is not available.

Aerobic bacteria – need oxygen to grow

Facultative anaerobic bacteria – can grow with or without free oxygen but have a preference

Microaerophilic organisms – can survive in a very little amount oxygen

How to Control Oxygen to Control the Growth of Microorganism

1. Bacteria grow in different oxygen requirement, it is difficult to control this condition.
2. Bacteria such as Clostridium Botulinum and Clostridium Perfringens live without

The presence of oxygen, it is important to cool foods in a shallow pan.

Moisture

Moisture is important factor in bacterial growth. The amount of water available for bacterial activity.

- Water Activity level – is the measure of the amount of water that is not available for bacterial to grow. (0- 10)
- Potentially hazardous foods (PHF) – foods that have a water activity level of .85 or higher.

How to Control Moisture to Control the Growth of Microorganism

1. Lower the amount of moisture in food through freezing, dehydrating, adding sugar or salt.

The growth pattern of a typical bacterial colony.

Bacteria growing in batch culture produce a growth curve with up to four distinct phases.

Batch cultures are grown in tubes or flasks and are closed systems where no fresh nutrients are added or waste products removed.

Lag phase occurs when bacteria are adjusting to the medium. For example, with a nutritionally poor medium, several anabolic pathways need to be turned on, resulting in a lag before active growth begins

In log or exponential phase, the cells are growing as fast as they can, limited only by growth conditions and genetic potential. During this phase, almost all cells are alive, they are most nearly identical, and they are most affected by outside influences like disinfectants.

Due to nutrient depletion and/or accumulation of toxic end products, replication stops and cells enter a stationary phase where there is no net change in cell number.

Death phase occurs when cells can no longer maintain viability and numbers decrease as a proportion.

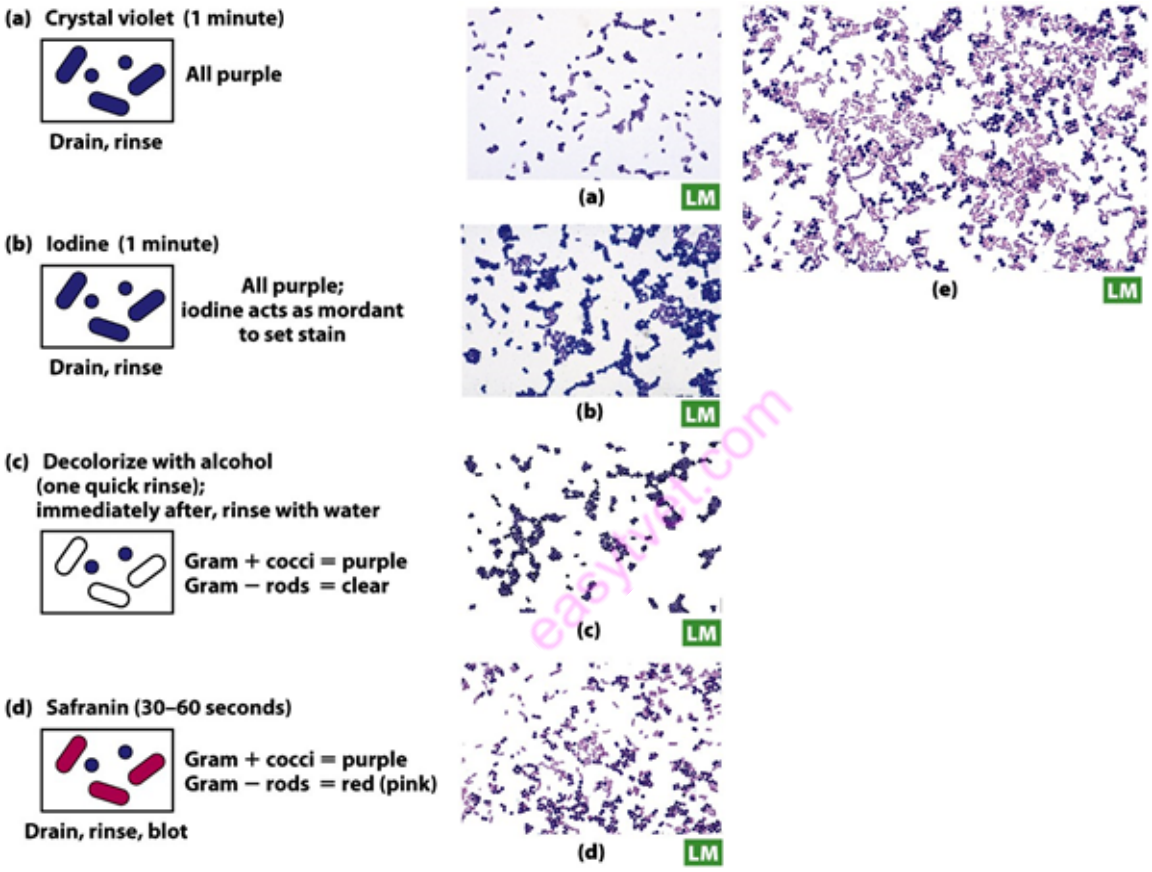
The gram stain method

i. Stain with crystal violet (1 minute). Drain and rinse with water

ii. Flood with iodine(mordant) for one minute

iii. Decolourise with alcohol(1 minute) and rinse with water immediately after

iv. Counterstain with safranin for about 30-60 seconds



AFB test

The Ziehl-Neelsen stain, also known as the acid-fast stain, was first described by two German doctors; Franz Ziehl (1859 to 1926), a bacteriologist and Friedrich Neelsen (1854 to 1894), a pathologist. It is a special bacteriological stain used to identify acid-fast organisms, mainly Mycobacteria. Mycobacterium tuberculosis is the most important of this group, as it is responsible for the disease called tuberculosis (TB). It is helpful in diagnosing Mycobacterium tuberculosis since its lipid rich cell wall makes it resistant to Gram stain. It can also be used to stain few other bacteria like Nocardia. The reagents used are Ziehl-Neelsen include carbolfuchsin, acid alcohol and methylene blue.

i. The sputum is spread evenly centrally over a slide about 20mm by 10mm in size.

ii. The slides are placed on a drier with smeared surface facing upwards, and air dried for about 30 minutes

iii. The smear is then fixed dried by heating

iv. The smear is then covered with carbol fuchsin

v. The smear is heated until there is vapor formation at about 60 degrees Celsius taking care not to overheat; avoiding boiling. And allow the stain to be on the side for about 5 minutes

vi. Clean water is then used to wash off the stain

vii. Cover the smear with 3% v/v acid alcohol for 2-5 minutes (or 20% sulfuric acid) or until the smear is sufficiently decolorized, i.e. pale pink.

Note: Check to see that no more red color runs off the surface when the slide is tipped. Add a bit more decoloriser for very thick slides or those that continue to "bleed" red dye

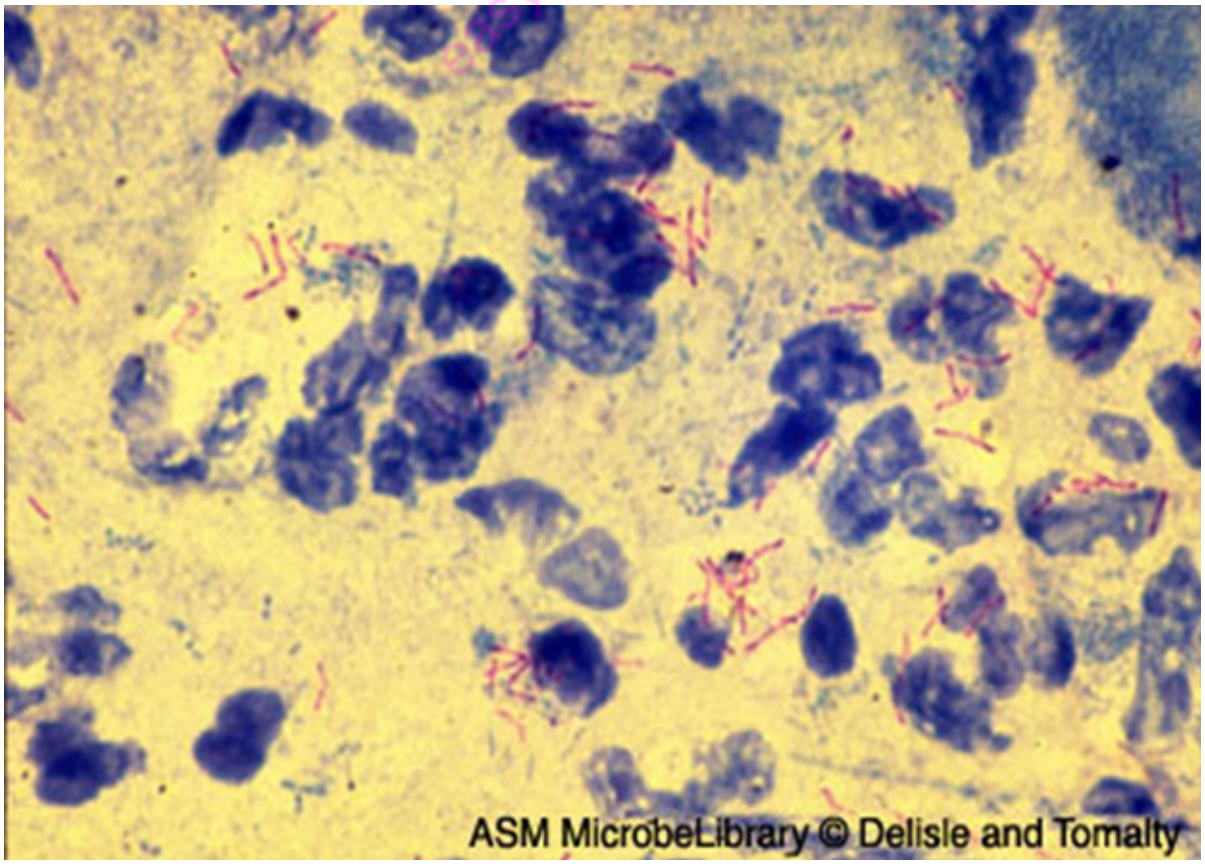
viii. The slide is washed with water

ix. The stain is covered with malachite green for 1 -2 minutes

x. Wash off stain with clean water

xi. Wipe the back of the slide clean, and place it in a draining rack for smear to air dry (DO NOT BOLT DRY).

xii. Examine the smear microscopically, using the 100x oil immersion objective (10X eye piece for a total of 1000X magnification) and scan the smear systematically.



Fungi

- Fungi are a group of organisms and micro-organisms that are classified within their own kingdom, the fungal kingdom, as they are neither plant nor animal.
- Fungi draw their nutrition from decaying organic matter, living plants and even animals.
- Many play an important role in the natural cycle as decomposers and return nutrients to the soil, they are not all destructive.
- Fungi usually reproduce without sex. Single-celled yeasts reproduce asexually by budding.

Examples of Fungi are:

1) Mold

- Eukaryotic cells
- Multicellular
- Non motile, filamentous and branched
- Composed of large numbers of filaments called hyphae which are aggregated and called mycelium.
- Reproduction occurs from spore formation.
- Mold cause spoilage in food and could cause illnesses
- They grow under almost any conditions, but grow well in sweet, acidic food with low water activity.
- Freezing temperatures prevent or reduce the growth of molds, but not destroyed
- Some molds produce called “aflatoxins”
- Example: penicllium spp.

2) Yeasts

- Eukaryotic cells
- Unicellular
- Round, oval (spherical) or elongated
- Non motile, can see budding formation
- Remain in yeast form at both room temperature (25°C) and body temperature (37°C)
- Reproduction is by asexual process called budding
- Yeast also cause food spoilage
- Yeast spoilage produce a smell or taste of alcohol. They appear in pink color discoloration
- They also grown well in sweet, acidic foods with low water activity level. Such as jellies, honey and fruit juices.

Example: Sacchromyces cerevisiae

Molds

Molds are multicellular filamentous fungi whose growth on foods usually is readily recognized by its fuzzy or cottony appearance. The main part of growth commonly appears white but may be colored, dark or smoky.

They are microscopic, plant-like organisms, composed of long filaments called hyphae. Mould hyphae grow over the surface and inside nearly all substances of plant or animal origin. Because of their filamentous construction and consistent lack of chlorophyll they are considered by most biologists to be separate from the plant kingdom and members of the kingdom of fungi. They are related to the familiar mushrooms and toadstools, differing only in not having their filaments united into large fruiting structures. For our purposes here, we shall consider as molds only fungi that are commonly encountered n the home and laboratory and that can be easily grown and studied.

When mold hyphae are numerous enough to be seen by the naked eye they form a cottony mass called a mycelium. It is the hyphae and resulting mycelia that invade things in our homes and cause them to decay.

Reproduction in fungi is complex and involves a great diversity of structures. At the most fundamental level we can say that most molds reproduce by spores. Spores are like seeds; they germinate to produce a new mold colony when they land in a suitable place. Unlike seeds, they are very simple in structure and never contain an embryo or any sort of preformed offspring. Spores are produced in a variety of ways and occur in a bewildering array of shapes and sizes. In spite of this diversity, spores are quite constant in shape, size, colour and form for any given mould, and are thus very useful for mould identification.

The most basic difference between spores lies in their method of initiation, which can be either sexual or asexual. Sexually initiated spores result from a mating between two different organisms or hyphae, whereas asexual spores result from a simple internal division or external modification of an individual hypha. The recognition of a mating and subsequent spore formation is often difficult for an observer, and is usually reserved for patient specialists. However, for practical purposes one can learn to recognize certain indications of the sexual process, namely, the four kinds of sexually determined spores that appear in mold fungi:

a. Oospores

b. zygospores

c. ascospores, and

d. basidiospores.

Viruses

- Microbes are single-celled organisms that can perform the basic functions of life — metabolism, reproduction, and adaptation.
- Viruses can't metabolize nutrients, produce and excrete wastes, move around on their own, or even reproduce unless they are inside another organism's cells.
- Viruses are the simplest and tiniest of microbes; they can be as much as 10,000 times smaller than bacteria.

- Viruses comes in many sizes and shapes
- Viruses consist of a small collection of genetic material (DNA or RNA) encased in a protective protein coat called a capsid.
- Some may survive in freezing and cooking
- They aren't even cells (non cellular entities).
- Most important are bacteriophages (bacterial viruses)

Parasite

- A parasite is an organism that lives by feeding upon another organism. Parasites living in the human body feed on our cells, our energy, our blood, the food we eat and even the supplements we take.
- There are several types of parasites: protozoa are single celled organisms that are only visible under a microscope, while worms come in all sizes from threadworms that measure less than one centimetre to tapeworms that grow up to 12 meters in length.
- They grow naturally in many animals such as pigs, cats and rodents
- They can be killed by proper cooking or freezing

How one gets parasites

- Contaminated or unfiltered water
- Contaminated soil
- Contaminated fruits and vegetables
- Raw or rare meat
- Pets Mosquitoes Contact with faeces
- Contact with someone with parasites

Factors that influence growth and activity of food microorganisms

Oxygen

Microorganisms have a range of oxygen requirement and on this basis can be grouped on the need for oxygen to grow.

Facultative anaerobic bacteria can grow in high oxygen or low oxygen content and are among the more versatile bacteria

In contrast, strictly anaerobic bacteria grow only in conditions where there is minimal or no oxygen present in the environment. Bacteria such as bacteroides found in the large bowel are examples of anaerobes.

Strict aerobes only grow in the presence of significant quantities of oxygen. Pseudomonas aeruginosa, an opportunistic pathogen, is an example of a strict aerobe.

Microaerophilic bacteria grow under conditions of reduced oxygen and sometimes also require increased levels of carbon dioxide. Neisseria species (e.g., the cause of gonorrhoea) are examples of microaerophilic bacteria.

Water Activity

Bacteria need water to dissolve the food they use for energy and growth. Water allows the food to get into the cells, is used for the many chemical reactions necessary for life and growth, and allows waste products to escape.

Food/Nutrients -- All bacteria require energy to live and grow. Energy sources such as sugars, starch, protein, fats and other compounds provide the nutrients.

Temperature:

Bacteria in general are capable of growing over a wide range of temperatures and are usually classified according to the temperature at which they grow.

Psychotropic bacteria are those that are capable of growing at 32°F - 45°F but their optimum is from 68°F to 86°F. They cause spoilage in foods stored under refrigeration.

Several pathogenic bacteria are psychotropic -- Yersinia and Listeria.

Mesospheric bacteria are bacteria are capable of growing at 60°F - 110°F and belong in this group. Most pathogenic bacteria grow at these temperatures.

Thermophilic bacteria grow at higher temperatures such as 110°F - 150°F.

Temperature is the most widely used method of controlling bacterial growth. Bacteria grow slowly at temperatures below 45°F and thermal destruction occurs at temperatures above 140°F. But in the temperature danger zone between 40°F and 140°F many bacteria are not controlled.

pH

pH is a measure of acid or alkali in a product. It is indicated on a scale from 0 to 14, with seven being neutral. If the pH value is below 7, the food is classified as acid; if it is above 7, the food is classified as alkaline. Most bacteria grow well at neutral pH, but many can reproduce in a pH range from 4.5 - 10.0.

4.3.3.3 Self-Assessment

1. The following are types of flagella arrangements except

   A. Filamentous
   B. Lophotrichous
   C. Amphitrichous
   D. Peritrichous

2. Spirillums _____

   A. Are curved in shape
   B. Have helical rigid shape
   C. Lack a cell wall
   D. Have branching filamentous

3. AFB test is used in diagnosis of

   A. Clostridium Botulinum
   B. Shigellosis
   C. Mycobacterium Tuberculi
   D. Listeriosis

4. Differentiate between a eukaryotic cell and a prokaryotic cell
5. Explain how the bacterial cells are classified
6. Distinguish between cocci and rod bacterials
7. Differentiate between gram positive and gram negative bacterias
8. Highlight the factors that influence growth and activity of microorganisms
9. Identify the basis of bacterial cells classification
10. Illustrate the structure of bacterial cell using a well labelled diagram
11. Explain how AFB test is performed and how positive results are presented
12. Discuss fungi as one of the microorganisms of interest to food

4.3.3.4 Tools, Equipment, Supplies and Materials

- Microscopes | Laboratory reagents | Stationery | Laboratory equipment | Laboratory

4.3.3.5 References

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4.3.4 Learning Outcome 3: Demonstrate the knowledge on microbiology of food fermentation

4.3.4.1 Activities

Learning activity	Special instructions
Identify and describe Terminologies in food fermentation and its importance are	Define various terms used in food fermentation • Fermentation
Identify and describe microorganisms in fermentation process	Identify microorganisms that cause fermentation Describe the role these microorganisms play in fermentation
Fermentation processes in different types of food are identified and described	Demonstrate ability to describe the fermentation process • Types of fermentation Identify the products of fermentation • Dairy products • Meat and fishery products • Non beverage plant products • Beverages and related products • Breads Process fermented foods

4.3.4.2 Information Sheet

Meaning of terms in food fermentation

Fermentation: a process in which chemical changes are brought about in an organic substrate through the action of enzymes elaborated by microorganisms

Starter culture: a preparation of living microorganisms, which are deliberately used to assist the beginning of fermentation, producing specific changes in the chemical composition and the sensorial properties of the substrate to obtain a more homogeneous product.

Probiotics: refers to the consumption of products that contain live organisms that are or are believed to be beneficial to the consumer.

Microorganisms in fermentation process

Fermentation involves any partial breakdown of carbohydrates taking place in the absence of oxygen. It is a metabolic process that converts sugar to acids, gases or alcohol. Biochemically, fermentation is the metabolic process in which carbohydrates and related compounds are partially oxidized with the release of energy in the absence of any external electron acceptors.

Fermenting organisms are dependent on intrinsic and extrinsic parameters of growth.

Types of fermentation

- Top fermentation: refers to the use of a yeast strain that carries out its activity at the upper parts of a large vat, such as in the production of ale
- Bottom fermentation: a type of fermentation that requires the use of a yeast strain that will act in lower parts of the vat, such as in the production of lager beer.

Importance of fermentation

a) Extended shelf life

b) Improved aroma and flavor characteristics

c) Increased vitamin content

d) Increased digestibility

e) Reduced toxicity from some foods e.g. fermentation of cassava to make gari

• Fermentation processes in different types of food; dairy products, grains, meats, fruits and vegetable and beverages

Products of fermentation

1. Dairy products
2. Meat and fishery products
3. Non beverage plant products
4. Beverages and related products
5. Breads
7. Dairy products

Milk Biota

The microorganisms in raw cow's milk consist of those that may be present on the cow's udder and hide and on milking utensils or lines.

Under proper handling and storage conditions, the predominant biota is gram positive.

Raw milk held at refrigerator temperatures for several days invariably shows the presence of several or all bacteria of the following genera: Enterococcus, Lactococcus, Streptococcus, Leuconostoc, Lactobacillus, Microbacterium, Oerskovia, Propionibacterium, Micrococcus, Proteus, Pseudomonas, Bacillus, and Listeria.

Studies have revealed the presence of psychrotrophic spore formers and mycobacteria in raw milk. Campylobacteriosis and salmonellosis are well established as illnesses that may be contracted from milk and milk products. Listeriosis and hemorrhagic colitis outbreaks have

also been traced to milk. Questions have been raised over the efficacy of milk pasteurization to destroy Mycobacterium paratuberculosis. The concern has to do with the fact that this bacterium causes Johne's disease in cattle, and appears to play a role in Crohn's disease of humans.

The spoilage of pasteurized milk products has two common origins;

1. First is the growth and metabolic activity of psychrotrophic organisms such as Pseudomonas, Alcaligenes, and Fiavobacterium spp. These gram-negative rods, which are usually lipolytic and proteolytic, are postpasteurization contaminants. The proteolytic organisms are able to cause a destabilization of the casein micelles and cause a "sweet-curdling" of the milk. However, the predominant spoilage is manifest by bitter and fruity off-flavors.
2. Second is the growth of heat-resistant organisms that are able to ferment lactose to lactic acid, and when the pH is reduced to about 4.6, the milk curdles. If mold spores are present, they may germinate and grow at the surface of the sour milk and elevate pH toward neutrality, thus allowing the more proteolytic bacteria such as Pseudomonas spp. to grow and bring about the liquefaction of the milk curd.

In extended-shelflife milk products (ultrahigh-temperature-pasteurized, UHT; spoilage by psychrotrophic spore formers is a significant problem. Organisms such as Bacillus cereus can survive the UHT process, and because of the longer shelf life, can initiate growth and produce toxins as well as causing "sweet-curdling" of the products.

Starter culture

- A lactic starter is a basic starter culture with widespread use in the dairy industry. For cheese making of all kinds, butter, cultured buttermilk, cottage cheese, and cultured sour cream
- Lactic starters always include bacteria that convert lactose to lactic acid, usually *L. lactis subsp. lactis, L. lactis subsp. cremoris*, or *L. lactis subsp. lactis biovar diacetylactis*.
- Where flavor and aroma compounds such as diacetyl are desired, the lactic starter will include a heterolactic such as *Leuconostoc mesenteroides subsp. cremoris, L. lactis subsp. lactis biovar diacetylactis, or Leuconostoc mesenteroides subsp. Dextranicum*
- Butter, buttermilk, and sour cream are produced generally by inoculating pasteurized cream or milk with a lactic starter culture and holding until the desired amount of acidity is attained.

1. Butter

- Milk cream is inoculated, the acidified cream is then churned to yield butter, which is washed, salted, and packaged.
- Butter undergoes fungal spoilage rather commonly by species of *Cladosporium, Alternaria, Aspergillus, Mucor, Rhizopus, Penicillium,* and *Geotrichum*, especially *G. candidum*

2. Buttermilk

- As the name suggests, it is the milk that remains after cream is churned for the production of butter.
- The commercial product is usually prepared by inoculating skim milk with a lactic or buttermilk starter culture and holding until souring occurs.
- The resulting curd is broken up into fine particles by agitation, and this product is termed cultured buttermilk.

3. Sour cream

- Cultured sour cream is produced generally by fermenting pasteurized and homogenized light cream with a lactic starter.
- These products owe their tart flavor to lactic acid and their buttery aroma and taste to diacetyl.

4. Yogurt (yoghurt)

- Produced with a yogurt starter, which is a mixed culture of *S. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* in a 1:1 ratio.
- The coccus grows faster than the rod and is primarily responsible for initial acid production at a higher rate than that produced by either when growing alone, and more acetaldehyde (the chief volatile flavor component of yogurt) is produced by *L. delbrueckii* subsp. bulgaricus when growing in association with S. thermophiles.
- The coccus can produce about 0.5% lactic acid and the rod about 0.6-0.8% (pH of 4.2-4.5
- The product is prepared either by reducing the water content of either whole or skim milk by at least one fourth (may be done in a vacuum pan following sterilization of milk), or by adding about 5% milk solids followed by water reduction (condensing).
- The concentrated milk is then heated to 82°-93°C for 30-60 minutes and cooled to around 45°C.46
- The yogurt starter is now added at a level of around 2% by volume and incubated at 450C for 3-5 hours followed by cooling to 5°C.
- The titratable acidity of a good finished product is around 0.85-0.90%, and to get this amount of acidity the fermenting product should be removed from 450C when the titratable acidity is around 0.65-0.70%.10. Good yogurt keeps well at 5°C for 1-2 weeks.

Process of cheese making

Step 1

- Milk is prepared and inoculated with an appropriate lactic starter.
- The starter produces lactic acid, which, with added rennin, gives rise to curd formation.
- The starter for cheese production may differ depending on the amount of heat applied to the curds.
- *S. thermophilus* is employed for acid production in cooked curds because it is more heat tolerant than either of the other more commonly used lactic starters; or a combination of *S. thermophilusand L. lactis subsp. lactis* is employed for curds that receive an intermediate cook.

Step 2

The curd is shrunk and pressed, followed by salting, and, in the case of ripened cheeses, allowed to ripen under conditions appropriate to the cheese in question.

Examples of fermented dairy products

Food products	Raw ingredients	Fermenting organism
Acidophilus milk	Milk	*Lactobacillus acidophilus*
Bulgarian buttermilk		*L delbrueckii subsp.bulgaricus*
Cheeses (ripened)	Milk curd	Lactic starters
Kefir	Milk	*Lactococcus lactis, Ldelbrueckii subsp. bulgaricus, "Torula" spp.*
Kumiss	Raw Marles milk	*Lactobacillus leichmannii,L. delbrueckii subsp. bulgaricus, "Torula" spp.*
Yogurt	Milk, milk solids	*L. delbrueckii subsp.bulgaricus, S.thermophilus*

Health benefits of fermented milks

a) Benefits to lactose-intolerant individuals

b) They lower serum cholesterol, and

c) Possess an anticancer activity

d) Probiotics

The objective on priobiotics is the ingestion of the organisms, and they consist generally of various lactic acid bacteria and/or bifidobacteria.

Non beverage plant products

These include

- Olives
- Pickels
- Sauerkraut

Olives

Olives to be fermented are done so by the natural biota of green olives, which consists of a variety of bacteria, yeasts, and molds. The olive fermentation is quite similar to that of sauerkraut except that it is slower, involves a lye treatment, and may require the addition of starters. The lactic acid bacteria become prominent during the intermediate stage of fermentation. L. mesenteroides and P. cerevisiae are the first lactics to become prominent, and these are followed by lactobacilli, with L. plantarum and L. brevis being the most important.

Pickels

Pickles are fermentation products of fresh cucumbers, and as is the case of sauerkraut production, the starter culture normally consists of the normal mixed biota of cucumbers.

In the natural production of pickles, the following lactic acid bacteria are involved in the process in order of increasing prevalence: L. mesenteroides, E. faecalis, P cerevisiae, L. brevis, and L. plantarum. Of these the pediococci and L. plantarum are the most involved, with L. brevis being undesirable because of its capacity to produce gas. L. plantarum is the most essential species in pickle production, as it is for sauerkraut.

Sauerkraut

Sauerkraut is a fermentation product of fresh cabbage. The starter for sauerkraut production is usually the normal mixed flora of cabbage.

The addition of 2.25-2.5% salt restricts the activities of gram-negative bacteria, while the lactic acid rods and cocci are favored. Leuconostoc mesenteroides and L. plantarum are the two most desirable lactic acid bacteria in sauerkraut product, with the former having the shorter generation time and the shorter life span.

Beverages and related products

This category of fermented products include;

- Beer
- Wines
- Distilled Spirits
- Ale
- Cider, and

Beer and Ale

These are malt beverages produced by brewing. An essential step in the brewing process is the fermentation of carbohydrates to ethanol.

Because most of the carbohydrates in grains used for brewing exist as starches, and because the fermenting yeasts do not produce amylases to degrade the starch, a necessary part of beer brewing includes a step whereby malt or other exogenous sources of amylase are provided for the hydrolysis of starches to sugars. The malt is first prepared by allowing barley grains to germinate. This serves as a source of amylases.

The process by which the malt and malt adjuncts are dissolved and heated and the starches digested is called mashing.

When lactic acid bacteria are present in beers, the lactobacilli are found more commonly in top fermentations, whereas pediococci are found in bottom fermentations.

Wines

Wines are normal alcoholic fermentations of sound grapes followed by aging. A large number of other fruits such as peaches, pears, and so forth may be fermented for wines, but in these instances the wine is named by the fruit, such as peach wine, pear wine, and the like. Because fruits already contain fermentable sugars, the use of exogenous sources of amylases is not necessary, as it is when grains are used for beers or whiskeys. Wine making begins with the selection of suitable grapes, which are crushed and then treated with a sulfite such as potassium metabisulfite to retard the growth of acetic acid bacteria, wild yeasts, and molds. The pressed juice, called must, is inoculated with a suitable wine strain of S. “ellipsoideus.” The fermentation is allowed to continue for 3-5 days at temperatures between 700F and 900F (210C and 32°C), and good yeast strains may produce up to 14-18% ethanol

Cider

Cider is a product that represents a mild fermentation of apple juice by naturally occurring yeasts. In making apple cider, the fruits are selected, washed, and ground into a pulp. The pulp “cheeses” are pressed to release the juice. The juice is strained and placed in a storage tank, where sedimentation of particulate matter occurs, usually for 12-36 hours or several days if the temperature is kept at 40° F or below. The clarified juice is cider. If pasteurization is desired, this is accomplished by heating at 170° F for 10 minutes. The chemical preservative most often used is sodium sorbate at a level of 0.10%. Preservation may be effected also by chilling or freezing. The finished product contains small amounts of ethanol in addition to acetaldehyde. The holding of nonpasteurized or unpreserved cider at suitable temperatures invariably leads to the development of cider vinegar, which indicates the presence of acetic acid bacteria in these products.

Distilled spirits

Distilled spirits are alcoholic products that result from the distillation of yeast fermentations of grain, grain products, molasses, or fruit or fruit products. Whiskeys, gin, vodka, rum, cordials, and liqueurs are examples of distilled spirits. Although the process for producing most products of these types is quite similar to that for beers, the content of alcohol in the final products is considerably higher than for beers. Rye and bourbon are examples of whiskeys. In the former, rye and rye malt, or rye and barley malt, are used in different ratios, but at least 51% rye is required by law. Bourbon is made from corn, barley malt, or wheat malt, and usually another grain in different proportions, but at least 51% corn is required by law. A sour wort is maintained to keep down undesirable organisms, the souring occurring naturally or by the addition of acid.

The mash is generally soured by inoculating with a homolactic such as *L. delbrueckii*, which is capable of lowering the pH to around 3.8 in 6-10 hours.57 The malt enzymes (diastases) convert the starches of the cooked grains to dextrins and sugars, and upon completion of diastatic action and lactic acid production, the mash is heated to destroy all microorganisms. It is then cooled to 75-80° F (24-27°C) and pitched (inoculated) with a suitable strain of *S. cerevisiae* for the production of ethanol. Upon completion of fermentation, the liquid is distilled to recover the alcohol and other volatiles, and these are handled and stored under special conditions relative to the type of product being made. Scotch whiskey is made primarily from barley and is produced from barley malt dried in kilns over peat fires. Rum is produced from the distillate of fermented sugar cane or molasses. Brandy is a product prepared by distilling grape or other fruit wines. Palm wine or Nigerian palm wine is an alcoholic beverage consumed throughout the tropics and is produced by a natural fermentation of palm sap. The sap is sweet and dirty brown in colour, and it contains 10-12% sugar, mainly sucrose. The fermentation process results in the sap's becoming milky-white in appearance due to the presence of large numbers of fermenting bacteria and yeasts.

Sake is an alcoholic beverage commonly produced in Japan. The substrate is the starch from steamed rice, and its hydrolysis to sugars is carried out by A. oryzae to yield the koji. Fermentation is carried out by Saccharomyces sake over periods of 30-40 days, resulting in a product containing 12-15% alcohol and around 0.3% lactic acid.

Bread

To make bread, flour and water are mixed with a live culture of yeast. The yeast uses ethanol fermentation to obtain energy form the sugars in the flour. It does so by breaking down the starch into glucose, which it can feed on. This energy gives yeast the energy it needs to live, and produces alcohol and carbon dioxide as waste. The yeast cells grow, the gluten protein pieces stick together to form networks, and alcohol and carbon dioxide are formed from the breakdown of carbohydrates (starch, sugars) that are found naturally in the flour. Enzymes present in yeast and flour also help to speed up this reaction. The carbon dioxide forms air pockets which then helps the dough to rise thereby making bread fluffy and light.

Meats

Fermented meat products are produced by first mixing meat, fat, salt, sugar, curing agents, and spices; filling the mixture in a casing; and fermenting it either naturally or by adding (during mixing) selected starter-culture bacteria.3 The acids produced by the starters during fermentation and the curing agents used help control the growth of pathogenic and spoilage bacteria that might be present in the meat. A good example of fermented meat product is sausages.

Benefits of fermentation

- Cholesterol synthesis inhaling
- Anticancer effects
- Decreases cooking time hence saves energy
- Prevention of infections
- Increased shelf life
- Adds microbes to the gut
- Improves flavor of food
- Eliminates antinutrients
- Increases micronutrients in food e.g. vit B
- Make food more digestible e. g in Lactose intolerance
- Produces carbon dioxide e.g. in bread, beer, champagne

4.3.4.3 Self-Assessment

1. Define the following terms;
   A. Fermentation
   B. Starter culture
   C. Probiotic
2. The microorganisms added to Swiss cheese to improve flavour and assist eye formation is
   A. *Lactobacillus cremoris*
   B. *Lactobacillus bulgaricus*
   C. *Streptococcus thermophillus*
   D. *Propionibacterium freudenreichii*

3. _____ are the most desirable lactic acid bacteria in sauerkraut product.

   A. *Leuconostoc mesenteroides* and *lactobacillus plantarum*

   B. Lactobacillus leichmannii and *l. Delbrueckii subsp.Bulgaricus*

   C. Lactobacillus acidophilus and *l. Delbrueckii subsp. Bulgaricus*

   D. S. Thermophiles and *L. lactis subsp. Lactis*

4. The yeast uses _____ to obtain energy form the sugars in the flour during bread making

   A. Top fermentation

   B. Bottom fermentation

   C. Ethanol fermentation

   D. Anaerobic fermentation

5. Explain the two types of fermentation
6. Highlight the importance of fermentation
7. Discuss the fermentation of dairy products
8. Outline the health benefits of fermented milks
9. Discuss the following non beverage plant products of fermentation

   A. Olives

   B. Pickles

   C. Sauerkraut

4.3.4.4 Tools, Equipment, Supplies and Materials

1. Equipped laboratory
2. Cold chains
3. Stationery
4. Staining reagents
5. Culture systems
6. Workplace procedures manual

4.3.4.5 References

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4.3.5 Learning Outcome 4: Demonstrate the knowledge of microbiological aspects of food safety

4.3.5.1 Learning Activities

Learning activity	Special instructions
Demonstrate understanding of terminologies in microbial aspects and in food safety	
Identify and describe microbial aspects of food safety	Apply microbial aspects of food safety • During production • Processing and labeling • Food handling • Distribution and storage • Food preparation • Food use

4.3.5.2 Information Sheet

Definition

Probiotic: Probiotic is a concentrated supplements of beneficial live bacteria culture taken orally intended to improve our health

Microbial aspects of food safety

Food microbiology focuses on all the microbial aspects of food spoilage and quality. During harvesting, food processing and downstream operations food may become contaminated with a wide range of microorganisms.

Food supply consists basically of plants and animals or product derived from them, it is understandable that our food supply can contain microorganism in interaction with food. These microorganisms use food supply as a source of nutrients for their own growth. These will cause two possibilities:

a. Food spoilage

b. Benefits to human

Microorganisms can cause deterioration of food;

- When they utilize the nutrients of the food, it involved changes in the food compound like synthesis a new compound that cause spoiling of the food or produced enzymatic changes and contributing off-flavours by mean of breakdown of product.

For fresh foods the primary food quality changes may be categorized as:

- Oxidation of lipids and pigments in fat-containing foods resulting in undesirable flavours, formation of compounds with adverse biological effects or discoloration
- Bacterial growth and metabolism resulting in possible pH-changes and formation of toxic compounds, off-odours, gas and slime-formation.

Important microorganisms in food

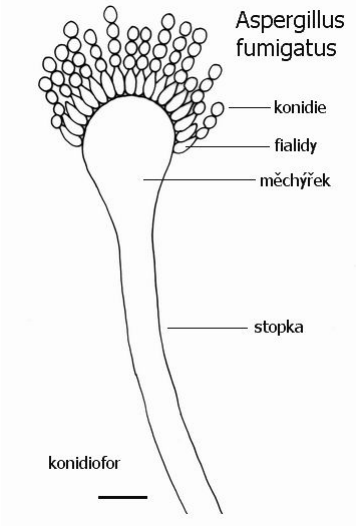
A. Important mold genera

1) Genus Aspergillus

- Widely distributed and contain many species important in food.
- Septate hyphae and produce asexual spores on conidia.
- Xerophilic; causing spoilage in grains, jams, nuts and vegetable.
- Example: *Aspergillus flavus* produce alfatoxin (a kind of mycotoxin)
- Strains used in food processing:

i. *A.oryzae:* hydrolyze starch in sake production.

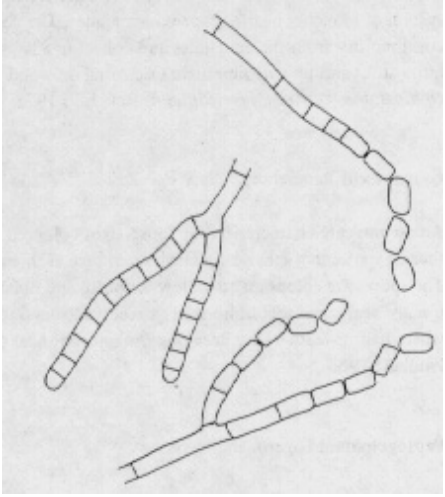
ii. *A.niger:* citric acid production.



Aspergillus

2) Genus Geotrichum

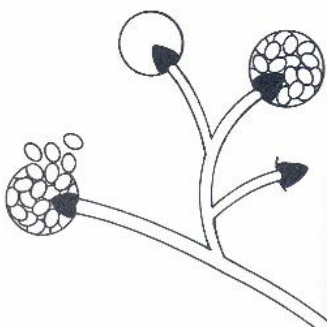
- *Septate hyphae* and produce arthrospore.
- Grow and forming a yeastlike cottony, creamy colony.
- Often grow on dairy products. Example: *Geotrichum candidum*



Geotrichum

3) Genus Mucor

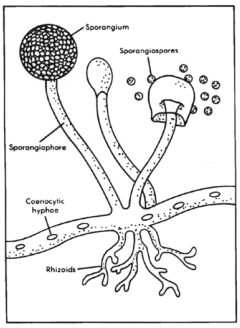
- Widely distributed
- *Nonseptate hyphae* and produce *sporangiophores*
- Some species are used in food fermentation and others can cause spoilage of vegetables e.g: *Mucor rouxii*



mucor

4) Genus Rhizopus

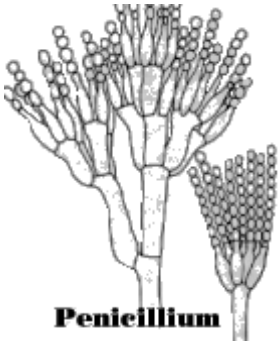
- Hyphae are aseptate and form sporangiophores
- Common in spoilage of foods and vegetables
- Rhizophus stolonifer : common black bread mould



rhizopus

5) Genus penicillium

- Widely distributed and contain many species
- Septate hyphae and form conidiophores on a brushlike conidia head. *Penicillium roquerfortii* and *Penicillium camembertii* are used in cheese production.
- Some species can cause spoilage in fruits, vegetables, grains, bread etc.
- The can also produce mycotoxin



B. Important yeast genera

1. Genus saccharomyces

- Cells may be round, ovate, and elongated.
- Reproduction is by budding or by acospore formation.
- S.cerevisiae is employed in many food industries e.g bread manufacturing, wines, alcohol etc.
- S.fragilis and s.lactis is important in milk and milk products because they are common spoilage microorganism.

2. Genus Torulopsis

- General spoilage yeast.
- Spoils a variety of food products e.g.: beer, milk products, fruit juices and some refrigerated foods.

3. *Genus Candida*

- Many spoil foods with high acid, salt and sugar form pellicle on the surface of liquids.
- Some can cause rancidity in butter and dairy products e.g: *Candida lipolytica*.
- Can form pseudohyphae or true hyphae with many budding cells.

4. *Genus Rhodotrula*

- Red, pink or yellow yeasts may cause discolourations on food such as in meat, fish and sauerkraut.

5. *Genus Pichia*

- Oval or cylindrical yeasts may form *pseudomycelia*.
- Ascospores are round or hat shaped.
- Form pellicle in beer, wine and brine.

C. Important bacteria genera

1. *Genus Bacillus*

- Different species may be mesophilic or thermophilic, lipolytic or proteolytic.
- Spores produce by this bacteria are generally heat-resistant.
- Some species may cause foodborne diseases *(Bacillus cereus)* and food spoilage in canned products *(Bacillus coagulans* and *Bacillus stearothermophillus)*
- The soil is an important source of this species.

2. *Genus clostridium*

- Rod shaped cells, anaerobic and form endospores.
- Found in soil, marine sediments, animal and plant products.
- Some are pathogens e.g.: *Clostridium botulinum* and *Clostridium perfingens* while others are important in food spoilage.
- *C.perfingens* cause stormy fermentation in foods (disruption of curd in milk)

3. *Genus Escherichia*

- Found in faeces, gram negative rod isolated from the intestinal tract of warm blooded animals.
- E.g. *Escherichia coli* used as an indicator of sanitation in the coliform and fecal coliform group.

- Many strains are non-pathogenic but some can be pathogenic to humans and animals (foodborne disease).

4. *Genus Lactobacillus*

- Rod shaped facultative anaerobic, non-motile, mesophilic.
- Can be homo or heterolactic fermentors.
- Found in plant sources, milk, meat and feces.
- Usage:
  i) Food bioprocessing: *L. bulgaricus, L. lactis*.
  ii) *Probiotics: L. acidophilus*
- Spoilage:
  i) Wine or beer production.
  ii) Cheese making.
  iii) Can survive pasteurization

5. *Genus Pseudomonas*

- Gram negative, aerobic, rod shaped, motile.
- Important in fish and meat spoilage.
- E.g: *P.aeruginosa* and *P.fluorescens*

6. *Genus Staphylococcus*

- S. aureus are frequently involved in foodborne diseases.
- It usually gives yellow to orange growth.
- Many beta haemolytic, coagulase positive strains are pathogenic and produce enterotoxin which causes food poisoning.

7. *Genus Streptococcus*

- *Streptococcus pyogenes* - important in foodborne diseases. A cause of human septic sore throat, scarlet fever. Can be found in raw milk.
- *Strep. Thermophillus* is important in cheese making and yogurt.

D. Groups of bacteria important in food

1. *Lactic Acid Bacteria*
    - Ability to ferment sugars to lactic acid e.g: important in cheese making but undesirable in term of spoilage of wines.
    - Major genera: *Leuconostoc, Lactobacillus, Streptococcus, Pediococcus*
2. *Acetic Acid Bacteria*
3. *Butyric Acid Bacteria*
4. *Propionic Acid Bacteria*
5. *Proteolytic Acid Bacteria*
    - Produce extracellular proteiases (enzymes which diffuse outside of the cells) and catalyzes the breakdown of protein.
    - Important genera : *Bacillus, Pseudomonas, Clostridium, Proteus*
6. *Lipolytic Acid Bacteria*
7. *Saccharolytic Bacteria*
8. *Thermophilic and Thermoduric Bacteria*
9. *Halophilic and Osmophilic Bacteria*
10. *Pigmented Bacteria*
11. *Slime or Rope Forming Bacteria*
12. *Gas Forming Bacteria*
13. *Fecal and Non-fecal Coliform group*
    - Definition: Short rods, aerobic or facultative anaerobes, gram negative, non-spore forming bacteria which ferment lactose with gas forming.
    - Major genera: Escherichia, Enterobacter
    - The fecal coliform groups includes coliforms capable of growth at an elevated temperature (44.5°C)

Importance of microorganisms in food

Good (desirable)	Bad (undesirable)
• Food bioprocessing	• Foodborne disease
• Food biopreservation	• Food spoilage
• Probiotics	

A. Desirable effects of microorganisms on food

1) Food bioprocessing: new food products are produced using biological process. In this process, food-grade microorganisms are used to produce different types of fermented food using raw materials from animal and plant sources (this process known as "starter culture"). Beside, microbial enzymes are also being used to produce food and food additives.

2) Food biopreservation: this is a food biological preservative by using antimicrobial metabolites (taken from certain microorganisms in order to control pathogenic and spoilage microorganisms in foods).

3) Probiotics: this is a concentrated supplement of beneficial live cells of bacteria (friendly bacteria) culture taken orally intended to improve our health by promoting our body's natural immunity and improving digestion system

Probiotics is a friendly bacteria which plays a vital role in keeping us fit and health. They improve digestion as well.

Examples of probiotics in food

Milk- baby milk nowadays is added with Lactobacillus acidophilus and Bifidus bacteria.

Yogurt- rich with live bacteria culture such as Lactobacillus bulgaricus and Streptococcus thermophillus.

Cheese- friendly bacteria that is added in cheese is Lactobacillus.

Buttermilk- Lactobacillus bulgaris

Advantages of probiotics

1. By increasing the absorption of mineral and vitamins and it also can improve digestion system especially of milk product. In our food, only vitamins that properly absorbed and digested are useful. Probiotics also improve lactose intolerance.
2. Taking probiotics can support out immune system which is fight bad bacteria and infection in keeping s cope from being run down. It produced antimicrobial substances that can deter various bad bacteria. It is so important because many of the disease begin in the intestinal tract.
3. Produced specific protein that act as antigen and stimulates the immune system.
4. Increase the absorption of calcium, important mineral in the prevention of osteoporosis.
5. Preventing intestinal tract infections that are cause by Candida spp. and Helicobacter pylori.
6. Normalising bowel elimination problems and promoting regularity.
7. Clean the colon and improve constipation.
8. Fights pathologicals moulds, yeast, fungu, viruses, parasites and bacteria.

9. Stimulates β─ lymphoctes and related antibody production.
10. Supporting healthy liver function.
11. Alleviating bowil wind, bloating and bleaching.
12. Assisting in cholesterol management.

Side effect of probiotics to human

Even though probiotic are beneficial bacteria, sometimes it can cause indirect or long term side effect especially if taken inappropriately.

Factors that can produce side effect:

b) Probiotics supplement in food are not safe or not working inside the body.

c) Product that has been taken (food) did not deliver probiotics to specific area to perform its function.

d) Not all probiotics product in market today are created sufficiently enough for body necessity. Some of them are over concentrated and some are less than minimal requirement.

Side effect that occurs include:

a) Excessive drainage syndromes.
b) Headache.
c) Diarrhea.
d) Bloating.
e) Constipation.
f) Production of intestinal gas.

Bacteria in the intestine

The variable and greatest number of bacteria lives in large intestine.

Lactobacillus acidophilus guard small intestine.

Bifidobacter protect large intestine.

Lactobacillus bulgarium is a travelling transient bacteria that aids the two it bases through our body.

B. Undesirable effects of microorganisms in food

1) Foodborne illnesses: a disease cause by consumption of contaminate during various stage of handling between production and consumption by many pathogenic microorganisms (bacteria, molds and viruses).

2) Food spoilage: a condition in which food is contaminated due to

   a. Growth of microorganisms in food

   b. The action of microbial heat stable enzymes

In this case, microorganisms use food as a source of nutrients for their own growth

Primary sources of microorganisms found in food

Microorganisms may be found everywhere in a very wide range of habitats. These may include;

- Soil and water
- Plants and plant products
- Intestinal tract of man and animals
- Food utensils
- Animal feeds and hides
- Air and dust

1. Soil and water

Grouped together because of atmospheric cycling. Soil and water are common sources of important pathogenic and spoilage microorganisms, which is why it is important to thoroughly wash raw foods with good quality water. Air and dust are important sources of microorganisms during food processing and can influence food quality in the home as well.

Soil contains several varieties of microorganisms. Soil is used to grow agricultural product and raise animals. Microbes can get into food from soil and multiply; their numbers can be very high. Soil contaminated with fecal material can be the source of enteric pathogenic bacteria and viruses in food.

Water is used to process, produce and store food. Other uses include: irrigation, drinking, washing, canning and freezing. It is also used as ingredients in many processed food. Therefore, water quality is greatly influence the microbial quality of foods.Chlorine treated potable water should be used in processing, washing, sanitation etc. because improperly treated water can contain pathogen and spoilage of microorganisms.

2. Air and dust

Air and dust are important sources of microorganisms during food processing and can influence food quality in the home as well.

3. Plants and plant products

The inside tissue of plant / animal is sterile. Some plants produce natural antimicrobial metabolites that can limit the presence of microbes. Fruits and vegetables harbour microorganisms on their surface. The type and number varies with soil condition, type of fertilizers and water used. Pathogens can be present if soil is contaminated with untreated sewage. Disease plants, damaged plants during harvesting, unfavorable storage condition can increase the number of microorganisms. Although most soil and water borne microbes will contaminate plants, very few types actually persist on them. Those that persist, such as lactic acid bacteria and some yeasts, must be able to adhere to the plant material and to utilize it for growth.

4. Food Utensils/equipment

This is another important source for cross contamination of raw and cooked foods e.g. cutting blocks, food trays where raw food was held. A wide variety of equipment is used in harvesting, processing, transportation and storage of foods. Many types of microorganisms from air, raw foods, water and personnel can get into the equipment and contaminate foods. When processing equipments are used continuously for a long period of time, microorganisms present initially can multiply and act as a source of contamination

5. Intestinal tracts of humans and animals

Poor sanitation practices (use of polluted water, poor personal hygiene) lead to contamination from these sources. And many pathogens are transmitted by this route. Animals and birds carry many types of microorganisms in the digestive system, in teat canal / udder, skin, hooves, hair and scales. Disease situation such as mastitis in cows can change the ecology of the microflora. Poor husbandry can lead to fecal contamination. Milk can be contaminated with fecal material on the udder surface. Meat contaminated with intestinal content of animals. Eggs can be contaminated from the eggshell

Steps to prevent this type of contamination

a. Good condition of animals and birds husbandry.

b. Properly slaughter.

c. Washing with clean water.

d. Removal of digestive system without contaminating other tissue.

e. Proper sanitation during entire processing.

f. Proper cleaning of udder before milking.

g. Collecting of eggs soon after laying. Washed and stored using recommended procedure.

h. Fish and marine product should be harvested from unpolluted water.

6. Food handlers /human

Food comes in contact with different people handling the foods, such as people processing the food, food handlers in restaurants, catering services, suppliers and producers. Personnel in food processing plants can contaminate foods during handling and processing. •
Human has been the source of pathogenic microorganisms in foods that later caused foodbourne diseases especially ready to eat foods. It is suggested that human beings shed 103 – 104 viable organisms per minute. The numbers and types of organisms shed are closely related to the subject's working environment. Microbiota on hands, garments, etc. reflects the habits of the individual. This can include microorganisms from virtually any environmental source.

Factors affecting this kind of contamination

a. Improperly cleaned hands
b. Lack of personal hygiene and aesthetic sense.
c. Dirty clothes and hair.
d. Presence of minor cuts.
e. Infections on hands and face.
f. Mild generalized diseases e.g.: flu and throat infection.

Prevention

a. Proper training of personnel in personal hygiene.
b. Regular check up.
c. Maintain efficient sanitary.

7. Animal feeds

Very important source of Salmonella in poultry and of Listeria monocytogenes (from silage) in dairy and meat animals.

Steps

8.Animal hides

E.g. Microbiota of raw milk influenced by that of the udder.

9. Sewage

If used as fertilizers in crops, microorganisms in sewage water can contaminate food especially enteropathogenic bacteria and viruses. Especially organically grown food and many imported fruits and vegetables.

Prevention:

a. Avoid using sewage as fertilizer
b. Washing
c. Treat sewage to kill pathogens

The concept of food safety and hygiene

Once food has been harvested, gathered or slaughtered, enzymes and bacteria become active in this food which cause it to deteriorate in texture and composition until it eventually becomes unfit for consumption. This deterioration is known as decay and leads to eventual food spoilage.

Food is considered safe for human consumption when it is free from substances like contaminants, toxins and micro-organisms that can cause undesirable reactions in the body when such foods are eaten. To ensure that food is safe for consumption, it should be:

- Protected from contamination by harmful bacteria, poison and other foreign bodies
- Prevented from having any bacteria present multiplying to an extent which would result in the illness of consumers or the early spoilage of the food
- For some foods: thoroughly cooked to destroy any harmful bacteria present
- Discarded when spoilt and/or contaminated

If food safety systems are not in place during processing. Hundreds if not thousands of consumers are at risk. A single incident of personal injury traced back to a specific food processor may put that company out business and result in criminal prosecution of the owners and management.

Systems which assure the safety and wholesomeness during food processing fall into three categories;

a) Good manufacturing practices (GMP's)
b) Sanitation procedures
c) Hazard Analysis Critical Control Points (HACCP)

Good manufacturing Practices

GMP's are guidelines to assure that food for human consumption is safe and has been prepared, packed and held under sanitary conditions. These guidelines deal with personnel involved in food processing, physical plant and grounds as well as facility construction and design.

Personnel GMP's: Personnel working in food processing can be a significant source of food contamination. This includes production employees, maintenance employees, supervisors and management. It is the responsibility of processing facility management to educate and train all food handlers about sanitary handling of food. Employees experiencing diarrhoea, vomiting, open - skin sores, boils, fever or disease must report these symptoms to their supervisor and must NOT be allowed to work with edible food products. All food handlers should have clean outer garments or aprons and thoroughly wash their hands before entering a food processing area, especially after using toilets. No jewellery (earrings, pendants. rings etc.) or wrist watches are allowed in the food processing areas as these items may fall into food products unnoticed. Clean, intact gloves as well hair restraints should be used by all personnel in the food processing area.

Sanitation procedures

Cleaning and sanitation are some of the most important programs in any food processing plant. Regular and scheduled equipment cleaning and sanitizing assures that food products are being processed under hygienic conditions. Adequate time must be given to the sanitation crew to allow for a thorough job. Cleaning and sanitation is best done by a specially trained sanitation and cleaning crew NOT by production personnel.

Cleaning and sanitizing involves five basic steps

- Physical debrils removal
- Rinse
- Detergent/mild abrasion
- Post rinse
- Sanitizing

HACCP

The prevention of physical, chemical and microbial contamination of produce during processing is essential to assuring the production of a safe product. A HACCP program is only effective if sanitation and good manufacturing processes are implemented and verified. It is recommended that each food processor identify one person in their operation to have formal HACCP training and be in charge of a team that is responsible for implementing the HACCP program. HACCP programs should be a simple as possible, without an excessive number of critical control points. Each HACCP program is unique and must be tailored to your specific operation's needs. A model for dried apples has been provided as an example of a HACCP program which can be used as a starting point for you to develop a HACCP program for your food processing operation.

General Do's and Dont's to Assure Food Safety During Processing

- Follow state regulations regarding the type of licenced facility you may use for food processing (for example, no home or farm kitchens).

- Educate and train employee's in proper food handling practices and personal hygiene.
- Strictly adhere to Good Manufacturing Practices (GMP's).
- Design food processing and storage areas to allow for easy cleaning and sanitation.
- Monitor raw material suppliers for adherence to Good Agricultural Practices.
- Keep processing facility grounds clean and free from clutter.
- Processing facilities should be completely enclosed from the outside environment by walls.
- Windows or other glass should not be present in the food processing area.
- Processing facility floors, walls and ceilings must be cleanable and in good repair.
- Adequate lighting should be rresent and be protected in case of breakage.
- Pipes, ducts and fixtures should not be suspended over processing areas.
- Use only potable (safe to drink) water.
- Monitor water quality regularly.
- Plumbing should be of adequate size and design for sanitary food processing (floor drains, separate sanitary sewers, etc.).

Indicators of food microbial quality and safety

Shelf life indicators are used to assess food sanitation and should meet the following criteria;

- They should be present and detectable in all foods whose quality (or lack thereof) is to be assessed.
- Their growth and numbers should have a direct negative correlation with product quality.
- They should be easily detected and enumerated and be clearly distinguishable from other organisms.
- They should be enumerable in a short period of time, ideally within a working day.
- Their growth should not be affected adversely by other components of the food flora.
- Managing microbial food spoilage

Identifying spoilt food

Food that is spoilt can be identified in different ways:

- Off odours: Foods tend to develop undesirable off-flavours and/or odours as they spoil
- Discolouration: Food undergoing spoilage normally changes in colour
- Slime / Stickiness: Gravy or soups sometimes become thick and slippery to touch
- Unusual taste: Food that is undergoing spoilage often changes in taste

- The production of gas: Some foods - especially when stored in sealed containers develop some gases which will be noticeable when opening the container
- Mould growth: Other foods, e.g. bread develop fungi like growth which is easy to see with the naked eye

Foods at high risk of food spoilage

Some foods are prone to faster spoilage by micro-organisms than others. Foods that spoil fast are usually referred to as “high risk foods.” Most often these are ready to eat foods or rich protein foods and require refrigerated storage. Examples of these foods are:

- (Cooked) meat, including poultry
- (Cooked) meat products including gravy, stews
- Milk and milk products
- Eggs and products made from raw eggs
- (Cooked) Fish

General guidelines for food storage

Foods should be stored differently on the basis of how fast they will spoil or develop off flavors. Foods can be categorized into 3 groups:

1. Perishable (e.g. milk, meat, raw fish)
2. Semi-perishable (e.g. vegetables and grains)
3. Non-perishable foods (tinned or dried food)

Perishable foods: e.g. eggs, milk, cream, fresh meat. These have the shortest shelf life and must be used within a few days. These should be stored in a clean cool place. In the absence of refrigerators, such foods can be placed in clean containers, saucepans or pots.

The containers can then be placed in a basin of cold water covered with a clean piece of cloth. In all circumstances, milk and meat should be consumed within 2 days.

Semi-perishable foods: e.g. bread, cakes, fresh fruit and vegetables. Breads and cakes should be stored in a bread bin or tin. Fruit and vegetables may be stored in a rack or basket. When put in storage, care should always be taken to remove and discard the particular foods that start showing signs of spoilage so as to avoid cross-contamination.

Non-perishable foods: e.g. dry, bottled and tinned foods can be stored in a cupboard on their own or in airtight containers

Further food categories and their storage methods

The recommended storage conditions for foods often vary; the variations even differ for the same foods depending on the freshness or dryness of the particular food.

Storage of cereals, bread, flour, and rice

- Bread needs to be stored in its original package at room temperature. It should be used within 5 to 7 days or else it will grow moulds (a sign of spoilage)

- Cereals - depending on the quantities and level of dryness - may be stored at room temperature in tightly closed containers to keep out moisture and insects. Properly dried cereals packaged in sacs can be stacked on racks in a dedicated food store. Due attention should be taken to keep out rodents (rats) that normally feed on stored grain
- Raw rice can be stored in closed containers at room temperature and used within one year. Once cooked, rice should be eaten immediately in the absence of refrigeration
- Storing fresh vegetables
- Proper storage of fresh vegetables helps to maintain their quality and retain nutrient value. Most fresh vegetables need to be stored under low temperatures in areas which are neither humid nor damp. If available, fresh vegetables can be stored in a clay pot fridge.
- Root vegetables (potatoes, sweet potatoes, onions, etc.), squashes and eggplant can be stored in a cool, well-ventilated place between layers of grass
- Onions should be left to dry thoroughly under the sun to avoid rotting in storage and when well dried can be kept for about 3 months
- Tomatoes continue to ripen after harvesting and should be stored at room temperature
- Storing fresh fruits
- All fresh fruits generally need to be stored in a cool area, preferably in a clay pot fridge
- Fruits have a tendency to either be contaminated by other foods and or to absorb odours from other foods. They therefore need to be kept separately
- Care must be taken to keep milk in clean covered containers that should be left to stand in a cool place. Unrefrigerated milk should be used within a day

Storing meat and fish

- Meat (including poultry), fish, eggs and milk are the best sources of proteins in the human diet. Given their high protein and moisture content, these products are highly perishable. It is for this reason that these products will spoil faster than others - however well prepared and stored. One big contributor to the faster spoilage of fresh cuts of meat is the fact that these usually contain spoilage bacteria on the surface that can grow quickly, producing slime and causing spoilage after a few days. Meat should be prepared and eaten within 24 hours of purchase/slaughter.
    - Ground and thinly cut pieces of meat are more susceptible to spoilage given the larger surface area for bacterial action. Meat and meat products should be used within a few days. If the meat cannot be used within a day, it is advisable to dry, smoke or salt it before storing it

- Like meat, fresh fish should be eaten immediately. Never store fish in water as this leads to loss of nutrients from the fish. In order to store fish for longer, it should be smoked.
- Storing Root Tubers (Cassava, Sweet Potatoes)
- Most root tubers may not be stored well for long after harvest, however root tubers keep longer than other vegetables, fruits, meat, milk, etc.
- When tubers will not be prepared within a few days, care should be taken to avoid bruising them. It is advisable to harvest cassava before it becomes fibrous, with part of the aerial stem still attached. This helps preserve the tubers in good condition.
- Cassava tubers can also be piled into heaps and watered daily to keep them fresh or coated with a paste of mud to preserve their freshness. They can keep for about 4-7 days.
- Unbruised sweet potatoes can be kept in a cool, dry place for up to 4-7 days. Care should be taken to remove any sprouting buds.
- In times of bumper harvests, tubers cannot be kept for long; it is advisable that these are peeled and sliced in small pieces and then sun dried on canvas or cleaned floors.
- Once well dried, the sliced dry tubers can be kept in sacks and stored for up to 3-4 months without spoiling.
- Storing milk and milk products
- Milk is a highly perishable food and yet very nutritious. To prolong its shelf life, milk should never be left at room temperature for a long time as it spoils quickly

Measures to prevent deterioration of food by microorganisms

- Minimize the contact between microorganisms and food
- Eliminate microorganisms from foods
- Understand about preservation of the food

4.3.5.3 Self-Assessment

1.Describe the primary sources of microorganisms

2.During processing, foods can get contaminated because of

A. Workers

B. Equipment

C. Packaging material

D. All of the above

3.Mechanical damage to fruits and vegetables by birds open the way

A. To microbial spoilage

B. To chemical spoilage

C. To spread infectious diseases

D. None of the above

4.The microbial load shed by a human being per minute is _____

A. 103 - 104

B. 101 - 103

C. 106 - 108

D. 104 - 105

5.Spoilage in foods may be due to

A. Insects

B. Physical changes

C. Growth and activity of microorganisms

D. All of the above

6. Explain the categories of food quality changes for fresh foods
7. Discuss the systems which assure the safety and wholesomeness during food processing
8. Classify foods based on the ease of spoilage
9. List the organisms involved in the breakdown of proteins
10. With aid of diagrams, discuss the molds important to food

4.3.5.4 Tools, Equipment, Supplies and Materials

1. Labs
2. Cold chains
3. Vaccines
4. Stationery
5. Staining reagents
6. Culture systems

4.3.5.5 References

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4.3.6 Learning Outcome 5: Demonstrate the knowledge on methods of detection, identification and enumeration of food microorganism

4.3.6.1 Learning Activities

Learning activity	Special instructions
Identify and describe terminologies in basic laboratory equipment and materials as per resource materials	Identify and describe basic laboratory equipment and materials • Equipment such as incubator, autoclave, petri dishes • Materials such as different types of media and reagents
Methods of detection, identification and enumeration of microorganisms are identified and described	Perform practical to detect, identify and enumerate microorganisms Wear PPEs during the practices • Lab coat • Gloves • Closed shoes • Masks

4.3.6.2 Information Sheet

Definitions

Enumeration: is counting of microorganisms present in a sample.

Introduction to basic laboratory equipment and materials

Laboratory equipment and materials	Use
Balance 	Used for measuring mass
Beaker 	Used to hold, mix, and heat liquids.

Beaker tongs 	Used to pick up beakers.
Bunsen burner 	Used as a heat source in the absence of flammable materials.
Buret 	Used for dispensing an accurate volume of a liquid.
Clay triangle 	Used to support a crucible during heating.
Crucible 	Used for holding chemicals during heating to very high temperatures.
Crucible tongs 	Used to hold crucibles.
Erlenmeyer Flask 	Used to hold and mix chemicals. The small neck is to facilitate mixing without spilling.
Evaporating dish 	Used to heat liquids for evaporation
Forceps 	Used to pick up or hold small objects.

Funnel 	Used to transfer liquids or fine-grained materials into containers with small openings. Also used for filtration.
Graduated cylinder 	Used to measure a precise volume of a liquid.
Microscope 	Used to magnify specimens
Mortar and pestle 	Used to crush and grind materials.
Petri dish 	Used to culture cells
Pipet bulb 	Used to draw liquids into a pipe
Ring clamp 	Used with a ring stand to hold glassware, such as a beaker or a funnel.
Ring stand 	Used to hold or clamp laboratory glassware and other equipment in place, so it does not fall down or come apart.

Stirring rod 	Used for stirring and mixing.
Test tube 	Used to hold a test tube, particularly when hot.
Test tube clamp 	Used to hold a test tube, particularly when hot.
Test tube rack 	
Thermometer 	Used to measure temperature in Celsius.
Utility clamp 	Used to secure glassware to a ring stand.
Volumetric flask 	Used to prepare solutions to an accurate volume.

Volumetric pipet 	Used to measure small amounts of liquid very accurately. Never pipet by mouth! Use pipetting aids.
Wash bottle 	Used to rinse pieces of glassware and to add small quantities of water.
Watch glass 	Used to hold solids while they are being weighed or to cover a beaker.
Wire gauze 	Used to support a container, such as a beaker, on a ring stand while it is being heated. May have a fiberglass or ceramic center.
Incubator 	Used to grow and maintain microbiological or cell cultures
Autoclave 	Used to sterilize laboratory instruments and nutrient agar

Methods of detection, identification and enumeration of microorganisms

Introduction

The detection and enumeration of pathogens in food and on food surfaces that come into contact with food are an important component of any integrate programme to ensure the safety of foods throughout the food supply chain.

Importance of detection and enumeration of microorganisms in food

- To determine the safety and quality of food
- To detect the presence of pathogens in raw and processed foods immediately

Ways to detect and enumerate microorganisms

- Testing for pathogenic bacteria
- Testing for staphylococci
- Testing for mesophilic bacteria
- Coliform test
- Total plate count

Enumeration

This is done to know the intense of presence of the spoilers in the spoiled food. It aims at detecting which type of organism is responsible for the spoilage.

This is mostly done using two important methods;

- Viable count: spread plate method
- Total count: Pour plate method

Viable count

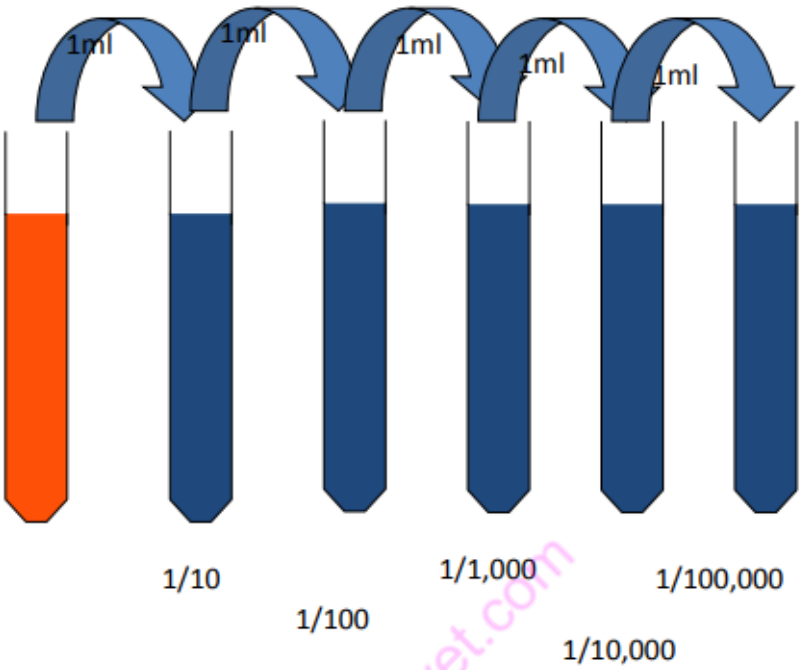
- A viable cell count allows one to identify the number of actively growing or dividing cells in a sample.
    - The plate count method or spread plate method relies on bacteria growing a colony on a nutrient medium.
    - Number of colonies can be counted.
    - Plate count agar is used for general count
    - MacConkey agar is used for Gram negative organisms.

Direct viable count:

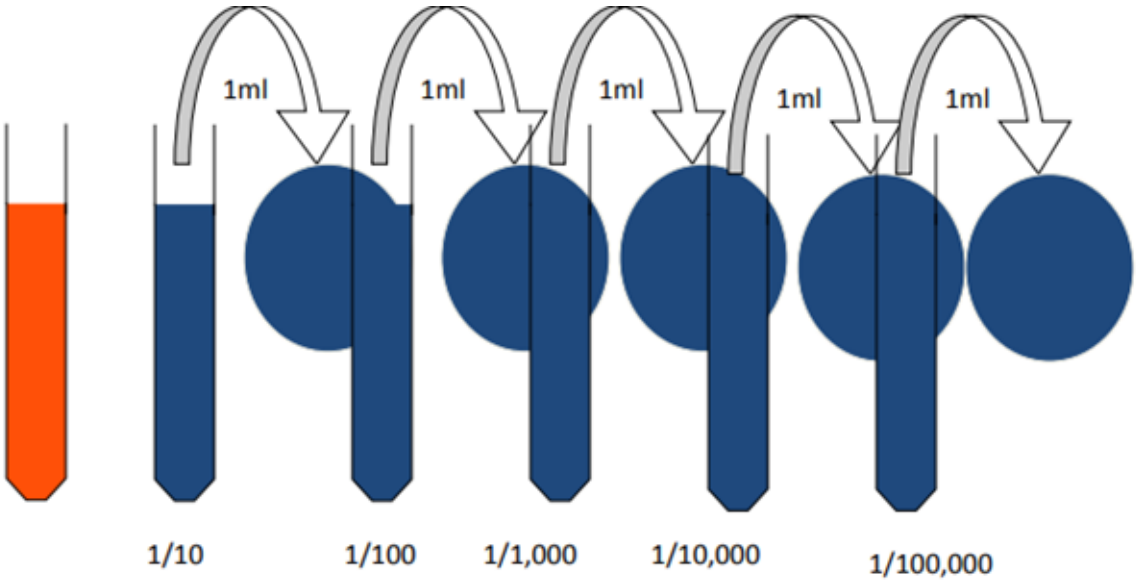
A direct viable count method involves a standard plate count, in which repeated dilutions of a sample.

- The sample is serially diluted as (1:10,1:100,1:1000 etc,) in sterile distilled water and cultivated on nutrient agar for bacteria.
- Potato dextrose agar or sabouraud's dextrose agar is used or fungal identification.

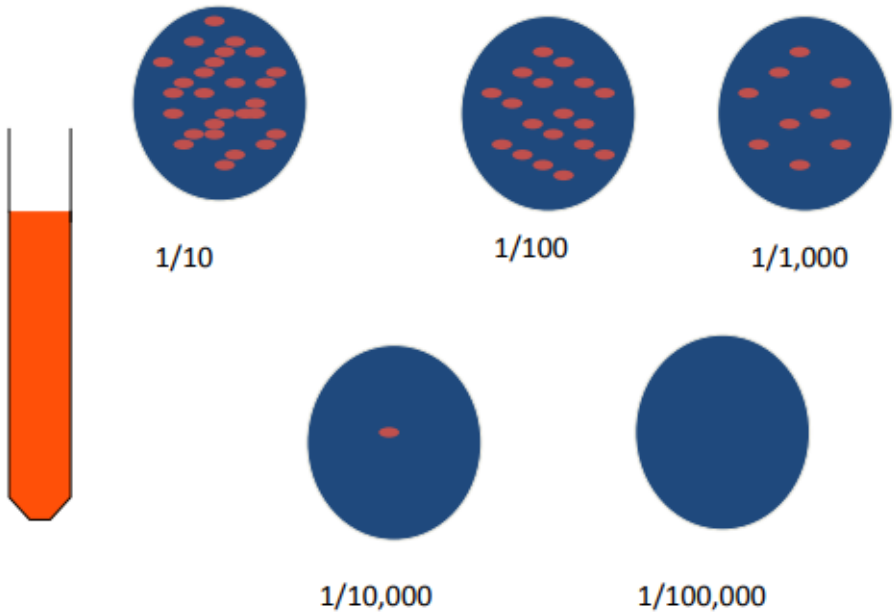
Dilution Series: dilution



Dilution series: plating



Dilution series: colony count



Total count

The initial analysis is done by mixing serial dilution of sample in liquid nutrient agar which is then poured into bottles.

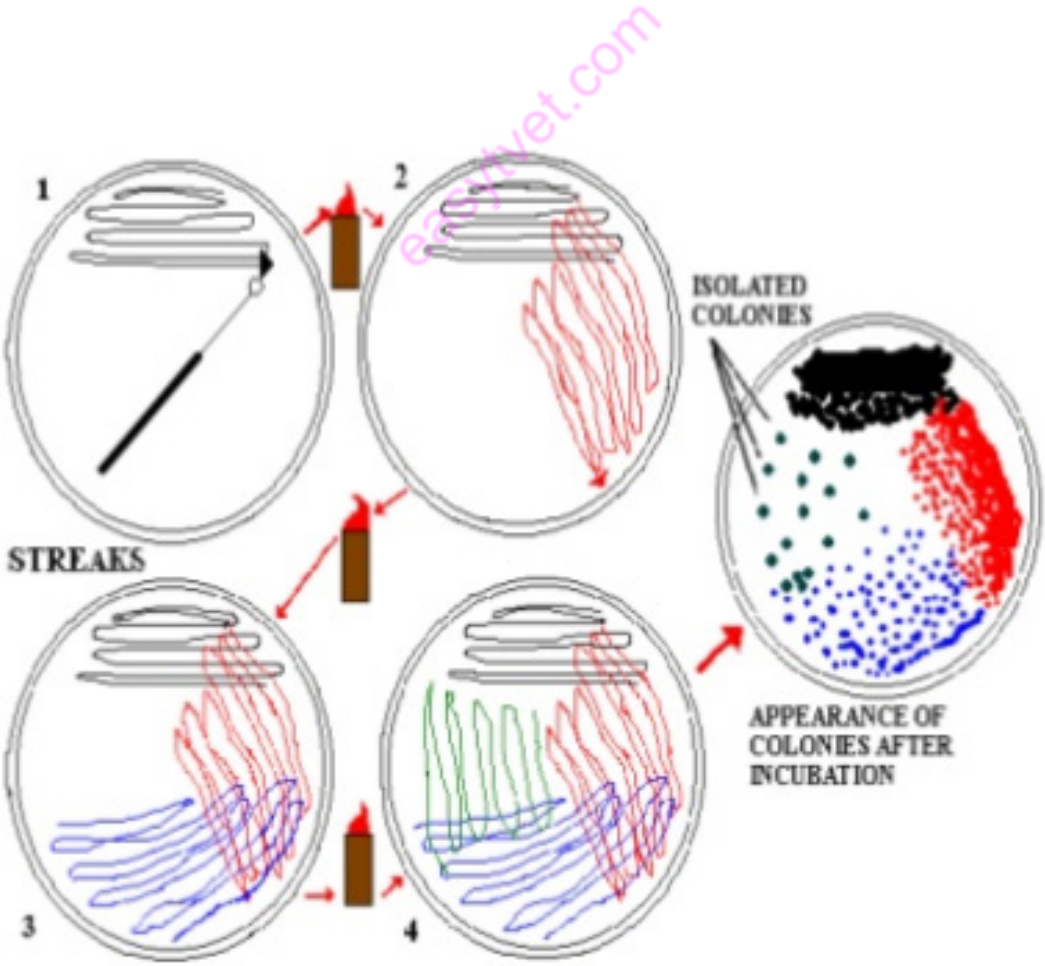
The bottles are then sealed and laid on their sides to produce a slopping agar surface.

The colonies are then counted by eye. The total number of colonies are said as Total Viable

Procedures involving plate counts;

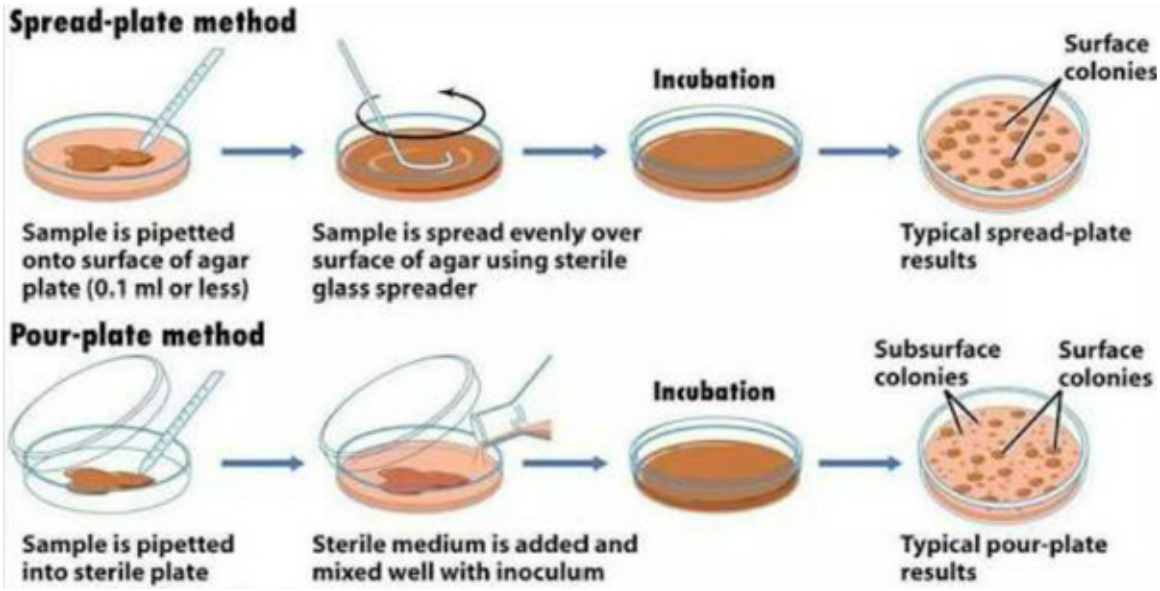
- Streak dilution plate
- Spread plate
- Pour plate

Streak dilution plate



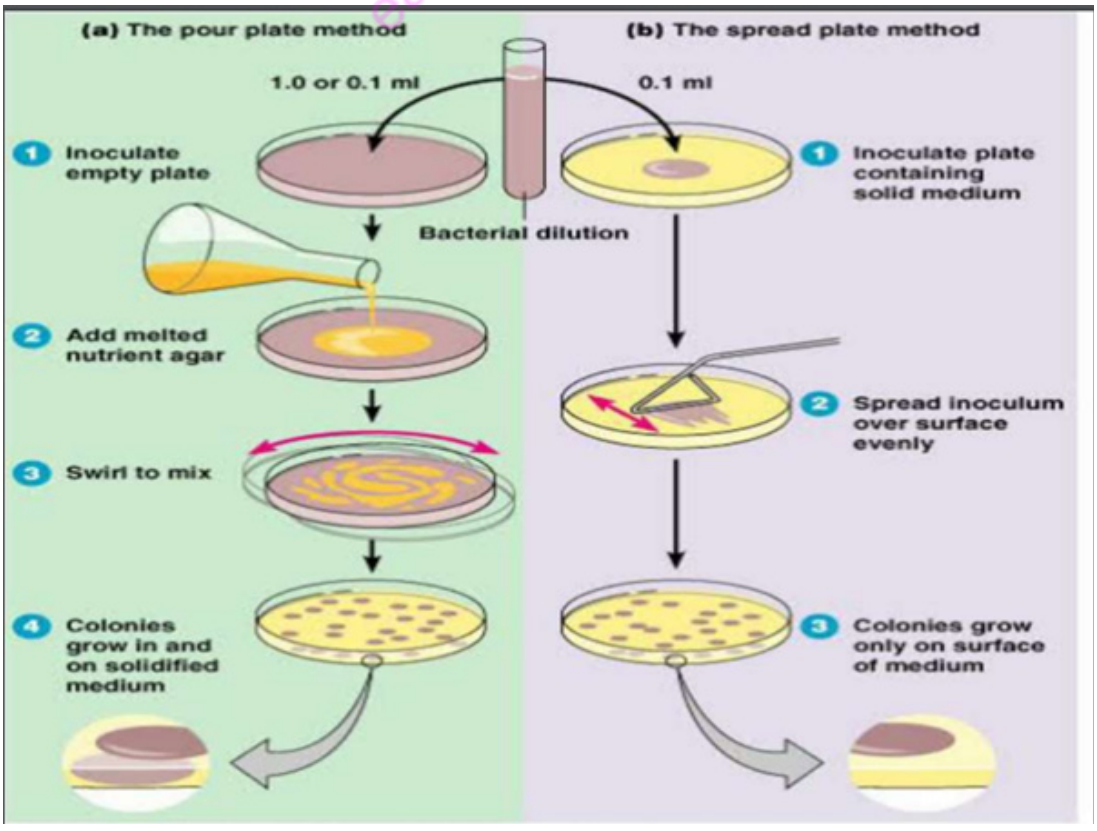
Spread plate

Method of quantifying the number of viable cells (or colony forming units) in a sample after appropriate dilution



Pour plate method

The same procedure is done for this till serial dilution. The serially diluted sample is then mixed with the molten nutrient agar, distributing the cells throughout the medium. Then poured onto the sterile petridish. Incubated under appropriate temperature and the colonies where counted.



Disadvantages

- Typical colony morphology seen in surface cultures will not be observed for those colonies that develop within the agar medium
- Some of the suspension may be left behind in the screw capped bottle

Microbial indicators assess food safety and should meet the following criteria:

i. be easily and rapidly detectable

ii. be easily distinguishable from other members of the food biota

iii. have a history of constant association with

iv. be present whenever the pathogen of concern is present

v. be an organism whose numbers ideally should correlate with those of the pathogen of concern

vi. be present only when there is a real danger of pathogen being present

vii. possess growth requirements and a growth rate equaling those of the pathogen

viii. have a die-off rate that at least parallels that of the pathogen and ideally persists slightly longer than the pathogen of concern

ix. be absent from foods that are free of the pathogen except perhaps at certain minimum numbers

Examples of organisms used as indicators

- E. coli
- Coliforms: has several strains; Citrobacter, Enterobacter, Escherichia, and Klebsiella.

Coliform test

The coliform bacteria are gram negative non-spore forming rods that occur in large numbers in human and animal feces. They are normally present on raw animal products, such as meats, milk, and eggs, and also occur naturally in soil, water, and surfaces of plants. They are heat sensitive and die rapidly during blanching or pasteurizing. Large numbers of coliforms after a heat process indicate an unacceptable degree of post-heating contamination or indicate time-temperature abuse of the food sufficient to permit growth. High coliform levels warrant investigations to determine the source of contamination or temperature mishandling.

Coliform test has been carried out to test the presence of enteropathogenic bacteria such as Salmonella and Shigella. This test normally uses coliform bacteria as indicator. As a result, testing for coliform bacteria can be a reasonable indication of whether other pathogenic bacteria are present.

The presence of Escherichia coli, member of the coliform group, in food usually indicates direct or indirect human or animal fecal contamination. Although this may be true in a broad sense, one must not assume a quantitative relationship between the numbers of E. coli and the degree of contamination with feces. E. coli grows well outside the animal body and thrives in unclean food handling equipment.

Reasons why coliforms are used as indicators for other bacteria

- They are able to survive for extensive periods of time in the environment
- Relatively easy to cultivate in the laboratory and numerous

Stages of coliform test

a) Presumptive tests

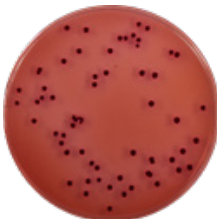
b) Confirmative tests

c) Completed tests

a) Presumptive test

Example in solid food sample using plating method;

- Add food into the petri dishes (in duplicate)
- Add molten violet red bile agar, mix then allow to harden
- Incubate the plate at 35C for 18-24 hours
- Positive result: dark red colonies with a surrounding zone of precipitated bile at least 0.5mm in diameter



b) Confirmation test

This tests should be carried out because gas formation in lactose broth is not only a characteristic for fecal Salmonella, Shigella and E. coli strains but also of non-fecal coliform like Enterobacter aerogenes and some Klebsiella species

In this second stage the presence of enteric bacteria is confirmed by re-culture of the positive result:

Positive result from plating method: Those colonies from violet red bile agar are transferred to a separate tube of brilliant green lactose bile broth and then incubated for 48 hours under 35°C before examined for the gas presence.

c) Completed test

This involves the positive tube from brilliant green. Lactose bile broth cultures are streaked and stabbed on slant of nutrient agar.

After incubation for 18-24 hours at 35°C, the slant is examined for the growth on the surface and in the stabbed portion of the slant.

Gram stain is then made from the agar slant and the positive result should show: Gram negative, non-sporing rods.

4.3.6.3 Self-Assessment

1. Define the term enumeration
2. Clay triangle is used
   A. For holding chemicals during heating to very high temperatures.
   B. To hold crucibles.
   C. To support a crucible during heating.
   D. To pick up beakers.
3. Which of the following media is used for identification of fungi?
   A. Potato dextrose agar
   B. Macconkey agar
   C. Nutrient agar
   D. Xylose Lysine Deoxycholate (XLD) agar
4. The following procedures involve plate counts except
   A. Streak dilution plate
   B. Spread plate
   C. Pour plate
   D. Serial dilution plate
5. Identify ways in which microorganisms can be detected and enumerated
6. Discuss the following methods of enumerating microorganisms

a. Viable count

b. Total count

7. Explain the procedure involving plate counts
8. Describe the characteristics of microbial indicators used to assess food safety
9. Explain the three stages of coliform tests

4.3.6.4 Tools, Equipment, Supplies and Materials

1. Equipped laboratory
2. Cold chains
3. Vaccines
4. Stationery
5. Staining reagents
6. Culture systems
7. Workplace procedure manual

4.3.6.5 References

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