FOOD MICROBIOLOGY

MCB 408

Course Lecturer

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Food Microbiology

Brief history of Food Microbiology. Flora and sources of microorganisms in food. Methods of detection and enumeration of microorganisms in foods. Intrinsic and extrinsic parameters of food that affects microbial growth and survival. Roles of microorganisms in spoilage, food borne diseases and in food products. Food preservation. Quality control and microbiological standards
BASIC PROCEDURES.

1. No eating or talking in food microbiology LAB.
2. All working benches, floor, and environment must be kept clean.
3. Licking of labels and mouth pipetting should be avoided.
4. Where there is a spillage or contamination material this should be covered with suitable materials and discarded appropriately.
5. Open wound or cut should be covered appropriately or dressed appropriately.
6. At strategic places fire extinguishers should be provided.
7. All microbiological procedures should be carried out under laminar airflow.
8. At all times LAB coats should be worn.
9. Nail vanishes and mouth polishing are not allowed in the LAB.
10. Hair, moustache, and beard must be covered.
11. When centrifuge are used, they must be allowed to stop on their own and generation of aerosols should be avoided.
12. After each operation, hand cleaning should be done.

EQUIPMENT AND MATERIAL USED.

1. INCUBATOR: - can be used for aerobic and anaerobic organism at desired temperature regimes.
2. DRYING OVEN/HOT AIR OVEN: - can be maintained at a temperature greater or equal to 120°C.
3. LAMINAR AIR- FLOW/INOCULATION HOOD: - Inoculation hood/ laminar airflow must be fitted with UV light. All microbiology processing must be undertaken in this environment.
4. MICROSCOPE: - an optical instrument used for enlarging images of minute objects using light and other radiations.
5. WATER: - BATH SHAKER.
6. COLONY-COUNTER: - for counting colonies.
7. BACTOMETER: - for counting colonies in food it takes precedence over the colony counter.

FAST METHODS OF FOOD MICROBIOLOGY.
- Use of DNA probes
- Use of impedance method - BACTOMETER.
- Use of kits.

Kits are used often to identify the organism causing food spoilage, e.g. RAPIDEC COLI/API system all of which are used for identification of unknown organisms.

THE IDENTIFICATION, ROLE AND SIGNIFICANCE OF MICROORGANISMS IN FOODS.
The main source of food to man are of the plant and animal origin. When one considers the types of microorganisms associated with plant and animal foods in their natural states, one can predict the general types of microorganisms to be expected on this particular food at some stages in its life history. Based on previous analyses and result obtained from many different laboratories. It has been shown that, untreated foods may contain varying numbers of bacteria, moulds or yeasts. It is therefore important to know nature and the general types of organism normally present under given conditions where foods are grown and handled.

BACTERIA: - The following consist of the most important genera of bacteria known to cause food spoilage and food poisoning. However some of these are highly desirable in certain foods.

Acetobacter    Lactobacillus
Acinetobacter   Leuconostoc
Alcaligenes     Micrococcus
Bacillus        Pedicoccus
Bacteroides    Proteus
Citrobacter     Pseudomonas
Clostridium     Salmonella
Corynebacterium Serratia
Enterobacter    Shigella
Escherichia    Staphylococcus
Flavobacterium Streptococcus
Kurthia         Streptomyces.

MOLDS: Some of the common genera of molds associated with food are as follows:

Alternaria    Geosporium
Aspergillus   Helminthosporium
Botrytis       Monilia (Neurospora)
Byssochlamys   Mucor
Cephalosporium Penicillium
YEAST: Some of the most common genera of yeast encountered in foods are as follows:

- \textit{Brettanomyces}, \textit{Hansenula}, \textit{Saccharomyces}
- \textit{Candida}, \textit{Kloeckera}, \textit{Schizosaccharomyces}
- \textit{Debaromyces}, \textit{Mycoderma}, \textit{Torulopsis (Torulas)}
- \textit{Endomycopsis}, \textit{Rhodotorula}, \textit{Trichosporon}

**PRIMARY SOURCES OF MICROORGANISM TO FOODS.**

The primary sources of microorganisms to food are from:

1. **SOIL AND WATER:**
   In these environment organisms are generally encountered which are often food-borne bacteria are: \textit{Alcaligenes}, \textit{Bacillus}, \textit{Citrobacter}, \textit{Clostridium}, \textit{Corynebacterium}, \textit{Enterobacter}, \textit{Micrococcus}, \textit{Proteus}, \textit{Pseudomonas}, \textit{Serratia} and \textit{Streptomyces} among others.
   For molds, some of the most commonly encountered in soils are \textit{Aspergillus}, \textit{Rhizopus}, \textit{Penicillium}, \textit{Trichotheccium}, \textit{Botrytis}, \textit{Fusarium}, and others.
   In the case of yeast, a large number of yeast genera are found in the soil, but their number are generally low in water.

2. **PLANTS AND PLANT PRODUCTS.**
   Bacteria that are often found to be associated with plants and plant products are \textit{Acetobacter}, \textit{Erwinia}, \textit{Flavobacterium}, \textit{Kurthia}, \textit{Lactobacillus}, \textit{Leuconostoc}, \textit{Pediococcus} and \textit{Streptococcus}. As for molds, the most important plant-borne genera are those that cause the spoilage of vegetables and fruits and these include \textit{Fusarium}, \textit{Aspergillus}, \textit{Botrytis}, \textit{Alternaria} etc. The most commonly encountered genera of yeast in plant products are the genus \textit{Saccharomyces}, \textit{Rhodotorula} and \textit{Torulas}.

3. **FOOD UTENSILS.**
   The types of food borne microbes that are found in food utensils depend largely on types of food handled, the care of these utensils, their storage and other factors. For example, utensils used in handling vegetables would be expected to have organisms that are associated with vegetables. Also utensils which have been cleaned with hot or boiling water will only as its micro flora those organisms that are able to with –stand the treatment. Utensil stored in open place where dust might collect should be expected to have air-borne bacteria, yeast and molds.

4. **INTESTINAL TRACT OF MAN AND ANIMALS.**
   These organisms, through fecal dissemination in man found their way directly into soil and water. From soil they may find their way to food utensils. The most encountered yeast in this case is the \textit{Candida}.
5. **FOOD HANDLERS.**
The organisms generally found on the hands and outer garments of food handlers are a function of the environment and the habit of the food handlers. Aside these, there are some bacteria that are specifically associated with hands Nasal cavities and mouth. These include *Micrococcus* and *Staphylococcus*. The genera Salmonella and *Shigella* are basically found in the intestine of man, they may be deposited onto foods and utensils by food handlers. If sanitary practices are not followed by each individual.

Yeast and Molds may be found on the hand, garment of individual depending upon the immediate history of individual.

6. **ANIMAL FEEDS.**
The types of organisms to be found in animal feed would actually depend on the source of the feeds, the treatment given them destroy microorganisms, the container in which they are stored and the like. Any one of the above mentioned genera of bacteria, yeast, and mold may be found in animal feeds, of particular importance, *Salmonella* sp. Which causes food poisoning?

7. **ANIMAL HIDES.**
Mostly organisms in soils, water, animal feeds, dust and faecal matter are often found on the hides of animal. From the animal hides, the microbes may find their ways into the air, hands of workers and directly into foods. Some members of the hide flora find their way into the lymphatic system of slaughtered animals from which they migrate into the muscle tissue of the slaughtered animal.

8. **AIR AND DUST.**
Majority of genera of bacteria mentioned above except for some pathogens are found in air and dust. Also, many genera of molds and yeast are found. Bacillus and Micrococcus spp are some of the notable bacteria often found in air and dust because of their ability to endure dryness to varying degrees.

**FOOD EXAMINATION**

Methods for the Microbiological Examination of Foods

This include the following:

Direct Examination

Cultural Technique

Enumeration methods such as (a) Plate count and (b) Most probable number

Alternative method

1. **DIRECT EXAMINATION**
   1. **Microbial Count**
      The use of optical microscope to examine microbes in food offer some advantages in a short while. For example, the observation of endospore of *Bacillus* species under
fluorescence microscope quickly gives an idea of the type of organisms in foods. The drawback here is that, the food debris often interfere with the heat fixing and care must be taken to prevent smear being washed off during staining. Also, this method does not distinguish between live and dead cells. The Direct Epifluorescent filter technique or DEFT is a microscopy technique that is used for the enumeration of microorganisms in a range of foods such as raw milk. It uses polycarbonate membrane filter which is stained with acridine orange and counted directly under the epifluorescence microscope. The acridine orange is a metachromatic fluorochrome, fluorescing either green or orange depending on the nature of the molecules within the cell to which it is bound. When bound to double-stranded DNA it fluoresces green by when bound to single-stranded RNA it fluoresces orange, as long as there is an adequate concentration of dye to saturate all binding sites.

Any of the following methods can be used to count microorganisms in food (a) Direct microscopic count (b) Standard plate count (c) Most Probable Number (d) Impedance measurement using a BACTOMETER (e) Dye reduction: this can be used to assess the keeping quality or otherwise the level of microbial contamination in a given food product particularly milk. The dyes normally used include the following: RESAZURIN or methylene blue.

In dye reduction test, properly prepare supernatant of food are added to standard solution of methylene blue or Resazurin and these are observed for colour reduction for e.g. methylene blue is reduced from blue to white, Resazurin is reduced from slate faint blue to pink or white. The number of microorganisms present in a given sample is proportional to the degree of reduction.

NOTE; the following are useful factors that affect microbial count.

(1) The sampling method used.
(2) The distribution of the microorganisms in the food sample.
(3) Nature of the food microflora.
(4) Nature of the food material biological component.
(5) Nutritional status of the culture media used.
(6) Incubation temp and the time used for the test.
(8) Extrinsic factor; - temperature, water vapour, partial pressure of gases of incubation.
(9) The type of diluents used.
(10) Hygienic nature of the handler.

II. CULTURAL METHOD

The art of propagating viable propagules to multiply in liquid or solid media is what that makes the cultural method. Agar is a polysaccharide with several remarkable properties which is produced by species of red algae. Although it is a complex and variable material a major component of agar is agarose which is made of alternating units of 1,4-linked 3,6-anhydro-L-galactose (L-galactose) and 1,3-linked D-galactose (or 6-O-methyl-D-galactose)
**Microorganisms EN-ISO 4833:2003**
Horizontal method for the enumeration of microorganisms. Colony-count technique at 30°C.

**Isolation medium**
Plate Count Agar (PCA)
Mass inoculation
**Incubation:** 30°C for 72 ± 3 h

**Bacillus cereus EN-ISO 7932:2004**
Horizontal method for the enumeration of presumptive Bacillus cereus. Colony-count technique at 30°C.

**Isolation medium**
Spread inoculum over the surface of Mannitol-Egg Yolk-Polymyxin (MYP) Agar
**Incubation:** 30°C for 18-24 h
Incubate for further 24 hours if colonies are not easily visible

**Confirmation**
8-haemolysis test on Sheep Blood Agar
(Blood Agar n. 2)
**Incubation:** 30°C for 24 ± 2 h
Staphylococcus aureus and other species EN-ISO 6888-1:1999
Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species).
Part 1: Technique using Baird-Parker Agar Medium.

Isolation medium
Spread inoculum over surface of Baird Parker Agar
Incubation: 37°C for 24-48 h

Confirmation
Coagulase

EN-ISO 6888-2:2000
Enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species).
Part 2: Technique that use Rabbit Plasma Fibrinogen (RPF) Agar Medium.

Isolation medium
Mass inoculation technique of Rabbit Plasma Fibrinogen Agar
Incubation: 35-37°C for 18-24 h
Incubate for a further 24 h if necessary

Confirmation
Is not necessary
**Escherichia coli** EN-ISO 16649-2:2001

Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli*.

Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide.

**Selective isolation medium**

Tryptone Bile X-Glucuronide Agar (TBX)

Mass inoculation

**Incubation:** 44°C for 18-24 h

In case of suspected damaged cells, incubate in anaerobic at 37°C for 4 h.
Enterobacteriaceae EN-ISO 8523:1991
General guidance for the detection of Enterobacteriaceae with pre-enrichment.

Pre-enrichment medium
Buffered Peptone Water
Incubation: 37°C for 16-20 h

Enrichment medium
Buffered Glucose-Brilliant Green Bile Broth (EE Broth)
Incubation: 37°C for 18-24 h

Isolation medium
Violet Red Bile Glucose Agar (VRBG)
Incubation: 37°C for 24 ± 2 h

Confirmation
Oxidase
Glucose fermentation
Glucose Bromocresol Purple
Incubation: 37°C for 24 ± 2 h

Enterobacteriaceae EN-ISO 4832:1991
General guidance for the enumeration of coliforms - Colony-count technique.

Isolation medium
Mesa inoculation with Violet Red Bile Lactose Agar (VRBL)
Incubation: 30°C, 35°C or 37°C for 24 ± 2 h

Characteristic colonies
Violet red with ø ≥ 0.5 mm
Listeria EN-ISO 11290-1:1996

**Enrichment media**
Primary enrichment
In Half-concentrated Fraser Broth
Incubation: 30°C for 24 ± 2 h

Secondary enrichment:
0,1 ml of primary culture
in 10 ml of Fraser Broth
Incubation: 36 or 37°C for 48 ± 2 h

**Isolation media**
Spread inoculum over surface of PALCAM Agar and OXFORD Agar
Incubation: Aerobic or microaerobic 30°C, 35°C or 37°C for 24 ± 2 h
and continue for another 24 ± 2 h if there are no colonies or they are small

**Confirmation**

- **Genus confirmation:**
  - Catalase
  - Gram
  - Motility

- **Species confirmation:**
  - Haemolysis
  - Fermentation of:
    - Rhamnose
    - Xylose
    - CAMP test

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**ENRICHMENT**

1. Fraser broth
2. 225 ml
3. 0,1 ml
4. Fraser broth
5. 225 ml

**ISOLATION**

- Oxford
- Palcam

[Diagram showing steps of enrichment and isolation]

**CONFIRMATION**

- TSYEA (35°C or 37°C for 18 or 24 h)

[Diagram showing steps of confirmation]

**Tests**

- Catalase
- Gram Stain (Gram -)
- Motility Broth (32°C for 48 h until 5 days if it is necessary)
- Haemolysis in Blood Agar n. 2 (32°C-24 ± 2 h)
- Rhamnose (30°C or 37°C until 5 days)
- Xylose (30°C or 37°C until 5 days)
- CAMP test in Blood Agar n. 2

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Note: This standard is being revised. The new version it is envisaged, will include as an isolation method a chromogenic medium, like ALCA Agar, or other lecithin based ones, and also another medium of the laboratories choice.
Yeasts and moulds EN-ISO 7954:1987
General guidance for enumeration of yeasts and moulds. Colony-count technique at 25°C.

Isolation medium
Chloramphenicol Glucose Agar, CGA (Yeast Extract Glucose Chloramphenicol Agar, YGC)
Mass inoculation technique
Incubation: 25°C with readings on day 3, 4 and 5

Confirmation
Microscopical observation if necessary

SAMPLE DILUTION   ISOLATION   CONFORMATION

1 ml  →  15 ml  →  Yeasts
1 ml  →  CGA

Penicillium or others
**Clostridium perfringens** EN-ISO 7937:2004 (UNE-EN 13401:2006)

Horizontal method for the enumeration of Clostridium perfringens. Colonies enumeration technique.

**Isolation medium**
Mass inoculation (poured-plate technique) in Sulfite-Cycloserine without Egg Yolk (SC)
(This was originally designated "Egg Yolk-free Tryptose Sulfite-Cycloserine without Egg Yolk", TSC)
**Incubation:** Under anaerobic conditions 37°C for 20 ± 2 h

**Confirmation: Two options**

**Option 1:**
- Lactose Sulfite Broth (LS)
  Incubation: Incubate anaerobically 46°C for 18-24 h in bath
  With prior inoculation in Thioglycollate Broth
  Incubation: Incubate anaerobically 37°C for 18-24 h

**Option 2:**
- Motility-Nitrate Medium
  Stab-inoculate colonies into freshly deaerated medium
  37°C for 24 h
  Incubation: Incubate anaerobically 37°C for 24 h
- Lactose-Gelatine Medium
  Incubation: Incubate anaerobically 38°C or 37°C for 24 h
  plus another 24 h to check Gelatin test, previous cooling 5°C for 1 h

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**SAMPLE DILUTION** | **ISOLATION** | **CONFIRMATION**
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1 ml | TSC | Option 1:
+ Lactose Sulfite Broth (LS)
Thioglycollate Broth
Option 2:
+ Motility-Nitrate Medium
Lactose Gelatin Medium

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Salmonella spp. EN-ISO 6579:2002
Horizontal method for the detection of Salmonella spp.

Pre-enrichment medium
Buffered Peptone Water
Incubation: 37°C for 18 ± 2 h

Enrichment media
Rappaport-Vassiliadis Soya Broth (RVS)
Incubation: 41,5°C for 24 ± 3 h

Isolation media
Isolation In Xylose Lysine Deoxycholate Agar (XLD)
and a second agar chosen by the laboratory
Incubation: 37°C for 24 ± 3 h

Confirmation
Biochemical Confirmation
Triple Sugar Iron Agar (TSI)
Urea Agar
Lysine Descarboxylation
β-galactosidase
Voges-Proskauer
Indole
Incubation: 37°C for 24 ± 3 h

Serological Confirmation
Antigens Confirmation O, H and Vi

<table>
<thead>
<tr>
<th>PRE-ENRICHMENT</th>
<th>ENRICHMENT</th>
<th>ISOLATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 g or 25 ml</td>
<td>0,1 ml</td>
<td>XLD and other second agar chosen by the laboratory</td>
</tr>
<tr>
<td>Buffered Peptone Water 225 ml</td>
<td>Rappaport-Vassiliadis Soya Broth</td>
<td>Brilliant Green Agar (BGA) Hektoen Agar Salmonella Shigella Agar (SS) Chromogenic</td>
</tr>
<tr>
<td>1 ml MKTTn</td>
<td></td>
<td>XLD and other second agar chosen by the laboratory</td>
</tr>
<tr>
<td></td>
<td>Brilliant Green Agar (BGA) Hektoen Agar Salmonella Shigella Agar (SS) Chromogenic</td>
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**CONFIRMATION**

- TSI Agar
- Urea Agar
- Lysine decarboxylation
- β-galactosidase
- Voges-Proskauer
- Indole
DIFFERENT TYPES OF CULTURE MEDIA

**Classification:**
Bacterial culture media can be classified in at least three ways; Based on consistency, based on nutritional component and based on its functional use.

1) **Classification based on consistency:**
Culture media are liquid, semi-solid or solid and biphasic.

A) **Liquid media:** These are available for use in test-tubes, bottles or flasks. Liquid media are sometimes referred as “broths” (e.g. nutrient broth). In liquid medium, bacteria grow uniformly producing general turbidity. Certain aerobic bacteria and those containing fimbriae (Vibrio & Bacillus) are known to grow as a thin film called 'surface pellicle' on the surface of undisturbed broth. *Bacillus anthracis* is known to produce stalactite growth on ghee containing broth. Sometimes the initial turbidity may be followed by clearing due to autolysis, which is seen in pneumococci. Long chains of Streptococci when grown in liquid media tend to entangle and settle to the bottom forming granular deposits. Liquid media tend to be used when a large number of bacteria have to be grown. These are suitable to grow bacteria when the numbers in the inoculum is suspected to be low. Inoculating in the liquid medium also helps to dilute any inhibitors of bacterial growth. This is the practical approach in blood cultures. Culturing in liquid medium can be used to obtain viable count (dilution methods). Properties of bacteria are not visible in liquid media and presence of more than one type of bacteria can not be detected.

B) **Solid media:** Any liquid medium can be rendered solid by the addition of certain solidifying agents. Agar agar (simply called agar) is the most commonly used solidifying agent. It is an unbranched polysaccharide obtained from the cell membranes of some species of red algae such as the genera *Gelidium*. Agar is composed of two long-chain polysaccharides (70% agarose and 30% a galactan). It melts at 95°C (sol) and solidifies at 42°C (gel), doesn’t contribute any nutritive property, it is not hydrolyzed by most bacteria and is usually free from growth promoting or growth retarding substances. However, it may be a source of calcium & organic ions. Most commonly, it is used at concentration of 1-3% to make a solid agar medium. New Zealand agar has more gelling capacity than the Japanese agar. Agar is available as fibres (shreds) or as Powders.

C) **Semi-solid agar:**
Reducing the amount of agar to 0.2-0.5% renders a medium semi-solid. Such media are fairly soft and are useful in demonstrating bacterial motility and separating motile from non-motile strains (U-tube and Cragie’s tube). Certain transport media such as Stuart’s and Amies media are semi-solid in consistency. Hugh & Leifson’s oxidation fermentation test medium as well as mannitol motility medium are also semi-solid.

D) **Biphasic media:**
Sometimes, a culture system comprises of both liquid and solid medium in
the same bottle. This is known as biphasic medium (Castaneda system for blood culture). The inoculum is added to the liquid medium and when subcultures are to be made, the bottle is simply tilted to allow the liquid to flow over the solid medium. This obviates the need for frequent opening of the culture bottle to subculture. Besides agar, egg yolk and serum too can be used to solidify culture media. While serum and egg yolk are normally liquid, they can be rendered solid by coagulation using heat. Serum containing medium such as Loeffler’s serum slope and egg containing media such as Lowenstein Jensen medium and Dorset egg medium are solidified as well as disinfected by a process of inspissation.

2) Classification based on nutritional component:
Media can be classified as simple, complex and synthetic (or defined). While most of the nutritional components are constant across various media, some bacteria need extra nutrients. Those bacteria that are able to grow with minimal requirements are said to non-fastidious and those that require extra nutrients are said to be fastidious. Simple media such as peptone water, nutrient agar can support most non-fastidious bacteria. Complex media such as blood agar have ingredients whose exact components are difficult to estimate. Synthetic or defined media such as Davis & Mingioli medium are specially prepared media for research purposes where the composition of every component is well known.

COMPLEX MEDIA.
Pasteur, Koch, and Tyndall typically prepared media by boiling animal or plant materials to extract nutritive molecules. Today, many modern complex media (such as Tryptic Soy Agar) contain extracts of beef (peptone), milk (tryptone), soybean meal (soytone), or yeast. The protein in these extracts are broken down into small peptides and amino acids. Although the specific amount of these molecules is not precisely determined, their wide assortment allows complex media to support a wide range of bacterial types.

DEFINED MEDIA.
Defined media are formulated from pure substances at predetermined concentrations. Thus, unlike complex media, the exact chemical composition of defined media is known precisely. Because the composition is precisely established, defined media are often used to determine the nutritional requirements of bacterial species.

SELECTIVE MEDIA. Complex or defined media may also be classified as selective (or ENRICHMENT) media, which support the growth of only certain types of bacteria. Media can be made selective through the addition of substances that enhance or inhibit the growth of particular types of bacteria. Media have been developed that are selective for an astonishing diversity of bacteria, and we will be using many of these media throughout the semester.

DIFFERENTIAL MEDIA.
Any of the above types of media might also be formulated as a differential medium. A differential medium reveals specific metabolic or metabolic characteristics of bacteria grown on it. Differential media are among the most powerful tools available to a microbiologist, revealing a wide range of information about an organism very quickly. Some media are both selective and
3) Classification based on functional use or application:

These include basal media, enriched media, selective/enrichment media, indicator/differential media, transport media and holding media.

A) **Basal media** are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar considered basal medium.

B) **Enriched media**: Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes them enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler’s serum slope etc are few of the enriched media.

C) **Selective and enrichment media** are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by addition of certain inhibitory agents that don’t affect the pathogen. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

D) **Enrichment media** are liquid media that also serves to inhibit commensals in the clinical specimen. Selenite F broth, tetrathionate broth and alkaline peptone water are used to recover pathogens from fecal specimens.

E) **Differential media or indicator media**: Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Examples: MacConkey’s agar, CLED agar, TCBS agar, XLD agar etc.

F) **Transport media**: Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart’s & Amie’s) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors. Cary Blair medium and Venkatraman Ramakrishnan medium are used to transport feces from suspected cholera patients. Sach’s buffered glycerol saline is used to transport feces from patients suspected to be suffering from bacillary dysentery.

G) **Anaerobic media**: Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation-reduction potential and extra nutrients. Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings can render a medium reduced. Robertson cooked meat that is commonly used to grow *Clostridium* spp medium contain a 2.5 cm column of bullock heart meat and 15 ml of nutrient broth. Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin. Methylene blue or
resazurin is an oxidation-reduction potential indicator that is incorporated in the thioglycollate medium. Under reduced condition, methylene blue is colourless.

FOR USE IN FOOD MICROBIOLOGY
While some media are good for isolation of bacteria in food. The same media cannot be used for the isolation of fungi. Essentially, microbiological culture media consist of Nitrogen source e.g. protein, amino acid etc. Water source which has to be pure, an energy source in form of carbohydrate and peptide and necessary growth factors like mineral salts and group B vitamins.

TYPES OF CULTURE MEDIA
On the basis of the essential component micro biological culture media can be grouped as follows;

- **GENERAL-PURPOSE CULTURE MEDIA**
  These are media that provide nutrition for growth of non-fastidious, heterotrophic microorganisms, such media include, Nutrient agar, blood agar and plate count agar for bacteria, or malt extract agar and potato/dextrose agar for fungi. These media do not contain any inhibitory agent however, they may not support the growth of fastidious organisms

- **ENRICHMENT CULTURE MEDIA**: these are formulated to stimulate the growth of certain organism by appropriate selection of condition e.g. Rapaport – vassilliade broth, selenite F. broth, Tetrathionate broth.

- **ELECTIVE CULTURE MEDIA**
  These groups of media satisfy the minimum nutritional requirement for growth for e.g. Lysine agar.

- **SELECTIVE CULTURE MEDIA**
  This group of culture media select certain organisms while at the same time inhibit the growth of others e.g. Salmonella- Shigella medium good for isolation of Salmonella in food, Preston selective medium good for *Campylobacter* in food.

- **DIAGNOSTIC CULTURE MEDIUM**
  This type of culture medium uses specific metabolic activity which is associated with the growth of some organism to produce recognizable changes in the medium for e.g. STARCH agar.

- **SELECTIVE AND DIAGNOSTIC (DIFFERENTIAL)**
  This type of culture medium differentiate one organism relative to all other organism present on the culture medium for e.g. on the basis of colour as obtained in MacConkey agar. This medium also utilizes lactose fermentation in the presence of a suitable indicator. On the basis of lactose fermentation some organism are reddish in colour i.e. lactose fermenters. Non-lactose fermenters appear as pale or colourless colonies.

CULTURING METABOLICALLY INJURED ORGANISM
When microorganisms are subjected to environmental stresses as in thermal processing many of the individual cell are known to undergo metabolic injury which can lead to their not being able to
be isolated on ordinary culture media. In order to be able to isolate these microorganisms it is necessary to pre-enriched them prior to isolation and for this purpose, it is recommended that non-selective culture media be used. Generally, the use of TSB (Trypticase Soy broth) incubated at 20-37°C for about 1-24 hours is ideal for isolation of yeast that has undergone sub-lethal heating processing, potato dextrose agar (PDA) at pH 8.0 is recommended for use.

**FAST METHODS IN USE FOR FOOD MICROBIOLOGY**

Because of improvement in biotechnology it is now possible to identify an unknown microorganism in food in relatively short period of time. Some of the methods are used at the molecular level and they can also be applied for routine use where the economy is buoyant e.g. of these methods include the following:

1. **Immunoassay**: whereby the organism is fluorescence labeled and can be identified by colour changes (fluorescence labeling).
2. **Other methods**
   - (a) Diagnostic kits e.g. for isolation of *Staph. aureus* kits such as the API system and OXI/FERM tubes (entero tubes) are used.
   - (b) Use of monoclonal antibody in ELISA (Enzyme linked immunosorbent assay).
3. **DNA probes**: in the DNA probe the bacteria are applied to a solid support, such as a nitrocellulose membrane filter, by spotting of filtration.

Thereafter the organisms are (a) Lysed and disrupted resulting in the release of double stranded DNA. (b) Denatured into single strand DNA that has been separated in form of strands is now (c) fixed to the solid support so that they would not be washed away during the next procedure. The strands are now (d) incubated in suitable fluids, after which the appropriate probe is mixed with the DNA on the filter. Unbound protein is washed away, and X-ray film of the preparation can be taken for comparative purposes (analysis). Radioactive colonies will appear as black dust on the film.

1. **Limulus Lysate assay**—this test is specifically useful for endotoxin and as such it is being adopted for use in detecting Gram –ve bacteria in food. For this test, aliquot part of the food suspension or any other small amount of the given material is added to the lysate after the preparation is incubated at 37°C for 1 hour. If there is endotoxin, there will be Gel formation.
2. **Use the Oxi/ferm tubes** – based in argnine, nitrogen gas hydrogen sulphide, xylose, AER-DEX, Urea citrate etc.

**FOOD FUNGI**

Like Bacteria, morphological characters are useful in the study of fungi. By far the easiest way to identify fungi in food is by using cultural technique on acidified media like – malt extract agar, potato dextrose agar or sabouraud dextrose agar. Food fungi are divided into yeast and mould and the study of these microorganisms are referred to as FOOD MYCOLOGY.

Like bacteria, fungi in food require nutrient, moisture, adequate temp. and atmosphere, as well as H+ concentration (pH) for survival in food.

Although some fungi are beneficial to man in terms of production of enzyme and by being used for food (edible fungi) quite a lot of them are known to spoil food matrices.
Yeasts on their own are larger than bacteria. They are single organism, which can be microscopically recognized.

(1) They are saprophytic in nature and can be found in dust, soil, and several other places.
(2) Industrially, yeast is important in the production of beer and several other alcoholic beverages.
(3) They are also useful in production of wine and also baking production.
(4) They are important in production of proteins fats and vitamins.
(5) They are important in production of Enzymes such as amylases.
(6) They can be use as food by man and animal.

Structurally, in the simplest form, a yeast cell has an outer cell wall made up of complex polysaccharides and a cell membrane all of which function in the same way as a bacterial cell.

MOULDS

Macromorphologically, the moulds are composed essentially of multifaceted hyphace (complex collection of hypha referred to as mycelium). These hyphae are thread like structure and serves as sources of nourishment for microorganism.

Some mould may be septated (i.e. have septae) while some do not have.

IMPORTANCE OF MOULDS

Generally,

(1) Moulds are very important in the shelf-life of grains, meats etc that are meant for storage,
(2) Some are very pathogenic for man and animal because they produce potent toxins such as aflatoxin, which are very lethal to man and animal – these toxins are referred to as mycotoxin. However, some moulds grow without much problem that is they require mineral nutrient.
(3) Some mould are also important in the production of some food products e.g. cheese e.g. Penicillium.
(4) Use in commercial production of proteases and acetic acid e.g. Aspergillus.
(5) Use in the production of antibiotics e.g. Penicillin, and conversion of starch to alcohol e.g. Rhizopus.
(6) Some moulds are very useful in the assay of some essential vitamins.

SOME METHOD OF PREVENTING FUNGAL DAMAGE

The following methods are recommended to minimize fungal damage of: -

(1) Harvest the crop when they are fully matured.
(2) Dry the grains as quickly as possible to a suitable moisture content level.
(3) Avoid bruises or physical damage to the grains at all stages of handling the grains.
(4) It is essential to check the moisture content before storage, particularly if they are meant to be stored for a long time.
(5) Avoid storing warm grain as this may retain heat and leads to mouldiness.
(6) Ensure that all the storage houses are in good shape particularly if the roofs are leaking to be mended.
(7) In storing the produce, the bag should be stored always away from the wall of the storehouse.
(8) Ensure good ventilation and even temp. in the store house. If polyethene bags are to be used for storing purposes they should be resistant to insect damage and as much as possible, contamination by insects, rodents and flies should be minimized. It is recommended that loading and off-loading of produce during the raining season should be carefully done. In fact if vehicles are to be used strong tarpaulin should be used to cover the vehicle. However, it is generally advisable not to load or unload during the raining season.

Factors that Influence Microbial Growth

Many factors must be evaluated for each specific food when making decisions on whether it needs time/temperature control for safety. These can be divided into intrinsic and extrinsic factors. Intrinsic factors are those that are characteristic of the food itself; extrinsic factors are those that refer to the environment surrounding the food. The need for time/temperature control is primarily determined by 1) the potential for contamination with pathogenic microorganisms of concern (including processing influences), and 2) the potential for subsequent growth and/or toxin production.

Most authorities are likely to divide foods among three categories based on an evaluation of the factors described below: those that do not need time/temperature control for protection of consumer safety; those that need time/temperature control; and those where the exact status is questionable. In the case of questionable products, further scientific evidence--such as modeling of microbial growth or death, or actual microbiological challenge studies--may help to inform the decision.

2. Intrinsic factors

2.1. Moisture content

Microorganisms need water in an available form to grow in food products. The control of the moisture content in foods is one of the oldest exploited preservation strategies. Food microbiologists generally describe the water requirements of microorganisms in terms of the water activity ($a_w$) of the food or environment. Water activity is defined as the ratio of water vapor pressure of the food substrate to the vapor pressure of pure water at the same temperature

$$a_w = \frac{p}{p_0}$$

where $p =$ vapor pressure of the solution and $p_0 =$ vapor pressure of the solvent (usually water). The $a_w$ of pure water is 1.00 and the $a_w$ of a completely dehydrated food is 0.00. The $a_w$ of a food on this scale from 0.00 - 1.00 is related to the equilibrium relative humidity above the food on a scale of 0 - 100%. Thus, % Equilibrium Relative Humidity (ERH) = $a_w \times 100$. The $a_w$ of a food
describes (a) the degree to which water is "bound" in the food, (b) its availability to participate in chemical/biochemical reactions, and (c) its availability to facilitate growth of microorganisms.

Most fresh foods, such as fresh meat, vegetables, and fruits, have $a_w$ values that are close to the optimum growth level of most microorganisms (0.97 - 0.99). Table 1 shows the approximate $a_w$ levels of some common food categories. The $a_w$ can be manipulated in foods by a number of means, including addition of solutes such as salt or sugar, physical removal of water through drying or baking, or binding of water to various macromolecular components in the food. Weight for weight, these food components will decrease $a_w$ in the following order: ionic compounds > sugars, polyhydric alcohols, amino acids and other low-molecular-weight compounds > high-molecular-weight compounds such as cellulose, protein or starch.

Microorganisms respond differently to $a_w$ depending on a number of factors. Microbial growth, and, in some cases, the production of microbial metabolites, may be particularly sensitive to alterations in $a_w$. Microorganisms generally have optimum and minimum levels of $a_w$ for growth depending on other growth factors in their environments. One indicator of microbial response is their taxonomic classification. For example, Gram (-) bacteria are generally more sensitive to low $a_w$ than Gram (+) bacteria.

Table 2 lists the approximate minimum $a_w$ values for the growth of selected microorganisms relevant to food. It should be noted that many bacterial pathogens are controlled at water activities well above 0.86 and only S. aureus can grow and produce toxin below $a_w$ 0.90. It must be emphasized that these are approximate values because solutes can vary in their ability to inhibit microorganisms at the same $a_w$ value. To illustrate, the lower $a_w$ limit for the growth of Clostridium botulinum type A has been found to be 0.94 with NaCl as the solute versus 0.92 with glycerol as the solute. When formulating foods using $a_w$ as the primary control mechanism for pathogens, it is useful to employ microbiological challenge testing to verify the effectiveness of the reduced $a_w$ when target $a_w$ is near the growth limit for the organism of concern.

<table>
<thead>
<tr>
<th>Animal Products</th>
<th>$a_w$</th>
</tr>
</thead>
<tbody>
<tr>
<td>fresh meat, poultry, fish</td>
<td>0.99 - 1.00</td>
</tr>
<tr>
<td>natural cheeses</td>
<td>0.95 - 1.00</td>
</tr>
<tr>
<td>Pudding</td>
<td>0.97 - 0.99</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.97</td>
</tr>
<tr>
<td>cured meat</td>
<td>0.87 - 0.95</td>
</tr>
<tr>
<td>sweetened condensed milk</td>
<td>0.83</td>
</tr>
<tr>
<td>Parmesan cheese</td>
<td>0.68 - 0.76</td>
</tr>
<tr>
<td>Honey</td>
<td>0.75</td>
</tr>
<tr>
<td>dried whole egg</td>
<td>0.40</td>
</tr>
<tr>
<td>dried whole milk</td>
<td>0.20</td>
</tr>
</tbody>
</table>
### Table 2. Approximate \(a_w\) values for growth of selected pathogens in food.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Minimum</th>
<th>Optimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em> <em>spp.</em></td>
<td>0.98</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium botulinum</em> type E*</td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Shigella</em> <em>spp.</em></td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vibrio vulnificus</em></td>
<td>0.96</td>
<td>0.98</td>
<td>0.99</td>
</tr>
<tr>
<td>Enterohemorrhagic <em>Escherichia coli</em></td>
<td>0.95</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> <em>spp.</em></td>
<td>0.94</td>
<td>0.99</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>
2.2. pH and acidity

Increasing the acidity of foods, either through fermentation or the addition of weak acids, has been used as a preservation method since ancient times. In their natural state, most foods such as meat, fish, and vegetables are slightly acidic while most fruits are moderately acidic. A few foods such as egg white are alkaline. Table 3 lists the pH ranges of some common foods. The pH is a function of the hydrogen ion concentration in the food:

\[ \text{pH} = -\log_{10} [\text{H}^+] \]

(a) \( pK_a \): Another useful term relevant to the pH of foods is the \( pK_a \). The term \( pK_a \) describes the state of dissociation of an acid. At equilibrium, \( pK_a \) is the pH at which the concentrations of dissociated and un-dissociated acid are equal. Strong acids have a very low \( pK_a \), meaning that they are almost entirely dissociated in solution. For example, the pH (at 25 °C [77 °F]) of a 0.1 M solution of HCl is 1.08 compared to the pH of 0.1 M solution of acetic acid, which is 2.6. This characteristic is extremely important when using acidity as a preservation method for foods. Organic acids are more effective as preservatives in the undissociated state. Lowering the pH of a food increases the effectiveness of an organic acid as a preservative. Table 4 lists the proportion of total acid undissociated at different pH values for selected organic acids. The type of organic acid employed can dramatically influence the microbiological keeping quality and safety of the food.

It is well known that groups of microorganisms have \( \text{pH optimum, minimum, and maximum} \) for growth in foods. Table 5 lists the approximate pH ranges for growth in laboratory media for selected organisms relevant to food. As with other factors, pH usually interacts with other parameters in the food to inhibit growth. The pH can interact with factors such as \( a_w \), salt, temperature, redox potential, and preservatives to inhibit growth of pathogens and other organisms. The pH of the food also significantly impacts the lethality of heat treatment of the food. Less heat is needed to inactivate microbes as the pH is reduced.

(b) Buffering capacity: Another important characteristic of a food to consider when using acidity as a control mechanism is its buffering capacity. The buffering capacity of a food is its ability to resist changes in pH. Foods with a low buffering capacity will change pH...
quickly in response to acidic or alkaline compounds produced by microorganisms as they grow. Meats, in general, are more buffered than vegetables by virtue of their various proteins.

(c) **Titratable acidity (TA)** is a better indicator of the microbiological stability of certain foods, such as salad dressings, than is pH. Titratable acidity is a measure of the quantity of standard alkali (usually 0.1 M NaOH) required to neutralize an acid solution. It measures the amount of hydrogen ions released from undissociated acid during titration. Titratable acidity is a particularly useful measure for highly buffered or highly acidic foods. Weak acids (such as organic acids) are usually undissociated and, therefore, do not directly contribute to pH. Titratable acidity yields a measure of the total acid concentration, while pH does not, for these types of foods.

<table>
<thead>
<tr>
<th>Food</th>
<th>pH Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dairy Products</strong></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>6.1 - 6.4</td>
</tr>
<tr>
<td>Buttermilk</td>
<td>4.5</td>
</tr>
<tr>
<td>Milk</td>
<td>6.3 - 6.5</td>
</tr>
<tr>
<td>Cream</td>
<td>6.5</td>
</tr>
<tr>
<td>Cheese (American mild and cheddar)</td>
<td>4.9; 5.9</td>
</tr>
<tr>
<td>Yogurt</td>
<td>3.8 - 4.2</td>
</tr>
<tr>
<td><strong>Meat and Poultry</strong></td>
<td></td>
</tr>
<tr>
<td>Beef (ground)</td>
<td>5.1 - 6.2</td>
</tr>
<tr>
<td>Ham</td>
<td>5.9 - 6.1</td>
</tr>
<tr>
<td>Veal</td>
<td>6.0</td>
</tr>
<tr>
<td>Chicken</td>
<td>6.2 - 6.4</td>
</tr>
<tr>
<td><strong>Fish and Shellfish</strong></td>
<td></td>
</tr>
<tr>
<td>Fish (most species)</td>
<td>6.6 - 6.8</td>
</tr>
<tr>
<td>Clams</td>
<td>6.5</td>
</tr>
<tr>
<td>Crabs</td>
<td>7.0</td>
</tr>
<tr>
<td>Oysters</td>
<td>4.8 - 6.3</td>
</tr>
<tr>
<td>Tuna Fish</td>
<td>5.2 - 6.1</td>
</tr>
<tr>
<td>Shrimp</td>
<td>6.8 - 7.0</td>
</tr>
<tr>
<td>Salmon</td>
<td>6.1 - 6.3</td>
</tr>
<tr>
<td>White Fish</td>
<td>5.5</td>
</tr>
<tr>
<td><strong>Fruits and Vegetables</strong></td>
<td></td>
</tr>
<tr>
<td>Apples</td>
<td>2.9 - 3.3</td>
</tr>
<tr>
<td>Apple Cider</td>
<td>3.6 - 3.8</td>
</tr>
</tbody>
</table>

Table 3: pH ranges of some common foods.
<table>
<thead>
<tr>
<th>Item</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bananas</td>
<td>4.5 - 4.7</td>
</tr>
<tr>
<td>Figs</td>
<td>4.6</td>
</tr>
<tr>
<td>Grapefruit (juice)</td>
<td>3.0</td>
</tr>
<tr>
<td>Limes</td>
<td>1.8 - 2.0</td>
</tr>
<tr>
<td>Honeydew melons</td>
<td>6.3 - 6.7</td>
</tr>
<tr>
<td>Oranges (juice)</td>
<td>3.6 - 4.3</td>
</tr>
<tr>
<td>Plums</td>
<td>2.8 - 4.6</td>
</tr>
<tr>
<td>Watermelons</td>
<td>5.2 - 5.6</td>
</tr>
<tr>
<td>Grapes</td>
<td>3.4 - 4.5</td>
</tr>
<tr>
<td>Asparagus (buds and stalks)</td>
<td>5.7 - 6.1</td>
</tr>
<tr>
<td>Beans (string and lima)</td>
<td>4.6 - 6.5</td>
</tr>
<tr>
<td>Beets (sugar)</td>
<td>4.2 - 4.4</td>
</tr>
<tr>
<td>Broccoli</td>
<td>6.5</td>
</tr>
<tr>
<td>Brussels Sprouts</td>
<td>6.3</td>
</tr>
<tr>
<td>Cabbage (green)</td>
<td>5.4 - 6.0</td>
</tr>
<tr>
<td>Carrots</td>
<td>4.9 - 5.2; 6.0</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>5.6</td>
</tr>
<tr>
<td>Celery</td>
<td>5.7 - 6.0</td>
</tr>
<tr>
<td>Corn (sweet)</td>
<td>7.3</td>
</tr>
<tr>
<td>Cucumbers</td>
<td>3.8</td>
</tr>
<tr>
<td>Eggplant</td>
<td>4.5</td>
</tr>
<tr>
<td>Eggs yolks (white)</td>
<td>6.0 - 6.3 (7.6- 9.5)</td>
</tr>
<tr>
<td>Lettuce</td>
<td>6.0</td>
</tr>
<tr>
<td>Olives (green)</td>
<td>3.6 - 3.8</td>
</tr>
<tr>
<td>Onions (red)</td>
<td>5.3 - 5.8</td>
</tr>
<tr>
<td>Parsley</td>
<td>5.7 - 6.0</td>
</tr>
<tr>
<td>Parsnip</td>
<td>5.3</td>
</tr>
<tr>
<td>Potatoes (tubers and sweet)</td>
<td>5.3 - 5.6</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>4.8 - 5.2</td>
</tr>
<tr>
<td>Rhubarb</td>
<td>3.1 - 3.4</td>
</tr>
<tr>
<td>Spinach</td>
<td>5.5 - 6.0</td>
</tr>
<tr>
<td>Produce</td>
<td>pH</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Squash</td>
<td>5.0 - 5.4</td>
</tr>
<tr>
<td>Tomatoes (whole)</td>
<td>4.2 - 4.3</td>
</tr>
<tr>
<td>Turnips</td>
<td>5.2 - 5.5</td>
</tr>
</tbody>
</table>

Table 4. Proportion of total acid undissociated at different pH values (expressed as percentages).

<table>
<thead>
<tr>
<th>Organic Acids</th>
<th>pH Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>98.5</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>93.5</td>
</tr>
<tr>
<td>Citric acid</td>
<td>53.0</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>86.6</td>
</tr>
<tr>
<td>Methyl, ethyl, propyl parabens</td>
<td>&gt;99.99</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>98.5</td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>97.4</td>
</tr>
</tbody>
</table>

Table 5. Approximate pH values permitting the growth of selected pathogens in food.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Minimum</th>
<th>Optimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>5.5 - 5.8</td>
<td>7.2</td>
<td>8.0 - 9.0</td>
</tr>
<tr>
<td><em>Vibrio vulnificus</em></td>
<td>5.0</td>
<td>7.8</td>
<td>10.2</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>4.9</td>
<td>6.0 - 7.0</td>
<td>8.8</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>4.9</td>
<td>6.5 - 7.5</td>
<td>9.0</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>4.9</td>
<td></td>
<td>9.3</td>
</tr>
<tr>
<td><em>Vibrio paraahaemolyticus</em></td>
<td>4.8</td>
<td>7.8 - 8.6</td>
<td>11.0</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em> toxin</td>
<td>4.6</td>
<td></td>
<td>8.5</td>
</tr>
<tr>
<td><em>Clostridium botulinum</em> growth</td>
<td>4.6</td>
<td></td>
<td>8.5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> growth</td>
<td>4.0</td>
<td>6.0 - 7.0</td>
<td>10.0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> toxin</td>
<td>4.5</td>
<td>7.0 - 8.0</td>
<td>9.6</td>
</tr>
</tbody>
</table>
Enterohemorrhagic *Escherichia coli* | 4.4 | 6.0-7.0 | 9.0  
Listeria monocytogenes | 4.39 | 7.0 | 9.4  
*Salmonella* spp. | 4.2\(^1\) | 7.0-7.5 | 9.5  
*Yersinia enterocolitica* | 4.2 | 7.2 | 9.6

\(^1\)pH minimum as low as 3.8 has been reported when acidulants other than acetic acid or equivalent are used.

### 2.3. Nutrient content

Microorganisms require certain basic nutrients for growth and maintenance of metabolic functions. The amount and type of nutrients required range widely depending on the microorganism. These nutrients include *water, a source of energy, nitrogen, vitamins, and minerals*.

Varying amounts of these nutrients are present in foods. Meats have abundant protein, lipids, minerals, and vitamins. Most muscle foods have low levels of carbohydrates. Plant foods have high concentrations of different types of carbohydrates and varying levels of proteins, minerals, and vitamins. Foods such as milk and milk products and eggs are rich in nutrients.

**Amino acids** serve as a source of nitrogen and energy and are utilized by most microorganisms. Some microorganisms are able to metabolize peptides and more complex proteins. Other sources of nitrogen include, for example, urea, ammonia, creatinine, and methylamines.

**Examples of minerals** required for microbial growth include phosphorus, iron, magnesium, sulfur, manganese, calcium, and potassium. In general, small amounts of these minerals are required; thus a wide range of foods can serve as good sources of minerals.

In general, the Gram (+) bacteria are more fastidious in their nutritional requirements and thus are not able to synthesize certain nutrients required for growth. For example, the Gram (+) foodborne pathogen *S. aureus* requires amino acids, thiamine, and nicotinic acid for growth. Fruits and vegetables that are deficient in B vitamins do not effectively support the growth of these microorganisms. The Gram (-) bacteria are generally able to derive their basic nutritional requirements from the existing carbohydrates, proteins, lipids, minerals, and vitamins that are found in a wide range of food.

### 2.4. Biological structure

Plant and animal derived foods, especially in the raw state, have biological structures that may prevent the entry and growth of pathogenic microorganisms. Examples of such physical barriers include *testa of seeds, skin of fruits and vegetables, shell of nuts, animal hide, egg cuticle, shell, and membranes*. 
Plant and animal foods may have pathogenic microorganisms attached to the surface or trapped within surface folds or crevices. Intact biological structures thus can be important in preventing entry and subsequent growth of microorganisms. Several factors may influence penetration of these barriers.

- The maturity of plant foods will influence the effectiveness of the protective barriers.
- Physical damage due to handling during harvest, transport, or storage, as well as
- Invasion of insects can allow the penetration of microorganisms.
- During the preparation of foods, processes such as slicing, chopping, grinding, and shucking will destroy the physical barriers.

Thus, the interior of the food can become contaminated and growth can occur depending on the intrinsic properties of the food. For example, *Salmonella* spp. have been shown to grow on the interior of portions of cut cantaloupe, watermelon, honeydew melons and tomatoes given sufficient time and temperature.

Fruits are an excellent example of the kind of food with good potential for pathogenic microorganisms to penetrate intact barriers. After harvest, pathogens will survive but usually not grow on the outer surface of fresh fruits and vegetables. Growth on intact surfaces is not common because foodborne pathogens do not produce the enzymes necessary to break down the protective outer barriers on most produce. This outer barrier restricts the availability of nutrients and moisture. Survival of foodborne pathogens on produce is significantly enhanced once the protective epidermal barrier has been broken either by physical damage, such as punctures or bruising, or by degradation by plant pathogens (bacteria or fungi). These conditions can also promote the multiplication of pathogens, especially at higher temperatures.

The egg is another good example of an effective biological structure that, when intact, will prevent external microbial contamination of the perishable yolk; contamination is possible, however, through transovarian infection. When there are cracks through the inner membrane of the egg, microorganisms penetrate into the egg. Factors such as temperature of storage, relative humidity, age of eggs, and level of surface contamination will influence internalization.

Heating of food as well as other types of processing will break down protective biological structures and alter such factors as pH and aw. These changes could potentially allow the growth of microbial pathogens.

### 2.5. Redox potential

*The oxidation-reduction or redox potential of a substance is defined in terms of the ratio of the total oxidizing (electron accepting) power to the total reducing (electron donating) power of the substance. In effect, redox potential is a measurement of the ease by which a substance gains or loses electrons.* The redox potential (Eh) is measured in terms of millivolts. A fully oxidized standard oxygen electrode will have an Eh of +810 mV at pH 7.0, 30 °C (86 °F), and under the same conditions, a completely reduced standard hydrogen electrode will have an Eh of -420 mV. *The Eh is dependent on the pH of the substrate*; normally the Eh is taken at pH 7.0.
The major groups of microorganisms based on their relationship to Eh for growth are aerobes, anaerobes, facultative aerobes, and microaerophiles. Examples of foodborne pathogens for each of these classifications include *Aeromonas hydrophila*, *Clostridium botulinum*, *Escherichia coli* O157:H7, and *Campylobacter jejuni*, respectively. Generally, the range at which different microorganisms can grow are as follows: aerobes +500 to +300 mV; facultative anaerobes +300 to -100 mV; and anaerobes +100 to less than -250 mV. For example, *C. botulinum* is a strict anaerobe that requires an Eh of less than +60 mV for growth; however, slower growth can occur at higher Eh values. The relationship of Eh to growth can be significantly affected by the presence of salt and other food constituents. The measured Eh values of various foods are given in Table 6. These values can be highly variable depending on changes in the pH of the food, microbial growth, packaging, the partial pressure of oxygen in the storage environment, and ingredients and composition (protein, ascorbic acid, reducing sugars, oxidation level of cations, and so on).

Another important factor is the poising capacity of the food. Poising capacity, which is analogous to buffering capacity, relates to the extent to which a food resists external affected changes in Eh. The poising capacity of the food will be affected by oxidizing and reducing constituents in the food as well as by the presence of active respiratory enzyme systems.

<table>
<thead>
<tr>
<th>Table 6. Redox potentials on some foods.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOOD</td>
</tr>
<tr>
<td>----------------------------</td>
</tr>
<tr>
<td>Milk</td>
</tr>
<tr>
<td>Cheese</td>
</tr>
<tr>
<td>Cheddar</td>
</tr>
<tr>
<td>Dutch</td>
</tr>
<tr>
<td>Emmenthal</td>
</tr>
<tr>
<td>Butter serum</td>
</tr>
<tr>
<td>Egg (infertile after 14 d)</td>
</tr>
<tr>
<td>Meats</td>
</tr>
<tr>
<td>Liver, raw minced</td>
</tr>
<tr>
<td>Muscle</td>
</tr>
<tr>
<td>Raw, post-rigor</td>
</tr>
<tr>
<td>Raw, minced</td>
</tr>
<tr>
<td>Minced, cooked</td>
</tr>
<tr>
<td>Cooked sausages and canned meat</td>
</tr>
<tr>
<td>Cereals</td>
</tr>
<tr>
<td>Wheat (whole grain)</td>
</tr>
<tr>
<td>Wheat (germ)</td>
</tr>
<tr>
<td>Barley (ground)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td><strong>Plant juices</strong></td>
</tr>
<tr>
<td>Grape</td>
</tr>
<tr>
<td>Lemon</td>
</tr>
<tr>
<td>Pear</td>
</tr>
<tr>
<td>Spinach</td>
</tr>
<tr>
<td><strong>Canned foods</strong></td>
</tr>
<tr>
<td>&quot;Neutral&quot;</td>
</tr>
<tr>
<td>&quot;Acid&quot;</td>
</tr>
</tbody>
</table>

NR = Not reported.

### 2.6. Naturally occurring and added antimicrobials

Some foods intrinsically contain naturally-occurring antimicrobial compounds that convey some level of microbiological stability to them. There are a number of plant-based antimicrobial constituents, including many **essential oils**, **tannins**, **glycosides**, and **resins**, that can be found in certain foods. Specific examples include eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allyl isothiocyanate in mustard, eugenol and thymol in sage, and carvacrol (isothymol) and thymol in oregano. Other plant-derived antimicrobial constituents include the phytoalexins and the lectins. Lectins are proteins that can specifically bind to a variety of polysaccharides, including the glycoproteins of cell surfaces. Through this binding, lectins can exert a slight antimicrobial effect. The usual concentration of these compounds in formulated foods is relatively low, so that the antimicrobial effect alone is slight. However, these compounds may produce greater stability in combination with other factors in the formulation.

Some animal-based foods also contain antimicrobial constituents. Examples include **lactoferrin**, **conglutinin** and the **lactoperoxidase system** in cow's milk, **lysozyme** in eggs and milk, and other factors in fresh meat, poultry and seafood. Lysozyme is a small protein that can hydrolyze the cell wall of bacteria. The lactoperoxidase system in bovine milk consists of three distinct components that are required for its antimicrobial action: lactoperoxidase, thiocyanate, and hydrogen peroxide.

It is also known that **some types of food processing** result in the formation of antimicrobial compounds in the food. The smoking of fish and meat can result in the deposition of antimicrobial substances onto the product surface. **Maillard compounds** resulting from condensation reactions between sugars and amino acids or peptides upon heating of certain foods can impart some antimicrobial activity. Smoke condensate includes phenol, which is not only an antimicrobial, but also lowers the surface pH. Some processors also lower the surface pH with liquid smoke to achieve an unsliced shelf-stable product.

**Some types of fermentations** can result in the natural production of antimicrobial substances, including **bacteriocins**, **antibiotics**, and **other related inhibitors**. Bacteriocins are proteins or peptides that are produced by certain strains of bacteria that inactivate other, usually closely-related, bacteria. The most commonly characterized bacteriocins are those produced by the
lactic acid bacteria. The antibiotic nisin produced by certain strains of *Lactococcus lactis* is one of the best characterized of the bacteriocins. Nisin is approved for food applications in over 50 countries around the world. Nisin's first food application was to prevent late-blowing in Swiss cheese by *Clostridium butyricum*. Nisin is a polypeptide that is effective against most Gram (+) bacteria but is ineffective against Gram (-) organisms and fungi. Nisin can be produced in the food by starter cultures or, more commonly, it can be used as an additive in the form of a standardized preparation. Nisin has been used to effectively control spore-forming organisms in processed cheese formulations, and has been shown to have an interactive effect with heat.

In addition to *naturally-occurring antimicrobial compounds* in foods, a variety of chemical preservatives and additives can extend the shelf life of food and/or inhibit pathogens, either singly or in combination. Added antimicrobial compounds can have an interactive or synergistic effect with other parameters of the formulation. One example is the interaction with pH. Many preservatives have an optimum pH range for effectiveness. Other factors include $a_w$, presence of other preservatives, types of food constituents, presence of certain enzymes, processing temperature, storage atmosphere, and partition coefficients. The effective use of combinations of preservatives with other physico-chemical parameters of a food formulation can stabilize that food against spoilage organisms or pathogens.

### 2.7. Competitive microflora

The potential for microbial growth of pathogens in temperature-sensitive foods depends on the combination of the intrinsic and extrinsic factors, and the processing technologies that have been applied. Within the microbial flora in a food, there are many important biological attributes of individual organisms that influence the species that predominates. These include: (a) the individual growth rates of the microbial strains (b) competition, (c) effects of growth inhibition and the mutual interactions or influences among species in mixed populations.

#### I. Growth

In a food environment, an organism grows in a characteristic manner and at a characteristic rate. The length of the lag phase, generation time, and total cell yield are determined by genetic factors. Accumulation of metabolic products may limit the growth of particular species. If the limiting metabolic product can be used as a substrate by other species, these may take over (partly or wholly), creating an association or succession. Due to the complex of continuing interactions between environmental factors and microorganisms, a food at any one point in time has a characteristic flora, known as its association. The microbial profile changes continuously and one association succeed another in what is called succession. Many examples of this phenomenon have been observed in the microbial deterioration and spoilage of foods.

As long as metabolically active organisms remain, they continue to interact, so that dominance in the flora occurs as a dynamic process. Based on their growth-enhancing or inhibiting nature, these interactions are either antagonistic or synergistic.
II. Competition

In food systems, antagonistic processes usually include competition for nutrients, competition for attachment/adhesion sites (space), unfavorable alterations of the environment, and a combination of these factors. An example of this phenomenon is raw ground beef. Even though *S. aureus* is often found in low numbers in this product, staphylococcal enterotoxin is not produced. The reason is that the *Pseudomonas-Acinetobacter-Moraxella* association that is always present in this food grows at a higher rate, outgrowing the staphylococci.

Organisms of high metabolic activity may consume required nutrients, selectively reducing these substances, and inhibiting the growth of other organisms. Depletion of oxygen or accumulation of carbon dioxide favors facultative obligate anaerobes which occur in vacuum-packaged fresh meats held under refrigeration.

Staphylococci are particularly sensitive to nutrient depletion. Coliforms and *Pseudomonas* spp. may utilize amino acids necessary for staphylococcal growth and make them unavailable. Other genera of Micrococccaeae can utilize nutrients more rapidly than staphylococci. Streptococci inhibit staphylococci by exhausting the supply of nicotinamide or niacin and biotin. *Staphylococcus aureus* is a poor competitor in both fresh and frozen foods. At temperatures that favor staphylococcal growth, the normal food saprophytic biota offers protection against staphylococcal growth through antagonism, competition for nutrients, and modification of the environment to conditions less favorable to *S. aureus*. Changes in the composition of the food, as well as changes in intrinsic or extrinsic factors may either stimulate or decrease competitive effects.

III. Effects on growth inhibition

Changes in growth stimulation have been reported among several foodborne organisms, including yeasts, micrococci, streptococci, lactobacilli and Enterobacteriaceae. Growth stimulating mechanisms can have a significant influence on the buildup of a typical flora. There are several of these mechanisms, a few of which are listed below:

- **Metabolic products** from one organism can be absorbed and utilized by other organisms.
- **Changes in pH** may promote the growth of certain microorganisms. An example is natural fermentations, in which acid production establishes the dominance of acid tolerant organisms such as the lactic acid bacteria. Growth of molds on high acid foods has been found to raise the pH, thus stimulating the growth of *C. botulinum*.
- **Changes in Eh or aw** in the food can influence symbiosis. At warm temperatures, *C. perfringens* can lower the redox potential in the tissues of freshly slaughtered animals so that even more obligately anaerobic organisms can grow.
- **There are some associations** where maximum growth and normal metabolic activity are not developed unless both organisms are present.

This information can be used in the hurdle concept to control microorganisms in temperature-sensitive foods.
3. Extrinsic factors

3.1. Types of packaging/atmospheres

Gases inhibit microorganisms by two mechanisms. First, they can have a *direct toxic effect* that can inhibit growth and proliferation. Carbon dioxide (CO\(_2\)), ozone (O\(_3\)), and oxygen (O\(_2\)) are gases that are directly toxic to certain microorganisms. This inhibitory mechanism is dependent upon the chemical and physical properties of the gas and its interaction with the aqueous and lipid phases of the food. Oxidizing radicals generated by O\(_3\) and O\(_2\) are highly toxic to anaerobic bacteria and can have an inhibitory effect on aerobes depending on their concentration. Carbon dioxide is effective against obligate aerobes and at high levels can deter other microorganisms.

A second inhibitory mechanism is achieved by *modifying the gas composition*, which has indirect inhibitory effects by altering the ecology of the microbial environment. When the atmosphere is altered, the competitive environment is also altered. Atmospheres that have a negative effect on the growth of one particular microorganism may promote the growth of another. This effect may have positive or negative consequences depending upon the native pathogenic microflora and their substrate. Nitrogen replacement of oxygen is an example of this indirect antimicrobial activity.

A variety of common technologies are used to inhibit the growth of microorganisms, and a majority of these methods rely upon temperature to augment the inhibitory effects. Technologies include *modified atmosphere packing* (MAP), *controlled atmosphere packaging* (CAP), *controlled atmosphere storage* (CAS), *direct addition of carbon dioxide* (DAC), and *hypobaric storage*.

Controlled atmosphere and modified atmosphere packaging of certain foods can dramatically extend their shelf life. The use of CO\(_2\), N\(_2\), and ethanol are examples of MAP applications. In general, the inhibitory effects of CO\(_2\) increase with decreasing temperature due to the increased solubility of CO\(_2\) at lower temperatures. Carbon dioxide dissolves in the food and lowers the pH of the food. Nitrogen, being an inert gas, has no direct antimicrobial properties. It is typically used to displace oxygen in the food package either alone or in combination with CO\(_2\), thus having an indirect inhibitory effect on aerobic microorganisms. Table 8 shows some examples of combinations of gases for MAP applications in meat, poultry, seafood, hard cheeses, and baked goods.

<table>
<thead>
<tr>
<th>Product</th>
<th>% CO(_2)</th>
<th>% O(_2)</th>
<th>% N(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh meat</td>
<td>30</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>15 - 40</td>
<td>60 - 85</td>
<td>0</td>
</tr>
<tr>
<td>Ingredient</td>
<td>0 - 10</td>
<td>0 - 20</td>
<td>0 - 50</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Cured meat</td>
<td>20 - 50</td>
<td>0</td>
<td>50 - 80</td>
</tr>
<tr>
<td>Sliced cooked roast beef</td>
<td>75</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Eggs</td>
<td>20</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Poultry</td>
<td>25 - 30</td>
<td>0</td>
<td>70 - 75</td>
</tr>
<tr>
<td></td>
<td>60 - 75</td>
<td>5 - 10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20-40</td>
<td>60-80</td>
<td>0</td>
</tr>
<tr>
<td>Pork</td>
<td>20</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>Processed Meats</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Fish (White)</td>
<td>40</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Fish (Oily)</td>
<td>40</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Hard cheese</td>
<td>0 - 70</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Cheese</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Cheese; grated/sliced</td>
<td>30</td>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td>Sandwiches</td>
<td>20 - 100</td>
<td>0 - 10</td>
<td>0 - 100</td>
</tr>
<tr>
<td>Pasta</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>70 - 80</td>
<td>0</td>
<td>20 - 30</td>
</tr>
<tr>
<td>Baked goods</td>
<td>20 - 70</td>
<td>0</td>
<td>20 - 80</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The preservation principle of antimicrobial atmospheres has been applied to fruits and vegetables, raw beef, chicken and fish, dairy foods including milk and cottage cheese, eggs, and a variety of prepared, ready-to-eat foods.

There are several intrinsic and extrinsic factors that influence the efficacy of antimicrobial atmospheres. These factors-including product temperature, product-to-headspace gas volume ratio, initial microbial loads and type of flora, package barrier properties, and biochemical composition of the food-all interact to determine the degree to which the microbial quality and safety are enhanced.

Temperature, the most important factor affecting the efficacy of antimicrobial atmospheres, directly affects growth rate, but also indirectly affects growth by affecting gas solubility. At
practical food storage temperatures, packaging configurations, especially the product-to-headspace volume ratio, play a major role in determining the magnitude of microbial inhibition.

In MAP, package barrier properties have a major effect on the microbial growth by influencing the time in which the selected modified atmosphere gases remain in contact with the product and the rate at which oxygen enters the package.

Water activity, salt content of the aqueous phase, pH, and fat content of foods also play a role in overall inhibitory effects of antimicrobial gases. As with temperature, the physical and chemical characteristics of the food have an effect on the solubility of the inhibitory gas. For example, increasing salt concentrations decreases CO₂ solubility.

3.2. Effect of time/temperature conditions on microbial growth

I. Impact of time

When considering growth rates of microbial pathogens, in addition to temperature, time is a critical consideration. Food producers or manufacturers address the concept of time as it relates to microbial growth when a product's shelf life is determined. Shelf life is the time period from when the product is produced until the time it is intended to be consumed or used. Several factors are used to determine a product's shelf life, ranging from organoleptic qualities to microbiological safety.

Under certain circumstances, time alone at ambient temperatures can be used to control product safety. When time alone is used as a control, the duration should be equal to or less than the lag phase of the pathogen(s) of concern in the product in question. For refrigerated food products, the shelf life or use-period required for safety may vary depending on the temperature at which the product is stored. In a similar manner, a pH increase from 4.5 to 6.5 decreases the lag time from 60 to 5 h. In conclusion, the safety of a product during its shelf life may differ, depending upon other conditions such as temperature of storage, pH of the product, and so on.

![Figure 1](image1.png)

**Figure 1**

Effect of temperature or pH on lag times of *Listeria monocytogenes* (2% NaCl, aₒ 0.989)
As stated earlier, time alone at ambient temperatures can be used to control product safety. When time alone is used as a control, the duration should be equal to or less than the lag phase of the pathogen(s) of concern in the product in question.

II. Impact of temperature

All microorganisms have a defined temperature range in which they grow, with a minimum, maximum, and optimum. Temperature has dramatic impact on both the generation time of an organism and its lag period.

At low temperatures, two factors govern the point at which growth stops: 1) reaction rates for the individual enzymes in the organism become much slower, and 2) low temperatures reduce the fluidity of the cytoplasmic membrane, thus interfering with transport mechanisms. At high temperatures, structural cell components become denatured and inactivation of heat-sensitive enzymes occurs. While the growth rate increases with increasing temperature, the rate tends to decline rapidly thereafter, until the temperature maximum is reached.

The relationship between temperature and growth rate constant varies significantly across groups of microorganisms. Four major groups of microorganisms have been described based on their temperature ranges for growth: thermophiles, mesophiles, psychrophiles, and psychrotrophs. Tables 9 and 10 list the temperature ranges for these four groups and for pathogens of concern.

The optimum temperature for growth of:

- **thermophiles** is between 55 to 65 °C (131 to 149 °F) with the maximum as high as 90 °C (194 °F) and a minimum of around 40 °C (104 °F).
- **Mesophiles**, which include virtually all human pathogens, have an optimum growth range of between 30 °C (86 °F) and 45 °C (113 °F), and a minimum growth temperature ranging from 5 to 10 °C (41 to 50 °F).
- **Psychrophilic** organisms have an optimum growth range of 12 °C (54 °F) to 15 °C (59 °F) with a maximum range of 15 °C (59 °F) to 20 °C (68 °F). There are very few true psychophilic organisms of consequence to foods.
- **Psychrotrophs** such as *L. monocytogenes* and *C. botulinum* type E are capable of growing at low temperatures (minimum of - 0.4 °C [31 °F] and 3.3 °C [38 °F], respectively, to 5 °C [41 °F]), but have a higher growth optimum range (37 °C [99 °F] and 30 °C [86 °F], respectively) than true psychrophiles. Psychrotrophic organisms are much more relevant to food and include spoilage bacteria, spoilage yeast and molds, as well as certain foodborne pathogens.

Growth temperature is known to regulate the expression of virulence genes in certain foodborne pathogens. For example, the expression of proteins governed by the *Yersinia enterocolitica* virulence plasmid is high at 37 °C (99 °F), low at 22 °C (72 °F), and not detectable at 4 °C (39 °F). Growth temperature also impacts an organism's thermal sensitivity. *Listeria monocytogenes*, when held at 48 °C (118 °F) in inoculated sausages, has an increase of 2.4-fold in its D value at 64 °C (147 °F).
Table 9. Temperature ranges for prokaryotic microorganisms.

<table>
<thead>
<tr>
<th>Group</th>
<th>Temperature °C (°F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
</tr>
<tr>
<td>Thermophiles</td>
<td>40 - 45 (104 - 113)</td>
</tr>
<tr>
<td>Mesophiles</td>
<td>5 - 15 (41 - 59)</td>
</tr>
<tr>
<td>Psychrotrophs</td>
<td>-5 - +5 (23 - 41)</td>
</tr>
</tbody>
</table>

Table 10. Approximate minimum, maximum and optimum temperature values in °C (°F) permitting growth of selected pathogens relevant to food.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Minimum</th>
<th>Optimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>5 (41)</td>
<td>28 - 40 (82 - 104)</td>
<td>55 (131)</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>32 (90)</td>
<td>42 - 45 (108 - 113)</td>
<td>45 (113)</td>
</tr>
<tr>
<td>Clostridium botulinum types A &amp; B*</td>
<td>10 - 12 (50 - 54)</td>
<td>30 - 40 (86 - 104)</td>
<td>50 (122)</td>
</tr>
<tr>
<td>Clostridium botulinum type E**</td>
<td>3 - 3.3 (37 - 38)</td>
<td>25 - 37 (77 - 99)</td>
<td>45 (113)</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>12 (54)</td>
<td>43 - 47 (109 - 117)</td>
<td>50 (122)</td>
</tr>
<tr>
<td>Enterotoxigen Escherichia coli</td>
<td>7 (45)</td>
<td>35 - 40 (95 - 104)</td>
<td>46 (115)</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>0 (32)</td>
<td>30 - 37 (86 - 99)</td>
<td>45 (113)</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>5 (41)</td>
<td>35 - 37 (95 - 99)</td>
<td>45 - 47 (113 - 117)</td>
</tr>
<tr>
<td>Staphylococcus aureus growth</td>
<td>7 (45)</td>
<td>35 - 40 (95 - 104)</td>
<td>48 (118)</td>
</tr>
<tr>
<td>Staphylococcus aureus toxin</td>
<td>10 (50)</td>
<td>40 - 45 (104 - 113)</td>
<td>46 (115)</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>7 (45)</td>
<td>37 (99)</td>
<td>45 - 47 (113 - 117)</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>10 (50)</td>
<td>37 (99)</td>
<td>43 (109)</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td>5 (41)</td>
<td>37 (99)</td>
<td>43 (109)</td>
</tr>
<tr>
<td>Vibrio vulnificus</td>
<td>8 (46)</td>
<td>37 (99)</td>
<td>43 (109)</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>-1 (30)</td>
<td>28 - 30 (82 - 86)</td>
<td>42 (108)</td>
</tr>
</tbody>
</table>

* proteolytic; ** non-proteolytic

3.3. Storage/holding conditions
It is well known that *increase in storage and/or display temperature will decrease the shelf life of refrigerated foods* since the higher the temperature, the more permissive conditions are for growth. At the same time, those foods that have been cooked or re-heated and are served or held hot may require appropriate time/temperature control for safety. For example, the primary organism of concern for cooked meat and meat-containing products is *C. perfringens*. Illness symptoms are caused by ingestion of large numbers (greater than $10^8$) of vegetative cells. The organism has an optimal growth range of 43 - 47 °C (109-116 °F) and a growth range of 12-50 °C (54 - 122 °F). Generation times as short as 8 min have been reported in certain foods under optimal conditions. Thus time/temperature management is essential for product safety.

The *effect of the relative humidity of the storage environment on the safety of foods* is somewhat more nebulous. Generally, foods that depend on a certain $a_w$ for safety or shelf life considerations will need to be stored such that the environment does not markedly change this characteristic. Foods will eventually come to moisture equilibrium with their surroundings. Thus, processors and distributors need to provide for appropriate storage conditions to account for this fact.

Packaging play a major role in the vulnerability of the food to the influence of relative humidity. But even within a sealed container, moisture migration and the phenomenon of environmental temperature fluctuation may play a role. It has been observed that certain foods with low $a_w$ can be subject to moisture condensing on the surface due to wide environmental temperature shifts. This surface water will result in microenvironments favorable to growth of spoilage, and possibly pathogenic, microorganisms. As a general guideline, the product should be held such that environmental moisture, including that within the package, does not have an opportunity to alter the $a_w$ of the product in an unfavorable way.

### 3.4. Processing steps

The processing steps are considered to be as important as pH and $a_w$ and as such foods are divided into two categories: Low-acid canned foods in a hermetically-sealed container do not require temperature control for safety. This rigid definition fails to address less processed foods, in less robust packaging, which still would not require temperature control for safety. Consider a baked product, such as a pie, with a pH of 5.5 and $a_w$ of 0.96. Since this product is baked to an internal temperature >180 °F (82 °C) to set the product structure of the pie, it will not contain any viable vegetative pathogens. Any pathogenic spores that survive the baking process will be inhibited by the pH and $a_w$ values listed above. If the product is cooled and packaged under conditions that do not allow recontamination with vegetative pathogens, the product is safe and stable at room temperature until consumed, or until quality considerations (that is, staling) make it unpalatable.

Scientifically sound criteria for determining whether foods require time/temperature control for safety should consider 1) processes that destroy vegetative cells but not spores (when product formulation is capable of inhibiting spore germination); 2) post-process handling and packaging conditions that prevent reintroduction of vegetative pathogens onto or into the product before packaging; and 3) the use of packaging materials that while they do not provide a hermetic seal, do prevent reintroduction of vegetative pathogens into the product.
4. Other factors

4.1. Intended end-use of product

In addition to carefully assessing how the product is produced and distributed, it is important to consider how the food will ultimately be prepared, handled, and/or stored by the end user. A food product that does not require time/temperature control for safety at one point in the food production or distribution chain may require time/temperature control at another point, depending on its intended use. For example, a thermally processed food that is hot-filled into its final packaging may not require refrigeration if spore-forming pathogens are not capable of outgrowth. However, once the food item is taken out of its original packaging, it may require time/temperature control for safety if the product is likely to be recontaminated during its intended use.

4.2. Product history and traditional use

The panel struggled with the concept of product history and traditional use as a means to determine the need for time/temperature control for safety. For example, there are foods which have a long history of safe storage use at ambient temperatures, yet have formulations, pH, and $a_w$ that would designate them as "temperature controlled for safety" (TCS) foods. Paramount among them is white bread, but products such as intact fruits and vegetables, other breads, bottled waters, and some processed cheeses have a history of being stored and used at ambient temperatures with no public health impact. In addition, moisture protein ratios (MPR) for shelf-stable fermented sausages were developed to ensure process control values for these sausages that also have a traditional history of safety as a non-TCS food. Moreover, an evaluation of the food characteristics provides a scientific explanation for the products to be safely stored at ambient temperatures. For example, baking of bread controls the growth of pathogens in the interior, and the low $a_w$ precludes the growth of pathogens on the outer surface, so that it can be stored safely at ambient temperatures. Clearly these products' traditional uses and histories provide a valid justification for a decision to be made based on history. Care must be observed, however, as this traditional history can be influenced by the intrinsic and extrinsic factors and any changes in product end-use, processes, formulation, physical structure, processing, distribution, and/or storage. Changes in any of these parameters may invalidate the sole use of history as a basis for decisions on whether a food needs temperature control for safety.

4.3. Interactions of factors

Traditional food preservation techniques have used combinations of pH, $a_w$, atmosphere, numerous preservatives, and other inhibitory factors. Microbiologists have often referred to this phenomenon as the "hurdle effect". For example, certain processed meat products and pickles may use the salt-to-moisture ratio (brine ratio) to control pathogens. USDA recognizes this strategy in designating as shelf-stable semi-dry sausages with a moisture-protein ratio of less than or equal to 3.1:1 and pH less than or equal to 5.0.
In salad dressings and mayonnaise-type products, the acid-to-moisture ratio along with pH is the governing factor for pathogen control. An acid:moisture ratio > 0.70 in combination with a pH < 4.1 is often used as the pathogen-control target level for these products. Usually, these ratios are combined with other factors such as pH or added antimicrobials to effect pathogen control. It is the interaction of these factors that controls the ability of pathogens to proliferate in foods.

**PRACTICAL**

(1) Gram staining  
(2) Spore staining  
(3) Test for extracellular enzyme  
   (i) Catalase  
   (ii) Coagulase  
   (iii) Oxidase  
(4) Detection of product of metabolism e.g. indole  
(5) Sugar fermentation.  
(6) Other biochemical procedure e.g. nitrase gluconate, decarboxylase.

**TEST OF EXTRACELLULAR ENZYME**

(1) Coagulase—coagulase is an extracellular enzyme produced by some disease causing strain of staphylococcus. Positive staph. Aureus
   (a) Slide technique: Emulsify the organism in normal saline (NaCl+ hydrogen), at the same time emulsify a control organism such as oxford streptococcus in another slide.
      - To the emulsified portion add a loopful of undiluted rabbit plasma. Mix and rock to mix.
      - Observe for the physical agglutination i.e. bisible agglutinate—which is visible to the naked eye. It appears as lines shown at the back of hand.
   (b) Tube Method/ techniques: this indicates coagulase positive.

\[
\text{Peptone H}_2\text{O} \\
+ \\
\text{Organism} \\
\text{(Add plasmal/10 diluted)} \\
\text{Incubated 37\textdegree C} \\
\text{1- 3hours or 4 hours.}
\]
(2) Oxidase Test: Add a redox dye to the culture of organism to be tested development of purple colouration indicates positive.

A redox dye is 1% aqueous tetramethyl- P- phenylene diamine dihydrochloride. It is capable of transferring electron between itself and the bacteria cells therefore effecting colour changes. Redox dye + Oxidase positive organism – purple colour.

A typical example of Oxidase positive organism is Pseudomonase

(b) Another method of carrying out the above is by spreading the culture of the organism on a filter paper. There will be instantaneous change if the organism is positive.

(iii) Catalase: - Is an extracellular enzyme that catalizes the splitting of H2O2 to produce O2. Add a few drops of H2O2 to the emulsified portion of the bacteria culture. Evolution of gas (O2) indicate catalase positive. 2H2O2 → 2H2O + O2 example of organism that are catalase positive is Micrococcus, staphylococcus, Pseudomonads and bacillus.

(4) Detection of products of metabolism
For e.g. Indole

Peptone water
Rich in tryptophane

+ Organism

37°C for 18 hours

Kovac’s reagent
Observe for a pinkish coloration of a supernatant
kovac's reagent contains:- P-amonibenzaldehyde - 5g
- Amyl alcohol - 75ml
- Hcl - 25ml

(2) Spore staining procedure
Emulsfy a bacteria suspension on a clean slide, air dry and Heat fix. Add drops of 5% malanchite green – steam in H2O for 5 sec.

Counter stain with dilute carbol fuchsins or 0.5% safranin.
Observation – spore appear green, while vegetative cell appear pink

ISOLATION OF FUNGI
(1) Observe the physical appearance of the food.
(2) Select the mouldy and non-mouldy ones and carry out the followings.
(3) Culture the selected mouldy ones on MEI/PDA.
(4) Culture at 22°C for about one week 35o – 37oC for 2 days.
(5) Observe and note the macuomorphological appearaned of each fungus on the culture media.
(6) It is essential to look at the front and back of the plate for proper morphological character on culture.
(7) Make wet mount and observe for fungal element.

Aspergillus spp  
- They produce septate mycelium.
- Aspergillus appear yellow – green to black in culture medium.
- While Penicillium appear blue-to-blue – green in culture.
- They both produce upright conidophore which terminate in a globose or clarate suelling in Aspergullius but not in penicillin.
- Asperbillus produces aflatoxin and proteases and acetic acid
- Penicilllin produces antibiotic penicillin.

Fusarium spp  
- Appear in culture with characteristic cottony mycelia with tinges of pink, purple or yellow
- Conidia from are both shape
- They produce mycotoxin.

Penicillium Spp

MICROBIOLOGY OF WATER

H₂O serves a lot of useful purposes in industries.
(1) Use as a heating medium
(2) Use as a cooling medium particularly when the H₂O has to be in contact with product, which has to be in contact with the coling medium.
(3) H₂O is also used as a cleaning medium particularly for floors and for use in disinfectant and toilet flush.
(4) H₂O form an essential constituent of some product for e.g. manufacture of soft drink.
(5) Important for Blanching (mild form of heating not too high or too low) done to inactivate enzymes, fix colour of the food and kill vegetative cells.

Test Done on Water

In order to ascertain the portability of a H₂O for use in the food industry physical, microbiological and chemical test are done. Also the source of H₂O has to be known. Microbiological test, chemical and physical test.
(1) microbiological test: The following test are carried out (1) Coliform test – ideally presumptive coliform test need to be done and this is carried out as follows:-

<table>
<thead>
<tr>
<th>Coliform test</th>
<th>Presumptive</th>
<th>Differential</th>
<th>Total</th>
<th>Coliform count</th>
</tr>
</thead>
</table>