FE 305 Food Microbiology
Microbial Metabolism of Foods

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Microbial Metabolisms

- Microbial activities; energy / nutrition is essential for their survival, growth, development and reproduction.
- Bacteria's ability to take advantage of foodstuffs depends on their ability to pass through the cell wall and cytoplasmic membrane.
- Molecules of substances useful to the cell in the external environment or in the environment cannot pass through the cell wall, since they are generally large.
- These need to be reduced to a level that can be passed by active transport.
- This task is performed by hydrolysing enzymes (exoenzymes) synthesized and excreted by bacteria.
- Food substances (protein, carbohydrate, lipid, etc.), which are reduced to sizes that can pass through cell membranes with the help of exoenzymes in the environment outside the microorganisms, can continue to decompose and decompose down to the smallest building blocks after they enter the cytoplasm.

Microbial Metabolisms

- Endoenzymes, which remain inside and are not released, catalyze the ongoing decomposition events in the cytoplasm (dissimilation-catabolism).
- Microorganisms subsequently synthesize the substances they need (protein, polysaccharide, lipid, enzyme, etc.) from these building blocks or larger molecules (assimilation-anabolism).
- These two and very important and successive biochemical events constitute metabolism.
- Metabolism, which is an integration of many enzymatic processes involved in the reproduction and development of microorganisms, includes all biochemical changes (anabolic, catabolic) that occur in living organisms.
- Most of the energy generated in metabolic chemical reactions is used in microbial activities and some is released.

Microbial Metabolisms

- A good understanding of microbial metabolic reactions provides many benefits.
- It is possible to examine metabolic events in three groups.
  - Digestion
    - Extracellular degradation
    - intracellular degradation
  - Respiration (respiration) and fermentation (Catabolism)
  - Biosynthesis (anabolism)

Microbial Metabolisms

- Metabolic events are managed step by step and sequentially by the catalytic effect of many enzymes. The smallest changes that may occur in enzymes cause these very important and serial biochemical events to deteriorate or change direction.
- Metabolism events in reproductive cells are continuous and interrelated.
  - With anabolism, the cell is refreshed, depleted and necessary compounds are synthesized.
  - In catabolism, the energy and building blocks required for synthesis are prepared.
- Food shortage, desiccation, sporulation, sub-minimal temperature, effect of chemical microbiostatic, etc. Metabolism will decrease for other reasons, as well as completely stop in freezing events.
- In catabolism events, a significant amount of energy is released with the decomposition of foodstuffs (exergonic reactions) and this energy is taken by ADP (adenosine diphosphate) and ATP (Adenosine triphosphate) in the form of high energy bonds and retained between their own phosphate bonds.
- The energy required for synthesis events is provided from these bonds (endergonic reactions).

Extracellular degradation

- Complex molecules are too large to enter the cell through the semipermeable microorganism cell membrane.
- Large complex molecules such as protein and starch are hydrolytically broken down to a size that can pass through the microorganism cell membrane outside the microorganism cell.
- These events are catalyzed by extracellular enzymes that are synthesized and secreted into the environment in the microorganism cell.
- The entry of organic molecules into the cell, which are fragmented to a size that can pass through the cell membrane, is not a simple diffusion event, but occurs as a result of an absorption event that takes place with the help of enzymes penetrating the cell. This process requires energy.

Intracellular degradation

- Further degradation of large organic molecules occurs within the cell.
- These events are carried out by digestive hydrolases.
- Nutrients formed as a result of the breakdown of intracellular (intracellular) digesting hydrolases are now available to the cell.
- The cell can use these nutrients for two interrelated vital events at the same time.
  - synthesis of new cellular components (growth = proliferation, development),
  - energy producing reactions (respiration = respiration and fermentation).

Intracellular degradation

- **Respiration (respiration) and fermentation events (Catabolism)**
  - The energy stored as the chief energy in the organic molecule released as a result of respiration and fermentation events in the cell is used in cell metabolism.

- **Biosynthesis (Anabolism) events**
  - They are complex processes in which more complex biomolecules are formed inside the cell using simple molecules.
  - synthesis of proteins from amino acids,
  - synthesis of fats from fatty acids.
  - The programming and realization of these synthesis events are provided by the genetic messages encoded in the DNA of the chromosomes.
  - As the final products of synthesis events; Microorganism cell walls, cell membranes, enzyme proteins, chromosomal materials, protoplasmic proteins, other components necessary for vitality and reproduction emerge.
  - Biosynthesis cannot be carried out without energy.
  - It occurs in conjunction with energy-producing reactions.

Biosynhtesis (anabolism)

- Bacterial metabolism is more intense and vigorous, and the metabolic rate is much higher than in animal cells.
  - A very small bacterial cell can metabolize large amounts in a short time, increasing its weight several times.
- To capture the metabolic activity in a bacterial cell, a person would need to consume half a ton of food per day.
- This very high metabolic activity of bacterial cells is due to their very small size, their relatively high surface area and their very short generation times.
- A single bacterial cell can reach millions in a day.
- Basically, all the changes that occur in the microorganism cell are carried out by enzymes, which are organic catalysts.
- Since the basis of the formation of many products and foodstuffs obtained by microorganisms is based on fermentation events, the main substances that are generally targeted in fermentations are carbohydrates.

Microbial Metabolism

Metabolism in microorganisms is examined in three main groups.

1) Carbohydrate metabolism,
2) Lipid metabolism,
3) Protein and amino acid metabolism.

Carbohydrate metabolism

- Microorganisms cannot metabolize complex carbohydrates directly.
- Under normal conditions, carbohydrate metabolism is generally understood as glucose metabolism.
  - Other types of sugar in the organism are converted to glucose to a large extent and enter the metabolism.
- Microorganisms ferment glucose in three main ways. Although there are mainly 7 metabolic pathways in the metabolism of monosaccharides, four of them, especially the EMP pathway, are more important.
  1. Decomposition of glucose via hexosediphosphate (Embden–Meyerhof–Parnas EMP pathway),
  2. Decomposition of hexose via oxidative pentosephosphate (HMP pathway),
  3. Breakdown of glucose by Entner - Doudoroff (ED pathway).
  4. Tricarboxylic acid cycle, TCA cycle, Krebs cycle, citric acid cycle

Carbohydrate metabolism

- Carbohydrates are organic compounds composed of carbon (C), hydrogen (H), and oxygen (O). These components came together at the rate of $(CH_2O)n$ among themselves.
- Carbohydrates serve as energy source for heterotrophic microorganisms and carbons serve as building blocks when synthesizing organic compounds.
- Carbohydrates are divided into parts (according to the number of carbon atoms) in their structure. Mono and disaccharides (sugars) are easily soluble in water and can be crystallized and dialyzed. On the other hand, polysaccharides are insoluble in water and are not dialyzable.
- **Monosaccharides**
  - Monosaccharides with the empirical formula $(CH_2O)n$ are classified among themselves according to their carbon atoms.
  - Triose ($C_3H_6O_3$): Glyceraldehyde;
  - Tetrose ($C_4H_8O_4$): Erythrose;
  - Pentose ($C_5H_{10}O_5$): Arabinose, xylose, rhamnose, ribose.
  - Hexose ($C_6H_{12}O_6$): Galactose, glucose, fructose, mannose.

Carbohydrate metabolism

Disaccharides ($C_{12}H_{22}O_{11}$)

- Disaccharides are formed by the condensation of two monomer monosaccharides by losing water.
  - Lactose: glucose + galactose; Maltose: glucose + glucose; Sucrose: Glucose + fructose

Trisaccharides ($C_{18}H_{32}O_{16}$)

- They are composed of three monosaccharides.
  - Raffinose: galactose + glucose + glucose

Polysaccharides ($(C_6H_{10}O_5)_n$)

- These are formed as a result of condensation of many monosaccharide molecules by losing water. Among the most important homopolysaccharides are dextrin, glycogen, inulin, starch, cellulose and mucopolysaccharides (hyaluronic acid) from heteropolysaccharides.

Alcohols

- Adonite, dulcite, erythrite glycerin, inosite, mannite

Carbohydrate Decomposition

A) Decomposition of polysaccharides: Polysaccharides are organic polymers with high molecular weight, consisting of monosaccharide units linked to each other by glycosidic bonds. Dissolution of the glycosidic bond between the monomers causes the polysaccharides to separate into their constituent units. This occurs in two main ways:

1- Hydrolysis: The glycosidic bond connecting monosaccharides is broken by the special enzymes (carbohydrase) in microorganisms by dissolving them through water. Thus, the polysaccharide decomposes.

For example,

$$\text{Starch} + H_2O \xrightarrow{\text{amylase}} \text{maltose}$$

$$\text{Maltose} + H_2O \xrightarrow{\text{maltase}} \text{glucose} + \text{glucose}$$

$$\text{Cellulose} + H_2O \xrightarrow{\text{cellulase}} \text{cellobiose}$$

$$\text{Cellobiose} + H_2O \xrightarrow{\text{cellobiase}} \text{glucuse} + \text{glucuse}$$

- Hyaluronic acid, a heteropolysaccharide, is hydrolyzed by the hyaluronidase enzyme found in many bacteria (*Streptococci, Staphylococci, Diplococci, Clostridium*, etc.).
- Lactose, a disaccharide, is decomposed by the beta-galactosidase enzyme found in *E. coli*.

Carbohydrate Decomposition

2- Phosphorylation

- Polysaccharide phosphorylase enzymes in microorganisms help to decompose polysaccharides.
- Since this reaction is reversible, it also takes part in the synthesis of carbohydrates.
- *Clostridium, Streptococcus, Neisseria, Chlorinebacter* and agro-bacteria have such enzyme.
- These enzymes catalyze the decomposition of polysaccharides by phosphorylation.

Example,

maltose Phosphorylase

Maltose+ H_3PO_4 ------------------------ beta - D-glucose -1- Phosphate + glucose

cellobiose phosphorylase

Cellobiose+ H_3PO_4 ---------------------------- alfa-D-glucose-1-phosphate + glucose

Carbohydrate Decomposition

B) Decomposition of Monosaccharides

- Glucose, one of the monosaccharides, has a very important role and place in the structure of other carbohydrates.
- After glucose enters from bacterial cell damage, it is either stored as glucose in special stores or continues to decompose, depending on the character of the decomposition (aerobic or anaerobic), until it is divided into final products.
- The decomposition style and the resulting end products vary widely among microorganisms. These are used to identify microorganisms.
- The final products formed by the decomposition of glucose by heterotrophic microorganisms:
  a. **Organic acids**: Acetic acid, butyric acid, formic acid, kojic acid, lactic acid, propionic acid, pyruvic acid, succinic acid, etc.
  b. **Neutral products**: Acetyl methyl carbinol, acetone, butyl alcohol, 2,3-butyleneglycol, diacetyl, ethyl alcohol, isopropyl alcohol, n-propyl alcohol, etc.
  c. **Gases**: Hydrogen, carbon dioxide, methane

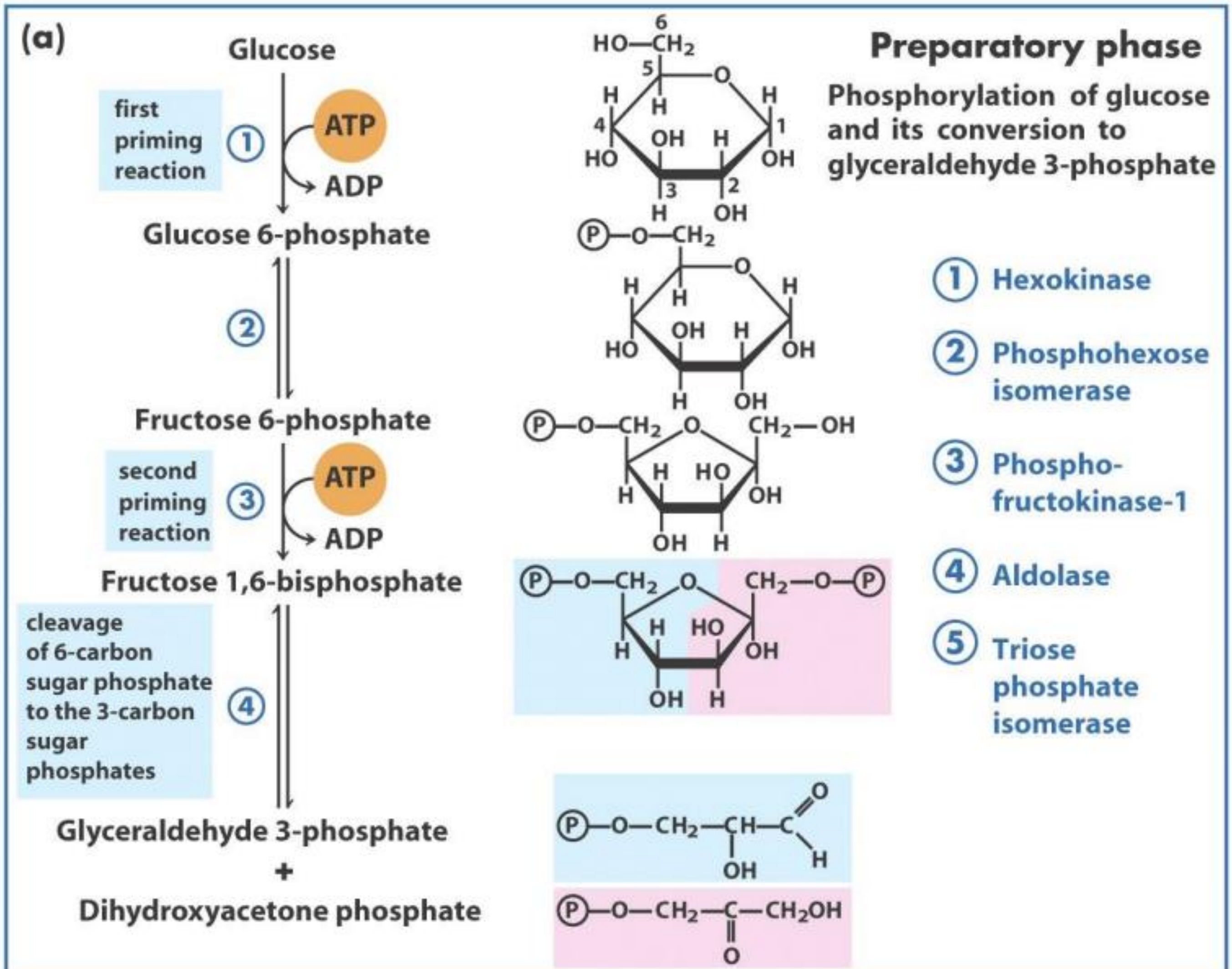
Carbohydrate Decomposition

- Aerobic, facultative and anaerobic microorganisms decompose glucose in different ways to form end products:
  - The decomposition of glucose to end products is not a single step, but involves a series of reactions.
  - Each step is independent and catalyzed by separate and specific enzymes.
  - Once the reaction is activated, the other steps follow one after the other.
- During the aerobic or anaerobic decomposition of glucose, an essential product, pyruvic acid, is formed.
  - For this reason, the divergences take their origin from here and continue until the advanced stages.
  - However, the decomposition of glucose to pyruvate is not a single step, but requires a series of biochemical reactions.
  - These steps showing the dissociation of glucose can be traced in the Embden-Meyerhof diagram.

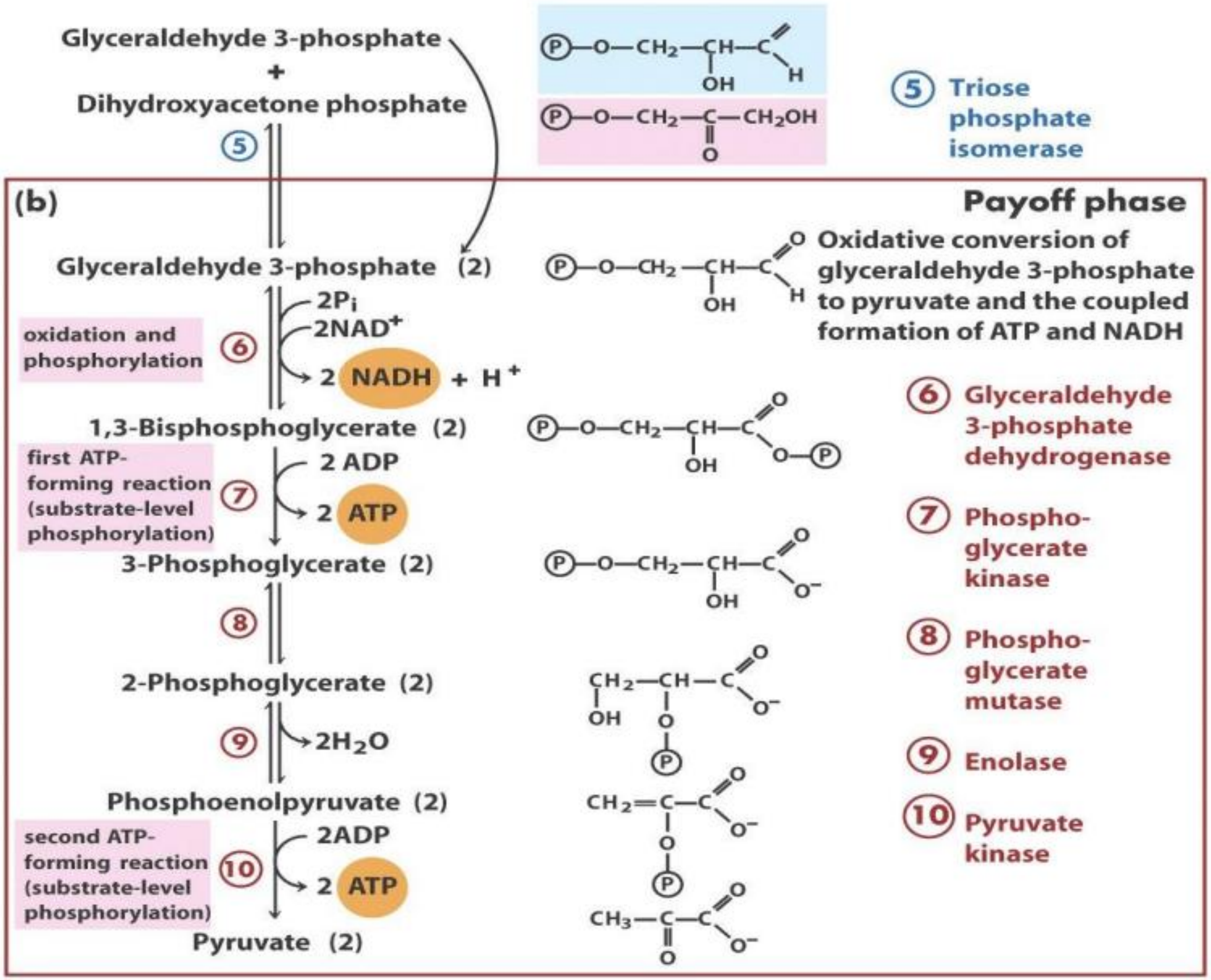
Embden–Meyerhof–Parnas (EMP) metabolic pathway, glycolysis

- EMP pathway is the most important way of anaerobic degradation of hexose in glycolysis and fermentation events that occur both in animal tissues and microorganisms (yeast and bacteria).
- This pathway is the largest for glucose catabolism in most cells.
- EMP path;
  - to ethyl alcohol and CO_2 as a result of ethyl alcohol fermentation of glucose by the yeast cell,
  - It shows the conversion of lactic acid in muscles by muscle cells.
- In addition, it explains the reduction of pyruvic acid and its conversion to lactic acid, thanks to the lactate dehydrogenase enzyme found in some microorganisms such as lactic acid bacteria.
- All these events continue in the cell under anaerobic conditions, so that the necessary energy is supplied to the cell without free oxygen.

Embden–Meyerhof–Parnas (EMP) metabolik yolu, glikoliz



Glycolysis



Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
5 Triose phosphate isomerase
(b)
Payoff phase
Glyceraldehyde 3-phosphate (2)
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
2Pi
2NAD+
oxidation and phosphorylation
6
2 NADH + H+
1,3-Bisphosphoglycerate (2)
6 Glyceraldehyde 3-phosphate dehydrogenase
first ATP-forming reaction (substrate-level phosphorylation)
7
2 ADP
2 ATP
3-Phosphoglycerate (2)
7 Phospho-glycerate kinase
8
2-Phosphoglycerate (2)
8 Phospho-glycerate mutase
9
2H2O
9 Enolase
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
10 Pyruvate kinase

Hexose Monophosphate (HMP) or Oxidative Pentose Phosphate Shunt

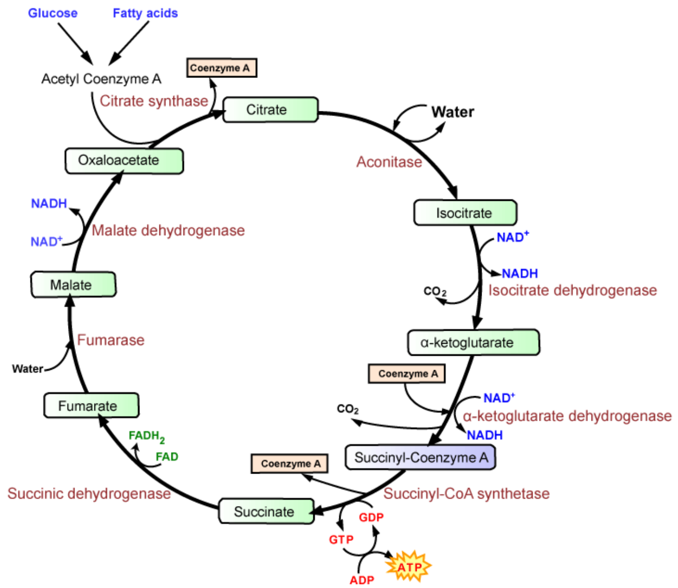
- The breakdown of carbohydrates through this pathway does not directly provide energy. However, the electrons of $NADPH_2$ formed in this way are transported to oxygen and ATP is formed again.
- on the way to HMP; It consists of nucleic acids, enzymes containing nucleotides in prosthetic groups, aromatic (ring structured) amino acids, starting materials required for the synthesis of some vitamins, and pyridine nucleotides used in biosynthesis. Except those; Amino acids such as leucine, isoleucine and valine can also be formed in this pathway.
- If monosaccharides are continuously introduced into the organism as nutrients, the HMP pathway is directed towards the formation of pyruvic acid.
- The main distinguishing features of the HMP pathway are;
    1. Unlike EMP, in HMP the aldehyde carbon of the sugar is separated as CO_2.
    2. In this way, hexoses are converted to 5-carbon sugars for nucleic acid formation and to 7-carbon sugars for amino acids synthesis.
    3. This pathway is especially important for autotrophic microorganisms, and the CO_2 determined by them can be converted into central metabolites in this way.
    4. It is another way to provide energy to the cell for the oxidation of glucose. It is a mechanism that provides energy from pentoses.

Entner Doudoroff (ED) Pathway

- Another way of anaerobic breakdown of glucose is the ED pathway.
- The pyruvic acid formed as a result of this pathway, according to the type of enzyme found in the organism; converts to lactic acid, ethyl alcohol or another fermentation product.
- In this way, 1 mole of ATP is formed from 1 mole of glucose.
- It is a less used pathway than the EMP pathway in microorganisms.
- This pathway is more common in some gram-negative bacteria and *Pseudomonas* sp.

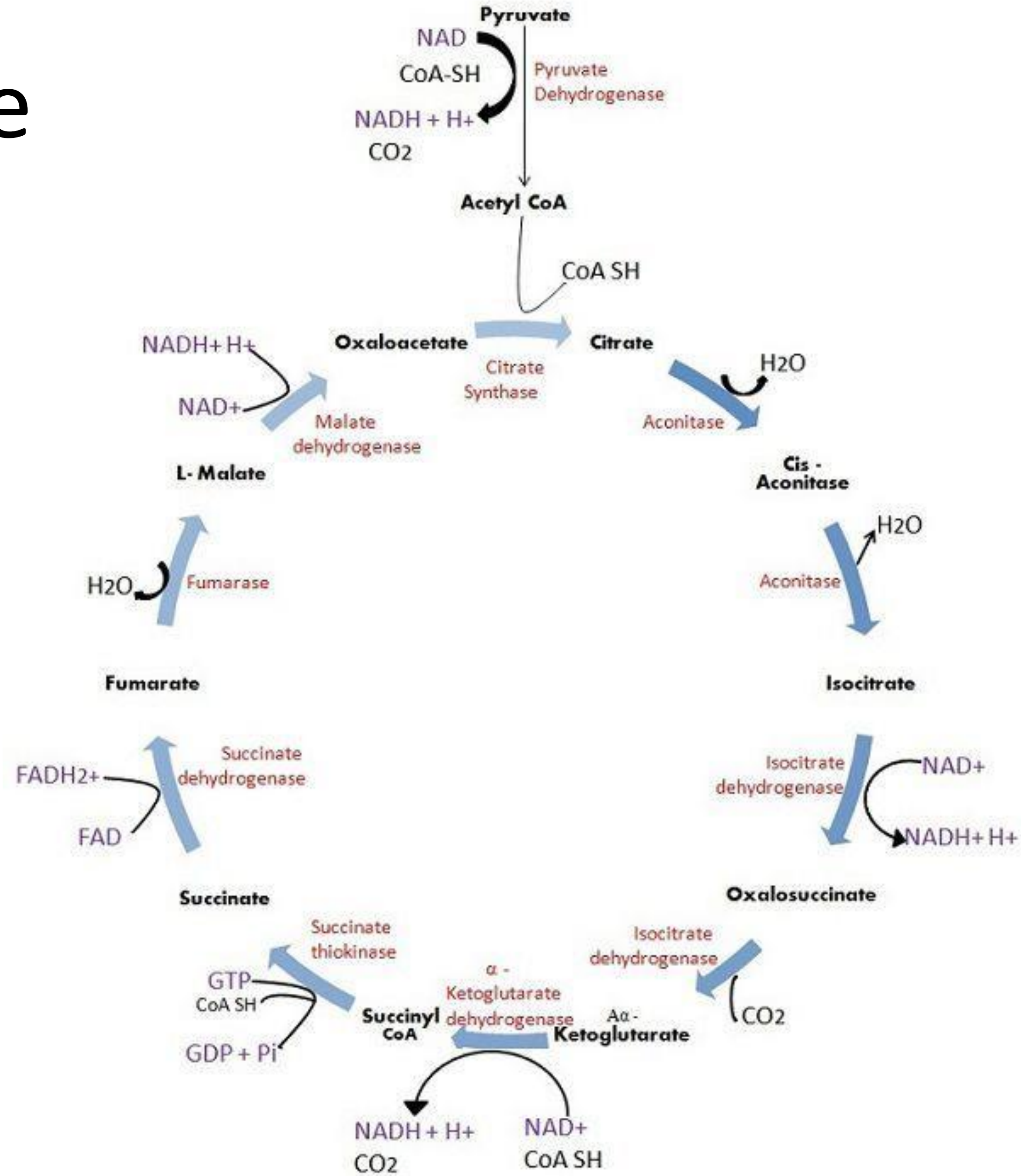
Tricaboxylic Acid Cycle, TCA

- The citric acid cycle is also known as the tricarboxylic acid cycle.
- The citric acid cycle is a cycle in which the acetyl groups of acetyl-CoA, which is the common end product of carbohydrate, fat and protein catabolism, are oxidized in cellular respiration. In aerobic cells, fuel molecules are oxidized by oxygen to CO_2.
- In each round of the cycle, the 2 C acetyl group enters as acetyl-CoA and two 13 molecules of CO_2 are released.
- The citric acid cycle begins with oxaloacetic acid and ends with the regeneration of oxaloacetic acid after eight successive reaction steps.



TCA cycle

- Each turn in the citric acid cycle produces an ATP molecule, but all four oxidation steps in the cycle provide significant electron flow to the respiratory chain.
    - In this cycle, 3 moles of NAD+ and 1 mole of FAD are reduced.
    - The resulting 3 moles of NADH and 1 mole of $FADH_2$ are oxidized in the respiratory chain.
    - As a result of the passing of 2 electrons from NADH to oxygen, 3 ATPs are synthesized, and as a result of passing through $FADH_2$, 2 ATPs are synthesized.



Total ATP production from glucose

- **Total ATP Yield**

02 ATP - glycolysis (substrate-level phosphorylation)

04 ATP - converted from 2 NADH - glycolysis

06 ATP - converted from 2 NADH - grooming phase

02 ATP - Krebs cycle (substrate-level phosphorylation)

18 ATP - converted from 6 NADH - Krebs cycle

<u>04 ATP</u> - converted from 2 $FADH_2$ - Krebs cycle

36 ATP - TOTAL

Lipid Metabolisms

Lipids are essential components of cell membranes.

Energy values are higher than carbohydrates and proteins.

- 90% of them are triacylglycerols. triacylglycerol + 3 fatty acids
- The basic lipid metabolism is the β-oxidation of fatty acids.
- Lipids are present as various compounds in the cell wall of microbes (especially in Gram-negatives), the cytoplasmic membrane, and intracellularly as food storage.
- In mycobacteria, waxy and large amounts of lipids are found in the cell wall.
- Lipids are usually
  - oils (glycerine esters of fatty acids),
  - beeswax (monoalcohol esters of fatty acids) and
  - they are in the form of complex lipids (phospholipid, lipopolysaccharide, lipoprotein, etc.).

Lipid Metabolisms

- Lipids are organic compounds composed of carbon (C) and hydrogen (H). They can also contain phosphorus (P), nitrogen (N) and sulfur (S).

Dissociation of Lipids

- Among the hydrolytic enzymes found in microorganisms, lipases decompose fats into their essential components. When lipids are hydrolyzed, fatty acids and glycerin are formed.

$$\text{Lipid} + H_2O \xrightarrow{\text{lipase}} \text{fatty acids} + \text{glycerine}$$

$$C_3H_5\,(O.CO.R)_3 + H_2O \xrightarrow{\text{Lipase}} C_3H_5\,(OH)_3 + 3\,R.COOH$$

- Lipopolysaccharides in Gram-negative bacteria and ribitol or glycerol teichoic acid synthesis in Gram-positive bacteria are important in terms of being surface macromolecules and determining the antigenic properties of these microorganisms.

Protein and Amino Acid Metabolisms

- Proteins are complex organic compounds and form the main structures of cells.
- In the composition of proteins, carbon (C) 50%, oxygen (O) 25%, nitrogen (N) 16% and hydrogen (H) 7% are found. Some proteins also contain up to 1% sulfur (S). Some may also contain phosphorus, iron, zinc and copper.
- Cells contain a wide variety of proteins in terms of their structure and functions.
- When proteins are high-molecular (5000-one million), they form colloidal solutions and are not dialyzed.
- They are broken down into their constituent amino acids by proteolytic enzymes or by acid hydrolysis.
- Amino acids are covalently joined to each other by peptide bonds. This peptide bond is formed between the carboxyl end of one amino acid and the α-amino group of the other amino acid.
- There are hundreds of amino acids in the polypeptide chain. Some proteins may have one polypeptide chain and the other more than one.

Nitrogen Metabolisms

- Proteins can be divided into two main parts according to their structure.
  1. Simple proteins: Only amino acids are formed from such proteins at the end of hydrolysis, no other organic or inorganic products are formed.
  2. Conjugated proteins: At the end of their hydrolysis, not only amino acids are formed, but also organic and inorganic components. (nucleoprotein, lipoprotein, phosphoprotein, flavoprotein, glycoprotein, etc.).
- Since proteins are composed of amino acids coming together and combining with each other, the dissolution of the bond between them causes the hydrolysis of proteins.

$$\text{Protein} \xrightarrow[+H_2O]{\text{proteinase}} \text{polypeptide} \xrightarrow[H_2O]{\text{peptidase}} \text{amino acids}$$

- Proteinases and peptidases are usually extracellular enzymes that hydrolyze proteins with large molecules outside the cell and decompose them into small units (amino acids).

Food Spoilage

1. Caused by microorganisms (bacteria, mold, yeast)
2. Damages caused by insects,
3. Activation of enzymes in foods,
4. Chemical reactions,
5. Freezing, burning, drying etc. physical factors

Microorganisms Used in the Production of Fermented Foods

Microorganisms	**Products**
Lactic acid bacteria	Pickle, Cheese, yoghurt
Saccharomyces cerevisiae	Wine, beer, bread
Saccharomyces carlbergensis	Beer
S. cerevisiae ve *Acetobacter aceti*	Vinegar
Penicillium camembertii	Camemberti Cheese
P. roqueforti	Rockfort Cheese

Microbial Spoilage in Foods

Foods are divided into 3 groups as;

1. Stable foods,
2. Foods that spoil more easily compared to stable foods,
3. Easily perishable foods.

- It is possible to divide the microorganisms in food into 3 according to their activities.
  1. Saprophytic microorganisms causing spoilage,
  2. Pathogenic microorganisms causing food poisoning and infections,
  3. Microorganisms involved in the production of fermented foods.

Microbial Spoilage in Foods

- Some features of foods arising from their natural structures prevent microbial development, and the processes applied to the raw material and the special environments provided can prevent or delay the deterioration of food by these creatures.
- Undesirable color, taste, odor, appearance and structure changes that occur as a result of the development of microorganisms in foods are called **microbial spoilage**.
  - Food disorder; It is a situation that causes a change in the appearance, smell and / or taste of the food and is unacceptable to the consumer.
- Spoilage of a food; It is an indication that microorganisms develop and reach high numbers during raw material supply, transportation, processing and storage.
- Most foods provide a suitable environment for microorganism growth, including some human pathogens.

Microbial Spoilage in Foods

- Foods are generally spoiled by microorganisms that contaminate the food.
- Depending on the nutritional value and water content of foods, their susceptibility to microbial growth is different.
- Stable, semi-stable and perishable foods have different and limited shelf-life.
- Various microorganisms induce spoilage, and food spoilage microorganisms can also be potential pathogens. Controlling microorganism in foods has 2 purposes;
  1. Delaying or completely preventing microbial spoilage in foods
  2. Prevention of diseases transmitted to humans by food.

Microbial Spoilage in Foods

Microorganisms that spoil foods use them as a source of nutrients and energy. Microbial hydrolytic exoenzymes play a major role in food spoilage.

Important hydrolytic exoenzymes produced by microorganisms

Enzyme	Influenced component
Pectinase	Pectin (Fruits and vegetables)
Celulase	Cellulose (Fruits and vegetables)
Protease	Protein (meat, fish etc.)
Amylase	Starch (Starchy foods)
Lipase	Oils (Oils and oily foods)

Proteolytic degradation

- As a result of the degradation of proteins in foods (proteolytic degradation), proteins are broken down into peptides and amino acids as hydrolysis. While it is desirable in cheese ripening, there is the formation of malodorous compounds in many foods.
- Especially as a result of anaerobic degradation of proteins, malodorous compounds such as H_2S, ammonia, mercaptan, indole, skatol occur.

Microbial Spoilage in Foods

Carbohydrate breakdown

- Di-, tri- and Polysaccharides can be used by microorganisms after hydrolysis to simple sugars.
- While glucose is broken down to water and CO_2 under aerobic conditions, many products can be formed under anaerobic conditions:
  1. to ethanol and CO_2 by yeasts
  2. to lactic acid by homofermentative Lactic Acid Bacteria (LAB)
  3. to lactic acid, other organic acids, alcohols etc by heterofermentative LAB.
  4. to organic acids and many compounds by coliform bacteria,
  5. to propionic acid and etc. by propionic acid bacteria,
  6. to butyric acid, acetic acid and etc. by anaerobic butyric acid bacteria

Microbial Spoilage in Foods

Decomposition of acetic acid

- They are oxidized to CO_2 and water by some aerobic microorganisms (film yeasts or oxidative yeasts).

Lipolytic disruption

- Fats in foods are broken down into glycerol and fatty acids by the effect of microbial lipase. Glycerol and fatty acids are also hydrolyzed and broken down into CO_2 and water.

Pect(in)olytic degradation

- Pectin in the walls of plant cells consists of galactronic acid polymers with methyl groups in their structure.
- The microbial pectinesterase enzyme hydrolyzes the methyl ester bonds to break down pectin into pectic acid and methanol.
- The enzyme polygalactronase hydrolyzes the bonds between pectin and galactronic acid molecules in pectic acid to form pectic acid and D-galactronic acid.

Microbial Spoilage in Foods

- These deteriorations in food are related to the water activity of the food. (aw)
- Water activity; It is the available water that can be used in metabolic processes.
  - Non-perishable foods have low water activity
  - The water activity of spoiled/semi-perishable foods is relatively higher.
- Fresh foods are spoiled by various bacteria and fungi, and each different type of fresh food is degraded by different microorganisms.
- For example: Included in enteric bacteria, all of which are potentially pathogenic;

  - *Salmonella*
  - *Shigella* ➡ It is rarely seen in fruit and vegetable spoilage.
  - *Escherichia* It is very common in meat spoilage.

- This contamination occurs when the intestinal contents leak and contaminate the meat during slaughter.

Microbial Spoilage in Foods

- The dominant microorganisms in dairy products are lactic acid bacteria.
- Pseudomonas species are highly associated with spoilage of fresh foods, as they are found in both soil and animals.
- The growth rate of the microorganism in the log phase depending on ;
  - temperature,
  - nutritional value of food and
  - other growth conditions.
- Once the microbial population reaches a certain level, the effect of food spoilage begins to be observed.
- However, during the exponential growth phase, population density is often low and no effect may be observed.
- Therefore, for most of the microbial growth period in foods, there is no visible and easily noticeable change in food quality.

Indicators of Microbial Spoilage in Foods

- If protective measures are not taken for foodstuffs and they are not consumed in a short time, they will be contaminated and spoiled by microorganisms.
- Undesirable quality characteristics (color, taste, texture, odor) occur as a result of spoilage of food.
- Pathogens and spoilage-causing microorganisms can contaminate foods due to inadequate hygiene and sanitation conditions at various stages of production, starting from the field and reaching consumers.
- In addition to threatening human health, these microorganisms cause deterioration in the storage process and, as a result, economic losses.
- It is not possible to isolate all microorganisms in order to understand whether foods are produced under hygienic conditions and the presence of pathogenic and spoilage microorganisms.
- For this reason, some microorganism groups and their metabolism products are considered as indicators for foods as an indicator of their presence or absence.
- In other words, the microorganisms that show whether the production conditions of the food are hygienic and whether the processes applied to make the food safe are carried out correctly are called indicator microorganisms.

Indicators of Microbial Spoilage in Foods

- The following features should be considered in the determination of microbial spoilage indicator microorganisms and their metabolites:
  1. The presence of microorganisms should be easily and quickly identified and counted,
  2. The growth of indicator microorganisms should not be affected by other microorganisms and should be easily distinguishable,
  3. It should be able to coexist with pathogens for which it is likely to be found.
  4. There should be a parallelism between the number of indicator microorganisms in the food and the number of pathogenic microorganisms.
  5. It should have characteristics similar to the growth needs and reproduction rate of the pathogen microorganism whose presence it indicates.
  6. The indicator microorganism must be as viable and durable as the pathogen
  7. The indicator microorganism should not be a natural contaminant in the analyzed food.
  8. Indicator microorganism should not have a high growth and death rate at storage temperature.
  9. In high quality fresh product, the number of microorganisms should be low and there should be no metabolites.
  10. When deterioration occurs, the main deterioration agent should be the indicator microorganism or metabolite.
  11. The presence and number of indicator microorganisms should be inversely proportional to the quality of the food.

Additional Criteria for Fecal Indicators

1. Ideally, fecal indicator should present only in intestinal environments.
2. They should present in very high numbers in feces,
3. They should show high resistance to external environment.
4. They should be easily in low numbers.

Indicators of Microbial Spoilage in Foods

- Type of food, composition, production method, packaging, storage conditions (temperature-time parameter, temperature fluctuation, etc.) affect microbial spoilage.
- In order to reduce food losses as a result of microbial spoilage, it is necessary to determine the shelf life and current microbial quality of foods.
- Indicators used to determine microbial product quality or shelf life are typical spoilage microorganisms or their metabolic products found in foods at certain levels.
- The increase in the number of spoilage microorganisms causes quality loss and shortens the shelf life of the product.
  - *Pseudomonas* species and some psychrophilic bacteria can be used as indicators in protein-rich foods such as meat, chicken, fish and milk at refrigerator temperature.
  - Yeasts and lactic acid bacteria appear to be the main cause of spoilage in fruit juices with high sugar content and low pH.
  - The total number of molds and yeasts, which can cause spoilage in foods where bacteria cannot grow due to their composition, is an important spoilage indicator.

Microbial Spoilage Indicators

Foods	Spoilage Indicators	Foods	Spoilage Indicators
Raw milk	Psychrophilic bacteria, Total number of bacteria	Fresh meat (without package)	Psychrophilic aerobic bacteria
Pasteurized milk	Psychrophilic bacteria, Total number of bacteria	Fresh meat (with package)	Psychrophilic lactic acid bacteria and enterobacteria
Butter	Lypolitic microorganisms	Processed meats (vacuum package)	Psychrophilic lactic acid bacteria and enterobacteria
Cream Cheese	Psychrophilic bacteria	Sea foods (fresh)	Psychrophilic bacteria
Drinks	Acid bacteria, Mold and Yeast	Salad dressings	*Lactobacillus* spp. Yeast

Microbial Spoilage Indicators

Foods	**Microorganisms**
Fresh meat, minced	*Pseudomonas fragi, P. fluorscens*
Fish and Fish products	*Shewanella putrefaciens, P. fragi*
Raw milk	*Lactococcus lactis*
Milk	*Bacillus cereus*
Butter	*P. putrefaciens*
Fresh fruit juice	*Acetobacter, Gluconobacter*
Fruit juice concentrate	Yeast
Dough	*Bacillus* spp.
Beer	*Pediococcus*
Wine	*Pediococcus, Penicillium, Aspergillus*
Mayonnaise and salad dressing	*Lactobacillus, Zygosaccharomyces*
Sugar (refined)	*Leuconostoc mesenteroides*

Indicators of Microbial Spoilage in Foods

- Along with the microorganisms in the food, some metabolites produced by these microorganisms also serve as spoilage indicators.
- Heat-stable lipase and proteinase enzymes produced by some psychrophilic bacteria growing in milk are important indicators in determining the shelf life of UHT milk.
- Ethanol produced by yeast in fruit products; Lactic acid produced by bacteria in canned vegetables acts as a spoilage indicator for these foods.

Foods	Metabolites
Meat and meat products	Cadaverine, putrescin, indole, H_2S
Fish and seafood	Cadaverine, putrescin, histamine...
Milk and dairy products	Proteinase, lipase
Apple juice	Fumaric acid, ethanol
Grape	Acetonine, ethanol
Frozen fruit concentrates	Diacetil
Canned vegetables	Lactic acid
Cereals	Ergosterol

Indicator Microorganisms

- Indicator microorganisms are evaluated as an indicator of whether production is carried out in accordance with the rules in the food industry.
- Indicator microorganisms provide sufficient information on raw material, production technology, good and correct production practice (GMP; Good Manufacturing Practice).
  - In other words, and briefly, indicator microorganisms are an indicator of quality.
- At this stage, indicator microorganisms and pathogens should not be mixed with each other.
- This group of microorganisms sought/counted to get an idea about food quality are microorganism groups such as total bacteria, total yeast and mold, total coliforms, fecal coliforms.
  - Even if there are pathogenic bacteria in the total bacteria (such as *Staphylococcus aureus*), they are only considered as total bacteria according to the analysis method.
  - Conversely, if a microorganism that is considered beneficial because it is used in the production of a food item (for example, *Penicillium roqueforti* used in the production of roquefort cheese) contaminates another food (for example, cheddar cheese), that product will be degraded, since mold will be found above the standards in the total yeast and mold analysis as an indicator microorganism.

Indicator Microorganisms

- All microorganisms are evaluated as indicators.
- Recently, in addition to microorganisms, it has been emphasized that products such as lactic acid, diacetyl, alcohol, which occur due to microbial growth, are also evaluated as microbial indicators.
- Indicator microorganisms specific to that enterprise in different enterprises of the food industry are emphasized.
  - For example, while the presence/number of lipolytic microorganisms is an important quality criterion in the butter plant, lipolytic bacteria are of no importance, for example, in the fruit juice industry.
  - While fecal contamination index bacteria is an important quality criterion for many foodstuffs, it is unnecessary to search for these bacteria in canned vegetables.
- Food businesses can determine different microorganisms as indicators starting from raw materials within the framework of their own quality programs.
- In addition, there are also indicator microorganisms determined by public control institutions.

Properties of Indicator Microorganisms

- Total bacteria, total yeast and mold, total Osmophilic and Osmotolerant yeasts, Xerophilic molds, total proteolytic bacteria, total coliforms etc. Different microorganisms, such as, are used as indicator microorganisms in determining the quality of different foods.
  - As a general principle, although indicator microorganisms should not be pathogenic, *Clostridium perfringens* is an exception.
- It was determined that there was mostly a linear relationship between pathogenic microorganisms and **fecal contamination**, and fecal-derived microorganisms were used as an **index (indicator) of sanitation in waters**.

Indicator Microorganisms

- The microorganism to be used as an indicator of fecal contamination should have the following characteristics in addition to the above-mentioned indicator microorganism criteria:
  - The bacteria must be of intestinal origin.
  - It must be able to survive outside the intestine.
  - It should be easy and reliable to detect, and it should be easily detectable even in low numbers.
  - It should be present in high numbers in stool, and its presence should be detectable when diluted.

- Indicator microorganisms can be examined under two headings:
  - Pathogen Indicators
  - Spoilage Indicators

Indicator Pathogen Microorganisms

- Pathogenic microorganisms are known for their disease-causing properties.
- The main pathogen indicators are:
  - Coliforms
  - Enterococci
  - Fecal Coliforms
  - Total number of viable microorganisms
  - Other security indicators

Coliform Bacteria

- They are gram-negative, non-spore-forming bacteria that can be inhibited at pasteurization temperature and that form gas in the environment as a result of the fermentation process.
- It was first used as an indicator of water safety, later as a possible fecal contamination in other foods and as an indicator of sanitation in the food industry.
  - They are Gram (-), non-spore stick, aerobic or facultative anaerobic bacteria belonging to the *Enterobacteriaceae* family, which form gas from lactose at 35°C within 48 hours. EMB and Endo form metallic colonies on agar. *Escherichia, Enterobacter, Klebsiella* and *Citrobacter* are the genera included in coliform bacteria.
  - Bacteria named as coli-aerogenes are E.coli and Enterobacter aerogenes. *Enterobacter cloacae, Klebsiella pneumonia* and *Citrobacter freun*dii are other important species.
- Since they are found in the human and animal intestinal tract, they are accepted as the best indicator of fecal contamination, but it has been determined that some coliforms are not only of fecal origin.
  - *Enterobacter cloacae, Klebsiella pneumonia* and *Citrobacter freundii* are common in nature.
- The presence of coliform bacteria in food is not a definitive indicator of fecal contamination or the presence of enteric pathogens. For this reason, coliform bacteria can be used more as a sanitation indicator.
  - Presence of coliforms in raw milk; indicates insufficient hygienic conditions during milking, transportation, storage and processing.
  - For frozen vegetables, especially because Enterobacter strains are frequently found in vegetables, coliforms are important only as an indicator of sanitation in the processing steps.
- These microorganisms can be detected using IMVIC tests.

Coliform Bacteria

a) Characteristics

- The term coliform represents a group of bacteria species: *E. coli, Enterobacter aerogenes, Klebsiella pneumoniae, Citrobacter freundii*.
  - Probably *Aeromonas hydrophila* and *Serratia mercescens.*
- The main reason for grouping them together is due to their characteristics.
  - They present in family Ent*erobacteriaceae*, Gram negative, non-sporeforming bacilli, aerobic or facultative anaerobic, relatively resistant to many disinfectant, ferment lactose to produce gas (and acid) within 48 h at 35°C, many are motile and able to grow in foods at pH between 4.0 and 7.5.
- They present in feces of humans, animals and birds.
- The sources of coliforms that contaminate foods are intestinal tract of man and animals, raw materials, equipments, workers, water, air, dust, additives and packaging materials.

Effects of Coliforms on Foods

b) Effects of coliforms on foods

- They are undesirable in foods due to their spoilage ability.
- They utilize carbohydrates to produce acids and gas.
- They hydrolyze proteins to produce off-flavors;
  - this would result with unclean or barny effect.
- *Enterobacter* and *Klebsie*lla cause ropiness and sliminess on foods.
- High numbers of coliforms are considered as poisons due to their end products and cellular components released from lysed cells.

Effects of Coliforms on Foods

c) Significance in foods

- Coliforms are most useful indicators.
- They present in many raw foods and food ingredients in high numbers.
- Their number may increase in refrigerated food due to growth of some species at low temperature.
- The presence of coliforms in heat-processed products indicates post-process contamination.
- The injured coliform cells in processed food can't be recovered in selective media,
  - they can grow during processing or storage periods.
- They will present in a limited viable-numbers in food while pathogens may present in foods.
- Coliforms can indicate the efficiency of treatment.
- Their presence in raw foods in high numbers (such as 10^3 cfu/g or mi) indicates:
  - Improper handling conditions,
  - Improper sanitation and
  - Possible presence of pathogens.

Fecal Coliforms

- Fecal coliforms are bacterial species in the coliforms
  - but they are specifically intestinal bacteria and fecal origin.
- This group includes mostly *E. coli, K. pneumonia* and *E. aerogenes*.
- Fecal coliforms grow at both 44.5°C and 35°C,
  - but non-fecal coliforms cannot grow at 44.5°C.
- They can present in animal foods due to contamination from fecal matter, soil and water.
- Fecal coliforms are easily destroyed by heat and
- They may die during freezing and frozen storage of foods.
- Contamination of a food with fecal coliform indicates a risk that other enteric pathogens may be contaminated and present in the food.

Fecal Coliforms

b) Significance in foods

- The presence of fecal coliforms in processed foods indicates:
  - improper sanitation,
  - possible fecal contamination and
  - presence of enteric pathogens.
- Their presence in raw foods in high numbers (such as 10^3 cfu/g or ml) indicates:
  - contamination of fecal matter,
  - improper handling conditions,
  - improper sanitation and
  - possible presence of enteric pathogens.
- Fecal coliforms are extensively used as indicators for foods of marina origin and more handled foods.

Fecal Contamination Indicator

- The most important group as indicator microorganisms are bacteria with fecal contamination index.
  - The presence of these is an indication that the food is directly or indirectly contaminated with sewage and faeces in one or more stages, starting from the raw material to the transportation of the food.
- Fecal coliforms, Enterococci and *Clostridium perfringens* are typical fecal contamination indexes.
- *E. coli* is commonly used instead of fecal coliforms. Of these, Enterococci are considered a better indicator than others for the determination of fecal contamination in waters.
- There are points to be considered on fecal contamination index and accordingly food quality.
  1. It must be proved that it is only found in feces.
  2. It should be present in the stool in high proportion.
  3. It should be resistant to adverse environmental conditions.
  4. It should be able to be detected relatively easily and reliably in foods, even in very low quantities.

Fecal Contamination Indicator

- Coliform, fecal coliform, *E.coli, Enterobactericeae, Enterococcus, Bifidobacterium, Pseudomonas, Clostridium, Staphylococcus,* coliphage and aerobic plaque counts are used as indicator tests.
- The presence of these bacteria in the analyzed material is a lack of hygiene.
  - Does this lack of hygiene come from the raw material or from the operating conditions?
- For example, defecation of birds on raw materials cannot be prevented practically and easily in field conditions. In this case, fecal contamination is natural in many spices.
- Conversely, if udder hygiene and milking conditions are controlled in milking, contamination of animal feces to raw milk can be completely prevented. In this case, there should be no fecal coliform in raw milk.
- In a product such as cheese made from pasteurized milk, all coliform bacteria die at the end of pasteurization.
- Therefore, the presence of fecal coliforms in these products is only due to the slurry at the end of pasteurization. The main responsible for this is that the employees in the enterprise do not pay attention to the minimum hygiene after the toilet.

E. coli

a) Characteristics

- *E. coli* confirm to the general characteristics described for coliform groups.
- Biochemically, it can be differentiated from other coliforms by
  - - IMVIC tests (indole from tryptone, methyl red reduction, Voges-Proskauer reaction and citrate utilization).
  - HOMoC tests (H_2S, ornithine utilization and motility).
- *E. coli* are used as indicators of fecal contamination and presence of enteric pathogens in foods.
- They are considered as nonpathogens and naturally present in the gastrointestinal tract of humans, animals and birds.
- Absence of *E. coli* in a food, however, does not ensure the absence of enteric pathogens.
- Animal origin raw foods mostly contain small numbers of *E. coli*,
  - because of the close association of these foods with the animal environment and the contamination of carcasses from fecal material, hides or feathers during slaughter.

E. coli

b) Significance in foods

- *E. coli* may die at a faster rate in dried, frozen and low pH product than some enteric pathogens.
- Some enteric pathogens are able to grow at low temperature (O to 2°C)
  - at which other *E. coli* strains can die.
- *E. coli* cells can be injured by treatments in higher degrees than some enteric pathogens,
  - may not be effectively detected by selective media.
- Heat processed foods should be free from *E. coli*,
  - their presence indicates recontamination.
- Presence of *E. coli* in a heat-processed food indicates;
  - process failure,
  - post-processing contamination from equipment, employees or contact with raw foods.
  - presence of enteric pathogens.

Enterobacteriaceae

- Some enteric pathogens do not ferment lactose, example *Salmonella*.
- Instead of only enumeration of coliforms or fecal coliforms in a food, enumeration of all the genera in *Enterobacteriaceae* family can be used as indicators.
- This family includes many genera that are enteric pathogens (*E. coli, Salmonella, Shigella, Vibrio* and *Serratia*) and nonenteric bacteria (Edwardsiella, *Erwinia*, *Citrobacter, Klebsiella, Enterobacter, Hafnia, Proteus* and *Marganella*).
- Enumeration of whole group can be used as a better indicator for level of sanitation, possible fecal contamination and possible presence of enteric pathogens.
- Enumeration method of this includes the counting on a selective
- Agar medium containing glucose instead of lactose.
- Many species of *Enterobacteriaceae* are not fecal origin.
- Many present naturally in the environment and on plants.
- In heat processed foods and ready-eat foods, their presence in the high numbers indicate
  - improper sanitation, presence of enteric pathogens and possible fecal contamination.

*Entero*coccus

There are 20 species of bacteria in the *Enterococcus* group.

a) Characteristics

- Common Enterococcus spp. used as indicator are
  - *E. faecalis* var. faecalis,
  - *E. faecalis* var. *zymogens*.
  - *E. faecalis* var. *liquefaciens* and
  - *E. faecium*.
- Enterococcus spp. are members of group D-streptococci, Gram-positive, nonsporeforming, nonmotile, cocci or coccobacilli, produce long or short chain, catalase-negative and facultative anaerobic.
- They can grow at a temperature ranging from 10 to 50°C.
- Some require 8 vitamins and amino acids for growth.
- Some survive at pasteurization temperature (thermodurics).
- They are more resistant to refrigeration, freezing, drying, low pH and salts than coliforms.

*Entero*coccus

- Compared to *E. coli, Enterococci* are more resistant to adverse environmental conditions (freezing, drying, low pH, etc.), although they are found in lower numbers in human feces, making them important as an indicator.
- *Enterococci* are used as indicators of fecal contamination and inadequate sanitation in frozen foods because of their resistance.
- The high number of *Enterococci*, which are also resistant to pasteurization, are detected in pasteurized foods, indicating that the microbial load of the food is high even when raw.

*Entero*coccus

- Sources of Enterococcus include fecal material from both warm-blooded and cold-blooded animals, plants and birds.
- Many present on equipment, processing environments, raw foods, sewage and water, especially polluted water and mud.
- *Enterococcus* differs from coliforms in that they
  - are salt tolerant (grow in the presence of 6.5 % NaCl and 40 % bile salt) and
  - relatively resistant to freezing.
- Certain *Enterococcus* spp. (*E. faecalis* and *E. faecium*) are also relatively heat resistant and may survive at pasteurization temperatures.
- *Enterococcus* is used as indicator bacteria in certain foods due to fairly salt-tolerant, and resistance to freezing and low pH characteristics.

b) Significance in foods

- Their presence in foods indicates fecal contamination, improper sanitation, and
- - possible presence of pathogens.
- Relation of Enterococcus to enteric pathogens is lower than coliforms.
- Their ability to survive at pasteurization temperature and in dried, frozen, refrigerated and low pH foods can place them in a favorable position as indicators for this characteristics of foods.
- Some strains may associate with foodborne gastroenteritis as opportinistic pathogens.

Total Viable Count

- **Total number of viable microorganisms**
  - Number of aerobic live bacteria
  - Number of anaerobic living bacteria
  - Number of psychrophilic live bacteria
  - Number of mesophyll viable bacteria
  - Number of thermophilic live bacteria
  - Cannot be used for fermented foods.

- **Yeast-mold count**

- The total number of microorganisms in the food;
  - The suitability of transportation and storage conditions,
  - Adequacy of sanitation in enterprises,
  - Determination of product shelf life,
  - The onset and level of microbial spoilage,
  - It is important in terms of showing the deterioration that occurs during the thawing of frozen foods.
- The majority of human and animal pathogens are mesophilic. Detection of mesophilic bacteria in food will be proof that the food was produced or stored in conditions that allow these pathogens to multiply.

Other Food Safety Indicators

- *Staphylococcus*: They are found in the mouth, nose, hands and skin of humans. There are pathogenic and non-pathogenic ones. The presence of $>10^6$/g, ml is considered a risk for health.
- *Pseudomonas aeruginosa*: Found in the intestinal tract of humans, but in lesser numbers than other fecal indicators.
- *Clostridium*: Found in the intestinal tract of humans, but also found in soil.
- *Mesophilic spore count*: its high amount in cold stored or dried foods increases the possibility of pathogenic bacteria such as *Clostridium perfringens, C. botulinum, Bacillus cereus*.
- *Thermophilic spore count*: In the canning industry, it can be used as an index of the efficiency of washing and cleaning processes of vegetables to be canned.
- *Bacteriodes*: Found in high numbers in fecal material. It is absolutely anaerobic and difficult to determine by routine testing.
- *Geotrichum candidum*: Known as machine mold. It loses its vitality in the heat treatments applied to the food. The presence of hyphae in processed foods is considered as an indicator of contamination and inadequate sanitation.
- *Kurthia zophii*: May develop in meats at temperatures above refrigerator temperatures. Its presence indicates a storage above the ideal storage temperature.