Power Point PMP No. 5

Microbial Growth & Microbial Growth Regulation

- **Growth**
- Growth of a cell is the culmination of an ordered interplay among all of the physiological activities of the cell. It is a complex process involving
- 1. Entrance of basic nutrients into the cell
- 2. Conversion of these compounds into energy and vital cell constituents
- 3. Replication of the chromosome
- 4. Increase in size and mass of the cell
- 5. Division of the cell into two daughter cells, each containing a copy of the genome and other vital components

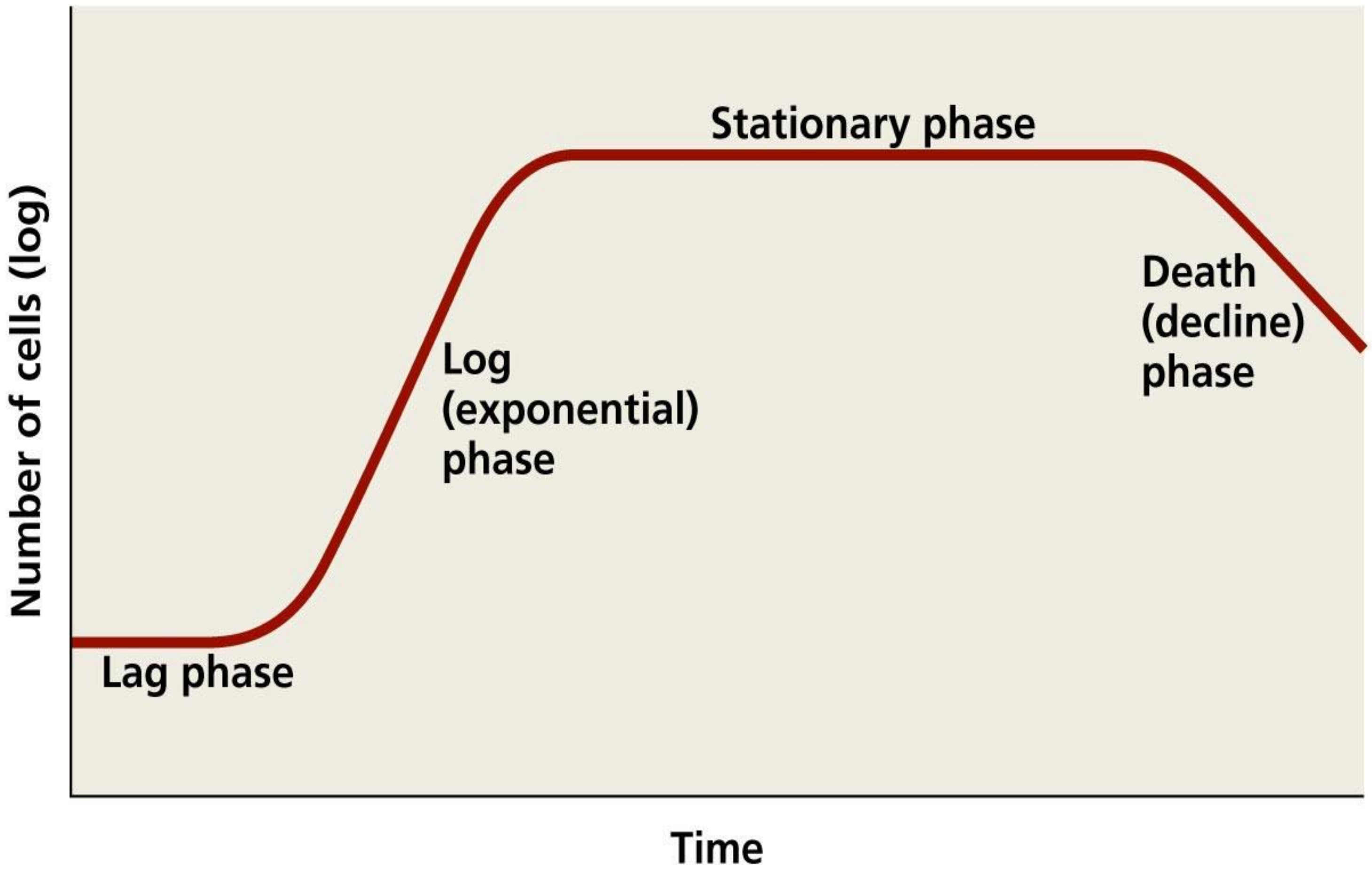
- Microbiologists usually consider the phenomenon of growth from the viewpoint of population increase. A study of the increase in population implies that each cell, as it is produced, is capable of producing new progeny.
- **Growth Cycle:** Growth cycle represent the following phases.
- **a- Lag phas**e: The cells initially experience a period of adjustment to the new environment, and there is a lag in the time required for all of the cells to divide. Actually, some of the cells in the initial inoculum may not survive this lag phase and there may be a drop in the number of viable cells.

- The surviving cells eventually adjust to the new environment and begin to divide at a more rapid rate. This rate will remain constant until conditions in the medium begin to deteriorate (e.g., nutrients are exhausted).This phase of growth is referred to as the **logarithmic phase (b)** or, more correctly, the **exponential phase**.
- All cultures of microorganisms eventually reach a maximum population density in the **stationary phase (c)**.

- Entry into this phase can result from several events. Exhaustion of essential nutrients, accumulation of toxic waste products, depletion of oxygen, or/ and development of an unfavorable pH are the factors responsible for the decline in the growth rate. Although cell division continues during the stationary phase, the number of cells that are able to divide (viable cells) are approximately equal to the number that are unable to divide (nonviable cells). Thus, the stationary phase represents an equilibrium between the number of cells able to divide and the number that are unable to divide.

- Eventually, the death of organisms in the population results in a decline in the viable population and the **death phase (d)** ensues. The exact shape of the curve during the death phase will depend on the nature of the organism under observation and the many factors that contribute to cell death. The death phase may assume a linear function such as during heat-induced death where viable cell numbers decline logarithmically.

Figure showing microbial growth phases



- **Factors regulating microbial growth**
- **Introduction:** Microbial growth is greatly affected by chemical and physical nature of their surroundings instead of variations in nutrient levels and particularly the nutrient limitation. For successful cultivation of microorganisms it is not only essential to supply proper and balanced nutrients but also it is necessary to maintain proper environmental conditions. Thus,
- understanding of environmental influences on the growth of microorganisms becomes mandatory (must be). As bacteria shows divers food habits, it also exhibits diverse response to the environmental conditions. Growth and death rates of microorganisms are greatly influenced by number of factors such as:

1-Nutrients

2-Oxygen

3-Carbon dioxide

4-Moisture

5-Radiation

6-Osmotic pressure and osmoregulators

7-Temperature

8-Sound

9- pH

- **1- Nutreint**

  All organisms have certain nutritional requirements; sources of carbon, nitrogen, energy, and growth essential factors (minerals and vitamins) are needed to support growth. Two main groups of organisms are classified on the basis of their ability to gain energy from certain sources and manner in which they satisfy their carbon and nitrogen requirements for growth:

- a- Lithotrophs: utilize carbon dioxide as the sole source of carbon and gain energy through the oxidation of inorganic compounds (chemolithotrophs) or light (photolithotrophs). Inorganic nitrogen is utilized for the synthesis of organic compounds.

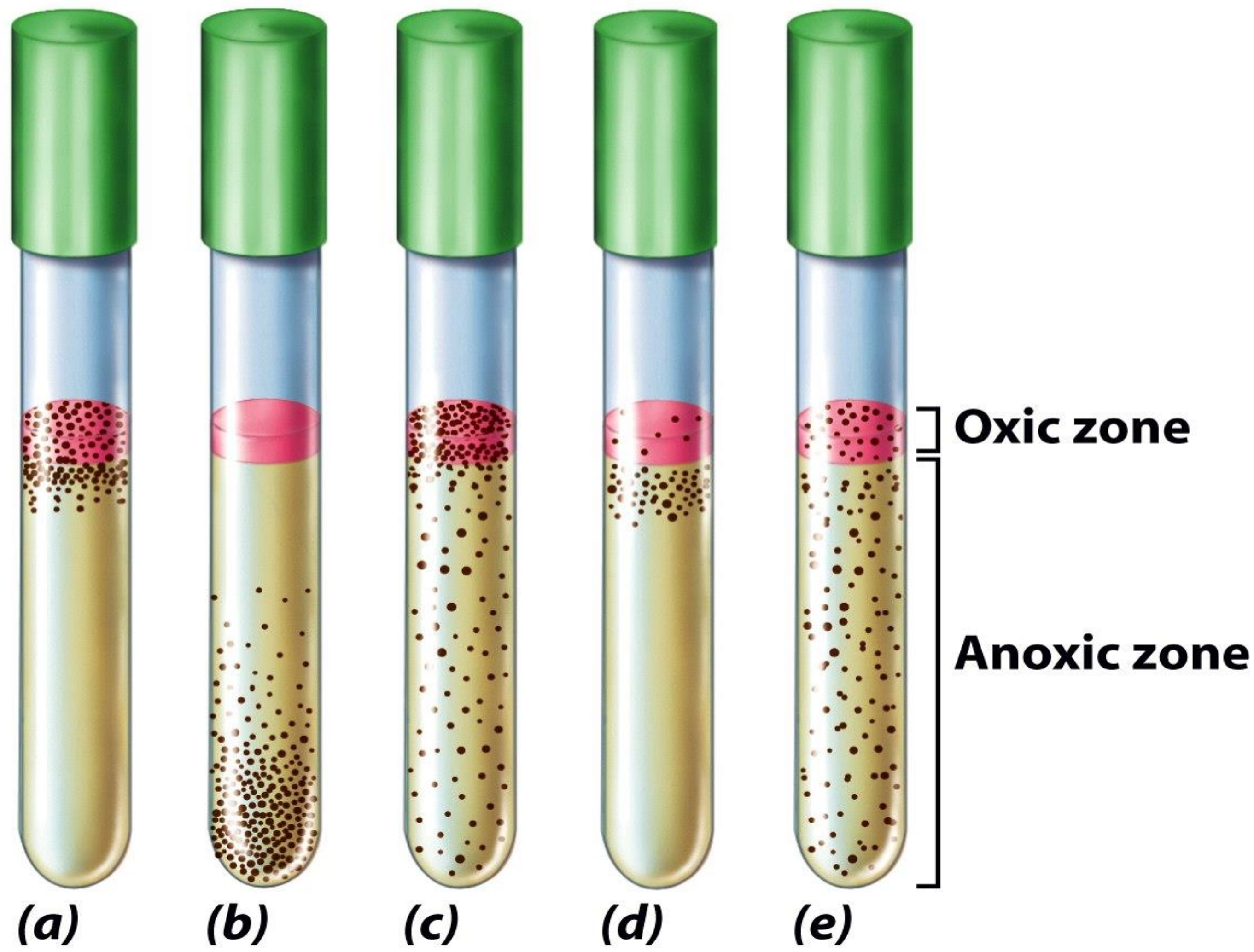
- b- Organotrophs: generally prefer organic substrates as a source of energy and carbon. Photoorganotrophs utilize light as a source of energy for assimilation of carbon dioxide as well as organic compounds. Chemoorganotrophs utilize organic compounds for growth. Organic nitrogen utilized as nitrogen source.
- Two categories of essential nutrients:
- 1-Macronutrients – required in large quantities; play principal roles in cell structure and metabolism (proteins, carbohydrates and lipids) such as oxygen, hydrogen, carbon and phosphorus.
- 2- Micronutrients or trace elements – required in small amounts involved in enzyme function and maintenance of protein structure: manganese, zinc, nickel, vitamins, co-factors.

- **Nutrients needed by microorganisms include:**
- **Carbon:** Carbon-containing compounds are needed as an energy source (eg. glucose). Component of cellular constituents
- **Nitrogen:**
- needed for amino acids and nucleotides;
- Some can synthesize all amino acids;
- Others have to have some provided in their medium.
- **Sulfur:** needed for amino acids (such as cystine and methionine) and coenzymes,
- **Phosphorus**: needed for ATP, phospholipids and nucleotides
- **Vitamins:** A vitamin is an organic substance that an organism requires in small amounts and bacteria in our bodies provide us with needed vitamins, such as vitamin K, that is typically used as a coenzyme. It is needed for blood clotting. Many bacteria make their own, but some are required in the medium;

- **2- Oxygen**
- Essential element for the metabolic activity,(Free atmospheric oxygen in air) although some organisms can utilize oxygen in C-H-O. Microorganisms are classified according to their oxygen requirements:
- a-Obligate aerobes (oxidative): microorganisms are completely dependent on atmospheric O2 for growth.
- b-Obligate (strict) anaerobes (fermentative): Oxygen is not needed for energy production, so there is no oxygen requirements and the oxygen may be either harmless or toxic to these kind of microorganisms.

- c-Facultative anaerobes: These kind of microorganisms can obtain energy for growth by both oxidation and fermentation processes, but they grow better in its presence of oxygen. In the absence of oxygen the facultative fungi can not synthesize some vitamins such as thiamine and nicotine as in the case of *Fusarium* and *Saccharomyces*.
- d-Microaerophiles: microbial cells are damaged by the normal atmospheric level of O2 (20%) but require lower levels (2 to 10%) for growth.
- e-Aerotolerant anaerobes: Ignore O2 and grow equally well whether it is present or not.

Type of Organism	Description	Oxygen Level	Example
Obligate aerobe	With O2	20%	*B. subtilis*
Obligate anaerobe	Without O2	0%	*C. botulinum*
Facultative anaerobe	With/without O2	0 - 20%	*E. coli*
Microaerophiles	With O2	2 - 10%	*Campylobacter*
Aerotolerant	With/without O2	0 - 20%	*Lactobacillus plantarum*


Oxic zone
Anoxic zone
(a)
(b)
(c)
(d)
(e)

- Aerobic organisms posses cytochromes and cytochrome oxidase, which are involved in the process of oxidative phosphorylation. Oxygen serve as the terminal electron acceptor in the sequence and water is one of the resultant products of respiration. Some of oxidation– reduction enzymes interact with molecular oxygen to give rise superoxide (O2-), hydroxyl radicals (OH•) and hydrogen peroxide (H2O2), all of which are extremely toxic:
- These varying relationships between microbes (especially the bacteria) and O_2 appear due to different factors such as protein-inactivation and the effect of toxic oxygen-derivatives. Bacterial enzymes can be inactivated when interact with oxygen. Nitrogen-fixing enzyme nitrogenase is very sensitive to oxygen and represents a good example of interaction between enzyme and oxygen.

- In order to survive, therefore, bacteria must be able to protect itself from oxidizing agents. All aerobes and facultative anaerobes contain two enzymes namely superoxide dismutase and catalase. These enzymes protect microbes against lethal effects of oxygen products. The enzyme catalase decomposes hydrogen peroxide into oxygen and water. The aerotolerant bacteria like lactic acid bacteria possess enzyme peroxidase instead of catalase to decompose the accumulated hydrogen peroxide.
- $2O_2^- + 2H^+$ -----Superoxide dismutase------ $O_2 + H_2O_2$
  $2H_2O_2$ -----Catalase------ $2H_2O + O_2$
  $H_2O_2 + NADH + H^+$ ----Peroxidase---- $2H_2O + NAD^+$

3- Carbon dioxide

The two most important gases that affect microbial growth are oxygen(O_2) and carbon dioxide (CO_2). Many organisms are dependent on the fixation of carbon dioxide . On a practical basis, certain organisms thrive (grow) better if they are grown in an atmosphere containing increased carbon dioxide , such organisms are called Capnophiles . *Haemophilus , Neisseria ,*and *compylobacter* , and many others require at least 5 to 10% carbon dioxide in the atmosphere for initiation of growth , particularly on solid media (eg. *Brucella and Mucor*). CO_2 react with water to produce carbonic acid. Increases in CO_2 concentration to 20% consider toxic

E.g. *Aspergillus nidulans* CO_2 fixation in glucose metabolism limited with increase bicarbonate due to the increases of CO_2 supply. Carbon dioxide is required to replenish intermediates in the TCA (tricarboxylic acid) cycle through the action of pyruvate carboxylase phosphoenolpyruvate carboxylase and malate enzymes.

- **4- Moisture**
- Of all the various factors that influence the growth of microorganisms , water may be considered the most important . Indeed , water may really be considered a nutrient since it form the bulk of the cellular substance (about 75%). Compared to higher organisms , which regulate their water content to some extent, microorganisms are dependent on the amount of water in the environment .
- When organisms are grown on surfaces such as an agar plate , high humidity can provide conditions favorable to the development of microorganisms . Water acts as a solvent , and most metabolic activities are conducted within an aqueous environment in the cell . Water also serves as a catalyst by aiding or actually entering into many enzymatic reactions.

Bacteria absorb food through their cell walls. However, their cell walls cannot pass solid food. Bacteria need moisture to break down solid food to the point where they can absorb it. Due to this, bacteria cannot grow well in a material with a moisture content of less than 15 percent.

Preserving techniques such as dehydration, concentration, freezing are based on making water unavailable for the microorganisms.

Mainly 3 methods for making water unavailable

1. Increasing the solute concentration; removing water, adding of solutes (salt, sugar).
2. Addition of hydrophilic (water-binding) colloids (gels, pectins, gums).
3. Bringing water to a solid phase (freezing)

- **5- Radiation**
- Various forms of radiant energy , such as gamma rays or ultraviolet radiation can cause mutations and/or kill bacteria. Generally, radiations in the UV region and long and short X rays have the most profound effects on biological systems. Near- UV light (325-400nm) and short visible light (above 400nm) also affect the viability of microorganisms or the effectiveness of transforming DNA; however, the mechanism(s) of action of light at these wavelengths has been less well studied . Some organisms have protective pigments or enzymes that can repair radiation-caused DNA damage. Organisms that produce special pigments (e.g., photosynthetic organisms) are able to absorb radiation at specific wavelengths and use the energy derived to sustain their growth and metabolic activities.

- Microbial photosynthetic pigments (chlorophyll, bacteriochlorophyll , cytochromes and flavins), sometimes, absorb light energy, become excited or activated, and act as photosensitizers. The excited photosensitizer (P) transfers its energy to oxygen which then results in **singlet oxygen** ($^1O_2$), The latter is very reactive and powerful oxidizing agent and quickly destroys a cell.

- **6- Osmotic pressure**
- Most microorganisms display optimum growth in a culture medium of low osmotic strength, in which, the cell cytoplasm possesses higher solute concentration in comparison to its environment. Thus, water always diffuses from a region of its higher concentration to a region of the lower concentration. This process is called **osmosis** which keeps the microbial cytoplasm in positive water balance.
- Many of microorganisms when placed in an environment containing high concentration of solute, fail to initiate growth. In hypertonic solution, the cell loses water and shrinks (plasmolysis). Microorganisms like *S. aureus* can survive over a wide range of water activity and are called as **osmotolerant** (as water activity is inversely related to osmotic pressure).

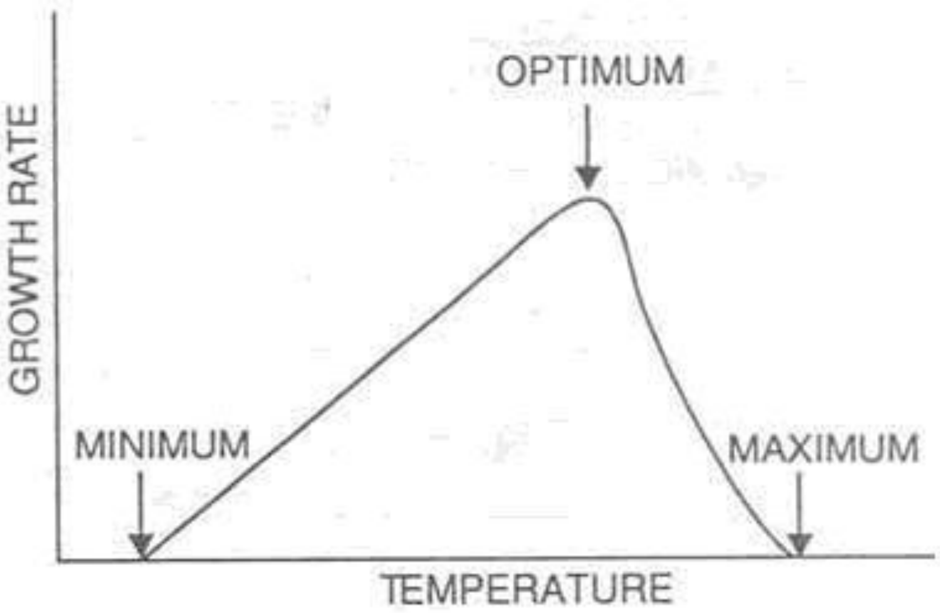
- However, most microorganisms grow well only near pure water activity (i.e. around 0.98-1).
- In hypotonic solution there may be swelling (plasmoptysis) and the cell actually burst if it sintegrity is not maintained by the rigid cell wall.
- Most living organisms respond to changes in the osmotic strength of the culture medium by accumulating compatible solutes that regulate the internal osmolarity of the cell. These solutes named **osmoregulatory compounds or osmoprotectants** may be inorganic cation, amino acids or amino acids derivatives, polyhydric alcohols, or carbohydrates

- Of these various compounds, betaines and proline are the most effective osmoprotectants for *E. coli* placed in culture media in inhibitory osmolarities. A number of halophilic bacteria accumulated betaine to increase in solute concentration such as *Acinetobacter , vibrio alcaligenes, pseudomonas , micococcus* and *chromobacterium.*
- Cyanobacteria accumulate different osmoregulatory compounds according to their salt tolerance and habitat, fresh water, marine and hypersaline. Fresh water strains accumulated simple saccharides such as sucrose and trehalose. Marine strains accumulated glycerol. The halophilic (hypersaline) strains accumulated sucrose and/or trehalose together with betaine or trimethyl-glutamate.
- Filamentous fungi such *Penicilliun chrysogenum* accumulated either glucose and fructose or KCl. Moreover, proline also used as a osmopretectant in *Phytophthora cinnamomi.* Lower fungi accumulated proline for this purpose (Oomycetes).

7-Temperature

All forms of life are greatly influenced by temperature. In fact, the microorganisms are very sensitive to the temperature since their temperature varies with that of environment **(poikilothermic).** Temperature influences the rate of chemical reactions and protein structure integrity thus affecting rates of enzymatic activity. At low temperature enzymes are not denatured, therefore, every 10°C rise in temperature results in rise of metabolic activity and growth of microorganisms. However, enzymes have a range of thermal stability and beyond it their denaturation takes place. Thus, high temperature kills micro- organism by denaturing enzymes, by inhibiting transport carrier molecules or by change in membrane integrity.

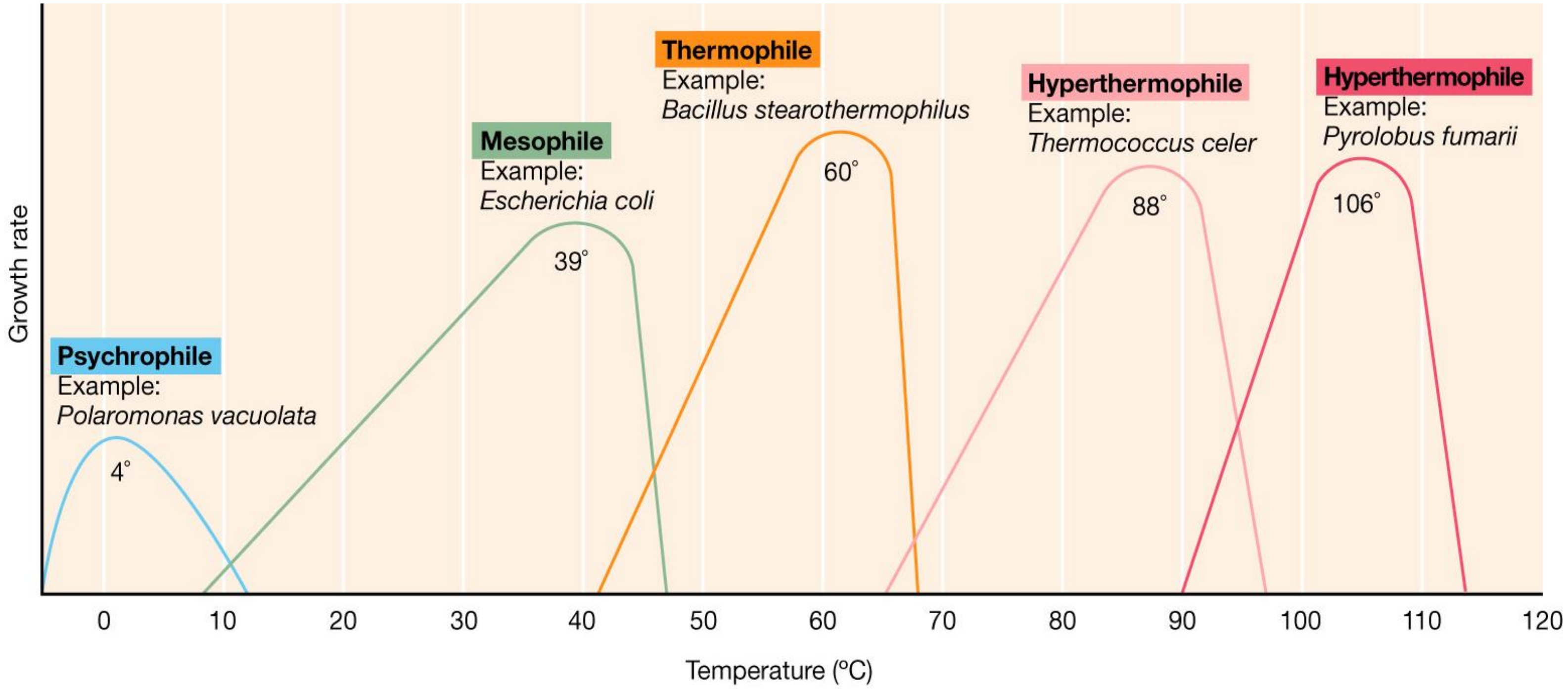
- Each microbe shows characteristic temperature dependence and possesses its own cardinal temperatures i. e. minimal, maximum and optimal growth temperatures. The values for cardinal temperature vary widely among bacteria. For convenience, bacteria isolated from hot springs can survive even at temperature of 100°C and above, while those isolated from snow can survive below -10°C. On the basis of susceptibility to the thermal conditions, microorganisms are classified into three categories: Thermophiles. Meshophiles and psychrophiles.



- **Thermophiles** are microorganisms that show growth optima at 55°C. They often have growth maxima of 65°C, while few can grow even at 100°C and higher temperatures. Their growth minima is 45°C. The vast majority of thermophiles belongs to prokaryotes although a few microalgae (e.g., *Cyanidium caldarium*) and microfungi (e.g. *Mucor pusillus*) are also thermophiles. A few microorganisms are **hyperthermophiles** as they possess growth optima between 80°C and about 113°C. Hyperthermophiles usually do not grow well below 55°C (e.g.. *Pyrococcus abyssi* and *Pyrodictium occultum).*

- **Mesophiles** are microorganisms that have growth minima between 15°C-20°C; optima between 20°C- 40°C and maxima at 45°C. Most microorganisms fall within this category. Almost all human pathogens are mesophiles as they grow at a fairly constant temp. of 37°C. **Psychrophiles** have optimum temperature for growth at 15°C, however, few can grow even below 0°C. The maximum growth temperature of psychrophiles is around 20°C. The spoilage of refrigerated food takes place because of psychrophiles. These are microorganisms that can grow at 0°C but have growth optima temperature up to 20C°.

- Three different effects of temperature contribute to the death of microbial cells:
- Denaturation of proteins (enzymes) by heat
- Intoxication due to accelerated metabolic reactions
- Changes in essential lipids. Melting points of the fats found in the organisms and temperature ranges of death are related.



Type of microorganism	Growth Range	Optimum
Psychrophiles	-5 - 20°C	10 - 15°C
Psychrotrophs	0 - 30°C	20 - 25°C
Mesophiles	20 - 50°C	35 - 40°C
Thermophiles	50 - 80°C	55 - 65°C
Hyperthermophiles	80 - 120°C	85.- 95°C

- **8- Sound**
- Sonic vibrations of less than 10,000 cycles/s have no observable effect upon microorganisms. At higher frequencies in the ultrasonic range, there may be profound effects. Sound at 100,000 cycles/s causes considerable variations in pressure over a very short interval of time (referred to as cavitation). These cavities grow in size and then collapse with a change in pressure, causing disruption of cell.
- Ranges of sound waves
  - Auditory- up to 50,000 Hz
  - Supersonic- up to 200,000 Hz
  - Ultrasonic- more than 200,000 Hz

- **9- pH**
- Microorganisms are able to grow in an environment with a specific pH. such as:
  - Acidophiles organism grow best between pH 0 and 5.5
  - Neutrophiles organism grow best between pH 5.5 and 8.0
  - Alkalophiles organism grow best between pH 8.5 and 11.5
- The pH may affect cell by :
- 1.Cell permeabillity
- 2. Other physiological activities e.g certain enzyme are produced only when pH reaches to a specific range .
  - Despite wide variations in habitat pH, the internal pH of most microorganisms is maintained near neutrality either by proton/ion exchange or by internal buffering
  - Sudden pH changes can inactivate enzymes and damage PMs.

Microorganisms are able to grow in an environment with a specific pH, as shown in this table

Microoganisms	Min. pH value	Opt. pH value	Max. pH value
Gram +ve bacteria	4.0	7.0	8.5
Gram –ve bacteria	4.5	7.0	9.0
Yeasts	2.0	4.0- 6.0	8.5- 9.0
Molds	1.5	7.0	11.0

- **Extremophiles**
- Microorganisms vary widely in their ability to initiate growth over certain ranges of temperature, hydrogen ion concentration and salt concentration. Organisms that function best under extreme environmental conditions are called **extremophiles**. Examples include bacteria found in hot springs and in the thermal vents on the ocean floor. These organisms prefer to grow at extremely high temperatures.

- Some microorganisms prefer to live in an acidic environment (**acidophilic** organisms) while others prefer an alkaline pH (**alkaliphilic** organisms). *E. coli* prefers a neutral pH environment and thus is classified as **neutralophilic**. (The older term, neutrophilic, is not consistent with the nomenclature of the other two groups and can be confused with neutrophiles, a form of white blood cell, and thus should not be used.) The ability of certain organisms to grow in extreme environments can be linked to the possession of specific membrane compositions and/or enzymes with unusual temperature or pH optima that are more suitable to their environment

- **Microbial Stress Responses**
- For **normalophiles**, meaning organisms that prefer to grow under conditions of 37 ◦C, pH 7, and 0.9% saline, variations in pH and temperature have a marked impact on enzyme activity and, ultimately, viability. Outside their optimal parameters, enzymes function poorly or not at all, membranes become leaky, and the cell produces compounds (e.g., superoxides) that damage DNA and other macromolecular structures. All of these factors contribute to cell death when the cell is exposed to suboptimal environments.

- However, many, if not all, microorganisms have built-in stress response systems that sense when their environment is deteriorating, such as when medium acidifies to dangerous levels. At this point, signal transduction systems perceive the stress and transmit instructions to the transcription/translation machineries to increase expression of specific proteins whose job is to protect the cell from stress.
-