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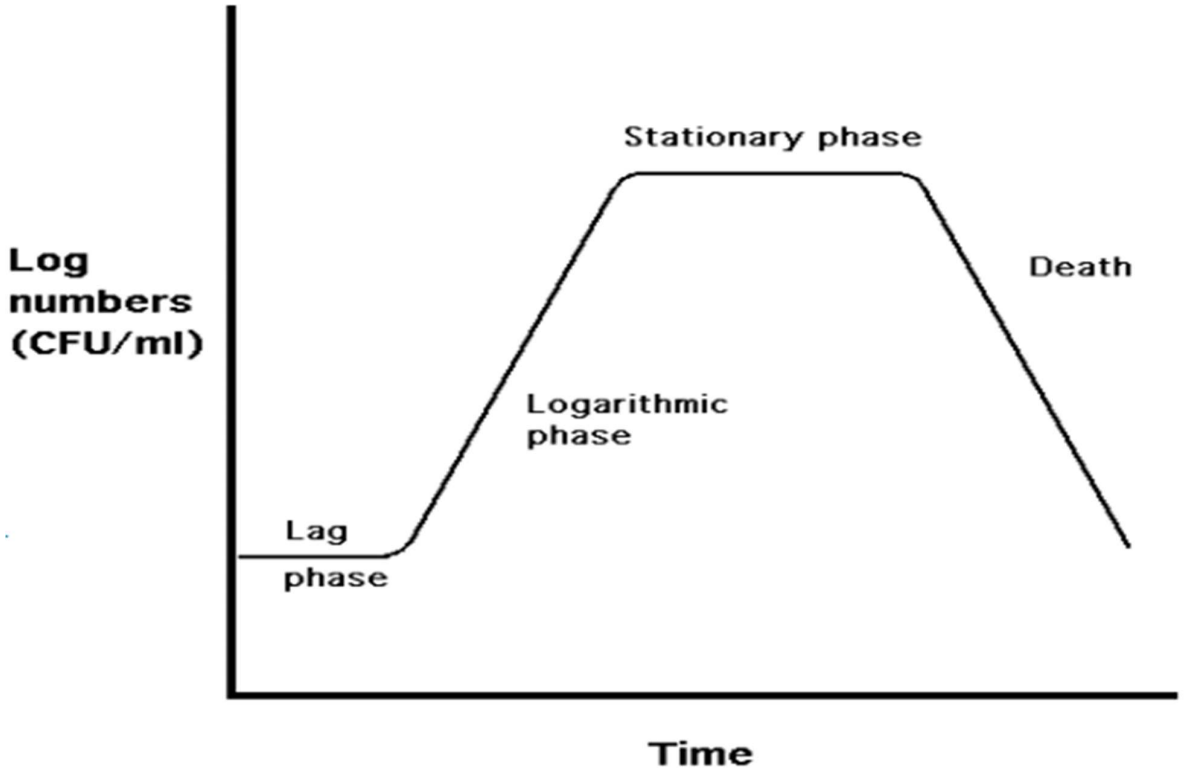
Milk Microbiology (Milk Microflora)

Milk has contained a full range of microorganisms which classified bacteria, viruses, and fungi with few protozoan pathogens such as Cryptosporidium and Giardia. **Bacteria** can be present in the milk in **three forms**: **pathogenic** bacteria, **spoilage** organisms, organisms that may be **conditionally beneficial** (e.g., lactic acid bacteria).

Bacteria reproduce **asexually by simple division** of the cell and its contents. The generation time can be as short as 20 min. Since each cell grows and divides at the same rate as the parent cell, this could under **favorable conditions** multiplication to an increase from one to 10 million cells in 11 hours! However, bacterial **growth** is limited by **lack of nutrients**, accumulation of toxins and metabolic waste, **unfavorable temperatures** and drying.

Bacterial populations are expressed as colony forming units (CFU) per gram or milliliter.

Bacterial growth generally takings through a series of phases:



Hypothetical bacterial growth curve

- **Lag phase:** time for microorganisms to become **accustomed** to their new environment. There is little or no growth during this phase.
- **Log phase:** bacteria logarithmic, or **exponential**, growth begins; the rate of multiplication is the most rapid and constant.
- **Stationary phase:** the rate of multiplication **slows down** due to lack of nutrients and build-up of toxins. At the same time, bacteria are constantly dying so the numbers remain constant.
- **Death phase:** cell numbers **decrease** as growth stops and existing cells **die off.**

The **shape** of the curve varies with **temperature**, **nutrient supply**, and other **growth factors**. This **exponential death** curve (thermal death) is also used in modeling the **heating** destruction of microorganisms.

Sources of Milk Microorganisms:

Milk production in **healthy** mammary glands is **sterile** and only becomes contaminated once it comes into contact with the external environment.

1. The contamination from milking animals.
2. The exterior of the udder.
3. The coat of the milking animals.
4. The surrounding air of the farm and storage.
5. The quality of milk handling equipment and storage tanks used.
6. Biofilms formation on diary plant pipes.

Difference Between Homofermentative and Heterofermentative Bacteria:

Homo-fermentation means the bacteria produce only lactic acid as a primary by-product in glucose fermentation without production of gas (CO_2) and flavor compounds.

Hetero fermentation means that **lactic acid** is the principal product of fermentation with significant amounts of **one or more** of the following metabolites:

- Carbon dioxide (CO_2) which causes small gas holes in cheeses.
- Short chain fatty acids such as **acetic acid** and **propionic acid**.
- Acetaldehyde, a principal component of yoghurt flavor.
- Diacetyl, a principal flavor, notes in sour cream, butter milk.
- Ethyl alcohol.

Conditionally Beneficial Bacteria:

1. ***Lactococcus*:**
   Lactococcus lactis is a Gram-positive bacterium, facultative anaerobic used extensively in the production of buttermilk and cheese. This genus is a **homofermentative** that ferments sugars, releasing only **one by-product**, **lactic acid**. *Lactococcus* genus is capable of **proteolysis**, a process which converts amino acids into flavor complexes, so it can cause **spoilage** of milk.

2. ***Bifidobacterium*:**

The *Bifidobacterium* genus (Gram-positive, facultative anaerobic bacteria) is an essential **probiotic** group in dairy products. Probiotics produce **bacteriocins**. Bacteriocins are bioactive peptides that display antimicrobial characteristics and thus may help in the preservation of fermented dairy products. In particular, ***Bifidobacterium lactis*** is the most predominant *Bifidobacteria* in milk. *Bifidobacteria* is a **heterofermentative** because it produces **acetic acid** as well as **lactic acid**, which is beneficial to human health.

3. ***Lactobacillus*:**
   This genus is extremely diverse, with over 170 species documented. *Lactobacilli* are prevalent in environments rich in carbohydrates such as milk. These microorganisms are extensively **employed in the dairy sector** as **starters** and produce **only lactic acid** as a by-product during the fermentation. Predominant lactobacilli species of industrial relevance include ***Lactobacillus bulgaricus***, ***L. acidophilus*** and ***Lactobacillus lactis***. Lactobacillus is a Gram-positive, facultative anaerobic, and **homofermentative**. ***Lactobacillus*** can cause **spoilage** of milk.

4. ***Streptococcus***:
   This genus consists of more than 90 species, the **majority** of which are **pathogenic** like *S. dysgalactiae.* Nevertheless, the ***S. thermophilus*** is of technological relevance in the dairy industry. This streptococci strain is defined as a **thermophilic** Lactic Acid Bacteria (LAB) and a vital starter in the fermentation of milk.
   The ability of *S. thermophilus* to rapidly ferment lactose, resulting in lowered pH, coupled with the production of essential metabolites such as **acetate.**
   S. thermophilus is always used in conjunction with *L. bulgaricus* in the production of **yogurts**.
   S. thermophilus is gram-positive bacterium, **heterofermentative**, and facultative anaerobic bacteria.

5. ***Propionibacterium***:
   Propionibacterium is a gram-positive, facultative anaerobic, and **heterofermentative** bacteria named for their unique metabolism. They are able to **synthesize propionic acid** by using unusual transcarboxylase enzymes. It is used mainly in cheese making because it **ferments lactose to acetate, propionate, and carbon dioxide** and at the same time enhance flavors through lipolysis of fatty acids.

6. ***Leuconostoc*:**
   The genus *Leuconostoc* is a gram-positive, facultative anaerobic, and **heterofermentative** bacteria. *Leuconostoc* species can produce metabolites such as **acetaldehyde, acetate, and lactate** of fermented milk products. These properties make *Leuconostoc* species an essential **nonstarter** LAB and primary source of aroma and flavor in cultured dairy products such as cultured buttermilk and creamery butter.

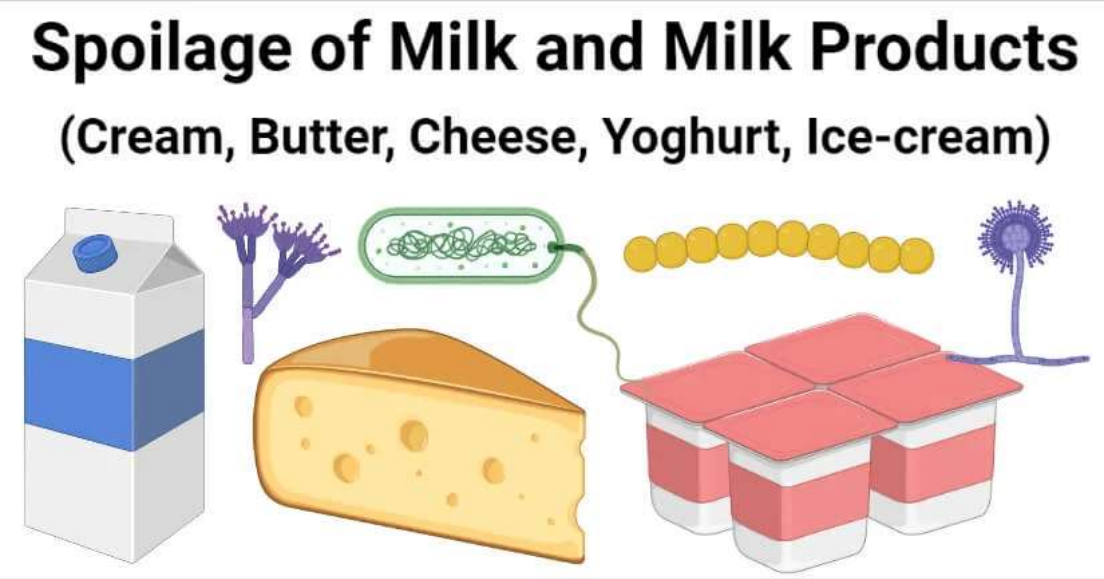
7. ***Enterococcus:***
   Enterococci are gram-positive, facultative anaerobic, and **heterofermentative** organisms. *Enterococci* are the most debatable group of LAB. This endless debate stems from the fact that different strains of *Enterococci* exhibit properties that are similar to **probiotics, starter cultures, pathogens, and spoilage microbes**. *Enterococci* also have a **psychrotrophic** properties and are able to proliferate in refrigerated milk.

Spoilage Microorganisms in Milk:

The microbial quality of raw milk is crucial for the production of quality dairy foods. **Spoilage** is a term used to describe the **deterioration** of a food's texture, color, odor or flavor to the point where it is unappetizing or unsuitable for human consumption.

Spoilage bacteria of food often involves the degradation of protein, carbohydrates, and fats by the microorganisms or their enzymes. In milk, the **spoilage microorganisms** that are principally involved in the spoilage are **psychrotrophic organisms**. Most psychrotrophs are **destroyed** by pasteurization temperatures. However, some spoilage bacteria like ***Pseudomonas fluorescens*, *Pseudomonas fragi*** can produce **proteolytic and lipolytic extracellular enzymes** which are **heat stable** and capable of causing spoilage.

Also, some species and strains of ***Bacillus***, ***Clostridium*, *Corynebacterium*, *Arthrobacter*, *Microbacterium*, and *Micrococcus*** can survive pasteurization and grow at refrigeration temperatures which can cause spoilage problems.



Fungal Population:

The prevalence of fungal populations in milk is dependent on the animal's health, feeding, and weather. Fungi are common contaminants of dairy products, which provide a favorable place for their growth. They are responsible for visible or non-visible defects, such as off-odor and -flavor, and lead to significant food waste and losses as well as important economic losses. Yeast genera include Candida, Cryptococcus, and Trichosporon. Molds, particularly of the Aspergillus, Geotrichum, Fusarium, Mucor, and Penicillium genera. However, it is worth mentioning that several fungal species such **as Debaryomyces, Saccharomyces** (beneficial fungi) have physiological characteristics, especially high proteolytic activity, which enhancing the aroma and flavor of fermented milk products.

Impact of Storage Conditions and Treatments on Milk Microorganisms:

1. Cold Storage:

Cold storage of milk is a typical method in many parts of the world. The reasoning behind this method is that at lower temperatures most bacteria cannot proliferate or grow and multiply at a reduced rate.

Cold storage is ineffective against psychrotrophs. Psychrotrophs are a major concern when refrigerating milk because they can bring about spoilage.

Under cold storage, *Pseudomonas* species dominate the microbial composition of milk. Cold storage of **raw milk** does little in enhancing the product's quality because of the increased biological activity of psychrotrophs, particularly *Pseudomonas* species. From this brief overview, it has been observed that cold storage of raw milk might minimize the growth of certain microbial populations. Nevertheless, lower temperatures favor the exponential growth and reproduction of psychrotrophs such as increasing the chances of spoilage.

2. Pasteurization:

Pasteurization was designed to minimize the total bacterial count of milk, resulting in reduced chances of spoilage. Pasteurization involves heat-treating milk over a short period. As such, microbial populations that cannot survive in extremely high temperatures are denatured by this process, resulting in an overall reduction in bacterial count. Moreover, **reintroduction** of certain microbial populations is done on pasteurized milk to achieve the **desired dairy product.**

Keep in mind that:

- Although pasteurization reduces the total bacterial count, it might promote the growth of spores. Heat treatment is only effective against psychrotrophs and mesophiles.
- Additionally, the possibility of microorganisms may transform into a viable but non-cultivable state, a condition in which they might not show up in culture-dependent testing.
- Viable but nonculturable (VBNC) bacteria refers to bacteria that are in a state of very low metabolic activity and do not divide but are alive and have the ability to become culturable once resuscitated.