### Inoculation and Milk Ripening:-

This stage is called **ripening the milk** and is done prior to renneting.

**Starters (Cultures):-**

**Basic Definition:-**

 Dairy starter cultures are pure cultures of microorganisms growing in sterilized skimmed milk that are on purpose added to milk in order to create a desired outcome in the final product, most often through their growth and “fermentation” processes. Starter cultures have essential roles to play during all phases of the cheese making and maturation (ripening) process.

**General Functions of Cheese Cultures: -**

**(1) to develop acidity; and (2) to promote ripening**. **Lactic acid cultures** contribute to both of these functions, while secondary **cultures** are added to help with the second function.

***A-* Development of Acidity:-**

 In controlled conversion of milk to fermented dairy products, a primary component of fermentation is development of acidity by lactic acid bacteria. Acid development in cheese making is essential to cheese flavor, cheese texture and cheese safety. Acid is required to:

* Assist coagulation. Lower pH results in faster coagulation and in acid coagulated cheese is the only factor which induces coagulation.
* Promote syneresis. This is a most critical means of controlling moisture content. Low acidity causes the protein matrix in the curd to contract and squeeze out moisture. That process of contraction is called syneresis.
* Prevent growth of pathogenic and spoilage bacteria(” (LAB) has ability to produce antimicrobial ,bacteriocins compounds such as Nisin). Proper rate and extent of acid development is the most important principle with respect to quality and safety of natural cheese.
* Develop cheese texture, flavor and color.

**B-Assist curing:-**

* Growth factors produced by lactic cultures are required for other non-starter microorganisms which contribute to the desired flavor and body of cheese
* Enzymes (both lipases and proteases) produced by lactic cultures contribute to interior ripening of cheese and are important to both flavor and texture development.

1**-Lactic acid cultures (primary starters):-**

**Classification of Lactic Acid Cultures: -**

It is helpful to classify lactic cultures according to general technological and growth characteristics. From that viewpoint, LAB is grouped by four criteria: -

**A- Principal metabolites *(end products of fermentation)*:homo and htero-fermentative:-**

**Homofermentative:** means that lactic acid is the principal metabolite without production of gas (CO2) and flavor compounds.

**Heterofermentative:** means that lactic acid is the principal end product of fermentation but significant amounts of one or more of the following metabolites are also produced.

* Carbon dioxide (CO2) which causes the small gas holes in Havarti, Gouda and other cheeses. Gassiness in most cheese varieties is a defect.
* Short chain fatty acids such as acetic acid and propionic
* Acetaldehyde, a principal component of yoghurt flavor
* Diacetyl, a principal flavor notes in sour cream, butter milk, Dutch cheese and Havarti cheese
* Ethyl alcohol

**B- Optimum growth temperatures: mesophilic and thermophilic***:-*

**Mesophilic cultures** prefer medium rangegrowth temperatures, (Cheddar, Dutch, Blue, surface mould and surface-smear families).

* Optimum growth range for mesophyllic cultures is 30 - 35C°.
* Acid production is slow or absent at temperatures less than 20C°.
* Growth is inhibited at temperatures greater than 39C°.
* Generally any cheese which does not require high temperatures to dry the curd will utilize mesophilic cultures. These include Cheddar, soft ripened cheese, most fresh cheese, and most washed cheese.
* Leuconostoc spp. are included in the starter for some cheese varieties, e.g. Dutch types; the function is to produce diacetyl and CO2, from citrate rather than acid production.

**Thermophilic cultures** are defined by their ability to grow at temperatures above 40C°. With respect to cheese making their important characteristics are:

* Optimum growth in the range of 39-50C°.
* Stay alive 55C° or higher .
* Minimum growth temperature is about 20C° below which cell counts decrease rapidly, so, bulk thermophilic cultures should not be stored at temperatures <20C°.
* Thermophilic starters are normally mixtures of cocci and rod cultures which at the time of inoculation are about equal in numbers. That is, the initial inoculum is 50% cocci and 50% rods.
* Rod/cocci blends grow together in a relationship referred to as 'mutualism' where the overall growth rate and acid production is faster than either culture on its own. The rods produce amino acids and peptides which stimulate the growth of cocci, and the cocci produce formic acid which is required by rods.
* The balance between the rods and cocci can be controlled by temperature and pH
	+ The cocci prefer higher temperatures (optimum about 46C°) than the rods (optimum about 39C°).
* The rods are more acid tolerant than the cocci, normally the cocci develop the initial acidity and outgrow the rods. But as the acidity increases the rods begin to grow faster than the cocci.
* Some thermophilic rod cultures have the ability to ferment galactose as well as glucose which is desirable in some cheese, especially Mozzarella. a thermophilic Lactobacillus culture is used, either alone (e.g. Parmesan) or with Streptococcus thermophilus (e.g. most Swiss varieties and Mozzarella).
* Although yoghurt cultures which include both rod and cocci, produce acetaldehyde which is the principal component of the characteristic yoghurt flavour, none of the thermophilic LAB are considered heterofermentative.

**Some lactic acid bacteria commonly used in cheese making.**

**1-Mesophilic Cultures**

- **Lactococcus lactis**

**-Streptococcus cremoris**

* **Used as a mixed blend these two form the most common mesophilic and homofermtative culture. Used for many low temperature varieties; Cheddar, Edam , Goad , Cottage.**

**-Leuconostoc citrovorum**

**-Leuconostoc lactis**

**- Streptococcus diacetylactis**

**Hetero cultures; ferment citrate; produce both CO2 for cheese with small holes such as Goad & Edam cheeses and diacetyl as flavor compound for traditional butter, butter milk and Cottage .**

**2- Thermophilic Cultures**

***-Streptococcus thermophilus***

***-Lactobacillus helveticus***

***- Lactobacillus bulgaricus***

***- Lactobacillus lactis***

**Commonly used coccus/rod blend for high temperature varieties, Swiss and Italian (Parmesan,Rommano,** **Emmental)**

**Commonly *Lactobacillus bulgaricus* blended with *S. thermophilus* for yoghurt.**

**Commonly *Lactobacillus lactis* blended with *S. thermophilus* for probiotic yoghurt.**

**C-Starter composition:-**

* **Single strain cultures** defined cultures are single strain microorganism cultured in skim milk and added separately to cheese vat for specific properties such as proteolytic characteristics.
	+ Have the advantages of uniform rate of acid development and uniform flavor profiles.
* **A mixed defined culture** is a blend of single strain cultures.
* Mixed cultures are nonspecific blends of cultures.
	+ Have the advantages of complex systems of phage resistance.
	+ Disadvantage is non uniform rates of acid development from vat to vat and non-uniform flavor profiles.
	+ Have resistance to scalding temperatures.
* Mixed cultures grow together in a relationship referred to as 'mutualism'e.g. mixed cultures used for Emmental(Streptococcus thermophilus+ Lactobacillus bulgaricus+ *Propioni bacterium shermaniee)* The Lactobacillus bulgaricus produce amino acids and peptides which stimulate the growth of Streptococcus thermophilus+ *Propioni bacterium shermaniee* which produce Large holes and the Streptococcus thermophilus produce formic acid which is required by Lactobacillus bulgaricus.

**D-Forms of Inoculation:-**

Cultures can be carried and prepared for cheese milk inoculation in one of three general formats:

* **Traditional starters** which need several scale up transfers. This system requires some microbiological facilities and knowledge and is only feasible for very large plants or perhaps for smaller plants which use mixed strain cultures.
* **Bulk set culture.** In this system, the culture supplier does all the purification and transfer work, and delivers a bulk set culture which is used to inoculate a bulk culture, which in turn is used to inoculate the cheese milk. Bulk cultures are the feasible in medium to large plants because the cost savings are significant.
* **Direct to the vat cultures** require no scale up at the cheese plant. Concentrated cultures ready to inoculate the cheese milk are supplied directly by the culture supplier.

**Technological properties of lactic acid cultures:-**

* Lactose metabolism. Most but not all LAB are able to metabolize lactose.
* Galactose metabolism.
* Proteolytic characteristics which determine cheese flavor development.
* Resistance to phage (bacterial viruses).
* The ability to metabolize citrate which is associated with flavor development (diacetyl or butter milk flavor) and gas formation.
* Production of bacteriocins, that is, antibiotics produced by bacteria against other bacteria.
* Resistance to bacteriocins.
* Antibiotic resistance.

**2-Secondary Cultures:-**

In addition to lactic acid cultures many special or secondary cultures are used to promote specific ripening (both flavour and texture) characteristics.

* Large holes: *Propioni bacterium freudenreichii subsp. shermaniee*
	+ Produce CO2 gas which forms the holes (or eyes) and the characteristic “nutty” flavor in Swiss.
* White moulds: *Penicillium camembertii, P. caseiocolum, and P. candidum*
* Blue/green moulds: *Penicillium roqueforti, Penicillium glaucum*
* Smears:
	+ yeasts and moulds
	+ Various coryneform bacteria including *Brevibacterium linens*, several species of *micrococci*, and several species of *Staphylocci* .

 **Culture Production, Distribution and Storage:-**

**Commercial culture preparation:-**

* Lactic cultures are grown in buffered media to facilitate maximum growth without acid inhibition .
* The cells are concentrated by centrifugation.
* The cell concentrate is fast frozen or freeze dried (lyophilized). Frozen (-40C) or lyophilized cultures can be stored for several months without large loss of activity. Lyophilized cultures usually require a longer "lag time", i.e. time between inoculations and rapid cell growth.

**Culture Practice in the Cheese Plant:-**

Direct to the vat cultures need only be stored under prescribed conditions and opened and delivered to the vat under aseptic conditions. The following comments relate to the preparation of bulk culture at the cheese plant.

* Culture preparation should take place in a separate culture room which is kept at positive air pressure with filtered air (0.2 µm filter).
* All surfaces in the culture room must be of a material that can be sterilized.
* Use sterile pipettes and sanitize surfaces and equipment with 200 ppm chlorine.
* Alternative culture media are:
	+ Milk, but care must be taken to avoid rancid milk, mastitic milk, milk containing antibiotics, and milk with high bacteria counts.
	+ 10 -12% reconstituted skim milk powder is adequate provided that the powder is tested and certified antibiotic free.
	+ Whey and reconstituted whey powder may be used, but may not achieve the same cell counts as skim milk (due to less buffer capacity).
* Addition of phosphates also confers phage resistance because phosphates bind calcium, and phage requires calcium to attach themselves to the bacterial cells.
* Culture media should be sterilized to destroy bacteria and some inhibitory substances. Heating also reduces the redox potential (lowers oxygen concentration) which encourages the growth of LAB.
* Optimum pH endpoint before cooling is between 4.5 and 5.0. At pH less than 4.5 some cultures will pass from growth (log) phase to stationary phase and will be less active when added to the cheese vat.
* Cell count can be increased by:
	+ Internal pH control using buffered media
	+ External pH control by adding sodium hydroxide to maintain pH at 5.0 - 5.5.
* Generally cultures should be cooled to 4C after the desired minimum pH and cell counts are obtained. However, the optimum storage temperature depends on the particular culture.

**Bacteriophage (bacterial viruses):-**

Bacteriophage is the stuff of a cheese maker's alarming. Like all viruses, bacteriophage are parasites, that is, part of their life cycle is dependent on the host bacteria.

**facts about their characteristics and how they can be controlled.**

* Extracellular phage, that is, phage particles existing separate from their bacterial hosts are called mature or resting particles.
* Resting particles are sperm shaped, < 1 micron in length.
* Resting particles consist of DNA (genetic material) and protein.
* The basic life cycle, called the **lytic cycle**, is:
* The phage attaches itself to the bacterial cell wall **by its tail**, bores a hole in the wall with the help of enzymes and injects its DNA into the cell. The protein sheath remains outside the cell.
* From the moment of attack the bacteria begins to reproduce phage DNA and protein in addition to its own.
* Nucleic acid and protein strands assemble themselves into new phage particles which finally lyse the cell (break it open) to release the phage particles into the medium. A new generation of resting phage are now available to repeat the lytic cycle .
* Culture growth will stop when phage levels reach **103 to 107 per ml.**
* Phage have a short latent period (reproduce as quickly as every **30 to 50 min**) and (**each lysed cell will release 50 to 100 new phage).**

