CHAPTER FOUR

Microorganisms Associated with Milk

Microenvironment of milk:

Milk, being considers a nutritious food for human beings, also serves as an ideal medium for the growth of different microorganisms particularly bacteria. The growth of microbes depends on the environment which includes both the substrate microenvironment (viz. properties of the substrate) and the outside conditions (of which temperature is by far the most important). Hence, in milk held at a favourable temperature, the growth of microorganisms is due to its congenial microenvironment. Milk consists of a variety of nutrients (including all the vitamins) which serve as sufficient raw material and essential factors for the growth of microorganisms under ambient conditions. For example, bacteria requiring lactose, milk fat, milk proteins etc.as nutrients can proliferate luxuriantly in milk. However, all bacteria growing in milk may not need the same nutrients. For example, some bacteria do not find lactose as a suitable energy source whereas others require nutrients which are either present in small amounts or are completely lacking. In such cases, an associative growth may occur, in which growth of one group of organisms provides nutrients for the growth of the other group. For example, those bacteria which rely on free amino acids as nitrogen source (which are present only in minute amounts in fresh milk) grow only after another group of bacteria (which hydrolyze milk proteins to release amino acids) have grown. Another such instance is the stimulation of the growth of lactobacilli due to production of CO_2 by some lactic Streptococci (the CO_2 which serves as a stimulant for lactobacilli can however, inhibit some Gram negative bacteria). Some microorganisms need elements which are either missing or are present in minute quantities, e.g. *Leuconostoc cremoris* needs Mn+2 (which often varies with season) for fermenting citric acid for flavor production. Besides possible lack or insufficiency of nutrients for some groups of bacteria, as discussed above, the milk may also possess some

unfavourable conditions for multiplication of other bacterial groups. The pH is limiting only for a few microbes but redox potential and O_2 pressure are mostly unfavourable except for the growth of obligate anaerobic bacteria. The growth of aerobic bacteria is favoured only in such locations where O_2 pressure is adequate, e.g. in cream layer. The microbial multiplication at ambient temperatures usually lowers both the pH and the O_2 pressure in milk and a few microorganisms get killed as they find conditions in milk to be completely unfavourable. Milk has also been known to possess certain antimicrobial substances which inhibit or kill certain groups of microorganisms. Milk presents an ideal nutritious medium for microbial growth and multiplication. Milk provides all the essential nutrients required such as nitrogen, Carbon, Oxygen, Sulfur and certain mineral salts and vitamins for microbial growth. Milk, in addition to being nutritious medium, presents a favorable physical environment for the multiplication of microorganisms. High moisture percentage in milk provides an ideal environment for microbial growth and for nutrients to be in solution in order to be taken into the microbial cell through the cell wall by osmosis. An ideal osmotic pressure and neutral pH in milk are optimum ideal factors for the multiplication of most bacteria, while yeasts and molds prefer acidified medium as in the fermented dairy products.

Microbiological quality of milk

Milk when secreted in udder is free from microorganisms i.e. sterile but as it passes through ducts and reservoirs of udder, bacteria are carried into it, especially from the walls of the teat canal. A variety of other sources during production, handling, transport and distribution of milk contribute various organisms to raw milk before it reaches the consumer or a processing dairy plant. The microorganisms entering milk may be pathogenic or nonpathogenic to human beings. The growth of microorganisms in milk affects the quality of milk by bringing about milk spoilages. The rate at which this spoilage occurs depends upon not only the initial microbial load but also on conditions under which milk is held and the length of time for which it is held. While these microorganisms growing on milk ingredients may release certain metabolites (like lactic

and other organic acids, gases, enzymes, flavouring compounds, pigments, toxins etc.) in the system which may be useful or harmful and thus directly affect its quality. Generally, these metabolites lead to various kinds of spoilages in the product and sometimes make it a potential health hazard. Lactose serves as the principal carbon source for majority of the spoilage causing microorganisms growing in milk.

Microorganisms in raw milk

The number and types of Microorganisms present in raw milk depend on the nature and extent of contamination which in turn varies with the conditions of milk production and the subsequent storage conditions. In other words, production of milk under hygienic conditions and subsequent cooling storage at low temperature (less than 4°C) restrict the contamination and proliferation of microorganisms.

Microbial numbers in raw milk

The microbial counts in raw milk may usually vary from less than one thousand per /ml to more than one million ($\mathbf{10^6}$)

per / ml. The total microbial load in raw milk may be estimated by standard plate count (SPC) by plating milk on tryptone dextrose agar or plate count agar and incubating at 37°C for 48 hours. This however, is not an exact estimate of the total bacterial content as there are other groups of microrganisms which require different cultivation conditions (media and incubation temperature) Hence, suitable test conditions are provided for enumerating the specific groups. For example psychrotrophs need incubation at 5-7°C for 10 days, thermodurics are enumerated by pasteurizing the milk sample before plating; the other groups, viz, Coliforms, Lactic acid bacteria, Gram negative rods, Lipolytic and proteolytic types, pathogenic organisms, etc. need selective media for their growth on the test plates. Besides enumeration by plating, the microbiological quality can be predicted by performing other routine tests like dye reduction time tests (Methylene blue reduction and resazurin reduction), presumptive coliform test etc. Based on the results of some of these tests, raw milk can be appropriately graded for its

microbiological quality. The routine grading tests like standard plate count do not reflect the sources of contamination and the production faults for which counts of specific groups of microorganisms such as psychrotrophs, thermodurics, coliforms, spores, streptococci etc. have to be determined. Such tests are therefore, mainly useful for advisory investigational and survey purposes.

Types of microflora in raw milk :

A heterogenous population of mesophilic, thermoduric, thermophilic, psychrotrophic and pathogenic organisms constitute the microflora of raw milk. The presence and relative proportions of the individual groups vary with the conditions of milk production and handling.

a- Mesophilic microflora

The microorganisms growing optimally at 30°C to 32°C are designated as "mesophilic". The different microorganisms forming the mesophilic microflora of raw milk as detected in plate count analysis at 32°C for 2-3 days are Micrococci, Staphylococci, Streptococci (faecal and mastitis causing Streptococci), Asporogenous Gram positive rods (Microbacterium spp.. Corynebacterium spp. Mycobacterium spp. and Lactbacillus spp.), Sporogenous Gram positive rods (Bacillus spp.) and Gram negative rods (Pseudomonas spp., Alcaligenes spp. and Coliforms).

b- Thermoduric microflora in raw milk: Thermoduric microorganisms are those which survive pasteurization conditions but cannot grow at pasteurization temperatures. A wide variation in the thermoduric count has been observed in raw milk supplies although most of them do not multiply faster at ambient temperatures. This indicates that the variations are due to differences in the extent of contamination from potent sources.

The genera of thermoduric bacteria in fresh raw milk are the following:

1-*Microbacterium lacticum* which is mostly derived from milking equipment.

2- Bacillus spores: they are generally higher in winter than in summer because these spores are mainly derived from the teat surfaces spoiled with bedding materials used for housing the cows. Milk cans could also serve as a source of Bacillus cereus spores.

3-Clostridum spores:

The clostridial spore count of raw milk is also higher in winter because these are mainly derived from silage (used in winter feeding) and bedding materials. Their number declines when cow starts going out for pasture feeding e.g. Cl. Tyrobutyricum

4-Micrococcus:

Micrococcus is almost exclusively derived from milking equipment and other utensils e.g. M. freudenreichit

C-Thermophilic microflora.

The term thermophilic applies to those bacteria which can readily grow at 55°C and most of them have an upper limit of growth at about 70°C. In dairy industry, therefore, the term is used for that group of organisms which not only survive pasteurization but also can grow at that temperature. The standard method of enumerating this group of organisms is to incubate agar plate at 55°C. A small number of thermophilic bacteria may gain access into raw milk mainly from soil, bedding, feeds and rarely from water supplies. The number increases due to their multiplication if raw milk is stored under warmer ambient conditions. The common members of thermophilic microflora of raw milk belong to aerobic or facultatively anaerobic sporeforming rods e.g. B. stearothermophilus, B. circulans, B. thermoacidurans and Lactobacillus thermophillus.

d-Pscychotrophic microflora:

Pscychotrophic organisms as understood in dairy industry are those which are able to grow at commercial refrigeration temperatures (2-7 °C) regardless of their optimum growth temperature. Usually the term"pscychtrophilic" is used for organisms that have an optimum

temperature below 20°C. In the psychrotrophic microflora of milk and milk products, very few are classical psychrophiles and majority of them are mesophilic (optimum growth temperature - 20 to 30°C) in nature. The Pscychotrophic bacteria detected (5-7 °C in 7-10 days) in raw milk belong to two broad categories, viz, Gram negative rods and Gram positive rods or cocci.

1- Gram negative rods:

This group is the most commonly occurring Psychrotrophic microflora in fresh raw milk and may comprise about 10-15% of the initial total microbial number. The psychrotrophic Gram negative bacteria belong to different genera namely Pseudomonas, Acinetobacter, Flavobacterium, Achromobacter, Alcaligenes and Enterobacter (Coliforms). Among these, Pseudomonas constitutes about half of the total Gram negative rods and the following species are prevalent:

a-Pseudomonas fluorescens (predominant).

b- Pseudomonas putida

c- Pseudomonas fragi

d- Pseudomonas aeruginosa

The species of the Coliform bacteria (mainly Enterobacter spp.) have been known to constitute 10-13 % of the Psychrotrophic microflora of raw milk. Some of the Gram negative psychrotrophs like Pseudomonas species can produce heat stable enzymes (lipases and proteinases) in refrigerated milk. During subsequent pasteurization, their vegetative cells get killed but these enzymes are not inactivated and may be responsible for fat and casein degradation causing milk spoilages. Improperly cleaned /sanitized milking equipment is the major source of psychrotrophic Gram negative rods although these may also be derived from animal's teat surfaces.

Il-Gram positive bacteria

Some strains of the following genera/ species of psychrotrophic Gram positive rods and cocci are also encountered in raw milk:

1-Bacillus spp. (spores) e.g. *B*. *coagulans B*. *circulans* and *B*. *Subtilis*

2-Arthrobacter spp.

3- Streptococcus spp.

e-Coliforms

The term "coliform bacteria" in milk denotes the aerobic and facultatively anaerobic, Gram negative, nonspore forming rod shaped bacteria which ferment lactose into acid and gas at 32°C within 48 hours The typical genera included under this group are Escherichia, Enterobacter and Klebsiella. The presence of Coliforms especially Escherichia coli in raw milk is no longer taken as an index of direct faecal contamination (unlike that in case of water supplies) as they may also come from other sources like improperly cleaned / sanitized milking equipment (where moist, milky residues allow the rapid building-up of Coliforms by multiplication). However, relating their absence to the use of properly cleaned milking equipment is also not necessarily true. Most of the strains of Enterobater species isolated from raw milk are psychrotrophic microorganisms.

f-Pathogenic microorganisms in raw milk:

Pathogens for man may gain entry into raw milk either due to their co secretion into milk as a result of diseased condition of the animal and / or its udder or due to contamination of milk from infected human handlers, faecal and non faecal sources, etc. A list of various pathogens reported to occur in raw milk along with their principal source of entery is presented in the following table:

Table 8: A composite picture of pathogens transmittable through raw milk

Pathogens	Principal cause/source
Brucella abourtus, *Brucella melitensis*, *Brucella suis*	Brucellosis in lactating animal
Mycobacterium tuberculosis *Mycobacterium bovis*	Tuberculosis in lactating animal
Streptococcus agalactiae *Staphylococcus aureus* *Escherichia coli*	Bovine mastitis (udder disease)
Listeria monocytogenes *Bacillus cereus* *Clostridium perfrigens*, *Pasteurella multocida*, Actinomyes species *Coxiella burnetii*	Bovine mastitis
Salmonella typhi *Salmonella paratyphi* *Escherichia coli* *Vibrio cholera* *Shigella flexneri* *Shigella dysenterae*	Faecal contamination of milk or Human handlers (infected or carriers)
Streptococcus pyogens	Human handlers (infected or carries)
Cryptococcus neoformans	Bovine mastitis (infrequent causative)
Adenoviruses (20 different types)	Human handlers (infected or carries)
Enterovirues, Hepatitis virus.	Human handlers (infected or carriers)
Tick borne encephalitis virus.	Animal which get tick-bites?
Foot and mouth disease (FMD) virus	Animal with FMD.