Milk Microbiology and Public Health Significance

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**Abstract :**

Milk is a complete food which is consumed by humans due to its high nutritional value. It contains complete nutrients that are a source of protein, lipids, carbohydrates, vitamins and minerals. It is a complex mixture of specific bioactive proteins, lipids and saccharides and contains numerous biologically active substances such as immunoglobulins, enzyme, antimicrobial, peptides, oligosaccharides, hormones, cytokines and growth factors (Pouliot and Gautheir, 2006). Fresh milk contains a mixture of antimicrobial agents that exhibit bacteriostatic and even bactericidal activities. Mammalian milk contains more than 60 different enzyme including digestive enzymes (proteinases, lipases, amylases, phosphatases) and enzymes with antioxidant and antimicrobial characteristics (Fox, 2003).

**Sources of milk microorganisms**

Milk in healthy udder cells is thought to be sterile (Tolle, 1980), but thereafter becomes colonised by microorganisms from a variety of sources, including the teat apex, milking equipment, air, water, feed, grass, soil and other environments in milk.

The bovine teat surface can contain a high diversity of bacteria (Braem *et al.,* 2012). In one particularly detailed study, culture-dependent methods revealed that the bacteria present could be classified at the phylum level as Firmicutes (76%), Actinobacteria (4.9%), Proteobacteria (17.8%) and Bacteroides (1.3%). Of those which could be identified, many corresponded to technologically important bacteria such as Lactobacillus, Leuconostoc and Enterococcus spp. Bacteria that can be involved in flavour, aroma and colour development in cheese such as coagulasenegative staphylococci as well as Arthrobacter, Brevibacterium and Corynebacterium spp. were also detected.

**The microbial composition**

Typically, cows' milk contains a significant LAB population that includes Lactococcus (8.2 × 101–1.4 × 104 CFU mL−1), Streptococcus (1.41 × 101–1.5 × 104 CFU mL−1), Lactobacillus (1.0 × 102–3.2 × 104 CFU mL−1), Leuconostoc (9.8 × 101–2.5 × 103 CFU mL−1) and Enterococcus spp. (2.57 × 101–1.58 × 103 CFU mL−1). A number of other microorganisms can be present in significant proportions. These include psychrotrophs such as Pseudomonas, Acinetobacter and Aeromonas spp., which flourish during cold storage (Raats *et al.,* 2011).

**Impact of milk microbiota**

The specific composition of the milk microbiota directly impacts on the subsequent development of dairy products (Fig. 1). Microorganisms can bring about the fermentation of milk through the production of lactate and have a variety of different impacts on the sensory, texture, flavour and organoleptic properties of resultant products (Wouters *et al.,* 2002). Microorganisms can also negatively impact on milk quality and shelf life; for example, psychrotolerant bacteria can proliferate during refrigeration and, through the production of extracellular lipases and proteases, result in spoilage. The microbial composition of milk can also have health-related implications in that the consumption of raw milk contaminated with pathogens can lead to, in some cases, severe illness (Oliver *et al.,* 2009). In contrast, it is claimed that other raw milk microorganisms can contribute to health by aiding digestion or by reducing the frequency of allergies, including asthma and atopic diseases, in individuals who consume raw milk during the early years of life.

**Technologically relevant bacteria of raw milk**

In dairy foods, Lactococcus lactis and Lactococcus lactis ssp. lactis and Lactococcus lactis ssp. cremoris in particular, are primarily known for their role as starter cultures for the cheese industry. While these microorganisms are naturally present in raw milk and artisanally produced cheeses, they are frequently added to pasteurised milk to facilitate the commercial manufacture of cheeses. Their primary role during cheese production is acidification through the production of L-lactate. However, they also contribute to proteolysis, the conversion of amino acids into flavour compounds (alcohols, ketones, aldehydes), citrate utilisation and/or fat metabolism (Smit *et al.,* 2005). Lactobacilli that are of particular importance within the dairy industry are L. helveticus, L. delbrueckii ssp. bulgaricus and L. delbrueckii ssp. lactis (the latter two species will be referred to as L. bulgaricus and Lactobacillus lactis hereafter). Streptococcus thermophilus is a thermophilic LAB widely used as a starter culture in the manufacture of dairy products. It is often regarded as the second most important industrial dairy starter after Lactococcus lactis. Its importance in dairy products is due to its ability to rapidly convert lactose to lactate; bringing about a rapid decrease in pH; and the production of important metabolites including low levels of formate, acetoin, diacetyl, acetaldehyde and acetate.

**Microbial spoilage of milk**

Milk is typically stored at refrigeration temperatures that reduce the growth of most bacteria, with the exception of psychrotolerant microorganisms that can proliferate under these conditions and become a major cause of milk spoilage (DeJonghe *et al.,* 2011). This is primarily a consequence of the production of extracellular enzymes, with lipases and proteases being most important. These lipases degrade milk fat causing rancidity, while proteases degrade casein producing a grey colour and bitter off-flavours (De Jonghe *et al.,* 2011). Investigations into seasonal variations of microbial growth in raw milk have, unsurprisingly, established that psychrotolerant bacteria exhibit better growth and protease production in winter milk rather than in summer milk. Pseudomonas spp., which are commonly found in raw milk, are the most common cause of milk spoilage. The Pseudomonas species most commonly detected in milk and cheeses are Pseudomonas fluorescens, Pseudomonas gessardii, Pseudomonas fragi and Pseudomonas lundensis (Mallet *et al.,* 2012). These bacteria can become the predominant microorganisms in raw milk stored at low temperatures, constituting up to 70–90% of the microbial population.

**Human health associations**

Milk and dairy products are important staples of a healthy diet. However, if pathogenic microorganisms are not removed by pasteurization, consumption of these products can represent a serious health risk. These pathogens can originate from the mammary gland or associated lymph nodes of cows suffering from systemic diseases or infections or from equipment, raw milk tankers and personnel. Ingestion of these microorganisms can lead to illnesses of varying severity. Typical symptoms can include fever, nausea, vomiting, diarrhoea and abdominal pains; in extreme cases, death can occur (Langer *et al.,* 2012). Indeed, food poisoning from consumption of raw milk and such products over a period of 13 years (1993–2006) in the United States resulted in 1571 reported incidences with 202 hospitalisations and 2 deaths. The main cause of illness was consumption of raw milk products contaminated with Salmonella spp., Listeria spp., E. coli, Campylobacter spp., Brucella spp. or Shigella spp. (Langer *et al.,* 2012).

**Contamination of milk:**

* Contamination of milk occurs two levels:
* **On farm base:**
	+ Freshly raw milk contains relatively few bacteria after all *Micrococcus*and *Streptococcus*are usually found in aseptically raw fresh milk.
	+ During normal milking process, milk is subjected to contamination from udder of animal and surrounding areas.
	+ Bacteria found in soil, water and manure contaminate are udder of animal from where they enter into the milk.
	+ Other possible source of contamination is hand and finger of milker or other dairy workers.
	+ Contamination also occurs from dairy utensils.
* **During transport and at processing plant base:**
	+ During transport and manufacturing, contamination occur through tanker, transfer pipes, sampling utensils and other equipment.
	+ Sometimes, pathogen may contaminate the milk from hand and finger of milk handler.

**Microbial Spoilage of milk and mik products:**

* Milk is an excellent culture media for growth of many microorganisms.
* Different types of microorganisms grow in it and cause spoilage.

**i. Spoilage of Milk and cream:**

* **Souring:**
	+ Evidence of souring of milk are sour flavor and then coagulation of milk to form solid like curd.
	+ Many lactic acid bacteria, coliform and other bacteria ferment sugar of milk and produce acid.
	+ At temperature of 10-37oC, *Streptococcus lactis*is most likely to cause souring with possible growth of *Coliform, Enterococci, Lactobacillus*and *Micrococcus.*
	+ At higher temperature, 37-50oC, *Streptococcus thermophilus*and *Streptococcus faecalis*may produce 1% acid and it may be followed by *Lactobacillus*which produces more acid.
	+ Little souring occurs in milk held at refrigeration temperature.
	+ Pasteurization of milk kills more active acid forming bacteria but permit survival of thermoduric lactic acid bacteria such as *Enterococcus, Streptococcus thermophilus, Lactobacillus,*etc.
	+ Bacteria other than lactic acid bacteria produce acid specially if conditions are unfavorable for lactic acid bacteria.
	+ For example: *coliform*produce acetic acid, formic acid, ethanol, CO2, H2etc.
	+ Similarly, *Clostridium*produce butyric acid.
* **Gas production (Strong fermentation of milk):**
	+ Sugar fermenting organism produce gas together with acid.
	+ Main gas formers, *Coliform, Clostridium, Heterofermentative lactic, Propianics bacillus,*etc.
	+ *Coliform, Clostridium,*and *Bacillus*produce both H2 and CO2, while others produce only CO2.
	+ Gas production in milk is evidenced by foam at top of liquid milk by gas bubble trapped in curd, by formation of curd.
	+ Excessive gas production causes cracking or breakdown of curd causing so called stormy fermentation of milk.
	+ *Clostridium perfringens*mainly causes stormy fermentation.
* **Proteolysis:**
	+ Proteolysis is facilitated by storage at lower temperature by destruction of lactic acid bacteria or by distribution of already produced acid by mold and yeast.
	+ Changed cause by proteolytic organism include:
	+ Acid proteolysis in which acid production and proteolysis occur simultaneously.
	+ Proteolysis with little acidity or even alkalinity.
	+ Sweet curdling which is caused by renin like enzyme of microorganisms.
	+ Slow proteolysis by intracellular enzyme of bacteria after their autolysis.
	+ Residual proteolytic activity of some heat stable proteinase.
	+ Acid proteolysis is caused by *Micrococcus , Streptococcus faecalis var liquefaciens*and some lactose fermenting proteolytic *Bacillus*species.
	+ Sweet curdling is caused by *Bacillus cereus.*
* **Ropiness/ sliminess:**
	+ Ropiness of milk occur both by bacterial and non-bacterial causes non-bacterial ropiness occurs due to thickness of cream or due to film of cousin or Lactalbumin during cooling.
	+ Bacterial ropiness is caused by slimy capsular material of bacteria which usually develop at low storage temperature.
	+ Bacteria producing ropiness in milk are *Alcaligenes viscolactis, micrococcus freudenreichii, Enterobacter aerogenes, Klebsiella oxytoca, E. coli.*
* **Change in milk fat:**
	+ Various bacteria, yeast and mold hydrolyses fat of milk and cause rancidity.
	+ Species of *Proteus, Pseudomonas fragi, Staphylococcus, Bacillus, Micrococcus, Clostridium,*etc. are lipolytic.
	+ *Pseudomonas fragi*and *Staphylococcus aureus*produce fairly heat resistant lipase.
* **Alkali production:**
	+ *Pseudomonas fluorescence*and *Alcaligene* *viscolactis* produce alkali.
	+ Alkali production is due to formation of ammonia from urea and formation of carbonate from organic acid.
* **Flavor defect:**
	+ **Acid flavor:**Acid flavor may be aromatic or sharp. Sharp flavor is caused by production of acetic acid formic acid, butyric acid etc. by *Coliform*and *Clostridium.*It is undesirable. Aromatic flavor is caused by *Streptococcus lactic*and *Leuconostoc*when they grow together. It is desirable.
	+ **Caramel or burnt flavor:**It is caused by *Streptococcus lactic var. maltigens.*
	+ **Bitter flavor**: It is caused by proteolytic organism.
	+ **Other flavor**: They include earthy flavor by *Actinomycetes,*fruity flavor by*Pseudomonas fragi,*soapiness by *Pseudomonas sapolactic*etc.
* **Color defect:** Growth of pigmented bacteria and other organism give undesirable color. Some examples include:
	+ Blue milk: It is caused by *Pseudomonas syncyaneum*
	+ Yellow milk: caused by *Pseudomonas synxantha*and also by flavobacterium.
	+ Red milk: caused by *Serratia marcescencs*and *Micrococcus roseus.*
	+ Brown milk: caused by *Pseudomonas putrefaciens*and by enzymatic oxidation of tyrosin by *Pseudomonas fluorescence.*

**Preservation of milk and milk products from microbial spoilage**

**1) Reducing contamination:**

* Keeping quality of milk is improved when contamination of milk is reduced.
* Udder and adjacent areas should be washed with water and if possible, by germicidal solution before milking.
* Hand and finger of milker should be clean.
* Dairy equipment and other milk processing equipment should be sanitized properly.
* Packaging of milk and milk products in can or packages also avoids contamination.

**2) Removal of microorganisms:**

* Microorganisms from milk are difficult to remove.
* High speed centrifugation (called bactofugation at 10,000 rpm removes about 99% spores and more than 50% of vegetative cell plus some protein.
* However, bactofugation is not done commercially for removing bacteria from milk.
* Mold can be removed physically from surface of cheese by scrapping or periodic washing.

**3) Use of heat:**

* For milk, pasteurization is used for preservation.
* Cream can be heated by injecting steam or by combination of steam injection and evacuation in a process called vacreation.
* Heat is also applied for cheese.
* Cooking at 65.6oC or higher in melting of cheese during manufacture reduces many spoilage microorganisms.
* Evaporated milk is canned and then heated by steam under pressure.

**4) Use of low temperature:**

* Refrigeration temperature is recommended for milk or similar product during storage in plant during transportation and during storage in home until consumption.
* Fermenter milk and cheese are chilled after their manufacture and kept chilled until they reach the consumption.
* Chilling storage is most commonly applied technique of preservation of milk and milk product in home for short term storage.

**5) Drying:**

* Many types of milk product are prepared by drying or removing moisture from them.
* There are two types of such product, one is condensed product and the other is dry product.
* One condensed product is evaporated milk, in which 60% moisture is removed, so that remaining high lactose concentration in solution is inhibitory to micro-organisms.
* Bulk condensed milk, sweetened condensed milk, semisolid butter are other examples of condensed product.
* Milk product prepared in dry form include dry milk, ice-cream mix etc.
* Milk can be dried by drum drying or spray drying.

**6) Use of chemical preservative:**

* There are two types of chemical preservatives i.e. developed and added preservative important in milk product.
* Developed acidity in many fermented milk product retards growth of many microorganisms.
* Propionic acid develop in swiss cheese is inhibitory to mold.
* Many chemical preservatives are added from outside in many milk products.
* Sorbic and propionic acid added in cheese and yoghurt prevent surface spoilage by mold.
* Sugar is added in sweeten condensed milk to reduce Aw.
* NaCl used in cheese during manufacturing gives flavor and also acts as preservative.
* The addition of H2O2 combine with mild heat treatment has been used for pasteurization of milk for certain kind of cheese. Some cheese is also smoked.

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