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Dairy microbiology

Milk is a complex biological fluid secreted in the mammary glands of mammals. Typically, bovine milk is composed of approximately 87% water, 3.7 - 3.9% fat, 3.2 - 3.5% protein, 4.8 - 4.9% carbohydrate (principally lactose), and 0.7% ash. Milk produced in the first few days' post parturition is known as colostrum. Colostrum has a very high protein content, and is rich in immunoglobulin to help protect the newborn against infections.

Microorganisms associated with raw milk

Milk is an ideal medium for microbial growth because of its high water content, neutral pH and biochemical composition. Therefore, raw milk may contain various kinds of microorganisms with variable characteristics in respect to classification, morphology and physiology. Very important for the quality of raw milk and dairy products are bacteria that predominate among all kinds of milk microorganisms. Bacteria in raw milk can be **spoilage** or **pathogenic** with **mesophilic**, **psychrophilic** or **thermophilic** behavior. Generation time in raw milk depends mainly on species or strains of bacteria as well as temperature, pH, level of oxygen, inhibitors and nutrients. Thus, the profile of initial microflora and the handling of raw milk regarding hygienic and temperature conditions are the determinative factors for raw milk quality before processing. Raw milk microflora is of critical importance for consumers' safety and quality and shelf-life of dairy products. Raw milk microflora could be grouped as **indigenous** or **contaminants** and also as **spoilage** or **pathogenic** microorganisms.

Indigenous microflora

Normally the udder of a healthy animal is habited by bacteria that belong to genera Streptococcus, Staphylococcus and Micrococcus which account for >50% of overall raw milk flora, followed by Corynebacterium, Escherichia coli and others Microbial counts of aseptically drawn milk is <100 cfu ml–1 (but in practice they usually range from <1000 cfu ml–1 to 20,000 cfu ml–1)

Contaminant microorganisms

After secretion, the initial microbial load of raw milk changes because microorganisms from different sources, i.e., environment, infected udder, milk equipment, etc., enter the milk. The contaminant microorganisms, which belong to different genera, are distributed as follows: Lactobacillus, Corynebacterium, Microbacterium, Pseudomonas, Escherichia, Alcaligenes, Acinetobacter, Bacillus, Clostridium, yeasts and moulds at levels of <10%, Lactococcus and Streptococcus at varying levels from 0 to 50% and Micrococcus and Staphylococcus at levels varying from 30 to 99% .

In general, both indigenous and contaminant microflora of raw milk are classified into two categories: (a) the spoilage and (b) the pathogenic microorganisms, which can cause dairy animal or human diseases. From technological point of view, spoilage microorganisms present in milk are grouped as: (i) **psychrotrophs** which can grow during milk storage at 6 C or less, (ii) **coliforms** and other Gram-negative bacteria which are associated with poor hygienic production, (iii) **thermoduric** bacteria which can survive pasteurization conditions, (iv) **sporeforming** bacteria which produce heat resistant spores, (v) **lactic acid bacteria** of which some are beneficial as they are used as starters in fermented dairy products, and (vi) **yeasts and moulds**.

Types of spoilage microorganisms present in raw milk

Psychrotrophic bacteria

The most effective measure for controlling the microbiological quality of raw milk is immediate cooling after milking and storage at low temperatures, i.e., at 2–7 C. Cooling decreases effectively the growth rate of the main spoilage microorganisms, but it also changes the microbiological profile of raw milk dramatically. Under these conditions, psychrotrophic bacteria predominate the milk microflora depending on the duration of the refrigerated storage. The most common psychrotrophic bacteria in raw milk are listed in psychrotrophs are microorganisms that can grow at temperatures between 0 and 7 C and produce visible colonies or turbidity within 7–10 days.

Table 1.2 Most common psychrotrophic bacteria in raw milk.

Groups	Pathogens[a]	Gram staining
Pseudomonas		–
Flavobacterium		–
Alcaligenes		–
Enterobacteriaceae	*Escherichia coli* O157:H7, *Yersinia enterocolitica*	–
Acinetobacter		–
Aeromonas	*Aeromonas hydrophila*	–
Bacillus	Strains of *B. cereus*	+
Clostridium		+
Arthrobacter[b]		+
Streptococcus[b]		+
Corynebacteria[b]		+
Lactobacillus		+
Micrococcus[b]		+
Others	*Listeria monocytogenes*	+

Due to their technological significance, the enzymes of psychrotrophs have been studied extensively for both their specificities and heat tolerance.

- At low temperatures, proteinases, lipases and phospholipases from Pseudomonas spp. are synthesized at the end of log phase of growth. In general, Pseudomonas produces only one type of lipase that can hydrolyze many natural oils and synthetic triglycerides. Also, phospholipase (lecithinase) produced by Pseudomonas can hydrolyze the milk fat globule membrane that leaves the milk fat unprotected against lipase action,
- Extracellular proteolytic activity, which is heat-stable at 140ºC for 5 s has been observed in Bacillus , Enterobacter , Serratia , Alcaligenes , Flavobacterium and Achromobacter strains or species. Similarly, lipase secreted by Bacillus ,Enterobacter , Serratia , Citrobacter , Moraxella and Achromobacter can with stand high heat treatments. Finally, heat-stable phospholipase are also produced by Bacillus, Flavobacterium, Alcaligenes and Aeromonas species or strains,
- Proteinases of Gram-positive and Gram-negative psychrotrophs exhibit different specificities against individual caseins. At first, their activities result in the development of off-flavor in dairy products, whereas gelation and sweet curdling are observed after the advancement of proteolysis.
- Various psychrotrophic Bacillus spp. induce flavor defects in milk stored at 7ºC when their counts exceed 107 cfu ml–1,

- Lipolytic activity of thermoduric psychrotrophs (e.g., Corynebacterium, Micrococcus) results often in rancid and fruity off-flavors in dairy products,
- Phospholipases, proteinases and glycosidases from Pseudomonas, Citrobacter and Enterobacter can damage milk fat globule membrane by synergistic action, B. cereus is a very important psychrotolerant for the dairy industry in relation to its ability to produce endospores that survive heat treatments. Vegetative cells of the most strains produce proteases causing sweet curdling, when their counts are >106 cfu ml–1. They also produce lipase and phospholipase acting against phospholipids causing fat accumulation defect in cream, also called " bitty cream" defect. On contrary, Gram-negative psychrotrophs do usually not cause bitty cream defect in dairy products. The majority of B. cereus strains do not grow on lactose but can ferment other carbohydrates, e.g., glucose, fructose, trehalose, N-acetyl glucosamine and mannose.
- Some strains of Bacillus spp., e.g., strains of B. amyloliquefaciens, B. claussi, B. subtilis and Paenibacillus polymyxa (formerly B. polymyxa) (an aerobic sporeformer) are able to reduce nitrate to nitrite, which is very important for controlling the growth of Clostridium in cheese.
- Recently, lecithinase (phospholipase) activity has been found in P. polymyxa , which can also produce gas from lactose fermentation.

Thermoduric bacteria

Under pasteurization conditions, i.e., heat treatment at 63ºC for 30 min or equivalent, non-sporeforming pathogens, yeasts and moulds, Gram negative and many Gram-positive bacteria are destroyed. However, thermodurics and thermophiles can survive under these conditions and along with sporeformers can decrease the shelf-life of dairy products kept under non-refrigerated conditions. Thermoduric bacteria can survive at high temperatures but do not necessarily grow at these temperatures opposite to thermophiles that require high temperatures for their growth.

They do not form spores and they can be a very important spoilage factor for pasteurized dairy products provided the psychrotrophic count of raw milk and recontamination have been efficiently controlled. Thermoduric bacteria are very important for cheese because cheese-making conditions are favorable for their growth. In general, a laboratory pasteurization count exceeding 500 cfu thermoduric bacteria per ml indicates major thermoduric problem in the raw milk production chain . Thermoduric species of raw milk include Microbacterium spp. (e.g., M. lacticum), Micrococcus spp., spores of Bacillus and Clostridium , Streptococcus(e.g., S. thermophilus), Corynebacterium spp., Enterococcus spp. (e.g., E. faecium)and Lactobacillus spp. The sources of thermodurics in raw milk are infected udder and outside udder and teats, as well as soil, water and milking machines.

They can grow fast along with lactic acid bacteria, when raw milk is kept under non refrigerated conditions. A healthy udder is also a source of thermodurics in raw milk since the predominant types of bacteria inside a healthy udder include Micrococcus (mesophilic, aerobic, Gram-positive cocci), Streptococcus(mesophilic, facultative anaerobic, Gram-positive cocci) and Corynebacterium (non-sporeforming, mesophilic, facultative anaerobic and Gram-positive irregular rods). Enterococcus spp. come mainly from animal environment. They are mesophilic, facultative anaerobes and Gram-positive cocci which are used as indicators of sanitation

Sporeforming bacteria

These microorganisms belong mainly to the genera of Bacillus, Clostridium and Geobacillus. They are Gram-positive, aerobic or facultative anaerobic, except for Clostridium spp. that are strictly anaerobic. The latter grow in cheese rather than in milk. C. tyrobutyricum causes late blowing in hard-type cheeses with high pH and low salt, fermenting lactic acid to produce butyric acid, CO_2 and H_2. C. sporogenes and C. butyricum are also involved in cheese defects such as putrid spots in the Swiss cheese. Although C. perfringens has not been widely associated with milk-based powdered products, owing to its survival under extreme conditions, it may pose a potential health risk in milk powder.

Table 1.3 Aerobic sporeforming bacteria occurring in raw milk.

Type	**Genera**	**Usual counts in raw milk**	**Most common species**	**Inactivation of endospores (indicative D-values)[d]**
Psychrotolerant	*Bacillus* *Paenibacillus*	0.003–3.5 spores ml^{-1}	*B. cereus*, *B. circulans*, *P. polymyxa*, *B. mycoides*, *B. thuringiensis*	$D_{100°C} \approx$ 0.3–10 min and $D_{121°C} \approx$ 0.04 min for *B. cereus*
Mesophilic	*Bacillus*	0–965 cfu ml^{-1}	*B. licheniformis*, *B. pumilus*, *B. subtilis*	$D_{100°C} \approx$ 20–103 min for *B. licheniformis;* $D_{121°C} \approx$ 0.03–0.5 min for *B. subtilis*
Facultative thermophiles or thermotolerant[a]	*Bacillus*	-	*B. licheniformis*, *B. coagulans*, *B. pumilus*, *B. sporothermodurans*, *B. subtilis*	$D_{100°C} \approx$ 14–160 min and $D_{121°C} \approx$ 2–3.5 min for *B. sporothermodurans*
Obligate thermophiles[a]	*Geobacillus*, *Anoxybacillus*	<10 cfu ml^{-1}	*Geobacillus stearothermophilus*,[b,c] *Anoxybacillus flavithermus*[b]	$D_{100°C} \approx$ 3000 min and $D_{121°C} \approx$ 2.5–4 min for *G. stearothermophilus*

[a] Consist the group of thermophilic sporeforming bacteria

[b] Formerly classified in the genus *Bacillus*

[c] Identified previously as *B. stearothermophilus*

[d] Time needed to reduce the number of spores by a factor of 10

Data compiled from: McKinnon and Pettipher (1983), Phillips and Griffiths (1986), Griffiths and Phillips (1990), Sutherland and Murdoch (1994), Sørhaug and Stepaniak (1997), Christiansson (2002), McGuiggan et al. (2002), Stepaniak (2003), Scheldeman et al. (2006), Walstra et al. (2006), Huck et al. (2008), Burgess et al. (2010).

Coliforms

The genera Escherichia, Enterobacter, Klebsiella, Proteus, Serratia, Hafnia and Citrobacter are grouped as coliforms. They are originated from the digestive tract of milking animal and their presence in raw milk is usually associated with the unhygienic conditions of the production line, although they can rapidly build up in biofilms on milking equipment. These microorganisms utilize proteins and lactose, and are able to produce CO_2, causing defects in cheese like early blowing of hard cheeses and poor structure of soft cheeses. They are Gram-negative asporogenous rods that can grow aerobically or facultative anaerobically at 37ºC, some of them are psychrotrophic and all are sensitive to pasteurization. Coliform counts in bulk tank raw milk vary considerably, i.e., from 0 to 4.7 $\log_{10}$ cfu ml^{-1}

Lactic acid bacteria

Lactic acid bacteria (LAB) originate from the gastrointestinal tract (GIT) of milking animals soon after the birth. They produce mainly lactic acid from lactose, causing souring of milk. Many of them are exploited by the dairy industry in making starter cultures for fermented products including yogurt, cheese and butter. The spoilage LAB belong mainly to the genera Lactobacillus , Lactococcus , Propionibacterium , Leuconostoc and Enterococcus and are usually heterofermentative causing off-flavors and texture defects in cheeses. Lactobacilli like .Lb. brevis and Lb. casei subsp. pseudoplantarum cause open texture in Cheddar cheese due to the production of gas. Lactobacilli are also responsible for forming white insoluble crystals of calcium lactate in ripened hard cheeses, sulphite or phenolic-like fl avors and pink discoloration Mesophilic and some thermophilic LAB are killed by low pasteurization, i.e., at 72ºC for 15 s.

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Yeasts and molds

Yeasts and molds originate usually from contaminated environment of the dairy farm or processing plant. They cause defects in cheese and yogurt. The most common yeasts found in milk are Debaryomyces hansenii, Kluyveromyces marxianus var . marxianus, K. marxianus var . lactis, Saccharomyces cerevisiae, Candida lacticondensi, C. famata, C. versatilis, C. lusitaniae and Yarrowia lipolytica (formerly Candida lipolytica) whereas molds belong to the genera Rhizomucor, Rhizopus and Aspergillus . It has been suggested that the ability of several yeasts to grow under refrigerated conditions in combination to the ability of some strains to produce proteinase and phospholipase may affect the quality of raw milk stored under low temperatures.

Table 1.1 Predominant spoilage microorganisms associated with raw milk.

Microorganism	Common sources	Spoilage
Sporeforming bacteria		
Bacillus cereus[a,c]	Feed, dung, soil, dust	Sweet curdling, bitty cream in pasteurized milk and cream
Bacillus subtilis[b]	Feed, dung, soil, dust	Spoil sterilized cream
Geobacillus stearothermophilus[b]	Feed, soil	Spoil evaporated milk
Clostridium tyrobutyricum[b]	Soil, silage, dung	Late blowing in cheese
Coliforms		
Escherichia coli[a,c]	Feces, milking utensils, contaminated water	Spoil milk and cheese
Klebsiella aerogenes	Feces, milking utensils, contaminated water	Spoil milk
Lactic acid bacteria		
Lactobacillus spp.	Milking utensils, parlor	Sour milk
Lactococcus spp.	Milking utensils, parlor	Sour milk
Leuconostoc spp.	Milking utensils, parlor	Sour milk
Streptococcus thermophilus[b]	Milking utensils, parlor	Sour milk
Enterococcus spp.	Feces	Spoil milk
Psychrotrophs		
Pseudomonas spp., *Achromobacter* spp., *Aeromonas* spp., *Alcaligenes* spp.,[b] *Chromobacterium* spp., *Flavobacterium* spp.	Milking utensils, cold-stored milk	Protein and fat hydrolysis in cold-stored milk
Thermoduring bacteria		
Micrococcus spp., *Microbacterium* spp.	Milking utensils	Growth on pasteurized products
Yeasts	Dust, milking utensils	Spoil cheese, butter, sweetened condensed milk
Molds	Dust, dirty surfaces, feed	Spoil yogurt, cheese, butter, sweetened condensed milk

Important pathogenic microorganisms present in raw milk

Raw milk can be a source of food-borne human diseases caused by pathogens. Their prevalence, like other non-pathogenic microorganisms, is affected by numerous factors such as farm size, number of animals, hygiene, farm management practices, geographical location and season .Pathogenic microorganisms can be transferred to raw milk either from animals, i.e., zoonotic pathogens or from contaminated environment, i.e., exogenous pathogens. Most of the pathogenic microorganisms in milk can cause the three types of microbial food-borne diseases: (a) milk-borne infection, (b) milk-borne intoxication and (c) milk-borne toxicoinfection .

Salmonella spp.

Salmonellae, a member of the family of Enterobacteriaceae, are natural inhabitants of the GIT of animals. They are Gram-negative, non-sporulating, facultative anaerobic rods. Salmonella spp. are mesophilic with optimum growth temperature of 35–37ºC, but can grow at the temperature range of 5–46ºC. They are sensitive to pasteurization, to low pH, e.g., pH <4.5, and do not multiply at aw <0.94, especially when combined with pH <5.5. Salmonella spp. were detected in 15% of raw bovine colostrum samples from dairy herds in Pennsylvania . More recently, about 28% of the dairy operations inspected, where raw milks were sampled from different parts of the dairy operations, were found to be contaminated with S. enterica, being more pronounced in milk filters.

Escherichia coli

Escherichia coli belongs to the family of Enterobacteriaceae and has been recognized as the most important indicator of fecal contamination of water and raw food products. E. coli strains are responsible for three main clinical syndromes, namely the enteric and diarrheal diseases, urinary tract infections and sepsis/meningitis, and thus they have grouped into enteroaggregative E. coli (EAggEC), enteroinvasive E. coli (EIEC), enteropathogenic E. coli (EPEC) and enterotoxigenic E. coli (ETEC). Strains of the latter group produce cytotoxins called verotoxins (VTEC) or shigatoxins (STEC) and colonize in the intestinal track of healthy animals. E. coli serotype O157:H7 (EHEC) is the most studied strain of E. coli, followed by 026 and 0111 serotypes belong to STEC group (also called enterohemorrhagic E. coli) . Most of E. coli strains are not heat-resistant and are readily destroyed by the pasteurization process; however, EHEC is an acid-resistant strain and thus can grow on acidified milk products such as yogurt and fresh acid cheese. Cattle feces are considered as a major reservoir of and hence surveys on its isolation from the bulk tank milk are being continuously carried out worldwide.

Yersinia enterocolitica

Yersinia enterocolitica belongs to the family of Enterobacteriaceae. This pathogen causes acute gastroenteritis, enterocolitis and mesenteric lymphadenitis as well as various extra-intestinal disorders .It is a psychrotrophic microorganism and thus is highly susceptible to pasteurization. Raw milk often contains Y. enterocolitica ,although dairy cattle are not considered reservoirs of this pathogen. It is thought to contaminate raw milk through contacting with animal feces or polluted water supplies. Prevalence of Y. enterocolitica in bulk tank milk was reported at rates varying from 1.2 to 6.1%.

Listeria spp.

Listeria spp., which belongs to the family of Listeriaceae, is commonly found in the dairy farm environment and thus raw milk can be contaminated through various sources. Listeria monocytogenes causes human listeriosis, a serious invasive disease that causes abortion in pregnant women and meningitis, encephalitis and septicemia in neonates and immunocompromised adults. Among 13 known serotypes of L. monocytogenes, the 4b, 1/2 and 1/2b are the most common ones that account for 89–96% of human listeriosis. L. monocytogenes is frequently isolated from raw farm milk or bulk tank milk samples throughout the world, since it can grow on steel and rubber surfaces and at refrigeration temperatures.

Staphylococcus spp.

Staphylococcus aureus belongs to the family of Micrococcaceae and is the most significant causative agent of mastitis in dairy cows. The enterotoxigenic strains produce enterotoxins that are classified according to serotypes into A-H groups and toxic shock syndrome toxin (TSST). Staphylococcal enterotoxin is heat-resistant and survives pasteurization. Enterotoxigenic S. aureus , that is coagulase-positive, was isolated from bulk tank milk samples from the USA dairies at levels varying from 27.4 to 37%, whereas colostrum was implicated in higher isolation rates, i.e., 42%.

Campylobacter spp.

Campylobacter jejuni belongs to the family of Campylobacteriaceae and is the most important causative agent of abortion in cattle and sheep and an etiological agent of human gastroenteritis. It is widely isolated from feces of infected cattle as well as from raw milk. C. jejuni is acid- and heat-sensitive, and thus is killed by pasteurization. Prevalence of C. jejuni in bulk tank milk was reported to range from 0.4 to 12.3% . Outbreaks of campylobacteriosis due to consumption of raw milk were reported in the USA Netherlands and Hungary during the last decade. Mycobacterium avium subsp. Paratuberculosis Mycobacterium avium subsp. paratuberculosis (MAP) is a member of the family of Mycobacteriaceae and is the causative agent of paratuberculosis which is a zoonotic disease also known as John's disease. MAP has also been linked to Crohn's disease (CD), a chronic human disease of the terminal ileum, although the relationship between MAP and CD is yet to be established

Others

Mycobacteriun tuberculosis is the causative agent of tuberculosis, one of the most pervasive and destructive human and animal disease in the past. It is the most heat-resistant non-sporeforming Gram-positive pathogen that is killed during pasteurization of milk at 72ºC for 15 s. Brucella abortus and B. melitensis are the members of the family of Brucellaceae and are responsible for the most prevalent bacterial zoonosis called brucellosis that still exists, especially in developing countries. They are killed easily by low pasteurization. Coxiella burnetii of Coxiellaceae family is responsible for Q fever.

Table 1.4 Groups of microbial pathogens possibly occurred in raw milk.

Organism	Disease	Main source of contamination
Enterobacteriaceae		
Escherichia coli, including 0157:H7	Gastroenteritis, hemolytic uremic syndrome, thrombotic thrombocytogenic purpua (TTP)	Feces, water, biofilms on the milking equipment
Salmonella	Gastroenteritis, typhoid fever	Feces, water
Shigella	Gastroenteritis	Feces, water
Yersinia enterocolitica[a]	Gastroenteritis	Feces, water
Other Gram-negative bacteria		
Aeromonas hydrophila[a]	Gastroenteritis	Cold-stored milk
Brucella spp.	Brucellosis (Bang's disease)	Sick animal
Campylobacter jejuni	Gastroenteritis	Feces
Pseudomonas aeruginosa[a]	Gastroenteritis	Cold-stored milk
Gram-positive spore-formers		
Bacillus cereus[b]	Intestinal intoxication	Soil, biofilms
Bacillus anthracis	Anthrax	Soil
Clostridium perfringens	Gastroenteritis	Silage feed, air
Clostridium botulinum	Botulism	Silage feed, air
Gram-positive cocci		
Staphylococcus aureus	Emetic intoxication	Mastitic udder
Streptococcus agalactiae	Sore throat	
Streptococcus pyogenes	Scarlet fever, sore throat	
Streptococcus zooepidemicus	Pharyngitis, nephritic sequelae	
Miscellaneous Gram-positive bacteria		
Corynebacterium spp.	Diphtheria	
Listeria monocytogenes[a]	Listeriosis	Dairy farm environment
Mycobacterium bovis	Tuberculosis	Sick animal
Mycobacterium tuberculosis	Tuberculosis	Sick animal
Mycobacterium paratuberculosis	Johne's disease (only for ruminants)	Feces
Spirochetes		
Leptospira interrogans	Leptospirosis	Sick animal
Rickettsia		
Coxiella burnetii	Q fever	Sick animal
Viruses		
Enterovirus, including polioviruses, rotoviruses, *Coxsackie* viruses	Enteric infection	Feces, water
FMD virus	Foot-and-mouth disease	Sick animal
Hepatitis virus	Infectious hepatitis	Feces
Fungi		
Molds	Mycotoxicosis	Air, feed
Protozoa		
Entamoeba histolytica	Amoebiasis	Water
Cryptosporidium muris	Cryptosporidiosis	Water
Cryptosporidium parvum	Cryptosporidiosis	Sick animal, feces
Giardia lamblia	Giardiasis	Water
Toxoplasma gondii	Toxoplasmosis	Water

Sources of contamination of milk

Milk after secretion from udder is immediately contaminated through various sources including the udder, environment and different milking practices. The hygienic control of these contaminating sources is crucial for microbiological quality of raw milk.

Udder hygiene-mastitis

The udder hygiene affects the microbial load of bulk tank milk, since poor teat cleanliness is associated with high bacterial counts. The numbers of bacteria in milk immediately after milking of healthy cow under hygienic conditions is about ≤10.000 cfu ml–. However in practice, raw milk has much higher bacterial counts due to probably poor hygienic conditions of milking. It has been calculated that the amount of transmitted dirt attached to the exterior of teat to milk is on average 59 mg l–1 .

Environment

The environment (air, soil, water, animal feces and feed) of dairy farm is the main contributor of contaminants and/or pathogenic microorganisms even in farms with milking room. In an extensive study, environmental samples (teat surface, air before and/or after work, air of milking room, hay of the day, hay of the month and settled dust) collected from 16 farms in France showed that fungi in the environment contained Aspergillus spp., Penicillium spp. , Eurotium spp. and white yeasts, while bacteria were Lactobacillus delbrueckii spp., Lb. paracasei, P. freudenreichii, Acinetobacter spp., Corynebacterium spp., Staphylococcus spp. and Streptococcus spp. Most of these microorganisms were also found in raw milk

Milking practices

Hand milking is of high risk for contaminating milk because milker may transfer the pathogens to milk, while milking machines, pipelines and milk filters act as reservoirs of high heat-resistant sporeforming microorganisms. Also, different hygienic milking practices can affect the balance between bacterial populations. Raw milks produced under high level of hygiene contain more Gram-positive non-lactic acid bacteria (Corynebacteriaceae and Micrococcaceae), while those milks produced under less intensive hygienic practices are dominated by Gram-negative bacteria and Lactococcus lactis or Brevibacterium lines and Leuconostoc mesenteroides. Poorly cleaned and disinfected milking equipment have residual milk which often contains bacterial load of ca. 109 cfu ml–1 and 1 ml of this residual milk is able to increase the bacterial load of 100 liters of the newly collected milk to 10,000 cfu ml–1. Biofilm formation on the surfaces of milking utensils has detrimental effect and must be controlled. Consequently, CIP protocol including prerinsing with water, circulation of alkaline/acidic and disinfectant solutions and finally rinsing with water.

Improving microbial quality of raw milk during storage

Anti-microbial factors of raw milk

Raw milk contains indigenous anti-microbial factors that belong to the fraction of minor serum proteins such as the enzymes lactoperoxidase and lysozyme, the iron binding protein lactoferrin and immunoglobulins (antibodies). All these proteins can act against a broad spectrum of microorganisms and viruses; thus they are potential inhibitors of microbial growth in raw milk during storage.

Lactoperoxidase-thiocyanate-hydrogen peroxide (LPO) system Lactoperoxidase (LPO, EC 1.11.1.7) is one of the most important indigenous milk enzymes in terms of both concentration and function. LPO is mainly responsible for the anti-microbial properties of raw milk in the presence of sufficient quantities of thiocyanate ion (SCN^-) and hydrogen peroxide

Lysozyme

Another indigenous milk enzyme with potential anti-microbial properties is lysozyme (EC 3.2.1.17), also called muramidase (peptidoglycan-N acetylmuranoylhydrolase), that cleaves the peptidoglycan in the bacterial cell-wall. It is an anti-bacterial agent present in many body fluids and in the milk of many mammalian species causing lysis of many types of bacteria. Its concentration in bovine milk is <0.03 mg ml–1 , increasing with increase in somatic cell count. It acts mostly against Gram-positive and a few Gram negative bacteria; however, its effect on raw milk's shelf-life is not significant due to its low concentration .

Lactoferrin

Lactoferrin (LF) is a non-hemic 80 kDa iron binding glycoprotein of the transferrin family, which is a very small fraction of milk serum proteins, i.e., 0.1–0.4 mg ml–1 . LF is a multifunctional protein that exhibits antimicrobial, anti-viral, anti-protozoan and anti-oxidant activities along with immunomodulation, modulation of cell growth and binding or inhibition of bioactive compounds

Immunoglobulins

Immunoglobulins (Igs) are antibodies distinguished in several classes. They are heat-sensitive glycoproteins with heterogeneous composition and IgG, IgA and IgM classes occur in milk serum. IgG predominates Igs in bovine milk (roughly 2/3 of total Igs). While Igs occupy 70–80% of the total protein in the bovine colostrums, their proportion in mature milk decreases to 1–2%. Concentrations of Igs in milk and colostrum differ from those in blood and are highly variable affected by species, breed, stage of lactation and health status of milking animals. They have many functions, i.e., augment leucocytes activities, activate complement-mediated bacteriolytic reactions, prevent the adhesion of microbes to surfaces, inhibit bacterial metabolism, agglutinate bacteria and neutralize toxins and viruses

Refrigeration during collection and storage of raw milk

Apart from milking procedures, the pre-storage conditions and transportation also affect the raw milk quality. According to European legislation the raw milk on the farm must be held at ≤8ºC immediately after milking or at ≤6ºC if it is not collected every day. Raw milk on most dairy farms is usually stored in a bulk tank but in the case of very small enterprises it is still collected and stored in cans (churns). Depending on the farm location, refrigerated milk is collected every day or every two or three days by tankers and is transported to the dairy factory for processing. Milk tankers are insulated to ensure that raw milk arrives at its destination at ≤5ºC; although, in practice this temperature limit is extended up to 6–9ºC or >10ºC .

Thermal treatments during storage of raw milk

In order to reduce the counts of microorganisms and avoid in particular the growth of psychrotrophs, a mild heat treatment with minimum heat damage of milk components should be applied to milk as soon as possible after its arrival at the dairy. This treatment is known as "thermization". Thermization usually allows milk to be stored 2–3 days after milking without deterioration. Typical temperature-time combinations for thermization are 68ºC for 10 s or 65ºC for 20 s. This heat treatment reduces the numbers of microorganisms by a factor of 3 log10 or 4 log10, while keeping alkaline phosphatase active. However, the final level of microorganisms will depend on their initial counts as well as on the thermization temperature. It was reported that thermization at 60ºC for 30 s had significantly inactivated Gram-negative bacteria, i.e., Listeria spp., Enterobacteria and coagulase-positive Staphylococci, but moderate effect against Gram-positive bacteria was reported under the same conditions.

Non-thermal treatments

Two centrifugation techniques, the clarification and bactofugation, are used for ‘cleaning’ milk. Both techniques operate to separate bacterial cells and milk components based on the differences in relative densities. Clarification removes mainly foreign particles, i.e., dirt, hairs, etc., whereas bactofugation removes bacterial spores. Bactofugation takes place at 60–65ºC and can remove up to 99% of the spores . Microfiltration, a membrane processing technique using 0.1–10 µm

pore-size membranes, improves microbial quality of raw milk by an average decimal reduction (DR) of 3.5, which means that if initial skim milk contains 20,000 cfu ml–1 , the microfiltered milk will contain less than 10 cfu ml–1 . For bacterial spores, the DR is higher than 4.5