



The Influence of Major Milk Microbiota on the Characteristics of Milk and Milk Products: A Review

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ABSTRACT

Milk is a nutrient-rich medium that encourages rapid microbial growth. The microorganisms associated with the milk of different milking animals and milk products have a significant impact on milk including milk from cows, buffalo, goats, and humans. Being nutritionally rich milk is a suitable growth medium for a variety of microorganisms and these have numerous positive and negative impacts on the properties of milk and their products. The high nutritional content of milk supports the growth of both beneficial and harmful microbiota. The entry of these microorganisms into the milk has various paths and sources, and, once in the milk, they start multiplication and increase their numbers. Many of these microbes show biological activities such as spore formation, toxin production, health promotion, biochemical alteration of medium, and other related activities. The authors are trying to present a detailed overview of the source, activity, and beneficial as well as harmful roles of microbes found in milk and milk products. The approach of authors to comprehensively review these topics is to examine the details of the microorganisms of milk to find out the activity and approach for improving milk processing and product stability for storage and sustainability in milk processing.

Key words: Dairy products, LAB, Milk microbiota, Milk.

Most of the essential nutrients for growth and metabolism of various microbes are found in milk, which is a substance secreted by the mammary glands in female mammals. The milk from cows, sheep and goats has been consumed for food for centuries. The characteristics of the milk microflora are directly related to its composition, which also has an impact on future dairy development in terms of new product development stability of processed products. Microorganisms have a variety of effects on the sensory parameters, texture, taste and organoleptic properties of the milk and resulting products by inducing the fermentation and biochemical changes of milk through the production of lactic acid. Microorganisms are also capable of degrading milk's quality as well as decreasing shelf life and are to blame for this (Quigley *et al.*, 2013). For instance, during refrigeration, hard bacteria multiply and can contribute to decomposition by releasing extracellular lipases and proteases. Because consuming raw milk contaminated with pathogens can result in disease, some of which can be severe, the microbial composition of milk can also have an impact on health (Wouters *et al.*, 2002). In contrast, other microbes found in raw milk may improve health by assisting with digestion, enhancing immunity and lowering the prevalence of allergies, such as asthma and atopic diseases, in people who consume raw milk early in life. Even though milk is thought to be sterile in healthy mammary cells, it is ultimately contaminated with microorganisms from a variety of sources, including teat tips, milking equipment, air, water, feed, hay, soil and other sources that come in the path of milk production. Milk contains important macro- and micronutrients, which is why it is considered beneficial during childhood and adolescence. However, the amount of saturated fat in it is relatively high and this raises concerns

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about potential negative effects, particularly on the cardiovascular system. Epidemiological, experimental and biochemical data frame our understanding of human health. As an example, consider the effects of milk (especially skim milk). The existing evidence is not conclusive, but there are some studies that show that milk and its derivatives are beneficial for humans. Milk and dairy products should be considered as part of a balanced diet unless there are obvious contraindications, although future research will help clarify their role in human health (Pereira, 2014).

Microbes in raw milk

Raw milk may contain different bacterial populations along with another category of microbes, some of which can cause spontaneous fermentation. In some instances, specific strains are so effective in this regard that they are purposefully kept apart from milk in order to give fermented products desirable properties. Bacteria are crucial if using pasteurized milk is required by law, so recovering milk

microbes can counteract the negative effects of population removal and affect the flavor of the final product. It is common knowledge that raw milk contains a diverse microbial community made up of many different microorganisms. The microbiota present in raw milk directly affects the subsequent development of dairy products (Braem *et al.*, 2012). Microorganisms can also produce extracellular lipases and proteases, which not only affect milk quality and shelf life but also cause spoilage. Additionally, the composition of the milk may cause health issues if abnormal microbes are present and ingesting pathogen-contaminated raw milk can result in serious illness.

Sources of microbes in milk and milk products

Freshly expressed milk is typically sterile, but it can occasionally become contaminated with microorganisms from different places. Although workers do not contribute much to most organisms, they are crucial because they have the potential to become human pathogens however the number and type of microorganisms from each source have a large effect on the milk. The way in which microorganisms enter milk or milk products is important. Internal sources of contamination include things like animal bodies, the environment, machinery and other things that are added to milk, dairy products, or the processes that produce them. If a production animal is not clean and its udder and teats are not given special hygienic treatment before milking, its body can become a source of attention (Braem *et al.*, 2012). Milk from cows or buffaloes with infected udders may contain large numbers of microorganisms. Hair, dust and dirt often get into the bucket or cup of a milking machine. Operators or milkers may act as sources of microbial contamination (Fig 1). They are involved in the milking process and they must be free from disease and in good health and maintain

a system of personal hygiene. Before starting work, they should wash their hands with an effective disinfectant solution and regularly wash and dry them with clean towels on the job Vacheyrou *et al.* (2011).

Bacteria in milk and milk products

Microorganisms can have a negative impact on the quality and shelf life of milk. For example, resistant bacteria grow during refrigeration and can contaminate milk (de Oliveira *et al.*, 2015). The microbial composition of milk can also affect health because consumption of raw milk contaminated with pathogens can lead to diseases, some of which can be serious for consumers. The continued development of dairy products is influenced by the unique composition of milk. Through the production of lactic acid, microorganisms can cause the fermentation of milk and have a variety of effects on the final product's flavor, texture and organic qualities. Unlike other raw milk pathogens, other raw milk microbes may contribute to health by aiding digestion and reducing the incidence of allergies, including asthma and atopic disorders, in individuals who consume raw milk early in life (Raats *et al.*, 2011).

Common harmful and pathogenic bacteria associated with milk and milk products

Milk can be contaminated with pathogenic bacteria or bacterial toxins, which can cause health problems like diarrhea, food poisoning, tuberculosis and salmonellosis. Several microorganisms, including *Salmonella species*, *Escherichia coli* and *Staphylococcus species*, are responsible for foodborne illnesses. Some pathogenic microorganisms can grow and thrive well in milk, particularly dangerous bacteria like *Salmonella* and *Escherichia coli*. These pathogens can act as sources of infection. But unlike

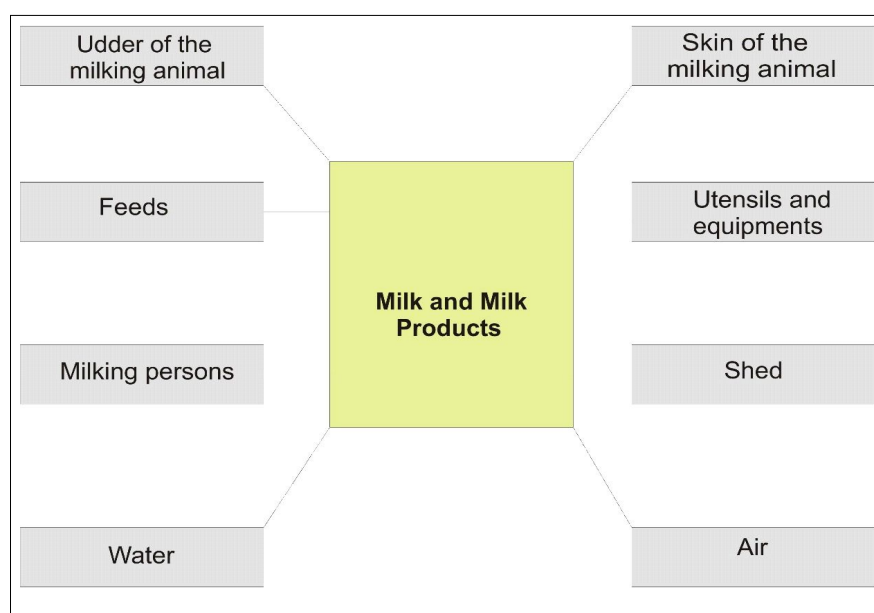


Fig 1: Sources and path of microbes to milk and milk products.

Salmonella and *E. coli*, bacteria like *Mycobacteria*, *Brucella* and *Leptospira* in milk, although pathogenic, do not freely reproduce in milk Chiodini *et al.* (2012).

Escherichia coli

Escherichia coli is the major coliform group responsible for many milk-derived infections in infants that cause gastroenteritis in infants. Depending on the disease's pathogenicity and the presence of virulence factors, there are different types of *E. coli* pathogens: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and enteroinvasive *E. coli* (EIEC), Shiga toxin-producing *Escherichia coli* (STEC), diffusely adherent *Escherichia coli* (DAEC) and enterohaemorrhagic *Escherichia coli* (EHEC). *Escherichia coli*, which causes hemolytic uremic syndrome in humans, can also enter milk through the feces of cows, udders, handlers and contaminated tools (Afzal *et al.*, 2010). Therefore, raw milk is associated with diarrhea caused by *E. coli*. Some outbreaks of *E. coli* causing diarrhea have been attributed to the consumption of unpasteurized milk and have been reported in several countries around the world.

Salmonella, a gram-negative, rod-shaped bacterium, is more likely to contaminate milk. Milk contamination with *Salmonella spp.* occurs mainly from infected people and the environment and rarely from the udder. It causes salmonellosis or typhoid fever and there are two species of *Salmonella*, *S. enterica* and *S. bongori*, of which only *S. enterica* is pathogenic and six subspecies (*enterica*, *Salamea*, *arizonae*, *diarizonae*, *houtenae* and *induced*). It causes dysentery and diarrhea in humans and is transmitted by the consumption of contaminated dairy products, which can be unpasteurized or partially pasteurized milk. According to Duthoit *et al.* (2003), the milk would have been contaminated by *Salmonella spp.* in certain regions. The feces of healthy cows excrete high levels of bacteria, which can be controlled by proper pasteurization and sanitation. *Salmonella* frequently spoils cheese made from raw milk in 34% of outbreaks, according to a report and is a common cause of foodborne outbreaks. Human salmonellosis is the most frequently reported zoonotic disease in the European Union.

The spherical, coccoid, Gram-positive, non-spore-forming bacterium *Staphylococcus aureus* is a member of the *Staphylococcus* genus. 32 species and subspecies make up the *Staphylococcus* genus, which is grouped together. *In vitro*, *S. aureus* can grow in media containing up to 10% salt. *Staphylococcus aureus* colonies have golden or light-yellow coloration Munsch-Alatossava and Alatossava (2007). *S. aureus* grows best at temperatures between 18 and 40°C, whether it is aerobic or anaerobic. Due to the exotoxins that *Staphylococcus aureus* produces, it has been established that this bacterium is the primary cause of food poisoning in humans. Enterotoxins are not destroyed by normal pasteurization or cooking because they are heat stable when stored (Monnet *et al.*, 2010). Thus, enterotoxin causes death even in the absence of live *S. aureus* cells.

Common beneficial and useful bacteria associated with milk and milk products

Numerous bacteria are present in raw milk, which is a well-known fact. Many of these bacteria can subsequently contribute to natural fermentation. Some strains have been so effective at this that they have been deliberately isolated from milk and added as starter cultures or adjuvants to fermented products in order to give them desirable properties. Reintroducing dairy microbes can counteract the removal of commensal populations and the resulting detrimental effect on the flavor of the final product. This is especially crucial when regulations demand the use of pasteurized milk. The most important known genera for a beneficial impact on milk and milk products belongs to Lactobacteria (Kahala *et al.*, 2008).

Lactococcus

For dairy products, *Lactococcus lactis* and *Lactococcus lactis ssp. Lactococcus ractis ssp. cremoris* is primarily known as the starter culture for the cheese industry Li *et al.* (2020). The production of taste-developing substances is another characteristic of *Lactococcus lactis sp. Lactis Biovardia Cetillactis* (Song *et al.*, 2017). Based on their use of arginine and citrate, growth temperature and salt tolerance, these microorganisms can be distinguished from one another (Smit *et al.*, 2005). Although these microorganisms exist naturally in raw milk and artisan cheese, they are frequently added to pasteurized milk to aid in the production of cheese for industrial use. By producing L-lactic acid, they primarily contribute to acidification during the cheesemaking process. However, they also play a role in the breakdown of proteins, the amination of amino acids to aromatics (alcohols, ketones and aldehydes), the use of citrates and/or the metabolism of fat.

Lactobacillus

According to the most recent estimates, the genus *Lactobacillus* is extremely diverse, with 174 different species and 27 subspecies. According to Broadbent *et al.* (2011) and Bernardeau *et al.* (2008), *lactobacillus lactobacilli* can be found in niches that are rich in carbohydrates, such as those that are connected to plants, animals, silage and raw milk. As the understanding of *Lactobacillus* biology deepened, *Lactobacillus* strains were used to expand the range of applications for industrial dairy products. Particularly, their proteolytic activity and capacity to create flavoring substances and exopolysaccharides may enhance the nutritional value and quality of dairy products (Bernardeau *et al.*, 2008). *Lactobacillus* of particular concern in the dairy industry is *L. helveticus*, *L. delbrueckii sp. bulgaricus* and *L. delbrueckii sp. ractis*.

Propionibacterium

Strains isolated from dairy and dairy products are called "dairy" or "classical" propionibacterium. In particular, milk propionibacterium is also said to have health-promoting

properties. The lactic acid bacteria group of Propionibacteria includes four species: *Propionibacterium freudenreichii*, *Propionibacterium acidipropionici*, *Propionibacterium jensenii* and *Propionibacterium thoenii* (Cousin *et al.*, 2011). *Propionibacterium freudenreichii* serves as an appetizer for Swiss cheese. It was first separated from Emmentaler cheese more than a century ago and it aids in the development of holes, or “eyes,” and flavors in these cheeses. Propionibacterium is typically isolated from milk and different kinds of cheese, but it can also be found in other dairy products. The fermentation of lactic acid to propionic acid, acetate and CO₂ is what distinguishes *P. freudenreichii*, but the related characteristic flavors come from the formation of fatty acids through lipolysis and the formation of branched-chain acids through the breakdown of amino acids. *P. freudenreichii* has GRAS status because it has a long history of being used in the production of cheese Falentin *et al.* (2010).

Together with LAB Numerous non-starter lactic acid bacteria (NSLAB) are present in dairy products and contribute to the development of flavour and other desirable qualities in milk-based products. The common NSLAB found in milk and milk products are *Pediococcus acidilactici*, *P. pentosaceus*, *Enterococcus durans*, *E. faecalis* and *E. faecium* and *Lactobacilli* (*Lactobacillus casei*, *L. paracasei*, *L. plantarum*, *L. curvatus*, *L. brevis* and *L. fermentum*) Settanni *L.*, (Moschetti, 2010). Dairy products may also contain bacteria as pathogenic agents or undesired food deterioration. The psychrotrophic bacteria (*Pseudomonas fluorescens* and *P. putrefaciens*), *Salmonella spp.*, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium botulinum*, *C. tyrobutyricum*, *C. perfringens* and *Vibrio cholera* are a few examples of these unwanted microorganisms (Machado *et al.*, 2013).

Common methods of detection of bacteria in milk

Many microbial communities are complex, that is, they consist of many different microbial taxa. An environment with a complex and diverse microbial population is present in raw milk, for instance. Much of what we know about the identification of microorganisms found in raw milk and the resulting dairy products, is acquired through the growth or “cultivation” of these microorganisms and the subsequent analysis (Vacheyrou *et al.*, 2011). Phenotypic and/or genotypic techniques are used for the final identification of these cultured microorganisms. Phenotypic methods are those that have historically been used to grow microorganisms in microbial media (general or selectively) and to characterize them using morphological, biochemical, or physiological means. These test procedures, which are still common in industrial settings, typically involve looking for specific pathogens or other microorganisms that can indicate contamination or counting all the bacteria present in the milk, which reflects its overall quality (Fricker *et al.*, 2011). Thermotolerant bacteria (pasteurization-resistant), sulfate-reducing *Clostridium*, *Listeria monocytogenes*, *Salmonella*, coagulase-positive *staphylococci*, *Escherichia coli*,

Enterobacteriaceae, coliforms, Bacillus-like bacteria wax, etc. are among the populations that are routinely tested. National and international accrediting organizations have approved these common practices. These tests often rely heavily on the use of microbial broths or agars that selectively support the growth of the target microbial population and often include additional confirmatory biochemical analysis (Loman *et al.*, 2012). These techniques tend to be low-tech and affordable, but they are also laborious and time-consuming and in some cases, they may not have enough discriminatory power, which could be a problem. Significant work has recently been put into creating high-throughput, quicker tests that use genotyping based on DNA. These methods typically rely on the use of polymerase chain reaction (PCR) techniques and can be used to verify the outcomes of conventional tests, but they are also increasingly common as alternatives to tests based on culture. Many microorganisms do not like to be isolated using common culture methods, which can seriously underestimate the community. This is one of the main benefits of replacing the culture step. When using these approaches, which are independent of culture, many factors must be considered. It is crucial to select a protocol that will effectively extract nucleic acids from the greatest number of microorganisms. Consideration should also be given to strategies such as the use of DNA binding agents or alternative RNAs of interest to limit the risk of false positives due to the DNA amplification of dead cells. This method might be able to separate milk with very low levels of bacteria, but it cannot separate milk with bacteria levels of, say, 200,000 per 1 cc. One point to remember is that the colonies appearing on the agar plates arise from groups of bacteria and from individual bacteria (Hagi *et al.*, 2010). It makes sense to add tests for shelf life and, given this particular importance, coliform organisms to the bacterial inspection because consumers judge the quality of milk based on the type and number of bacteria present. Although it is possible to obtain good results, the time required to obtain them is as long, if not longer, than the plate method. It appears that while the Breed and Frost methods are well suited to quickly classify milk into broad grades, their accuracy is necessarily reduced by their nature since the results obtained are more dependent on calculations than the plate method.

Common methods of inactivation of bacteria from milk

Bactofugation is the process of using centrifugal force to remove microorganisms from milk. To sterilize milk at a lower temperature-time combination, a unique isolated form of microorganisms is used, primarily sporulates (*Bacillus/Clostridia*) (Monnet *et al.*, 2010). By pasteurizing, most microorganisms are rendered inactive. However, very heat-resistant spores survive the pasteurization process. Proteolysis, lipolysis and gas formation can cause serious quality issues in hard and semi-hard cheeses as well as other products with a long shelf life. Therefore, the primary

method of production for these products involves sterilization. The objectives of sterilization are to improve the hygienic quality of milk, avoid heat-resistant bacteria without using excessive heat and guarantee extremely high bacterial purity in milk.

It removes live and dead bacteria from the processed substance, while conventional heat treatment kills the bacteria and leaves them in the food. Most of the microorganisms that spoil milk, reduce the quality of fermentation in milk powder and butter and cause foaming in cheese are spore-forming bacteria (Loman *et al.*, 2012). Foods contaminated by bacteria that produce heat-stable endotoxins require bactericidal action to be effective.

The interest in the system in the dairy industry has grown as a result of two key factors. First, alterations in consumer purchasing patterns have resulted in an increase in the number of people who only purchase milk once per week and ask to consume it six to seven days later, even if the milk was not stored in a suitable manner during this time. Second, milk processing is concentrated in a few ultra-large dairies, resulting in an extensive distribution chain with airtight roasting, dairy farms can add days to the shelf life of their dairy products (Masoud *et al.*, 2011). This response to consumer demand for a longer shelf life of fresh produce after leaving the store. Dairies can improve their product using established technology at a reasonable cost by using the Hermetic Sterilizer, a development of the market-leading Hermetic Separator.

The common mode of storage and conditions on milk microbiology

Refrigerated storage

It is important to make the point clear that even in cold storage there will be scope for changes in the microflora of the raw milk when it is stored or is in processing. Milk usually stored at cold temperatures reduces the growth of most bacteria, except for psychrotolerant microorganisms that can proliferate under these conditions and become a major cause of the spoilage of milk (de Oliveira *et al.*, 2015). Generally, the psychrophilic bacteria are not present in the fresh milk after just milking but the psychrophilic bacteria become the part of bulk and start growing with time and start affecting the milk composition and texture biochemically. This results primarily from the production of extracellular enzymes, the most significant of which are lipase and protease. These lipases cause milk fat to become rancid and casein-degrading proteases result in a gray color and an unpleasant taste. The study of seasonal variation in microbial growth in raw milk revealed that psycho-bacteria exhibited better growth and protease production in winter milk compared with summer milk. *Pseudomonas* spp., commonly found in raw milk, is the most common cause of milk spoilage. According to Meng *et al.* (2017) *Pseudomonas fluorescens*, *Pseudomonas gessardii*, *Pseudomonas fragi* and *Pseudomonas lundensis* are the *Pseudomonas* species most frequently found in milk and cheese. These bacteria can make up between 70 and 90 per cent of the

microorganism population in fresh milk that is kept at low temperatures. While many other psychrotolerant microorganisms are found in milk, *Pseudomonas* is typically more significant for milk spoilage.

Pasteurization

Pasteurization of raw milk is done to reduce the microbial load in milk and particularly to limit the number of spoilage microorganisms and prevent foodborne illnesses. The procedure, though, also results in fewer microorganisms than are typically responsible for the milk's desirable organoleptic properties. In these cases, the original cultures are known to produce the desired flavor and aroma, which are added to the milk after pasteurization. The Effect of different methods of pasteurization on the bactericidal action of human milk was summarized by (Patil *et al.*, 2019) Typical milk pasteurization treatment is a "high temperature, short time" (HTST) approach involving heating at 72°C for 15 s. Several countries have increased temperatures and/or exposure times. Although this can further reduce bacterial counts and eliminate microorganisms of concern, including *Mycobacterium avium* ssp. *Paratuberculosis* and *L. monocytogenes*, it has also been suggested that this approach may promote spore activation, which may be inactive in milk. Milk heat treatment generally reduces psychrophilic and many mesophilic populations, leaving two main groups to be considered later, thermophilic microorganisms and bacteria introduced by post-pasteurization contamination. After pasteurization, some microorganisms may become "viable but not culturable," which means they may be underestimated compared to traditional farming methods.

CONCLUSION

This work contains details about the microflora present in milk and milk products, with an emphasis on useful and harmful microorganisms. The source of microbes in milk starts at the site of milking and continues up to the utilization of final milk and milk product consumption. In order to produce a clear conclusion about the association and effect of microbes on milk and its products, the occurrence, application, as well as effect added to the milk and milk products by these organisms, were summarized here.

Conflict of Interest

The authors are declaring that there is no conflict of interest.

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