

## DIVERSITY OF LACTIC ACID BACTERIA IN RAW MILK

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### Abstract

In this study we described the diversity of lactic acid bacteria and their representatives in raw and thermally treated milk, focusing on their potential in cheese production influencing cheese quality. The aim of the present study was to analyse the concentrations and representatives of lactic acid bacteria in raw milk and to detect the changes of lactic acid bacteria microflora during thermal treatment of cheese milk at a dairy processing plant. The analysis carried out in the study showed a seasonal variation in the microbial composition and quantity of raw milk. The most frequently isolated lactic acid bacteria: lactococci, lactobacilli, leuconostoc were found at low level in raw milk (mean  $9.27 \times 10^3$  CFU mL<sup>-1</sup>) and the most frequently identified species were *Lactococcus lactis*, *Lactobacillus brevis* and *Lactobacillus fermentum*. The microflora of raw and pasteurised milk is similar to the analysed lactic acid bacteria representatives in the samples. Interestingly, we found the same species in raw milk and pasteurised milk, for example, *Lactobacillus brevis* and *Lactobacillus fermentum* were detected in the same samples in raw milk and pasteurised milk. Our study showed that lactic acid bacteria concentration was quite low in pasteurised milk (0-76 CFU mL<sup>-1</sup>), but they grow rapidly in cheese during ripening; therefore the definition of limits of the non-starter lactic acid bacteria colony forming units in milk should be reasonable for selection of appropriate raw milk quality for cheesemaking.

**Key words:** lactic acid bacteria, total plate count, lactobacilli, raw milk.

### Introduction

Raw milk is a natural growth medium for microorganisms. The composition and quality of raw milk microflora are determined not only by hygienic observation in the places of milk production and processing, rapidity of milk cooling and temperature, but also by microflora in the air of dairy environment, and on the surfaces of equipment and premises. An integral part of raw milk microflora is lactic acid bacteria – *Lactobacillus casei* subsp. *paracasei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus curvatus*, *Lactobacillus brevis*, *Lactobacillus fermentum*; *Leuconostoc lactis*, *Leuconostoc cremoris*; *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus durans* and *Pediococcus* spp.: *P. pentosaceus*, *P. acidilactici*.

Hygienically produced raw milk may contain  $10^2$  lactobacilli mL<sup>-1</sup>. Pasteurization regime selected in dairy industry is able to destroy essential microflora, enzymes and pathogens in milk. It should be noted that inactivation level of microorganisms depends on the count of microorganisms, growth phase and other factors. Although lactobacilli are inactivated by pasteurisation, some strains may survive the heat treatment and proliferate in dairy products production and storage (McSweeney et al., 1999; Jordan and Cogan, 1999).

Non-starter lactic acid bacteria (NSLAB) are found in cheeses made from raw and pasteurized milk. In cheese made from pasteurized milk, they are normally present in relatively low numbers, probably  $< 10^3$  CFU g<sup>-1</sup> at the beginning of ripening, but they grow rapidly during ripening to levels of  $\sim 10^8$  CFU g<sup>-1</sup> within 2 to 4 months, depending on the species, cheese, and the ripening temperature (Coppola et al., 1997).

Bactofugation, microfiltration, and application of food additives, cannot significantly decrease the proportion of *Lactobacillus* spp. and *Leuconostoc* spp. in milk. Defects caused by non-starter lactic acid bacteria are found in all dairy products, but the most problematic they are in cheeses.

Taking into account the impact of non-starter lactic acid bacteria on the formation of cheese flavour, various solutions are recommended for manufacturers to assure the quality of cheeses and one of the solutions is the definition of limits of the non-starter lactic acid bacteria colony forming units in milk. The critical limits of mesophilic non-starter lactic acid bacteria are stated  $10^2$  CFU in 1 mL of milk (Fox et al., 2000). In Latvia mesophilic non-starter lactic acid bacteria are not detected in raw milk, we do not have critical limits for NSLAB.

According to the study of A. Mikelsona (2011) and her research conclusions, the representatives of *Lactobacillus* genus and its colony forming units differ between same cheese varieties manufactured at different plants. This proved that manufacturing and ripening conditions at cheese plant have a significant impact on the diversity of microflora however, the main source of cheese microflora still remains raw milk. From this point of view, the aim of the present study was to analyse the concentrations and representatives of lactic acid bacteria in raw milk and detect the changes of microflora of lactic acid bacteria during thermal treatment of cheese milk at a dairy processing plant.

### Materials and Methods

Research was performed from January 2014 to February 2015 at:

- ✓ the Laboratory of Microbiology of the Department of Food Technology of Latvia University of Agriculture;
- ✓ the laboratory of the dairy processing company “Latvijas piens” Ltd.

*Object of the research*

In order to study the critical limits for non-starter lactic acid bacteria in raw milk, bulk milk samples were analysed (n = 19) in the dairy company. The samples were analysed twice per month over one year period. The samples were taken from raw milk tanks in the dairy company. Bulk milk was kept at 2-4 °C prior treatment.

For better understanding the proliferation of lactic acid bacteria representatives during thermal treatment, raw milk was pasteurized at 74 °C 30 s in the dairy company. Treated milk samples (n = 22) were taken from cheese vats before renneting.

*Methods of analyses*

Determination of total plate count (TPC) was performed in all analyzed bulk milk samples according to LVS EN ISO 4833-1:2014 using PCA (plate count agar) (OXOID, UK). Sample dilutions were performed according to ISO 6887-5:2010 using salt-peptone solution. The chosen parameters for cultivation of bacteria in PCA agar were 72 hours at 30 °C. The cultivation media were prepared according to LVS CEN ISO/TS 11133-1:2009.

Determination of *Lactobacillus* spp. was performed in all analyzed samples according to LVS ISO 15214:1998 using MRS agar (de Man Rogosa and Sharpe with Tween) media (OXOID, UK). Media

were prepared according to LVS CEN ISO/TS 11133-1:2009. Sample dilutions were performed according to ISO 6887-5:2010 using salt-peptone solution.

The chosen parameters for cultivation of lactic acid bacteria in MRS agar were 72 hours at 37 °C, taking as a basis regimes recommended in the scientific literature (Coeuret et al., 2003).

Taking into account the fact that lactic acid bacteria belong to facultatively anaerobic representatives group, anaerobic cultivation of lactic acid bacteria using AnaeroGEN™ Compact system was provided, too.

Identification of *Lactobacillus* spp. colonies was performed taking as a basis the fermentation of carbohydrates using API 50 CHL (BioMerieux, France). The program APILAB Plus version 4.0 (BioMerieux) was used for identification of the isolated colonies up to species.

Data mathematical treatment was performed by using Microsoft Excel programs. The mean and the standard deviation of experimental data were determined.

**Results and Discussion**

A useful indicator for monitoring the sanitary conditions during the production, collection and handling of raw milk is the total plate count. Its sole value is to indicate changes in the production, collection, handling, and storage environment. Raw milk quality will influence the quality of the processed products. The total plate and lactic acid bacteria count of bulk milk samples is reported in Figure 1.

The microbiological quality of raw milk is strictly related to the management practice, such as equipment and environment hygiene, cow welfare, etc (Little

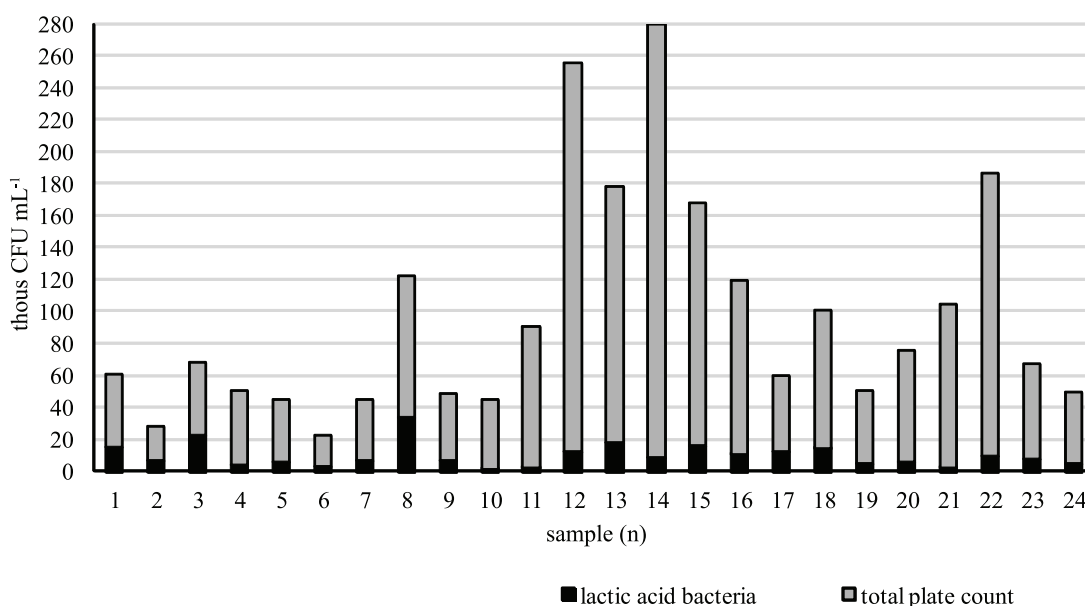


Figure 1. The total plate and lactic acid bacteria count (thous CFU mL<sup>-1</sup>) in analysed samples.

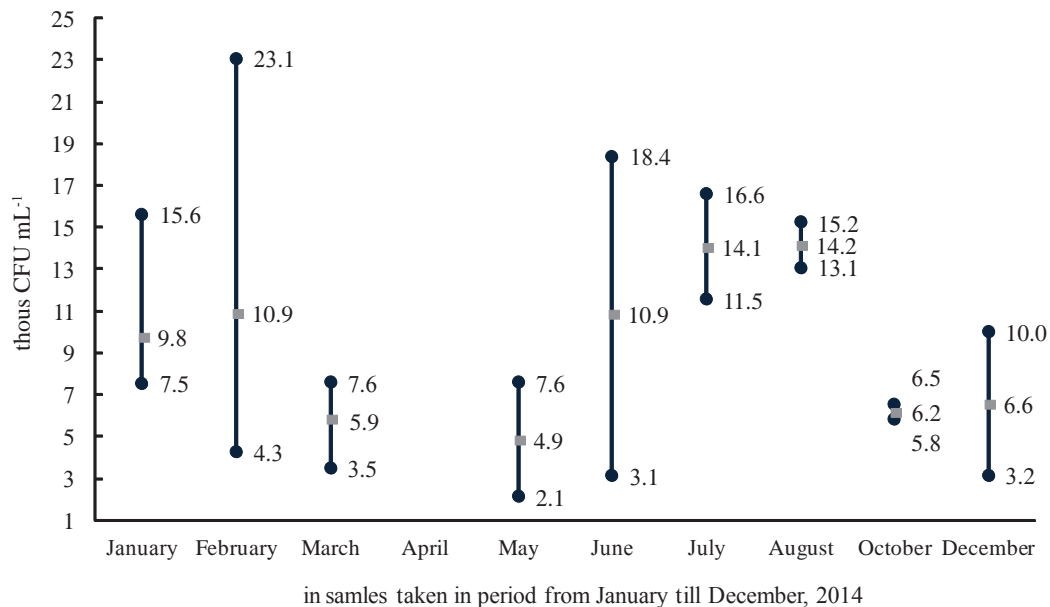


Figure 2. The lactic acid bacteria CFU in analysed raw milk.

et al., 2008). In Latvia, the current regulatory plate count for raw milk is  $< 100\ 000\ \text{CFU mL}^{-1}$  (at  $30\ ^\circ\text{C}$ ) and for raw milk used for dairy products production is less than  $300\ 000\ \text{CFU mL}^{-1}$  ( $30\ ^\circ\text{C}$ ) before processing, as specified in Regulation (EC) 853/004 laying down specific hygiene rules on the hygiene of foodstuffs (Regulation 853/2004). Our results for TPC determined in samples of raw milk indicated that bulk milk samples satisfy official requirements for raw milk hygiene before processing.

According to E. Franciosi et al. (2009) study that LAB/TPC ratio is almost 1, showing LAB dominating role in the bacterial population of raw milk. Our data (it was 0.16) contradicted the E. Franciosi and co-authors study. The microflora of raw milk depends on hygiene of milking, but the cooling rate and temperature, as well as the storage temperature and time, are the main factors taken into account when explaining the results. The microflora of raw milk has changed during the last decades due to milking equipment and storage facilities modernisation. In modern commercial practice, raw milk is normally cooled to  $4\ ^\circ\text{C}$  immediately after milking and may be held at about this temperature for several days at a farm and factory. It means that dominating microflora of raw milk is psychrotropic representatives, such as *Pseudomas* spp., *Alcaligenes* spp., etc. The main differences in ratio between the total plate count and LAB we should explain with the previously mentioned considerations. The min, max and average value of lactic acid bacteria in raw milk samples are showed in Fig. 2.

The analysis carried out in the study showed a seasonal variation in the microbial composition and quantity of the milk. This is an important factor when considering the ultimate use of the milk as,

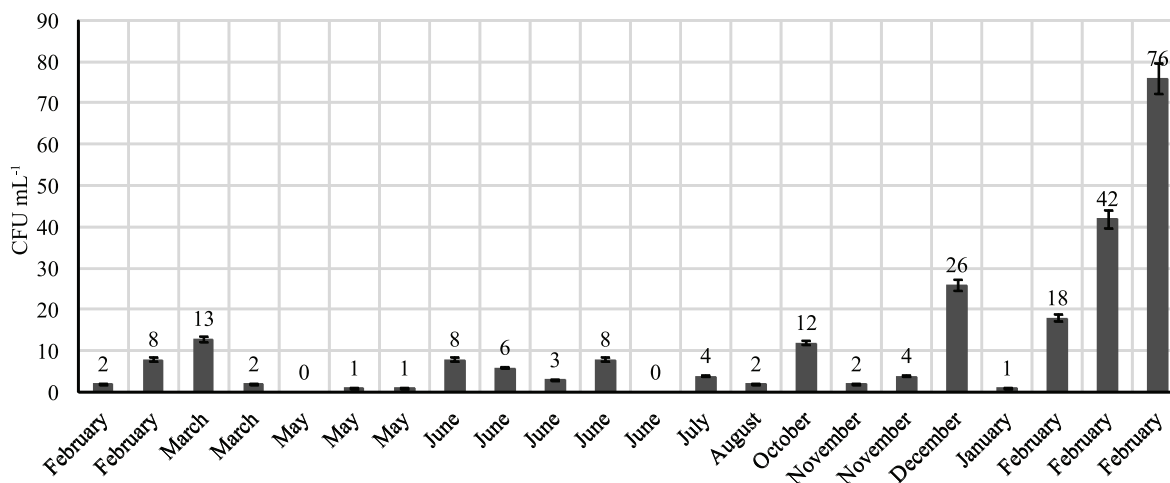
for example, some variations of LAB may affect the flavour development of cheese (Randazzo et al., 2010).

The mean population of LAB was  $9.27 \times 10^3\ \text{CFU mL}^{-1}$  in the raw milk. Almost all of LAB isolates were lactococci and lactobacilli and the most frequently identified species were *Lactococcus lactis*, *Lactobacillus brevis* and *Lactobacillus fermentum*. According to the findings of E. Franciosi et al. (2010), almost 94% of isolates belong to *E. faecalis*, *E. durans* and *Lactococcus lactis* and only 6% of isolates include *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, etc. in raw milk.

In the present study, microorganisms are identified based on phenotypical criteria, and the obtained results also highlighted the absence of isolates in some analysed raw milk samples, therefore, in Table 1 we summarized all LAB isolates. The microflora of pasteurised milk is primarily of bacterial nature, and bacteria commonly isolated from pasteurised milk are of the same type that is found in raw milk (Table 1).

Table 1  
Lactic acid bacteria species isolated from raw and pasteurised milk

Raw milk	Pasteurised milk
<i>Lactobacillus paracasei</i>	<i>Lactobacillus paracasei</i>
<i>Lactobacillus brevis</i>	<i>Lactobacillus brevis</i>
<i>Lactobacillus fermentum</i>	<i>Lactobacillus fermentum</i>
<i>Lactobacillus curvatus</i>	<i>Lactobacillus curvatus</i>
<i>Lactococcus lactis</i>	<i>Lactococcus lactis</i>
<i>Lactobacillus plantarum</i>	<i>Lactobacillus acidophilus</i>
<i>Lactobacillus rhamnosus</i>	<i>Leuconostoc lactis</i>



in samples taken in period from February (2014) till February (2015)

Figure 3. Lactic acid bacteria CFU in analysed pasteurised milk samples during the study period.

The microflora of raw and pasteurised milk is similar to the analysed lactic acid bacteria representatives in the samples. Interestingly, we found the same species in raw milk and pasteurised milk—for example, *Lactobacillus brevis* and *Lactobacillus fermentum* were detected in the same samples of raw milk and pasteurised milk.

*Lactococcus lactis* was the acidifying bacterium, thus preventing both alteration and growth of potentially pathogenic bacteria. *Lactobacillus plantarum* was rarely detected in milk unlike *Lactobacillus acidophilus*, which was frequently present in raw milk samples. Notably, the species *Lactococcus lactis* and *Leuconostoc lactis* were frequently presented in milk (Casalta and Montel, 2008). Strains of these species have been recognized as an important starter composition in many dairy products and cheeses.

The lactic acid bacteria concentration in pasteurised milk is showed in Figure 3.

Despite the fact that pasteurisation eliminates the most of vegetative cells, some species such as thermophilic microorganisms can survive and subsequently propagate in the final product disturbing quality of dairy products.

Our study showed that lactic acid bacteria concentration was quite low in cheese milk and from

this point of view we should not predict potential defects of produced cheeses. According to R. Coppola and co-authors study, mesophilic lactobacilli are present in relatively low numbers in pasteurised milk and in this study it was from 0 to 76 CFU mL<sup>-1</sup> however, they grow rapidly in cheese during ripening. Therefore in a further study, it would be interesting to determine the impact of lactic acid bacteria on cheese quality starting with raw milk, thermally treated cheese milk and cheese, analysing their influence on the cheese ripening and final product quality.

### Conclusions

The most frequently isolated lactic acid bacteria were found at low level in raw milk and the most frequently identified species were *Lactococcus lactis*, *Lactobacillus brevis* and *Lactobacillus fermentum*. The microflora of raw and pasteurised milk is similar to the analysed lactic acid bacteria representatives in the samples. Our study showed that lactic acid bacteria concentration was quite low in pasteurised milk (0-76 CFU mL<sup>-1</sup>), but they grow rapidly in cheese during ripening; therefore the definition of limits of the non-starter lactic acid bacteria colony forming units in milk should be reasonable for the selection of appropriate raw milk quality for cheesemaking.

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