

## TYPES OF PSYCHROTROPHIC BACTERIA IN RAW MILK COLLECTED IN A DAIRY FARM COOLING BASIN

ANKA KASALICA and IVANKA OTENHAJMER

*Dairy Institute, Beograd, Yugoslavia*

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*The psychrotrophic microflora from raw milk cooled in a dairy farm cooling basin for two days was isolated and identified. A standard method enabling an increase of the psychrotrophic bacteria by incubation of the inoculated Petri plates at 7°C for 10 days as well as at 21°C for 25 h was applied. A total of 286 strains of psychrotrophic microorganisms were isolated from 14 raw milk samples. Namely, 144 of them were isolated after incubation at 7°C for 10 days whereas 142 strains were isolated after incubation at 21°C for 25 h. The dominant microflora on the former plates (7°C for 10 days) was composed of Gram-negative rods (39,59%), whereas those incubated at 21°C for 25 h were dominated by Gram-positive cocci (48,59%). The largest proportion of strains isolated at 7°C after 10 days pertained to the coryneform group of bacteria (19,45%) as well as to the genus *Streptococcus* (13,20%) and *Flavobacterium* (12,50%) whereas those isolated at 21°C for 25 h belonged to the genus *Streptococcus* (37,33%), followed the coryneform group of bacteria (10,57%) and the genus *Bacillus* (9,86%).*

*Key words: Types of psychrotrophic bacteria, raw milk.*

### INTRODUCTION

Psychrotrophic microorganisms are widely spread throughout nature. Their natural dwelling-places are soil, water, vegetation, animals, as well as equipment (Thomas, 1958; Winter, 1961; Stokes et al., 1966; Tomàs et al., 1966; Thomas and Thomas, 1973).

Thomas (1966) claims that most of the psychrotrophs penetrate into milk and dairy products out of soil, water and vegetation. Since these microorganisms are known to be capable of multiplying at low temperatures, the count of psychrotrophs present in cooling basins for milk increases throughout the cooling process. While multiplying psychrotrophs tend frequently to release undesirable metabolites and enzymes (lipase and proteinase) which leads to deterioration in the quality of raw milk. Many of them are thermoresistant (Adams,

1975; Gerbre- Egziabher, 1980), which means that they are quite capable of spoiling both milk and dairy products.

This examination was undertaken with the purpose of isolating the psychrotrophic microflora from milk cooled in the farm chilling basins for two days and identifying them.

#### MATERIAL AND METHODS

Milk originating from four milkings was collected and cooled with the purpose of isolating and identifying psychrotrophs throughout July on one test-farm. Altogether 14 samples were analysed.

Two tests were performed. Samples of the cooled milk were taken after each milking while the milk was cooled for 12, 18 and 24 hours down to 6°C. Nutrient agar ("Torlak") was used as the medium for isolation of the characteristic colonies.

The inoculated Petri plates were incubated using a standard method securing the growth of psychrotrophic bacteria, namely, at 7°C for 10 days (American Public Health Ass., 1978) as well as at 21°C for 25 h (Oliveria and Parmelee, 1976). Upon conclusion of the incubation, characteristic colonies were picked out of the Petri plates and transferred into nutrient broth ("Torlak").

The following criteria were used for identification of the isolates at the level of genera staining according to morphology, formation of spores, motility, synthesis of catalase and oxidase, reaction to litmus milk, pability of forming pigments (King), oxidation - fermentation of carbohydrates (Hugh-Liefson).

The IMVC test was used as well for the determination of strains originating from the Enterobacteriaceae family. Bacterial strains of Streptococcus and Micrococcus were determined according to Bergery (1991). Representative strains were identified using the API SYSTEM (API 20 E, API 20 NE and API 50 CH).

#### RESULTS AND DISCUSSION

The results of an earlier examination (Kasalica and Otenhajmer, 1995) indicated poor quality of cooled raw milk due to the high share of psychrotrophic microorganisms in it. The undesirable consequences of their biochemical activity is wellknown. However, for proper assessment of the quality of milk the detection of specific bacterial species is far more important than the total count of contaminants which may only slowly disintegrate the components of milk. A total of 286 strains of psychrotrophic microorganisms were isolated from the 14 milk samples. Thus, 144 strains were isolated after incubation at 7°C for 10 days and 142 strains after incubation at 21°C for 25 h.

According to some authors (Oliveria and Parmelee, 1976; Grifitis et al., 1980) the time needed for incubation of the psychrotrophic bacteria might be reduced by applying incubation-related temperatures of growth for most of the psychrotrophs. However, the examination conducted by Lawton and Nelson (1954) Bauman and Reinbold (1963) Piton and Rangbaux-Gida (1990) and

Kasalica and Otenhajmer (1995) have not confirmed this because the growth of psychrotrophs at temperatures ranging from 21°C – 25°C were higher than temperatures between 5°C and 10°C. This is supported by the results (Table 1) relating to the different percentage of Gram-positive and Gram-negative psychrotrophic microorganisms in raw milk after incubation at 7°C or 21°C.

Table 1. Participation of psychrotrophic microorganisms in raw milk (Gram-stained)

	Relative numbers (%)	
	7°C for 10 days	21°C for 25 days
Gram-negative rods	39.58	25.05
Gram-positive rods	38.90	25.36
Gram-positive cocci	21.52	48.59

After incubation at 7°C for 10 days the dominant microflora was composed of Gram-negative rods (39,59%) whereas after incubation at 21°C for 25 h Gram-positive cocci (48,59%) were dominant.

Such a high percent of cocci (*Streptococcus*) isolated at 21°C was in agreement with the findings of Miller (1966), who claimed that certain species such as *S. cremoris*, *S. lactis* and *S. faecalis*, were able to grow at a higher rate at temperatures (>10°C) than at lower ones.

Taken together, Gram-positive microorganisms were recorded in higher relative numbers after incubation-related temperatures. Thus, the percentage of Gram-positive rods and cocci isolated at 21°C for 25 h amounted to 73,95% and at 7°C for 10 days to 60,42%. The high proportion of Gram-positive microorganisms in the milk indicates that the production conditions on the farm were unsuitable.

Table 2. Identification of strains of psychrotrophic microorganisms isolated from raw milk

Species	Relative numbers (%)	
	7°C for 10 days	21°C for 25 days
<i>Flavobacterium</i> sp.	12.50	8.45
<i>Alcaligenes</i> sp.	9.03	6.34
<i>Pseudomonas</i> sp.	7.64	2.11
<i>E. coli</i>	10.42	9.15
<i>Lactobacillus</i> sp.	10.42	4.93
<i>Brevibacterium</i> sp.	10.50	2.85
<i>Microbacterium</i> sp.	4.17	6.34
<i>Kurtia</i> sp.	2.78	1.41
<i>Bacillus</i> sp.	9.03	9.96
<i>Streptococcus</i> sp.	6.25	21.13
<i>S. lactis</i>	4.17	7.75
<i>S. faecalis</i>	2.78	8.45
<i>Micrococcus</i> sp.	8.33	9.15
Yeast	—	2.11

Individual identification of the isolated strains (Table 2) revealed that coryneform bacteria (19,45%) were the largest group isolated at 7°C after 10 days followed by the genus *Streptococcus* (13,20%) and *Flavobacterium* (12,50%). After incubation at 21°C for 25 h genus *Streptococcus* (37,33%) was predominant followed by the coryneform group of bacteria (10,57%) and the genus *Bacillus* (9,86%).

According to other authors (Tinuoye and Harmon, 1975 and Washam et al., 1977) the thermoresistant psychrotrophic bacteria were usually Gram-positive rods and cocci pertaining to the genera *Arthrobacter*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Lactobacillus*, *Microbacterium*, *Micrococcus* and *Streptococcus*.

On the basis of the data in Table 1 we may conclude that a large percent of isolated microorganisms were thermoresistant microorganisms. Such raw milk might be a source of contamination of pasteurized milk by thermoresistant bacteria if some bacteria survive the pasteurization temperatures and thereby trigger rapid spoilage of products even when they are stored at low temperatures. This would mean that the shelflife of the product would be reduced (Credit et al., 1972; Grosskopf and Harper, 1974; 1977; Mikolajcik and Simon, 1978; Champagne and Gardner, 1990; Griffiths, 1990; Baker et al. 1992). Further sources of contamination of raw milk, include incorrectly applied preventive measures for cleaning and disinfection of the milking equipment on the farm (Wintter, 1961; Thomas and Druce, 1969; Thomas and Thomas, 1966). Thomas and Thomas (1977) suggest that an unsuitable way of washing the milking apparatuses leads to the highest contamination of milk kept in the farm's collecting tanks.

Identification of psychrotrophic bacteria strains (Table 2) indicate that the hygienic conditions on the test-farm were inadequately maintained resulting in a high contamination of the examined milk by Gram-positive psychrotrophic bacterial strains. Some Gram-positive psychrotrophs are thermoresistant proteolytic and lipolytic microorganisms, which can produce proteases and lipases, survive pasteurization and grow in milk so affecting the keeping quality of milk. Also, it is known that *Bacillus* sp. strains can produce emetic and diarrheagenic toxins and outbreaks of infection due to contamination of milk products.

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## TIPOVI PSIHROTROFNIH BAKTERIJA MLEKA U RASHLADNIM BAZENIMA FARME

ANKA KASALICA I IVANKA OTENHAJMER

### SADRŽAJ

Vršena je izolacija i identifikacija psihrotrofne mikroflore iz dvodnevno hlađenog mleka u rashladnim bazenima farme. Inkubacija zasejanih Petri ploča

rađena je standardnom metodom za porast psihrotrofnih bakterija 10 dana pri 7°C (American Public Health Ass., 1978) kao i 25 h pri 21°C (Oliveria and Parmelee, 1976). Iz 14 uzoraka mleka izolovano je 286 sojeva psihrotrofnih mikroorganizama, od kojih 144 soja inkubacijom na 7°C za 10 dana i 142 soja inkubacijom na 21°C za 25 h. Inkubacijom zasejanih ploča na 7°C za 10 dana dominantnu mikrofloru činili su Gram-negativni štapići (39,59%), a pri inkubaciji na 21°C za 25 h Gram-pozitivne koke (48,59%). Najveći procenat izolovanih sojeva na 7°C za 10 dana pripadao je grupi korineformnih bakterija (19,45%) i rodovima *Streptococcus* (13,20%) i *Flavobacterium* (12,50%), a sa 21°C za 25 h rodu *Streptococcus* (37,33%), grupi korineformnih bakterija (10,57%) i rodu *Bacillus* (9,86%).