

Bacterial Population of Raw Milk and Their Proteolytic and Lipolytic Activities

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Abstract: Raw milk samples (n=75) were collected from various local milk collection centers in different parts of Kanchipuram District, South India to enumerate and to identify bacteria with proteolytic and lipolytic activity. The total bacterial count (TBC) of 26.7% exceeded the hygienic limit in raw milk. Six bacterial isolates, *Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Staphylococcus aureus* were identified based on the phenotypic characteristics from the raw milk samples. Out of 75 raw milk samples collected, a highest number of 11 samples (14.7%) showed the presence of *S.aureus* followed by *E.coli* (9 samples, 12.0%), *P.aeruginosa* (7 samples, 9.3%), *B.cereus* (5 samples, 6.7%), *M.luteus* (5 samples, 6.7%) and *S.marcescens* (3 samples, 4.0%). Though *S.aureus* recorded highest presence in milk samples, it did not show any proteolytic activity, but showed lipolytic activity. All the isolates of *E.coli* were found to have neither proteolytic nor lipolytic activity.

Keywords: Raw milk, bacterial isolates, total bacterial count, *Staphylococcus aureus*, *Escherichia coli*, phenotypic characteristics

INTRODUCTION

Milk is one of the widely consumed nutrient foods and also it is an excellent culture medium for the growth and reproduction of microorganisms. Such balanced diet milk becomes contaminated with several types of microorganisms which originate from the soil, water or skin and the hair of the animals or utensils or from the milk handlers^[1]. Temperature plays a major role in the diversification of microorganisms and spoilage of milk during transport, processing and storage. During storage under low temperature the milk undergoes spoilage due to proteinases and lipases released by psychrotrophic bacteria^[2,3,4,5]. The psychrotrophs are able to grow at refrigeration temperatures and alter the milk by producing heat resistant proteolytic enzymes which induce degradation of casein^[6,7,8]. The spoilage of milk thus results in the production of many off-flavours which are characterized as fruity, musty, bitter, rancid and even putrid. They are most commonly encountered as members of the genera, *Acinetobacter*, *Aeromonas*, *Alkaligenes*, *Bacillus*, *Bifidobacterium*, *Escherichia*, *Flavobacterium*, *Lactobacillus*, *Proteus* and *Pseudomonas*^[9,10,11,12]. The hygienic limit for TBC in the raw milk samples collected from different localities of Kanchipuram District (South India) exceeded the permissible limit

and the correlation between total bacterial count (TBC) and total coliform count (TCC) was highly significant^[13]. But, no work has been carried out with respect to proteolytic and lipolytic bacteria in raw milk samples of the present sampling area. In view of this, the present study has been carried out to enumerate and to identify proteolytic and lipolytic bacteria in raw milk samples collected from local milk collection centers in different areas of Kanchipuram District, South India.

MATERIALS AND METHODS

A total of 75 raw milk samples were collected from various local milk collection centers in different areas of Kanchipuram District, Tamil Nadu during the year 2003. The samples were collected under aseptic conditions in sterile containers and transported in thermo-boxes with cooling inserts and processed immediately after delivery to the laboratory. Each milk sample was serially diluted individually with sterile saline from 10⁻¹ to 10⁻⁵. One ml of each sample was separately plated in to plate count agar in three replicates with control for total bacterial count (TBC) and the plates were incubated at 37°C for 24-48 hrs. Then the bacterial colonies were subjected to morphological characterization such as Gram staining and motility.

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Table 1: Phenotypic characteristics of bacteria isolated from raw milk samples.

Sl. No.	Characteristics	Bacterial isolates					
		I	II	III	IV	V	VI
1.	Motility	-	+	-	+	+	+
2.	Gram reaction	+	-	+	-	+	-
3.	Growth on nutrient agar	Golden yellow colonies	Bluish green colonies	Lemon yellow colonies	Red colonies	Luxuriant growth	Luxuriant growth
4.	Growth on MSA	Yellow colonies	-	-	-	-	-
5.	Growth on MYPA	-	-	-	-	Clear zone around colonies	-
6.	Growth on EMB	-	-	-	-	-	Metallic sheen
7.	Growth on CA	-	Bluish green colonies	-	-	-	-
8.	Catalase	+	+	+	+	+	+
9.	Oxidase	+	+	+	-	+	-
10.	Indole	-	+	-	-	-	+
11.	Methyl red	+	-	+	-	-	+
12.	Voges-Proskauer	+	-	+	+	+	-
13.	Citrate	+	+	-	+	-	-
14.	Coagulase	+	-	-	-	-	-
15.	Urease	+	-	-	-	-	-
16.	Sugar fermentation						
	(a) Glucose	+	+	-	+	+	+
	(b) Lactose	+	-	-	-	-	+
	(c) Mannitol	+	-	-	-	+	-
	(d) Sucrose	-	-	-	+	-	+

+ = positive; - = negative;

Based on the morphological characters, the bacteria were inoculated into selective media, Mannitol salt agar (MSA), Eosin Methylene Blue agar (EMB), Mannitol Egg Yolk Phenol Red agar (MYPA), Cetrimide agar (CA) and Nutrient agar for isolating different bacterial strains. Then the plates were incubated at 37°C for 24-48 hrs. The identification of bacterial isolates were confirmed by biochemical tests such as Indole, Methyl Red, Voges-Proskauer and Citrate (IMVIC), catalase, oxidase, coagulase, urease and sugar fermentation tests⁽¹⁴⁾. Since *Lactobacillus* group of bacteria are the common microflora of milk, they were left out for the above studies. Finally the bacterial isolates were subjected to proteolytic and lipolytic activities using

Skim Milk agar and Tributyrin agar respectively and were incubated at 37°C for 24-48 hrs⁽¹⁵⁾.

RESULTS AND DISCUSSIONS

The total bacterial count (TBC) in a total of 75 raw milk samples showed a range of 6.0×10^3 to 1.59×10^5 CFU/ml. The hygienic limit for TBC was exceeded in 26.7 percent of samples. It is higher than the percentage obtained in a study which reported 20.8 percent of bulk milk samples that exceeded the hygienic limit⁽¹⁶⁾. A recent study also showed that the hygienic limit for TBC exceeded in 17.9, 36.4 and 20.0% of the raw milk samples collected from three

Table 2: Presence of bacterial isolates as single growth in raw milk samples (n=75).

Sl. No.	Bacterial isolates	Presence in	
		No. of samples	Percentage
1.	<i>Staphylococcus aureus</i>	11	14.7
2.	<i>Escherichia coli</i>	9	12.0
3.	<i>Bacillus cereus</i>	5	6.7
4.	<i>Pseudomonas aeruginosa</i>	7	9.3
5.	<i>Serratia marcescens</i>	3	4.0
6.	<i>Micrococcus luteus</i>	5	6.7

Table 3: Presence of bacterial isolates as mixed growth in raw milk samples (n=75).

Sl. No.	Bacterial isolates	Presence in	
		No. of samples	Percentage
1.	<i>S. aureus</i> + <i>P. aeruginosa</i> + <i>B. cereus</i>	9	12.0
2.	<i>S. aureus</i> + <i>P. aeruginosa</i> + <i>E. coli</i>	8	10.7
3.	<i>P. aeruginosa</i> + <i>E. coli</i>	11	14.7
4.	<i>B. cereus</i> + <i>M. luteus</i> + <i>S. marcescens</i>	4	5.3
5.	<i>P. aeruginosa</i> + <i>M. luteus</i> + <i>S. aureus</i>	3	4.0

Table 4: Proteolytic and lipolytic activity of bacteria isolated from raw milk.

Sl. No.	Bacterial isolates	Proteolytic activity	Lipolytic activity
1.	<i>Pseudomonas aeruginosa</i>	+	+
2.	<i>Bacillus cereus</i>	+	+
3.	<i>Serratia marcescens</i>	+	+
4.	<i>Micrococcus luteus</i>	+	+
5.	<i>Staphylococcus aureus</i>	—	+
6.	<i>Escherichia coli</i>	—	—

(— = No activity; + = activity present)

different sets of samples respectively and the correlation between total bacterial count (TBC) and total coliform count (TCC) was highly significant⁽¹³⁾. This may be attributed to the poor maintenance of hygienic conditions during milking, utensils and containers used for transporting raw milk^[17,18].

A total of six bacterial isolates (I, II, III, IV, V and VI) were categorized based on phenotypic characteristics. Based on the biochemical tests and growth on selective media, these isolates were identified as *Bacillus cereus* (V), *Escherichia coli* (VI), *Micrococcus luteus* (III), *Pseudomonas aeruginosa* (II), *Serratia marcescens* (IV) and *Staphylococcus aureus* (I) (Table 1). The bacterial isolates in raw milk samples were observed singly and mixed growth in different degrees of their presence as indicated in Tables 2 and 3. In the bacterial isolates obtained as single growth, *S.aureus* showed a higher percentage of presence of 14.7 raw milk samples than the rest of the isolates which followed the hierarchy of *E.coli* > *P.aeruginosa* > *B.cereus* = *M.luteus* > *S.marcescens*. *P.aeruginosa* showed its presence to a higher contribution of respectively 46.7 and 50.7 percent in single and mixed growth.

The proteolytic and lipolytic activities of the isolates are shown in Table 4. *M.luteus* showed slow proteolytic activity when compared with *B.cereus*, *P.aeruginosa* and *S.marcescens*. Except *E.coli*, all the bacterial isolates exhibited lipolytic activity. This kind of proteolytic and lipolytic activity of different bacterial isolates in milk has also been reported^[5,8,11]. Putative *Pseudomonas species* were isolated from raw and processed milk and were characterized by the production of proteases, lipases and lecithinases^[19]. The bacterial isolates with proteolytic and lipolytic activity has been isolated from raw milk samples in the present study and this shows the level of contamination of milk before exposing the milk to refrigeration temperature. Proteolysis of milk proteins can be attributed to both native proteases and proteases produced by psychrotrophic bacteria during storage of fresh raw milk^[2,20]. The bacterial and enzyme action in the stored raw milk was greater than that in fresh raw milk^[3]. It is evident from these studies that there might be a possibility of alteration of milk by the activity of the bacterial isolates in the present study, if the raw milk is stored at refrigeration temperature. The findings of the present study clearly indicate the presence of

high number of contaminant bacteria in raw milk samples and their lipolytic and proteolytic activities. The high number of bacteria and their activity may result in the degradation of milk during transport and storage, hence utmost care is to be taken while handling raw milk to avoid spoilage.

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