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RESEARCH ARTICLE

STUDIES ON THE MICROBIAL FLORA IN SOME TRADITIONAL FERMENTED DAIRY PRODUCTS

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ABSTRACT

This investigation was carried out to isolate and identify the predominant microbial flora in milk product samples collected from different parts of the Tamil Nadu. A total of 30 samples were collected from Thanjavur, Trichy and Madurai, in sterile bottles and transported in a cool box at 4ºC for analysis. The samples were subjected to microbiological examination (total viable bacteria, Staphylococcus aureus, *Salmonella* sp., lactic acid bacteria and yeasts and moulds). The results showed the occurrence of coliform bacteria, *S. aureus*, lactic acid bacteria, yeasts and moulds in the samples, while *Salmonella sp*. was not detected. Coliform bacteria were not detected in samples collected from Madurai area, and detected in 30 and 20% of samples collected from Trichy and Thanjavur areas respectively. *S. aureus* was detected in 40, 60 and 20% of samples from Madurai, Trichy and Thanjavur respectively while yeasts and moulds were detected in 100, 90 and 90% of samples from Madurai, Trichy and Thanjavur areas, respectively .Lactic acid bacteria were detected in all samples under study.

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INTRODUCTION

The nature of fermented dairy products is different from one region to another depending on the local indigenous micro flora. While Leuconostoc is responsible for traditional fermentation of milk in temperate climates, Lactobacillus and Streptococcus are responsible for fermentation in tropical and subtropical climates (Kurmann, 1994). Environmental conditions in each country affect the properties of the predominant native microflora limiting the use of some universal starters, and the rational solution is the selection of starter cultures from the native flora that could be used successfully in the dairy industry. Many pathogenic microorganisms were isolated from tradition ally fermented dairy products of different parts of the world. These organisms are S. aureus, Bacillus cereus, Klebsiella, coliforms (Beukes *et al*., 2001; Lore *et al*., 2005; Uzeh *et al*., 2006). The main reason for the isolation of pathogenic organisms from traditionally fermented dairy products is the method of manufacture which involves the use of unpasteurized milk. The raw milk and other dairy products are sometimes contaminated by human pathogenic organisms Streptococcus, Staphylococcus and Clostridium botulinum etc., due to insufficient thermal processing and post-process contamination during milk pasteurization treatment. As a result toxic substances like neurotoxin A, B, E, and F are secreted causing human botulism which causes serious illness and paralysis of muscles. Hence the present study aimed to isolate and identify the predominant microflora in some traditional fermented dairy products from different areas in Tamil Nadu.

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MATERIALS AND METHODS

Collection of samples

A total of 30 dairy product samples (lazy, curd, yoghurt and butter milk etc.,) were obtained from three areas in Tamil Nadu namely Thanjavur, Trichy and Madurai. The samples were collected in sterile bottles, transported to the laboratory in a cool box at 4ºC before analysis. Samples were analyzed within 48 h of collection.

Microbial enumeration and isolation

Eleven grams of dairy product samples were transferred aseptically into a sterile bottle containing 99 ml sterile peptone water and mixed thoroughly. Serial dilutions (10^{-1} -10^{-8}) were made for each sample and 1 ml of the appropriate dilution was transferred into a sterile Petri dish and pour plated using the appropriate culture media (for yeasts and moulds, 0.1 ml was transferred into solidified medium and spread plated). Agar plate was used for the enumeration of total viable bacteria, and

the plates were incubated at 32ºC for 48 h. Mannitol salt agar medium was used for the enumeration of *S. aureus* and the plates were incubated at 37ºC for 48 h. *Salmonella* Shigella agar medium was used for detection of *Salmonella* sp. and incubated at 35ºC for 48 h. McConkey agar medium was used for the numeration of coliform bacteria and the plates were incubated at 32ºC for 24 h. Potato dextrose agar medium was used for the enumeration of yeasts and moulds and plates were incubated at 30ºC for 5 days. Biochemical tests were carried out according to Barrow and Feltham (1993).

Statistical analyses

The samples were analyzed for total viable bacteria, *S. aureus*, coliform bacteria, lactic acid bacteria and yeasts and moulds count using Statistical Analysis Systems (SPSS).Means were separated using Duncan multiple range test with $p<0.05$.

RESULTS

Total viable bacteria count

Results showed that total viable bacteria count was significantly ($p<0.05$) affected by the area from which samples were collected, with the highest count being in samples collected from Trichy area and the lowest count in samples collected from Thanjavur area (Table 1). Total viable bacteria count ranged from Log10 7.53 to Log10 8.47 cfu/ml in Trichy, Log10 7.34- 7.69 cfu/ml in Thanjavur and Log10 7.24-8.69 cfu/ml in Madurai area. All samples tested were positive (Table 2, 3 and 4).

Table 1. M icrobiological profile of fermented dairy product samples from three different areas in Tamil Nadu

Organism	Area of study			
	Trichy	Thanjavur	Madurai	SL
Total viable bacteria count	8.14±0.40a	7.56±0.40b	8.07±0.40b	*
Coliform bacteria	5.60±2.62 a	5.70±2.62a	ND	NS
Staphylococcus aureus	6.15±3.15 a	6.18±3.15 a	6.00±3.15 a	*
Salmonella sp.	ND	ND	ND	-
Lactic acid bacteria	7.80±0.57 a	7.09±0.57 b	7.51±0.57ab	*
Yeasts and moulds	5.53±1.41 a	4.64±1.41 a	5.50±1.41 a	NS

Means within each row bearing the same superscripts are not significantly different ($p>0.05$). *: $p<0.05$, NS = Not Significant, SL = Significance Level, SE = Standard error, ND = Not detected

Coliform bacteria count

Mean coliform bacteria count was not significantly affected by the area, and no coliform bacteria were detected in samples from Madurai area (Table 1). Coliform bacteria were detected in 30% of samples from Trichy and 20% of samples from Thanjavur area, and the total coliform count ranged between Log10 6.00 - Log10 6.30 cfu/ml in Trichy and Thanjavur areas (Table 2, 3 and 4).

S. aureus count

S. aureus count did not show any significant difference in the three areas under study, although the highest count was in samples from Thanjavur area (Table 1). The organism was detected in 40% of samples collected from Trichy area, 60% in Thanjavur area and 20% in Madurai area. The count ranged between Log10 6.00 - Log10 6.90 cfu/ml and Log 10 6.00 - Log10 6.60 cfu/ml in Trichy, and Thanjavur areas, respectively (Tab le 2, 3 and 4).

Salmonella sp.

The organism was not detected in all samples collected from the three areas under study.

Lactic acid bacteria count

Mean lactic acid bacteria count was significantly ($p<0$ .05) higher in Trichy area, while the lowest mean count was in Thanjavur area (Table 1). Lactic acid bacteria were detected in all samples, and the count ranged between Log10 7.04 and Log10 8.29 cfu/ml in Trichy, Log10 6.78 - Log10 7.28 cfu/ml in Thanjavur and Log10 5.70 - Log10 7.81 cfu/ml in Madurai area (Table 2,3and 4).

Yeasts and moulds count

There was non-significant variation in the count of yeasts and moulds in the areas under study; however, the highest count was in samples from Trichy area (Table 1). Yeasts and moulds were detected in 100, 90 and 90% of samples collected from Trichy, Thanjavur and Madurai areas respectively, with the range being Log10 4.00 - Log10 5.97 cfu/ml Log10 4.30 - Log10 4.90 cfu/ml and Log 10 3.70 – Log10 6.21 cfu/ml, while

Table 2. Microbiological profile (Log10 cfu/ml) of fermented dairy product samples and percent positive samples (% +ve) from Trichy area

Sample No	TVBC	*Salmonella* sp	Coliform bacteria	*S. aureus*	Yeasts and moulds	Lactic acid bacteria
1	7.53	ND	ND	ND	4.00	7.18
2	8.29	ND	6.0	6.0	5.83	8.06
3	8.19	ND	6.0	ND	5.97	7.48
4	7.64	ND	6.3	ND	5.20	7.04
5	7.89	ND	ND	ND	5.08	7.66
6	7.76	ND	ND	6.60	4.70	7.26
7	8.24	ND	ND	6.00	5.65	7.92
8	8.34	ND	ND	6.90	5.90	7.98
9	8.47	ND	ND	ND	5.15	8.29
10	8.19	ND	ND	ND	4.85	7.40
Mean ± SE	8.14±0.40	ND	5.60±2.62	6.15±3.15	5.53±1.41	7.80±0.57
% + ve	100	0	30	40	100	100

TVBC = Total viable bacteria count, ND = Not detected,
% +ve = Percent positive samples

Table 3. Microbiological profile (Log10 cfu/ml) of Fermented dairy product samples and percent positive samples (% +ve) from Thanjavur area

Sample No	TVBC	*Salmonella* sp	Coliform bacteria	*S. aureus*	Yeasts and moulds	Lactic acid bacteria
1	7.37	ND	ND	ND	4.60	6.78
2	7.43	ND	ND	ND	4.78	6.90
3	7.46	ND	ND	6.30	4.30	7.08
4	7.54	ND	ND	ND	4.78	6.95
5	7.52	ND	ND	6.30	4.60	7.00
6	7.54	ND	ND	6.00	4.30	7.20
7	7.69	ND	ND	6.30	4.90	7.28
8	7.65	ND	ND	ND	4.90	7.15
9	7.72	ND	6.00	6.60	ND	7.26
10	7.53	ND	6.30	6.60	4.60	7.08
Mean± SE	7.56±0.40	ND	5.70±2.62	6.18±3.15	4.64±1.41	7.09±0.57
% +ve	100	0	20	60	90	100

TVBC = Total viable bacteria count, ND = Not detected,
% +ve = Percent positive samples

Table 4. Microbiological profile (Log10 cfu/ml) of Fermented dairy product samples and percent positive samples (% +ve) from Madurai area

Sample No	TVBC	*Salmonella* sp	Coliform bacteria	*S. aureus*	Yeasts and moulds	Lactic acid bacteria
1	7.37	ND	ND	ND	4.00	6.60
2	7.46	ND	ND	ND	3.70	5.70
3	7.87	ND	ND	ND	5.40	7.40
4	7.37	ND	ND	ND	4.60	6.60
5	7.33	ND	ND	ND	ND	6.65
6	8.48	ND	ND	6.00	5.81	7.81
7	7.24	ND	ND	ND	4.30	6.30
8	8.00	ND	ND	ND	5.51	7.51
9	8.06	ND	ND	ND	5.40	7.40
10	8.69	ND	ND	6.00	6.21	8.21
Mean±SE	8.07±0.40	ND	ND	6.00±0.00	5.50±1.41	7.51±0.57
% +ve	100	0	0	20	90	100

TVBC = Total viable bacteria count, ND = Not detected,
% +ve = Percent positive samples

yeasts and moulds were not detected in one sample from each of Thanjavur and Trichy (Table 2, 3 and 4).

DISCUSSION

Due to absence of heat treatment of milk prior to fermentation in addition to utilizing natural fermentation, it is expected that total bacterial count is high in all areas sampled. The results of microbiological examination indicate that this product is highly contaminated with microorganisms of public health concern. The high number of total bacterial count, *S. aureus* and coliform bacteria indicates unhygienic conditions during production of milk and further processing into milk products without heat treatment (Hussain, 2010; Uzeh *et al*., 2006). Similar results of total bacteria count were reported for different traditional dairy products (Beukes *et al*., 2001; Mathara *et al*., 2004; Savadogo *et al*., 2004; Lore *et al*., 2005; Al-Tahiri, 2005; Hassan *et al*., 2008). The detection of coliform bacteria and *S. aureus* in high number is a public health concern since it indicates faecal contamination during production or processing of this product. These organisms were isolated by different researchers in other fermented dairy products (Savadogo *et al*., 2004; Al Tahiri, 2005, Lore *et al*., 2005; Uzeh *et al*., 2006). The results of lactic acid bacteria count show that fermentation is mainly carried out by lactic acid bacteria in uncontrolled conditions of fermentation. Similar results were reported by Abdelgadir *et al*., (2001), Beukes *et al*., (2001), Mathara *et al*., (2004), Savadogo *et al*., (2004), El-Baradei *et al*., (2008), Hassan *et al*., (2008) and Jokovic *et al*., (2008). *Salmonella* sp. was not detected in all samples tested. Dairy product samples were highly contaminated with yeasts and moulds. This might be possibly due to poor processing conditions and/or uncontrolled fermentation which lead to contamination with yeasts and moulds, and this is obvious by alcoholic fermentation resulting in alcohol production in additionto lactic acid. Abdelgadir *et al*., (2001), Ali *et al*., (2002), Mathara *et al*., (2004), Savadogo *et al*., (2004), Al-Tahiri (2005), Lore *et al*., (2005) and Uzeh *et al*., (2006) detected yeasts and moulds in different traditional fermented dairy products.

Conclusion

In conclusion, the high number of coliform bacteria and *S. aureus* indicate the unhygienic conditions during processing and subsequent handling of the product, while high number of lactic acid bacteria indicate that the natural microflora of milk are responsible for producing lactic acid, in addition to yeasts which might produce alcohol during fermentation converting the product into slightly alcoholic.

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