

INCIDENCE OF *PSEUDOMONAS* SPECIES IN PASTEURIZED MILK*

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ABSTRACT

Pasteurized milk samples were collected from different sources aseptically and analyzed for total bacterial count and psychrotrophic count. The predominant psychrotrophic bacteria were Pseudomonas fluorescens (42.42%), Pseudomonas fragi (24.24 %) and Pseudomonas aeruginosa. (12.12 %). Pseudomonas species were analyzed for protease and lipase production. 64.28 and 42.85 % of the P. fluorescens, 62.50 and 50 % of the P. . fragi were positive for protease and lipase production respectively. Presence of Pseudomonas species in pasteurized milk indicates post-processing contamination. It can be concluded that there is a high incidence of spoilage flora in pasteurized milk. Hence, hygienic practices should be followed to reduce the Pseudomonas load and milk should be processed as early as possible to reduce the extent of spoilage.

Key words: Pasteurized milk – *Pseudomonas* species - proteolytic and lipolytic activity.

INTRODUCTION

The predominant microorganisms limiting the shelf life of processed fluid milk at 4°C are *Pseudomonas* spp. these species are able to grow to high numbers during refrigerated storage, and also produce heat-stable extracellular lipases, proteases, and lecithinases which can further contribute to milk spoilage. Many of these enzymes remain active, even following thermal processing. Degradation of milk components through various enzymatic activities can reduce the shelf life of processed milk. Not all *Pseudomonas* strains are equally capable of producing degradative defects in processed milk. Objectives in this study were as follows: (i) To assess total bacterial count and Psychrotrophic count of pasteurized milk (ii) To determine the incidence of *Pseudomonas* species in pasteurized milk and III). To determine the spoilage potential of *Pseudomonas* flora in pasteurized milk.

MATERIALS AND METHODS

Pasteurized milk samples from 9 different brands sold in the market were aseptically collected weekly over a period of 8 weeks and 8 laboratory pasteurized milk samples were used in this study. These samples were placed in ice in the thermos jar under aseptic precautions and were transported to the laboratory and analyzed for total bacterial count and psychrotrophic counts (Marshall, 1993).

Colonies developed on the plates were picked randomly and streaked for purification. Sixty two isolates were characterized according to Gram staining results, oxidase and catalase activities. The colonies that were confirmed as putative *Pseudomonas* spp. were further characterized by biochemical tests (Buchanan and Gibbons, 1974).

To determine the proteolytic and lipolytic activities, *Pseudomonas* colonies were streaked on

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plate count agar and then single colonies were transferred to agar plates containing the appropriate substrates. The proteolytic activity was tested by inoculating the strains on 50 per cent skim milk agar. Then plates were incubated at 5°C for 10 days. The presence of clear zone of hydrolysis around the colonies confirmed as positive (APHA, 1978). The lipolytic activity was tested by culturing the strains on tributyrin agar. The plates were incubated at 25°C for 3 days and screened for the presence of clear zone of hydrolysis (Harrigan and Mc Cance, 1976). The data were analyzed statistically as per Snedecor and Cochran 1989.

RESULTS AND DISCUSSION

The analyzed data on the mean total bacterial count of pasteurized milk and psychrotrophic count are presented in Table 1. The laboratory pasteurized milk samples had the lowest mean total bacterial count (\log_{10} cfu/ml) of 0.99 ± 0.17 . Among the different brands studied high total bacterial count of $4.44 \pm 0.08 \log_{10}$ cfu/ml was observed in the brand 2. The laboratory pasteurized milk samples had the low mean total bacterial count (\log_{10} cfu/ml) of 0.99 ± 0.17 . These results concur with the total bacterial count reported by Gopi *et al.* (2001) and Valbuena *et al.* (2004).

The laboratory pasteurized milk had the lowest psychrotrophic count of 0.41 ± 0.11 . Similar to total bacterial count the highest psychrotrophic count was observed in brand 2 compared to other brands. There was a significant ($P < 0.01$) difference in psychrotrophic count between different brands of pasteurized milk samples and the laboratory pasteurized milk. The high total bacterial count of different brands of pasteurized milk samples may be attributed to post-pasteurization contamination, temperature fluctuation during storage and transport and presence of thermotolerant microbial flora. The lowest count in the laboratory pasteurized milk may be attributed to the hygienic practices adopted during heat treatment.

Among the total bacterial population the percentage of psychrotrophs in pasteurized milk ranged from 14.19 to 32.92 per cent. Kuzin *et al.* (1992) reported similar results for pasteurized milk. This may be due to the low temperature of storage of pasteurized milk, which might have supported the growth of psychrotrophs as reported by Holm *et al.* (2004).

Out of 33 isolates obtained from pasteurized milk, 14, 8, 4, 2, and 5 isolates were identified as *P. fluorescens*, *P. fragi*, *P. aeruginosa*, *Flavobacterium aquatile* and *Bacillus cereus* representing 42.42, 24.24, 12.12, 6.06 and 15.15 per cent, respectively (Table 2). This result agrees with the findings of Saleha (1992) and Valbuena *et al.* (2004).

The *Pseudomonas* species accounted for 78.78 per cent of the psychrotrophic isolates identified. This finding agrees with the findings of Saleha (1992) and Dogan and Boor (2003).

About 64.28 per cent of the *P. fluorescens* isolates from pasteurized milk were found to be proteolytic in nature. Similarly, 62.50 per cent of *P. fragi* isolates from pasteurized milk were found to have proteolytic activities (Table 3)

Nearly, 42.85 per cent and 50 per cent of the *P. fluorescens* and *P. fragi* isolates respectively from pasteurized milk exhibited lipolytic activities

Dogan and Boor (2003) and Alatosava and Alatosava (2005) reported that more than 80 per cent of *Pseudomonas* to have proteolytic potential.

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Table 1

Mean (± SE) total bacterial and psychrotrophic counts of pasteurized milk

Source of milk	Total bacterial count	Psychrotrophic Count	Psychrotrophs (%)
Brand 1	4.21 ^{bc} ± 0.04	3.35 ^b ± 0.05	14.19
Brand 2	4.44 ^c ± 0.08	3.91 ^d ± 0.03	32.02
Brand 3	4.29 ^{bc} ± 0.02	3.58 ^{b c} ± 0.11	23.26
Brand 4	4.35 ^{bc} ± 0.04	3.65 ^{c d} ± 0.11	24.62
Brand 5	4.37 ^{bc} ± 0.04	3.80 ^{cd} ± 0.08	29.09
Brand 6	4.34 ^{bc} ± 0.04	3.71 ^{cd} ± 0.05	25.21
Brand 7	4.15 ^b ± 0.09	3.62 ^{b cd} ± 0.13	30.30
Brand 8	4.22 ^{bc} ± 0.04	3.73 ^{c d} ± 0.09	32.92
Brand 9	4.32 ^{bc} ± 0.07	3.65 ^{c d} ± 0.07	24.51
Laboratory Pasteurized milk	0.99 ^a ± 0.17	0.41 ^a ± 0.11	26.30

Means (n=8) bearing different superscripts in a column differ significantly (P<0.01)

Table 2
Incidence of psychrotrophs in pasteurized milk

S.No	Name of the bacteria	No. of isolates	Percentage	No. of isolates	
				Laboratory pasteurized milk	Brands
1.	<i>Pseudomonas fluorescens</i>	14	42.42	2	12
2.	<i>Pseudomonas fragi</i>	8	24.24	2	6
3.	<i>Pseudomonas aeruginosa</i>	4	12.12	1	3
4.	<i>Flavobacterium aquatile</i>	2	6.06	0	2
5.	<i>Bacillus cereus</i>	5	15.15	1	4
	Total	33	100.00	6	27

Table 3
Proteolytic and lipolytic activity of *Pseudomonas* species from pasteurized milk

Table 3
Proteolytic and lipolytic activity of *Pseudomonas* species from pasteurized milk

S.No.	Species	Proteolytic activity			Lipolytic activity		
		No. of isolates screened	No. of isolates positive	Positive (%)	No. of isolates screened	No. isolates positive	Positive (%)
1.	<i>P.fluorescens</i>	14	9	64.28	14	6	42.85
2.	<i>P.fragi</i>	8	5	62.5	8	4	50.00