

# CHARACTERIZATION OF *BACILLUS* SPECIES ISOLATED FROM PASTEURIZED MILK\*

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## ABSTRACT

Sixty pasteurized milk samples were collected aseptically from different processing dairies and analyzed for total viable count and aerobic spore count. *Bacillus subtilis* was found to be the predominant aerobic spore former (33.33%) followed by *B. megaterium* (24.44%), *B. licheniformis* (17.77%), *B. cereus* (8.88%), *B. coagulans* (6.66%), *B. pumilus* (4.44%), *B. sphaericus* (2.22%), and *B. circulans* (2.22%). The total viable count ( $\log_{10}$  cfu per ml) varied between  $4.18 \pm 0.095$  and  $4.43 \pm 0.081$  among nine different processing dairies and it was the lowest in lab-pasteurized samples ( $3.65 \pm 0.078$ ). The mean aerobic spore count ( $\log_{10}$  cfu per ml) varied between  $3.56 \pm 0.051$  and  $3.87 \pm 0.112$  among the processing dairies and the lowest values were obtained in lab pasteurized milk samples at  $3.09 \pm 0.085$ . Out of forty five *Bacillus* species isolated, twenty five isolates were positive for proteolysis and seventeen for lipolysis and twelve isolates for both proteolysis and lipolysis.

**Key words :** Pasteurized milk, *Bacillus* species, Proteolytic and Lipolytic activity

## INTRODUCTION

*Bacillus* species play a very important role in the keeping quality of milk and dairy products. These organisms survive heat treatment and high temperature used for processing of the products, activates spore germination and out growth, resulting in spoilage of products. *Bacillus cereus* is important as it affects the shelf life of pasteurized milk and heat treated dairy products. The organism is associated with defects such as off flavours, sweet curdling and bitty cream caused by proteinase, lipase and phospholipase enzymes (Meer *et al.*, 1991). In addition to causing these defects in dairy products, *B. cereus* has also been associated with out breaks of food poisoning (Johnson, 1984; Kraemer and Gilbert, 1989).

The objectives of this research work include studies on the incidence of various *Bacillus* species in pasteurized milk, their biochemical characterization and proteolytic and lipolytic activity.

## MATERIALS AND METHODS

Nine different processing dairies were selected randomly and 6 pasteurized milk samples from each dairy were collected in an aseptic manner at weekly intervals for 6 weeks and brought to the laboratory under refrigerated condition for further analysis. Six lab pasteurized milk samples were also used in the study.

The total viable count and aerobic spore count were carried out as per the standard methods

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for examination of dairy products APHA, (1978).

Representative colonies that had developed on the plates after incubation were isolated and inoculated in to the nutrient broth. The purification of the culture was done by placing and sub culturing in nutrient broth. The cultures were examined under the microscope for purity. The presence of spores was confirmed by spore staining technique (Cowan and Steel, 1974). The isolates were identified by morphological, cultural and biochemical tests following the methods described by Buchanan and Gibbons (1974) and they were assigned serial numbers.

The proteolytic activity was tested by culturing the strains on 50% skim milk agar and incubating the plates at 37°C for 24 – 48 h. Presence of clear zone of hydrolysis around the colonies was taken as positive for proteolysis (APHA, 1978).

The lipolytic activity was tested by culturing the strains in tributyrin agar and incubating the plates at 37°C for 3 days and screening the plates for the presence of clear zone of hydrolysis as described by Harrigan and McCance (1976).

The data thus obtained were analyzed statistically as per Snedecor and Cochran (1989).

## RESULTS AND DISCUSSION

The mean total viable count and aerobic spore count are presented in Table 1. The total viable count of pasteurized milk samples ( $\log_{10}$  cfu per ml) varied between  $4.18 \pm 0.095$  and  $4.43 \pm 0.081$  among nine different processing dairies and it was the lowest in lab-pasteurized samples ( $3.65 \pm 0.078$ ). There are highly significant differences ( $P < 0.01$ ) in total viable counts between different processing dairies.

The values obtained in this study are slightly higher than the report of Giffel *et al.* (1996) who found 50 to 5,000 cfu per ml in 334 pasteurized milk samples. However, Kikuchi *et al.* (1996)

obtained a standard plate count of 100 to 1000 cfu per ml ( $2$  to  $3 \log_{10}$  cfu per ml) in pasteurized milk samples and the thermophilic bacterial count ranged between 10 and 100 cfu per ml ( $1$  to  $2 \log_{10}$  cfu per ml) which was lower than that obtained in the present study.

The mean aerobic spore count of pasteurized milk samples ( $\log_{10}$  cfu per ml) varied between  $3.56 \pm 0.051$  and  $3.87 \pm 0.112$  among the processing dairies. It was the lowest in lab pasteurized samples at  $3.09 \pm 0.085$ . There are highly significant differences ( $P < 0.01$ ) between different processing dairies with regard to the mean aerobic spore count.

Larsen and Jorgensen (1996) analyzed 458 samples of pasteurized milk and cream from 3 Danish dairies and found the total viable count of *Bacillus cereus* ranging from  $10^3$  to  $3 \times 10^5$  cfu / ml ( $3 \log_{10}$  cfu per ml to  $5.47 \log_{10}$  cfu per ml). These values were within the range obtained in the present study. The values obtained by the Shah *et al.* (1996) were lower when compared to the aerobic spore count found in this study.

The number of *Bacillus* isolates positive for proteolysis and lipolysis is given in Table 2. Out of a total 45 isolates, *Bacillus subtilis* contributed to the highest incidence (33.33%) and the least incidence was noted in *Bacillus sphaericus* and *Bacillus circulans* each contributing one isolate (2.22%).

Meer *et al.* (1993) concluded that 57% of the *Bacillus* isolates obtained from 59 Grade A milk samples in Oregon, US were lipolytic. Almeida *et al.* (2000) detected that 92% of the *Bacillus* isolates were found to have proteolytic and or lipolytic activity. In this study, on an average 55.55% and 37.77% of total *Bacillus* species isolated are found to be proteolytic and lipolytic respectively.

Matta and Punj (1999) obtained 59 lipolytic *Bacillus* isolates and showed that *Bacillus cereus*

(32.2%) was the predominant lipolytic organism whereas the present study concluded that *Bacillus pumilus* is the predominant lipolytic organism.

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