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# Study the incidence of *Bacillus cereus* isolates from dairy foods

**Vinay Kumar Singh, Sangeeta Shukla and Anil Chaturvedi**

**Abstract**
Among 200 selected dairy samples 50 each of pasteurized milk, cream, butter and paneer were analyzed for the presence of *Bacillus cereus* the paneer samples were found to have the highest incidence of *Bacillus cereus* (52.94%) followed by cream sample (29.41%) and pasteurized milk sample (17.64%) however none of the butter samples were found to be contaminated with *Bacillus cereus*. These isolates were found to be gram positive bacilli with spores, they were found to ferment sucrose, glucose, dextrose; they gave the positive result for $NO_3$ reduction, oxidize, catalase, and for starch hydrolysis.

**Keywords:** *Bacillus cereus*, Pasteurized milk, Butter, Cream.

## 1. Introduction
The genus *Bacillus* is comprised of a diverse array of Gram-positive, aerobic and facultative anaerobic and facultatively anaerobic endospore forming rods. *Bacillus* species are found in a wide range of habitats, and include species possessing environmental, industrial and clinical significance. Many species are common soil inhabitants and may frequently contaminate foods, including dairy products, meats, infant food, rice dishes, vegetables, spices and cereals (Christiansson *et al*., 1999) [5]. Members of the *Bacillus* genus are ubiquitous soil microorganisms and are generally considered harmless contaminants. The vegetative cells range from 0.5 by 1.2 to 2.5 by 10 µm in diameter and can grow at optimal temperatures ranging from 25 to 37 °C, although Thermophile and psychrophilic members are capable of growth at temperatures as high as 75 °C or as low as 3 °C. Some species can flourish at extremes of acidity and alkalinity, ranging from pH 2 to 10. *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 outgrowth, 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) [15]. In addition to these defects in dairy products, *B. cereus* has also been associated with outbreaks of food poisoning (Johnson1984; [12] Kramer and Gilbert, 1989) [13].
*Bacillus cereus* is a widely distributed bacteria, have been isolated from rice, spices, meat, and egg and dairy products. The presence of *Bacillus cereus* spores in milk is unavoidable. This pathogen produces both a toxin and spore capable of surviving pasteurization. The growth of *B. cereus* significantly spoils the quality of dairy products, causing sweet curdling and "bitty" cream.
*Bacillus cereus* causes problems in the food industry both by deteriorating the products (TeGiffel *et al.,* 1996; [20] Pirijarvi *et al.,* 2000; [16] Eneroth *et al*., 2001), [8] and by endangering people's health upon consuming contaminated foods (Granum *et al*., 1993; [10] Ghelardi *et al.,* 2002) [9]. Under certain conditions, strains of this species produce haemolysins, phospholipases C, also emetic toxins and enterotoxins that cause food poisoning (Rusul and Yaacob, 1995; [17] Andersen-Borge *et al*., 2001; [2] Agata *et al*., 2002) [1]. It causes two different types of food poisoning: the diarrhoeal type and the emetic type. The diarrhoeal type of food poisoning is caused by different enterotoxin complexes produced during the growth of *Bacillus cereus* in the small intestine while the emetic toxin is produced by the growing cells in food. The organism has ability to adapt to different chemical (Willinghan *et al*., 1996; [24] Ultee *et al*., 2002) [22]. Heat (Browne and Dowds, 2001) [3] and cold environment (TeGiffel *et al*., 1997), [21]

as well as by toxin production (Andersen-Borge *et al*., 2001) [2]. The ability of spores of *B. cereus* to resist against the high pasteurization temperature, which very frequently contaminates the dairy products. The food poisoning caused by *B. cereus* is categorized by a number of different syndromes (emetic and diarrheal) with a variable intensity (Christian-Son *et al*., 1989) [4]. *B. cereus* produces a number of toxic products that are important virulence factors and participate in the course of foodborne human gastrointestinal diseases.

## 2. Materials and Methods

### 2.1 Place of Work

The present study entitled "**Study the incidence of *Bacillus cereus* isolates from dairy foods"** was conducted in the lab of Dairy Microbiology, Warner school of Food & Dairy Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences Deemed- to – be University, Allahabad.

### 2.2 Collection of Sample

Two hundred samples of dairy products were collected which include 50 samples each of cream, butter, paneer and pasteurized milk from the local market of Allahabad and Student's Dairy in Sam Higginbottom Institute of Agriculture, Technology and Sciences Deemed- to – be University, Allahabad.

### 2.3. Isolation of *Bacillus cereus*

Isolation of *Bacillus cereus* was performed by streaking the samples on nutrient agar. These plates are incubated at 37°C for 24-48 hrs.

### 2.4 Identification of The Isolates

The isolates observed from the nutrient agar were identified on the basis of cultural, morphological and biochemical characteristics given in Bergey's Manual of Systematic Bacteriology ***(Holt et al.,* 1984)** [11].

## 3. Results and Discussion

### 3.1 Incidence of *Bacillus cereus*

Among the 200 dairy samples which include 50 samples each of Paneer, Pasteurized milk, Cream and Butter sample, screened for growth of *Bacillus cereus* on nutrient agar plate, 17 (8.5%) *Bacillus cereus* was isolated. The incidence of *Bacillus cereus* was 17 (8.5%) in which 9 (52.94%) were isolated from Paneer sample, 3 (17.64%) were isolated from Pasteurized milk sample, 4 (29.41%) were isolated from Cream sample, and no *Bacillus cereus* isolated from Butter sample. However a lower incidence of *B. cereus* was recorded in the study of Wong *et al*., (1987) [25] and *Schlegelove et al*., (2003) [18]. In comparison to the incidence of *B. cereus* recorded in the selected dairy products in present study a higher incidence was reported in the study of *Schlegelove et al*. (2003) [18] in spreading butter (65.07%). In the present study a higher incidence of *B. cereus* was recorded which was in agreement with the studies of De Santis *et al*., (2008) [7]. However Vaishnavi *et al*., (2001) [23] reported contrasting results.The higher incidence of *B. cereus* in pasteurized milk could be attributed to the contamination of teats with soil (Christiansson *et al*., 1999), [5] or the entry of *B. cereus* spores in raw milk through air after pasteurization process (Lin *et al*., 1998) [14]. Similarly the higher level of contamination with *B. cereus* in cream and paneer sample tested in the present study could be occurred due to poor processing condition (TeGiffel and Beumer, 1998). [19] However, none of the butter samples tested showed the presence of *B. cereus*. Since the high salt- in moisture content is found in butter, it would have had the cidal effect on the cells (Champagne *et al*., 1994), [6] or it may be due to over pasteurization of the samples also during the homogenization of spreading butter, and 65-70ºC, the contaminating micro flora is eliminated (*Schlegelove et al*., 2003) [18].

**Table1:** Incidence of *Bacillus cereus* from selected dairy samples

| Total samples | No. of isolates | Incidence of bacillus cereus (%) | | | |
|---|---|---|---|---|---|
| | | Pasteurized milk\ | Cream | Butter | Paneer |
| 200 | 17 (8.5%) | 3(17.64%) | 5(29.41%) | 0 (0) | 9(52.94%) |

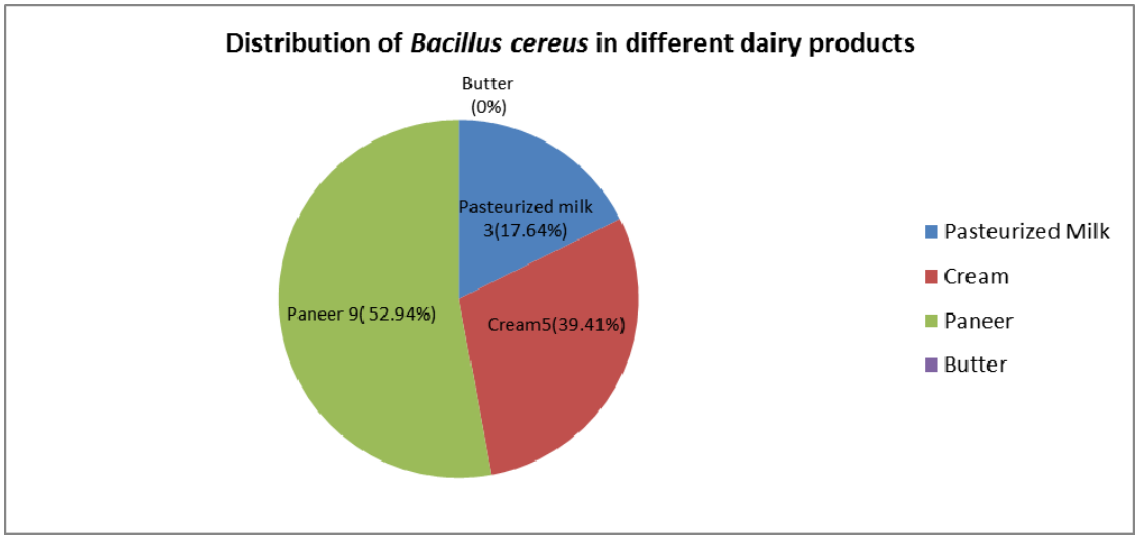


**Fig 1:** Distribution of *Bacillus cereus* in different dairy products

### 3.2. Conclusion

In conclusion the fairly higher incidence of Bacillus cereus recorded in the different dairy products is quite alarming as these organisms are causative agents of food poisoning and spoilage. It is a major threat in food industry both by deteriorating the products and by endangering people's life upon consumption. Additionally, the products contaminated by these organisms, may be, vectors of resistance to antibiotics. Since it is of vital concern, it is highly recommended that proper control measures during various stages of production and processing should take to control the entry of this ubiquitous organism in the dairy industry. It is further emphasized that implementation of HACCP in the different dairy industry is the best way to assure the lowest possible risk of Bacillus cereus.

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