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# Prevalence of *Bacillus cereus* in Milk and Milk Products

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***Abstract:*** **A total of 30 different commercially manufactured Milk and Milk Products (MMP) from different manufacturers were analyzed for the presence of *B. cereus*. Out of the tested samples, 26 number of Dried/powdered milk products (65.4%) showed the presence of *B. cereus* and zero percent prevalence in the liquid samples. The study was undergone as per Food Safety and Standard Authority of India (FSSAI) requirements under safety criteria. Analyzed final products of different milk and milk products were found to be in the range as per Food Safety and Standard Regulation (FSSR), 2011 Annexure B and thus were reported as consumer safe.**

***Keywords: B. cereus*, Milk and Milk Products, Prevalence.**

## I. INTRODUCTION

*Bacillus* is the largest genus in the Bacillaceae family, with at least 226 species, the vast majority of which are saprophytes that are widely distributed in the environment and commonly isolated from soil, air, water, plants, and animals. *Bacillus cereus sensu lato* (or *B. cereus* group) consists of eight formally recognised species*: B. anthracis, B. pseudomycoides, B. mycoides, B. thuringiensis, B. weihenstephanensis, B. cytotoxicus, B. toyonensis, and B. cereus sensu stricto* (Dufrenne *et al.*, 1995). *B. cereus* represents spore-forming Gram-positive bacteria that are optionally motile and facultative anaerobic saprophytes (Coorevits *et al.*, 2008). It is also a well-known food poisoning organism capable of producing one or more enterotoxins. Some are food pathogens that can cause both diarrheal and emetic gastrointestinal syndromes in humans (Pluta *et al.*, 2015).

Milk and milk products (MMP) have been a major part of human food and have played an important role in the diet since ancient times (Pal *et al.*, 2016). Milk based product preserves the nutritional value of milk and makes it more appealing to consumers (Das *et al.*, 2015. Most of the essential components are found in milk and its products, making it the most significant source of nutrition for humans (Pfeuffer and Watzl, 2017). India is the world's largest producer of milk. India ranks first in milk production, accounting for 23% of global milk production. Dairy cooperative milk procurement increased by 7.9 percent between 2020 and 2021.The increase in milk procurement was not proportional to the increase in milk sales by dairy cooperatives (Dairy Food Processing Sector Report, 2021). The increased use of dairy products has a wide range of consequences for food and nutrition security. Millions of smallholder farmers, who produce roughly 70% of the nation's milk, rely on the milk sector for a living (Kumar *et al.*, 2011). The rising demand for milk and milk products provides opportunities for smallholder farmers to increase their income by improving milk production efficiency. Smallholders can increase their income by improving milk production efficiency as demand for milk and milk products rises. Increased milk production comes with the risk of cross contamination, leading transfer of harmful organisms to food that can occur from hands to food, equipment to food, raw food to processed.

A variety of agents predominantly bacteria are considered major causes of milk borne illnesses worldwide. Milk is understandably an important constituent of human diet and raw milk is an ideal growth medium for several microorganisms. Milk and milk products are considered vehicles for *Bacillus cereus* infection in humans (Velusamy *et al.*, 2010). Since 1916, *B. cereus* has been reported in milk, it is a common contaminant of raw milk produced in some dairy farms. Furthermore, the bacterium has been found in high concentrations in dairy products. Contamination of milk powder with

*B. cereus* is also a major food safety concern. Milk powder can be stored for long period of time due to their low water activity, due to this; bacterial spores can remain dormant until more favourable conditions are present, at which point spore germination and outgrowth can occur (Setlow *et al*., 2016).

In India, the Food Safety and Standards Authority of India (FSSAI) developed and implemented various food safety standards, as well as tested and ensured the quality and hygiene of various food products. The Bureau of Indian Standards (BIS) regulates various standards, including lab testing and lab practices for chemical and microbial analysis of food and dairy products. The BIS develops standards in accordance with the International Organization for Standardization (ISO) to match global dairy product testing standards.

## II. MATERIALS AND METHOD

***Sample Analysis***: For the present study, 30 different Milk & Milk Products (MMP) samples were analyzed. Samples were received from various food industries/manufacturers, between the periods of August 2022 to November 2022 across India. Out of 30 samples, 24 number of Skimmed Milk Powder (SMP), 4 number of Milk, and 2 number of Infant Food (IF) samples were analysed in accordance with Indian Standards at the Centre for Analysis and learning in Livestock & Food (CALF) Laboratory, NDDB, Anand (Gujarat).

***Enumeration of B. cereus:*** For the analysis of solid samples, 10 g of sample was weighed and homogenized and transferred to 90 ml of diluent i.e., 0.1% peptone salt solution (PSS) (HiMedia, Mumbai). For liquid samples, 1 ml of sample was transferred to 9 ml diluent. Tenfold dilutions were prepared by serial dilution, 1 ml of the required dilution was spread plated on Mannitol Egg yolk Polymyxin B (MYP) agar (HiMedia, Mumbai) in duplicate plate and incubated at 30ºC for 48h (IS 5887-Part 6).

***Confirmation of B. cereus:*** For confirmation, 5 presumptive positive typical colonies from MYP agar plates were selected and spotted on commercially available blood agar plates (HiMedia, Mumbai) and incubated at 30ºC for 24h.

## III. RESULT

***Enumeration of B. cereus:*** Large, pink colonies surrounded by the zone of precipitation were considered as typical presumptive positive colonies (Fig. I A).

***Confirmation of B. cereus***: The presumptive positive colonies were counted, and spotted on sheep blood agar for showing of β- haemolysis (β- hemolysis is the complete lysis of RBCs, resulting in a distinct, clear, colorless zone surrounding the colony) (Fig. I B). Calculation was done by using the formula.

**Formula for Calculation:**

$$\mathbf{N} = \frac{\sum \boldsymbol{C}}{\boldsymbol{V} \times \{\boldsymbol{n_1} + (\mathbf{0.1} \times \boldsymbol{n_2})\} \times \boldsymbol{d}} \times \frac{\boldsymbol{x}}{\mathbf{5}}$$

Where:

**N** = Total viable colony counts;

**∑C** = Sum of colonies counted from all plates;

**V**= Inoculum volume;

**$n_1$** = Number of plates counted at first dilution;

**$n_2$** = Number of plates at second dilution;

**C** = Number of colonies counted;

**d** = Dilution factor from which the first counts obtained (least counted dilution);

**x** =Total no. of colonies, showing β– haemolysis.

Results are represented in colony forming unit (CFU $g^{-1}$ or $ml^{-1}$) Table I.

**Prevalence of *Bacillus cereus***

***Statistical Data Analysis:*** CFU was converted into $Log_{10}$ value using Microsoft Office Excel 2007 enterprise and data were analyzed statistically by using descriptive statistics

In the present study, out of 26 powdered milk products (SMP & Infant Food) tested, *B. cereus* was enumerated in 17 samples (65.3%) and none of the milk samples tested showed any prevalence (0%). *B. cereus* counted in milk products ranged from 1.0 to approximately 3.0 log CFU $g^{-1}$. Among analyzed MMP, *B. cereus* has been most frequently found in SMP and infant food (dried or powdered products) than milk products (Liquid sample).

## IV. DISCUSSION

In the present study prevalence of *B. cereus* in milk and milk products demonstrates a significant difference depending on the products, i.e., skimmed milk powder & infant food has a 65.3% of prevalence rate and milk has no prevalence rate at a level of 1-3 log CFU $g^{-1}$. The prevalence rate of *B. cereus* was mainly found in dried milk powder. In contrast to this, in Poland, 30% of the pasteurized milk samples were found to be contaminated by *B. cereus* at a level of 1–2 log CFU/mL (Bartoszewicz *et al.*, 2008). Another study in milk powder, it showed 52% prevalence at a level of 2–3 log CFU/g. In Chile, Reyes *et al.*, (2007) found 10% of milk powder samples were contaminated with *B. cereus* at a level of up to 3 log CFU/g. In a similar study, conducted with the samples from retail outlets in Mumbai, India, *B. cereus* was prevalent in 40% of the unpackaged samples and 27% of the packaged samples, at a level of 1–3 log CFU/mL (Warke *et al.*, 2000). 16% of Jammu and Kashmir market milk samples were reported contaminated with *B. cereus* at a level 2–3 log CFU/mL *(*Altaf *et al.*, 2011*)*. Jayachandra *et al*. (2010) were reported distribution of *B. cereus* as 12.3% in milk samples collected from Srinagar street vendors, dairy plants, organized dairies and villages at a level of 1–2 log CFU/mL, while as Ahmed *et al.* (2000) reported a prevalence of 21% in raw milk and present study have 0% prevalence in milk. Among others Abdel khaliq *et al*. (1996) found 40% and 30% of raw and pasteurized milk samples at a level of 4 log CFU/mL, respectively contaminated with *B. cereus*, while as Sharma *et al.*, *(2003*) reported 66% contamination of milk with *B. cereus* at a level of 4–5 log CFU/mL. The high incidence of *B. cereus* in milk powder samples is likely due to monospecies biofilm formation on the milk evaporators, which can be a source of recurrent contamination of the final product, as stated by Burgess *et al.*, (2009). The difference in the percentage of contaminated samples and level of contamination in different studies may be attributed to the degree of post-pasteurization contamination, storage, and seasonal variation in sampling. Because milk powder contains an elevated level of carbohydrates and minerals, which can promote *B. cereus* cell proliferation and enterotoxin production when they are reconstituted and held at ambient temperature for extended periods, even low levels of *B. cereus* in milk powder can act as potential vehicles for foodborne diseases (Reyes *et al.*, 2007). India being a tropical country, the average load of *B. cereus* encountered in many food samples was higher than that reported from other parts of the world having colder climates (Bhatnagar *et al.*, 2007).

## V. CONCLUSION

Milk and Milk Products (MMP) are the basic nutritional food source for infants and adults. It is a major concern to check the quality of the products we consume. This study gives an outlook of the presence of *B. cereus* in MMP, which would cause health hazards to the consumers as, *B. cereus* is a spore-producing pathogen that causes food poisoning with symptoms of vomiting and diarrhea, exhalation of toxins may damage liver tissue, and inflammatory diseases such as gastroenteritis and meningitis. The study was undergone as per Food Safety and Standard Authority of India (FSSAI) requirements under safety criteria. Analyzed final products of different milk and milk products were found to be in the range as per Food Safety and Standard Regulation (FSSR), 2011 Annexure B and thus were reported as consumer safe.

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## APPENDICES - A

### TABLE I. ENUMERATION OF *B. CEREUS* FROM MMP

| Sr. no. | Sample Name | Sample Code | Dilution | Presumptive Colony | | Haemolysis (out of 5) | CFU $g^{-1}$ or $ml^{-1}$ |
|---|---|---|---|---|---|---|---|
| | | | | Plate 1 | Plate 2 | | |
| 1 | SMP | S1 | $10^{-1}$ | 0 | 0 | NA* | <10 |
| 2 | SMP | S2 | $10^{-1}$ | 11 | 11 | 1 | 22 |
| 3 | SMP | S3 | $10^{-1}$ | 0 | 0 | NA* | <10 |
| 4 | SMP | S4 | $10^{-1}$ | 0 | 0 | NA* | <10 |
| 5 | Infant food | IF1 | $10^{-2}$ | 116 | 106 | 5 | 11000 |
| 6 | Infant food | IF2 | $10^{-2}$ | 8 | 2 | 2 | 200 |
| 7 | SMP | S5 | $10^{-1}$ | 11 | 7 | 3 | 54 |
| 8 | Milk | M1 | $10^{0}$ | 0 | 0 | NA* | <10 |
| 9 | Milk | M2 | $10^{0}$ | 0 | 0 | NA* | <10 |
| 10 | Milk | M3 | $10^{0}$ | 0 | 0 | NA* | <10 |
| 11 | SMP | S6 | $10^{-1}$ | 4 | 3 | 1 | 7 |
| 12 | Milk | M4 | $10^{0}$ | 0 | 0 | NA* | <10 |
| 13 | SMP | S7 | $10^{-1}$ | 23 | 23 | 5 | 230 |
| 14 | SMP | S8 | $10^{-1}$ | 7 | 5 | 1 | 12 |
| 15 | SMP | S9 | $10^{-1}$ | 0 | 0 | NA* | <10 |
| 16 | SMP | S10 | $10^{-1}$ | 0 | 0 | NA* | <10 |
| 17 | SMP | S11 | $10^{-1}$ | 0,0 | 0 | NA* | <10 |
| 18 | SMP | S12 | $10^{-1}$ | 0,0 | 0 | NA* | <10 |
| 19 | SMP | S13 | $10^{-1}$ | 50 | 50 | 4 | 400 |
| 20 | SMP | S14 | $10^{-1}$ | 11, | 11 | 3 | 66 |
| 21 | SMP | S15 | $10^{-1}$ | 5 | 5 | 1 | 10 |
| 22 | SMP | S16 | $10^{-1}$ | 78 | 78 | 5 | 780 |
| 23 | SMP | S17 | $10^{-1}$ | 0 | 0 | NA* | <10 |
| 24 | SMP | S18 | $10^{-1}$ | 12 | 10 | 3 | 66 |
| 25 | SMP | S19 | $10^{-1}$ | 11 | 9 | 2 | 40 |
| 26 | SMP | S20 | $10^{-1}$ | 3 | 2 | 3 | 15 |
| 27 | SMP | S21 | $10^{-1}$ | 81 | 81 | 5 | 810 |
| 28 | SMP | S22 | $10^{-1}$ | 0 | 0 | NA* | <10 |
| 29 | SMP | S23 | $10^{-1}$ | 5 | 5 | 2 | 20 |
| 30 | SMP | S24 | $10^{-1}$ | 3 | 7 | 1 | 10 |

### TABLE II. PREVALENCE OF *B. CEREUS* IN MILK AND MILK PRODUCTS

| Sr. no. | Sample Name | Sample Code | Results | |
|---|---|---|---|---|
| | | | CFU $g^{-1}$ or $ml^{-1}$ | $Log_{10}$ CFU $g^{-1}$ or $ml^{-1}$ |
| 1 | SMP | S1 | 0 | 0 |
| 2 | SMP | S2 | 22 | 1.34 |
| 3 | SMP | S3 | 0 | 0 |
| 4 | SMP | S4 | 0 | 0 |
| 5 | Infant food | IF1 | 11100 | 4.04 |
| 6 | Infant food | IF2 | 200 | 2.30 |
| 7 | SMP | S5 | 54 | 1.73 |
| 8 | Milk | M1 | 0 | 0 |
| 9 | Milk | M2 | 0 | 0 |
| 10 | Milk | M3 | 0 | 0 |
| 11 | SMP | S6 | 7 | 0.84 |
| 12 | Milk | M4 | 0 | 0 |
| 13 | SMP | S7 | 230 | 2.36 |
| 14 | SMP | S8 | 12 | 1.07 |
| 15 | SMP | S9 | 0 | 0 |
| 16 | SMP | S10 | 0 | 0 |
| 17 | SMP | S11 | 0 | 0 |

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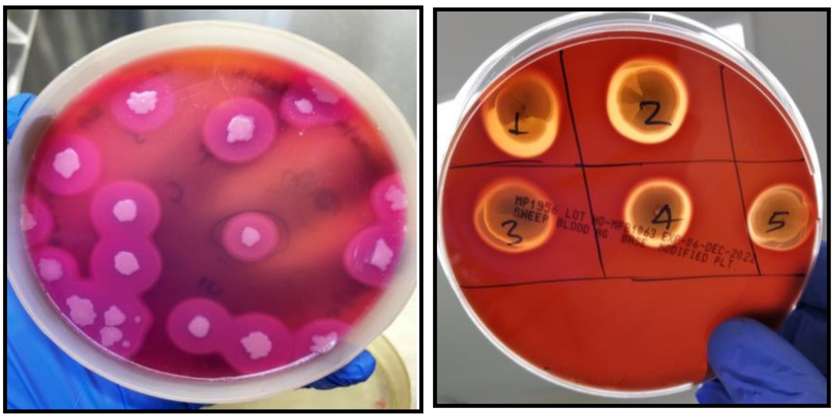
| | | | | |
|---|---|---|---|---|
| 18 | SMP | S12 | 0 | 0 |
| 19 | SMP | S13 | 400 | 2.60 |
| 20 | SMP | S14 | 66 | 1.81 |
| 21 | SMP | S15 | 10 | 1 |
| 22 | SMP | S16 | 780 | 2.89 |
| 23 | SMP | S17 | 0 | 0 |
| 24 | SMP | S18 | 66 | 1.81 |
| 25 | SMP | S19 | 40 | 1.60 |
| 26 | SMP | S20 | 15 | 1.17 |
| 27 | SMP | S21 | 810 | 2.90 |
| 28 | SMP | S22 | 0 | 0 |
| 29 | SMP | S23 | 20 | 1.30 |
| 30 | SMP | S24 | 10 | 1 |
| **Percentage Prevalence** | | | **65.3%** | |




**A.** **B.**

**Figure I. (A) *B. cereus* colonies on MYP Agar, (B) β- Haemolysis on Sheep Blood Agar.**

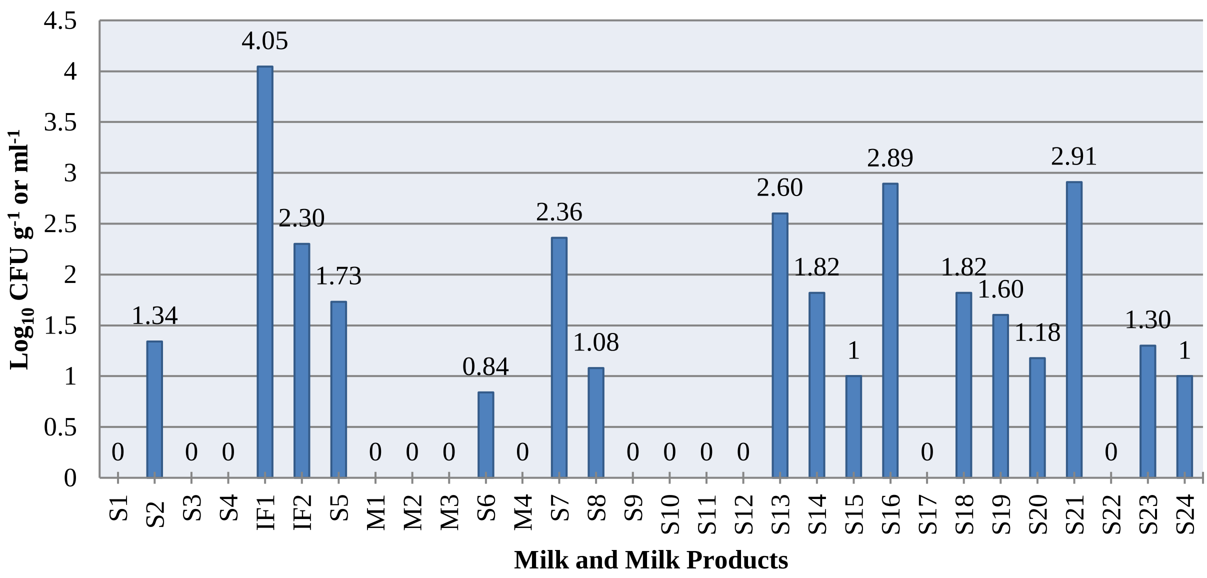



**Figure II. Enumeration of *B. cereus* in MMP ($Log_{10}$ CFU $g^{-1}$ or $ml^{-1}$)**