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Biochemical characterization of *Bacillus cereus* isolated from dairy products

Ridhima Mahadeva, Surendra and Abhishek Gaurav

Abstract
Food safety is a scientific discipline that describes how to handle, prepare, and store food to avoid food-borne illness. On the analysis of all the 120 dairy products samples collected from market available brands and local vendors/retail shops from Udaipur city (Rajasthan) it was observed that on the basis of cultural and biochemical characterization, 17 isolates were considered as presumptive *Bacillus cereus*. Out of the 120 dairy products samples including ice creams (n=40), lassi (n=40) and flavored milk (n=40), it was found that 9, 7 and 1 samples were positive for *B. cereus* giving a prevalence rate of 22.5%, 17.5% and 2.5%, respectively. In total, the prevalence of *B. cereus* was found to be 14.1% (17/120).

Keywords: *Bacillus*, dairy products, food-borne organism

Introduction
Food safety is a scientific discipline that describes how to handle, prepare, and store food to avoid food-borne illness. The organisms which can infect intestines when ingested along with the food and produce toxins in situ to bring about symptoms of poisoning are known to cause toxi-infections. Eating food, infected with bacteria, viruses, parasites, or chemical substances such as heavy metals can cause over 200 diseases. Consumption of infected food causes foodborne diseases, which may occur at any point in the food processing, distribution, and consumption chain. This can be caused by a variety of environmental contaminations, such as pollution of the water, soil, or air, as well as improper food storage and processing (WHO, 2019).

Bacillus cereus is a Gram positive, facultative anaerobic, spore forming, motile bacterium (Tallent *et al.*, 2012) [31] isolated from rice, spices, meat, egg and dairy products (Johnson, 1984) [14]. The optimal temperature for the growth is between 28 ºC and 35 ºC; ranging from 4 ºC to 48 ºC. *B. cereus* can grow at pH values from 4.9 to 9.3 (Jay *et al*., 2005) [13].

Bacillus cereus is widely distributed pathogen. Bacillus is a Latin word that means “long rod”, and cereus is a Latin word meaning “wax-like”. When examined under a microscope or on blood agar plates, *B. cereus* seems to have an easily identifiable morphology. The organisms and strains of this genus have a broad variety of growth properties, ranging from psychrophilic to thermophilic and acidophilic to alkaliphilic.

B. cereus is transmitted either by the ingestion of a large number of bacterial cells in infected food (Diarrheal type) or by the ingestion of pre-formed toxins contaminated food (emetic type). Consumption of infected foods, inappropriate food handling/storage, and improper cooling of cooked foodstuffs all contribute to the spread of this bacterium. The ingestion of rice and pasta has been linked to the emetic form of food poisoning. While, the diarrheal type is spread predominantly through milk products, vegetables, and meat.

Materials and Methods
In the present study, attempts were made to determine the prevalence of *Bacillus cereus* isolated from dairy products. The various materials and methods used in conducting the study are described below:

In total, 120 samples of dairy products were collected twice in a week from milk parlours, retail shops and street vendors from Udaipur city, Rajasthan.

The samples were collected in sterile containers and transported to the laboratory within 2 hours in chilled condition by using ice packs. The samples were collected from different localities of Udaipur city. The different types of samples and their sample size are described in Table 1.

Table 1: Different types of samples collected for the isolation of *Bacillus cereus* from dairy products

S. No.	Type of Sample	No. of Samples
1.	Ice Cream	n=40
2.	Lassi	n=40
3.	Flavored Milk	n=40
Total		120

Sample collection and preliminary isolation of *Bacillus cereus*

In total, 120 samples of dairy products were collected from Udaipur city (Rajasthan). The samples of dairy products were collected twice in a week from the market available brands and local vendors/retail shops. The samples were collected in sterile container and transported to the laboratory within 2 hours in chilled condition by using ice packs. The sample (1 ml/1 gm) was homogenized in brain heart infusion broth (9 ml) and incubated at 37 °C for 24 hours. Then, the enriched broth was streaked on selective media Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue Agar (PEMBA) and incubated at 37 °C for 24 hours.

Biochemical characterization

Various biochemical tests were performed to confirm the suspected *B. cereus* isolates *viz*., indole production test, methyl red test, voges-proskauer test, citrate utilization test, catalase test, oxidase test and urease test. Further, colony morphology, egg yolk reaction, hemolysis pattern, motility characteristics and nitrate reduction reactions were also observed.

Results and Discussion

Out of 120 isolates, 17 isolates showed peacock blue-coloured colonies on PEMBA (Fig. 1) and were selected for further identification. Gram's staining (Fig. 1) and various biochemical tests were performed on the 17 putative isolates which were morphologically identified as Gram-positive bacilli arranged singly or in chain.

After preliminary isolation, all the 17 putative isolates were subjected to different biochemical tests. All the suspected isolates (n=17) when tested for indole & MR test, showed no development of red coloured ring on the top and red colour, respectively. Similarly, on testing for voges-proskauer test (Fig. 1) and citrate test (Fig. 1), development of red color in all the isolates and blue color in some of the isolates was observed, respectively. This indicated that all the suspected isolates were negative for indole and MR test, while positive for voges-proskauer test and variable for citrate test.

The isolates were stabbed in motility agar medium and incubated at 37 °C for 24 hours. After 24 hours, the motility agar medium was observed with growth extending away from line of inoculation (Fig. 1). This indicated that the isolates were motile. Similarly, the isolates when streaked on urea agar slant and incubated at 37 °C for 24 hours were observed, no change in colour was seen, which indicated negative reaction.

A loopful of fresh culture from all the isolates was mixed with a drop of 3% hydrogen peroxide over a clean glass slide. There was production of gas bubbles within few seconds which was considered as catalase positive. Similarly, all the isolates when rubbed on the surface of oxidase disc, showed the absence of colour development or delay in appearance of any colour and were considered as negative for oxidase test. An isolated colony when streaked on the 5% sheep blood agar and incubated at 37 °C for 24 hours showed complete hemolysis (Fig. 1). Similarly, all the isolates were inoculated in nitrate broth and incubated at 37 °C for 24 hours. After incubation, 1 ml of sulfanilic acid and 1 ml of α-naphthylamine were added to the broth. There was development of red color in some of the isolates indicating positive reaction, while in other isolates zinc was added. Development of red color indicated a negative reaction. The result for nitrate reduction test was found to be variable in all the isolates tested (Fig. 1).

All the isolates were streaked on PEMBA media and incubated at 37 °C for 24 hours to observe the lecithinase activity (Fig. 1). All the isolates showed lecithinase activity. Similarly, the isolates when streaked on urea agar slant and incubated at 37 °C for 24 hours were observed, no change in colour was seen, which indicated negative reaction for the urease test (Table No. 2).

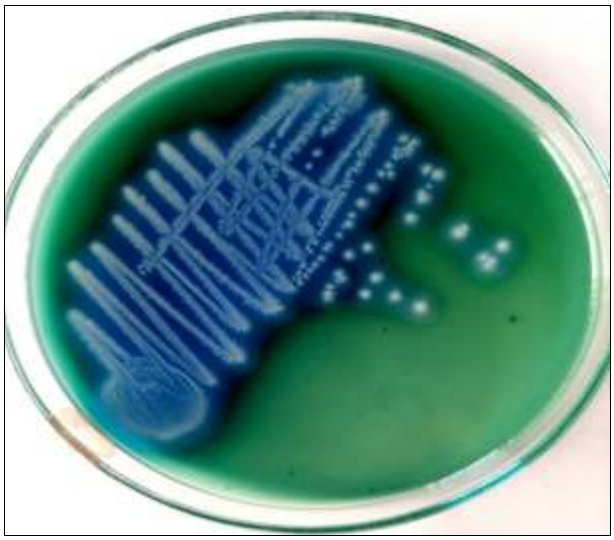

Fig 1: Growth of the test culture on polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA)

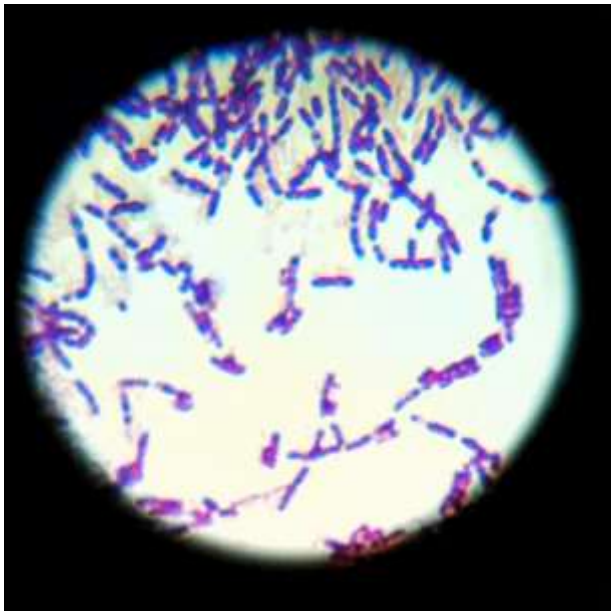

Fig 2: Gram's staining of the isolates (Gram positive rods)

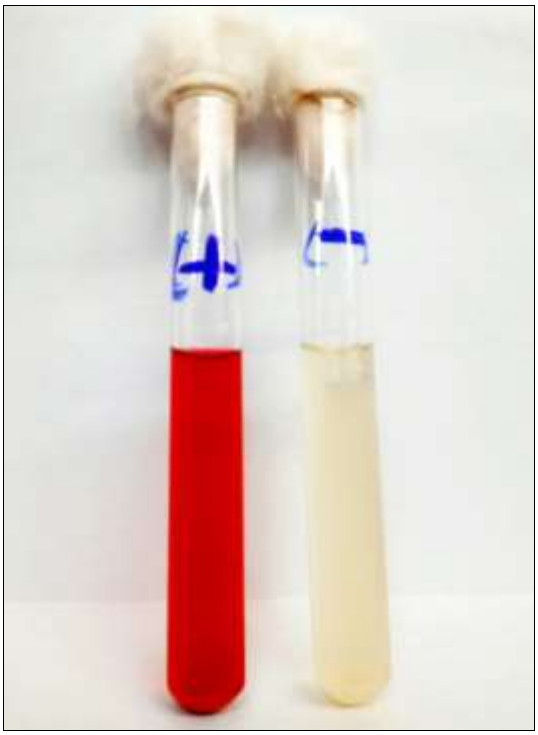

Fig 3: Voges- Proskauer test of the isolates

Fig 4: Citrate test of the isolates

Fig 5: Motility test of the isolates

Fig 6: Nitrate reduction test of the isolates

Fig 7: Hemolysis pattern of the isolates

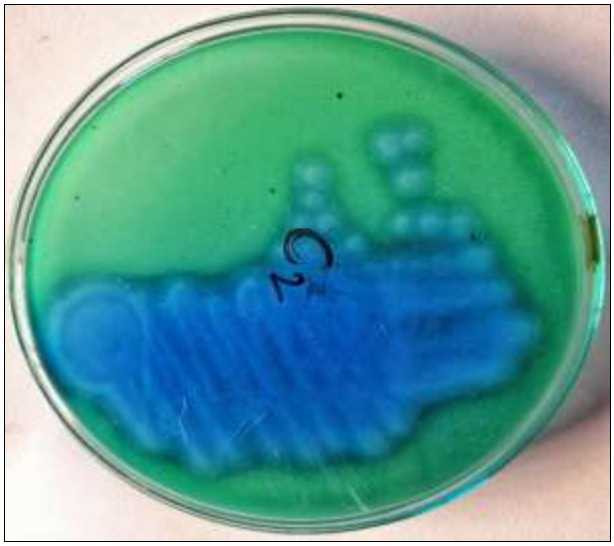

Fig 8: Lecithinase activity/ Egg yolk reaction of the isolates

Table 2: Biochemical reactions of the test isolates

S. No.	Biochemical Tests	Reactions
1.	Indole test	Negative
2.	Methyl red test	Negative
3.	Voges-Proskauer test	Positive
4.	Citrate test	Variable
5.	Hemolysis pattern	β-Hemolytic
6.	Urease test	Negative
8.	Catalase test	Positive
9.	Oxidase test	Negative
10.	Motility test	Motile
11.	Nitrate Reduction Test	Variable
12.	Lecithinase activity	Positive

Prevalence of *Bacillus cereus* in dairy products

On the analysis of all the 120 dairy products samples collected from market available brands and local vendors/retail shops from Udaipur city of Rajasthan including ice creams (n=40), lassi (n=40) and flavored milk (n=40) it was found that 9, 7 and 1 samples were positive for *B. cereus* giving a prevalence rate of 22.5%, 17.5% and 2.5% respectively. In total, the prevalence of *B. cereus* was found to be 14.1% (17/120) (Table No. 3).

Table 3: The prevalence of *B. cereus* isolated from dairy products

S. No.	Dairy product samples	Total No. of samples	No. of samples positive for *B. cereus*	Prevalence
1.	Ice Cream	n=40	9	22.5%
2.	Lassi	n=40	7	17.5%
3.	Flavored Milk	n=40	1	2.5%
	Total	N=120	17	14.1%

In the present study, the prevalence of *Bacillus cereus* in ice cream, lassi and flavored milk was found to be 22.5%, 17.5% and 2.5%, respectively. Higher prevalence of *Bacillus cereus* in ice cream was reported by Ahmed *et al*., 1983 [1]; Wong *et al*., 1988 [33]; Kamat *et al*., 1989 [15]; Abdallah, 1997 [35]; Ombui *et al*., 2008 [3]; Messel hauser *et al*., 2014 [20], Hassan *et al*., 2010 [36] and Hussein *et al*., 2015 [12] as 48%, 52%, 87%, 44%, 33%, 62.7%, 48% and 55%, respectively. While, a lower prevalence rate was reported by Masud, 1989 and Fadihl *et al*., 2019.as 4% and 9%, respectively. Apart from ice cream, dairy products like lassi were also found to be contaminated with *Bacillus cereus* organism (Sharma *et al*., 2014 and Rana *et al*., 2020) [28, 25]. The *Bacillus cereus* isolates were identified in 10.5% of the milk-based beverages (Rana *et al*., 2020) [25]. On the other hand, Biva *et al*. and 2019 [3] have also reported the contamination of almond milk, strawberry milkshake and chocolate milk samples, respectively with *Bacillus cereus* strains.

Conclusion

To control *B. cereus*, it is very important to trace the presence of spores throughout the food chain. The storage temperature is the most important factor in keeping *B. cereus* numbers to a minimum. Besides this, food poisoning generally occurs as a result of poor hygiene and food handling practice. Hence, it is important to educate food handlers about their responsibilities for food safety and train them on personal hygiene policies and basic practices for safe food handling. (Tewari and Abdulla, 2015) [32]. There is still the need for the implementation of good manufacturing practices and maintenance of sanitary conditions during the production, transportation and storage of these products.

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