

Original Research Article

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Isolation and Identification of *Bacillus cereus* from Milk and Milk Products in Udaipur, Rajasthan, India

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ABSTRACT

Keywords

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The present study was envisaged with the aim to isolate and identify *Bacillus cereus* from milk and milk products. A total of 160 samples which comprise of raw pooled market milk (n=20), pasteurized milk (n=20), dahi (n=20), paneer (n=20), khoa (n=20), milk powder (n=20), ice cream (n=20) and butter (n=20) were collected and processed in the laboratory. Out of the 160 samples screened, *Bacillus cereus* could be isolated from 44 samples of different milk and milk products employing culture and biochemical assays. Also, the representative phenotypically confirmed isolates (n=10) were further subjected for genotypic confirmation by using PCR. On molecular analysis, *gyrB* gene could be detected in 100% (10/10) isolates, while 60% (6/10) and 40% (4/10) of the isolates were found positive for the *cytK* and *hblA* genes, respectively. Presence of enterotoxigenic genes (*cytK* and *hblA*) in the isolates possesses a potential health threat for the public. Keeping in the view, there is an insistent need for elaborative study with more number of samples from different part of the region.

Introduction

Bacillus cereus is a Gram positive, facultative anaerobic, spore forming, motile bacterium (Tallent *et al.*, 2012) which is widely distributed in nature and contaminates almost every agricultural commodity (Khudor *et al.*, 2012). The bacterium is isolated from numerous foods, including dairy products, eggs and meat (Kramer and Gilbert, 1989; Ombui *et al.*, 2008). *Bacillus cereus* can grow in maximum foods at a pH above 4.5 and

temperatures above 4°C (Faria-Reyes *et al.*, 2001; Svensson *et al.*, 2007). *B. cereus* associated food-borne illness occurs as two distinct intoxication syndromes; emetic and diarrhoeal (Oh *et al.*, 2012). The diarrhoeal type of *B. cereus* food poisoning is caused by enterotoxins such as haemolysin BL (HBL), nonhaemolytic enterotoxin (NHE) and cytotoxin K (CytK) (Ankolekar *et al.*, 2009, Ngamwongsatit *et al.*, 2008).

Globally, the safety of dairy products in

respect to food-borne diseases is a great concern. Food safety is a scientific discipline describing handling, preparation, and storage of food in ways that prevent food borne illness. It includes numerous techniques that should be followed to escape potentially severe health hazard. It is mainly true in developing countries where production of milk and several milk products prepare under unhygienic conditions and poor production practices (Tewari *et al.*, 2012 and Kumari and Sarkar, 2014).

Rapid detection of *B. cereus* in food is important to facilitate the application of quality control measures to eradicate *B. cereus* from food and improve diagnosis of food poisoning outbreaks (Swaminathan and Feng, 1994; Rambabu and Kaiser, 2005). Best of our knowledge, studies in relation to detection of *B. cereus* in milk and milk products in Rajasthan region has not been attempted so far. Keeping this in the view the present study was envisaged to isolate and identify the *B. cereus* in the milk and milk products.

Materials and Methods

Collection of samples

A total of 160 milk and milk products samples comprising of raw pooled market milk (n=20), pasteurized milk (n=20), dahi (n=20), paneer (n=20), khoa (n=20), milk powder(n=20), ice-creams (n=20), and butter samples (n=20), were collected from dairies and sweet shops of Udaipur city, Rajasthan. The sample were collected aseptically in sterile sampling vials and transported on ice packs to the laboratory immediately.

Isolation and identification

After collection of samples, 1ml/1gm of the milk and milk product sample was

homogenized in 9 ml of brain heart infusion broth and incubated at 37°C for 24 hours. Then a loopful of inoculum was streaked on selective medium polymyxin pyruvate egg yolk mannitolbromothymol blue agar (PEMBA) and incubated at 37°C for 24 hours. After 24 hours, the plates were observed for the presence of peacock blue coloured colonies. Suspected colonies were further confirmed by biochemical tests *viz*; colony morphology, egg yolk reaction, haemolysis pattern, motility characteristics, catalase, urease, nitrate reduction, sugar fermentation, oxidase, indole, methyl red, vogesproskauer and citrate test.

Molecular characterization of *Bacillus cereus*

Bacillus cereus isolates were subjected to PCR for finding out the presence of the *gyrB* gene, *cytK* gene and *hblA* gene. The primers designed by Tewari *et al.*, (2013) (F-5'TCATGAAGAGCCTGTGTACG3'; R-5'CGACGTGTCAATTCACGCGC3') were used in this study for detection of *gyrB* gene for differentiation and confirmation of *B. cereus*. The primers used in the present study for detection of *cytK* gene were designed by Kwarteng *et al.*, (2017) (F-5'ACAGATATCGGGTCAAATGC3'; R-5'TCCAACCCAGTTATGCCAGTTC3'), while for *hblA* gene primer designed by Das *et al.*, (2009) (F-5'GCTAATG TAGTTT CACCTGTAGCAAC3'; R- AATCATGCCA CTGCGTGGACATATAA3').

Results and Discussion

The isolation and identification results are depicted in Table 1. Out of the 160 samples screened, *Bacillus cereus* could be isolated from 44 samples of different milk and milk products employing culture and biochemical assays. Also, the representative phenotypically confirmed isolates (n=10)

were further subjected for genotypic confirmation by using PCR. On molecular analysis, *gyrB* gene could be detected in 100% (10/10) isolates, while 60% (6/10) and 40% (4/10) of the isolates were found positive for the *cytK* and *hblA* genes, respectively.

Out of 160 samples screened, the positivity of *Bacillus cereus* was recorded in 30% (6/20) raw pooled market milk, 20% (4/20) pasteurized milk, 5% (1/20) dahi, 25% (5/20) paneer, 45% (9/20) khoa, 25% (5/20) milk powder, 30% (6/20) ice-cream and 40% (8/20) of butter samples.

The findings of the present study are in

accordance with the earlier studies, wherein the prevalence of the *Bacillus cereus* in raw market milk sample was around 30 % (Khudor *et al.*, 2012; Abraha *et al.*, 2017; Ali *et al.*, 2016; Yusuf *et al.*, 2018). However, higher prevalence rate were revealed in the study conducted by Kwarteng *et al.*, (2017) and Gundogan and Avci, (2014) in which *Bacillus cereus* was found in 47% and 90% of raw milk samples, respectively, while a lower prevalence rate of 11%, 9.8% and 9.84% were also recorded for *Bacillus cereus* contamination in raw milk by Tewari *et al.*, (2012), Cui *et al.*, (2016) and Fossi *et al.*, (2017), respectively.

Table 1 Results of isolation and identification of *Bacillus cereus* in milk and milk products

Positivity	Raw milk	Pasteurized milk	Dahi	Paneer	Khoa	Milk powder	Ice cream	Butter
Phenotypically	30%(6)	20%(4)	5%(1)	25%(5)	45%(9)	25%(5)	30%(6)	40%(8)
<i>GyrB</i> gene	100%	100%	ND	ND	ND	ND	ND	ND
<i>CytK</i> gene	66.66%	50%	ND	ND	ND	ND	ND	ND
<i>HblA</i> gene	50%	25%	ND	ND	ND	ND	ND	ND

*ND = Not Done

As far as the pasteurized milk is concerned Yiber *et al.*, (2017) reported 26% prevalence of *Bacillus cereus* which was slightly higher to the prevalence observed in our study (20%). A higher prevalence of *Bacillus cereus* was revealed in pasteurized milk samples as 57.14%, 100% and 55% by Reis *et al.*, (2013), Chitov *et al.*, (2008) and Kumari and Sarker (2014), respectively.

In conclusion, this study reveals high level of contamination of *Bacillus cereus* in milk and milk products which is sufficient to produce food poisoning. The presence of psychotropic as well as heat resistant *Bacillus cereus* represents a potential risk for dairy products stored under refrigeration. High level of

contamination in milk products is a great public health concern. So there is a need for thorough food inspection and frequent bacteriological surveillance by food inspection agencies. Keeping in the view, there is an insistent need for elaborative study with more number of samples from different part of the region. Also, it would be necessary to educate the farmer about clean milk production practices.

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