Article 106

A Study on the Biotyping of *Bacillus cereus* Emetic Strains Isolated from Raw Milk and Milk Products in Kashmir Valley

Altaf Mosvi, Mehboob Willayet, Manzoor Ahmad, Asif Iqbal* and Rayaz Chauhan

The present investigation pertains to the starch adulteration of raw milk and milk products and to study the biotyping of Bacillus cereus emetic strain isolated from raw milk and milk products in Kashmir valley. The study was conducted during October 2009-June 2010. A total of 175 samples comprising of 50 raw milk and 125 of different milk products (25 each of burfi, rasgulla, rasmalai, ice-cream and cheese/paneer) were tested. Starch/rice flour was detected in 7, 6, 5, 4, 3 and 2 samples of raw milk, rasgulla, burfi, ice-cream, rasmalai and cheese/paneer samples, respectively. Bacillus cereus emetic strains were isolated from 8 of the raw milk and 20 of the milk product samples, (6, 5, 4, 3 and 2 samples out of 25 each of rasgulla, burfi, ice-cream, rasmalai and cheese/paneer, respectively). B. cereus emetic strains were isolated from 16 percent of the raw milk. Raw milk, burfi and ice-cream revealed biotypes 2, 5 and 7, whereas, rasmalai and cheese/paneer revealed only biotype 5. Biotypes 2 and 5 were recovered from rasgulla.

KEY WORDS
Bacillus cereus emetic strains, raw milk, rasgulla, burfi, rasmalai, cheese, ice-cream, Biotyping.

INTRODUCTION

The incidence of foodborne disease has increased during recent years despite improvement in hygiene. [1], Food- and waterborne diseases are a significant cause of morbidity and mortality throughout the world. The reasons for this increase may lie in recent trends in global food production and changes in food technology in the industrialized countries. Reporting and diagnostic methods have also developed. Despite improved levels of hygiene and sanitation in handling and preparation of food items, the incidence of food-borne infections is increasing, owing to better reporting and diagnostic techniques, changes in eating habits and identification of new human pathogens [2]. *Bacillus cereus* is an endospore-forming bacterial species and a common cause of food poisoning in many countries. It is a gram positive, spore forming bacterium is wide spread in environment (soil, water and dust), and easily contaminates the foods of both plant and animal origin such as cereals, vegetables, milk and milk products, meat and meat products etc, thereby causing food borne illnesses in humans [3]. Outbreaks of food poisoning due to *Bacillus cereus* have been described since the beginning of the last century with the first confirmed report in Norway in 1948 [4]. Since then many food-borne outbreaks were reported [5-6]. Milk, being ideal medium for growth of microorganisms, makes it suitable for multiplication of *B. cereus* as well as elucidation of its toxin in it. With the advent of increased number of psychrotolerant *B. cereus* strains, the dairy industry has witnessed increased reports of food poisoning outbreaks due to this organism [7].

Two distinct types of gastrointestinal disorders caused by *B. cereus* in humans viz; an early "emetic syndrome" and a late onset "diarrheal syndrome" involving two different types of enterotoxins, have been recognized [8]. The

Division of Veterinary Public Health,
Faculty of Veterinary Sciences & Animal Husbandry,
SKUAST-K, Srinagar.

emetic syndrome, a food borne intoxication, caused by preformed *B. cereus* emetic enterotoxin (BCEET) in food has a rapid onset (1-5 hours) characterized predominantly by nausea and vomition, resembling closely to staphylococcal food poisoning [9]. In contrast the diarrhoeal syndrome is caused due to production of *B. cereus* diarrhoeal enterotoxins (BCDET) during the vegetative growth of bacteria in the foods or in the intestines following ingestion, has a longer incubation period (12-24 hours) and is characterized by symptoms like diarrhoea, abdominal pain and rectal tenesmus, resembling closely *Clostridium perfringens* type A food poisoning [8-9]. Apart from gasteroenteritis *B. cereus* is also involved in a variety of non-GIT infections like meningitis, endopthalmitis, endocarditis, periodonitis, osteomyelitis, wound infection and septicaemia in humans [10]. It is also emerging as potential pathogen of serious concern in animals owing to increased reports of its role in diseases like osteomyelitis, middle ear infections, abortions and mastitis [11]. These reports unfold its explosive pathogenic role in various infections of animals. The biological effects of *B. cereus* toxins have been studied extensively. The diarrhoeal enterotoxin produced during the exponential growth phase of the organism is destroyed at 56^{o}C in 20 minutes, a temperature which is far less than the temperature attained in conventional cooking. However, emetic enterotoxin, commonly called as cerulide, produced in the stationary growth phase, is highly heat stable (126^{o}C for 90 minutes), withstanding extremes of pH (2-11) and is unaffected by the temperature attained during conventional cooking process. As a result the episodes of food poisoning out breaks due to *B. cereus* emetic toxin outnumber the diarrhoeal ones. The production of enterotoxins by *B. cereus* is dictated by the type of food rather than the strain involved. Studies on food ingredients have indicated rice and rice flour containing a high percentage of *B. cereus* emetic strains whereas diarrhoeal strains are found in almost all foods of animal origin. During growth, harvesting, milling, and other agricultural operations rice can variably become contaminated with *B. cereus* spores from a wide variety of environmental sources including soil, dust, sediment, and water [12]. The spores survive normal cooking temperatures [13]. and proliferate when the cooked rice is stored at room temperatures for long time leading to the episodes of intoxication (emetic type) or toxi-infection (diarrhoeal type) due to consumption of such temperature-abused rice. The emetic enterotoxin is selectively produced in rice and vegetable sprouts, milk and milk products, the later mostly being adulterated by the addition of rice powder/flour. Thus keeping in view above points the present investigation was undertaken.

MATERIALS AND METHODS

Sampling

i) Milk

A total of fifty samples of market milk (100 ml each) were collected in sterile urocols from local milk vendors of five arbitrary zones of Srinagar city viz. north (Hazratbal and surrounding areas), south (Sonawar and Dalgate), east (Harwan, Shalimar and Nishat), west (Qamarwari and adjacent areas) and central (Habbakadal and adjacent areas). The samples (Table 1) were brought to the laboratory on ice and processed within two hours of collection.

ii) Milk Products

A total of 125 samples of milk products, comprising of Burfi, Rasgola (white), Rasmalai, Ice cream, and Cheese (25 each) were collected aseptically from various retail outlets of the afore said zones of the Srinagar city (Table 1). The samples were brought to the laboratory on ice and processed within two hours of collection.

Biotyping

The isolates were biotyped on the basis of their ability to ferment Ammonium salt sugars (ASS) viz. Xylose, Salicin and Cellubiose (Table-2) as per the scheme proposed by Jha et al [14].

RESULTS

The isolates were biotyped as per the scheme proposed by Jha et al [14] by demonstrating their fermentation reaction in ammonium salt sugars viz; xylose, salicin and cellubiose. Of the seven isolates of *Bacillus cereus* emetic strains recovered from market milk 3 fermented cellubiose, 2 xylose and 2 salicin. Similarly, out of 20 isolates of *Bacillus cereus* emetic strains recovered from different milk products, five biotypes from barfi and four biotypes from ice-cream fermented cellubiose. All the biotypes recovered from rasmalai and cheese fermented salicin but not ferment cellubiose. All the 6 biotypes recovered from rasgulla fermented xylose.

In the present study the field isolates of *Bacillus cereus* emetic strains recovered from milk and milk products were grouped into 7 of the 9 existing biotypes. The predominant biotypes recovered were biotype 5 (3 out of 7) followed by biotype 7 (2 out of 7), biotype 2 (2 out of 7). Biotypes 2 and 5 were recovered from rasgulla while as barfi and ice-cream revealed 2, 5, and 7 biotypes. Cheese and rasmalai revealed biotype 5 only. The results are presented in Table- 3 and 4.

DISCUSSION

Biotyping scheme for *Bacillus cereus* proposed by Jha et al [14] has been used exclusively for locating the source of contamination of different foods [15-16] but to make the scheme more reliable and expressive; more number of samples from all possible sources needs to be screened.

In the present study, the biotyping pattern of isolates from the market milk revealed predominance of biotypes 5, 7 and 2 with 42.85 per cent isolates typed as biotype 5, 28.571 per cent as biotype 7 and 28.571 as biotype 2. Barfi and ice-cream also revealed 2, 5 and 7 as the most common biotypes. Rasgulla revealed only biotypes 2 and 5 while as cheese and rasmalai were contaminated with biotype 5 only. Previously, Yadav [15], reported predominance of biotypes 7, 6 and 5 from fried rice and while biotyping the heat resistant isolates from boiled rice, Willayat [16] found biotypes 7, 6, 5 and 1 as the most common biotypes. Javed [17] studied the predominance of biotypes 1, 7, 5 and 2 in boiled rice and 3, 4 and 2 in the market milk. Biotype 7 and 5 as such, appear to have more affinity to starchy foods. Interestingly, biotype 2, 5 and 7 were the common biotypes isolated from milk and different milk products, indicating adulteration of milk and milk products with rice flour. Cheese, being used as a base for rasmalai manufacturing revealed the common biotype (biotype 5).

Six isolates from the most common biotypes from milk and milk products along with standard *Bacillus cereus* strain (NCTC 11143) were cultured to produce emetic enterotoxin by shake culture method using rice culture broth as a basal medium. The nature and type of food has been related to the amount of enterotoxin production by many workers [16, 18]. This is also substantiated by the fact that food poisoning outbreaks due to *Bacillus cereus* are restricted to the consumption of particular food items more so in case of cooked rice where emetic strains are predominantly found and frequently produce emetic enterotoxins.

ACKNOWLEDGEMENT

The authors express their thanks to the Dean, FVSc and A.H, Shuhama, Alusteng, Srinagar, for providing necessary facilities at time of research.

REFERENCES

1. Varnam A.H. and Evans M.G. 1991. Food poisoning: medical and microbiological overview; Bacillus. In foodborne pathogens, an illustrated text. Mosby year book, London, UK, pp: 9-19; 267-288
2. Crerar, S. K., Dalton, C. B., Longbottom, H. M. and Kraa, E. Foodborne diseases: current trends and future surveillance needs in Australia. Med J Aust 1996; 165: 672-675.
3. Larsen, H. D.and Jorgeusen, K. The occurrence of *Bacillus cereus* in Danish Pasteurised milk. Inter J Food Micro 1996; 34: 179-186.
4. Hauge, S. Food poisoning caused by aerobic spore forming Bacilli. J App Bact 1995; 18: 591-95.
5. Lund, T., DeBuyser, M. L. and Granum, P. E. A new cytotoxin from *Bacillus cereus* that may cause necrotic enteritis. Mole Micro 2000; 38: 254-261.

6. Hussain, S. A., Munshi, Z. H. and Hussain, I. Food poisoning due to Bacillus cereus. A case report. J. Vet Pub Health 2006; 5:57-59.
7. Granum, P. E., Brynestad, S., O'Sullivan, K. and Nissen, H. Enterotoxin from Bacillus cereus: production and biochemical characterisation. Neth Milk Dairy J 1993; 47: 63-70.
8. Kramer, J. M. and Gilbert, R. J. *Bacillus cereus* and other Bacillus species. M.P Doyle (ed.) Food-borne Bacterial pathogens. Marcel Dekker, New York. pp 1989; 21-70.
9. Adams, M. R. and Moss, M. O. Royal Society of Chemistry Great Britain, England. Food Micro 2007; 3:60-64.
10. Schoeni, J. L. and Wong, A. C. L. *Bacillus cereus* food poisoning and its toxins. J Food Prot 2005; 68: 636-48.
11. Schiefer, B., Macdonald, K. R., Klavano, G. G. and Van-Dreumel, A. A. Pathology of *Bacillus cereus* mastitis in dairy cows. Cana Vet J 1976; 17: 239-43.
12. Johnson, K. M., Nelson, C. L. and Busta, F. F. Influence of temperature on germination and growth of spores of emetic and diarrheal strains of *Bacillus cereus* in a broth medium and in rice. J. Food Sci 1984; 48: 286–287.
13. Vijaylakshmi, G., Dwarkanath, C. T. and Sreenivasa Murthy, V. Studies on *Bacillus cereus* contamination of rice and rice preparation. J Food Sci Technol 1981; 18, 231-234.
14. Jha, N. K. and Narayan, K. G. 1995. Biotyping of *Bacillus cereus* isolates. Journal of Food Science Technology 32: 231-32.
15. Yadav, R. 1993. Biotyping and serotyping of *Bacillus cereus* isolated from rice and chowmein. Phd. Thesis submitted to Birsa Agricultural University, Ranchi (Bihar).
16. Willayat, M. M. 1997. "Studies on *Bacillus cereus* enterotoxins and their pathogenesis".Ph.D. thesis submitted to Birsa Agricultural University, Ranchi (Bihar).
17. Javed, A. H. 2001. "Studies on prevalence of heat stable strains of *Bacillus cereus* in milk and rice". M.V.Sc thesis submitted to SKUAST-Kashmir, J & K.
18. Shinagawa, K. 1993. Serology and characterization of toxigenic Bacillus cereus. Bulletin of the International Dairy Federation. 287: 42-49.

---

*Address for correspondence:
Asif Iqbal, Division of Veterinary Public Health, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-K, Shuhama, Alusteng, Srinagar
e-mail address: asifent2008@gmail.com

TABLES

Table 1: Source and detail of milk and milk products collected from different zones of Srinagar city.

Sample	Central	Eastern	Western	Northern	Southern	Total
Raw milk	10	10	10	10	10	50
Burfi	5	5	5	5	5	25
Rasgola (white)	5	5	5	5	5	25
Rasmalai	5	5	5	5	5	25
Cheese/paneer	5	5	5	5	5	25
Ice cream	5	5	5	5	5	25
Total	35	35	35	35	35	175

Table 2: Biotyping scheme as proposed by Jha and Narayan (1995).

Biotype	Xylose	Salicin	Cellubiose
1	-	-	-
2	+	-	+
3	-	+	-
4	+	-	-
5	+	+	+
6	-	-	+
7	-	+	+
8	+	+	-

Table 3: Biotyping of isolates of *Bacillus cereus* emetic strains recovered from raw milk.

Biotype	**Isolate No**	**Sugar Fermentation (Ammonium Salt).**			**Total**
		Xylose	Salicin	Cellubiose	
2	Mj2, MJ 9	+	-	+	2
5	Mj22, MJ44, MJ29	+	+	+	3
7	Mj13, Mj8	-	+	+	2
	Total positive	5	5	7	7

Table 4: Biotyping of isolates of *Bacillus cereus* emetic strains recovered from different milk products.

Biotype	Milk product					Total
	Burfi	Ice-cream	Rasmalai	Rasgulla	Cheese	
2	Br 13, Br 7	Ic 11, Ic 23	___	Rg 9, Rg 14, Rg 16	___	7
5	Br 9, Br 6	Ic 2	Rm 7, Rm 10, Rm 23	Rg 5, Rg 22, Rg24	Ch 3, Ch 18	11
7	Br 23	Ic 9	___	___	___	2
Total	5	4	3	6	2	20