

## Incidence and Enterotoxin Gene Profile of *Bacillus cereus* Strains Isolated from Human Stool Samples from Uttarakhand, India

Anita Tewari\* and S.P. Singh

Department of Veterinary Public Health and Epidemiology, Collage of Veterinary and Animal Sciences, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Distt. U. S. Nagar -263145, India.

(Received: 20 December 2014; accepted: 15 January 2015)

The present investigation was undertaken to study the incidence and enterotoxigenic profile of *Bacillus cereus* in human stool samples. A total of 311 stool samples of apparently healthy human were collected and screened for the presence of *B. cereus*. Recovered *B. cereus* isolates were confirmed by PCR for *gyrB* gene and further evaluated for enterotoxin genes. Organism was isolated from 23 samples making the percent incidence of 7.4%. Screening of isolates by multiplex polymerase chain reaction revealed the overall distribution of various enterotoxin genes *hbl/DAC* complex, *nheABC* complex, *cytK* and *entFM* as 39.1, 95.6, 69.5 and 100%, respectively. Incidence rate (7.4%) was found low in population but majority of isolates harboring all the enterotoxin genes. *B. cereus* isolates of human stool origin were found to be strongly hemolytic and showed the presence of at least two enterotoxigenic genes. *B. cereus* is a very common food borne pathogen but a very limited data are available regarding the enterotoxin gene profile of *B. cereus* strains isolated from human stool. The presence of large number of enterotoxigenic *B. cereus* strains in stool samples must be considered as a potential health hazard as well as a source of contamination to environment.

**Key words:** *Bacillus cereus*; enterotoxin gene; human stool; Incidence.

*Bacillus cereus* is an important cause of foodborne illness in humans and bacterium is one of the pathogens frequently responsible for human diarrhoea. Acute diarrhoea is an endemic disease in many parts of India and a major cause of morbidity and mortality in infants and young ones especially in developing countries (1).

*B. cereus* is a gram-positive, rod shaped, endospore forming, motile facultative anaerobic bacterium. It can survive in any given situation because of its ubiquitous nature and ability to grow in a different type of foods. It has shortest mean

incubation period (0.8 h) and onset of illness within 8 h which makes *B. cereus* more potent pathogen than the other enteric organisms (2). There are two types of *B. cereus* food poisoning syndromes which are caused by two different toxins. Diarrheal syndrome is caused by enterotoxin(s), results in diarrhoea and emetic syndrome is characterized by nausea and vomiting (3). Sometimes both types of symptoms are produced probably due to the synergistic effects of one or more enterotoxins (4). The onset of infection occurs in about 8-16 h, lasts for 12-24 h, and is mostly associated with abdominal pain, profuse watery diarrhoea and tenesmus and later nausea and vomiting. *B. cereus* produce four enterotoxins: hemolysin BL or Hbl, nonhemolytic enterotoxin or Nhe, Cytotoxin K or *cytK* (5) and enterotoxin FM or *entFM* (6). These

---

\* To whom all correspondence should be addressed.  
Tel: +91-9914885634  
E-mail: id- anita\_vet@rediffmail.com,  
dranitatewari@gmail.com

toxins are considered as the primary virulence factors in *B. cereus* diarrhoea. The haemolysin BL consists of a binding component B and two lytic components L1 and L2 responsible for enterotoxigenicity of *B. cereus*. The B and L (L1 and L2) components are encoded in the genes *hblA*, *hblD* and *hblC*, respectively. These three components may be present in a different composition in *B. cereus*, and all the components together are necessary for the expression haemolysis to occur. Non-haemolytic enterotoxin also consists of three different proteins, A, B and C with the corresponding encoding genes *nheA*, *nheB* and *nheC*, respectively (7).

The organism is present in human faeces (8), although there is no existing evidence. It is possible that farm animals harbor the organism in their faeces and, similar to other pathogens such as *Listeria monocytogenes*, may contribute to the presence of the organism in the soil environment by the persistent cycle of faecal-oral transmission (9). As far as Indian conditions are considered, people are mostly uneducated and they do not nurture good sanitation habits which makes relatively higher chances of fecal oral and human to human transmission. Besides, the use of domestic wastewater and night-soil in agriculture is a centuries-old practice that is getting renewed attention with the increasing scarcity of fresh water resources in many arid and semi-arid regions of the world (10). All these facts increase the chances of contamination of vegetables and other agricultural products with *B. cereus*.

Keeping in view the above facts a study was undertaken to determine the presence of *B. cereus* in fecal samples of population of Uttarakhand and to characterize these isolates to understand their virulence features.

## MATERIALS AND METHODS

A total of 311 (n) human stool samples of mid-age group (from 25-40 years) and both sexes were randomly collected from different localities of Uttarakhand, India. Details of the samples are given in Table 1. The sterilized containers were distributed to target population in evening and collected early in the next morning. All the samples aseptically brought to the laboratory immediately after collection maintaining the proper cold chain

for processing.

### Isolation and Identification

All the samples were processed for isolation and identification of *B. cereus* as per the methodology of Shinagawa (11) with suitable modifications. Each of the collected stool samples was aseptically transferred into 10 ml of Brain heart infusion broth (BHI) supplemented with polymyxin B and incubated at 35°C for 24 h. Then after, a loop full of broth culture was streaked onto Polymyxin Pyvurate Egg Yolk Mannitol Bromothymol Blue Agar (PEMBA). All the inoculated plates were incubated at 35°C for 24 h. The typical crenate to fimbriate peacock blue colored colonies (3-5 mm) on PEMBA surrounded by blue zone of egg yolk hydrolysis were presumptively identified to be *B. cereus*. All the presumptive colonies of *B. cereus* were subjected to morphological and biochemical tests for identification of species.

### DNA Template

One typical colony from each of the isolates was picked up and inoculated in 5ml of Brain Heart Infusion (BHI) broth and incubated overnight at 35°C. The broth culture was subjected for DNA extraction using the Hi-Pura Bacterial and yeast genomic DNA purification kit (Hi-Media, Mumbai) as per the instructions of manufacturer. The DNA samples were diluted to a concentration of 50ng  $\mu\text{l}^{-1}$  prior to its amplification.

### Molecular Characterization of *B. cereus* Isolates Primers and PCR assay for confirmation

The primers (Forward-BCJH-5'TCATGAAGAGCCTGTGTACG3'; Reverse-BCJH-5'CGACGTGTCAATTCACGCGC3') for detection of *gyrB* gene (encoding the subunit B protein of DNA gyrase) for differentiation and confirmation of *B. cereus* used in this study were got synthesized from M/S Aldrich, USA. PCR was performed for molecular characterization of *B. cereus* by using reaction condition as described by Park *et al.* (12) with suitable modifications. A 25  $\mu\text{l}$  PCR reaction mixture was made consisting of 0.5  $\mu\text{l}$  DNA template (25 ng), 2.5  $\mu\text{l}$  PCR buffer with  $\text{MgCl}_2$  (1X), 0.5  $\mu\text{l}$  dNTPs (100  $\mu\text{M}$ ), 0.20  $\mu\text{l}$  forward primer (460 pmol/ $\mu\text{l}$ ), 0.40  $\mu\text{l}$  reverse primer (230 pmol/ $\mu\text{l}$ ) and 0.4  $\mu\text{l}$  Taq DNA polymerase (1 U/ $\mu\text{l}$ ).

The optimized PCR was setup in a 25  $\mu\text{l}$  volume reaction mixture and the cycling conditions consisted of an initial denaturation at 94°C for 5

min, 30 cycles of amplification with denaturation at 94°C for 30 sec, annealing at 63°C for 30 sec, an extension at 72°C for 30 sec, and final extension of the incompletely synthesized DNA at 72°C for 5 min.

#### Multiplex PCR Protocol

The primers used for the detection of enterotoxin genes (*hblADC*, *nheABC*, *cytK* and *entFM*) in the current study are described by Ngamwongsatit *et al.* (13). Two sets of multiplex PCR were employed to amplify the genes under study. Briefly, in first reaction; *hblC*, *hblD*, *hblA* and *cytK* genes were amplified while in second reaction; *nheA*, *nheB*, *nheC* and *entFM* genes were targeted using specific primers. In first set of reaction, 5 µl (50 ng µl<sup>-1</sup>) aliquot of bacterial genomic DNA was combined with 3.0 µl of reaction buffer (2 µl of 1X PCR buffer, 1 µl of 1.5 mM MgCl<sub>2</sub>), 0.4 µl of deoxynucleoside triphosphate (dNTP) mixture (concentration of each dNTP, 200 µM), 6.0 µl of primers (0.4 µM for each of *hblC*, *hblD*, *hblA* and 0.3 µM of *cytK*), and 1 µl of Taq DNA polymerase (5U µl<sup>-1</sup>). Then after, the volume was made upto 20 µl by addition of triple glass distilled water in final mixture. The second set of reaction was similar to first set except having 3.2 µl of primers (0.2 µM for each of *nheA*, *nheB*, *nheC* and *entFM*). Both the sets of reactions were amplified under same cycling conditions. Amplification was performed in a thermocycler (GeneAmp PCR system 9700, Applied biosystems) involving an initial denaturation at

95°C for 5 min., followed by 30 cycles of denaturation at 94°C for 45 s, primer annealing at 54°C for 1 min. and extension at 72°C for 2 min. A final extension at 72°C for 5 min. was also given. The PCR products were electrophoresed in 1.5% agarose gel and analysed using Gel documentation system (Alpha Innotech).

#### Statistical analysis

The comparison of the prevalence of *B. cereus* between male and female population and diarrheal and non-diarrheal samples was made by the Chi square test. The value of P<0.05 was considered significant.

## RESULTS

Among 311 samples, *B. cereus* was isolated from 23 (7.4%) of the samples. Stool samples of 7.0% male and 7.9% females of targeted population were found positive for *B. cereus*. 9.5% diarrheal while 7.1 % non-diarrheal samples were reported positive for *B. cereus*. All the isolates were motile and 20 were hemolytic on 5% sheep blood agar. All presumptive *B. cereus* isolates were subjected to PCR targeting *gyrB* gene by using species-specific primers. All isolates produced PCR product of 475 bp on agarose gel (Fig 1), which was specific to *B. cereus*.

#### Enterotoxigenic Profile

The isolates were screened by multiplex PCR for the presence of eight enterotoxin genes

**Table 1.** Details of sample population

	Male	Female	Diarrhoeal	Non- diarrhoeal
Stool Sample*	13/185	10/126	4/42	19/269
Percentage	7.0%	7.9%	9.5%	7.1%
Total	23/311 (7.4%)	23/311		

\* Number of positive samples out of total no. of samples in each head

**Table 2.** Distribution of enterotoxigenic isolates of *B. cereus* in different groups

Group	Gene/ genes present	Number of Isolates	% isolates in each group
G1	HBL, <i>entFM</i> , <i>cytK</i> , NHE complex	3 + 1 (IC*)= 4	17.4
G2	HBL complex, <i>entFM</i> , NHE complex	5	21.7
G3	<i>entFM</i> , <i>cytK</i> , NHE complex	10 + 1 (IC*)= 11	47.8
G4	<i>entFM</i> , NHE complex	2	8.7
G5	<i>cytK</i> , <i>entFM</i>	1	4.3

\*IC- incomplete isolates means lacked atleast one gene of NHE complex

(*hblADC* complex, *nheABC* complex, *cytK* and *entFM*), having the predicted size of 1,018, 935, 884, 759, 695, 618, 565 and 486 for *hblD*, *nheB*, *hblA*, *nheA*, *hblC*, *nheC*, *cytK* and *entFM*, respectively (Fig. 2).

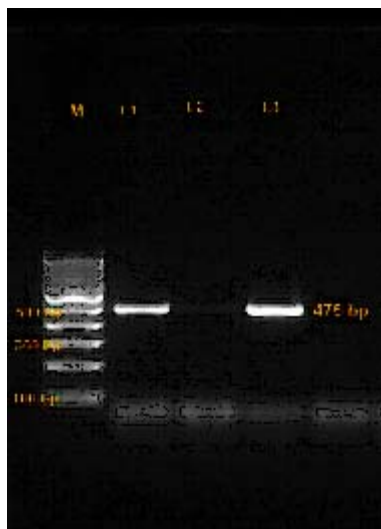
The polymerase chain reaction products in base pairs in figure indicate the enterotoxin gene i.e. 1,018, 935, 884, 759, 695, 618, 565, 486 indicates *hblD*, *nheB*, *hblA*, *nheA*, *hblC*, *nheC*, *cytK* and *entFM*, respectively.

On the basis of the presence or absence of enterotoxins, *B. cereus* isolates were divided into 5 groups (G1-G5). Group-1(G1) isolates showed the presence of *cytK*, *entFM*, NHE and HBL complex but one member was devoid of at least one gene of NHE complex. Isolates of Group-2 (G2) lacked only *cytK* gene. Group 3 (G3) comprised of isolates lacking HBL complex but one isolate of

this group was also devoid of at least one gene of NHE complex. Members of the Group 4 (G4) were devoid of *cytK* and HBL complex while isolates lacking HBL and NHE complex were placed in Group 5 (G5). Of the total isolates, 4 (17.4%), 5 (21.7%), 11(47.8%), 2 (8.7%) and 1 isolates (4.3%) were categorized into G1, G2, G3, G4 and G5, respectively (Table 2). The *entFM* gene was the most common enterotoxin gene found in all isolates (100%), followed by NHE complex and *cytK* with 22 (95.6%) and 16 isolates (69.5%), respectively. The HBL complex gene was detected in only 9 isolates (39.1%) of *B. cereus* (Table 3).

## DISCUSSION

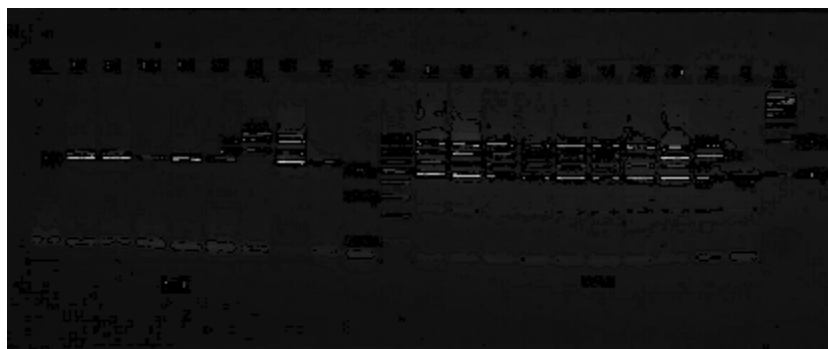
The isolates were confirmed as *B. cereus* based on various morphological and biochemical tests as described by Rhodehamel and Harmon (14), and the results were in accordance with previous reports (15), (16). *B. cereus* was isolated from 23 of the 311 human stool samples screened making an overall percent incidence of 7.4%. Almost similar reporting was done by Hassan and Nabbut (17) who also reported 7% *B. cereus* in normal stool samples while, Ghosh (8) found 14% prevalence of



**Fig. 1.** Molecular characterization of *B. cereus* by using *gyrB* gene (475 bp)

**Table 3.** Distribution of enterotoxin genes in total isolates

Gene	Isolate having the gene	Total number of isolate	%
NHE	22	23	95.6
HBL	9	23	39.1
<i>entFM</i>	23	23	100
<i>cytK</i>	16	23	69.5



**Fig. 2.** Agarose gel electrophoresis of multiplex PCR products amplified from genomic DNA of *Bacillus cereus* for grouping on the basis of enterotoxin gene

*B. cereus* in stool sample in two studies conducted on British population. Turnbull and Kramer (18) however, reported a higher prevalence (25-40%) in normal stool specimen harbouring *B. cereus*, whereas, Volkova (19) and Banerjee et al. (2) reported relatively very less prevalence in stool samples i.e. 0.35% and 3.5% respectively. The possibly explanations of this variation may be type of diet taken by individuals. The higher isolation rate might be expected in that group whose diet is mainly rice and other (20). Another factor for the variation might be the stage of the stool sample collection after taking *B. cereus* laden diet, as according to the Ghosh (8) excretion of *B. cereus* probably lasted <2 weeks in healthy individuals. The target population in the present study was apparently healthy but 7.4% population was found to have the organism. This may occur because the organism can function as a commensal in the gastrointestinal tract of certain individuals, or there may be a critical inoculum that one must ingest to produce disease. Some authors believe that large numbers of organisms must be present in the ingested food to result in the diarrheal form of *B. cereus* food poisoning (21).

No special association was noted between incidence rates of *B. cereus* in male and female population. *B. cereus* was isolated about equally from both male and female in the population. Nor was there any obvious association between diarrheal and non-diarrheal samples. Similarly Turnbull and Kramer (18) reported non-significant relationship between isolation rate of *B. cereus* from stool samples and sex or age.

All of the *B. cereus* isolates carried at least one of the enterotoxin genes out of 4 encoding virulence factors investigated in this study, similar to findings of Yang Ichen et al. (22) and Tewari et al (23). NHE complex was present in 22 isolates (95.6%) of *B. cereus*, whereas, Noori et al. (24) reported less prevalence (80%) of this gene in their study. However, NHE complex was found in almost all tested isolates in previous study of Stenfors Arnesen et al. (25). He also explained that because of the variability in the NHE operons, these unexpected results were found. The enterotoxigenic profile of the isolates revealed the presence of HBL complex (*hbl*/DAC) in 9 isolates (39.1%). On the contrary of this finding, Souza and Abrantes (26), Guinebreiere et al. (27) and Thaenthanee et al. (28)

reported higher frequency of HBL complex in samples i.e. 52.9%, 73% and 65.5% respectively. In the present study, the genes of HBL complex were found in lesser frequency than NHE complex, this finding was in accordance with Moravek et al. (29) and Al-khatib et al. (30).

According to the reports of Ngamwongsatit et al. (13) and Vyletelova and Banyko (31) the three genes of NHE complex occur together in operon. But the present study indicates that the genes in an operon can occur independently of each other, as 2 of the isolates showed the absence of one or two genes in NHE complex (Table 2). Thus, the genotype and incidence of enterotoxin genes may vary in different geographical locations and source of origin as is reported by various authors above.

Twenty isolates produced beta haemolysis on sheep blood agar. As per the result of multiplex PCR 9 isolates out of 23 harbored HBL complex which indicated gene function. Eleven isolates lacked HBL complex, but interestingly, they produced positive haemolysis reaction on blood agar. There is a possibility that such haemolytic effect might be produced by other toxins such as haemolysin I (Cereolysin O), haemolysin II, Haemolysin III or *cytK* of *B. cereus* (32).

The *entFM* gene was the common enterotoxin gene found in all *B. cereus* isolates in this study, Ngamwongsatit et al. (13) and Rahimi et al (33) also found this gene in all tested isolates of *B. cereus*. The gene for *cytK* was found in 16 (69.5%) of the isolates whereas Wijnands et al. (34), Ngamwongsatit et al. (13), Chitov et al. (16) and Rather et al. (35) detected it in 50, 88, 70.40 and 65.98 % of their isolates, respectively.

## CONCLUSION

The wide distribution of *B. cereus* in nature and in various foods including milk (36), rice (37), vegetables (38), and meat (24) and poultry (35), these organisms are unavoidably ingested frequently in small numbers and contribute to the momentary member of intestinal flora. But the presence of enterotoxigenic *B. cereus* must be considered as serious health hazard. There is high probability of the potential transmission of enterotoxigenic bacteria from human to environment to food and vice versa.



## ACKNOWLEDGEMENTS

The authors are gratefully thankful to Dean, College of Veterinary and Animal Sciences and Director Research, G.B. Pant University of Agriculture and Technology, Pantnagar for providing necessary funds and facilities to carry out this work.

## REFERENCES

- Gordon, J.E., Behar, M., Scrimshaw, N.S. Acute diarrhoeal disease in less developed countries- An epidemiological basis for control. *Bull. World Health Organ.*, 1964; **31**(1): 1-7.
- Banerjee, M., Nair, G.B., Ramamurthy, T. Phenotypic & genetic characterization of *Bacillus cereus* isolated from the acute diarrhoeal patients. *Ind. J. Med. Res.*, 2011; **133**: 88-95.
- Tewari, A., Abdullah, S. *Bacillus cereus* food poisoning: international and Indian perspective. *J. Food Sci. Technol.*, 2014; DOI 10.1007/s13197-014-1344-4.
- Andersson, M.A., Mikkola, R., Helin, J., Andersson, M.C., Salkinoja, S.M. A novel sensitive bioassay for detection of *Bacillus cereus* emetic toxin related depsipeptide ionophores. *Appl. Environ. Microbiol.*, 1998; **64**: 1338-43.
- Clair, G., Roussi, S., Armengaud, J., Duport, C. Expanding the Known Repertoire of Virulence Factors Produced by *Bacillus cereus* through Early Secretome Profiling in Three Redox Conditions. *Mol. Cell. Proteomics.*, 2010; **9**:1486- 98.
- Asano, S.I., Nukumizu, Y., Bando, H., Iizuka, T., Yamamoto, T. Cloning of novel enterotoxin genes from *Bacillus cereus*, *Bacillus thuringiensis*. *Appl. Environ. Microbiol.*, 1997; **63**: 1054-57.
- Fagerlund, A., Lindback, T., Granum, P.E. *B. cereus* cytotoxins Hbl, Nhe and CytK are secreted via the Sec translocation pathway. *BMC Microbiol.*, 2010; **10**: 304.
- Ghosh, A.C. Prevalence of *Bacillus cereus* in the faeces of healthy adults. *J. Hyg. Camb.*, 1978; **80**: 233-35.
- Altayar, M., Sutherl, A.D. *Bacillus cereus* is common in the environment but emetic toxin producing isolates are rare. *J. Appl. Microbiol.*, 2006; **100**: 7-14.
- Egun, N.K. Effect of Channelling Wastewater into Water Bodies: A Case Study of the Orogodo River in Agbor, Delta State. *J. Hum Ecol.*, 2010; **31**(1): 47-52.
- Shinagawa, K. Analytical methods for *Bacillus cereus*, other *Bacillus* species. *Int. J. Food Microbiol.*, 1990; **10**: 125-42.
- Park, S., Kim, H., Kim, J., Kim, T., Kim, H. Simultaneous Detection and Identification of *Bacillus cereus* Group Bacteria Using Multiplex PCR. *J. Microbiol Biotechnol.*, 2007; **17**(7): 1177-82.
- Ngamwongsatit, P., Buasri, W., Pianariyanon, P., Pulsrikarn, C., Ohba, M., Assavanig, A., Panbangred, W. Broad distribution of enterotoxins genes (*hblCDA*, *nheABC*, *cytK*, *entFm*) among *Bacillus thuringiensis* and *Bacillus cereus* as shown by novel primers. *Int. J. Food Microbiol.*, 2008; **121**: 352-56.
- Rhodehamel, E.J., Harmon, S.M. Bacteriological Analytical Manual. U.S. Food and Drug Administration. 1998; <http://www.cfsan.fda.gov/~ebam/bam-14.html#authors>.
- Sharma, C.S., Sharma, D.K., Gill, J.P.S., Aulakh, R.S., Sharma, J. *Bacillus cereus* from foods of animal origin in India, its public health significance. *Acta. Vet. Sc.*, 2003; **44**: 118.
- Chitov, T., Dispan, R., Kasinrer, W. Incidence and diarrheagenic potential of *Bacillus cereus* in pasteurized milk and cereal products in Thailand. *J. Food Saf.*, 2008; **28**: 467-81.
- Hassan, G., Nabbut, N. Prevalence, characterization of *Bacillus cereus* isolates from clinical and natural sources. *J. Food Prot.*, 1995; **59**(2): 193-96.
- Turnbull, P.C.B., Kramer, J.M. Intestinal carriage of *Bacillus cereus*: fecal isolation studies in three population groups. *J. Hyg.*, 1985; **95**: 629-39.
- Volkova, R.S. *Bacillus cereus* contamination of foods and environment at institutional feeding points. *Gigiena-i-Sanitariya.*, 1971; **36**(2): 108-09.
- Kramer, J.M., Gilbert, R.J.: *Bacillus cereus*, other *Bacillus* species. In: *Foodborne Bacterial Pathogens* (Doyle MP, ed). Marcel Dekker. New York USA, 1989; pp 21-77
- DeBuono, B.A., Brondum, J., Kramer, J.M., Gilbert, R.J., Opal, S.M. Plasmid, serotypic, enterotoxin analysis of *Bacillus cereus* in an outbreak setting. *J. Clin. Microbiol.*, 1988; **26**(8):1571.
- Yang, I.C., Chih, S.Y., TsuiPing, H., YunPu, H., JanYi, W., TzuMing, P. Establishment of a novel multiplex PCR assay and detection of toxigenic strains of the species in the *Bacillus cereus* group. *J. Food Prot.*, 2005; **68**(10): 2123-30.
- Tewari, A., Singh, S.P., Singh, R. Incidence and

- enterotoxigenic profile of *Bacillus cereus* in meat and meat products of Uttarakhand, India. *J. Food Sci. Technol.*, 2013; DOI 10.1007/s13197-013-1162-0.
24. Noori, E., Deilami Khiabani, Z., Bigdeloo, Y., Rahnama, M., Shapouri, R. Study Genes of NHE complex in *Bacillus Cereus* Isolated from Common Rice Samples in Iran. *Modares J. Med. Sci. (Pathobiology)*, 2012; **14**(4): 88-94.
  25. Stenfors Arnesen, L.P., Fagerlund, A., Granum, P.E. From soil to gut: *Bacillus cereus*, its food poisoning toxins. *FEMS Microbiol Rev.*, 2008; **32**: 579–606.
  26. Souza, C.M.O., Abrantes S.M.P. Detection of enterotoxins produced by *B. cereus* through PCR analysis of ground and roasted coffee samples in Rio de Janeiro, Brazil. *Food Sci. Technol. (Campinas)*, 2011; **31**(2). <http://dx.doi.org/10.1590/S0101-20612011000200025>
  27. Guinebretiere, M.H., Broussolle, V., Nguyenhe, C. Enterotoxigenic profiles of food poisoning, food-borne *Bacillus cereus* strains. *J. Clin Microbiol.*, 2002; **40**: 3053–6.
  28. Thaenthanee, S., Wong, A.C.L., Panbangred, W. Phenotypic and genotypic comparisons reveal a broad distribution and heterogeneity of hemolysin BL genes among *Bacillus cereus* isolates. *Int. J. Food Microbiol.*, 2005; **105**: 203–12.
  29. Moravek, M., Dietrich, R., Burk, C., Broussolle, V., Guinebretiere, M.H., Granum, P.E., Nguyenhe, C., Martlbauer, E. Determination of the toxic potential of *Bacillus cereus* isolates by quantitative enterotoxin analyses. *FEMS Microbiol. Lett.*, 2006; **257**: 293–8.
  30. Al-Khatib, M., Khyami, H., Badran, E., Shehabi, A. Incidence and characterization of diarrheal enterotoxins of fecal *Bacillus cereus* isolates associated with diarrhea. *Diag. Microbiol. Infect. Dis.*, 2007; **59**: 383-7.
  31. Vyletelova, V., Banyko, J. Detection of HBL and NHE enterotoxin genes in *Bacillus cereus* using Multiplex PCR. Paper presented at 12th International Symposium on Microbial Ecology (ISME12), Cairns: Australia. 2008.
  32. Ramarao, N., Sanchis, V. The Pore-Forming Haemolysins of *Bacillus cereus*: A Review. *Toxins (Basel)*, 2013; **5**(6): 1119-39.
  33. Rahimi, E., Abdos, F., Hassan, M., Zienab, T.B., Mohammad, J. *B. cereus* in infant foods: prevalence study and distribution of enterotoxigenic virulence factors in Isfahan Province, Iran. *Sci. World J.*, 2013; 292571. doi:10.1155/2013/292571.
  34. Wijnands, S.L.M., Dufrenne, J.B., Rombouts, F.M., Veld, P.H., Leusden, F.M. Prevalence of potentially pathogenic *Bacillus cereus* in food commodities in The Netherlands. *J. Food Prot.*, 2006; **69**(11): 2587-94.
  35. Rather, M.A., Aulakh, R.S., Gill, J.P.S., Ghatak, S. Enterotoxin gene profile and antibiogram of *Bacillus cereus* strains isolated from raw meats and meat products. *J. food Safety.*, 2012; **32**: 22-8.
  36. Tewari, A., Singh, S.P., Singh, R., Kumar, R. Characterization of *Bacillus cereus* isolates from raw milk. *J. Vety. Pub. Health*. 2013; **11**(2): 145-148.
  37. Ankolekar, C., Rahmati, T., Labbe, R.G. Detection of toxigenic *Bacillus cereus* and *Bacillus thuringiensis* spores in U.S. rice. *Int. J. Food Microbiol.*, 2009; **128** (3):460-6.
  38. Ezeonu, I.M., Okonkwo, O. Influence of incubation temperature, pH and food substrate on production of *Bacillus cereus* extracellular proteins and toxins. *African J. Microbiol. Res.*, 2013; **7**(19): 2050-56.