Australian Journal of Basic and Applied Sciences, 4(21): 5887-5893, 2010
ISSN 1991-8178

Relatedness among Clusters of Native Food Isolates of *Bacillus cereus* Based on Isolation Sources and Potent Toxigenic Traits

Shivalingsarj V. Desai and Mandyam C. Varadaraj

Human Resource Development, Central Food Technological Research Institute
(Council of Scientific & Industrial Research), Mysore 570020, India

Abstract: Genetic relatedness among 12 native isolates of *Bacillus cereus* obtained from a diversified range of food products including traditional fast foods were evaluated using 4 selected arbitrary primers in RAPD-PCR. The total amplified products of the 4 primers were 168 of 100-1000 bp. The genetic similarity coefficient for these isolates ranged from 0.040 to 0.470. The data analysis generated a dendogram with 3 clusters covering 11 isolates and the remaining one isolate was of non-cluster type. The cluster patterns could distinguish the isolates of *B. cereus* based on source of isolation, growth temperature and prevalence of toxigenic traits like haemolysin and sphingomyelinase, besides phosphatidylinositol. The present study clearly envisages the usefulness of RAPD as a molecular based analytical tool to understand the relationship between toxigenicity and source of isolation of native isolates of *B. cereus* occurring as opportunistic pathogen in a wide range of foods.

Key words: *Bacillus cereus*, RAPD-PCR, Clustering pattern, Traditional fast foods, Genetic similarity, Dendogram.

INTRODUCTION

Strains of *Bacillus cereus* are known to be opportunistic foodborne pathogen of public health significance and cause two types of illnesses through elaboration of enterotoxins (Smith *et al*., 2004; Guven *et al*., 2006; Roy *et al*., 2007). The unique properties of *B. cereus* in exhibiting heat resistance (endospores), survival and growth at varied temperatures (low to high) and toxigenic potential have directed the focus of research towards understanding phenotypic and genotypic characteristics of this bacterial species known to cause health hazards (Warke *et al*., 2000; Guinebretiere et al., 2002; Svensson *et al*., 2004; Valero *et al*., 2007 and Abou Zeid, 2009). The changing scenario of food habits has given impetus on the use of processed foods with minimal processing and prolonged storage at low temperatures.

The multifaceted dimensions of *B. cereus* has emphasized on the significance of epidemiological investigations and molecular typing of the organism with a clinical perspective. Conventional techniques have limited application in microbial sub-typing since genotypic differences do not often encode differences in phenotypic traits like antigen, enzymes, phage typing, metabolic profile or antibiotic susceptibility. Among the molecular methods of typing, randomly amplified polymorphic *D*NA – PCR (RAPD-PCR) is the most widely used analytical tool. This technique also called as arbitrarily-primed PCR (AP-PCR) is a simple molecular-based analysis, which uses single short random primers (8-10 bases) not targeted at any specific sequence in the genome. Based on the template DNA and primers, the proximity number and position of the primer sites vary between individual strains. Accordingly, the DNA fingerprinting with different banding profile are generated by which a dendrogram is deduced, depicting the relative position and distance of the strains with one another. The technique assesses the degree of genotypic relatedness among the strains and assists in differentiating the dissimilar isolates (Welsh and McClelland, 1990 and Williams *et al*., 1990). In view of the uniqueness of this analytical tool, RAPD-PCR has been proposed as a method for large scale typing of *B. cereus* (Nilsson *et al*., 1998).

In this study, 12 native isolates of *B. cereus* obtained from a varied range of Indian traditional fast foods were subjected to RAPD analysis as a means to assess the isolates for their genetic relatedness / diversity among the cluster patterns based on the source, toxigenic potential of cultures and growth at low temperatures.

Corresponding Author: Mandyam C. Varadaraj, Human Resource Development, Central Food Technological Research Institute (Council of Scientific & Industrial Research), Mysore 570020, India
Tel.: +91-821-2514310; Fax: +91-821-2517233
E-mail: varadaraj@cftri.res.in

MATERIAL AND METHODS

***Bacterial cultures*:**

The present study included 12 potent toxigenic native food isolates of *B. cereus* (CFR 1506, 1508, 1521, 1525, 1529, 1530, 1532, 1533, 1534, 1535, 1536 and 1540) selected from the Department collection of bacterial cultures that have been isolated from a range of Indian traditional fast foods collected from the local markets of Mysore City, India. These isolates had shown positive amplification with primers of phosphatidylinositol phospholipase (Pi-PLC), haemolysin BL (Ha-1) and sphingomyelinase (Sph). The cultures were individually maintained at 6°C on Brain Heart Infusion (BHI) agar slants (Hi Media Labs., Mumbai, India) and the cultures were propagated in BHI broth for 18 h at 37°C, prior to use in experimental trials.

***Growth at low temperatures*:**

All the 12 isolates of *B. cereus* were evaluated individually for their ability to grow at 6 and 10°C, respectively, by inoculating one loopful of overnight BHI culture broths into 10 ml BHI broth aliquots and incubated at the above-mentioned two temperatures in a BOD incubator (Sub Zero, Industrial Laboratory Tool Corporation, Chennai, India). The growth of bacterial cultures was monitored for 8 d through visual observation for turbidity in inoculated BHI broth.

***Isolation of genomic DNA from bacterial cultures*:**

The total genomic DNA of individual isolates of *B. cereus* was extracted from 1.5 ml aliquots of BHI culture broth by Phenol-chloroform method and ethanol precipitation (Schraft and Griffiths, 1995). The dried DNA was then resuspended in TE buffer of pH 8.0 and determined for quality and quantity at 260 nm in a UV-VIS Spectrophotometer (Genesys 5, Milton Roy Co., USA). This DNA was used as template for amplification by the primers of random primers in RAPD-PCR.

***PCR primers and conditions for RAPD analysis*:**

Approximately 35 arbitrary primers for the RAPD reactions were initially screened for amplification against genomic DNA of *B. cereus* isolates. Based on good amplification pattern and reproducibility of initially screened primers, finally 4 primers designated as RA08, RA14, RA18 and RA26 were selected for the final experimental trials in RAPD-PCR. The nucleotide sequences of selected primers were RA08 – 5¢ TGGCCGTGTG 3¢, RA14 – 5¢ TTCGAGCCAG 3¢, RA18 – 5¢ GATGACCGCC 3¢ and RA26 – 5¢ GGACACCACT 3¢. All the oligonucleotide primers were decamers with 2 dinucleotide repeats each and synthesized from a commercial company (Sigma Aldrich, Bangalore, India).

***RAPD-PCR reactions*:**

The protocol for RAPD-PCR was in accordance with an earlier documented procedure (Williams *et al*., 1990). Amplification of DNA was performed in a total reaction volume of 25 µl, which consisted of 2.5 µl of assay buffer (10 mM Tris-HCl of pH 9, 1.5 mM $MgCl_2$, 50 mM KCl and 0.01% gelatin of 10X concentration), 1 U of Taq DNA polymerase, 0.2mM dNTPs (Bangalore Genei, Bangalore, India), 2 µl of primer (0.2 µM) and 4 µL (~ 25-50 ng) of template DNA and 16.7 µl of Milli Q water (A10 Elix 3, Millipore Corporation, Billerica, USA). The thermocycling programme for the RAPD reaction was an initial denaturation under conditions of 94°C for 4 min, followed by 30 cycles of 94°C for 1 min, 36°C for 1 min, 72°C for 1 min and final extension at 72°C for 5 min performed in an automated DNA Thermal Cycler as described previously. The PCR amplified products in aliquots of 15 µL were run along with 100 bp DNA ladder in 2% agarose gels in 1X Tris acetate buffer for 2 h at 100 V and stained in 0.5 µg/ml ethidium bromide solution (Sambrook and Russel, 2001), followed by documentation in Gel Documentation System (Vilber Lourmat, France). The size of amplification products was determined using a 100 bp DNA ladder (Bangalore Genei, Bangalore, India).

***RAPD data analysis*:**

The well-resolved fragments in RAPD profiles, ranging from 100-1000 bp were scored as present (1) or absent (0) for each analysis. Bands with the same migration distance were considered homologous. By taking into consideration the total number of distinct DNA-amplified fragments corresponding to different sizes, a pair-wise similarity matrix was computed and analyzed with Numerical Taxonomy System (NTSYS) version 2.02 (Rohlf, 1998) using the simple matching coefficient (Sokal and Michener, 1958). The similarity matrix was used to construct a dendrogram by the unweighed pair-group method with arithmetical averages (UPGMA) to determine the genetic relatedness between the isolates.

RESULTS AND DISCUSSION

The ability of native isolates of *B. cereus* to grow at low temperatures showed that 4 isolates (33.3%) grew at 6°C and the other 8 isolates (66.7%) could grow at 10°C (table 1). Similarly, evaluation of isolates of *B. cereus* for potent toxigenic traits by PCR (data not shown) revealed that 4 isolates were positive for all the 3 traits tested in this study, while another 4 isolates were positive for only haemolysin and phosphatidylinositol phospholipase C and one each for sphingolyelinase and *pi-plc*. The remaining 3 isolates were positive only for PI-PLC (Table 1). These characteristics exhibited by *B. cereus* isolates have been linked with the source of their isolation, which happens to be food products (table 1). The ability of native food isolates selected in this study did show the ability to grow at low temperatures, wherein foods subjected to cold storage are not devoid from causing health hazards.

The potential of these native food isolates in being toxigenic / virulence was evidenced by the presence of either 3 or 2 traits in them by PCR. Even the 3 isolates exhibiting positive PCR amplification for PI-PLC are to be viewed as a concern of health hazard, in that phospholipase is known to cause degradation of cell and mucous membranes, which are rich in phospholipids leading to necrosis and is considered as virulence factor (Gilmore *et al*., 1989 and Beecher and Wong, 2000). Although molecular biology studies of many strains of *B. cereus* cluster are documented and available in public domain, the same is not true with the identity and traceability of culture to its source / origin (habitat and geographical distribution), an aspect of high importance in microbial diversity and establishing relatedness among cultures in a phylogram.

In the background of reproducibility and reliability of RAPD-PCR in differentiating strains of *B. cereus* (Nilsson *et al*., 1998), preliminary screening of nearly 35 arbitrary primers was performed with two *B. cereus* isolates CFR 1521 and CFR 1534, as a means to assess for their ability to amplify DNA fragments. Based on the visual scoring pattern of bands in two independent sets of experimental trials, 4 primers were selected for RAPD-PCR with all the 12 native isolates of *B. cereus*. The RAPD finger prints of these isolates with 4 primers can be visualized in gel documented photographs (photo 1a, b, c & d)). Each of the 4 primers used generated good number of polymorphic bands and the positions of these bands varied between the isolates. The total amplified products of the 4 primers was 168 (average of 42 bands per primer), which ranged from almost 100 to 1000 bp (table 2).

The genetic similarity (GS) coefficient for the 12 isolates of *B. cereus* resulting from RAPD analysis ranged from 0.040 (between the isolates CFR 1534 and CFR 1533) to 0.470 (between the isolates CFR 1532 and CFR 1535), except CFR 1540 (table 3). This isolate was different from the other isolates, wherein GS coefficient ranged from 0 (with respect to CFR 1529, CFR 1533 and CFR 1535) to 0.187 (with respect to CFR 1525). Based on the GS coefficient values, the relatedness among 4 potent toxigenic isolates of *B. cereus* namely CFR 1529, 1530, 1534 and 1536 was charted out (data not shown) and the GS coefficient was in the range of 0.160 to 0.286. The purpose of highlighting this aspect was due to the fact that these isolates were harbouring all the 3 toxigenic traits assessed in this study.

Table 1: Characteristics of *B. cereus* isolates with respect to source, growth at low temperatures and toxigenic factors

Isolates of *B. cereus*	Source	Growth at low temp. (°C)		Toxigenic potential Amplification in PCR		
		6	10	Pi-PLC	Ha-1	Sph
CFR 1506	Bread sandwich	+	+	+	+	-
CFR 1508	Bhel puri[a]	+	+	+	+	-
CFR 1521	Pani puri[b]	-	+	+	+	-
CFR 1525	Churi muri[c]	+	+	+	+	-
CFR 1529	Cooked spicy rice	-	-	+	+	+
CFR 1530	Cooked spicy rice	-	-	+	+	+
CFR 1532	Vegetable salad	-	+	+	-	-
CFR 1533	Ice cream	-	-	+	-	-
CFR 1534	Ice cream	+	+	+	+	+
CFR 1535	Khoa[d]	-	-	+	-	+
CFR 1536	Pani puri[b]	-	+	+	+	+
CFR 1540	Raw milk	-	+	+	-	-

[a-c]Processed rice/wheat based foods added with spices and vegetable salads

[d]Heat desiccated milk product used as a base in the preparation of Indian milk sweets

Table 2: RAPD-PCR analysis of native isolates of *B. cereus* with selected arbitrary primers

Primer identity	Sequence	No. of bands formed	Amplicon size (bp)
RA-08	TGGCCGTGTG	31	350-1000
RA-14	TTCGAGCCAG	54	210-1000
RA-18	GATGACCGCC	43	200-1000
RA-26	GGACACCACT	40	100-1000

Table 3: Genetic similarity coefficient among the native food isolates of *B. cereus* based on RAPD-PCR profiles

Isolates of	Isolates of *B. cereus* (CFR)											
B. cereus	1506	1508	1521	1525	1529	1530	1532	1533	1534	1535	1536	1540
CFR 1506	1.000											
CFR 1508	0.417	1.000										
CFR 1521	0.120	0.360	1.000									
CFR 1525	0.120	0.308	0.333	1.000								
CFR 1529	0.227	0.179	0.227	0.080	1.000							
CFR 1530	0.125	0.138	0.227	0.125	0.182	1.000						
CFR 1532	0.182	0.185	0.238	0.130	0.250	0.250	1.000					
CFR 1533	0.043	0.200	0.091	0.043	0.095	0.095	0.100	1.000				
CFR 1534	0.250	0.200	0.250	0.200	0.208	0.160	0.273	0.040	1.000			
CFR 1535	0.125	0.100	0.174	0.080	0.238	0.182	0.470	0.150	0.208	1.000		
CFR 1536	0.214	0.290	0.360	0.259	0.222	0.222	0.185	0.071	0.286	0.222	1.000	
CFR 1540	0.055	0.136	0.055	0.187	0.000	0.059	0.062	0.000	0.105	0.000	0.087	1.000

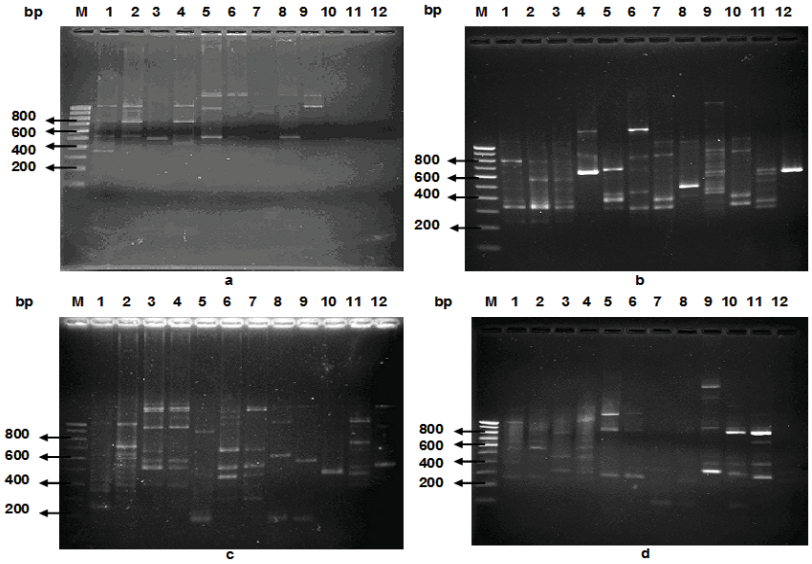

Photo (1 a, b, c & d): RAPD-PCR profiles of 12 native food isolates of B. cereus with randomly selected primers of RA-08 (a), RA-14 (b), RA-18 (c) and RA-26 (d).Lane M, DNA ladder of 100-1000 bp; Lanes 1-12, Native isolates of B. cereus CFR 1506, 1508, 1521, 1525, 1529, 1530, 1532, 1533, 1534, 1535, 1536 and 1540.

A broad degree of genetic diversity was observed among the isolates of *B. cereus* subjected to RAPD-PCR analysis, wherein isolates, CFR 1532 and CFR 1535 with highest degree of GS coefficient (0.470) were obtained from two different types of food items namely vegetable salad and a traditional heat desiccated milk product, respectively. However, isolates, CFR 1533 and CFR 1534 with least degree of GS coefficient (0.04) were obtained from the same type of food sample, ice-cream, but were placed apart. Similarly, CFR 1529 and CFR 1530 were isolated from processed rice based product and had a low degree of GS coefficient (0.182). The RAPD-PCR pattern provides evidence toward the existence of relatedness and diversity. In a few of the earlier studies, RAPD-PCR enabled detection of contamination of *B. cereus* in food ingredients, finished product and processing plant based on genetic diversity (Christiansson *et al*., 1999; Svensson *et al*., 1999 and Sorokulova *et al*., 2003). The present study revealed that irrespective of isolation source of *B. cereus* cultures (Table 1), there did exist genetic relatedness among 4 native isolates of *B. cereus*, which harboured all the 3 potent toxigenic traits as determined by PCR.

The dendrogram generated based on GS coefficients of RAPD analysis showed clear distinction into major and minor clusters (fig 1) with the presence of 3 major clusters A, B and C and sub-clusters within the major clusters. The sub-clusters, A1 and A2 had 2 and 4 isolates, respectively, while B1 had 3 isolates and B2 one isolate. Cluster C had only one isolate. The native isolate of *B. cereus* CFR 1540 was not present among the clusters generated and remained as a non-cluster pattern. In the present study, as a means to understand the possibility of any linkage factors, an attempt is being made to bring out certain relationships among the native food isolates of *B. cereus*.

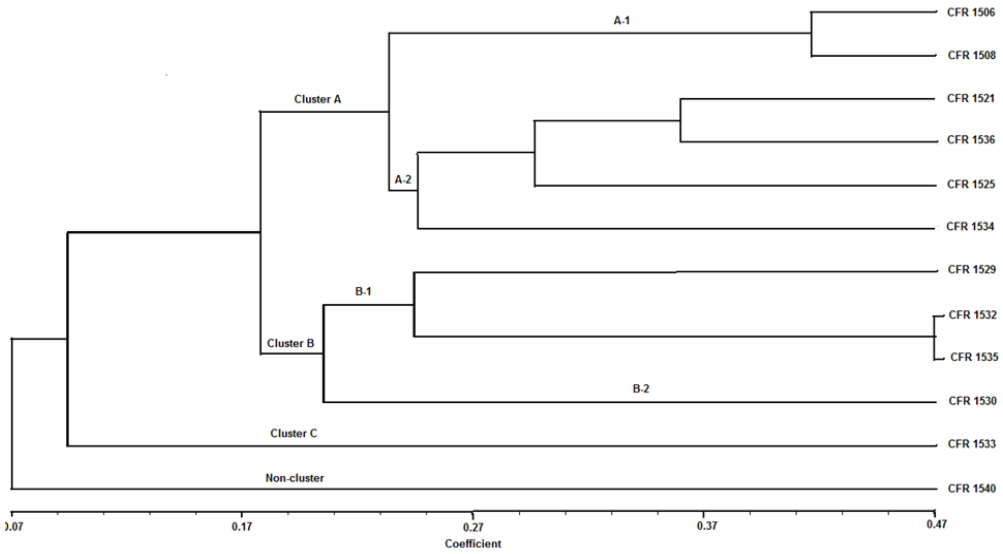

Fig. 1: Dendogram showing genetic relatedness/diversity among the native isolates of B. cereus based on the cluster analysis of RAPD-PCR data.

One of the attributes being addressed is that of processing criteria in the food products that have been source of isolation (table 1). Nearly 66.6% of isolates in cluster A were obtained from a similar type of food product, which was based on processed rice/wheat added with spices and vegetable salads and devoid of any heat processing, but mere mixing of ingredients at the point of consumption. An almost identical situation did exist in case of 2 other isolates of cluster A. In cluster B, 75% of the isolates were obtained from foods, which had undergone at least one step of heat processing and the remaining isolate was from a product devoid of any heat processing. Similarly, isolate in cluster C and the one under non-cluster have been from food products not exposed to any heat processing. In an identical scenario, RAPD has served as a useful genotyping technique to identify specific subtypes associated with specific source and location with respect to epidemiological investigations relating to strains of *Salmonella enteritidis* and *S. typhimurium* occurring in Italy (De Cesare *et al*., 2001).

Another aspect of approach was the low growth temperature of *B. cereus* isolates (table 1). All the isolates of *B. cereus* in cluster A were able to grow at 6 and/or 10°C, indicating almost psychrotrophic nature of these cultures. Except for *B. cereus* CFR 1532 (growth at 10°C) in cluster B, other remaining isolates in cluster B and the one isolate of cluster C were mesophilic. The isolate of *B. cereus* CFR 1540 present under non-cluster had a growth temperature of 10°C. As a means to interlink the basis of source of isolation and growth temperature, it becomes evident that isolates of *B. cereus* obtained from foods which had a unit operation of heat processing (cluster B) were mesophilic. Similarly, *B. cereus* cultures obtained from foods which did not involve heat processing at the time of consumption (cluster A) were psychrotrophs. In a similar approach to the present study, RAPD analysis using 3 different primers could clearly distinguish psychrotolerant strains of *B. cereus* group which resulted in proposing a new species *B. weihenstephanensis* sp. nov. (Lechner *et al*., 1998).

A more probable basis of relatedness has been the prevalence of toxigenic traits among the native isolates of *B. cereus*, which were positive with primers of Pi-PLC, Ha-1 and Sph (table 1). It is of interest to record that all isolates of *B. cereus* in clusters A and B did harbour the toxigenic trait of *hbl* and/or *sph*. However,

isolates in cluster C and non-cluster were negative for both the toxigenic traits. The findings did bring out that the presence of one or more of these toxigenic attributes in the isolates is a difficult proposition to analyze and there appears to be no single directional approach. Earlier research investigations on similar lines have clearly revealed that RAPD-PCR analysis could differentiate groups of isolates of *B. cereus*, *B. anthracis*, *B. thuringiensis* and other species based on combination of phenotypic and genotypic traits (Ghelavdi *et al*., 2002; Gaviria Rivera and Priest, 2003 and Levy *et al*., 2005).

In an approach towards projecting the importance of clustering pattern, a hypothetical scheme was prepared (fig 2) based on prevalence of toxigenic traits in the isolates of *B. cereus* and the source of their isolation (food products). The scheme projects the significance of clustering pattern, wherein it is evident that isolates of *B. cereus* obtained from specific types of traditional fast foods did harbour potent toxigenic traits and occurrence under one cluster like cluster A. However, milk based foods did not reveal any specific linkage with cluster pattern as isolates of *B. cereus* obtained from such foods did include clusters A, B, C and non-cluster. To a certain extent, spicy and vegetable based foods did have relatedness with *B. cereus* isolates of cluster B pattern. The scheme presented here may have limitations for wider applications as large number of isolates and specific type of foods associated with their isolation are to become the subject of study.

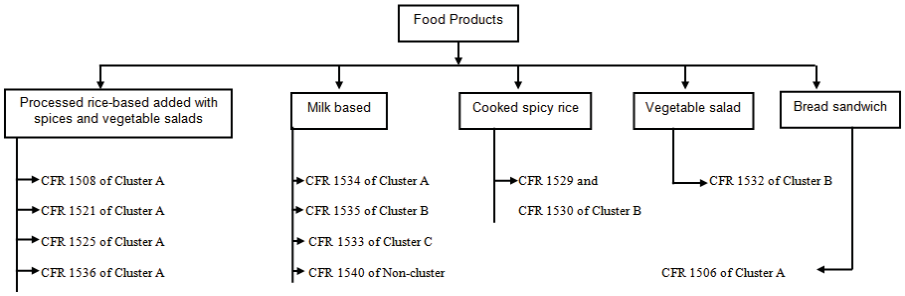

Fig. 2: Hypothetical representation of clustering pattern among potent native toxigenic isolates of B. cereus in relationship to their source.

In the background of growing emphasis on microbial diversity, the present study has clearly established the usefulness of four selected arbitrary primers in RAPD-PCR analysis to evolve cluster patterns among potent toxigenic isolates of *B. cereus* which do prevail in traditional fast foods.

ACKNOWLEDGEMENT

The authors are thankful to Dr. V. Prakash, Director, CFTRI, Mysore for providing the facilities and interest in present work. The first author is grateful to Council of Scientific and Industrial Research, New Delhi for awarding Research Fellowship.

REFERENCES

Abou Zeid, A.A., 2009. Molecular characterization and enterotoxin genes typing of local strains of *Bacillus cereus*. Australian J. Basic Appl. Sci., 3: 160-166.

Beecher, D.J. and A.C.L. Wong, 2000. Cooperative, synergistic and antagonistic haemolytic interactions between haemolysin BL, phosphatidylcholine phospholipase C and sphingomyelinase from *Bacillus cereus.* Microbiology, 146: 3033–3039.

Christiansson, A., J. Bertilsson and B. Svensson, 1999. *Bacillus cereus* spores in raw milk: Factors affecting the contamination of milk during the grazing period. J. Dairy Sci., 82: 305-314.

De Cesare, A., G. Manfreda, T.R. Dambaugh, M.E. Guerzoni and A. Franchini, 2001. Automated ribotyping and random amplified polymorphic DNA analysis for molecular typing of *Salmonella enteritidis* and *Salmonella typhimurium* strains isolated in Italy. J. Appl. Microbiol., 91: 780-785.

Gaviria Rivera, A.M. and F.G. Priest, 2003. Molecular typing of *Bacillus thuringiensis* serovars by RAPD-PCR. Syst. Appl. Microbiol., 26: 254-261.

Ghelardi, E., F. Celandroni, S. Salvetti, C. Barsotti, A. Baggiani and S. Senesi, 2002. Identification and characterization of toxigenic *Bacillus cereus* isolates responsible for two food poisoning outbreaks. FEMS

Microbiol. Letters, 208: 129-134.

Gilmore, M.S., A.L. Cruz-Rodz, Leimeister-Wachtes, J. Kieft and W. Goebel, 1989. A *Bacillus cereus* cytolytic determinant, cerolysin AB, which comprises the phospholipase C and sphingomyelinase genes: Nucleotide sequence and genetic linkage. J. Bacteriol., 171: 744-753.

Guinebretiere, M., V. Broussolle and C. Nguyen-The, 2002. Enterotoxigenic profiles of food poisoning and foodborne *Bacillus cereus* strains. J. Clin. Microbiol., 40: 3053-3056.

Guven, K., M.B. Mutlu and O. Avci, 2006. Incidence and characterization of *Bacillus cereus* in meat and meat products consumed in Turkey. J. Food Safety, 26: 225-228.

Hsieh, Y.M., S.J. Sheu, Y.L. Chen and H.Y. Tsen, 1999. Enterotoxigenic profiles and polymerase chain reaction detection of *Bacillus cereus* group cells and *B. cereus* strains from foods and foodborne outbreaks. J. Appl. Microbiol., 87: 481-490.

Lechner, S., R. Mayr, K.P. Francis, B.M. PrüB, T. Kaplan, E. WieBner-Gunkel, G.S.A.B. Stewart and S. Scherer, 1998. *Bacillus weihenstephanensis* sp. nov. is a new psychrotolerant species of the *Bacillus cereus* group. Int. J. Syst. Bacteriol., 48: 1373-1382.

Levy, H., M. Fisher, N. Ariel, Z. Altboum and D. Kobiler, 2005. Identification of strain specific markers in *Bacillus anthracis* by random amplification of polymorphic DNA. FEMS Microbiol. Letters, 244: 199-205.

Nilsson, J., B. Svensson, K. Ekelund and A. Christiansson, 1998. A RAPD-PCR method for large-scale typing of *Bacillus cereus*. Letters in Appl. Microbiol., 27: 168-172.

Rohlf, F.J., 1998. NTSYS-pc.Numerical taxonomy and multi-variate analysis system Version 2.02 Exeter software. State University of New York.

Roy, A., B. Moktan and P.K. Sarkar, 2007. Characteristics of *Bacillus cereus* isolates from legume-based Indian fermented foods. Food Control, 18: 1555-1564.

Sambrook, J. and D.W. Russel, 2001. Molecular Cloning: A Laboratory Manual. 3rd Edn. Cold Spring Harbor Laboratory Press.

Schraft, H. and M.W. Griffiths, 1995. Specific oligonucleotide primers for detection of lecithinase positive *Bacillus* spp. by PCR. Appl. Environ. Microbiol., 61: 98-102.

Smith, D.P., M.E. Berrang, P.W. Feldner, R.W. Phillips and R.J. Meinersmann, 2004. Detection of *Bacillus cereus* on selected retail chicken products. J. Food Protect., 67: 1770-1773.

Sokal, R.R. and C.D. Michener, 1958. A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull., 38: 1409-1438.

Sorokulova, I.B., O.N. Reva, V.V. Smirnov, I.V. Pinchuk, S.V. Lapa and M.C. Urdaci, 2003. Genetic diversity and involvement in bread spoilage of *Bacillus* strains isolated from flour and ropy bread. Letters in Appl. Microbiol., 37: 169-173.

Svensson, B., A. Eneroth, J. Brendehaug and A. Christiansson, 1999. Investigation of *Bacillus cereus* contamination sites in a dairy plant with RAPD-PCR. Int. Dairy J., 9: 903-912.

Svensson, B., K. Ekelund, H. Ogura and A. Christiansson, 2004. Characterization of *Bacillus cereus* isolated from milk silo tanks at eight different dairy plants. Int. Dairy J., 14: 17-27.

Valero, M., Hernandez-Herrero, L.A. and M.J. Giner, 2007. Survival, isolation and characterization of a psychrotrophic *Bacillus cereus* strain from a mayonnaise-based ready-to-eat vegetable salad. Food Microbiol., 24: 671-677.

Warke, R., A. Kamat, M. Kamat and P. Thomas, 2000. Incidence of pathogenic psychrotrophs in ice creams sold in some retail outlets in Mumbai, India. Food Control, 11: 77-83.

Welsh, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. Nuc. Acids Res., 18: 7213-7218.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rofalski and S.V. Tingey, 1990. DNA polymorphism amplified by arbitrary primers are useful as genomic markers Nuc. Acids Res., 18: 6531-6535.