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Original Article

Characterization of *Bacillus cereus* isolated from contaminated foods with sequencing of virulence genes in Egypt

Caracterização de *Bacillus cereus* isolado em alimentos contaminados com sequenciamento de genes de virulência no Egito

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Abstract

The current study describes the presence of *Bacillus cereus* (*B. cereus*) in contaminated foods of animal source and ready for human consumption with highlighting on their virulence contributing factors by detection of its virulence genes in addition to identification of their sequencing.

Three hundred sixty food samples categorized as (228) meat products and (132) milk products were examined for *B. cereus* isolation and all of these isolates were confirmed by biochemical tests. Eighteen strains obtained from different food samples were examined for the attendance of a number of virulence genes (*nheA*, *cytK*, *entFM*, *bceT* and *hblC* genes) using uniplex PCR method. Furthermore, the *B. cereus* strains were valued for the sequencing of described genes.

Generally 24.44% (88/360) food samples classified as 11.11% (40/360) meat products and 13.33% (48/360) milk products carried *B. cereus* according to cultural and biochemical properties, with geometric mean ($1.5\times10^7\pm0.15$ CFU/g or mL) . The highest counts (above 10^5 CFU/g or mL) were originated from milk products (with geometric mean $2.2\times10^7\pm0.22$ CFU/g or mL) more than meat products (with geometric mean $1\times10^7\pm0.19$ CFU/g or mL). The results revealed that all of our isolates had one or more virulence (enterotoxin) genes. In our research, the most predominant genes were *nheA* (100%), followed by *cytK* (61.11%), *entFM* (33.33%), *bceT* (11.11%) then *hblC* (5.56%). Molecular method detected that overall, 5 strains (27.78%) harbored only 1 gene (*nheA*), 7 strains (38.88%) harbored 2 genes which classified as 5 strains (27.78%) (*nheA* and *cytK*), 2 strains (11.11%) have (*nheA* and *entFM*). Moreover, 5 strains (27.78%) have 3 genes classified as 3 strains (16.67%) harbored (*nheA*, *cytK* and *entFM*), 1 strain (5.56%) had (*nheA*, *cytK* and *hblC*), and 1 strain (5.56%) had (*nheA*, *cytK* and *bceT*). Only 1 strain (5.56%) carried 4 tested virulence genes (*nheA*, *cytK*, *entFM* and *bceT*) genes. The most prevalent gene in meat and dairy foods was *nheA* (100%). The nucleotide sequences of (*bceT*, *cytK*, *entFM*, *hblC* and *nheA* genes) of *B. cereus* strains were deposited in GenBank under accession no. (MW911824, MW911825, MW911826, MW911827 and MW911828), respectively.

Our study was established to indicate the presence of virulent *B. cereus* in meat and milk products ready for human consumption as a result of deficient hygienic actions. So, a plain for good hygienic measures should be modified to avoid causing serious health problems to human due to ingestion of such products.

Keywords: *Bacillus cereus*, *nheA*, *cytK*, *entFM*, *bceT*, *hblC*.

Resumo

O presente estudo descreve a presença de *Bacillus cereus* em alimentos contaminados de origem animal e prontos para consumo humano, com destaque para seus fatores de contribuição de virulência por meio da detecção de seus genes de virulência, além da identificação de seu sequenciamento. Trezentas e sessenta amostras de alimentos categorizados como produtos cárneos (228) e produtos lácteos (132) foram examinadas para isolamento de *B. cereus*, e todos esses isolados foram confirmados por testes bioquímicos. Dezoito cepas obtidas de diferentes amostras de alimentos foram examinadas para a presença de uma série de genes de virulência (genes *nheA*, *cytK*, *entFM*, *bceT* e *hblC*) usando o método de PCR uniplex. Além disso, as cepas de *B. cereus* foram avaliadas para o sequenciamento dos genes descritos. De forma geral, 24,44% (88/360) das amostras de alimentos classificados como produtos cárneos (11,11%; 40/360) e produtos lácteos (13,33%; 48/360) transportavam *B. cereus*, de acordo com as propriedades culturais e bioquímicas, com média geométrica de $1,5\times10^7\pm0,15$ CFU/g ou mL. Os resultados revelaram que todos os nossos isolados tinham um ou mais genes de virulência (enterotoxina). Em nossa pesquisa, os genes mais predominantes foram *nheA* (100%), seguidos de *cytK* (61,11%), *entFM* (33,33%), *bceT* (11,11%) e *hblC* (5,56%). O método molecular detectou que, no geral, 5 cepas (27,78%) apresentavam apenas 1 gene (*nheA*) e 7 cepas (38,88%) continham 2 genes que foram classificados como 5 cepas (27,78%) (*nheA* e *cytK*), 2 cepas (11,11%) possuíam

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(*nheA* e *entFM*). Além disso, 5 cepas (27,78%) continham 3 genes classificados como 3 cepas (16,67%) hospedados (*nheA*, *cytK* e *entFM*), 1 cepa (5,56%) tinha (*nheA*, *cytK* e *hblC*) e 1 cepa (5,56%) teve (*nheA*, *cytK* e *bceT*). Apenas 1 cepa (5,56%) carregava 4 genes de virulência testados (*nheA*, *cytK*, *entFM* e *bceT*). As sequências de nucleotídeos (genes *bceT*, *cytK*, *entFM*, *hblC* e *nheA*) de cepas de *B. cereus* foram depositadas no GenBank sob o número de acesso (MW911824, MW911825, MW911826, MW911827 e MW911828), respectivamente. Nosso estudo foi estabelecido para indicar a virulência de *B. cereus* em carnes e produtos lácteos prontos para consumo humano como resultado de ações higiênicas deficientes. Portanto, deve ser estabelecido um plano com boas medidas de higiene para evitar sérios problemas de saúde humana por causa da ingestão de tais produtos.

Palavras-chave: *Bacillus cereus*, *nheA*, *cytK*, *entFM*, *bceT*, *hblC*.

1. Introduction

Bacillus cereus which categorized as an important bacterial species belongs to the genus *Bacillus* is described as large Gram-positive rods, facultative anaerobic, motile, strongly beta-hemolytic, have the ability to form spores in unfavorable circumstances and widely distributed in nature. It is usually found in soil and implicated in several foods (Felis et al., 2009), induced food poisoning symptoms in human and the level of contamination of meat products with *B. cereus* constitutes serious problems for consumers (Tawab et al., 2020). *B. cereus* contamination depends upon the production of several exogenous enzymes as hemolysins, phospholipases, proteases, and the ability to form biofilms, in addition to the presence of toxin-encoding genes which play an important role in its pathogenicity (Tirloni et al., 2020).

Various toxins produced by *B. cereus* play an important role in food safety as it cause health problems to consumers as a result of their ability to spoil food and causing diseases (Owusu-Kwarteng et al., 2017), these enterotoxins act on the epithelial cells of the small intestine through damaging its plasma membrane and causing diarrhea as a result of these tissue destructive proteins (Senesi and Ghelardi, 2010). Abdominal pain and diarrhea are *B. cereus* food poisoning symptoms which appears 8 - 16 hours subsequently to contaminated food consumption, then disappears generally within 12 - 24 hours. On the other hand more serious cases necessitating hospitalization have been defined; endocarditis; meningitis in addition to fatal cases were recorded (Logan and Rodríguez-Díaz, 2006). Foodborne outbreaks of *B. cereus* are generally benign and spontaneously resolved, however, also sometimes it may cause hospitalization of immunosuppressed persons or even death may occur (Bennett et al., 2013).

B. cereus enterotoxins included entFM which showing typical characteristic enterotoxin (Shinagawa et al., 1991). Another enterotoxin, bceT, was reported to be a diarrheal enterotoxin with biological activities related to diarrheal enterotoxins, such as fluid accumulation and vascular permeability in rabbit ileal loops and cytotoxic activities (Agata et al., 1995). Isolates of *B. cereus* strains that were implicated in many food poisoning outbreaks had a cytotoxic protein (CytK) of 34 kDa which was reported to cause necrotic enteritis accompanied with severe symptoms and bloody diarrhea (Lund et al., 2000). There are two *B. cereus* enterotoxins; hemolysin BL (Hbl) and non-hemolytic enterotoxin (Nhe); involved in food poisoning (Granum, 2001). Hemolysin BL consists of three antigenically different proteins which are: B, a binding factor and (L1and L2), which are two "lytic" factors. This *B. cereus* toxin causes the diarrheal type of food poisoning syndrome and other necrotizing infections like endophthalmitis (Beecher et al., 1995). The highest biological activity of Hbl was believed to be achieved by a 1:1:1 ratio of its three components (Schoeni and Wong, 1999). Non-hemolytic enterotoxin (NHE) is complex, consisting of three protein components which are: two lytic factors(nheA, nheB) and a binding factor (nheC) with molecular masses of 39, 45, and 105 kDa, respectively, and cytotoxicity can occur in low concentrations of this enterotoxin (Lund and Granum, 1996).

B. cereus pathogenicity could be related to a large number of secreted cytotoxins which may be associated with diarrheal disease, that is elicited by three pore-forming heat-labile enterotoxins; the two enterotoxin complexes which are (non-hemolytic enterotoxin, nhe) and (haemolysin BL, hbl), beside single-component toxin cytK "cytotoxin K" (Pfrunder et al., 2016). *B. cereus* is commonly documented as food poisoning agents, but strains can also play a role in causing localized wound and eye infections in addition to other systemic diseases (Ehling-Schulz et al., 2019). The food source used for isolation of *B. cereus* strains affect its cytotoxicity. The combinations of various *B. cereus* enterotoxin genetic factors are expressively related to the cytotoxic potential of this bacterium (Amor et al., 2019). The genomic sequencing (GS) has been applied in many clinical settings and researches as one of the current medical practices, also (GS) is well-known as an important portion for clinical diagnosis, research findings and ever more, precision medicine (Wynn et al., 2018).

For that reason, the goal of this work was to detect the incidence of *B. cereus* in contaminated food ready for human consumption with highlighting on their virulence contributing factor through detection of virulence factors (*nheA*, *cytK*, *entFM*, *bceT* and *hblC* genes) by using uniplex PCR and recognition of their sequencing.

2. Material and Methods

2.1. Ethical approval

In this research, we didn't use live animals; thus ethical approval was not necessary. Food (meat and milk products) samples were collected from various supermarkets and retail outlets in various zones at Dakahlia Governorate, Egypt.

2.2. Sampling

A total of 360 food samples categorized as 228 meat products (chicken luncheon, beef luncheon, kofta, minced meat, beef burger and sausage) (38 for each) and 132 milk products samples (pasteurized milk, raw milk, cheese and yoghurt) (33 for each) were obtained from January 2019 to December 2020 from various supermarkets and retail outlets in various areas at Dakahlia Governorate, Egypt. The food samples were weighed, marked clearly, collected separately in plastic bags, and transported in a sterile cool container to the laboratory. Samples were subjected to bacteriological examination. The time limit for carrying out microbiological analyzes after collecting the samples was within 1 to 2 days.

2.3. Bacteriological analysis

Twenty-five grams from each food sample was homogenized in 225 mL of 0.1% bacteriological peptone water (Oxoid, LTD England) and incubated at 37°C for 2h. Later, after incubation, serial dilutions were made and 0.1mLfrom each diluted sample was spread onto the surface of *Bacillus cereus* selective agar (BCSA) (Lab M, England) and incubated at 37°C for 48 h for counting of colonies on plates. The colonies with pink color (mannitol negative) and surrounded by heavy precipitate indicating lecithinase activity on BCSA and showing distinct β- hemolytic on blood agar were considered as *B. cereus*. The suspected colonies were identified by the following tests: Gram's staining (Gram-positive), presence of spores (positive), β-hemolytic activity on blood agar (positive), catalase test (positive), nitrate reduction test (positive) and Voges-Proskauer test (positive).

2.4. Molecular identification of B. cereus virulence genes

Uni-plex polymerase chain reaction assays were applied for the recognition of five enterotoxin virulence genes of *B. cereus* strains (*nheA*, *cytK*, *entFM*, *bceT* and *hblC* genes). DNA extraction from purified suspected colonies was performed using the conventional boiling method (Zinathi et al., 2015). The amplification reaction was performed using specific primers and profiles (as shown in Table 1). Each amplification process was performed in a 25 μL reaction mixture containing 12.5 µL of PCR Master Mix (enzynomics, Korea), 1 µL of forward primer, 1µL of reverse primers(Metabion international AG, Germany), 5.5 µLof nuclease free water and 5 µL of DNA template . The PCR conditions were: initial denaturation at 95ºC for 5 min followed by 30 cycles of secondary denaturation at 94ºC for 1 min for (*nheA*, *entFM* and *bceT*) and 94ºC for 45 sec for (*cytK*, *hblC*), annealing temperature depending on the primer pair used (Table 1), extension was made at 72ºC for 2 minutes. A final extension step was carried out at 72ºC for 7 minutes. The analysis of PCR products was applied by 1% agarose gel electrophoresis (iNtRON Biotechnology, Inc) in 1x TBE buffer stained with ethidium bromide, followed by visualization on UV trans-illuminator.

2.5. Sequencing of virulence genes of B. cereus

The purification of amplified products for five examined virulence genes (*nheA*, *cytK*, *entFM*, *bceT* and *hblC* genes) was established from one representative *Bacillus cereus* strain by a QIAquick PCR Product extraction kit (Qiagen Inc. Valencia, CA), and was sequenced with Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer, Foster city, CA), Kit used for purification of the sequence reaction (Centrisep, spin column), using an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA) according to the instructions of the manufacture.

The sequences were applied by Elim biopharmaceuticals (USA). A BLAST® analysis (Basic Local Alignment Search Tool) (Altschul et al., 1990) was initially made to identify sequence identity to GenBank accessions. Phylogenetic analyses were performed using maximum likelihood, neighbour joining and maximum parsimony in MEGA6 (Tamura et al., 2013).

2.6. Nucleotide accession number

In this investigation, the nucleotide sequences of the *B. cereus* strain, comprising the *bceT*, *cytK*, *entFM*, *hblC* and *nheA* genes were deposited in GenBank under accession no. (MW911824, MW911825, MW911826, MW911827 and MW911828), respectively.

Table 1. Primer sequences and annealing temperature of *B. cereus* virulence genes.

Target gene	Oligonucleotide sequence	Annealing temp (°C)	Product size (bp)	References
nheA	**F-5′** TACGCTAAGGAGGGGCA **′3**	51°C		Hansen and Hendriksen, 2001.
	R-5′ GTTTTTATTGCTTCATCGGCT **′3**	45 sec.	499	
entFM	**F-5′** ATGAAAAAAGTAATTTGCAGG **′3**	50°C		Asano et al., 1997.
	R-5′ TTAGTATGCTTTTGTGTAACC **′3**	45 sec.	1269	
bceT	**F-5′** TTA CAT TAC CAG GAC GTG CTT′**3**	55°C		Agata et al., 1995
	R-5′ TGT TTG TGA TTG TAA TTC AGG **′3**	45 sec.	428	
hblC	**F-5′** CCTATCAATACTCTCGCAA **′3**	54°C		Ngamwongsatit et al., 2008
	R-5′ TTTCCTTTGTTATACGCTGC **′3**	1 min.	695	
cytK	**F-5′** CGACGTCACAAGTTGTAACA **′3**	58°C		
	R-5′ CGTGTGTAAATACCCCAGTT **′3**	1 min	565	

3. Results

3.1. Prevalence of B. cereus in contaminated food samples

The prevalence of *B. cereus* was screened in the present study in food samples in accordance to cultural and biochemical properties. Of 360 samples, *B. cereus* strains were identified in 88 (24.4%) food samples. The *B. cereus* strains were observed in 11.1% (40/360) of meat products [12 chicken luncheon (31.6%), 9 sausage (23.7%), 7 beef burger (18.4%), 5 kofta (13.2%),4 beef luncheon (10.5%) and 3 minced meat (7.9%)] and 13.3%(48/360) from milk products [16 cheese (48.48%), 14 yoghurt (42.42%), 12 raw milk (36.36%) and 6 pasteurized milk (18.18%)]. The contamination level of *B. cereus* strains in examined food samples reveals that the bacterial concentrations were ranged from 3.3×10^6 to 6.4×10^7 CFU/g or mL in the examined food samples (Table 2) with geometric mean (1.5×10^7 ±0.15 CFU/g or mL). The highest counts (above 10^5 CFU/g or mL) were originated from milk products (with geometric mean 2.2×10^7±0.22 CFU/g or mL) more than meat products (with geometric mean 1×10^7±0.19 CFU/g or mL).

3.2. Molecular identification of B. cereus virulence genes

According to uniplex PCR assays, different virulence genes (*nheA*, *cytK*, *entFM*, *bceT* and *hblC* genes) of 18 different *B. cereus* strains were identified (Figure 1). Overall, 5 (27.78%) strains harbored only 1 gene (*nheA*), 7 strains (38.88%) harbored 2 genes which classified as 5 strains (27.78%) (*nheA* and *cytK*), 2 strains (11.11%) have (*nheA* and *entFM*), 5 strains (27.78%) have 3 genes classified as 3 strains (16.67%) harbored (*nheA*, *cytK* and *entFM*), 1strain (5.56%) had (*nheA*, *cytK* and *hblC*), 1 strain (5.56%) had (*nheA*, *cytK* and *bceT*). Particularly, only 1 strain (5.56%) carried 4 tested virulence genes (*nheA*, *cytK*, *entFM* and *bceT*) genes. From these results, all examined 18 (100%) strains had one or more enterotoxin virulence genes of *B. cereus* (Table 3). The most prevalent genes in meat products were *nheA* (100%), *cytK* (57.14%), *entFM* (42.86%), *bceT* (28.57%) and *hblC* (14.29%), while milk products showed prevalence of *nheA* (100%), *cytK* (63.64%), *entFM* (27.27%) and absence of both *bceT* (0%) and *hblC* (0%).

After identification of previous virulence genes of *B. cereus* using PCR technique, sequencing of all detected genes applied by Elim biopharmaceuticals (USA). In this study analysis of sequence data and submission were made and GenBank accession numbers for nucleotide sequence are as the following: BankIt2449539 Bacillus (MW911824), BankIt2449541 Bacillus (MW911825), BankIt2449542 Bacillus (MW911826), BankIt2449543 Bacillus (MW911827) and BankIt2449545 Bacillus (MW911828) (Figure 2).

Table 2. Total count of *B. cereus* in various food samples.

CFU/g or ml	Sample source	Sample ID
5.2×10^6	Chicken luncheon	NF1
6.1×10^7	Raw milk	NF3
3.3×10^6	Pasteurized milk	NF4
7.8×10^6	Yoghurt	NF5
4.4×10^7	Sausage	NF11
6.5×10^6	Minced meat	NF13
5.2×10^6	Kofta	NF16
3.8×10^7	Raw milk	NF19
3.4×10^7	Yoghurt	NF32
4.1×10^7	Chicken luncheon	NF36
3.6×10^6	Beef luncheon	NF97
6.4×10^7	Cheese	NF98
G (Geometric mean)=1.5×10^7 ±0.15 CFU/g or ml		

4. Discussion

Food poisoning in humans caused by virulent *B. cereus* strains was categorized as a serious public health worldwide originated from food of meat and milk origin (Owusu-Kwarteng et al., 2017; Tawab et al., 2020) so this study was done to detect *B. cereus* in such foods . In the present work, isolation of *B .cereus* strains from 360 different food samples showing that milk products have higher *B. cereus* prevalence than meat products, which may be due to contamination of udder and teats with large quantities of spore-forming bacteria (Scheldeman et al., 2005). The highest prevalence in meat products was detected in chicken luncheon while milk products showed the highest prevalence in cheese. This result indicates relatively middle contamination level of *B .cereus* in contaminated food intended for human consumption in Mansoura city, Dakahlia, Egypt. Similar results were noted down by other investigators who found that food samples had 23.35%, 24.3%, 26% and 27% of *B. cereus* strains (Abbas et al., 2014; Shawish and Tarabees, 2017; Gao et al., 2018; Tawab et al., 2020), respectively. In contrast, (Rahimi et al., 2013; Hwang and Park, 2015; Owusu-Kwarteng et al., 2017) found 42%, 47% and 50.51% of *B. cereus* in food, respectively. Whatever, the food must be free from *B. cereus* to be considered fit for human consumption, so, it is generally advised to food

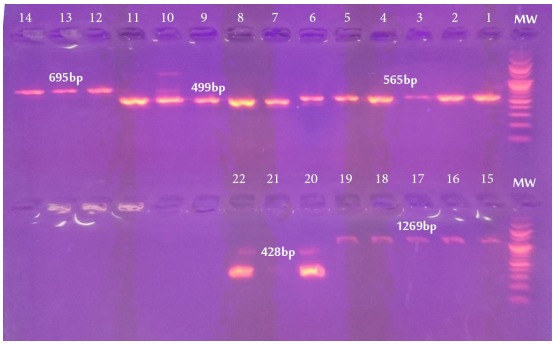

Figure 1. PCR analysis of virulence genes of *B. cereus*. Lanes: MW-DNA ladder (100bp); *cytK* (565bp) 1-NF1; 2-NF11; 3-NF3; 4-NF32; 5-NF36; 6-NF14; *nheA*(499bp) 7- NF1; 8-NF11; 9-NF5; 10-NF32; 11-NF98; *hblC*(695bp)12-NF98; 13-NF98;14-NF98; *entFM*(1269bp)15- NF1; 16-NF11;17 –NF19; 18-NF32; 19-NF14; *bceT*(428bp) 20-NF1;21- NF36; 22-NF1.

Table 3. Pattern of virulence genes of *B. cereus* obtained from various food samples.

Virulence genes					Sample source	Sample ID
bceT	***hblC***	***cytK***	***entFM***	***nheA***		
-	-	-	-	+	Sausage	NF43
-	-	-	-	+	Kofta	NF16
-	-	+	+	+	Sausage	NF11
-	-	-	+	+	Beef luncheon	NF97
-	+	+	-	+	Cheese	NF98
-	-	-	-	+	Cheese	NF99
-	-	+	-	+	Yoghurt	NF17
-	-	+	+	+	Yoghurt	NF14
-	-	-	-	+	Yoghurt	NF35
-	-	+	-	+	Yoghurt	NF5
-	-	-	+	+	Raw milk	NF19
	-		-	+	Raw milk	NF3
	-					
	+					
-	-	-	-	+	Cheese	NF25
-	-	+	-	+	Minced meat	NF13
+	-	+	+	+	Chicken luncheon	NF1
-	-	+	-	+	Pasteurized milk	NF4
-	-	+	+	+	Yoghurt	NF32
+	-	+	-	+	Chicken luncheon	NF36
2 (11.1%)	1 (5.56%)	11 (61.11%)	6 (33.33%)	18 (100%)	**Total (%)**	

industries to put this point in their considerations as foods that have 10^5 CFU of *B. cereus* per gram are categorized as unfit for consumption because the emetic and diarrheal syndromes of *B. cereus* can take place when the *B. cereus* concentration level is high (Public Health England (PHE), 2009), so according to our results *B. cereus* obtained can cause both emetic and diarrheal syndromes. The implicated foods that have been involved in most of *B. cereus* outbreaks worldwide have concentrations more than 10^5 CFU/g (Louisiana Department of Health and Hospitals, 2013). Food-borne outbreaks in humans caused by the opportunistic pathogen *Bacillus cereus* after ingestion of contaminated foods, which have count in excess of 10^4–10^5 vegetative cells or spores of *B. cereus* per gram, so, gastrointestinal diseases can occur in two forms, which are emesis and diarrhea (Bamnia and Kaul, 2015). RTEs (Ready- to- Eat Foods) were classified according to bacterial concentration of *B. cereus* into four classes which are: acceptable, satisfactory, unsatisfactory and potentially harmful (>10^4 CFU/g) (NSW Food Authority, 2009). Also, in England "unsatisfactory" RTE foods are described as those of >10^5 CFU of *B. cereus* per gram (Health Protection Agency, 2009). Hong Kong categorizes RTE foods according to the presence of *B. cereus* as: satisfactory (<10^3 CFU/g), acceptable (10^3–10^5 CFU/g), or unsatisfactory (>10^5 CFU/g), and the "unsatisfactory" RTE foods sale must be forbidden (Centre for Food Safety, 2014). Therefore, proper temperature control must be taken in considerations, so even during food service; cold foods must be preserved cold (lower than 40 degrees F) while hot foods have to be kept hot (more than 140 degrees) to avoid contamination of food with *B. cereus* (Medeiros and LeJeune, 2015).

In another study, *B. cereus* isolates had *cytK* (90%), *hbl* (70%) and *nhe* (80%) enterotoxigenic virulence genes, respectively, (Tawab et al., 2020) that were (*cytK* and *hbl*) higher while nhe was lower than this study. The same as our study *nheA* was detected in all (100%) of the isolates, while none of the isolates amplified the gene, *hblC*, which was detected at very low incidence in this study (Zinathi et al., 2015). Other investigators detected *entFM*, *nheA*, *hblC* and *bceT* enterotoxigenic genes isolated from foods were detected with the frequencies of 61.90%, 44.04%, 34.52% and 19.04%, respectively (Rahimi et al., 2013) which are higher than results obtained in this study except *nheA* which is lower than that we obtained. Also *cytK* gene was detected in all strains (100%) while *hblC* in 90% of strains (Shawish and Tarabees, 2017) which were found to be higher than this study. The presence of both *cytK* and *hblC* genes (66.7%) was dominant between *B. cereus* enterotoxin virulence genes, followed by *cytK* (25%) then *hblC* (8.3%) (Tharwat et al., 2020).

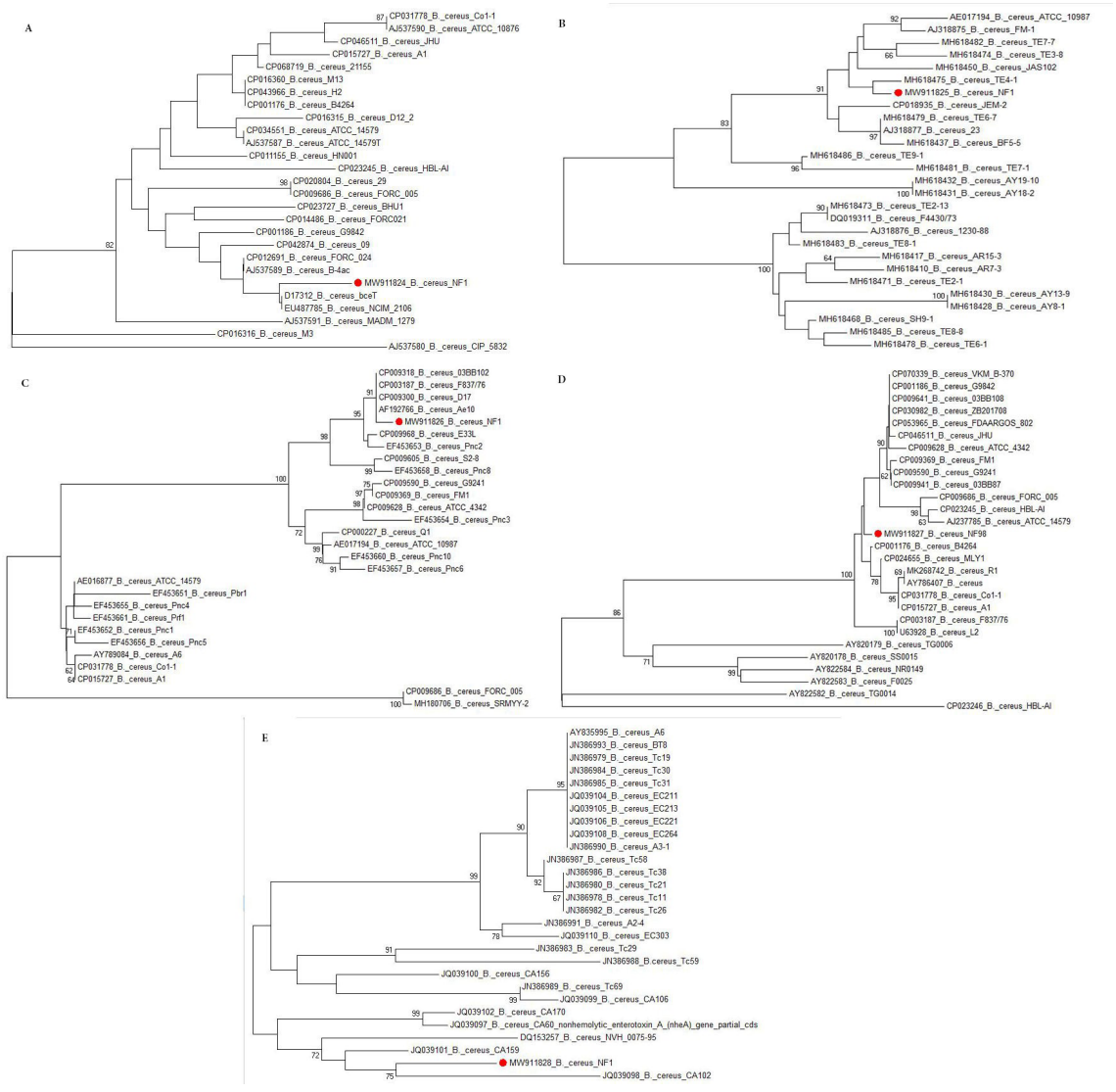

Figure 2. Phylogenetic trees viewing the genetic relatedness among *B. cereus* strains according to nucleotide sequence analysis of (A) *bceT* gene (MW911824 -B.-cereus.-NF1), (B) *cytK* gene (MW911825 -B.-cereus.-NF1), (C) *ent FM* (MW911826 -B.-cereus.-NF1), (D) *hbl C* (MW911827 -B.-cereus.-NF98) and (E) *nhe A* (MW911828- B.-cereus.-NF1). The circles in red indicate our strains accession number and its position in comparison to other isolates according to the nucleotide sequence in the GenBank.

PCR for *B. cereus* strains showed that *hblC*, *bceT* and *entFM* were detected at different percentages 64%,57% and 94%, respectively (Kim and Batt, 2008) which were higher than this study. Other profiles revealed that 52.5% of *B. cereus* strains had *B. cereus* genes (*hblC* and *nheA*) (Samapundo et al., 2011).

Of all the tested food samples, 35% were *B. cereus* positive, with 49 and 89% of the isolates had the enterotoxin-encoding genes of *hblC* and *nheA*, respectively. Also, all strains of *B. cereus* carried *entFM* gene while *cytK* gene was found in 68% of the obtained isolates (Yu et al., 2020) which are higher than our results except *nheA*.

Various foods were categorized as a source of *B. cereus* isolates (Yu et al., 2019) that causes foodborne diseases (Marrollo, 2016) and produces different virulence factors that could pass into the gastrointestinal tract through consumption of such foods, as a result, it can cause diarrhea and vomiting (Song et al., 2019). Four different *B .cereus* enterotoxins which are, hemolysin BL (HBL) that encoded by *hbl* gene, non-hemolytic enterotoxin (NHE) that encoded by *nhe*, while enterotoxin FM (EntFM) encoded by *entFM* and the cytotoxin K (CytK) encoded by *cytK* were involved in causing diarrhea (Berthold-Pluta et al., 2019). In addition to food poisoning, *B. cereus* strains are also involved in many severe infections as endocarditis, bacteremia, endophthalmitis, pneumonia, osteomyelitis and necrotizing fasciitis (Ikeda et al., 2015). So, the extensive studies have been applied to detect different virulence genes of *B. cereus*, as *hblC*, *nheA*, *entFM* and *cytK* by PCR (Yu et al., 2019) and *bceT* (Agata et al., 1995). In our study,

the virulence genes concerning *nheA*, *cytK*, *entFM*, *bceT* and *hblC* were detected in *B. cereus* strains obtained from contaminated food samples. Hence, serious illness as food poisoning (diarrheal and emetic type) can occur from the ingestion of such foods in humans. Furthermore, the *bceT* gene sequence in our isolated *B. cereus* was seem to be identical by (95.2% to 99.5%), while *cytK* gene (94.4% to 98.8%), *entFM* gene (89% to 99.8%), *hblC* gene (82.3% to 99.5%) and *nheA* gene (92% to 96%) identity with the other *B. cereus* strains according to Gene Bank sequences.

5. Conclusion

Foods contamination by virulent bacteria such as *B. cereus* is a major food safety concern; therefore, it is essential to screen and characterize *B. cereus* as a source of foods contamination and important food poisoning agent. So, it is essential to increase hygienic measures during the handling of meat and milk products to reduce public health hazards. Also, coordinated measures are essential to decrease or prevent the risks caused by *B. cereus* at different stages in the food chain.

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