REVIEW ARTICLE AGROBIOLOGICAL RECORDS
ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

PATHOGENICITY OF FEED-BORNE *BACILLUS CEREUS* AND ITS IMPLICATION ON FOOD SAFETY

Md Atiqul Haque [1, 2], Hongkun Quan [1], Zonghui Zuo [1], Ahrar Khan [3], Naila Siddique [4] and Cheng He [1,*]

[1]Key Lab of Animal Epidemiology and Zoonoses of Ministry of Agriculture, College of Veterinary Medicine, China Agricultural University, Beijing, 100193, China
[2]Department of Microbiology, Faculty of Veterinary & Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur, 5200, Bangladesh
[3]Shandong Vocational Animal Science and Veterinary College, Weifang, 261061, China
[4]National Reference Lab for Poultry Diseases (NRLPD) Animal Sciences Institute, National Agricultural Research Centre, Islamabad, Pakistan

∗Corresponding author: *hecheng@cau.edu.cn*

ABSTRACT

Bacillus cereus (*B. cereus*) is a novel emerging pathogen contaminated extensively in animal feed and food chains, posing a huge economic loss for animal industry and high risk for human health. This pathogen is a robust omnipresent heat resistant spore former, able to form biofilm and isolated from different environments such as food and atmosphere that occur all year round without any particular geographic distribution. The potential of survival for *B. cereus* spores in unfavorable conditions pose a considerable threat to food safety and also cause economic losses to the food industry. *B. cereus* aggravates acute diarrhea and malnourishment in poultry by inducing gizzard erosion and ulceration (GEU). It will facilitate persistent other bacterial infection in the lungs via damaging gastric intestine tract. Also, it can cause serious food safety because it seems difficult to fully prevent their presence in food. It may cause gastrointestinal diseases that trigger emetic and diarrheal symptoms as well as general and local infections related to the respiratory tracts of immunologically threatened individual and newborns. *B. cereus* produces a wide range of potential virulence factors, including heat stable/labile toxins (cerulide, NHE, HBL, CytK, Ent-FM, bc-D-ENT, CLO, HlyII, HlyIII) and tissue-destructive enzymes (PI-PLC, PC-PLC, SMase, β-lactamase, InhA1, NprA), but their roles and molecular mechanism in specific infections have not been clarified yet. This review provided a historical record of possible risk factors and pathogenesis of animal industry and highlights the implications for animal industry and food safety by ingestion of the feed-borne *Bacillus cereus*.

Keywords: *Bacillus cereus*; emetic toxin; enterotoxin; pathogenicity; food safety

Article History (2020-0621) || Received: 23 Jun 2020 || Revised: 18 Jul 2020 || Accepted: 29 Jul 2020 || Published Online: 11 Aug 2020

1. Introduction, Background and Taxonomy

The term "bacillus" implies a "small rod," whereas the Latin word "cereus" refers to "wax-like" whereby the easily identifiable *Bacillus cereus* phenotype can be easily recognized under a microscope or on agar and other blood-containing selective media (Arnesen et al. 2008). It was originally isolated from air in a cowshed by Frankland and Frankland in 1887 and was discovered in 1906 by Plazikowski related to food poisoning in Europe (Griffiths and Schraft 2017). The genus *Bacillus* was formed in 1920 and has undergone major taxonomic changes over the past 30 to 40 years, and several of its members belong to many seemingly diverse classes of rDNA sequences including "*B. subtilis* group", "*B. cereus* group" and "*B. sphaericus* group" (Logan 2011). Phenotypic characteristics of *B. cereus* group species used for taxonomic classification (e.g., motility and hemolysis) differ in and across different species. In addition, the genomic determinants subject to certain phenotypes are regulated by plasmid, in particular production of anthrax toxin/capsular proteins, bio-insecticidal crystal proteins, and synthetase proteins of the emetic toxin (cereulide). These features may be lost, retrieved, heterogeneous within one species, or present in many kinds (Carroll et al. 2020). Carroll et al. recently proposed a genomics-based bacterial taxonomy of *B. cereus* group which are i) genomospecies as, *B. pseudomycoides, B. paramycoides, B. mosaicus, B. cereus s.s., B. toyonensis, B. mycoides, B. cytotoxicus,* and *B. luti* ii) putative genomospecies as, *B. bingmayongensis, B. gaemokensis, B. manliponensis*, and *B. clarus* iii) Subspecies as, *B. mosaicus* subsp. *anthracis*, *B. mosaicus* subsp. *cereus* iv) Biovars as Biovar Anthracis,

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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Biovar Emeticus, Biovar Thuringiensis. Another such subcluster includes *B. amyloliquefaciens*, *B. licheniformis*, and other close relatives of *B. subtilis* – the *B. subtilis* group (Logan and Halket 2011). *B. cereus* was the fourth factors of foodborne outbreaks reported in the European Union and the second leading cause of confirmed foodborne outbreaks in France (Zhang et al. 2017). In China, it is recognized as the second agent of foodborne diseases, 4342 cases of *B. cereus* outbreaks were reported between 2011 and 2016 (Cui et al. 2016a; Liu et al. 2019; Ducrest et al. 2019). It ranks the third pathogen after the species *Salmonella* and *Staphylococcus aureus* among cumulative food poisoning infections, and this opportunistic pathogen contributes to vomiting and diarrheal syndromes in animal and humans (Zhang et al. 2019).

2. Prevalence of *B. cereus*-derived Food Poisoning

The worldwide distribution of *B. cereus* can be identified in a number of ready-to-eat (RTE) foods, raw milk and dairy products, meat and meat products, spices, peas, beans, dried foods, raw and cooked vegetables, potatoes, eggs, vanilla, sauces, custards, soups, ice cream and in various cereals especially in rice (Eglezos et al. 2010; Abdou et al. 2012; Abraha et al. 2017; Cadirci et al. 2018). The prevalence of food poisoning cases in humans worldwide are summarized in Table 1.

Table 1: Prevalence of human case of *B. cereus* food poisoning in Asia, America, Australia and European countries

Country/region	Year	Prevalence %	References
China mainland	1992-2001	6.8	Zhang et al. (2017)
China	1986-1995	18.0	Tewari and Abdullah (2014)
	1994	14.9	Kotiranta et al. (2000)
	1991-2005	11.2	Raddadi et al. (2010)
Japan	1973-1985	0.7	Kotiranta et al. (2000)
USA	1972-1982	1.3	Kotiranta et al. (2000)
	1982-1997	1.0	Raddadi et al. (2010)
	1993-1997	0.8	Schoeni and Wong (2005)
	1998-2008	1.74	Bennet et al. (2013)
North America	1998-2000	7.0	Tewari and Abdullah (2014)
Brazil	2003-2013	3.1	Lentz et al. (2018)
Australia	1980-1995	7.4	Bamnia and Kaul (2015)
	2001-2007	2.4	Eglezos et al. (2010)
Canada	1973-1985	2.2	Kotiranta et al. (2000)
Denmark	2017	6.3	Anonymous (2018)
European Union	2007	17.1	Tewari and Abdullah (2014)
	2007-2014	27.6	EFSA (2016)
England	1973-1985	0.7	Schoeni and Wong (2005)
France	1998-2000	4-5	Tewari and Abdullah (2014)
Finland	1973-1985	17.8	Schoeni and Wong (2005)
	1992	22	Adam and Moss (2005)
Germany	2008-2009	21	Pichner et al. (2014)
Italy	2014-2015	26.8	Proroga et al. (2019)
Scotland	1973-1985	0.8	Schoeni and Wong (2005)
Norway	1988-1993	33.0	Adam and Moss (2005)
	2000	32.0	Osman et al. (2018)
Iceland	1985-1992	47.0	Adam and Moss (2005)
Netherlands	1973-1985	11.5	Kotiranta et al. (2000)
	1991	8.5	Adam and Moss (2005)
	1991-1994	19.0	Schoeni and Wong (2005)
	1993-1998	12.0	Arnesen et al. (2008)
	2006	5.4	Osman et al. (2018)
Hungary	1960-1968	15.0	Kotiranta et al. (2000)

3. Morphology, Colony and Biochemical Characteristics, and Pathogenesis

3.1. Morphology, Colony and Biochemical Characterization

Bacillus cereus is commonly regarded as a mesophilic body growing at a temperature of 10 to 50 °C (ideal, 35-40 °C), with a pH of 4.9-9.3 and a moisture content of 0.92-1.0, their cells are somewhat larger (1.0-1.2 µm widths; 3.0-5.0 µm lengths) rod (Fig. 1), forming large (3-8 mm in breadth), gray colonies having a very flat and "ground-

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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glass" appearance, often with rough edges varying from circular, entire and fimbriate producing zones of β-hemolysis surrounding colonies on agar media; dull gray or greenish colored colonies with dull and a raw matted surface aerobically on 5% sheep blood agar at 37°C (Fig. 2); straight or somewhat curved slender rods, grouped individually in shorter chains with even edges in broth cultures (Schoeni and Wong 2005; Arnesen et al. 2008; Bottone 2010; Abraha et al. 2017). Spores are central, ellipsoidal or cylindrical in shape and do not cause swelling in the sporangium appear green in a red vegetative cytoplasm cell that contains black lipid globules in intracellular lipid stain (Schoeni and Wong 2005; Adams and Moss 2005). *B. cereus* creates a precipitation zone around suspected colonies due to hydrolysis of egg yolk (phospholipase activity) and appears pink (Fig. 3) or peacock blue (Fig. 4) color on MYPA (mannitol–egg yolk–phenol red–polymyxin agar) and PEMBA (polymyxin–pyruvate-egg yolk–mannitol–bromothymol blue agar) respectively (Arnesen et al. 2008). Biochemical test is to check whether *B. cereus* is able to generate acid from glucose but not from mannitol, xylose and arabinose; oxidase negative, motility positive, catalase positive, citrate utilization positive, casein hydrolysis positive, nitrate reduction positive, and Voges-Proskauer (VP) reaction positive, l-tyrosine reduction positive, and growth in 0.001% lysozyme positive (Adams and Moss 2005; Vilas-Boas et al. 2007; Griffiths 2010).

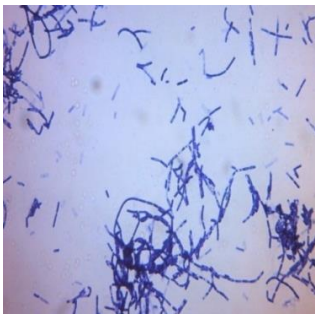

Fig. 1: *Bacillus cereus* on gram stain (100X)

Fig. 2: *Bacillus cereus* on blood agar

Fig. 3: *Bacillus cereus* on MYPA agar

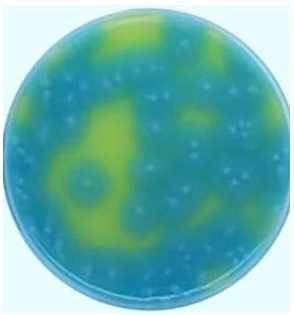

Fig. 4: *Bacillus cereus* on PEMBA agar

3.2. Pathogenesis

Bacillus cereus produces various virulence factors that are summarized in Table 2, however, the actual contribution and importance of these diseases-causing factors is largely unknown. It may cause two different and distinct forms of foodborne diseases: the emetic (vomiting) type that resembles food poisoning from *Staphylococcus aureus* is due to emetic toxin known as cereulide, whereas the diarrheal type that resembles from food poisoning *Clostridium perfringens* is due to many enterotoxins (Forghani et al. 2014). Both forms of diseases are generally self-limiting, but some serious or extreme cases have been reported (EFSA 2005). The virulence potency of *B. cereus* increases at an ever greater population rate, due to the production of a large number of virulence factors, including enterotoxins and depsipeptide toxin cereulide (*Ces*) triggered by the master controls, Pleiotropic regulator (PlcR), a chromosome-encoded transcriptional regulatory protein, and a key sporulation regulator, Spo0A (Ehling-Schulz et al. 2019). PlcR is an important global pleiotropic regulator of at least 45 genes, including pathogenic-related enterotoxins and phospholipases that may induce sporulation of bacteria in the formation of biofilms (EFSA 2016; Ehling-Schulz et al. 2019). When sporulation occurs in bacteria, the Spo0A transition state regulator suppresses plcR transcription and hence PlcR-regulated gene expression, and the formation of biofilm facilitates the generation of adhesive spores and contributes to a high resistance (Guillemet et al. 2009). *B. cereus* virulence is also closely linked to flagella and motility, aeration, the oxidation–reduction potential (ORP), and nutrients like the carbon source or iron involving the environmentally-sensitive proteins Fnr, ResDE, CcpA, and CodY including another, to date least defined, two element regulatory regimes (Ceuppens et al. 2011). Moreover, CodY is also a key pleiotropic regulator involving the growth and survival of this bacteria in various settlements including soil, food, insect guts and the human body and it performs an essential function in regulating and timing the expression of virulence gene within the *B. cereus* group (Ehling-Schulz et al. 2019). Furthermore, the flagellar protein FlhF is necessary for optimal virulence, possibly due to the effect on protein synthesis and excretion, as well as the ability of gastric mucosa to sustain bacterial cells and probably to preserve enterotoxins from intestinal decay (Rossi et al. 2018).

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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Table 2: Virulence factors of *B. cereus*

Virulence factors	Characteristic	Encoded gene
Toxins		
Emetic toxin		
Cereulide (Ces)	Heat-stable; a cyclic dodecadepsipeptide (1.2 kDa) toxin that is resistant to heat, pH, and proteolysis but is not antigenic	*ces (cesA* and *cesB)*
Enterotoxin		
Hemolysin BL /HBL (HblACD)	Heat-labile, three component proteins, a binding component, B (35 kDa), & two lytic components, L1 & L2 (36 & 45 kDa); pore-forming toxin;	*hblA, hblC, hblD*
Nonhemolytic enterotoxin (NheABC)	Heat-labile, three component proteins NheA, NheB & NheC (39, 45 & 105 kDa) pore-forming toxin	*nheA, nheB, nheC*
Cytotoxin K (CytK)	Heat-labile, single enterotoxic protein (34 kDa), β-barrel pore-forming toxin; two different forms, CytK-1 and CytK-2	*cytK1, cytk2*
Enterotoxin FM (Ent-FM)/ CwpFM	Single enterotoxic protein (45 kDa), cell wall peptidases	*entFM*
Enterotoxin T (bc-D-ENT)	Single enterotoxic protein (40/41 kDa), diarrheal toxigenicity	*bceT*
Hemolysins		
Hemolysin I (HlyI) / Cereolysin O (CLO)	Heat-labile, thiol-activated hemolysin, cholesterol-binding pore-forming toxin	*clo*
Hemolysin II (HlyII)	Heat-labile, β-barrel channel-forming toxin	*hlyII*
Hemolysin III (HlyIII)	Hemolytic activity via pore formation on erythrocytes	*hly-III*
Enzymes		
Phosphatidylinositol hydrolase/ phosphatidylinositol specific phospholipases C (PI-PLC)	Breakage of the protein anchorage on plasma membranes	*piplC*
Phosphatidylcholine hydrolase/ lecithinase / Phosphatidylcholine specific phospholipases C (PC-PLC)	General hydrolytic action	*pcpIC*
Sphingomyelinase (SMase)	Hemolytic protein that binds to sphingomyelin on erythrocytes	*sph*
Cerolysin AB	Two components (PC-PLC+SMase) cytolysin, which acts synergistically in lysing human eythrocytes	*cerAB*
Beta-lactamases		
β-lactamase I	Class A β-lactamases and is an extracellular penicillinase with a serine in the active site	*bla1*
β-lactamase II	Class B β-lactamase, is activated by binding Zn (II) and Co(II) ions	*bla2*
β-lactamase III	Class A membrane bound lipoprotein also having a secreted form	*Blm*
Camelysin	A cell-bound metalloprotease	-
Immune inhibitor A1 (InhA1)	A zinc metalloprotease	*inhA*
Bacillolysin/ NprA	A metalloprotease	*nprA*
Neutral metallopeptidases	Proteolytic activity	*Npr*
IlsA	Iron-regulated, leucine-rich surface protein, iron deprivation in the host	*ilsA*

References: Aragon-Alegro et al. (2008); Bhunia (2008); Cadirci et al. (2018); Cadot et al. (2010); Chaves et al. (2017); Cui et al. (2019); EFSA (2016); Ehling-Schulz et al. (2019); Fedhila et al. (2006); Guillemet et al. (2009); Heini et al. (2018); Kotiranta et al. (2000); Lindback (2004); Mallozzi et al. (2010); Martínez-Blanch et al. (2011); Minnaard et al. (2007); Mohkam et al. (2020); Montanhini et al. (2013); Nduhiu et al. (2009); Özdemir and Arslan (2019); Raddadi et al. (2010); Rouzeau-Szynalski et al. (2020); Tahmasebi et al. (2014); Tran et al. (2010); Vilas-Boas et al. (2007); Zhao et al. (2020).

The majority of *B. cereus* virulent factors belong to the PlcR, triggered during beginning of the stationary period by a small guiding peptide (PapR) being a quorum-sensing agent regulating the expression of many *B. cereus* toxins such as Nhe, Hbl, CytK, hlyI, SMas and PC-PLC, that may lead to the diarrhea by rupturing the epithelium (Fedhila et al. 2006; Guillemet et al. 2009; Tran et al. 2010; Ceuppens et al. 2011; Ehling-Schulz et al. 2019). Many of these toxins are predominant in diarrheal strains and PlcR-regulated genes (*hbl*, *nhe* and *cytK*) appear to show a significant role in pathogenesis at the time of *B. cereus* gastroenteritis and related diseases. Obliteration of *plcR* decreases, but does not eradicate, bacterial virulence in different infection models (rabbit, mice and insect), indicating that other PlcR-independent factors, such as EntFM, InhA1, HlyII, and IlsA, have been found to contribute in *B. cereus* pathogenicity (Fedhila et al. 2006; Tran et al. 2010). The emetic toxin, Ces is generated by only some *B. cereus* strains (*B. cereus s.s.* and *B. weihenstephanensis*), and is chemically associated with the potassium ionophoric antibiotic, valinomycin which is non-antigenic and highly acid-resistant, as well as proteolysis and heat, tolerates gastric acid,

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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intestinal proteolytic enzymes, and by reheating foods stored at room temperature following a first heating (Arnesen et al. 2008; Griffiths 2010; Chaves et al. 2017; Mohkam et al. 2019).

Finally, identification of *B. cereus* by biochemical test, microscopic observation and target genes by PCR and various lesions and the risk of toxins produced by *B. cereus* has been shown in Table 3 and 4, respectively.

Table 3: Identification of *B. cereus* by biochemical test, microscopic observation and target genes by PCR

Biochemical tests		**Target gene by PCR**			**References**
Tests	Result	Gene		Prevalence %	
Catalase	+	*hbl*	*hblA*	29–92	Ehling-Schulz et al. (2005); Vilas-Boas et al. (2007); Aragon-Alegro et al. (2008); Ceuppens et al. (2011); Abdou et al. (2012); Jonkuvienė et al. (2012); Di Pinto et al. (2013); Abraha et al. (2017); Chaves et al. (2017); Carter et al. (2018); Cui et al. (2019); Adame-Gómez et al. (2020)
Motility	±		*hblB*		
Nitrate reduction	±		*hblC*		
Tyrosine degradation	+		*hblD*		
Lysozyme resistance	+	*nhe*	*nheA*	84– 100	
Egg yolk hydrolysis	+		*nheB*		
Glucose utilization (anaerobic)	+		*nheC*		
Voges–Proskauer	+	*cytK*	*cytK-1*	37–89	
Starch hydrolysis	+		*cytK-2*		
Hemolysis	+	*entFM*		84– 100	
Citrate utilization	+	*bceT*		12–75	
Gelatin hydrolysis	+	*hlyII*		19–56	
Casein hydrolysis	+	*Ces*	*CesA*	1.5-32.8	
Oxidase	-		*CesB*		
Acid from mannitol	-	Microscopic feature			
Xylose fermentation	-	Gram + (purple colored), rod shaped with short to long chains			

Positive = +; Usually positive but occasionally may be negative = ±; Negative = -

4. Pathogenesis of Gizzard Erosion and Ulceration (GEU) and Hemorrhagic Pneumonia

4.1. Gizzard Erosion and Ulceration (GEU)

Although animal feeding experiments suggest that its mechanism is receptor-mediated, the mechanism behind cereulide activity in humans is not yet fully clarified. Upon absolve through the stomach towards duodenum, the toxin binds to serotonin 5-HT3 receptors acts as an ionophore cation (K+) and accelerates mitochondrial K+ absorption, H+ efflux, and decrease in intramembrane capacity, detention of respiratory activity, impairment of ATP production and ease of proapoptotic or necrotic factors thus inhibiting mitochondrial activity and fatty acid oxidation, causing liver failure (Arnesen et al. 2008; Griffiths 2010; Popoff 2011). The emetic syndrome is rather serious than the diarrhea and the diarrheal syndrome is very mild but the enterotoxins (Nhe, Hbl, and CytK) released destroy the intestinal epithelial layer and trigger diarrhea (Vaughan et al. 2003; Tsilia et al. 2016).

In vitro experiment it is observed that *B. cereus* is typically immune to low pH in the stomach and adheres to intestinal surfaces thus could rise the bacterial propagation time leading to enterotoxin production near the epithelial layer (Tsilia et al. 2016). In our previous study, it is observed that feed-borne *B. cereus* co-infection with avian influenza virus (H9N2) has produced significant gizzard erosions and ulceration (GEU) in all bird groups that contribute to damage to the epithelium of the digestive tract, which facilitates other susceptible pathogens (Zhang et al. 2019). Importantly, chickens exposed to *B. cereus* co-infection with *Chlamydia psittaci* developed a severe GEU suggesting that damage to the koilin layer of the gizzard with its toxins, may instantly affect the gizzard membrane, while intra-esophageal *C. psittaci* infection also promotes the development of the GEU. In addition, two secreted toxins Hbl and Cytk damage the koilin layer of the gizzard, resulting in long term ulceration inducing pores in epithelial cells, necrosis, mucosal damage and contributing to diarrhea by destroying the digestive tract (Zuo et al. 2020). *B. cereus* strains have *B. anthracis* pX01 toxin genes (Hoffmaster et al. 2006; Bottone 2010).

4.2. Hemorrhagic Pneumonia

Research reports have shown that in apparently healthy welders suffered from the life-threatening pneumonia led by *B. cereus* (Hoffmaster et al. 2006). In our previous study, it was also shown that cross-contamination of *B. cereus* with avian influenza (H9N2) virus induced typical hemorrhagic pneumonia where feeds were mostly found to be contaminated with *B. cereus* and possibly immunosuppression due to bacteria aggravated respiratory infections, indicating that *B. cereus*, as a primary or permanent latent infection may provoke lung inflammation *in vivo*, and can enhance other-pathogens susceptibility (Zhang et al. 2019). In our recent, study exposure to feed borne *B. cereus*

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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Table 4: Different lesions and risk of toxins produced by *B. cereus*

Toxin/enzymes	Lesions and toxicity	References
Ces	Hepatocyte degeneration, liver damage acute meningoencephalitis, respiratory distress, bioaccumulation in kidney, liver, muscles and fat tissues, cerebral effects, induction of diabetes, mitochondrial swelling in Hep-2 cells, necrotic cell death in porcine pancreatic Langerhans cells, emesis, beta cell dysfunctions, inhibit human natural killer cells, immune system impairment	Kotiranta et al. (2000); Lindback (2004); Ehling-Schulz (2005); Sergeev et al. (2006); Minnaard et al. (2007); Arnesen et al.)2008); Bhunia (2008); Cadot et al. (2010); Griffiths (2010); Tran et al. (2010); Ceuppens et al. (2011); Martínez-Blanch et al. (2011); Popoff (2011); Oda et al. (2012); Sastalla et al. (2013); Forghani et al. (2014); Cui et al. (2016a); EFSA (2016); Bartoszewicz and Czyżewska (2017); Tausch et al. (2017); Yang et al. (2017); Bauer et al. (2018); Cui et al. (2019); Ehling-Schulz et al. (2019); Mohkam et al. (2020); Özdemir and Arslan (2019); Nguyen and Tallent (2019)
HBL	Intestinal fluid secretion, disrupting osmotic equilibrium, pore formation, hemolysis, Cycotoxicity to Vero cells, retinal tissue, Chinese hamster ovary cells, dermonecrotic and vascular permeability activities	
NHE	Intestinal fluid secretion, transmembrane pore formation, lysed Vero cells, Osmotic lysis and cell death	
CytK	Bind to the cell membrane, forming transmembrane pores, hemolysis, fatal bloody diarrhea, necrosis, highly toxic to Caco 2 and Vero cells)	
EntFM	High doses fluid accumulation in rabbit and mouse ligated intestinal loop, capillary permeability, hemolysis, cytotoxic to Vero cells	
bc-D-ENT	Cytotoxicity, capillary permeability	
HlyII	Apoptosis to different monocytes and macrophages and pore formation, activates caspase 3- and 8-dependent pathways	
InhA1	Counteract the host immune system; digests various substrates, including extracellular matrix proteins, and cleaves tissue components such as fibronectin, laminin, and type I and IV collagens; pivotal for escape of *B. cereus* from the macrophages endosome after phagocytosis and induce cell mortality	
NprA	Cleave a variety of host cell components and regulatory proteins such as fibronectin, laminin, and collagen	
PC-PLC	Hemolysis, hydrolyses phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine	
PI-PLC	Hemolysis, cleaves phosphatidylinositol and glycosylated derivatives of phosphatidylinositol which anchor many proteins to the surface of the plasma membrane to generate a messenger for intracellular signal transduction	
SPH	Hemolysis, reduction in phagocytosis, contribute to the evasion from immune response by macrophages at early stages of infections	
DNase	Suppress both macrophage bactericidal activity and TLR9-mediated innate immune response	
Camelysin	Ability to hydrolyze hemoglobin, albumin and casein have a role in non-gastrointestinal infections	
Collagenase	Degraded soluble and insoluble collagens, Azocoll, gelatin and bradykinin	

further intensifies bird pneumonia after chlamydial infection, hemorrhagic lesions in the lungs leading to respiratory stress and breathing dysfunction that can suppress bird immunity (Zuo et al. 2020). Beside its potential for foodborne gastrointestinal infections, *B. cereus* group members are also acknowledged as a nosocomial pathogen that causes many systemic and local infections, especially neonates, IV medication abusers, seriously injured patients and those with inhabited catheters, in immunosuppressed and immune-skilled individuals (Kotiranta et al. 2000; Bottone 2010; EFSA 2016). Local infections lead to orbital abscess, keratitis, endophthalmitis and panophthalmitis, traumatic and surgical wound infections, gingivitis and periodontitis, whilst systemic infections include septicemia, severe hemorrhagic meningoencephalitis, urinary tract infections, endocarditis, pneumonia, fatal hepatic failure, and gas gangrene-like cutaneous infections (Kotiranta et al. 2000; Bottone 2010; Di Pinto et al. 2013; Ehling-Schulz et al. 2019).

5. Food Related Contamination and Food Safety Issues

5.1. Food Related Contamination

Since *B. cereus* is widely distributed throughout the environment, contamination can be transmitted across various substrates, including soil, plant-based foods (like rice, potatoes, cereals, grains, spices), salads, pasta, fish, stew, poultry, fried rice, meat and meat products, dairy products and equipment and the subsequent growth of spores in both raw and heat-treated items namely ready-to-eat (RTE) meals, RTE-vegetables, pasteurized liquid eggs, milk,

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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then germination of spores during cooling process enabling the bacteria to grow in the food and/or secrete large quantities of toxins depending on the strain(s) present can result in either emetic or diarrheal food poisonings (Ceuppens et al. 2011). The possible transmission of *B. cereus* from environment to human food chain has been shown in Fig. 5. The extremely stable emetic toxin, Ces will withstand a thermal treatment (121° C for 2 h at pH 7.0), frying, roasting, and microwave cooking and accumulation of Ces in food presents a potential risk since it is not destroyed at the time of subsequent preparation or manufacturing of foodstuffs, or after passing in the gastrointestinal tract (Ceuppens et al. 2011). Additionally, *B. cereus* can form biofilms on various surfaces of food processing machines, including stainless steel, glass, storage tanks, processing lines, plastics and other sites, and it has been shown that food isolated strains can consist of up to 90 percent of spores as well as vegetative ones (Hussain et al. 2018). Biofilm formation of this bacteria is therefore highly crucial for the food sector and considerable economic consequences of human health (Vaughan et al. 2003; Hussain et al. 2018). *B. cereus* spores were also identified in the paper manufacturing sectors and in packaging applications which may constitute an added route to food contamination (EFSA 2005). The extent of *B. cereus* contamination in different foods worldwide varies which are summarized in Table 5. This bacterium is also of particular concern in powdered infant formula (PIF), resulting in severe disease and death in young children attributable to illnesses of various pathogens, of which *B. cereus* is regarded as being the predominant microorganisms involved in PIF contamination (Di pinto et al. 2013; Heini et al. 2018).

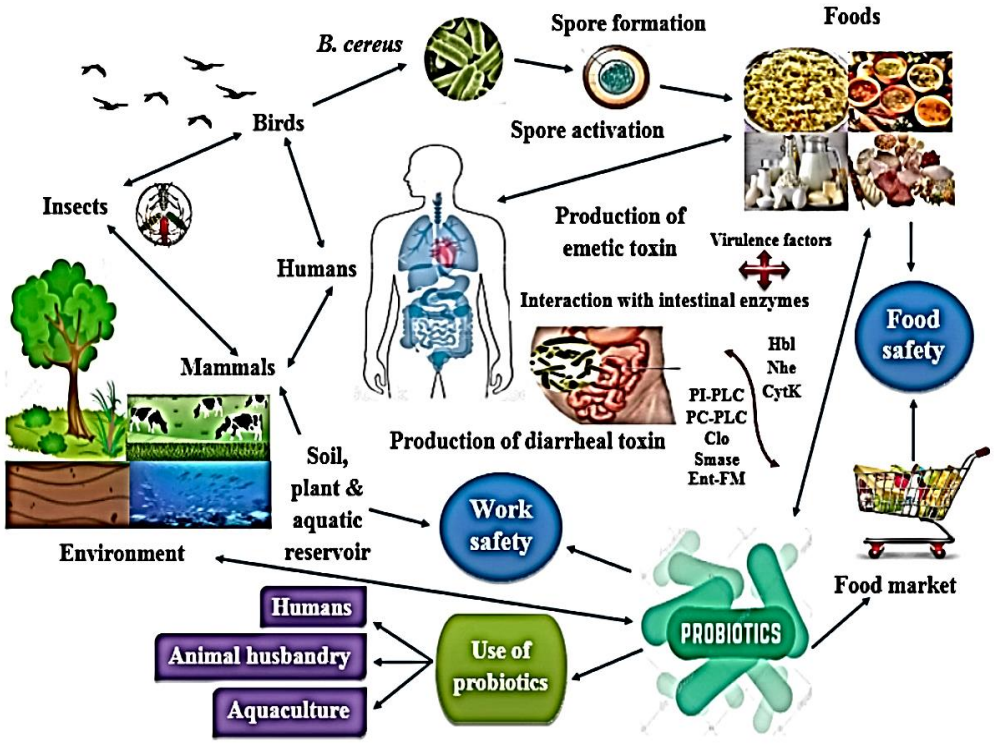

Fig. 5: *Bacillus cereus* transmission from environment to human food chain (Bamnia and Kaul 2015; Chen et al. 2018; Cui et al. 2019; Ehling-Schulz et al. 2019; Fu et al. 2020).

5.2. Food Safety Issue

Bacillus cereus isolates in bedding, waste, feed, wet manure and raw milk were 93.3%, 78.9%, 41.2%, 100.0% and 9.8%, respectively in ten native dairy farms (Cui et al. 2016b). European Food Safety Authority (EFSA) recommends that the amount of *B. cereus* spores in PIF and dried dietary foods require as small as possible all through processing and so, a standard of hygiene need to be set through effective measures aimed to minimize the error between processing and distribution (Di pinto et al. 2013). Maximum tolerable limit of *B. cereus* contamination in foodstuffs was summarized in Table 6. On other hand, other members in this genus such as *B. subtilis, B. licheniformis, B. pumilus, B. amyloliquifaciens, B. mojavensis, B. firmus, B. circulans, B. lentus, B. thuringiensis, B. megaterium, B. simplex, B. fusiformis, B. brevis* and *B. coagulans* were classified as negligible and ignored in episodes of food poisoning, but their existence and subsequent toxins output (Table 7) similar to emetic and enterotoxins have been increasingly documented for food safety concerns (HPA 2009; Griffiths 2010; Raddadi et al. 2010; Logan 2011; Osman et al. 2018). In addition, facultative alkaliphilic strains, *B. lentus* isolated frequently from raw and pasteurized

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Table 5: Contamination of *Bacillus cereus* in milk, meat, egg, fish and their products

Country/ authority	Food	Contamination level			References
		Minimum	Maximum	Total	
Malaysia	Formula milk	<3 MPN/g	>1100 MPN/g	-	Lesley et al. (2017)
	UHT milk	<3 MPN/g	>1100 MPN/g	-	
Lithuania	Meat dishes	$1.0x10^1$cfu/g	$5.0x10^2$ cfu/g	-	Jonkuvienė et al. (2012)
	Whole milk powder	$3.0x10^1$cfu/g	$2.1x10^4$ cfu/g	-	
	Skimmed milk	$6.0x10^1$cfu/g	$4.0x10^2$ cfu/g	-	
	Dry whey	$1.0x10^1$cfu/g	$1.0x10^2$ cfu/g		
	Fish dishes	$1.0x10^2$cfu/g	$1.3x10^2$ cfu/g		
Italy	Infant milk powder	-	-	$1x10^6$ cfu/g	Di Pinto et al. (2013)
	Dairy products	$\leq10^3$ cfu/g	$>10^5$ cfu/g	-	Proroga et al. (2019)
Canada	Pasteurized milk	-	-	$>10^5$ cfu/ml	Saleh-Lakha et al. (2017)
China	Pasteurized full fat milk	-	-	11.7 MPN/ml	Zhou et al. (2008)
	Prepackaged infant formula	10^3 cfu/g	10^4 cfu/g	-	Zhang et al. (2017)
	Infant powdered formula	$\geq1x10^1$ cfu/g	$>1x10^2$ cfu/g	-	Pei et al. (2018)
	Egg	-	-	1.3×10^8 cfu/g	Zhang et al. (2019)
	Cooked meat	<3 MPN/g	≤1100 MPN/g	-	Yu et al. (2020)
Costa Rica	Powdered milk	3 MPN/g	>100 MPN/g	-	Rojas et al. (2014)
India	Meat	-	-	$9.65x10^4$ cfu/g	Rao Vemula et al. (2012)
	Raw milk, skimmed milk powder			$>10^5$ cfu/g	Tewari and Abdullah (2014)
Brazil	Dairy products	$<10^2$ CFU/g	$>10^4$ cfu/g	-	Aragon-Alegro et al. (2008)
	Pasteurized milk	$1.5x10^1$ cfu/ml	$4.3x10^3$ cfu/ml	-	Chaves et al. (2017)
	Ultrahigh-temperature milk	$1.2x10^3$ cfu/ml	$5x10^3$ cfu/ml	-	
England	Meat pie	-	-	$1x10^8$ cfu/g	Mclauchlin et al. (2016)
	Liver pate	-	-	$2.6x10^5$ cfu/g	Mclauchlin et al. (2017)
Austria	Pork goulash	-	-	$1x10^4$ cfu/g	Schmid et al. (2016)
Iran	Beef burger	-	-	$>10^6$ cfu/g	Soleimani et al. (2017)
	Infant food	$3x10^1$ cfu/g	$9.3x10^1$ cfu/g	-	Rahimi et al. (2013)
Germany	Marinated pork	$<1x10^1$ cfu/g	$1x10^3$ cfu/g	-	Pichner et al. (2014)
	Cooked pork	-	-	1.0×10^2 cfu/g	Messelhäusser et al. (2014)
Netherland	Meat and meat products	-	-	$<10^5$ cfu/g	Wijnands et al. (2006)
	Milk and milk products	$<10^5$ cfu/g	$\geq10^5$ cfu/g	-	
Tunisia	Dairy products	-	-	$<10^3$ cfu/g	Gdoura-Ben Amor et al. (2018)
	Poultry meat	$<10^3$ cfu/g	10^4 cfu/g	-	
Mexico	Artisan cheese	$4.5x10^4$ cfu/g	$5.2x10^5$ cfu/g	-	Adame-Gómez et al. (2020)
Ghana	Raw milk	-	-	$1.9x10^3$ cfu/g	Owusu-Kwarteng et al. (2017)
	Cheese	-	-	$3.9x10^2$ cfu/g	
	Yoghurt	-	-	$6.3x10^1$ cfu/g	
Libya	Beef	7×10^3 cfu/g	9×10^3 cfu/g	-	Naas et al. (2019)
	Chicken	9.4×10^2 cfu/g	1.5×10^3 cfu/g	-	
	Camel meat	3.5×10^3 cfu/g	8.5×10^4 cfu/g	-	
Denmark	Meat for open sandwiches	$<10^3$ cfu/g	$\geq10^4$ cfu/g	-	Rosenquist et al. (2005)
	Ice-cream with milk products	$<10^3$ cfu/g	$\geq10^4$ cfu/g	-	
Egypt	Beef luncheon	1×10^2 cfu/g	3×10^2 cfu/g	-	Abdou et al. (2012)
	Defatted cheese	1×10^2 cfu/g	3.3×10^2 cfu/g	-	
	Double cream cheese	1.1×10^2 cfu/g	3×10^2 cfu/g	-	
	Turkish cheese	1.1×10^2 cfu/g	5×10^2 cfu/g	-	
	Raw milk	4×10^1 cfu/g	6×10^2 cfu/g	-	
	Raw meat	1.7×10^2 cfu/g	3.3×10^3 cfu/g	-	
Ethiopia	Raw milk			$>10^5$ cfu/ml	Abraha et al. (2017)
EU	Liquid egg yolk pasteurized	-	-	$<10^3$ cfu/ml	EFSA (2005)
	Pasteurized milk	$<10^3$cfu/ml	$>10^5$cfu/ml	-	
	Pasteurized milk after 8 days	10^3cfu/ml	$3x10^5$cfu/ml	-	

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	Milk powder	4 spores/g	40 spores/g	-	
	Powdered infant formulae	0.04 mpn/g	1 mpn/g	-	
Spain	Raw tuna fish	-	-	$4.5x10^5$ cfu/g	Domenech-Sanchez et al. (2011)
	Cooked tuna fish	-	-	$8x10^6$ cfu/g	
Australia	Cooked meat pies	$1.5x10^2$ cfu/g	$5.0x10^2$ cfu/g	-	Eglezos et al. (2010)
	Cooked sausage rolls	$3.9x10^2$ cfu/g	$7.9x10^2$ cfu/g	-	
	Processed meats	$1.9x10^3$ cfu/g	$1.9x10^3$ cfu/g	-	
	Raw diced chicken	$1.9x10^4$ cfu/g	$6.3x10^4$ cfu/g	-	
Turkey	Ice-cream	$2.0x10^1$ cfu/g	$4.0x10^2$ cfu/g	-	Cadirci et al. (2018)
Japan	Egg and its products	-	-	$3.8x10^7$ cfu/g	Agata et al. (2002)
	Meat and its products	-	-	$2.8x10^6$ cfu/g	
	Milk (stationary)	-	-	$9.1x10^7$ cfu/g	
	Milk (shaking)	-	-	$4.3x10^8$ cfu/g	

EU = European union, cfu = Colony forming unit, MPN = Most probable number, g = Gram, ml = Milliliter

milk. *B. coagulans* is responsible for flat-sour spoilage in certain processed foods and the manufacture of tea-based drinks, while commercial bioinsecticides, *B. thuringiensis* have been isolated from a variety of foods shown cytotoxicity and enterotoxicity identical to enterotoxigenic *B. cereus* strains (Kotiranta et al. 2000; Griffiths 2010; Logan 2011). Other pathogenic *Bacillus* spp with some *B. cereus*-like features can therefore be controlled and authorities granting the use of *Bacillus* species as biopesticides must be concerned. The national survey in China recently found that 33.7% of animal-used probiotics were contaminated with human-risk pathogens (*Klebsiella pneumonia*, *Cronobacter sakazakii*, *Shigella sonnei*, *Enterococcus faecium*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp) and human intestinal anthrax toxin gene *cya* was transmitted from nearby chicken and fish farm (Fu et al. 2020). Therefore, the presence of pathogenic *Bacillus* spp and other emerging disease agents in animal-used probiotics poses an emerging threat to food safety.

6. The Antimicrobial Resistance Issues

Antimicrobial medication is the primary means for eliminating foodborne pathogens, like *B. cereus*, in persons with food poisoning where antimicrobial resistance has now arisen for *B. cereus* as a result of antibiotic misuse or development of resistance genes via horizontal gene transfer resulting in failure of antibiotic therapy (Yu et al. 2019). *B. cereus* group species displayed resistance to β-lactam antibiotics such as penicillin, ampicillin, amoxicillin, oxacillin, cephalothin, cefoxitin, neomycin and rifampin that correlated with the ability of the strains to synthesize ß-lactamase, the antibiotic degradation enzymes (EFSA 2016; Abraha et al. 2017; Yu et al. 2019). Most members of the *B. cereus* group are also resistant to co-trimoxazole, fosfomycin, streptomycin, tetracycline, trimethoprim, and ceftriaxone depending on the strains that tend to be resistance to ciprofloxacin, clindamycin, tetracycline and levofloxacin, while being entirely susceptible to chloramphenicol, clindamycin, vancomycin, erythromycin, and gentamicin (Kotiranta et al. 2000; EFSA 2016). Antibiotic resistance to bacteria is a human health challenges and thus, it is necessary to evaluate a variety of antibiotics by antimicrobial susceptibility testing to screen effective antibiotics for ensuring a better control of foodborne *B. cereus* illness. Additionally, the development of drug resistance is multifactorial, which can be due to the frequent use of these agents in sublethal doses in medical and veterinary practices to prevent or treat infections, decrease or disappear the efficacy of the drug against the bacteria, leading to the emergence of drug-resistant species and failure of clinical treatment (Yu et al. 2019). As a possible source of both resistant bacteria and resistant genes, the antimicrobials may also be transferred to humans directly via food chain (Jonkuvienė et al. 2012). Recently, probiotic bacterial species have been linked with clinical infections, as well as the dissemination of toxin genes and genes with antibiotic resistance, such as aminoglycosides (*aadD2*), macrolides (*erm34*), β-lactams ($bla_{BCL\text{-}1}$), chloramphenicol (cat_{Bcl}), tetracycline (*tetM*) and erythromycin (ermD and ermK) (Mingmongkolchai and Panbangred 2018).

In China's national survey between 2016 and 2018, over one-third of probiotics containing antibiotic resistance and susceptible pathogens, thus probiotics existing in commercial food items (meats and seafood) can be possible candidate for transferring antibiotic-resistant pathogens from food animals to humans, posing an increasing risk to public health and food safety (Fu et al. 2020). Therefore, it is important to track *B. cereus* group multiple drug resistance in food chain, in probiotics for human and animal, providing adequate understanding of the safety of consumable foods contaminated with foodborne disease and contributing to the investigation of this urgent problem worldwide. Given the broad and unregulated use of probiotics, and antimicrobial resistance profiles, stringent regulations to minimize the risks of feed-borne or food-borne *B. cereus* contamination are urgently needed.

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Table 6: *Bacillus cereus* reference maximum tolerable limit (MTL) in different foods

Authority/ Country	Food items	Limit (cfu/g)				References
		Satis-factory	MTL	Unsatisfactory/ Unfit for consumption	Potentially hazardous	
Austria	RTE food	-	-	$>10^4$	$>10^5$	EFSA (2016)
Finland	RTE food	-	-	$>10^4$	-	
	Fresh vegetables	-	-	$>10^5$	-	
	Dried herbs and spices	-	-	$>10^5$	-	
France	Dried infant formulae and dried dietary foods for special medical purposes intended for infants below 6 months of age	50	10^2	-	-	
Ireland	RTE food	$<10^3$	10^3 - $<10^4$	10^4 to $<10^6$	-	SCESF (2003)
HPA	RTE food	$<10^3$	10^3 - $\leq10^5$	$>10^5$	-	HPA (2009)
Denmark	RTE food	$<10^3$	-	10^3 - 10^4	$>10^4$	Rosenquist et al. (2005)
FSANZ	RTE food	$<10^2$	10^2 - $<10^3$	$10^3 \leq10^5$	$>10^5$	FSANZ (2018)
Korea	RTE food	$<10^2$	10^2 - $<10^3$	$10^3 \leq10^5$	-	Chon et al. (2015)
CFSHK	RTE food	$<10^3$	10^3 - $\leq10^5$	$>10^5$	-	CFS (2014)
USA	Spices, Herbs, Coffee and Tea	$<10^4$	-	-	$\geq10^4$	NACMCF (2015)
	Meals and Entrees—RTE, sous-vide, cook and chill, deli salads, sandwiches, heat-eat meals, sushi, Grain-based products-RTE, Baked items, Egg Products-Pasteurized, Processed	$<10^3$	-	-	$\geq10^4$	
	Dairy-Dried Products	$<10^2$	-	-	$\geq10^4$	
	Dried fruit and coleslaw	$<10^2$				Prakitchaiwattana and Det-udom (2017)
	Powdered infant formulae	≤50				
	Breakfast cereal	≤10				
UK	RTE food	$<10^3$	10^3 - $<10^4$	10^4 - $<10^5$	$\geq10^5$	Gilbert et al. (2000)
	Dried herbs and spices	$<10^3$	10^3 - $<10^4$	$>10^4$		Sagoo et al. (2009)
	Ready-to-eat meat pies	$<10^3$	$\geq10^3$ - $<10^5$	-	$\geq10^5$	Mclauchlin et al. (2016)
Philippine	Frozen entrees containing rice or corn flour as main ingredient	-	10^2	-	$>10^4$	FDA (2013)
	Tofu	-	10^2	-	$>10^3$	
	Cereal base foods for infants	-	10^2	-	$>10^4$	

FSANZ= Food Standards Australia New Zealand, HPAUK=Health Protection Agency United Kingdom, CFSHK=Centre for Food Safety, Hong Kong, RTE food = Ready to eat food.

7. Comprehensive Control Measures and Outlook

Corn and soybean meal are the main ingredients of poultry and animal diets which have been fermented by microorganisms to improve the feed quality and gut ecology thus lowering anti-nutritional contents and toxins in the feeds, also better in feed conversion ratio (FCR) of broiler and piglet (Wongputtisin et al. 2012). Nevertheless, contaminations may lead to overgrowth of harmful bacteria particularly *B. cereus*, as its spores are resistant to various stresses and excellent adhesion to food surfaces. Fermented soybean foods for human consumption such as Douchi (China), doenjang (Korea), natto (Japan), Rabadi (Pakistan), Gari (Africa), Soibum (India), Ugba (Nigeria) may have probiotic carriers of *Bacillus* (Lee et al. 2019). This can be controlled by standardized fermentation criteria, including structure and properties of the sampling materials, initial culture method, fermentation parameters, post-fermentation strategies and use of bacterial peptides, bacteriocins with wide-spectrum antimicrobial action against *B. cereus* used

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as ex and in situ food additive (Ceuppens et al. 2011; Balciunas et al. 2013; Dai et al. 2020]. In soybean and buckwheat fermented with *B. amyloliquefaciens* RD7-7, the production and toxin expression of *B. cereus* have been substantially reduced and may serve as a basis for developing biological control agents to boost the safeguard of fermented soybean products (Eom and Choi 2016). Additionally, *Bacillus* species are commonly used as animal feed additives,

Table 7: Food poisons induced by different Bacillus species and the related toxins

Species	Toxin	Characteristics and functions	References
B. subtilis	Surfactins	Heat-stable, similarity to cyclic lipopeptides possess hemolytic activity, create pores in epithelial cells, toxic to sperm cells, inhibit sperm motility, damaged cell membrane integrity, depleted cellular ATP, and caused swelling of the acrosomes of spermatozoa	Griffiths (2010); Raddadi et al. (2010); EFSA (2011); Carlin and Nguyen-The (2013)
	Fengycin		
B. licheniformis	lichenysin A		
B. pumilus	Pumilacidin		
B. fusiformis	Lipopeptides		
B mojavensis	Amylosin		
	Fengycin		
B. amyloliquefaciens	Amylosin		
B. firmus	Lipopeptides		
B. megaterium	Lipopeptides		
B. simplex	Lipopeptides		

generating biologically active substances or improving the general conditions. For example, *Bacillus CIP* 5832 is still used as human medicinal additives and Paciflor, *Bacillus CIP* 5832 Toyerocin, *B. cereus var. toyoi* as animal feed for poultry, rabbits, cattle, and swine to increase yield and FCR (Cui et al. 2019; Zuo et al. 2020). Moreover, use of herbal medicine like *Dryopteris uniformis* (Makino), *Wedelia chinensis Osbeck* (Asteraceae), *Syzygium polyanthum* L. extract, *Melia azedarach* L. extract, *Eriocephalus L.* species, *Rhodomyrtus tomentosa* (Ait.) Hassk. leaf extract, *Punica granatum*, *Zingiber officinales*, *Hibiscus sabdariffa* (Roselle), *Rosmarinus officinalis* (rosemary), *Syzygium aromaticum* (Clove), and *Thymus vulgaris* (thyme) as natural antibacterial agents may be potential candidates for the development of new strategies to control the spread of *B. cereus* in food industry (Das et al. 2017; Darah et al. 2013; Ramli et al. 2018; Sen and Batra et al. 2012; Njenga et al 2005; Voravuthikunchai et al. 2010; Mostafa et al. 2017; Gonelimali et al. 2018). To prevent contamination in food chain, regulation will concentrate on *B. cereus* group food safety limits (maximum tolerable limit, MTL) of 10^3 cfu/g in dairy products for the general public, and 10^2 cfu/g in infant formula, 10^3 cfu/g in ready-to-eat meat and egg products.

Classification of *Bacillus* strains within species-level often relies on analysis of the 16S rDNA sequence. However, it cannot be uniquely identified different closely related *Bacillus* species. Random amplification of polymorphic DNA (RAPD)-PCR, REP (repetitive extragenic palindromic), ERIC (enterobacterial repetitive intergenic consensus)-PCR, or BOX-PCR, variable-number tandem repeats (VNTR) test may be used for molecular typing of various strains of *Bacillus* species. Among these methods, BOX-PCR fingerprinting is relatively rapid method for distinguishing *Bacillus* members (Banyko and Vyletelova 2009). In contrast, multi locus sequence typing (MLST) based methods of *B. cereus s.l.* strains were successfully applied for inferring genetic relationships, for example soil, insects, food, and humans. However, the laborious sequencing efforts required by MLST lessens its usefulness for high-performance research instead of amplified fragment length polymorphism (AFLP) being the standard tool. Moreover, Fourier transform infrared spectroscopy (FTIR) has successfully been used in microbial and epidemiological studies to classify *B. cereus* group strains that could be an alternative genetic method for subtyping and monitoring contamination source (Ehling-Schulz et al. 2013). Additionally, Pulsed-field gel electrophoresis (PFGE) recognizes large fragments of DNA with high resolution, high repeatability, and strong comparability for *B. cereus* typing can help us perform epidemiological traceability and provide a scientific basis for epidemiology and disease control (Liu et al. 2016). Future research will be centered on developing new quantitative tools to measure the diversity of emetic and diarrheal toxins in food and feed products, toxic dose, and the relationship between *B. cereus* counts and toxin production.

Conclusion: In this review, we summarized the pathogenicity of *B. cereus* with respect to its various toxins and other virulence factors, the prevalence and consequences in the food chain presenting health risks to humans and the environment. *B. cereus* group carry enterotoxin genes showing great differences in nature and the quantity of toxin production thus posing a hazard in the food chain. *B. cereus s.s.* causes modest to acute food poisoning, primarily due to enterotoxin secretion, resulting diarrhea and emetic toxins furthermore by certain pathways that are yet not elucidated. Other group (*B. subtilis*) species are also posing common potential risks of food poisoning. With better understanding virulence and toxin production of *Bacillus spp*, both feed-borne and food-borne *B.cereus* contamination have to be minimized risk for sustainable animal industry and human health.

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
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Competing interest: The authors declare no competing interests.

Funding: This work was supported by the Taishan Scholar Foundation of Shandong Province under Grant [No. ts201511084], High-level Innovation and Entrepreneurship Talent of Jiangsu Province.

ORCID

Md Atiqul Haque https://orcid.org/0000-0002-0978-4484
Hongkun Quan https://orcid.org/0000-0002-6767-6641
Zonghui Zuo https://orcid.org/0000-0002-3582-2210
Ahrar Khan https://orcid.org/0000-0001-5492-4266
Naila Siddique https://orcid.org/0000-0001-5569-7456
Cheng He https://orcid.org/0000-0002-1223-9639

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