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FEED-BORNE *BACILLUS CEREUS*: AN EMERGING THREAT TO FOOD CHAIN RELATED HAZARD, SAFETY AND PATHOGENIC POTENTIALITY

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INTRODUCTION

Bacillus cereus is a Gram-positive, rod-shaped, motile (flagellated), aerobic or facultative anaerobic, spore and biofilm forming bacterium, commonly found in nature. It belongs to a group of genetically similar forms assigned to the genus *Bacillus*, consisting of several closely related species. This opportunistic pathogen is often isolated from food and gastrointestinal disorders, as well as from non-gastrointestinal infections. Furthermore, it leads to vomiting and diarrheal syndromes in both animals and humans, which are linked to quickly fatal systemic and local infections, notably in neonates and immunosuppressed hospitalized patients. The ability of these pathogens to sporulate, and production of lipases and thermostable proteases allows them to withstand the common cleaning procedures in the food industry, resulting in finished product defects and food poisoning outbreaks.

B. cereus has also been used as a probiotic in human medicine and livestock production, but due to low standards of safety evaluation, toxins production, transfer of toxins and antibiotic resistance gene (ARG) in humans via the food chain, it is potentially posing a new threat to food safety. Consequently, feed-borne *B. cereus* contamination worsens extreme diarrhea and malnutrition in poultry by causing gizzard erosion and ulceration (GEU) syndrome, as well as hemorrhagic inflammation in lungs and immunosuppression, when co-infected with other pathogens. Considering the pathogenic potential of the entire *B. cereus* group, it is critical to gain insight into their genomes through whole-genome sequencing and gene analysis. This chapter includes an overview of the historical data on possible risk factors and pathogenesis of feed-borne *B. cereus* from animal feed to the human food chain, along with their implications for the food industry, focusing on food safety risks, classical and molecular analysis, advanced diagnostic methods, and their diversity, sensitivity, and ability to discover toxic and nontoxic bacteria.

Background and Taxonomy

The word "bacillus" means a "small rod," while the Latin word "cereus" refers to "wax-like", mostly used interchangeably with any "aerobic endospore-forming bacterium" (AEFB), which was first isolated from air in a cowshed in 1887 by Frankland and Frankland and discovered in 1906 by Plazikowski in connection with food poisoning in Europe (Fritze and Pukall 2011; Haque et al. 2021). *B. cereus* was first linked to food poisoning in the 1950s, when outbreaks of vanilla sauce poisoning were reported in Norway (Eglezos and Dykes 2014). *B. cereus* spores can last for years, even surviving during cooking due to their resistance to extreme temperatures, their growth is optimal in the presence of oxygen, but it can also thrive in anaerobic conditions, or at very low or high temperatures (Lutpiatina 2020).

There are currently 376 species in the *Bacillus* genus, with the *B. subtilis* and *B. cereus* group being two of the most common, however, several gene structures and regulatory mechanisms vary between these two groups of bacteria (Yin et al. 2020). *B. amyloliquefaciens*, *B. atrophaeus*, *B. licheniformis*, *B. mojavensis*, *B. paralicheniformis*, *B. pumilus*, *B. subtilis*, *B. tequilensis*, *B. vallismortis*, and *B. velezensis* are all members of *B. subtilis* group, while *B. cereus* group includes *B. anthracis*, *B. cereus sensu stricto (s.s.)* (usually referred to as *B. cereus*), *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis*, *B. weihenstephanensis*, *B. cytotoxicus*, and *B. toyonensis* (Lindbäck and Granum 2019; Yin et al. 2020; Haque et al. 2021). Currently, *B. cereus* group has been divided into: i) genomospecies such as, *B. pseudomycoides*, *B. paramycoides*, *B. mosaicus*, *B. cereus s.s.*, *B. toyonensis*, *B. mycoides*, *B. cytotoxicus*, and *B. luti;* ii) putative genomospecies such as, *B. bingmayongensis*, *B. gaemokensis*, *B. manliponensis*, and *B. clarus;* iii) subspecies such as, *B. mosaicus* subsp. *anthracis*, *B. mosaicus* subsp. *Cereus;* iv) Biovars such as Biovar *Anthracis*, Biovar *Emeticus* and Biovar *Thuringiensis* (Carroll et al. 2020). Even though the *B. cereus* group is phylogenetically heterogeneous in general, single strains with highly similar 16S and 23S rRNA sequences, especially *B. cereus* or *B. cereus s.s.*, *B. thuringiensis*, *B. anthracis* and *B. toyonensis* isolates, can be considered as single species due to transfer of virulence factors through plasmids;these are also subsumed under '*B. cereus sensu lato*' (Ehling-Schulz and Messelhäusser 2012; Griffiths and Schraft 2017; Lindbäck and Granum 2019). *B. thuringiensis* produces δ-enterotoxin (BT toxin), which appears as a crystalline parasporal inclusion body and is insecticidal, making it a biopesticide; *B. anthracis* is the causative agent of anthrax in human and animals; spores of this organism may be used in bioterrorism; *B. toyonensis* is the current species designation for *B. cereus*

var. *toyoi* strain that has been used as a fattening farm animals feed additive under the brand name of Toyocerin (Pontieri 2016; Griffiths and Schraft 2017).

B. cereus s.s. has been increasingly recognized as an evolving foodborne pathogen, with enterotoxins capable of causing emetic or diarrheic gastroenteritis in recent years (Pontieri 2016). It may also lead to local skin and wound infections, ocular infections (panophthalmitis, endophthalmitis, and keratitis), fulminant liver failure, and pervasive disease in cancer patients, such as endocarditis, osteomyelitis, pneumonia, brain abscess, meningitis myelodysplasia and extreme bacteremia.

Characteristics of the organism, growth and reservoirs

B. cereus are ubiquitous bacteria that can be found in decaying organic matter, air, dust, fresh and marine water, rhizosphere, animal and plant materials, vegetables, fomites, invertebrates' guts, beddings, feed and feedstuffs, pasture, with their adhesive spores can tolerate adverse conditions, like average cooking temperature, heat, dehydration, radiation and other physical stresses (Kumari and Sarkar 2016; Ramarao et al. 2020). Bacillus cells range in size from 0.5 × 1.2 to 2.5 × 10 µm and contain oval or cylinder-shaped spores that do not disclose the sporangia clustered in individual or short chains and located centrally, subterminally, or terminally (Fig. 1). The hydrophobic structure of the spores and presence of protrusions (1-30 in number of 0.45-3.8 µm x 13.6 nm) on the exterior result in strong adhesion to food processing surfaces, such as stainless steel. *Bacillus* species bacteria quickly sporulate in most media after 1 to 3 days (Kumari and Sarkar 2016; Grutsch et al. 2018; Lindbäck and Grnum 2019). They grow best at temperatures between 28-40°C, while they can multiply at a temperature between 4-50°C. Thermophilic varieties, on the other hand, grow best at 65°C. A water activity (a_w) ≥ 0.91, a pH of 4.0-9.3 (optimal 7.0) and a NaCl concentration <10% are also required for their development. Under ideal conditions, growing time is between 12 and 27 minutes (Eglezos and Dykes 2014). *Bacillus* spp. colony morphology varies by species, but they all grow on common agar media, like nutrient agar (NA) or plate count agar (PCA), producing large colonies (3-8 mm in diameter) with a flat, greyish and 'ground-glass' appearance, sometimes with irregular borders. They metabolize organic substrates, like amino acids, organic acids, and sugars through aerobic/anaerobic respiration, or fermentation, depending on species and environment (Kumari and Sarkar 2016; Grutsch et al. 2018; Ramarao et al. 2020).

Mode of transmission and contamination

B. cereus is a common soil saprophyte that can be found in a variety of environmental habitats, as well as man-made settings, such as food manufacturing plants, food handling and processing facilities, transportation vehicles and hospital environments, where they may constitute reservoirs for the disease. Such habitats can provide a favorable environment for *Bacillus* spp. production, or may still harbor spores, which can quickly be transmitted to different raw foods, such as grain and cereals products, dried herbs, spices, eggs, milk and dairy products, fruit, vegetables, meat products, sauces, puddings, sprouts, rice and other carbohydrate-rich foods, as well as commercial RTE meals and products, which may become contaminated, resulting in transient colonization of the animal and human intestine (Fritze and Pukall 2011; Eglezos and Dykes 2014). *B. cereus* spores can become contaminant in the dairy sector when they come in contact with cows' udders during pasture or by feed or bedding material, then move into the raw milk. The spores may also withstand pasteurization, dehydration, γ radiation, and other physical stresses (Grutsch et al. 2018; Lindbäck and Grnum 2019). The ability of *B. cereus* to bind firmly to surfaces and form biofilms, which shield their cells and spores against the antimicrobial action of sanitizers, accounting for their survival in food processing environments (Grutsch et al. 2018). Intake of food or air or a wound in the body contaminates spores or vegetative cells (Ramarao et al. 2020).

Virulence factors and Pathogenicity

B. cereus is most commonly associated with food poisoning and other serious systemic and local infections, owing to the synergistic effects of a range of virulence factors that foster intestinal cell disruption and/or immune system tolerance in the host. Table 1 summarizes the substances formed by *B. cereus* during bacterial growth, primarily enterotoxins, hemolysins, phospholipases and emetic toxin.

Incidence of illness and outbreak data

The exact incidence of the *B. cereus* food-borne poisoning is mysterious for many reasons: a) it is widely underestimated because the symptoms of the disease are intermittent (<24h), slight, and self-limiting, so people do not seek treatment; b) most of the community is partially covered by resistance gained through chronic exposure; c) large numbers are needed to induce infection; d) symptoms are usually misdiagnosed with clostridial or *Staphylococcus aureus* intoxications (Griffiths and Schraft 2017). *B. cereus* appears to be responsible for 1.4-12% global food-borne illness outbreaks (Grutsch et al. 2018). Tables 2 and 3 summarize the outbreak and occurrence data for *B. cereus* poisoning over the last two decades.

Pathogenesis of the diseases

B. cereus and other members of the *B. cereus* group induce two forms of food poisoning: emetic syndrome and diarrheal illness. The emetic (vomiting) syndrome, which is similar to *Staphylococcus aureus* poisoning, is exacerbated by a ready toxin found in cooked rice and other cereal-based foods that resist high temperatures, trypsin, pepsin, and pH; whereas the diarrheal illness, which is similar to *Clostridium perfringens* poisoning,

Table 1: Virulence factors of *B. cereus*

Virulence factors	Properties	Encoded gene	Reference
Emetic toxins			Carlin and Nguyen-The (2013); Lindbäck and Granum (2015); Visiello et al. (2016); Ehling-Schulz et al. (2019); Haque et al. (2021)
Cereulide (Ces)	Thermo-stable, Cyclopeptide (1.2 kDa), hepatic and immune dysfunction, toxic in various mammalian cell lines, cerebral effects, bioaccumulation in vital organs and necrotic cell death	*ces*	
Enterotoxins			
Hemolysin BL (Hbl)	Thermo-labile, protein, 3 components (35, 36, & 45 kDa), pore formation, hemolysis, cytotoxicity, dermonecrotic and capillary permeability	*hblA, hblB, hblC, hblD*	
Nonhemolytic enterotoxin (Nhe)	Thermo-labile, protein, 3 components (39, 45, & 105 kDa), pore formation, intestinal fluid secretion, osmotic and Vero cell lysis, cell death	*nheA, nheB, nheC*	
Cytotoxin K (CytK)	Thermo-labile, single-cell protein (34 kDa), pore formation, hemolysis, cytotoxicity and necrosis	*cytK1, cytK2*	
Enterotoxin FM (entFM)/ CwpFM	Single-cell protein (45 kDa), cell wall peptidase, hemolysis, capillary permeability and cytotoxicity	*entFM/ cwpFM*	
Enterotoxin T (entT)	Single-cell protein (40/41 kDa), diarrheal toxigenicity, capillary permeability and cytotoxicity	*bceT*	
Hydrolytic enzymes			
Hemolysin I (HlyI)/ Cereolysin O	Thermo-labile, cholesterol-binding and thiol dependent hemolysin, pore formation	*Clo*	
Hemolysin II (HlyII)	Thermo-labile, cholesterol-independent, cytotoxicity, pore formation, apoptosis in macrophages (caspase-3,8 pathways)	*hlyII*	
Hemolysin III (HlyIII)	Hemolysis, transmembrane pore formation	*hly-III*	
Phospholipase C/ Cerolysin A	Degradation of neutrophils	*plC*	
Phosphatidylinositol specific phospholipases C (PI-PLC)	Destroying of protein harborage on plasma membranes	*piplC/ plcA*	
Phosphatidylcholine specific phospholipases C (PC-PLC)	A small, monomeric enzyme (28.5 kDa), general hydrolytic action, hemolysis, involved in substrate binding and necessary for enzymatic activity and protein formation	*pcplC/ plcB*	
Sphingomyelinase (SMase)/Cerolysin B	Hemolytic protein that binds to sphingomyelin on erythrocytes, hemolysis, decrease in phagocytosis, dodging macrophage in initial phases of infection	*sph*	
Cerolysin AB	2 components (PC-PLC+Smase) cytolysin, that function together to lyse human erythrocytes	*cerAB*	
Camelysin	A cell-bound metalloprotease, capability to cleave hemoglobin, albumin and casein in non-gastrointestinal infections	-	
Immune inhibitor A1 (InhA1)	A zinc metalloprotease, efficient escape from macrophages	*inhA*	
Bacillolysin	A metalloprotease	*nprA*	
Neutral metallopeptidases/ Neutral protease	Proteolytic activity	*Npr/ nprB*	
IlsA	Iron-regulated, leucine-rich surface protein, iron deprivation in the host	*ilsA*	
Collagenase	Degraded soluble and insoluble collagens, Azocoll, gelatin and bradykinin	*cola*	
Antibiotic resistance			
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*	

is provoked by a complex enterotoxin throughout vegetative growth of *B. cereus* in the small intestine, mainly linked to proteinaceous foods (Eglezos and Dykes 2014; Haque et al. 2021). Table 4 shows the key characteristics of *B. cereus* poisoning found in food and feed. According to our earlier studies, feed-borne *B. cereus* caused GEU, as well as hemorrhagic inflammation in lungs of chicken. Co-infection with other pathogens, such as avian influenza virus (H9N2) and *Chlamydia psittaci* worsened acute diarrhea and led to the development of GEU and immunosuppression in birds. Importantly, Hbl and Cytk, enterotoxins of *B. cereus,* disrupt the koilin layer of the gizzard, causing long-term ulceration, necrosis, mucosal damage and diarrhea by damaging the digestive tract (Zhang et al. 2019; Zuo et al. 2020).

In a recent study, it was found that stomach ulceration caused by feed-borne *B. cereus* in conjunction with severe diarrhea, and co-infection with *Aspergillus fumigatus* alleviated gastric lesions and immunosuppression in weaned piglets (Li et al. 2020).

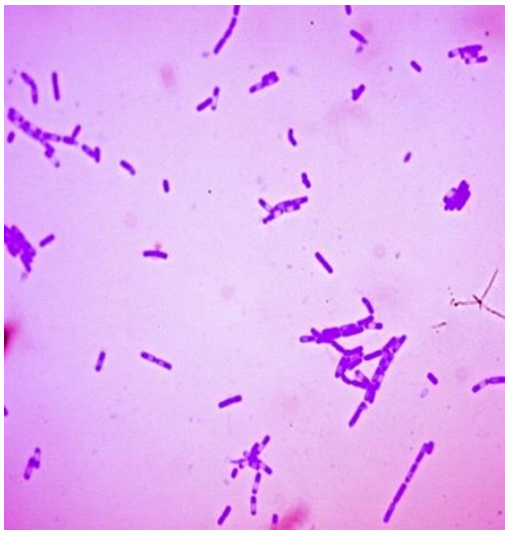

Fig. 1: *Bacillus cereus* on gram staining (Photo by Md Atiqul Haque). **Fig. 2:** *Bacillus cereus* on MYPA (Photo by Hongkun Quan).

Table 2: Food poisoning outbreaks due to *B. cereus* worldwide from 2000 to 2020

Year	Country /Region	Food	Affected persons/ consequences	Contamination level (cfu/g)	Attack rate (%)	Type of poisoning	Reference
2000	Italy	Cakes	173 people; N and D (watery), 23 patients hospitalized	10^2	n.a.	EPP	Osimani et al. (2018)
2003	Belgium	Pasta salad	Family outbreak; 5 children hospitalized; V, LF, 1 death	1.0×10^7 –1.0×10^8	n.a	EP, EPP	Dierick et al. (2005)
	Greece	No information	A 72-year-old woman, V, A, LF, death	n.a. n.a.	n.a	EP EP	Latsios et al. (2003)
1991-2005	Canada	Mainly Asian food, followed by raw food	39 outbreaks, V, A, N and D		32		McIntyre et al. (2008)
2005	Spain	Seafood cocktail and fried shrimp	100 people	3.8×10^4	95	EP, EPP EP, EPP	Osimani et al. (2018)
	Finland	Pasta and meat dish	2 persons; V and D	$1.3\text{-}1.8\times10^5$	n.a. n.a.		Pirhonen et al. (2005)
	Korea	Cooked and fried rice	37 persons; V, A and headache	n.a.	n.a.	EP	Kim et al. (2010)
2004-2006	Korea	Not specified	Sporadic food poisoning case	n.a.	n.a.	EP EP	Chon et al. (2012)
2006	Germany	Rice dish, cooked cauliflower	18 people (17 children, 1 adult), V, collapsed with hospitalization	1×10^4	n.a		Osimani et al. (2018)
	Italy	Ricotta cheese	57 persons	n.a.		EPP	
2007	Germany	Ready-to-eat rice pudding	46 people (43 children, 3 adults), V	n.a.	30	EP	
	Spain	Cooked tuna fish	5 persons, G	8.0×10^6	n.a.	EP	
1998-2008	USA	Rice, meat and poultry dishes	235 outbreak, 2050 people, 17 hospitalizations, D, A,V	n.a.	n.a.	EP, EPP	Haque et al. (2021)
2006-2008	India	Not specified	42 case, D	n.a. n.a.	n.a. n.a.	EPP	Banerjee et al. (2011)
2008	France	Pasta	A 15-year-old boy; V, A, LF	n.a.	n.a.	EP	Saleh et al. (2012)
	Belgium	Spaghetti meal (pasta)	A 20-year-old man; death	n.a. n.a.		EP	Naranjo et al. (2011)
	Oman	Hospital meal	58 people; D,V	n.a.	14.1	EPP	Al-Abri et al. (2011)
	Switzerland	Pasta	A 9-year-old girl; A, V, LF, shock	n.a. n.a.	n.a.	EP	Posfay-Barbe et al. (2008)
	Korea	Raw fish	8 persons, family outbreak	n.a.	n.a.	EP, EPP	Kim et al. (2009)
	Brazil	Fruits and vegetables	93 cases, N, V, D, A		n.a.	EP, EPP	Elias et al. (2018)
2009	Brazil	Fruits and vegetables	21 cases, 3 hospitalized, D, A		n.a.	EP, EPP	
2010	Korea	Lunch buffet	43 persons, D, A		20.3	EPP	Choi et al. 2011
	Germany	Lunch buffet	4 persons, acute V	2.8 x 10^4 CFU/g	n.a. n.a. n.a.	EP EP EP	Ehling-Schulz and Messelhäusser (2012)
	Japan	Fried rice	An 11-year-old boy; G, LF, acute E,	n.a. n.a.	n.a.		Ichikawa et al. (2010)

	Japan	Reheated fried rice	3 persons; V, acute E, 1 death				Shiota et al. (2010)
2011	Germany	Mixed lunch (pasta)	22 persons (20 children, 2 adult), 4 hospitalized, acute D	2.2 x 10^6 CFU/g		EPP	Ehling-Schulz and Messelhäusser (2012)
	Belgium	Rice-based dishes	8 people; 1 hospitalized	2.8×10^5 –2.4×10^7	88	EP	Osimani et al. (2018)
2008-2012	France	n.s.	39 people, 8 death	n.a. n.a.	n.a	EPP	
2012	Italy	Basmati rice	12 people, V, N, A, D		92	EP	
	UK	Dried haricot beans	200 people (182 children, 18 adults) V	2.0×10^6	63.2	EP	
	Belgium	Mashed rice-cucumber-chicory	20 children, V	> 1.5×10^7	90.9	EP	
2001-2013	Australia	Multiple foods	6 outbreaks, 114 cases, 1 emetic and 5 diarrheal; V, D	n.a.	n.a. n.a.	EP, EPP	May et al. (2016)
2003-2013	Brazil	Mainly cereals, sauce	346 people; 3 hospitalized, D, A, V	n.a.	n.a.	EPP	Lentz et al. (2018)
2007-2013	Germany	Multiple foods	Several affected people, V	≤1×10^2 –6.1×10^7		EP	Messelhausser et al. (2014)
2013	UK	Ready-to-eat meat pie	5 people	1.5×10^6 –1.0×10^8	n.a.	EP	McLauchlin et al. (2016)
	Australia	Buffet lunch	125 people, D, A	n.a.	n.a.	EPP	Sloan-Gardner et al. (2014)
	Austria	Mashed potatoes Dish	14 people, 3 hospitalized	2.1×10^5 –3.4×10^5	44.0	EP, EPP EP, EPP	Osimani et al. (2018)
		Pancake strip soup	14 people	1.0×10^2 –1.0×10^4	22.2	EP, EPP	
		Fruit salad	106 people	n.a.	29.3		
2007–2014	France	Starchy food and Vegetables	74 outbreaks, 911 cases, A,V, D	4.0×10^2 –1.0×10^9	n.a. n.a.	EP, EPP EP,EPP	
2014	China	Fermented black beans (douchi)	139 people, N, V, D	1.6×10^7 –2.3×10^7	n.a. n.a.	EP,EPP EP,EPP	Zhou et al. (2014)
	EMS	Mixed food	287 outbreaks, 3,073 cases, 257 hospitalized	n.a. n.a.	n.a. n.a.		EFSA and ECDC (2016)
2015	EMS	Mixed food	291 outbreaks, 3131 cases, 101 hospitalized	n.a. n.a.	n.a.		
	Norway	n.s.	4 outbreaks,17 cases		n.a.	n.a.	
	Argentina	Cooked chicken	A 39-year-old healthy woman, hospitalized, V, D (watery)	n.a. n.a.	n.a.	EP, EPP	Lopez et al. (2015)
	Germany	Rice meal	A 13-month-old boy; V, A, LF	n.a.	n.a. n.a.	EP	Tschiedel et al. (2015)
2016	France	Human milk	3 infant cases, hospitalized, sepsis, brain hemorrhage,2 death	n.a. n.a.	n.a.	EP, EPP	Rigourd et al .(2018)
	USA	Refried beans	179 people, V, D	n.a. n.a.		EP,EPP	Carroll et al. (2019)
2011-2017	Korea	n.s.	50 outbreak, 491 people	n.a.		n.a.	Kim and Kim (2021)
	USA	n.s.	69 outbreak,1389 people			n.a.	
2017	Indonesia	Sardines	22 people, V, D			EPP EPP	Depo et al. (2018)
	India	Sweetened curd	204 people, A,V		44.0		Sahu et al. (2021)
2018	EMS	n.s.	98 outbreak, 1539 people, 111 hospitalize, 1 death		n.a.	EP, EPP EP, EPP	Rodrigo et al. 2021
	Australia	Multi-course-dinner (Beef)	15 people, V, D	3.5×10^3 –1.9×10^4	37.0		Thirkell et al. (2019)
	China	School canteen food and drink	209 people, V, D, A, fatigue, dizziness, fever, headache	10 - 1.6 × 10^5	3.9-12.5	EPP	Chen et al. (2019)
2019	Germany	Buck wheat	A 57-year-old woman; massive V, D, esophageal perforation, Boerhaave syndrome	n.a. n.a.	n.a. n.a	EP	Dichtl et al. (2019)
2020	China (Taiwan)	Breast milk	A 1490-g female infant was hospitalized, A, V, tachycardia, hyperglycemia, and elevated C-reactive protein			n.a	Liao and Tsai (2020)

EMS= European member states; n.s.= Not specified; n.a.= Not available; N=Nausea; V= Vomiting; D=Diarrhea; A= Abdominal pain; LF=Liver failure; E= Encephalopathy, G=Gastroenteritis; EP= Emetic poisoning; EPP= Enteropathgenic poisoning

Bacillus spp. as probiotics/feed additives, food safety implication and antibiotic resistance

Strains of *Bacillus* species have long been used as probiotics in human, veterinary, aquaculture, plant and environmental applications. Probiotic strains are used in animal production, either directly as microbial feed additives, or as a source of other feed additives, especially enzymes (EFSA 2011; Cui et al. 2019). Spores of *Bacillus* strains are used in human, veterinary, and aquaculture applications due to their probiotics characteristics, and the bacteria can then spread in food after ingestion (Carlin and Nguyen-The 2013). *Bacillus*-based probiotics can have a beneficial impact on poultry production by strongly activating immune-related components, controlling pathogenic bacteria, modulating immune responses, fostering gut integrity, raising feed conversion rate (FCR), acting as a growth factor and improving disease resistance and health (Bilal et al. 2020; Arsene et al. 2021). *B. subtilis* is a common food supplement in animal industry, particularly in poultry and fish farming (Arsene et al. 2021). In the swine industry, it is used as a replacement for antibiotics to treat diarrhea in weaning piglets; *Bacillus* spp.-fermented (notably *B. subtilis, B. licheniformis, B. amyloliquefaciens*) feed additives have been found to reduce morbidity and mortality rates, ameliorate enteritis, have a beneficial impact on the lessening diarrheal incidence and increasing the growth efficiency of weaning piglets (Lin and Yu 2020; Arsene et al. 2021). However, some bacteria of *B. cereus* and other group may cause problems by producing different enterotoxins and emetic toxins (Table 5), and carrying ARG, which can be transmitted to humans via the food chain or the environment. In light of the data about the above noted probiotic candidates, especially those belonging to the *B. cereus* group, it appears that they have no toxic potential (Cui et al. 2019).

Antibiotics are a common way to control or prevent bacterial infections in farming, and the widespread use of antibiotic growth promoters (AGP) in animal feed has led to a rise in livestock production. However, inappropriate and abusive antibiotics use can spread antibiotic residues in animal-derived foods, such as milk, meat, and eggs, as well as the environment, might spread antibiotic resistance in animal microbial communities, with the possibility of ARG being transferred from animal to human microbiota (Mingmongkolchai and Panbangred 2018; Arsene et al. 2021). Resistance determinants for β-lactams (bla_{BCL-1}), chloramphenicol (cat_{Bcl}), aminoglycosides (*aadD2*), macrolides (*erm34*), tetracycline (*tetM* and *tetK*) and erythromycin (*ermD* and *ermK*) have been found in probiotic strains of *B. cereus, B. clausii, B. subtilis* and *B. licheniformis* (Mingmongkolchai and Panbangred 2018). Consequently, global public health authorities have raised concerns about AGP and their role in the increased multidrug-resistant bacteria, with adverse effects on consumer health (Mingmongkolchai and Panbangred 2018; Arsene et al. 2021). Since the use of antibiotics in animal feeds has been banned in several countries, an alternative approach that has proved useful is the application of probiotics in consideration of the safety evaluation of new probiotics. Fig. 5 depicts the main route of transmission and development of antibiotic resistance from the feed and food chain to humans.

Isolation and Identification

B. cereus can be isolated and identified from food and other samples, using a variety of methods. Table 6 displays the advantages and limitations of various such approaches.

Traditional Approaches

B. cereus isolation and enumeration from foods, environment and clinical settings are usually done with conventional selective plating media. Food authorities suggest two standard media for *B. cereus* identification: Mannitol-egg yolk-polymyxin agar (MYPA) and Polymyxin-egg yolk-mannitol-bromothymol blue (PEMBA) for their characteristic colonies, Pink color and Peacock blue color precipitation zones of egg yolk hydrolysis, respectively (Figs. 2 and 3). Finally, to distinguish hemolytic and nonhemolytic *B. cereus* strains, a hemolysis test is conducted on 5% sheep blood agar plates at 37°C, which produces dull gray and opaque colonies with a rough matted surface (Fig. 4) (Eglezos and Dykes 2014; Pontieri 2016; Griffiths and Schraft 2017; Ramarao et al. 2020; Haque et al. 2021). Several biochemical and microscopic tests are performed after bacterial isolation, including glucose, mannitol, xylose, arabinose, oxidase, motility, catalase, citrate utilization, casein hydrolysis, nitrate reduction, Voges-Proskauer (VP) reaction, l-tyrosine reduction, and growth in 0.001% lysozyme to validate and distinguish various phylogenetically close *Bacillus* spp. (Table 7). The miniaturized API 50CHB test package (bioMerieux), which evaluates the capacity to assimilate 49 carbohydrates, is a quick *Bacillus* identification method based on conventional biochemical tests. This system is believed to be capable of classifying possible emetic strains, but it does not distinguish *B. cereus* and *B. thuringiensis* (Eglezos and Dykes 2014; Griffiths and Schraft 2017).

Molecular Methods

Molecular approaches for confirming *Bacillus* spp. identification include a variety of techniques, which are summarized in Fig 6. Genes encoding major enterotoxins (nhe, hbl, cytK, entFM, bceT, hlyII) and emetic toxin (cesA, cesB) at various levels of production are much more relevant with respect to species determination (Table 8) in *B. cereus* toxin gene profiling by PCR detection protocols, particularly in outbreak situations. Furthermore, diagnostics should focus more on determining toxin or virulence genes, as well as toxin output quantification (Pontieri 2016; Ramarao et al. 2020). PFGE is one of the most effective fingerprint typing methods for *B. cereus* outbreak in the epidemiological investigation, because it splits large pieces of genomic DNA and allows for precise

Table 3: Incidence of *B. cereus* food poisoning in different foods worldwide from 2000 to 2020

Year	Country/Region	Food	Incidence (%)	Reference
1998-2000	Germany	Mass catering food	60	Ehling-Schulz and Messelhäusser (2012)
	France	n.s.	4-5	Tewari and Abdullah (2015)
2000	Norway	n.s.	32	Haque et al. (2021)
2001-2002	Turkey	Meat and meat products	22.4	Tewari and Abdullah (2015)
2003	Czech Republic	Dairy products	31.0	Schlegelova et al. (2003)
		Meat products	28.0	
2004	USA	Retail chicken products	45.0	Smith et al. (2004)
1991-2005	China (Taiwan)	n.s	11.2	Raddadi et al. (2010)
2005	Spain	Seafood cocktail and fried shrimp	5.0	Hernandoa et al. (2007)
	India	Milk and milk products	53.8	Tewari and Abdullah (2015)
	Chile	Dried milk products	45.9	Kumari and Sarkar (2016)
2006	Netherland	n.s.	5.4	Haque et al. (2021)
	China	Pasteurized full-fat milk	71.0	Kumari and Sarkar (2016)
2006-2007	Australia	Chilled raw diced chicken	2.4	Haque et al. (2021))
	USA	Raw rice	46.6	Ankolekar et al. (2009)
2007	EU	Not specified	17.1	Tewari and Abdullah (2015)
	Belgium	Commercial food products	56.3	Samapundo et al. (2011)
1998-2008	USA	Rice, meat and poultry dishes	19.0	Haque et al. (2021)
2001-2008	Korea	Raw fish	3.7	Chon et al. (2012)
2006-2008	India	n.s.	3.5	Banerjee et al. (2011)
	Korea	Cooked rice	37.5	Chang et al. (2011)
2008	Turkey	Cheese	12.0	Kumari and Sarkar (2016)
	Austria	Ice cream	62.7	Heydarzadeh et al. (2020)
2007-2009	Korea	n.s.	1.5	Gwack et al. (2010)
2008-2009	Germany	Marinated pork products	21.0	Haque et al. (2021)
	Jordan	Various foods	23.3	Batchoun et al. (2011)
	Korea	Brown rice and glutinous rice	37.0	Lutpiatina (2020)
	Turkey	Milk and meat products, Boza	66.0	Güven and Mutlu 2009
2009	India	Traditional food	46.0	Tewari and Abdullah (2015)
	Egypt	Raw milk	30.0	Kumari and Sarkar (2016)
2009-2010	USA	Raw commingled silo milk	8.9	Liu et al. (2020)
2010	Scotland	Cheese	32.0	Heydarzadeh et al. (2020)
2011	Belgium	Cooked rice	18.5	Delbrassinne et al. (2012)
2010-2012	Korea	Fermented soybean products	67.9	Kim et al. (2015)
	Brazil	Milk and dairy products	24.2	Heydarzadeh et al. (2020)
2012	Mexico	Vegetables	57.0	Flores-Urban et al. (2014)
	India	Meat and meat products	30.8	Tewari et al. (2012)
		Raw milk	11.0	
2003-2013	Brazil	Cereals or sauces	3.1	Haque et al. (2021)
2007-2013	Germany	Multiple foods	10.0	Messelhausser et al. (2014)
2012-2013	Singapore	Sushi	5.1	Yap et al. (2019)
2013	Iran	Infant food	42.0	Lutpiatina (2020)
2007-2014	EU	n.s.	27.6	Haque et al. (2021)
2008-2014	Brazil	Fruits and Vegetables	6.6	Elias et al. (2018)
2013-2014	China	Raw milk	9.8	Cui et al. (2016)
	Iran	Powdered milk infant formula	67.2	Dallal et al. (2017)
	Iran	Beef Burger	31.2	Soleimani et al. (2017)
	EU	Not specified	5.5	Food safety authority of Ireland (2016)
2014	India	Various dairy products	32.0	Kumari and Sarkar (2016)
	Cambodia	Fermented vegetables	31.0	Chrun et al. (2017)
	Malaysia	Formula milk	41.7	Lesley et al. (2017)
		UHT milk	30.0	
	China	Infant formula	3.5	Zhang et al. (2017)
2013-2015		Rice flour	1.0	
	Nigeria	Retailed foods	36.8	Adesetan et al. (2019)
2014-2015	Canada	Pasteurized Fluid Milk	5.5	Saleh-Lakha et al. (2017)
	Italy	Dairy products	26.8	Haque et al. (2021)
	Egypt	Meat products	43.7	Mohamed and Ghanyem (2015)
2015	Ghana	Raw milk	46.6	Heydarzadeh et al. (2020)
	Nigeria	Milk-based infant food	3.0	Ranjbar and Shahreza (2017)
	Korea	RTE vegetables	48.0	Chon et al. (2015)
	Iran	Raw and cooked meat	14.5	Zeighami et al. (2020)
2002-2016	Italy	Ricotta cheese	15.9	Scatassa et al. (2018)
		Retail aquatic products	25.4	Zhang et al. (2020)

		Pasteurized milk	27.0	Liu et al. (2020)
2011-2016	China	Meat and meat products	26.3	Kong et al. (2021)
		Vegetables	50.0	Yu et al. (2019)
		RTE foods	35.0	Yu et al. (2020)
	China	Goat milk powder infant formula	36.1	Liu et al. (2018)
2016	Brazil	Pasteurized Milk	28.6	Chaves et al. (2017)
	Mexico	Artisan cheeses	29.4	Adame-Gomez et al. (2020)
2007-2017	Poland	Commercial food products	38.8	Berthold-Pluta et al. (2019)
2011-2017	Korea	n.s.	6.5	Kim and Kim (2021)
	USA	n.s.	3.5	
		Boiled milk	50.0	
2017	Egypt	Pasteurized milk	15.0	Abou Zeid and Yassin (2017)
		UHT milk	15.0	
		Beef products	26.0	Shawish and Tarabees (2017)
	Turkey	Milk and cheese	10.4	Yibar et al. (2017)
2017-2018	Malaysia	Ready-to eat cooked rice	34.0	Navaneethan and Esah (2020)
	China	Raw milk	16.0	Liu et al. (2020)
	Switzerland	PIF	78.0	Heini et al. (2018)
2018	China	School canteen food and drink	4.1	Chen et al. (2019)
	EU	n.s.	1.9	Rodrigo et al. (2021)
	Thailand	Mixed food stuffs	21.0	Sornchuer and Tiengtip (2021)
2018-2019	China	Dairy products	10.8	Liu et al. (2020)
	Iran	Dairy products	10.6	Heydarzadeh et al. (2020)
	Egypt	Buffalo milk	12.9	Abouelhag et al. (2021)
2019	Italy	Fried rice meals	7.8	Tirlonia et al. (2019)
	Indonesia	Cooked rice (yellow rice)	21.0	
2019-2020	Iraq	Soft cheese	67.0	Al-Jobory and Abdulaal (2020)
	Egypt	Various RTE food	5.0-10.0	Enan et al. (2020)
		Milk powder	6.9	Abdeen et al. (2020)
	Egypt	Ras-cheese	8.5	
2020		Meat and Chicken Products	21.5	Gharib et al. (2020)
	Pakistan	Different milk	20.0	Rafique et al. (2020)
	Malaysia	UHT chocolate milk	24.3	Ubong et al. (2020)
	China	Rice/ noodles	50.0	Lutpiatina (2020)
	Colombia	Powdered food	11.0	Sanchez-Chica et al. (2020)

n.s.= Not specified; RTE= Ready to eat; PIF=Powdered infant formula; UHT= Ultra heat treatment.

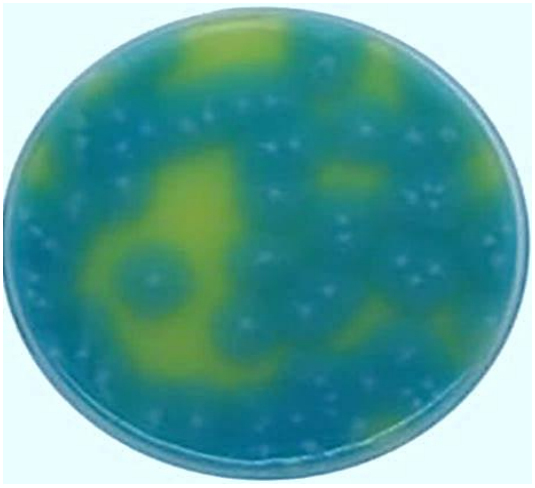

Fig 3: *Bacillus cereus* on PYMBA (Photo by Md Atiqul Haque).

Fig. 4: *Bacillus cereus* on Blood agar (Photo by Hongkun Quan).

resolution of minor variations in genomic sequences for bacterial group studies. The RAPD-PCR method is a reliable and widely used for molecular typing of diverse *Bacillus* spp; it uses specific primers to randomly amplify segments of target DNA. It can be used to distinguish emetic strains from other *B. cereus* strains and is thus commonly shown in the laboratory as a screening process. MLST is considered the "gold standard" for typing of *B. cereus* group strains, showing the sequences of many basic or housekeeping genes clustered across the chromosomes, occurring in three major *Bacillus* spp. clades.

For certain applications, AFLP may be preferable to classify various *B. cereus* strains into different phylogenetic classes (Pontieri 2016; Griffiths and Schraft 2017; Grutsch et al. 2018).

Spectrometry

MALDI-TOF-MS has been widely adopted and applied in clinical microbiology for routine pathogen detection at the species level. The mass/charge ratio of microbial proteins ionized from intact cells collected from pure culture is graphed as a peak in mass spectrometry results. The mass spectral profile is defined by comparing it to a reference database. MALDI-TOF–MS was used to detect enterotoxins (CytK1 and Nhe) produced by pathogenic strains and was found to be an effective risk assessment technique in routine pathogen detection for determining the presence of *B. cereus* strains in food-borne outbreaks (Pontieri 2016).

Biosensors

Biosensors have proven to be effective in detecting food-borne pathogens, such as *B. cereus*. Several biosensors-based techniques for detecting *B. cereus* have been developed and published so far. DNA-based biosensors, in particular, have shown a great success because they allow for the selective identification of different *B. cereus* strains. As an alternative to DNA probes, mono or poly-clonal antibodies targeting *B. cereus* cells can be used as identification elements in biosensors (Ramarao et al. 2020).

A biosensor incorporates rabbit polyclonal anti-*B. cereus* antibodies that have been shown to have high sensitivity, detecting *B. cereus* at concentrations as low as 10^1 CFU/ml, and rapidity with a detection period of just 6 minutes (Raddadi et al. 2010).

Detection and Quantification of toxins

Detection and Quantification of Cereulide (*Ces*)

For the detection and quantification of cereulide (Ces) toxin in various food matrixes, a variety of assays are now available. Generally, cell culture-based assays, using various cell lines (HEp-2, Hep-G2, CaCo-2, HeLA cells), and sperm-based assays in which the biological effects of Ces can be assessed by inhibition of mitochondrial function, cellular vacuolization and loss of motility of boar spermatozoa, have been used. These assays, however, are not so precise, since other mitochondrial toxins are also susceptible to them and impair sperm motility (Raddadi et al. 2010; Cui et al. 2019). Instrumental methods, such as high-performance liquid chromatography (HPLC), HPLC linked to ion trap mass spectrometry (HPLC-MS), and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) are used to detect Ces toxin (Cui et al. 2019). A new reversed - phase chromatography (RPC) was developed recently to identify and quantitatively measure Ces toxin existence, using ultra performance liquid chromatography-mass spectrometry (UPLC–MS/MS) (Kalbhenn et al. 2021).

Detection and Quantification of Enterotoxins (Nhe, Hbl, and CytK)

Various tests, such as the vascular permeability reaction (VPR), the ligated rabbit ileal loop, and cell cytotoxicity assays, are currently available and widely used for the detection of diarrheal toxins (Raddadi et al. 2010). VPR

Table 4: Main features of food-borne diseases associated with *B. cereus*

Properties	Diarrheal syndrome	Emetic syndrome	Reference
Type	Toxico-infection	Intoxication	Raddadi et al. (2010), Lindbäck and Granum (2019), Kumari and Sarkar (2016), Griffiths and Schraft (2017), Lindbäck and Granum (2019)
Mode of action	Receptor unknown, however Hbl, Nhe and Cytk specific receptors are suggested; causes hemolysis, cytolysis, demonecrosis and vascular permeability activity	Binds to serotonin 5-HT3 receptors; causes emesis by action on vagus afferent, inhibition of mitochondrial fatty acid oxidation and T (NK) cells	
Regulatory gene	PlcR	SpooA and AbrB	
Infective dose	10^5–10^7 cells (total) or cfu/g	10^5–10^8 cells (per g/ml substrate) or 0.02–1.83 µ g/kg of body weight	
Toxin production	In the small intestine of the host	Preformed in foods	
Toxin involve	Cereulide	Hbl, Nhe and CytK	
Nature of toxin	Protein(s)	Cyclic peptide	
Heat stability	Labile, inactivated 56°C, 5 min	Extremely stable 121°C, 90 min	
pH stability	Unstable < 4 and > 11	Stable 2- 11	
Requirements for illness	Vegetative cells of spore production in food to an infectious dose, consumption of which leads to infection and formation of toxins inside of a host	Cereulide production in food at high concentration outside of host resulting illness due to consumption of pre-formed toxin	
Incubation time	8–16 h (occasionally >24 h)	0.5–6 h	
Duration of illness	12–24 h (occasionally >24 h)	6–24 h	
Symptoms	Abdominal pain, watery diarrhea (occasionally bloody type), sometimes with nausea and lethality	Nausea, vomiting, malaise (sometimes followed by diarrhea), in some cases fatal liver failure	
Foods commonly implicated	Proteinaceous foods: meat products, fish, poultry, soups, vegetables, puddings, sauces and stews, milk and milk products	Farinaceous/Starch-rich foods; fried and cooked rice, pasta, potatoes, bread, pastries and noodles	
Assays available for detection	Hep-2 cell bioassay, rapid sperm bioassay, HPLC-MS and PCR-based assays	BCET-RPLA, Tecra BDE-VIA kit, and Duopath Cereus Enterotoxins test assay	

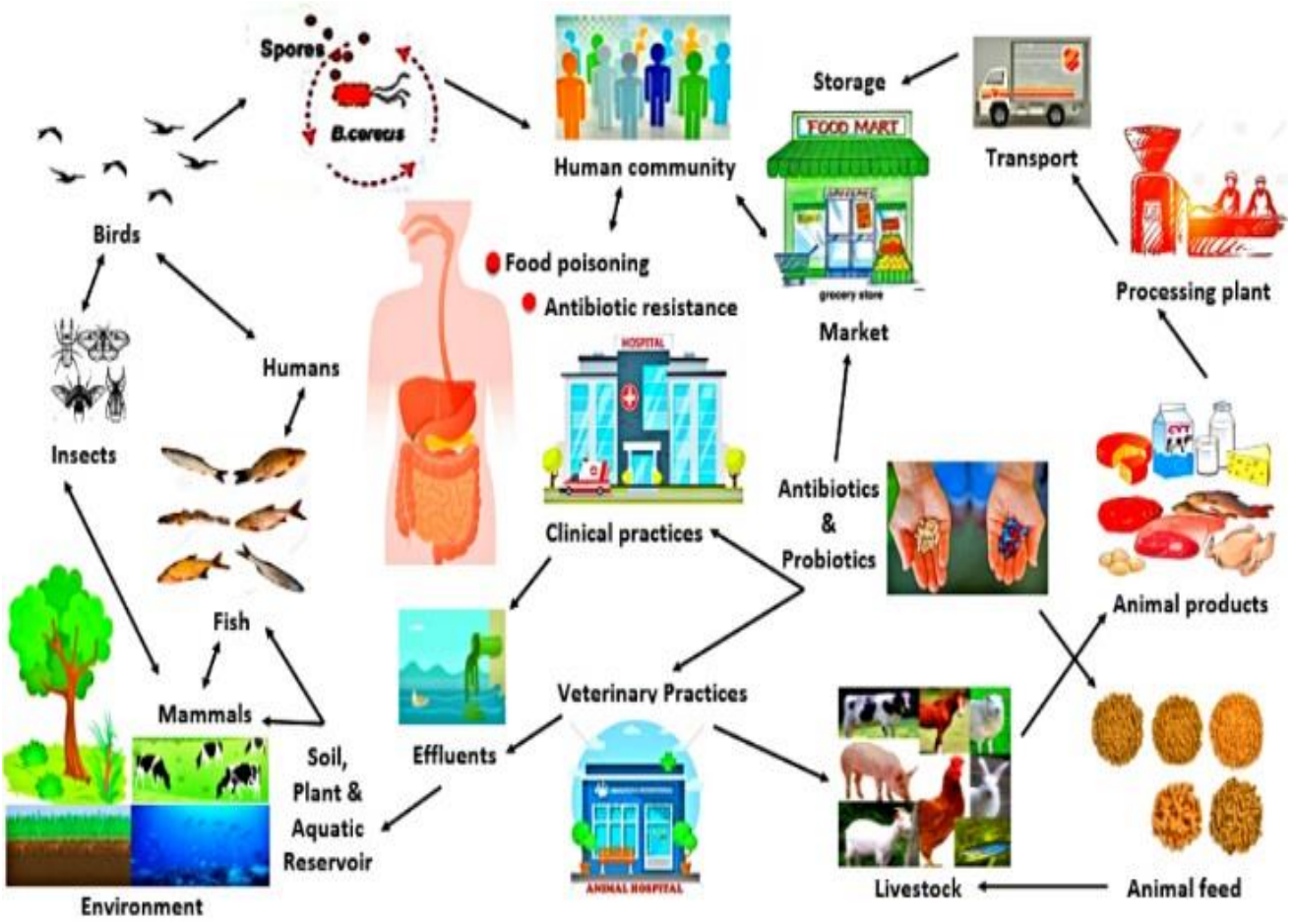

Fig 5: Flowchart showing the presence of antibiotic residues arising from the use of probiotic and antibiotic in animal feed (Arsene et al. 2021; Haque et al. 2021; Hassan et al. 2021).

Table 5: *Bacillus* spp. implicated in food-borne infections and the related toxins

Species	Toxins	Features	References
B. thuringiensis	Enterotoxins	Heat-labile, cytotoxicity, risk of food-borne intoxication implicated	Griffiths (2010); EFSA (2011); Delbrassinne and Mahillon (2016); Mingmongkolchai and Panbangred (2018); Haque et al. (2021);
B. weihenstaphanensis	Cereulide	Heat-stable, risk of food-borne intoxication implicated	
B. subtilis	Amylolysin, fengycin	Surfactin-like components, heat-stable, inhibition of boar sperm motility, cytotoxicity, implicated in food-borne gastroenteritis	
B. licheniformis	Lichenysins	Heat-stable lipopeptide, inhibition of boar sperm motility, implicated in food-borne gastroenteritis, fatal cases reported (dairy and infant food), also involved in local and systemic infections	
B. pumilus	Pumilacidins	Complex lipopeptides, heat-stable, inhibition of boar sperm motility, implicated in food-borne poisoning, also involved in local and systemic infections	
B. fusiformis	Cytotoxins	Lipopeptides; heat-stable, cytotoxicity	
B. mojavensis	Amylolysin, fengycin	Surfactin-like components, heat-stable, cytotoxicity, inhibition of boar spermatozoa motility, implicated in food-borne poisoning	
B. amyloliquefaciens	Amylosin	Heat-stable lipopeptide, connected with food poisoning	
B. firmus	Cereulide-like toxins	Lipopeptides; heat-stable, inhibition of boar sperm motility	
B. simplex	Cereulide-like toxins	Lipopeptides; heat-stable, inhibition of boar sperm motility	
B. circulans	Cytotoxins	Implicated in food-borne poisoning, also involved in local and systemic infections	
B. lentus	Cytotoxins	Toxin production observed, but no food poisoning case reported yet	
B. megaterium	Cereulide-like toxins	Lipopeptides, implicated in food-borne poisoning	

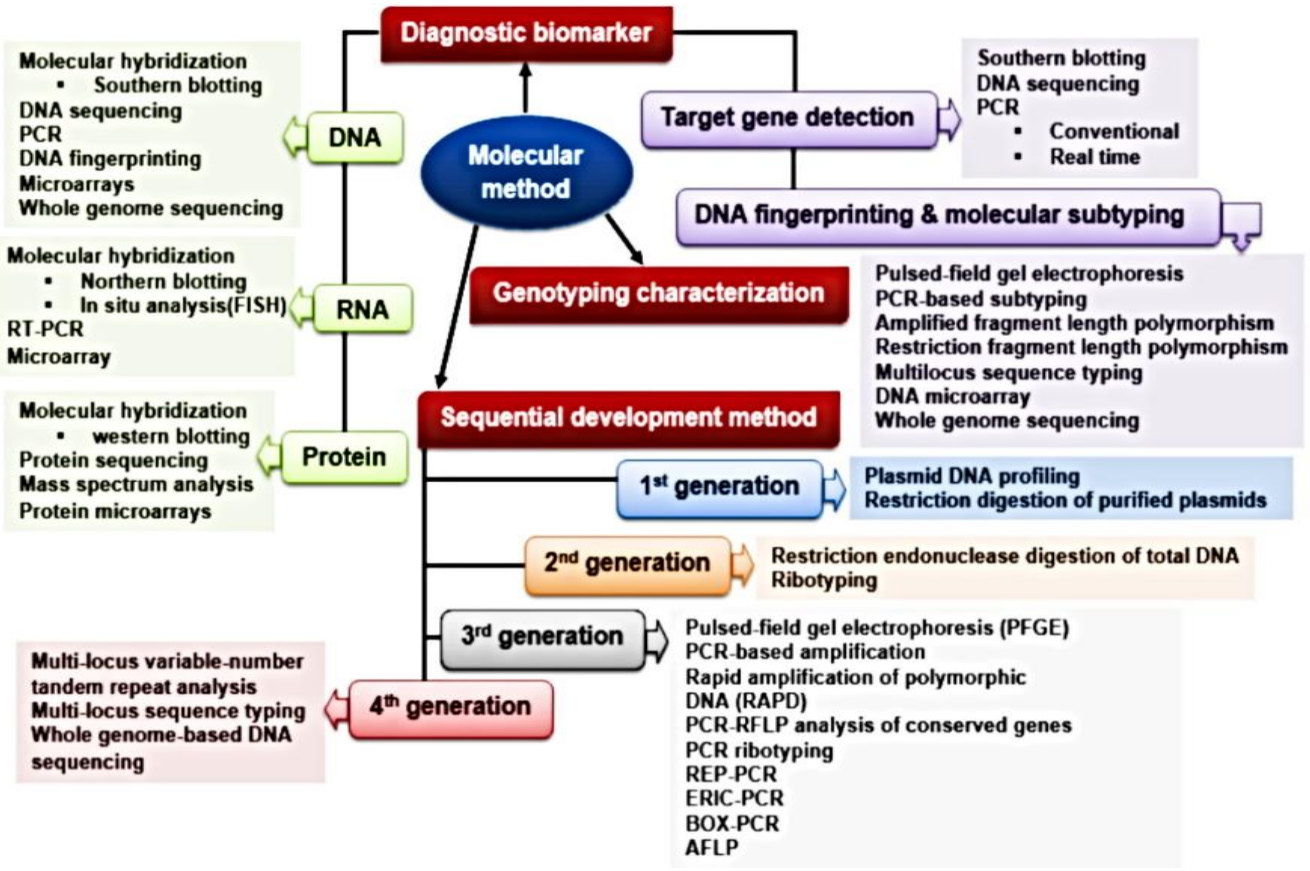

Fig. 6: Molecular methods for the isolation and identification of bacteria (Raddadi et al. 2010; Quinn et al. 2016).

Table 6: Advantages and disadvantages of different diagnostic methods

Methods		Advantages	Disadvantages	References
Traditional method	Culture medium-based method and microscopic observation with Biochemical assay	Simple, cheap and easy handling Able to recognize a single bacterial strain Identification of viable cells Optimal toward suitable media For phenotypic drug susceptibility testing Inexpensive equipment	Time-consuming, laborious and resource demand Poor sensitivity and specificity Not possible to detect injured or VBNC cells Can lead to misidentification Risk of contamination Require qualified personnel	Raddadi et al. (2010); Pontieri (2016); Ehling-Schulz et al. (2011);
Immunological method	ELISA, RPLA and immunofluorescence assay	Precise and reproducible results Inexpensive equipment Onsite application	Relatively low sensitivity and selectivity/ specificity Immunodeficient host may not be able to respond	Abbasian et al. (2018); Grutsch et al. (2018); Bao et al. (2020);
Molecular method	PCR	Highly accurate Relatively high sensitivity and specificity	Not real-time Not able to distinguish dead and alive cells Not for qualification Sophisticated, expensive equipment and costly Sometimes amplification errors or false-negative results Require enrichment step in case of a lower number of pathogens Gel electrophoresis is laborious, time-consuming and low-resolution False-positive results due to laboratory contamination.	Kim and Kim (2020); Mishra et al. (2020); Ramarao et al. (2020); Vidic et al. (2020); Kalbhenn et al. (2021)
	Real-time PCR	Highly accurate Quantitative Strong sensitivity and specificity Very precise and robust Easy and fast data processing	Not real-time Not able to distinguish dead and alive cells Require enrichment step in case of a lower number of contaminants Risk of contamination with genomic DNA Possibility of false-positive results	

	Multiplex PCR	Detection of multiple pathogens or toxins simultaneously Small quantities of DNA extracted Economic and time saving	Intrusion by the existence of polymorphism Possibility of false-negative results Only qualitative, not for quantification Further confirmation test is required
	Nested PCR	High sensitivity and specificity Decreases non-specific target sequence amplification	Costly and time-consuming Risk of cross-contamination
	ERIC- PCR	Highly reliable Easy and first Inexpensive	Low discrimination
	RAPD	Very simple and fast Inexpensive	Extremely laboratory-based Requires carefully designed laboratory protocols for reproduction
	AFLP	Strongly distinguishable Sensitive and specific High resolution and sampling power Reproducibility Genetic heterogeneity detection	Costly More reaction phage and reagents
	RFLP	Strong discrimination and typification Clarity	Lower sensitivity for specific mutant Laborious and time-consuming
	MLST	Rapid tool for large population analysis Repeatable and indisputable Fully automated analysis	Expensive Requires intensive sequencing efforts
	NGS	Highly accurate Quantitative Detection of multiple pathogens in one sample	Expensive Not real-time Required bioinformatics analysis
	PFGE	Stability, Reproducibility More discriminatory	Time-consuming Expensive Labor intensive
	LMAP	Highly accurate High specificity and sensitivity Rapid, simple and efficient Without expensive equipment and complicated thermo-cycling	Strict primer design principle Possibility of false-positive results
	CAMP	Highly accurate Relatively more rapid, specific and sensitive Easy, reliable and simpler primer design	Possibility of false-positive results
	Hybridization	High sensitivity and specificity Low cost Fast detection	Costly and time-consuming Focused on DNA assay
	DNA microarray	High efficiency Multiple genes analysis	Confounding first time users Findings are not reproducible
Spectrometry and chromatographic method	MALDI-TOF-MS	Highly accurate Fast, easy and reliable Cost-effectiveness	Pure cultures are needed Quantification error
	LC-MS	Highly accurate	Expensive MS equipment required
	SIDA–MS/MS	Highly accurate	Sophisticated and expensive high-end MS equipment required
	RPC	Fast, easy and robust Cost-effective	Require trained personnel
Biosensors	Gold nanoparticle (AuNPs) aggregation	Onsite application Easy, rapid and detection by the naked eye	Costly and relatively low affinity Not easy to apply in solid or liquid-solid matrix

VBNC=Viable but not-culturable; PCR=Polymerase chain reaction; ERIC-PCR=Enterobacterial Repetitive Intergenic Consensus-PCR; ELISA=Enzyme-linked immunosorbent assay; RPLA=Reverse passive latex agglutination; LMAP=Loop-mediated isothermal amplification; CAMP=Competitive annealing mediated isothermal amplification; MLST=Multilocus sequence typing; NGS=Next generation sequencing; MALDI-TOF-MS=Matrix-assisted laser desorption ionization time-of-flight mass spectrometry; LC-MS=Liquid chromatography coupled to mass spectrometry; SIDA–MS/MS=Stable isotope dilution assay tandem mass spectrometry; RPC=Reversed-phase chromatography; RAPD=Random amplified polymorphic DNA; AFLP=Amplified fragment length polymorphism; RFLP=Restriction fragment length polymorphisms; PFGE= Pulsed-field gel electrophoresis.

Table 7: Phenotypic criteria to differentiate members of the *B. cereus* group

Features	*B. cereus*	*B. anthracis*	*B. thuringiensis*	*B. mycoides*	*B. pseudomycoides*[a]	*B. weihenstepanensis*[b]	*B. cytoticus*[c]	*B. toyonesis*[d]	References
Gram stain	+	+	+	+	+	+	+	+	Fritze and Pukall (2011); Jimenez et al. (2013); Eglezos and Dykes (2014); Pontieri (2016); Lindbäck and granum (2019); Ramarao et al. (2020); Haque et al. (2021)
Colony morphology	White	White	White/gray	Rhizoid	Rhizoid	White	White	white	
Hemolyis	+	-	+	(+)	(+)	+	+	+	
Motility	±	-	±	-	-	+	+	+	
Susceptibility to penicillin	-	+	-	-	-	-	-	-	
Parasporal crystal	-	-	+	-	-	-	-	-	
Growth temperature range (°C)	10-45	>10-<50	10-45	15-40	10-40	5-37	20-50	10-45	
Lysis by gamma phage	-	+	-	-	-	-	-	-	
Catalase	+	+	+	+	+	+	+	+	
Citrate utilization	+	-	+	-	-	-	-	+	
Lecithinase activity (Egg yolk reaction)	±	(+)	±	±	(+)	+	(+)	+	
Acid from mannitol	-	-	-	-	-	-	-	-	
Glucose anaerobic utilization	+	+	+	+	+	+	+	+	
Reduction of nitrate	±	+	+	+	+	+	+	+	
VP reaction	+	+	+	+	+	+	(+)	+	
Tyrosine decomposition	+	(+)	+	(+)	+	+	+	+	
Resistance to lysozyme	+	+	+	+	+	+	+	+	
Anaerobic growth	+	+	+	+	(+)	-	(+)	+	
Starch	+	+	+	+	+	+	-	+	
Indole	-	-	-	-	-	-	-	-	

[a]Differentiated from *B. mycoides* based on fatty acid composition and 16S RNA sequence; [b]Differentiated from *B. cereus* based on growth at <7°C and not at 43°C; it can be identified rapidly using rRNA gene- or cspA (cold shock protein A gene)-targeted PCR; [c]Differentiated from *B. cereus* by maximum growth at 50°C and minimum growth at 20°C, by the absence of starch hydrolysis, and by the absence of growth on synthetic media without tryptophan; [d]distinguished from other *B. cereus* group members by pairwise calculation of the average nucleotide identity; +, Positive; -, negative; (+), weakly positive; ±, usually positive but occasionally may be negative; VP, Voges-Proskaurer

involves injecting 0.1 ml of cell-free culture supernatant intra-dermally into 2.5–3.0 kg rabbits. After 3 hours, a 4 ml intravenous injection of 2% Evans blue dye solution is given. After 1 hour the perpendicular diameters of the light and dark blue areas, as well as any necrosis, are measured. The ligated rabbit ileal loop assay involves injecting bacterial culture supernatant into a 5 cm ileal loop of female New Zealand White rabbits. If the amount of fluid retention to loop tube diameter is >0.5, the test is positive. In cytotoxicity assays, filtered supernatant is added to a cell line, and the treatment effects on the cells are assessed. The diarrheal toxins can affect a variety of cell lines, such as Vero (monkey kidney), Chinese hamster ovary (CHO), HeLa S3, Human Embryonic Lung (HEL) and McCoy cell lines (Raddadi et al. 2010).

Commercial kits/immunoassays

Enterotoxins can be detected by using different commercial immunological assays. The presence of the L2 portion of HBL and NheA is measured by the BCET-RPLA Toxin Detection kit (Oxoid, UK) and Tecra BDE-VIA kit (Tecra Diagnostics, Australia), respectively. The Duopath Cereus Enterotoxins (Merck KGaA Chemicals, Germany) test assay simultaneously detects both HBL and Nhe (Raddadi et al 2010; Lindbäck and Granum 2015).

Control and prevention

B. cereus and its latent spores are ubiquitously present in nature, so they can easily contaminate various types of food and degrade the organoleptic properties of food (especially eggs, meat and milk based products), affecting their market quality. This is a serious public health issue, as well as a significant economic risk for the food industry. However, due to the absence of any legislation for the systematic screening of food items for pathogen contamination, limits on the quantity of *B. cereus* cells in foodstuffs have been set in various nations and regions, based on standard recommendations (Table 9). The majority of *B. cereus* of food-borne outbreaks have been linked to bacterial concentrations >10^5 CFU/g of food material, while some instances are linked to number as low as 10^3 CFU/g. Furthermore, determination of a safe limit is difficult, as the pathogenicity is not only assessed by the quantity of bacterial cells. Regulation will focus on *B. cereus* group food safety, with a maximum tolerable limit (MTL) of 10^3 cfu/g in dairy products for the general population, 10^2cfu/g in infant formula, 10^3 cfu/g in RTE meat and 10^5 cfu/g in egg products. Food processors should guarantee that *B. cereus* counts of 10^3-10^5/g are not reached (or surpassed) at the point of consumption under normal storage and handling settings, which must also be

Table 8: Diagnostic marker genes for *Bacillus* spp.

Species	Target gene by PCR			References
	Gene		Prevalence (%)	
B. cereus	*ces*	*cesA*	1.5-32.8	Raddadi et al. (2010); EFSA (2011); Haque et al. (2021)
		cesB		
	hbl	*hblA*	29–92	
		hblB		
		hblC		
		hblD		
	nhe	*nheA*	85– 100	
		nheB		
		nheC		
	cytK	*cytK-1*	40–89	
		cytK-2		
	entFM		84– 100	
	bceT		12–75	
	hlyII		19–56	
B. cereus group	*gyrB*		-	
	groEL		-	
B. subtilis group	*gyrase A*		-	
	gyrase B		-	
B. weihenstephanensis	*cspA (heat shock protein)*		-	
B. cytoticus	*cytK-1*		-	

Table 9: Maximum tolerable limit (MTL) of *B. cereus* contamination in different foodstuffs

Country/authority	Food items	MTL (cfu/g)	Reference
EU	DIF	50	Chon et al. (2015); McLauchlin et al. (2016); EFSA (2016); Osimani et al. (2018); Ramarao et al. 2020; Haque et al. (2021)
CAC, FAO, WHO	IF	10^2	
FSANZ, Korea	RTE food	10^2 - $<10^3$	
Ireland	RTE food	10^3 - $<10^4$	
UK	RTE food, Dried herbs and spices	10^3 - $<10^4$	
HPAUK, CFSHK	RTE food	10^3 - $<10^5$	
FDA	Dairy products, cheese and cheese products	$<10^4$	
	DIF and DDF intended for infants <6 months of age	10^2	
France	Cooked ham and salami, CFP, cooked meat-based products, RTE meals, sauces, cold starters, salads containing raw vegetables and cheese, fish- or meat-based starters, cooked starters, cured meats served hot or cold, RTE cooked pastries	$<10^2$	
	Starch-rich food	10^5	
Philippine	Frozen entrees containing rice or corn flour as main ingredient, Tofu, CBF for infants	10^2	
Italy	Pasta or rice salads, cheese meals, pizza, bread, cooked products and cold served foods, pastries and biscuits, fish-based products, honey, cereals, RTE vegetables, cooked ham and salami, cheese made from pasteurized milk, gastronomic products, fresh pastry, egg-based pasta, RTE dishes	$<10^2$	
	Raw vegetables, spices, herbs, sandwiches, salads containing uncooked ingredients	$<10^3$	
Spain	Teas and derivates, herbs and spices	$<10^3$	
Germany	Herbs and spices, tofu	$<10^3$	
	CBP, sandwiches, sprouts, RTE hot products	$<10^2$	
Portugal	Sashimi	$<10^2$	
Croatia	Puddings, heat-treated dairy desserts and related products	$<5\times10^2$	
	RTE dried foods for infants	<10	

EU=European Union, CAC= Codex Alimentarius Commission, FAO= Food and Agriculture Organization of the United Nations, WHO= World Health Organization, FSANZ= Food Standards Australia New Zealand, UK=United Kingdom, HPAUK=Health Protection Agency United Kingdom, CFSHK=Centre for Food Safety, Hong Kong, FDA=Food and Drug Administration, RTE=Ready to eat, DIF=Dried infant formulae, IF= Infant formulae, DDF=Dried dietary foods, CPF=Cooked food products, CBF=Cereal base foods, CBP=Cereal based products.

applied to rehydrated foods reconstituted with hot water before intake (Blackburn and McClure, 2009; Zhang et al. 2020; Ramarao et al. 2020; Yu et al. 2020; Haque et al. 2021). While *B. cereus* is present in many foods, its vegetative form is inhibited by most cooking methods, it still challenges with spores survival and later outgrowth remains in damp protein-based foods and rice.

Cooked foods should either be kept at a temperature above 60°C or quickly cooled and refrigerated below 4°C to prevent the growth of *B. cereus* spores (Eglezos and Dykes 2014). *Bacillus* strains also have the potential as promising probiotics to enhance human and animal health by consuming large amounts of live cells directly. Probiotic *Bacillus* spp. may possess toxicity and transmit

ARGs between probiotics and opportunistic or pathogenic bacteria in GITs. Toxicity testing is a primary safety concern for probiotics candidates that are to be consumed by humans and livestock, thus the absence of *B. cereus* toxin and susceptibility to antibiotics in *Bacillus* spp. intended for use as feed additives must be thoroughly investigated. To reach a consensus on the phenotypic and genotypic characteristics of targeted *Bacillus* spp. and their correlation with those having generally recognized as safe (GRAS) status, the entire genome should be sequenced and analyzed to look for genes that are responsible for the production of enterotoxins and the emetic toxin.

The necessity of strain-level identification, on the other hand, is essential for detecting and removing any causative relationship between probiotics and strains obtained from immune-compromised hosts. As a result, it is critical to remember that clinical studies of these regimens should include a large proportion of the target population, including persons with poor immunity. Therefore, more work is needed to be done in terms of monitoring virulence factors, toxins and antibiotic resistance determinants in probiotic *Bacillus* spp. (Elshaghabee et al., 2017; Mingmongkolchai and Panbangred 2018; Cui et al. 2019; Deng et al. 2021). In addition, widespread antibiotic use can result in the development of antibiotic-resistant bacterial strains, with the potential for resistance genes to be passed on to other pathogenic and nonpathogenic bacteria, as well as the human food chain. So, it is critical to use legal antimicrobials in food-produing animals by registered health experts, maintaining withdrawal periods, limiting antimicrobials products as feed additives, to ensure proper sewage treatment of human and veterinary hospital effluents, and prohibiting the use of poultry offal, litters and livestock waste in aquaculture (Hassan et al. 2021). Antimicrobial resistance profiles and other virulence factors of *Bacillus* spp. have recently been evaluated, using next generation sequencing. This method could change probiotic exploration, because it can detect other probiotic characteristics, such as bacteriocin production, adhesion-ability, and signaling pathways at the genome level, in addition to safety hazards (Ramlucken et al. 2020). Organic acids (acetic, butyric, citric, formic, lactic, propionic, malic, and sorbic acids) and their salts (sodium acetate, sodium butyrate, sodium citrate, sodium formate, sodium lactate, and sodium propionate) have also been used as acidifiers in animal feeds to improve gut health and performance, as well as weight gain, survival, and FCR. Acidifiers have a similar effect to antibiotics in that they significantly regulate gut bacterial populations and boost immune response. Acidifiers coated salts are now commercially available for usage in food animals, particularly pigs and poultry. Combining organic acids with other antimicrobial substances, such as phytochemicals or permeabilizers, in an effort to use possible synergy to more efficiently combat pathogenic bacteria, fungi or mold in feed prophylactic measure, is a new emerging strategy to modulate gut microflora and reduce pathogens in the gut (Pearlin et al. 2020). Fermented feed ingredients (soybean and corn) in herd and poultry diets, as well as soybean food for human consumption, may contain *B. cereus* vehicles that can be regulated using uniform fermentation principles, such as the structure and composition of the testing products, the basic culture technique, fermentation criteria, post-fermentation methods and the utilization of bacterial peptides, bacteriocins and other antimicrobials (Haque et al. 2021). Since the emergence of antibiotic-resistant bacteria, phage endolysins, especially LysB4EAD-LysSA11, hybrid endolysin have piqued interest as a promising alternative to antibacterial agents for the simultaneous control of multiple bacteria, including *B. cereus*. Furthermore, this strategy would allow for the development of multifunctional and highly specific antimicrobials, thereby reducing the prevalence of multidrug-resistant bacteria (Son et al. 2020). Besides this, natural antibacterial agents, such as Makino, Asteraceae, Roselle, Rosemary, clove, thyme and others, may be possible candidates for the production of new strategies to combat the spread of *B. cereus* in the food and feed industry (Haque et al. 2021).

REFERENCES

Abbasian F et al., 2018. Microbiological sensing technologies: A review. Bioengineering 5: 1-33.

Abdeen EES et al., 2020. Prevalence of virulence determinants among *Bacillus cereus* from milk products with potential public health concern. Pakistan Journal of Biological Sciences 23: 206-212.

Abou Zeid MAM and Yassin SA, 2017. Detection of some virulence factors of *Bacillus cereus* in heat-treated milk. Alexandria Journal of Veterinary Sciences 53: 72-78.

Abouelhag HA et al., 2021. Prevalence, antibiogram pattern and virulence genes profile of *Bacillus cereus* isolated from buffalo milk. International Journal of Veterinary Science. 10: 234-239.

Adame-Gomez R et al., 2020. Prevalence of the strains of *Bacillus cereus* group in artisanal Mexican cheese. Foodborne Pathogens and Disease 17: 8-14.

Adesetan TO et al., 2019. Biochemical characterization and antimicrobial susceptibility of *Bacillus cereus* isolates from some retailed foods in Ogun state, Nigeria. Journal of Microbiology, Biotechnology and Food Science 9: 616-621.

Al-Abri SS et al., 2011. A hospital acquired outbreak of *Bacillus cereus* gastroenteritis, Oman. Journal of Infection and Public Health 4: 180–186.

Al-Jobory LSA and Abdulaal NI, 2020. Checkerboard pattern of green tea and rosemary extracts on multi drugs resistant *Bacillus cereus* recovered from soft cheese in Kirkuk, Iraq. Plant Archives 20: 6799-6805.

Ankolekar C et al., 2009. Detection of toxigenic *Bacillus cereus* and *Bacillus thuringiensis* spores in U.S. rice. International Journal of Food Microbiology 28: 460–466.

Arsene MMJ et al., 2021. The use of probiotics in animal feeding for safe production and as potential

alternatives to antibiotics. Veterinary World 14: 319-328.
Banerjee M et al., 2011. Phenotypic and genetic characterization of *Bacillus cereus* isolated from the acute diarrheal patients. Indian Journal of Medical Research 133: 88-95.
Bao H et al., 2020. Rapid and simple detection of *Bacillus cereus* in milk by real-time competitive annealing mediated isothermal amplification. The Analyst 145(20): doi: 10.1039/DoANo0965B.
Batchoun R et al., 2011. Molecular characterization of *Bacillus cereus* toxigenic strains isolated from different food matrices in Jordan. Foodborne Pathogens and Disease 8: 1153–1158.
Berthold-Pluta A et al., 2019. Prevalence and toxicity characterization of *Bacillus cereus* in food products from Poland. Foods 8: 1-12.
Bilal M et al., 2020. Effects of novel probiotic strains of *Bacillus pumilus* and *Bacillus subtilis* on production, gut health and immunity of broiler chickens raised under suboptimal conditions. Poultry Science 100: 1-11.
Blackburn CDW and PJ McClure, 2009. Pathogenic Bacillus species: Foodborne Pathogens, 2nd Ed., Woodhead Publishing Series in Food Science, Technology and Nutrition, pp: 844-888.
Carlin F and Nguyen-The C, 2013. Pathogen update: *Bacillus* species: Advances in Microbial Food Safety, Woodhead Publishing Limited; pp: 70–96.
Carroll LM et al., 2019. Characterization of emetic and diarrheal *Bacillus cereus* strains from a 2016 foodborne outbreak using whole-genome sequencing: Addressing the microbiological, epidemiological and bioinformatic challenges. Frontiers in Microbiology 10: 1-20.
Carroll LM et al., 2020. Proposal of a taxonomic nomenclature for the *Bacillus cereus* group which reconciles genomic definitions of bacterial species with clinical and industrial phenotypes. mBio 11: 1-20.
Chang HJ et al., 2011. Prevalence of the levels of *Bacillus cereus* in fried rice dishes and its exposure assessment from Chinese-style restaurants. Food Science and Biotechnology 20: 1351–1359.
Chaves JQ et al., 2017. Molecular characterization and risk assessment of *Bacillus cereus sensu lato* isolated from ultrahigh-temperature and pasteurized milk marketed in Rio de Janeiro, Brazil. Journal of Food Protection 80: 1060–1065.
Chen D et al., 2019. A foodborne outbreak of gastroenteritis caused by Norovirus and *Bacillus cereus* at a university in the Shunyi district of Beijing, China 2018: A retrospective cohort study. BMC Infectious Diseases 19: 1-6.
Choi KB et al., 2011. Epidemiological investigation for outbreak of food poisoning caused by *Bacillus cereus* among the workers at a local company in 2010. Journal of Preventive Medicine and Public Health 44: 65-73.
Chon JW et al., 2012. Prevalence, phenotypic traits and molecular characterization of emetic toxin-producing *Bacillus cereus* strains isolated from human stools in Korea. Journal of Applied Microbiology 112: 1042–1049.
Chon JW et al., 2015. Quantitative prevalence and toxin gene profile of *Bacillus cereus* from ready-to-eat vegetables in South Korea. Foodborne Pathogens and Disease 12: 795–799.
Chrun et al., 2017. Microbiological hazard contamination in fermented vegetables sold in local markets in Cambodia. Biocontrol Science 22: 181-185.
Cui Y et al., 2016. Characterization of *Bacillus cereus* isolates from local dairy farms in China. FEMS Microbiology Letters 363: 1-6.
Cui Y et al., 2019. Multifaceted toxin profile, an approach toward a better understanding of probiotic *Bacillus cereus*. Critical Reviews in Toxicology 49: 342-356.
Dallal SMM et al., 2017. To determine the frequency of *Bacillus cereus* in powdered milk infant formula consuming in neonatal intensive care unit (nicu) in Tehran hospitals in 2013-14. Iranian South Medical Journal 19: 982-988.
Delbrassinne L and Mahillon J, 2016. Bacillus: Occurrence: Encyclopedia of Food and Health, Elsevier Ltd. pp: 307–311.
Delbrassinne L et al., 2012. Prevalence and levels of *Bacillus cereus* emetic toxin in rice dishes randomly collected from restaurants and comparison with the levels measured in a recent foodborne outbreak. Foodborne Pathogens and Disease 9: 809–814.
Deng F et al., 2021. Antimicrobial resistance, virulence characteristics and genotypes of Bacillus spp. from probiotic products of diverse origins. Food Research International 139: 1-9.
Depo M et al., 2018. *Bacillus cereus* contamination of donated food for an orphanage in Gunung Kidul district, Indonesia, 2017. Proceedings of "9th TEPHINET Bi-regional Conference" Lao PDR, 2018.
Dichtl K et al., 2019. Food poisoning: An underestimated cause of Boerhaave syndrome. Infection 48: 125-128.
Dierick K et al., 2005. Fatal family outbreak of *Bacillus cereus*-associated food poisoning. Journal of Clinical Microbiology 43: 4277–4279.
Ehling-Schulz M et al., 2019. The *Bacillus cereus* group: *Bacillus* species with pathogenic potential. Microbiology Spectrum 7: 1-35.
Ehling-Schulz M and Messelhäusser U, 2012. One pathogen but two different types of foodborne outbreak: *Bacillus cereus* in catering facilities in Germany: Case Studies in Food Safety and Authenticity. Woodhead Publishing series in Food Science, Technology and Nutrition 63–70.
Ehling-Schulz M et al., 2011. *Bacillus cereus* in milk and dairy production. In: Hoorfar J (editor). Rapid Detection, Identification, and Quantification of Foodborne Pathogens. ASM Press, Washington, DC; USA, pp: 275-289.
EFSA, 2011. Panel on additives and products or substances used in animal feed (FEEDAP): Technical guidance on the assessment of the toxigenic potential of Bacillus species used in animal nutrition. EFSA Journal 9: 2445.
EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and

Control), 2016. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA Journal 14: 4634.
Eglezos S and Dykes GA, 2014. Microbiological safety of meat, *Bacillus cereus*: Encyclopedia of Meat Sciences, 2nd Ed., Elsevier Ltd. pp: 324–329.
Elias SDO et al., 2018. Foodborne outbreaks in Brazil associated with fruits and vegetables: 2008 through 2014. Food Quality and Safety 2: 173–181.
Elshaghabee FMF et al., 2017. Bacillus as potential probiotics: Status, concerns, and future perspectives. Frontiers in Microbiology 8: 1-15.
Enan G et al., 2020. Incidence of *Bacillus cereus* in Egyptian foods and its control by probiotics. Bioscience Research 17: 550-559.
Flores-Urban KA et al., 2014. Detection of toxigenic *Bacillus cereus* strains isolated from vegetables in Mexico City. Journal of Food Protection 77: 2144–2147.
Food safety authority of Ireland, 2016. https://www.scribd.com/document/390600011/*Bacillus-Cereus*-Factsheet-2016-FINAL-ACCESSIBLE
Fritze D and Pukall R, 2012. Culture media for *Bacillus* spp. and related genera relevant to foods. In: Corry JEL, Curtis GDW, Baird RM (editors). Handbook of Culture Media for Food and Water Microbiology. 3rd Ed., Royal Society of Chemistry, pp: 90-114.
Gharib AA et al., 2020. Multiplex polymerase chain reaction for detection of toxin genes of *Bacillus cereus* group isolated from meat and chicken products. Zagazig Veterinary Journal 48: 457-470.
Griffiths MW, 2010. *Bacillus cereus* and other *Bacillus* spp. In: Juneja VK, Sofos JN (editors). Pathogens and Toxins in Foods: Challenges and Interventions; ASM Press, Washington, DC, USA; pp: 1-19.
Griffiths MW and Schraft H, 2017. *Bacillus cereus* Food Poisoning: Foodborne Diseases, 3rd Ed., Academic Press, Elsevier Inc. pp: 395–405.
Grutsch AA et al., 2018. *Bacillus* spp. as pathogens in the dairy industry: Foodborne Diseases, Academic Press, Elsevier Inc. pp: 193–211.
Güven K and Mutlu MB, 2009. Properties of *Bacillus cereus* collected from different food sources. Turkish Journal of Biology 33: 101-108.
Gwack J et al., 2010. Trends in water and foodborne disease outbreaks in Korea, 2007–2009. Osong Public Health and Research Perspectives 1: 50–54.
Haque MA et al., 2021. Pathogenicity of feed-borne *Bacillus cereus* and its implication on food safety. Agrobiological Records 3: 1-16.
Hassan MM et al., 2021. Residual antimicrobial agents in food originating from animals. Trends in Food Science and Technology 111: 141–150.
Heini N et al., 2018. Characterization of *Bacillus cereus* group isolates from powdered food products. International Journal of Food Microbiology 283: 59–64.
Hernandoa V et al., 2007. Investigation of a foodborne intoxication in a high-density penitentiary center. Gaceta Sanitaria 21: 452-457.
Heydarzadeh M et al., 2020. Detection of emetic toxin (ces) gene and antimicrobial susceptibility of *Bacillus cereus* isolates from Iranian traditional dairy products. Research Square. 2020: 1-13.
Ichikawa K et al., 2010. Acute encephalopathy of *Bacillus cereus* mimicking Reye syndrome. Brain and Development 32: 688–690.
Jimenez G et al., 2013. Description of *Bacillus toyonensis* sp. nov., a novel species of the *Bacillus cereus* group, and pairwise genome comparisons of the species of the group by means of ANI calculations. Systematic and Applied Microbiology 36: 383–391.
Kalbhenn EM et al., 2021. Detection and isolation of emetic *Bacillus cereus* toxin cereulide by reversed phase chromatography. Toxins 13: 1-14.
Kim CW et al., 2015. Prevalence, genetic diversity and antibiotic resistance of *Bacillus cereus* isolated from Korean fermented soybean products. Journal of Food Science 80: 123–128.
Kim JB et al., 2010. Food poisoning associated with emetic-type of *Bacillus cereus* in Korea. Foodborne Pathogens and Disease 7: 555–563.
Kim JH et al., 2009. A case of emetic toxin producing *Bacillus cereus* strains isolated from outbreak. Korean Journal of Clinical Microbiology 12: 48-52.
Kim SO and Kim SS, 2020. Bacterial pathogen detection by conventional culture-based and recent alternative (polymerase chain reaction, isothermal amplification, enzyme linked immunosorbent assay, bacteriophage amplification and gold nanoparticle aggregation) methods in food samples: A review. Journal of Food Safety 2020: 1-12.
Kim SO and Kim SS, 2021. Recent (2011–2017) foodborne outbreak cases in the Republic of Korea compared to the United States: A review. Food Science and Biotechnology 30: 185–194.
Kong L et al., 2021. An investigation on the occurrence and molecular characterization of *Bacillus cereus* in meat and meat products in China. Foodborne Pathogens and Disease 2021: 1-9.
Kumari S and Sarkar PK, 2016. *Bacillus cereus* hazard and control in industrial dairy processing environment. Food Control 69: 20–29.
Latsios G et al., 2003. Liver abscess due to *Bacillus cereus*: A case report. Clinical Microbiology and Infection 9: 1234-1237.
Lentz SAM et al., 2018. *Bacillus cereus* as the main causal agent of foodborne outbreaks in Southern Brazil: Data from 11 years. Cadernos de Saúde Pública 34: 1-9.
Lesley MB et al., 2017. Detection of *Bacillus cereus* in formula milk and ultra-high temperature (UHT) treated milk products. International Food Research Journal 24: 985-989.
Li X, 2020. Gastric ulceration and immune suppression in weaned piglets associated with feed-borne *Bacillus cereus* and *Aspergillus fumigatus*. Toxins 12: 1-13.
Liao SL and Tsai MH, 2020. *Bacillus cereus* bacteremia in a preterm infant caused by consumption of contaminated breast milk. Pediatrics and Neonatology 62: 337-338.

Lin KH and Yu YH, 2020. Evaluation of *Bacillus licheniformis*-fermented feed additive as an antibiotic substitute: Effect on the growth performance, diarrhea incidence, and cecal microbiota in weaning piglets. Animals 10: 1-16.

Lindbäck T and Granum PE, 2015. *Bacillus cereus* phospholipases, enterotoxins, and other hemolysins: The Comprehensive Sourcebook of Bacterial Protein Toxins, 4th Ed., Academic press, Elsevier Ltd. pp: 839–857.

Lindbäck T and Granum PE, 2019. *Bacillus cereus*. In: Doyle MP, Diez-Gonzalez F and Hill C (editors). Food Microbiology: Fundamentals and Frontiers, 5th Ed., ASM Press, Washington, DC, USA, pp: 541-554.

Liu Y et al., 2018. Detection of *Bacillus cereus sensu lato* from environments associated with goat milk powdered infant formula production facilities. International Dairy Journal.83: 10-16

Liu XY et al., 2020. Characterization of *Bacillus cereus* in dairy products in China. Toxins 12: 1-18.

Lopez AC et al., 2015. A case of intoxication due to a highly cytotoxic *Bacillus cereus* strain isolated from cooked chicken. Food Microbiology 46: 195–199.

Lutpiatina L, 2020. Pathogens transmitted through contaminated rice, In: Recent Advances in Rice Research. IntechOpen. doi:10.5772/intechopen.93757

May FJ et al., 2016. Epidemiology of bacterial toxin-mediated foodborne gastroenteritis outbreaks in Australia, 2001 to 2013 Communicable Diseases Intelligence 40: 460-469.

McIntyre L et al., 2008. Identification of *Bacillus cereus* group species associated with food poisoning outbreaks in British Columbia, Canada. Applied and Environmental Microbiology 74: 7451–7453.

McLauchlin J et al., 2016. Assessment of the microbiological quality of meat pies from retail sale in England 2013. Journal of Food Protection 79: 781–788.

Messelhausser U et al., 2014. Emetic *Bacillus cereus* are more volatile than thought: Recent foodborne outbreaks and prevalence studies in Bavaria (2007–2013). BioMed Research International 2014: 1–9.

Mingmongkolchai S and Panbangred W, 2018. Bacillus probiotics: An alternative to antibiotics for livestock production. Journal of Applied Microbiology 124: 1334-1346.

Mishra SS et al., 2020. Biotechnological tools in diagnosis and control of emerging fish and shellfish diseases. In: Genomics and Biotechnological Advances in Veterinary, Poultry and Fisheries, Academic Press pp: 311–360.

Mohamed WS and Ghanyem HR, 2015. Effect of some preservatives on *Bacillus cereus* isolated from some meat products. Assiut Veterinary Medical Journal 61: 1-7.

Naranjo M et al., 2011. Sudden death of a young adult associated with *Bacillus cereus* food poisoning. Journal of Clinical Microbiology 49: 4379–4381.

Navaneethan Y and Esah EM, 2020. Prevalence, toxigenic profiles, multidrug resistance, and biofilm formation of *Bacillus cereus* isolated from ready-to eat cooked rice in Penang, Malaysia. Food Control 121: 1-10.

Osimani A et al., 2018. *Bacillus cereus* foodborne outbreaks in mass catering. International Journal of Hospitality Management 72: 145–153.

Pearlin BV et al., 2020. Role of acidifiers in livestock nutrition and health: A review. Journal of Animal Physiology and Animal Nutrition 104: 558-569.

Pirhonen TI et al., 2005. Biochemical and toxic diversity of *Bacillus cereus* in a pasta and meat dish associated with a food-poisoning case. Food Microbiology 22: 87–91.

Pontieri E, 2016. *Bacillus cereus* group diagnostics. In: Savini V (editor), The Diverse Faces of *Bacillus cereus*. Academic Press, Elsevier Inc. pp: 15–33.

Posfay-Barbe KM et al., 2008. Food poisoning as a cause of acute liver failure. The Pediatric Infectious Disease Journal 27: 846–847.

Quinn et al., 2016. Concise Review of Veterinary Microbiology. 2nd Ed., Wiley Blackwell, West Sussex, UK.

Raddadi N et al., 2010. Bacillus. In: Liu D (editor), Molecular Detection of Foodborne Pathogens, CRC Press Taylor & Francis Group, Boca Raton, London, New York; pp: 129-144.

Rafique A et al., 2020. Multiplex PCR based detection of toxin producing *Bacillus cereus* from different milk samples retailed in Pakistan. Pakistan Journal of Agricultural Sciences 57: 887-891.

Ramarao N et al., 2020. Advanced methods for detection of *Bacillus cereus* and its pathogenic factors. Sensors 20: 1-23.

Ranjbar R and Shahreza MHS, 2017. Prevalence, antibiotic-resistance properties and enterotoxin gene profile of *Bacillus cereus* strains isolated from milk-based baby foods. Tropical Journal of Pharmaceutical Research 16: 1931–1937.

Rigourd V et al., 2018. Recent actuality about *Bacillus cereus* and human milk bank: A new sensitive method for microbiological analysis of pasteurized milk. European Journal of Clinical Microbiology and Infectious Diseases 37: 1297–1303.

Rodrigo D et al., 2021. Risk of *Bacillus cereus* in relation to rice and derivatives. Foods 10: 1-11.

Ramlucken U et al., 2020. Advantages of Bacillus based probiotics in poultry production. Livestock Science 241: 104215; doi:10.1016/j.livsci.2020.104215.

Sahu R et al., 2021. Acute gastroenteritis outbreak in a school associated with religious ceremony in Mirzapur district, Uttar Pradesh, India. Indian Journal of Public Health 65: 18-22.

Saleh M et al., 2012. *Bacillus cereus*, an unusual cause of fulminant liver failure: Diagnosis may prevent liver transplantation. Journal of Medical Microbiology 61: 743–745.

Saleh-Lakha S et al., 2017. A study to assess the numbers and prevalence of *Bacillus cereus* and its toxins in pasteurized fluid milk. Journal of Food Protection 80: 1085–1089.

Samapundo S et al., 2011. Incidence, diversity and toxin gene characteristics of *Bacillus cereus* group strains

isolated from food products marketed in Belgium. International Journal of Food Microbiology 150: 34–41.
Sanchez-Chica J et al., 2020. Genetic and toxigenic diversity of *Bacillus cereus* group isolated from powdered foods. Journal of Food Science and Technology. 58: 1892-1899.
Scatassa ML et al., 2018. Retrospective study on the hygienic quality of fresh ricotta cheeses produced in Sicily, Italy. Italian Journal of Food Safety 7: 68-71.
Schlegelova J et al., 2003. The prevalence of and resistance to antimicrobial agents of *Bacillus cereus* isolates from foodstuffs. Veterinary Medicine-Czech 48: 331–338.
Shawish R and Tarabees R, 2017. Prevalence and antimicrobial resistance of *Bacillus cereus* isolated from beef products in Egypt. Open Veterinary Journal 7: 337-341.
Shiota M et al., 2010. Rapid detoxification of cereulide in *Bacillus cereus* food poisoning. Pediatrics 125: 951–955.
Sloan-Gardner TS et al., 2014. An outbreak of gastroenteritis linked to a buffet lunch served at a Canberra restaurant. Communicable Diseases Intelligence 38: 273-278.
Smith DP et al., 2004. Detection of *Bacillus cereus* on selected retail chicken products. Journal of Food Protection 67: 1770–1773.
Soleimani M et al., 2017. Occurrence of *Bacillus cereus* in beef burger marketed in Tehran, capital of Iran. Journal of Food Quality and Hazards Control 4: 70-73.
Son B et al., 2020. Simultaneous control of *Staphylococcus aureus* and *Bacillus cereus* using a hybrid endolysin LysB4EAD-LysSA11. Antibiotics 9: 1-11.
Sornchuer P and Tiengtip R, 2021. Prevalence, virulence genes, and antimicrobial resistance of *Bacillus cereus* isolated from foodstuffs in Pathum Thani Province, Thailand. Pharmaceutical Sciences Asia 48: 194-203.
Tewari A and Abdullah S, 2015. *Bacillus cereus* food poisoning: International and Indian perspective. Journal of Food Science and Technology 52: 2500–2511.
Tewari A et al., 2012. Prevalence of multidrug resistant *Bacillus cereus* in foods and human stool samples in and around Pantnagar, Uttrakhand. Journal of Advanced Veterinary Research 2: 252-255.
Thirkell CE et al., 2019. An outbreak of *Bacillus cereus* toxin-mediated emetic and diarrheal syndromes at a restaurant in Canberra, Australia 2018. Communicable Diseases Intelligence 43: 1-9.
Tirlonia E et al., 2019. *Bacillus cereus* in fried rice meals: Natural occurrence, strain dependent growth and haemolysin (HBL) production. LWT - Food Science and Technology 114: 1-7
Tschiedel E et al., 2015. Lifesaving liver transplantation for multi-organ failure caused by *Bacillus cereus* food poisoning. Pediatric Transplantation 19: 11–14.
Ubong A et al., 2020. Prevalence of *Bacillus cereus s.l.* in ultra-high temperature chocolate milk from selected milk manufacturers in Malaysia. Food Research 4: 982 – 990.
Visiello R et al., 2016. *Bacillus cereus* hemolysins and other virulence factors. In: The Diverse Faces of *Bacillus cereus*. Elsevier Inc. pp: 35–44.
Vidic J et al., 2020. Food Sensing: Detection of *Bacillus cereus* spores in dairy products. Biosensors 10: 1–16.
Yap M et al., 2019. Microbial quality and safety of sushi prepared with gloved or bare hands: Food handlers' impact on retail food hygiene and safety. Journal of Food Protection 82: 615–622.
Yıbar A et al., 2017. Prevalence, enterotoxin production and antibiotic resistance of *Bacillus cereus* isolated from milk and cheese. Kafkas Universitesi Veteriner Fakultesi Dergisi 23: 635-642.
Yin W et al., 2020. Cyclic di-GMP signaling systems in the gram-positive *Bacillus cereus* group. In: Microbial Cyclic Di-Nucleotide Signaling, Chou et al. (editors). Springer Nature Switzerland AG, pp: 261-275.
Yu P et al., 2019. *Bacillus cereus* isolated from vegetables in China: Incidence, genetic diversity, virulence genes, and antimicrobial resistance. Frontiers in Microbiology 10: 1-10.
Yu S et al., 2020. A Study on prevalence and characterization of *Bacillus cereus* in ready-to-eat foods in China. Frontiers in Microbiology 10: 1-11.
Zeighami H et al., 2020. Frequency of hemolysin BL and non-hemolytic enterotoxin complex genes of *Bacillus cereus* in raw and cooked meat samples in Zanjan, Iran. Toxicology Reports 7: 89–92.
Zhang Y et al., 2017. Quantitative prevalence, phenotypic and genotypic characteristics of *Bacillus cereus* isolated from retail infant foods in China. Foodborne Pathogens and Disease 14: 564-572.
Zhang Q et al., 2019. Contaminated feed-borne *Bacillus cereus* aggravates respiratory distress post avian influenza virus H9N2 infection by inducing pneumonia. Scientific Reports 9: 1-9.
Zhang Y et al., 2020. Prevalence, virulence feature, antibiotic resistance and MLST typing of *Bacillus cereus* isolated from retail aquatic products in China. Frontiers in Microbiology 11: 1-9.
Zhou G et al., 2014. Characterization of three *Bacillus cereus* strains involved in a major outbreak of food poisoning after consumption of fermented black beans (Douchi) in Yunan, China. Foodborne Pathogens and Disease 11: 769–774.
Zuo Z et al., 2020. Feed-borne *Bacillus cereus* exacerbates respiratory distress in birds infected with *Chlamydia psittaci* by inducing hemorrhagic pneumonia. Avian Pathology 49: 251-260.