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REVIEW

Insights into *Bacillus cereus* prevalence, risk factors, and prevention in Malaysian food products

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Abstract

Bacillus cereus is a significant foodborne pathogen responsible for diarrheal and emetic food poisoning syndromes. Ingesting preformed emetic toxins such as cereulide produced by *B. cereus* in contaminated food can cause severe illness or even death. In Malaysia, *B. cereus* is known to be prevalent in rice-based dishes, but the actual number of outbreaks is underreported. As *B. cereus* continues to pose a risk to public health, understanding its microbiological characteristics and implementing control measures in food safety practices are vital for reducing foodborne illnesses. This review discusses the bacteriological characteristics, detection methods, and prevalence of *B. cereus* in food samples in Malaysia. It also highlights the importance of enhanced surveillance and proper food handling practices to mitigate the risk of *B. cereus*. Lastly, implementing effective preventative measures during food preparation is essential to controlling the spread of this pathogen.

1. Introduction

Bacillus cereus was first isolated in 1887 from the air environment in a cowshed in the United Kingdom (Frankland and Frankland, 1887). *B. cereus* is a rod-shaped, Gram-positive, aerobic or facultative anaerobic, spore-forming bacterium commonly found in soils, sediments, and plants. Other members of the *Bacillus* group, which have similar genetic elements, include *B. anthracis*, *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis*, and *B. weihenstephanensis.*

In Malaysia, *B. cereus* has been isolated from various food products, including rice noodles, wheat noodles, spices, grains, legumes, fried noodles, cooked rice, and cereals (Rampal *et al*., 1984; Rusul and Yaacob, 1995; Sandra *et al*., 2012; Lesley *et al*., 2013). Ingestion of food contaminated with *B. cereus* can result in two types of illnesses, for example, diarrheal and emetic illness. Diarrheal is primarily caused by ingestion of vegetative cells where the enterotoxin may produce in the small intestine by vegetative cells which are resistant to the low pH gastric environment and degrading enzymes in the stomach. Meanwhile, emetic illness is caused by the ingestion of the preformed toxin produced by *B. cereus* in food. Three key toxins produced by *B. cereus* have been identified and characterised as causes of illness: haemolysin BL (*Hbl*), non-haemolytic enterotoxin (*Nhe*), and cytotoxin K (*Cyt K*). Additionally, the heat-resistant cereulide toxin produced by *B. cereus* can cause emetic illness within 0.5-5 hours after ingestion (Granum and Lund, 1997). The spore-forming ability might also allow *B. cereus* to survive under harsh environmental conditions. For example, spores that endure mild heat treatment may become more heat-resistant upon subsequent exposure. Survival and germination of *B. cereus* spores may lead to large-scale food poisoning outbreaks. Furthermore, *B. cereus* acts as a food spoilage microorganism, specifically in dairy products, where it can cause curdling and the formation of bitty cream in pasteurised milk.

The characteristics, reservoirs, and pathogenicity of *B. cereus* are elaborated in this review to provide a better understanding of this foodborne pathogen. Various identification and detection approaches for *B. cereus* approaches are also reviewed. Additionally, this review discusses various aspects of *B. cereus* in Malaysia, including prevalence studies, food poisoning outbreaks, fatal cases, antibiotic resistance profiles, and risk assessments related to its role in foodborne illness.

1.1 Characteristics of B. cereus

B. cereus is a Gram-positive, large rod-shaped, aerobic or facultative anaerobic, spore-forming bacterium with a variable size typically 1.0 µm × 3.0 to 5.0 µm (Adams and Moss, 2008). Sporadically, *B. cereus* may appear Gram-negative in older cultures due to the deterioration of cell walls (Rajkowski and Bennett, 2003). *B. cereus* is able to produce catalase, which breaks down hydrogen peroxide into water and oxygen.

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Table 1. Characteristics of *B. cereus* (Winn and Koneman, 2006).

Biochemical characteristics	*B. cereus*
Lecithinase (egg yolk agar)	+
Arginine dihydrolase[a]	V
Nitrate reduction[a]	V+
Gelatin hydrolysis[a]	+
Starch hydrolysis	+
Casein hydrolysis	+
Production of acid from:[b]	
Arabinose	-
Mannitol	-
Salicin	+
Trehalose	+
Inulin	-
Glycerol	+
Glycogen	+
β-lactamase production	+

[a]Reaction determined by Analytical Profile Index 20 Enterobacteriaceae identification system (API 20E)
[b]Reaction determined by Analytical Profile Index 50 Metabolism of Carbohydrate by Bacillus (API 50CHB)
+: positive reaction
-: negative reaction
V: variable reaction
V+: variable reaction (most strains positive)

In the oxidase activity test, the absence of oxidase activity prevents the oxidation of Wurster's blue (tetramethyl-p-phenylenediamine) into a blue-violet colour indicating a negative result. Additionally, *B.* cereus is capable of decomposing tyrosine and forming a clearing zone around colony on a tyrosine agar plate. Other biochemical characteristics of *B. cereus* are listed in Table 1.

The optimum intrinsic parameters for the growth of *B. cereus* are 30-40°C, pH 6.0 - 7.0, and a water activity (a_w) of 0.93 (ICMSF, 1996). In term of temperature growth conditions, some *B. cereus* strains are capable of growing at low temperatures. For example, Choma *et al*. (2000) demonstrated that *B. cereus* can grow at 5°C and 10°C in cooked-chilled vegetable products. Other characteristics that make *B. cereus* difficult to eradicate in food products is its ability to form biofilms and spores. *B. cereus* forms biofilms, an extracellular polymeric substance (EPS), on food matrices and processing equipment that make them resistant to chemicals disinfectants such as detergent and physical cleaning methods like washing or flushing. Furthermore, some *B. cereus* spores are resistant to heating, freezing, drying, and radiation (Kotiranta *et al*., 2000). Spores that survive these eradication treatments can rapidly germinate into vegetative cells once favourable growth conditions are present. Additionally, the hydrophobic characteristics of *B. cereus* spores facilitate better adhesion to hydrophobic surfaces, such as processing system. Andersson *et al*. (1998) also reported that hydrophobic *B. cereus* spores enhanced their adhesion to epithelial cells of the small intestine which might be an additional virulence mechanism.

The cell envelope structures (known as the S-layer) composed of identical protein or glycoprotein subunits covering *B. cereus* cell surface has been described by Sidhu and Olsen (1997), Kotiranta *et al*. (1998), and Mignot *et al*. (2001). Sleytr and Messner (1988) found that S-layer can be multifunctional for cell shape determination and maintenance, protective coats, molecular sieves and ion traps, cell adhesion, and surface recognition. Kotiranta *et al*. (1999) reported that *B. cereus* with S-layer was 2.6 times more resistant to gamma rays than *B. cereus* without S-layer. The presence of an S-layer on the surface of *B. cereus* therefore poses additional challenges for the food industry.

1.2 Reservoir of B. cereus

B. cereus is commonly found in soil, sediment, and plants. One gram of soil sample has been shown to contain 10^5 to 10^6 *B. cereus* spores (Andersson *et al*., 1995). *B. cereus* is also found in many other farinaceous foods such as noodles, rice, and cereals (Rampal *et al*., 1984; Sandra *et al*., 2012; Lesley *et al*., 2013). In the food industry, food processing equipment may serve as a reservoir for *B. cereus* through the production of biofilms on equipment surfaces. In healthy individuals, a low number of *B. cereus* can be found in stool samples (Curtis and Lawley, 2003). Nevertheless, the presence of *B. cereus* in stool samples from healthy individuals is transient and depends on their dietary intake (Turnbull and Kramer, 1985).

Table 2. Characteristics of diarrhoeal and emetic syndromes caused by *B. cereus.*

	Diarrhoeal syndrome	Emetic syndrome
Infective dose	10^5–10^7 total cells/ spores	10^5–10^8 cells per gram
Toxin produced	In the small intestine of the host	Preformed in foods
Type of toxin	Enterotoxin (Hbe and Nhe)	Emetic toxin (Cereulide)
Incubation period	8–16 hours (occasionally > 24 hours)	0.5–5 hours
Duration of illness	12–24 hours (occasionally several days)	6–24 hours
Symptoms	Watery diarrhoea, abdominal pain and cramp	Nausea and vomiting
Implicated foods	Meat products, soups, vegetables, puddings/ sauces, milk/milk products	Fried and cooked rice, pasta, pastry, and noodles

Source: Granum and Lund, 1997

1.3 Gastrointestinal illness characteristics

Gastrointestinal illness caused by *B. cereus* tends to be underreported due to mild symptoms, its self-limiting

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nature, and the fact that laboratory analysis of food samples implicated in the food poisoning case is not always possible. Diarrhoeal and emetic syndromes are the distinct food poisoning diseases caused by *B. cereus*. The characteristics of diarrhoeal and emetic syndromes are shown in Table 2.

Watery diarrhoea, abdominal pain, and cramps are the common medical signs of diarrhoeal syndromes. The incubation time is about 8–16 hours after ingestion of *B. cereus* contaminated foods and the syndrome can last up to 1–2 days. Besides, any *B. cereus* which survives from gastric acidic conditions in the stomach and adheres to the intestinal epithelium cells may subsequently produce enterotoxins in the small intestine.

Nausea and vomiting are characteristics of the emetic syndrome caused by *B. cereus*. The emetic syndrome usually has a rapid onset of 0.5–5 hours. Duration of emetic syndrome can last up to 6–24 hours. Ingestion of preformed emetic toxin, such as cereulide produced by *B. cereus* in foods, is the main cause of emetic illnesses.

1.4 B. cereus in food industry

The formation of biofilms, the hydrophobic characteristics of *B. cereus* spores, and resistance of *B. cereus* spores to physical and chemical treatments are major concerns for the food industry. For example, the presence of *B. cereus* is particularly problematic in the dairy industry which may cause sweet curdling and bitty cream in milk. Andersson *et al*. (1995) reported the presence of *B. cereus* is hard to avoid in the dairy industry as the contamination sources start at the farm or cowshed in which the udder of cows often contaminated with soil and dung.

2. Pathogenicity of *B. cereus*

Toxins produced by *B. cereus* are the main causative agents of food poisoning. Two types of enterotoxins, hemolysin BL (*Hbl*) and non-hemolytic enterotoxin (*Nhe*), are known to cause diarrhoeal syndrome. Cytotoxin K (*CytK*) has also been identified as a pathogenicity factor responsible for food poisoning in some cases. Cereulide emetic toxin produced by *B. cereus* is known to induce emetic syndrome. Additionally, phospholipase C, beta-lactamases, collagenase, and proteases have been identified as potential virulence factors of *B. cereus* in some clinical cases (Kotiranta *et al*., 2000). Despite the various proposed pathogenic factors, the widely known causative agents of *B. cereus* are the *Hbl*, *Nhe*, *Cytk*, and cereulide.

2.1 Hemolysin BL

Hbl is a three-component protein complex that consists of B, L1, and L2 subunits. The B subunit is referred to as the binding protein component and is encoded by the *hblA* genes. The L1 and L2 subunits are referred to as lytic components and encoded by the *hblD* and *hblC* genes. Beecher and Macmillan (1991) reported that B subunit is responsible for the initial step of cell binding activity before the L1 and L2 can induce cell lysis. A combination of all three enterotoxin *Hbl* components has been demonstrated to exhibit hemolytic, cytotoxic, dermonecrotic, and vascular permeability activities (Beecher *et al*., 1995).

Beecher and Wong (1994a) demonstrated that when the supernatant of some *B. cereus* isolates was added into sheep and calf blood agar, a discontinuous hemolytic pattern appeared within 2 to 2.5 hours of incubation at 37°C. In addition, Prüß *et al*. (1999) found that 22 out of 23 (95.7%) *B. cereus* strains obtained from pasteurised milk, turmeric root, soil, and rice exhibited strong beta-hemolysis with ring effect around bacterial cell on blood agar plates. In terms of cytotoxic activitiy, Sastalla *et al*. (2013) reported that *Hbl* caused rapid cell death in vitro to human fibroblasts (HT1080), neutrophils (hPMNs), hamster ovary cells (CHO), and mouse macrophages (RAW264.7), with a half-maximal effective concentration (EC50) of approximately 0.5% of sterile culture supernatant. Besides, Sastalla *et al*. (2013) also reported that an in-vivo model showed that 50% of cell death occurred within 10 minutes after the injection of sterilised culture supernatant into the mouse peritoneal cavity. Furthermore, haemolytic enterotoxin demonstrated dermonecrotic vascular permeability (VP) in rabbit skin and caused rapid fluid accumulation in ligated rabbit ileal loops (RIL) assay (Beecher and Wong, 1994b; Beecher *et al*., 1995).

2.2 Non-hemolytic enterotoxin

Non-hemolytic enterotoxin (*Nhe*) is a three-component protein complex similar to *Hbl* but does not possess haemolytic activity. It consists of *NheA*, *NheB*, and *NheC* subunits encoded from one operon containing *nheA*, *nheB*, and *nheC* genes. Lindback *et al*. (2004) reported that maximal cytotoxic activity in Vero cells was achieved when the *NheA*, *NheB*, and *NheC* at the molar ratio of 10:10:1. Fagerlund *et al*. (2008) described the enterotoxin *Nhe* caused colloid-osmotic lysis in epithelial plasma membranes after pore formation in lipid bilayers. Lindback *et al*. (2010) also reported enterotoxin *Nhe* induced cytotoxicity required a specific binding order to the cell surface and *NheC* was the first subunit in the binding process followed by *NheB* and *NheA*.

2.3 Cytotoxin K

Cytotoxin K (*CytK*) is a β-barrel pore-forming toxin that has been reported to be responsible for necrotic enteritis, haemolytic activity, and cytotoxic against human intestinal epithelial cells (Lund *et al*., 2000;

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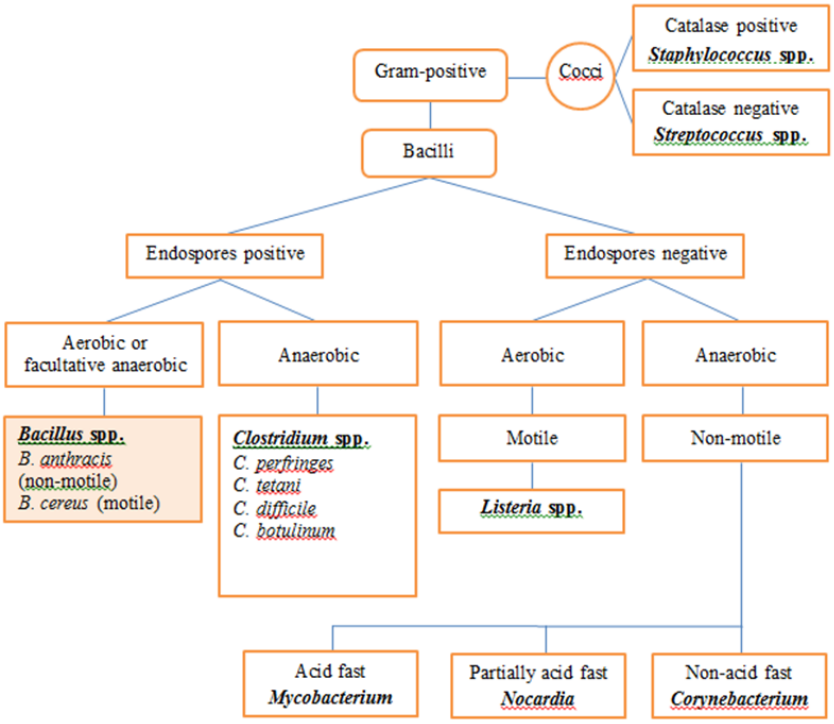

Figure 1. Flowchart of the bacterial identification scheme (Locke *et al*., 2013).

Hardy *et al*., 2001; Fagerlund *et al*., 2004). *CytK* has been identified and classified into *CytK-1* and *CytK-2*. Both *CytK-1* and *CytK-2* are haemolytic and cytotoxic to human intestinal epithelial cells and Vero cells, but *CytK -2* is found to be less toxic than *CytK-1*. This may be due to relatively small differences in sequence and smaller pore size than *CytK-1* (Fagerlund *et al*., 2004).

2.4 Cereulide

Cereulide is an emetic cyclic peptide synthesised by non-ribosomal peptide synthesis complexes. It tolerates high temperature, a wide range of pH between 2 and 11, and resists proteolytic enzymes cleavage. Rajkovic *et al*. (2008) demonstrated that the cereulide was inactivated by heat at 121°C for 80 minutes and 150°C for 60 minutes in high pH environment, while cereulide stability remained unharmed at pH 7.0 after more than 2 h at 121°C. Agata *et al*. (2002) reported bacterial growth and production of cereulide was inhibited in foods that cooked with vinegar, mayonnaise, and ketchup but the production of this emetic toxin was detected high in starchy foods compared to eggs, meat, milk, and soymilk. In terms of toxic dose, Jaaskelainen *et al*. (2003) reported cereulide at the concentration of 8 µg/kg of bodyweight in humans caused emetic syndrome.

The pathogenicity of cereulide leads to an emetic syndrome is only partially known. Bhunia (2008) reported that cereulide may bind to vagus nerve receptor (5-HT_3 receptor) in the stomach and leads to the emetic syndrome. The disturbance of ionic equilibrium and transmembrane potential, for example, the loss of K^+ gradient leading to mitochondrial swelling and dysfunction has been suggested as well (Kroten *et al*., 2010).

3. Identification and detection methods

B. cereus can be differentiated from other bacteria through conventional methods such as observation of colony growth characteristics on agar plate, cell staining with dyes, and biochemical tests. The selective media can be used to detect and identify *B. cereus* via distinct colony colour appearance and other characteristics on the agar plate. Commercial identification kits provide convenience and less labour-intensive methods for detection of *B. cereus* and their production of enterotoxins. Molecular approaches such as enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and deoxyribonucleic acid (DNA) barcoding enable highly sensitive detection by identifying specific protein molecules or DNA sequences present in the microorganisms.

3.1 Conventional methods

Colony morphology, staining approach, and biochemical test are the most common identification methods used in the past. Information gathered from all these approaches provides details about growth characteristics (e.g. colony shape, colony edge, elevation, surface, opacity, and pigmentation on agar plate), cellular structures (e.g. shape, size, spore-bearing, Gram-negative/positive through staining), and intracellular and extracellular enzymatic activities (e.g. nitrate reduction, presence of catalase and starch hydrolysis). Results from these conventional methods are therefore used to identify the bacterial species by referring to the Bergey's Manual of Determinative Bacteriology. An example of a bacterial identification scheme is shown in Figure 1.

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Table 3. Examples of PCR primer sequences used for the detection of *B. cereus*.

Genes profile	Primer	Primer sequences	Product Size (bp)	References
phospholipase C	S1-Forward S2-Reverse	5'-GAG TTA GAG AAC GGT ATT TAT GCT GC-3' 5'-CTA CTG CCG CTC CAT GAA TCC-3'	411 bp	Schraft and Griffiths, 1995; Lee *et al*., 2009
gyrase B	BCJH-Forward BCJH-Reverse	5'-TCA TGA AGA GCC TGT GTA CG-3' 5'-CGA CGT GTC AAT TCA CGC GC-3'	475 bp	Sandra *et al*., 2012
entFM	*entFM*-Forward *entFM*-Reverse	5'-ATG AAA AAA GTA ATT TGC AGG-3' 5'-CGT GCA TCT GTT TCA TGA AA-3'	1269 bp	Nooratiny and Sahilah, 2013
hblA	*hblA*-Forward *hblA*-Reverse	5'-GCT AAT GTA GTT TCA CCT GTA GCA AC-3' 5'-AAT CAT GCC ACT GCG TGG ACA TAT AA-3'	874 bp	Nooratiny and Sahilah, 2013
hblD	*hblD*-Forward *hblD*-Reverse	5'-AGG TCA ACA GGC AAC GAT TC-3' 5'-CGA GAG TCC ACC AAC AAC AG-3'	205 bp	Jawad *et al*., 2015
ces (emetic gene)	*ces*-Forward *ces*-Reverse	5'-GGT GAC ACA TTA TCA TAT AAG GTG-3' 5'-GTA AGCGAA CCT GTC TGT AAC AAC A-3'	665 bp	Jawad *et al*., 2015

3.2 Selective medium

Mannitol-Egg Yolk-Polymyxin (MYP) agar and Polymyxin-Pyruvate-Egg Yolk-Mannitol-Bromothymol Blue agar (PEMBA) are selective media used to identify *B. cereus*. These two media rely on the *B. cereus* lecithinase enzyme activity and mannitol fermentation ability to form a precipitate and distinct colour change in the selective media (Schraft and Griffiths, 2011). Lecithinase produced by *B. cereus* hydrolyses egg yolk lecithin and forms a zone of opacity or precipitation in MYP and PEMBA (Levin, 2009). The absence of mannitol fermentation results in pink colonies on MYP agar while yellow colonies result from mannitol fermentation by other species than *B. cereus*. In PEMBA, bromothymol blue is used as a pH indicator to detect mannitol fermentation. *B. cereus* growth in PEMBA will show a turquoise to peacock blue colonies due to the inability to ferment mannitol (Levin, 2009).

Brilliance™ *Bacillus cereus* (BBC) agar (also known as Chromogenic *Bacillus cereus* (CBC) agar) and chromogenic *B. cereus* group plating medium are examples of chromogenic selective agar facilitate the identification of *B. cereus* based on their enzymatic activity in hydrolysing the chromogenic substance and resulting in a distinct colour change in the agar. BBC/ CBC agar incorporates the 5-bromo-4-chloro-3-indolyl-β -glucopyranoside chromogenic substrate, which is cleaved by β-D-glucosidase and gives a white with blue-green centre colonies (Fricker *et al*., 2008). Chromogenic *B. cereus* group plating medium agar contains 5-bromo-4 -chloro-3-indoxyl myoinositol-1-phosphate chromogenic substrate, which is cleaved by phosphatidylinositol phospholipase C and results in blue-turquoise colour with or without halo colonies (Peng *et al*., 2001). Several reports revealed that chromogenic selective medium was performed well and given superior results compared to commonly used selective medium and Analytical Profile Index (API)® identification kits (Peng *et al*., 2001; Fricker *et al*., 2008; Jeong *et al*., 2010; Chon *et al*., 2014).

3.3 Commercial identification kits

Analytical Profile Index (API) identification test strip is a conventional biochemical test kit for identification of unknown microorganisms. API 20E combined with API 50 CHB identification system is the commonly used method for the identification and confirmation of *B. cereus* (Choma *et al*., 2000; Oguntoyinbo and Oni, 2004; Østensvik *et al*., 2004; Aruwa and Olatope, 2015). In addition, BBL Crystal™ Gram-positive identification kit is another commonly used commercial kit which employs fluorogenic and chromogenic substrates to identify aerobic Gram-positive bacteria. For example, Lesley *et al*. (2013) used this kit to confirm *B. cereus* isolates from ready-to-eat (RTE) cereals.

Bacillus diarrhoeal enterotoxin visual immunoassay (BDE-VIA™, Tecra) and *B. cereus* enterotoxin reversed passive latex agglutination (BCET-RPLA, Oxoid) kit are the examples of *B. cereus* enterotoxin detection kit. BDE -VIA™ is used to detect the non-hemolytic enterotoxin subunit A (*NheA*) whereas BCET-RPLA is used to detect the L2 component of hemolysin BL (*Hbl*). Both BDE-VIA™ and BCET-RPLA have been widely used to detect the presence of enterotoxins from *B. cereus* (Beecher and Wong, 1994c; Rusul and Yaacob, 1995; Lund and Granum, 1997; Fletcher and Logan, 2002; Ceuppens *et al*., 2012). Moreover, the Duopath® Cereus Enterotoxins test is another type of immunoassay kit that detects non-hemolytic enterotoxin subunit B (*NheB*) and L2 component of Hbl independently within the same kit (Krause *et al*., 2010).

3.4 Molecular approaches

Enzyme-linked immunosorbent assay (ELISA) is commonly used to detect the presence of an antigen or antibody in a sample. For foodborne pathogen detection, ELISA can be used to detect specific antigens exhibited on the surface of foodborne microorganisms. For examples, Mikami *et al*. (1990) and Murakami *et al*. (1991) utilised ELISA to detect *B. cereus* using the monoclonal antibody against *B. cereus* flagellar antigen. Chen *et al*. (2001) developed a rapid identification method to detect *B. cereus* with antibodies against the 28.5-kilodalton *B. cereus* cell surface antigen.

Polymerase chain reaction (PCR) offers several

Table 4. Prevalence studies of *B. cereus* in food samples from Malaysia.

Sampling date	Sampling locations	Detection methods	Food samples and prevalence	*B. cereus* counts	References
*N/A	Retail stores, wet markets, and campus canteen at Universiti Putra Malaysia in Serdang, Selangor	*B. cereus* selective agar	Rice noodles (3/3)=100% Wet wheat noodles (2/2)=100% Dried wheat noodles (10/10)=100% Grains (8/8)=100% Spices (4/4)=100% Legumes (11/11)=100% Legume products (3/3)=100% Cooked foods (17/28)=60.7%	2×10^2 to 3×10^3 CFU/g 2×10^3 to 3×10^4 CFU/g 2×10^2 to 5.2×10^3 CFU/g 3.2×10^2 to 4×10^3 CFU/g 7×10^2 to 9×10^3 CFU/g 2×10^2 to 1.2×10^6 CFU/g 8×10^3 to 1×10^4 CFU/g 1×10^2 to 5×10^2 CFU/g	Rusul and Yaacob, 1995
April 2005 to September 2005	Thirty-three school hostels kitchens and canteens previously involved in food poisoning outbreaks from 2000 to 2004 in Pahang	*N/A	Five (1.9%) out of 264 RTE food samples were found to have unsatisfactory *B. cereus* counts. Fried rice Nasi lemak with egg Spinach Boiled drinking water Plain rice	1×10^2 CFU/g 6×10^2 CFU/g 2.2×10^3 CFU/g 2.4×10^4 CFU/g 1.2×10^4 CFU/g	Jeyaletchumi *et al*., 2006
*N/A	Local supermarkets (Sampling areas were not mentioned in the previous studies)	MPN-PCR	Infant cereals (25/30)=83.3% Raw cereals (15/17)=88.2% Pre-mixed cereals drinks (14/15) =93.3% Breakfast cereals (25/41)=61.0% Cereals bars (8/8)=100%	30 to >2400 MPN/g 30 to 2900 MPN/g 30 to 530 MPN/g 61 to >2400 MPN/g 90 to >2400 MPN/g	Lee, 2009; Lee *et al*., 2009
*N/A	Restaurants, retail food stores, and supermarkets in Selangor	MPN-PCR	Nasi lemak (38/54)=70.4% Biryani rice (Persian rice) (10/20) =50% Chicken rice (20/20)=100% White rice (16/21)=76.2% Beef burgers (22/53)=41.5% Chicken burgers (21/79)=26.6% Fish burgers (30/65)=46.2%	3 to >1100 MPN/g 9 to >1100 MPN/g 3 to 460 MPN/g 3.6 to >1100 MPN/g 3 to >1100 MPN/g 3 to >1100 MPN/g 6 to >1100 MPN/g	Sandra, 2012; Sandra *et al*., 2012
December 2009 to February 2010	Supermarkets in Kuching and Kota Samarahan, Sarawak	*B. cereus* selective agar	Breakfast cereals (3/19)=15.8% Cereal drinks (0/4)=0% Original cereals (0/3)=0% Corn flakes (0/2)=0% Oat (0/1)=0% Instant oatmeal (1/1)=100%	*N/A	Lesley *et al*., 2013
*N/A	Restaurants, fast food restaurants, roadside stalls, wet markets, and supermarkets in Kota Bharu, Kelantan	PCR	Cooked chicken meats (9/30)=30.0% Raw chicken meats (1/30)=3.3%	*N/A	Aklilu *et al*., 2016
*N/A	Twenty local indigenous rice grains were collected in Sarawak, and twenty imported rice grains were purchased from retail shops and hypermarkets in Malaysia.	MPN-PCR	Local indigenous rice grains (17/20) =85.0% Imported rice grains (20/20)=100%	3 to >1100 MPN/g >1100 MPN/g	Bilung *et al*., 2016

advantages over conventional culture and biochemical test methods. PCR detection of foodborne pathogens is more sensitive than conventional methods because specific primers are designed to target the DNA sequences in foodborne microorganisms. Many gene profiles of *B. cereus* such as *hblA*, *hblC*, *hblD*, *nheA*, *nheB*, *nheC*, *cytK*, *ces*, *entFM*, and *bceT* gene can be used to detect the presence of *B. cereus* in food samples (Mantynen and Lindstrom, 1998; Chaves *et al*., 2012; Nooratiny and Sahilah, 2013). Several examples of PCR primer sequences used for *B. cereus* detection are listed in Table 3.

Multiplex PCR allows the amplification of two or more genes in a single reaction. The use of multiplex PCR provides a time- and cost- saving method for detecting different pathogenic and/or housekeeping genes in foodborne microorganisms. For example, Forghani *et al*. (2014) developed a multiplex PCR to detect *B. cereus sensu lato* group (*groEL*), four enterotoxins (*entFM*, *hblC*, *nheA*, and *cytk*), and one emetic toxin (*ces*) gene using six primer pairs.

Real-time PCR (also known as quantitative PCR) enables the monitoring of gene amplification in real-time using florescent dye probes. The increase in amplicons during the PCR cycle is indicated by the increasing fluorescence intensity, which is detected by a detector device and analyses using computer software. Furthermore, agarose gel electrophoresis and visualisation of PCR products through gel staining are not required in real-time PCR therefore allowing for faster sample analysis. For example, Fricker *et al*. (2007) employed a real-time PCR assay to detect the presence

of *B. cereus* cereulide emetic toxin in food samples within two hours. Wehrle *et al*. (2010) also developed a multiplex real-time PCR assay for the simultaneous detection of *nheA*, *hblD*, *cytK1*, and *ces* genes.

DNA barcoding is another technique used for identifying unknown biological species through a short DNA sequence. For DNA barcoding of bacteria, 16S ribosomal RNA (16S rRNA) universal primer is the most widely used genetic marker for amplifying the 16S rRNA genes in bacteria. Next, DNA sequencing is performed to determine the order of nucleotide bases in the short DNA sequence. Finally, the sequencing results are analysed using an online database such as National Center for Biotechnology Information-Basic Local Alignment Search Tool (NCBI-BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the unknown bacterial species.

4. Current scenario of *B. cereus* in Malaysia

4.1 Prevalence studies of B. cereus

To date, there are only a few studies reported the detection of *B. cereus* in food samples. It looked inconspicuous as *B. cereus* is not isolated, characterised, and study extensively in Malaysia compare to other countries. Examples of the prevalence studies of *B. cereus* from food samples in Malaysia are shown in Table 4.

4.2 B. cereus food poisoning outbreaks

The first reported *B. cereus* foodborne outbreak in Malaysia was reported by Rampal *et al*. (1984). Food poisoning caused by the consumption of *B. cereus* contaminated fried noodles occurred in a secondary school hostel in Klang, Selangor. One hundred and fourteen female students were affected in this food poisoning outbreak. The majority of the affected students experienced abdominal pain (85.1%), nausea (78.1%), vomiting (71.9%), and giddiness (70.2%) within 1 – 3.5 hours after consuming the contaminated fried noodles. The bacteriological analysis revealed that the *B. cereus* count was 2.3×10^6 per gram in the fried noodles.

There are a limited number of reports on *B. cereus* food poisoning outbreak in Malaysia. This may be due to several reasons such as causative agents in food poisoning cases not being identified, and the food remnants not being available for sampling. It is important not to underestimate the danger of this foodborne microorganism because the high prevalence of *B. cereus* has been widely detected in starchy foods (Rampal *et al*., 1984; Sandra *et al*., 2012; Lesley *et al*., 2013).

In fact, numerous reports indicate that food poisoning outbreaks in Malaysia were mainly due to the consumption of rice-based foods. For example, nine hundred students, consisting of 700 girls and 200 boys, suffered from stomach cramps, diarrhoeal, and vomiting after consuming nasi lemak in a school hostel in Sungai Petani, Kedah, Malaysia (The Star Online, 2006). In 2015, ten members of a family from Sungai Petani, Kedah, also experienced diarrhoea and vomiting after consuming parboiled rice (also known as pullungul arisi in Tamil) (The Star Online, 2015a). Nonetheless, we cannot simply conclude that these two food poisoning outbreak cases were caused by *B. cereus* as the identification of the causative agent was unpublished and it could have been caused by other foodborne microorganisms.

4.3 Deadly food poisoning cases

To date, there is a dearth of information regarding fatal cases caused by *B. cereus* in Malaysia. However, there are a few reports describing fatal food poisoning cases from the consumption of starchy foods in Malaysia, but the identification of lethal agents in the food poisoning cases has not been clarified. For example, a seven-year-old girl died and 25 people experienced food poisoning syndromes after eating nasi lemak and murtabak (pan fried bread that usually made with egg, garlic, onion, and minced meat) bought from a night market in Tangkak, Johor, Malaysia (The Star Online, 2012). In addition, a 38-year-old woman died and her younger sister recovered from diarrhoea and vomiting, and the food poisoning sources were suspected from the nasi lemak and fried noodles bought from a food stall in Kota Kinabalu, Sabah, Malaysia (The Star Online, 2015b). Again, it was not possible to confirm that these two deadly food poisoning cases were related to the consumption of starchy foods caused by *B. cereus* as the laboratory analysis and autopsy results were not reported. However, based on the previous prevalence studies, *B. cereus* has a high possibility of acting as the causative agent in these few deadly outbreaks.

In other countries, *B. cereus* has been confirmed as the etiologic agent in few deadly food poisoning cases. For example, a family of five children aged 7 to 14 years, from Belgium suffered from emetic syndrome after consuming pasta salad (Dierick *et al*., 2005). The 7-year-old girl was the youngest victim of this food poisoning case and died after experiencing vomiting, respiratory distress, coma, diffuse bleeding, and severe muscle cramps. *B. cereus* was detected in the pasta salad and vomiting sample with the concentration of 10^7–10^8 CFU/g and 2.0×10^2 CFU/g, respectively. All four other children gradually recovered after emergency treatment and hepatic function monitoring.

Another deadly food poisoning case was caused by the consumption of contaminated spaghetti in Belgium (Naranjo *et al*., 2011). A 20-year-old man ate spaghetti

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Table 5. Antibiotic resistant profiles of *B. cereus* obtained from different food samples in Malaysia.

Number of isolates	Food samples	Antibiotics	Resistant	Intermediate	Moderate susceptible	Susceptible	References
164	Dried rice noodles, wet wheat noodles, dried wheat noodles, spices, grains, legumes, legume products, and cooked foods	Cloxacillin (1 µg)	100%	-	-	-	Rusul and Yaacob, 1995
		Ampicillin (10 µg)	98.8%	-	0.6%	0.6%	
		Gentamycin (10 µg)	-	-	-	100%	
		Streptomycin (10 µg)	0.6%	0.6%	-	98.8%	
		Erythromycin (15 µg)	1.2	21.3%	-	77.4%	
172	RTE cereals	Ampicillin (10 µg)	100%	-	-	-	Lee, 2009; Lee *et al*., 2009
		Metronidazole (5 µg)	100%	-	-	-	
		Imipenem (10 µg)	-	-	-	100%	
		Teicoplanin (30 µg)	-	-	-	100%	
		Furazolidone (15 µg)	-	-	-	100%	
		Quinupristin (15 µg)	-	-	-	100%	
		Enrofloxacin (5 µg)	-	-	-	100%	
		Erythromycin (15 µg)	1%	-	-	99%	
		Oxytetracycline (30 µg)	-	1%	-	99%	
		Spectinomycin (100 µg)	1%	5%	-	94%	
92	Cooked rice and burgers	Ampicillin (10 µg)	98.9%	-	-	1.1%	Sandra, 2012
		Penicillin (10 µg)	97.8%	-	-	2.2%	
		Erythromycin (15 µg)	-	14.1%	-	85.9%	
		Cephalotin (30 µg)	-	1.1%	-	98.9%	
		Norfloxacin (10 µg)	1.1%	-	-	98.9%	
		Streptomycin (10 µg)	1.1%	1.1%	-	97.8%	

with tomato sauce that had been prepared and left at room temperature for 5 days. Although the spaghetti was warmed in a microwave oven before eating, the young man suffered from headache, abdominal pain, and nausea within 30 minutes of ingestion. Subsequently, he vomited profusely and had two episodes of watery diarrhoea. Lastly, he was found dead approximately 10 h after eating the spaghetti. The report showed that *B. cereus* was found in the spaghetti with the high concentration of 9.5×10^7 CFU/g. One gram of the spaghetti was also found to contained 14.8 µg of cereulide toxin. The toxin level was much higher than other food samples implicated in emetic food poisoning outbreaks with cereulide toxin level ranged from 0.01 to 1.28 µg/g (Agata *et al*., 2002).

5. Antibiotics resistant profiles

B. cereus isolates from different geographical origins and types of food may exhibit different antibiotic resistance patterns due to genetic mutation and/or the acquisition of antibiotics resistance genes from other bacteria. In this section, the antibiotics resistance profiles of *B. cereus* isolates obtained from different food samples in Malaysia are summarised in Table 5.

6. Microbiological risk assessment of foods in Malaysia

Food poisoning outbreaks are unpredictable. However, the expected number of foodborne infections per year in a population and the likelihood of individuals contracting an illness from consuming specific foods contaminated with certain foodborne pathogens can be estimated through microbial risk assessment. For instance, Lee (2009) conducted a microbial risk assessment to evaluate the infection risk associated with consuming one serving of cereal products on a weekly basis. Results from the risk assessment study revealed that there will be an estimated 48 cases of illness per year among 1.32 million people out of Malaysia's 27-million population who consumed RTE cereals.

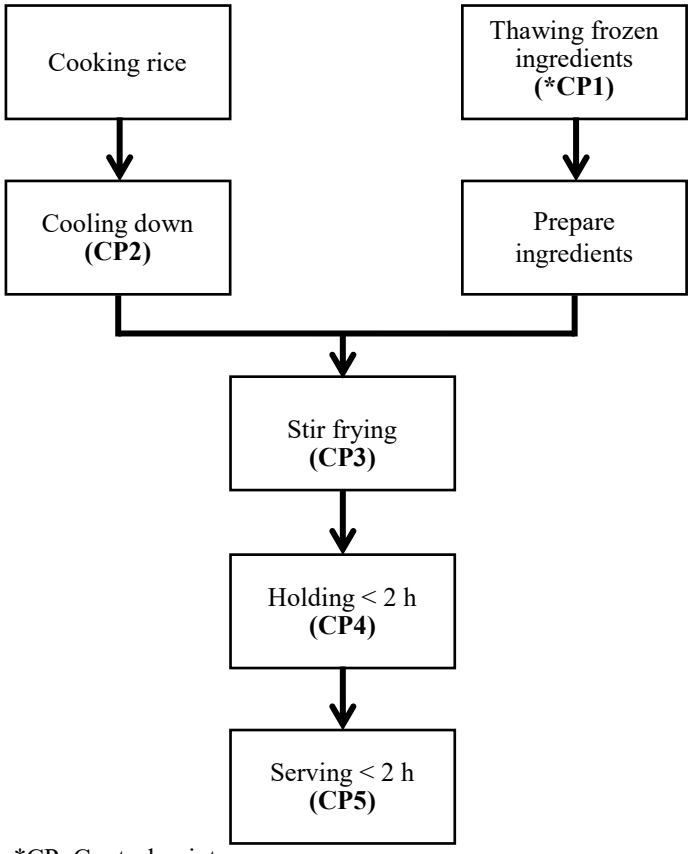
Sandra (2012) also conducted a microbial risk assessment of *B. cereus* in cooked rice based on the assumption that 75% of Malaysia's population (28.7 million) consumed white rice and nasi lemak, chicken rice, and biryani rice on daily, weekly, and monthly basis, respectively. Findings showed that white rice had the highest estimated illness cases with 1,800 cases per year followed by nasi lemak with 1,660 cases per year, chicken rice with 336 cases per year, and biryani rice with 38.7 cases per year.

7. Risk factors and prevention

Biofilm control remains a great safety concern for the dairy industry. Formation of biofilms and hydrophobic characteristics of *B.* cereus spores will allow *B. cereus* to adhere to milking equipment, storage tank, and processing pipelines. Hence, pasteurisation process is often used by dairy industry to eliminate foodborne pathogens. Nonetheless, Andersson *et al*. (1995) reported that although pasteurisation significantly reduces and eliminates most foodborne microorganisms, it remains insufficient to kill the spores. To ensure safe production, the amount of *B. cereus* spores in farm tank milk (FTM) should be minimised to below the maximum spore limit (MSL) of 3 $\log_{10}$ spores per litre (Vissers *et al*., 2007). Bacteria cells and spores present in raw milk can be removed through centrifugal force by using bactofugation. Pasteurisation process with four times decimal reduction time (D-value) must be applied in order to eliminate 10^3 *B. cereus* spores per millilitre of milk (Andersson *et al*., 1995). Lastly, preventing processed milk products from being re-contaminated by *B. cereus* is the ultimate challenge and this can be enforced by setting a bacteriological quality standard for milk products. For example, according to Sweden's legal

standards, milk must not exceed 10 spores per 100 milliliter at packaging line and must contain fewer than 10^4 spores per millilitre after 6 days at +8°C (Ronner and Husmark, 1992).

In Malaysia, food poisoning cases frequently occur in school and hostel canteens. According to the Minister of Health Malaysia, a total of 5,265 out of 12,122 food poisoning cases in 2014 were caused by food prepared at school or hostel canteens in Malaysia (The Star Online, 2014). To ensure food safety in school and hostel canteens, the Ministry of Health Malaysia has published food preparation guidelines (MOH, 2014). Some of the handling practices in the preparation of fried rice and nasi lemak that may contaminate with *B. cereus* are discussed in this section.



*CP: Control point
Figure 2. Control points in fried rice preparation.
Source: MOH, 2014

7.1 Risk factors and prevention measures during the preparation of fried rice

Raw ingredients for fried rice consist of white rice, anchovies, chili paste, chicken/beef, garlic, vegetables, and cooking oil. In general, white rice, anchovies, chili paste, and garlic are foods commonly associated with pathogenic *B. cereus* (MOH, 2014). A flowchart of fried rice preparation with integrated control points is shown in Figure 2. Meanwhile, control points, potential risks, and proper handling practices in the preparation of fried rice are summarised in Table 6.

Table 6. Control points, potential risks, and proper handling practices in the preparation of fried rice.

Control point (CP)	Potential risks	Proper handling practices
CP1: Thawing Frozen Ingredients	Pathogenic bacteria present in the ingredients	Fully thaw before cooking.
CP2: Cooling Down	*B. cereus* spores that survived begin to germinate	Separate the cooked rice into smaller portions. Smaller amounts of food will cool down quickly than larger amounts.
CP3: Stir Frying	Pathogenic bacteria may survive.	Stir-fry all the ingredients thoroughly at 63°C and above
CP4 & CP5: Holding and Serving	*B. cereus* that survived stir frying start to proliferate	Ensure the holding and serving time is less than 2 hours

Source: MOH 2014

7.2 Risk factors and prevention measures during the preparation of nasi lemak

Raw ingredients for nasi lemak normally consist of white rice, anchovies, chilli paste, egg, cucumber, and peanut. *B. cereus* may be found in white rice, anchovies, and chilli paste, while *Salmonella* may also be found in the egg. A flowchart of nasi lemak preparation is shown in Figure 3.

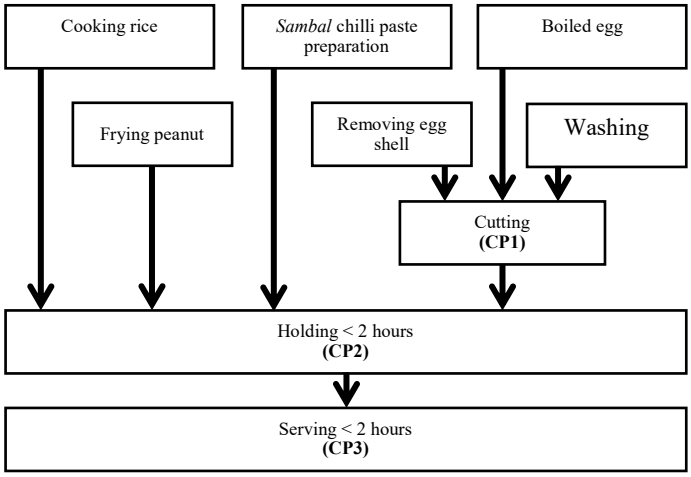


Figure 3. Control points in nasi lemak preparation.
Source: MOH, 2014

For rice preparation, the rice should never be kept at a temperature below 63°C after cooking to prevent bacterial growth. Besides, the sambal chilli paste must be fully cooked and early preparation should be avoided. It should be stored in the refrigerator (0-4°C) for multiple uses, and leftover sauce should not be mixed with freshly prepared sambal chilli sauce. In CP1, the food handlers should follow proper hygiene practices and avoid handling cooked food ingredients with bare hands. Moreover, food handlers should avoid using banana

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leaves as food packaging to prevent cross-contamination. In CP2 and CP3, nasi lemak must be served within 2 hours. Control points, potential risks, and proper handling practices in the preparation of nasi lemak are summarised in Table 7.

Table 7. Control points, potential risks, and proper handling practices in the preparation of nasi lemak.

Control point (CP)	Potential risks	Proper handling practices
CP1: Cutting	Cross-contamination of *Staphylococcus aureus* from food handlers	Wash hands thoroughly before handling food
CP2: Holding	*B. cereus* spores that survived start to germinate	Avoid early preparation.
	S. aureus and *Salmonella* that survived start to proliferate.	
CP3: Serving	*B.* cereus, *S. aureus*, and *Salmonella* start to proliferate.	Serve hot and ensure serving time is less than 2 hours.

Source: MOH 2014

Conclusion

The risk posed by *B. cereus* is undoubted as it can cause severe illness in humans through the production of enterotoxins and emetic cereulide toxin. High *B. cereus* counts resulting from spore outgrowth and high concentrations of emetic cereulide toxin in foods will eventually cause a fatal food poisoning. Rice and noodles are known as the staple foods consumed by the majority of Malaysians. The high prevalence of *B. cereus* in starchy food products as reported by several researchers in Malaysia indicates that there is a high possibility of *B. cereus* infection occurring. However, the prevalence of *B. cereus* in food samples and incidence of the foodborne outbreak caused by *B. cereus* is not extensively reported and published in Malaysia. It is important to identify and sampling of foods associated with food poisoning outbreak to find out the causative agents, and thus, effective precaution measures can be established to prevent it from happening again. Although foodborne pathogens such as *B. cereus* are sometimes difficult to eradicate in food processing, with the understanding of bacteriological characteristics of *B. cereus*, there are no problems we cannot solve together.

Conflict of interest

The author declares no conflict of interest.

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