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Review Article

Pathogenic *Bacillus cereus*, an Overlooked Food Contaminants in Southeast Asia

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ABSTRACT

Bacillus cereus is a food-borne pathogenic bacterium that can cause infection and intoxication to human beings. Its ability to form spores and produce toxins are significant contributory factors to making it a great health risk for the consumer. This paper aims to provide an overview of the occurrence of emetic and diarrhoeal food poisoning caused by *B. cereus* in Southeast Asia. It concerns foods commonly consumed by Southeast Asia citizens, such as fresh food, beverages and traditional food. Rice is the food most associated with *B. cereus* contamination. Methods used in detecting and quantifying *B. cereus* and enterotoxins as well as cereulides are compiled in this paper. *Bacillus cereus* can be identified using biochemical tests or commercially available kit. The methods used to detect the emetic-producing *B. cereus* are HEp-2 cell vacuole formation, polymerase chain reaction (PCR), commercial kit and High-Performance Liquid Chromatography (HPLC). On the other hand, diarrhoeal-producing *B. cereus* can be detected using a commercial kit and real-time PCR. The food safety laws and regulations implemented in Southeast Asian countries are also included and precautionary steps are suggested. Food poisoning due to *B. cereus* is always overlooked because it has a short duration of illness and the symptoms are usually mild.

Keywords: *Bacillus cereus*, diarrhoeal toxin, emetic toxin, enterotoxin, food poisoning, Southeast Asia

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INTRODUCTION

Food is a basic human need. However, it may be contaminated with microorganisms that can affect one's health. Food-borne illness is defined as any form of an unhealthy condition suffered by the patient after the consumption of food contaminated with pathogens, viruses and parasites (Adley & Ryan, 2016). *Bacillus cereus* is a Gram-positive, spore-forming bacterium that is commonly associated with food-borne illnesses (Ankolekar et al., 2009; Wang et al., 2014). *Bacillus cereus* is currently ranked as the second food-borne pathogen in France and the third in China (Gao et al., 2018). It can be found in many types of foods, such as rice (Sawei & Sani, 2016), meat (Aklilu et al., 2016), vegetables (Seung Kim et al., 2017) and dairy products (Chitov et al., 2008; Lesley et al., 2017). Foods contaminated with *B. cereus* do not usually show signs of spoilage because *B. cereus* does not change the appearance or taste of the food, it can easily go unnoticed until too late (Tewari & Abdullah, 2015).

Bacillus cereus belongs to human pathogen Risk Group 2 (RG2) according to the classification of the Health and Safety Executive (HSE) (2013). Group 2 is classified as a biological agent that can cause diseases to humans and may present hazard to people who work with it (HSE, 2013). This biological agent rarely spreads to the community while treatment and prophylaxis are available in case of infection (ACDP, 2013). According to the World Health Organization (WHO), *B. cereus* is an etiological agent of both emetic and diarrhoeal type of food poisoning (Wang et al., 2014). The emetic type is caused by a cereulide toxin, which is pre-formed inside the contaminated foods; infected persons will experience vomiting and nausea a few hours after ingesting the contaminated foods (Jawad et al., 2015). Contamination is usually related to time and temperature abuse, which includes cooking the food a few hours before consumption or storing the food at room temperature (Bennett et al., 2013). The incubation period of the cereulide toxin of *B. cereus* is 30 minutes to 5 hours (Jawad et al., 2015). The diarrhoeal type of food poisoning caused by an enterotoxin is formed inside the intestine of the host (Toh et al., 2004). The symptoms of the diarrhoeal type infection arise about 12 hours after food consumption, with an intoxication which can last about 24 hours (Jawad et al., 2015). The incubation period of the enterotoxin is between 8 to 16 hours and the symptoms include nausea, abdominal pain and diarrhoea (Kim et al., 2010).

The region of Southeast Asia includes Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam. The tropical climate of these countries can contribute to the rapid reproduction of bacteriological contaminants. According to Bennett et al. (2013), food in Asia is commonly associated with *B. cereus* outbreaks (31%). Although *B. cereus* was not among the top 10 food-borne related illnesses in Southeast Asia, it should not be underestimated. *Bacillus cereus* was first identified as a food-borne pathogen responsible for food poisoning

in 1955. Chitov et al. (2008) noted that *B. cereus* gastroenteritis in many countries was not often medically evaluated and hence its incidence was probably underrated. In Southeast Asian countries, official data regarding *B. cereus* food-borne illness has been underreported and this paper attempts to update the current status of *B. cereus* exposure in food. Nevertheless, the degree of the discussion of how the pathogen has affected each country may vary due to limited information from some of those countries.

Bacillus cereus

Bacillus cereus is an aerobic, rod-shaped, Gram-positive bacterium that grows singly or in a chain and can usually be found in the soil and a variety of foods (Aklilu et al., 2016). Six (6) species are categorized under the genus of *Bacillus*: *B. cereus*, *B. mycoides*, *B. thuringiensis*, *B. anthracis*, *B. weihenstephanensis* and *B. pseudomycoides* (Jawad et al., 2015). Table 1 shows some of the tests and expected results to differentiate the members in the *B. cereus* group. Some *B. cereus* species are psychrophiles while others are mesophiles. Physiological and biochemical tests can be conducted to further confirm the identity of the particular *B. cereus*. According to the bacteriological analytical manual of Food and Drug Administration (FDA) (1998), a series of biochemical tests for the identification of *B. cereus* have been suggested. The tests include Gram staining, cell morphology, spore-formation, swollenness of sporangia, motility, nitrate broth, tyrosine agar, lysozyme broth, anaerobic utilization of glucose, Voges-Proskauer (VP) medium, rhizoid growth, hemolysis and crystal toxin production. A commercial identification kit is also available to identify *B. cereus*; namely, the BBL Crystal Identification Systems Gram-Positive ID kit supplied by Becton- Dickson, USA (Lesley et al., 2013).

Table 1
The comparison results of some biochemical test for the identification of Bacillus cereus*,* B. anthracis*,* B. thuringensis*,* B. mycoides*,* B. weihenstephanensis *and* B. pseudomycoides

Biochemical test	*Bacillus cereus*	*Bacillus anthracis*	*Bacillus thuringiensis*	*Bacillus mycoides*	*Bacillus weihenstephanensis*	*Bacillus pseudomycoides*
Colony morphology	White	White	White/grey	Rhizoid	Can be differentiate from other members of *B. cereus* group by ability to growth at < 7˚C but not at 43˚C. *B. weihenstephanensis* can also be detected by using PCR targeting the rDNA or cspA (cold shook protein A).	Have similar physiological and morphological characteristic with *B. mycoides*. Can only be differentiate on fatty acid composition and 16 RNA sequence.
Hemolysis	Positive	Negative	Positive	Positive		
Mobility	Positive	Negative	Positive	Negative		
Susceptible to penicilin	Negative	Positive	Negative	Negative		
Parasporal crystal inclusion	Negative	Negative	Positive	Negative		

Source: Lindback and Granum (2008)

Bacillus cereus can form spores under unfavourable conditions, such as lack of nutrients and unsuitable pH. Unlike other microorganisms that can easily be killed during cooking or the pasteurization process, *B. cereus* can withstand normal cooking temperatures and dry storage. The spore is thermoduric and therefore difficult to kill. Also, it can remain dormant for many years (Lesley et al., 2013). Hence, it is able to survive through various methods of food processing. Once the conditions become favourable again and the germinant receptor detects the presence of nutrients, the process of germination starts and causes *B. cereus* to reactivate and multiply as a vegetative cell (Van der Voort & Abee, 2013). Despite that, in a study of rice in which the maximum *B. cereus* spore level was 10^5 CFU/g, Azanza, and Centeno (2007) claimed that the *B. cereus* spore is moderately heat resistant and that the spore can be reduced by about 90% after heating at 100°C for 5 minutes.

On the farm level, *B. cereus* contamination could be introduced through the plantation soil or the irrigation water. Contamination can also occur during the processing, transport or storage of food.

Exposure to *Bacillus cereus*

Since the symptoms are mild, self-limiting and self-treated, the illness caused by *B. cereus* is often underestimated. This type of food poisoning is also rarely fatal. Most infected persons self-treat instead of seeking medical treatment. However, the elderly and children, with weaker immune systems, might face a higher risk. Currently, there are no reported deaths caused by *B. cereus* food poisoning in Southeast Asian countries. Yet, several fatal cases in children and adults due to mitochondrial toxin have been reported in other parts of the world. In 2003, a child past away in Belgium after eating *B. cereus* contaminated pasta salad (Biesta-Peters et al., 2010). In 1998, three people died in France due to *B. cereus* food poisoning (Lindback & Granum, 2008).

In certain countries of the Southeast Asian region, food safety is not an important concern for either the government or citizens, indicating an indifference probably related to the poverty and economic situation of these nations. Hence, throughout the region, laws concerning the permissible limit of *B. cereus* in foods have not been passed.

The first outbreak of *B. cereus* related food poisoning happened in 1982 at a military camp in Jurong, Singapore (Tay et al., 1982). The source of contamination was believed to be the fried rice for their breakfast. Fourteen army personnel were sick. The symptoms included vomiting (89.5%), abdominal cramps (52.6%), diarrhoea (47.4%), headache (47.4%) and fever (10.5%). The rice was believed to be cooked the evening before and stored inside the rice cooker. Egg and roast pork were then added to the rice and cooked together before being sold.

In September 2017, a food poisoning incident in a school canteen in Singapore had occurred. Fourteen students were ill after eating the fried rice sold in that canteen. The contaminated fried rice was prepared quite similarly to the incident

back in 1982 at the military camp. The fried rice was prepared by firstly the rice being cooked and kept warm for three hours in the rice cooker. After that, the rice was fried along with egg and crab meat. Subsequently, the fried rice was put inside the insulated container for two hours at room temperature before being sold at the school canteen.

The first outbreak in Malaysia was reported in 1984 and occurred in a school hostel in Klang (Rampal et al., 1984). The fried noodles served were contaminated with 2.3×10^6 CFU/g of *B cereus*. Later, on 15 February 2012, another outbreak due to *B. cereus* happened in a primary school canteen at Kota Kinabalu (Jeffree & Mihat, 2016). The source of *B. cereus* food poisoning outbreak was identified as the *nasi kuning* (turmeric rice), which was prepared using untreated water and poor hygienic practices.

The Thailand Bureau of Epidemiology received three reports of *B. cereus* outbreaks in 2008 and 2009, each of which occurred in schools. Moreover, in December 2009, an emetic *B. cereus* food poisoning outbreak associated with sweet stewed egg and pork occurred in a kindergarten located in Bangkok, Thailand (Santayakorn et al., 2012).

Emetic Food Poisoning

The emetic food poisoning is often associated with consumption of cooked or fried rice from a Chinese restaurant and thus it is also known as 'Fried Rice Syndrome' or 'Chinese Restaurant Syndrome' (Sandra et al., 2012; Schoeni & Wong, 2005). Emetic food poisoning is caused by the cereulide toxin. Cereulide is a cyclic dodecadepside, [D-0-Leu-Ala-O-Val-L-Val]3, which is produced by a nonribosomal peptide synthetase (NRPS) that is resistant to heat, pH and proteolysis (Guérin et al., 2017). The symptoms of infection include vomiting and nausea that occur 1 to 5 hours after ingestion (Häggblom et al., 2002). Emetic toxin-producing *B. cereus* usually grows on farinaceous foods such as pasta, noodles and rice, and ingestion of this toxin can lead to fatal liver failure (Mahler et al., 1997). While the mechanism of emetic toxin in the human body is still unknown, researchers believe the cereulide toxin binds to the 5-HT_3 receptor which stimulates the afferent vagus nerve, resulted in vomiting (Agata et al., 1995; Ehling-Schulz et al., 2004). The emetic producing *B. cereus* strain is unable to break down starch or fermenting salicin due to the lack of the hemolysin BL gene (Van der Voort & Abee, 2013). Based on animal studies, the emetic dose is between 9-12 µg/kg (Jeffree & Mihat, 2016). The result was recorded based on the minimum dose of cereulide to cause tested monkeys to vomit (Shinagawa et al., 1995). According to Bilung et al. (2016), the cell dose of *B. cereus* that leads to the emetic syndrome is between 10^5-10^7 cells/g. This is the range of viable cells of *B. cereus* needed for the cereulide to be detectable in the contaminated food.

Method of Detecting the Emetic Toxin. Traditionally, many researchers have utilized a human larynx carcinoma cell (HEp-2)

vacuolation assay to detect the emetic toxin-producing *B. cereus*. This assay depends on the vacuole-forming ability of the cereulide. However, this technique was found to be laborious, subjective and unreliable because the presence of the toxin can easily be missed (Hughes et al., 1988). Researchers have also used an assay in which boar sperm motility is inhibited by damage to the mitochondria caused by the cereulide. Recently, the polymerase chain reaction (PCR) method seems to be preferred by researchers and evidently, PCR detection kit for the emetic toxin is now commercially available. To detect the emetic toxin with the PCR method, the primer is designed to target the NRPS gene sequence since cereulide is produced by the NRPS complex and this gene sequence is only present in the cereulide-producing *B. cereus* (Toh et al., 2004). The primers used were BEF (5' ACT TAG ATG ATG CAA GAC TG-3') and BER (5'- TTC ATA GGA TTG ACG AAT TTT-3') with the amplicon size of 850 bp (Toh et al., 2004).

Currently, two commercial kits for emetic toxic detection are commercially sold. The first kit is Single-path Emetic Tox Mrk, developed by Merck. This is an immunochromatography kit. The sensitivity and specificity of this kit were evaluated by Nakayama et al. (2012), who found that this kit provided the result of high sensitivity and specificity by giving no false-negative result and only three strains of false positive. Another kit, one for detecting cereulide-producing *B. cereus*, is Swiftgene by KAINOS Laboratories, Inc. This kit helps to identify cereulide-producing *B. cereus* by combining the Nucleic Acid Sequence-Based Amplification (NASBA) and Nucleic Acid Chromatography (NAC) method. The NASBA method will amplify the cesPTABCD (ces) mRNA and Internal Control (IC) by using the specially designed primers provided inside the kit. The amplicon will then be determined by a single NAC strip.

In recent years, there has been an increasing interest in the quantification of cereulide. The first attempt to quantify cereulide was made by Häggblom et al. (2002). He used high-performance liquid chromatography (HPLC) connected to ion trap mass spectrometer. This method was attractive because it was sensitive and produced a precise result. The detection limit of cereulide by HPLC-mass spectrophotometry was 10 pg per injection. Ever since this method was first reported, many scientists have shown an interest in developing more sensitive and more rapid methods. Several methods currently exist for the measurement of cereulide including the usage of real-time PCR (Fricker et al., 2007). The micellar electrokinetic chromatography-capillary electrophoresis (MEKC-CE) method was next developed (Oh & Cox, 2010), followed by LCMS, LC-MS^2 and UPLC-ESI-MS/MS (Delbrassinne et al., 2012; Rønning et al., 2015; Zuberovic Muratovic et al., 2014). In most recent studies, cereulide has been successfully measured using the droplet digital PCR (Porcellato et al., 2016). It has been reported that this technique requires a lesser sample volume and has a lower cost of operation.

Diarrhoeal Food Poisoning

Diarrhoeal food poisoning is caused by enterotoxins. Four types of toxins have been identified to be responsible for diarrhoeal food poisoning. The toxins are haemolysin BL (*hblA*, *hblC*, *hblD*), non-haemolytic BL (*nheA*, *nheB*, *nheC*), enterotoxin FM (*entFM*) and cytotoxin K (*cytK*). Unlike the other toxins, the *EntT* toxin is believed to be unrelated to food-borne outbreaks (Lynn et al., 2013). According to Chitov et al. (2008), the most frequently isolated enterotoxin was *nheB* (80.80%) while the least frequently isolated was the enterotoxin gene *nheA* (59.20 %).

The intoxication of 10^5-10^7 cells or spore per gram can lead to diarrhoeal food poisoning (Bilung et al., 2016). These toxins are produced in the intestinal tract of the host and are unstable to heat. Diarrhoeal toxins are produced in conditions of inadequate refrigeration and improper reheating of leftover food. Proteinaceous food has been identified as the main vehicle for enterotoxin-producing *B. cereus*.

Method of Detecting the Diarrhoeal Toxin. Two commercially available kits can be used for the identification of the diarrhoeal toxin. The Tecra *Bacillus* diarrhoeal enterotoxin immunoassay (Tecra-VIA) kit detects the *nheA* gene, while the Oxoid *Bacillus cereus* Enterotoxin-Reversed Passive Latex Agglutination (Oxoid BCET-RPLA) kit can detect the L_2 component from the *hbl* gene. *Hbl* is made up of binding component B and the two lytic component L_1 and L_2 (Häggblom et al., 2002). A study performed to establish the effectiveness of these kits where Beecher and Wong (1994) found that each detected different antigen, as there was a lack of cross-reactivity between the positive controls provided in each kit. However, the Tecra-VIA kit has the capability to identify enterotoxins that have been heat-denatured, and the result is correlated with a Chinese hamster ovary (CHO) cell toxicity assay. The lowest concentration of the L_2 component detected by Oxoid BCET-RPLA kit was 0.6 ng/ml.

Salihah et al. (2018) investigated the effect of two different real-time PCR fluorescence strategies for the detection and quantification of the enterotoxin. The fluorescence methods tested were the ZEN double-quenched probe and improved SYBR Green dye. Of the two, the improved SYBR Green dye provided a much better result.

Prevalence of *Bacillus cereus* in Foods

Rice. *Bacillus cereus* is commonly associated with food poisoning from rice and rice-based foods (Lynn et al., 2013). Rice is a staple food of many Southeast Asian countries, such as Malaysia, Myanmar and the Philippines. At an average, Filipinos consume 4-5 servings of rice every day. *Bacillus cereus* has been identified as normal microflora of rice grain. Thus, it is essential to perform studies to establish that this food is safe for consumption.

In one of the studies conducted in Selangor, out of 25 samples of local Malaysian varieties of rice grain (*Keladi Halus Wangi*, *Keladi Wangi*, *Kanowit Halus*

Wangi, *Lansam Halus Wangi* and *Bario*), 100% of the samples tested were positive with *B. cereus* (Sandra et al., 2012). Both *Keladi Halus Wangi* and *Lansam Halus Wangi* had a maximum value of more than 1,100 MPN/g. These two varieties were considered too risky for consumption. In the same study, the presence of *B. cereus* was tested in varieties of cooked rice samples (*nasi lemak*, *nasi biryani*, *nasi ayam* and *nasi putih*). The total prevalence of cooked rice samples tested positive was 73.04%. The highest percentage of contamination with *B. cereus* was found in *nasi ayam* (100%), *nasi putih* (76.2%), *nasi lemak* (70.4%) and *nasi biryani* (50%). The cooked rice samples, except *nasi ayam*, had a maximum value of more than 1100 MPN/g. Sandra et al. (2012) used a combination of the most probable number-polymerase chain reaction (MPN-PCR) for the detection of the *gyrB* gene present in *B. cereus*. Using a similar method, another study in Malaysia showed that 85% (17/20) of the local indigenous and 100% (20/20) of imported rice grains tested positive for the presence of *B. cereus*, with a value of more than 1100 MPN/g (Chitov et al., 2008).

Enumeration of *B. cereus* in local Thai rice grain (Happy Rose Quality Thai Fragrant Rice, Liansin Butterfly, Liansin Mr Thai, Liansin Cap Amoi Super Siam, Happy Bamboo Thai, Liansin Beras Pulut Susu and NutriRice Thai Fragrant Brown) and Vietnamese rice grain (Tulip Vietnam Premier Rice, Teana Sargon Super Imported Rice and Lap Padi Emas Premium Quality White Rice) sold in Malaysia showed that all the samples had more than 1100 MPN/g (Tan et al., 2015). These studies showed a high percentage of *B. cereus* on all varieties. However, these studies did not test whether the identified *B. cereus* produced toxins.

Testing of 24 unhusked local rice samples (10 storage samples and 14 fresh samples) from Sarawak, Malaysia showed that in every sample, MPN results were more than 1,100 MPN/g (Bilung et al., 2018). The results indicated that unhusked rice might be the possible source for food-borne illness associated with *B. cereus*. The diarrhoeal syndrome was shown to be caused by 10^3 CFU/g of *B. cereus*, while the emetic syndrome was caused by 10^5 CFU/g (Guinebretière & Brousollee, 2002).

Aside from rice, the ready-to-eat (RTE) cereal distributed and sold in Sarawak was also tested for the presence of toxin-producing *B. cereus* (Lesley et al., 2013). The study found that *B. cereus* was present in 15% of the breakfast cereal (3/19) and 100% of the instant oatmeal (1/1) samples. The contamination was believed to occur due to cross-contamination in the processing line. Cross-contamination can also occur between the equipment used for food preparation and raw food materials. This result suggested that a processed food product might be contaminated with *B. cereus* even when it underwent strict processing conditions and continuous surveillance. The same study also tested a cereal drink, an original cereal, cornflakes and oat cereals, which were found to be free of *B. cereus*. The result of this study might not be significant because the sample size was small, and the amount of *B. cereus* found in the food was not mentioned.

Rice noodles, fresh wheat noodles and dried wheat noodles from Malaysia were found to be 41%, 17.6% and 34 % positive for *B. cereus* respectively (Rusul & Yaacob, 1995). Using the Tecra-VIA and BCET-RPLA, 178 (91.8%) and 164 (84%) of the isolated strains were identified as enterotoxin- producing *B. cereus* respectively.

Fresh Food. A study found that 3.33% (1/30) of the raw and 50% (15/30) of the cooked chicken meat samples in Kota Bharu, Malaysia tested positive for *B. cereus* (Aklilu et al., 2016). *Bacillus cereus* was found to be more prevalent in cooked than in raw chicken meat. It is likely that the contamination was due to the cross-contamination from the cooking utensils, food additives and ingredients used to cook the chicken meat. In this study, the CFU/g was not counted.

The study by Ananchaipattana et al. (2012a) offered the data concerning *B. cereus* contamination in meat and fish or seafood in Thailand. This study found that 2% (1/51) of the tested meat and 35% (13/37) of the fish or seafood were contaminated with *B. cereus*. All the positive samples of meat were obtained from open markets. Meanwhile, for fish or seafood, 7 positive samples were from open markets and the rest were from the supermarket.

Ananchaipattana et al. (2012b) investigated the soybean curd (tofu) in Thailand and found that 41% of the tofu samples were positive for *B. cereus*. Out of the 54 isolates, five were identified as enterotoxin-producing *B. cereus*. *Bacillus* spp. was isolated from 5% (2/38) of the green leafy vegetable samples obtained from independent farms and company farms (Ananchaipattana et al., 2012b). The number of samples contaminated with *Bacillus* spp. was the lowest compared to the other tested microbial contaminants such as *Escherichia coli*, *Pseudomonas* spp., *Staphylococcus* spp. and others.

Beverages. *Bacillus cereus* resides not only in foods but can also contaminate beverages. In Vietnam, common street drinks such as iced tea, sugarcane juice and corn milk were tested for the presence of microorganisms. The result showed that all the drinks contained *B. cereus* along with other pathogenic bacteria. The testing was conducted by Healthplus magazine in collaboration with the Centre for Evaluation and Conformation under the Vietnam Institute of Dietary Supplements (VIDS) of the Ministry of Health.

Additionally, 41.7% (5/12) of formula milk and 3% (6/20) of ultra-high temperature (UHT) milk tested in Malaysia was positive for *B. cereus*. The samples used for formula milk were infant formulas, follow-up formulas and young-child formulas. Of these, only the infant formula samples were negative for *B. cereus* contamination. For the UHT milk, the types of samples tested were whole milk, full cream milk, low-fat milk, skimmed milk and fresh milk. The MPN count showed more than 1100 MPN/g for all these products (Lesley et al., 2013).

Although in Malaysia the food poisoning cases due to milk spoilage had happened a few times, no report indicates the cause to be *B. cereus* contamination. This is probably because the milk normally spoils before *B. cereus* contamination and it is enough to cause gastrointestinal illness (Lesley et al., 2017).

Traditional Food. In Thailand, *B. cereus* has not been documented as a major microbial enteropathogen that causes outbreaks (Adley & Ryan, 2016). However, a study was conducted to investigate the presence of *B. cereus* on pasteurized milk (100%), rice (brown rice and sticky rice; 41.9%), cereal flour (33.3%), noodles (18.7%) and quick-cooked cereal-based meal (62.5%) (Chrun et al., 2017). All samples were contaminated with *B. cereus* at 0.5×10^2 to 1.7×10^3 CFU/g. The enterotoxin gene from the isolated *B. cereus* was profiled using PCR and the result showed that *nheB* (80.8%), *cytK* (70.4%) and *nheC* (69.9%) were the most common enterotoxins.

Another study in Thailand discovered the presence of *B. cereus* in 15% of tested Thai pandan custard, which then increased to 25% and 45% after one–day and two-day storage respectively (Puangburee et al., 2016). The source of the contaminant appeared to be the pandan leaves, 95% of which were positive for *B. cereus* with an average of 3.4 log CFU/g.

Fermented rice noodle is a local food in Myanmar that is commonly consumed during breakfast and as a snack. It was assumed that *B. cereus* contaminated the rice noodle during the fermentation process. Three samples of *B. cereus* isolated from the fermented rice noodle were used to study the enterotoxin gene using molecular assay (Lynn et al., 2013). The result confirmed that the isolates were enterotoxin-producing *B. cereus*.

In Cambodia, the contamination rate of *B. cereus* in local fermented mixed vegetables and fermented single-type vegetables was investigated. The results showed that the fermented single-type vegetables had a higher percentage of *B. cereus* contamination, 35% (17/48), compared to the fermented mixed vegetables, which had 20% (4/20) (Chrun et al., 2017).

Ananchaipattana et al. (2012a) reported that fermented meat and fish in Thailand had higher numbers of *B. cereus* compared to non-fermented meat and fish. Of the fermented meat and fish samples, 82% (9/11) were positive with *B. cereus*. The *B. cereus* likely originated from the cooked rice used as one of the fermentation ingredients.

There is no information on other countries including Malaysian on *B. cereus* food poisoning in traditional foods due to the lack of studies. Further work is needed to determine the prevalence of *B. cereus* in traditional food.

The Potential Use of *Bacillus cereus* as Antimicrobial Bacteriocins. Though responsible for food-borne illness, *B. cereus* can also act as an antimicrobial bacteriocin; this has been demonstrated with *B. cereus* isolated from *budu* in West Sumatra, Indonesia. *Budu* is a local food

made up of fermented fish. It was found that *B. cereus* can inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* Typhi, *Bacillus subtilis* and *Listeria monocytogenes* (Yusra et al., 2014). This antimicrobial bacteriocin was found to be stable at a pH range of 2 to 1 and could withstand heat treatment of 121˚C for 15 minutes.

Regulations. *Bacillus cereus* can cause illness only if it is ingested in high concentrations. Different countries have set varying maximum level limits for *B. cereus*. Food Standards Australia New Zealand has set the allowable amount of *B. cereus* in food to be below 10^2 CFU/g, with 10^4 CFU/g being considered risky. However, in the United Kingdom, the limit is 10^3 CFU/g and 10^5 CFU/g are regarded as unsafe (Bilung et al., 2016).

In Malaysia, food must undergo quality control based on the Food Regulations 1985 and the Food Act 1983 (Bilung et al., 2016). Under these regulations, there is no specific microbiological standard for *B. cereus* (Food Act, 1983). Instead, the limit is set at a plate count of less than 10^6 CFU/g at 37˚C for 48 hours. The Ministry of Agriculture and Agro-Based Industry (MOA) and the Ministry of Health (MOH) are the agencies responsible for food safety and hygienic control in Malaysia. MOA oversees the safety and hygienic control of production and primary processing. Imported and processed foods are under the responsibility of the MOH.

The Agri-Food and Veterinary Authority (AVA) of the Ministry of National Development is the administrative body responsible for food standards, safety and hygiene control in Singapore (Hiroaki, 2013). AVA has determined that less than 2.0×10^2 CFU/g for ready-to-eat (RTE) food is considered safe and represents a low risk to the public. Food that exceeds the permissible limit is considered unsafe and must be recalled immediately for further inspection.

The main administrative bodies responsible for food safety, food standards and hygienic control in Thailand are the Ministry of Public Health and the Ministry of Agriculture and Cooperatives (Hiroaki, 2013). The law responsible for protecting the consumers is the Food Act of B. E.2522 (1979) and the government has set the limit of *B. cereus* in food at 100 per 1 g or 1 ml.

Head of the National Agency for Drug and Food Control of the Republic of Indonesia is the government body that is responsible for controlling food contaminant in Indonesia. The government of Indonesia passed Act Number 7 on Food in 1996 (Hiroaki, 2013). Under the Selected Indonesian National Standards (SNI) 2897:2008 for microbial testing in foods such as meat, eggs and milk as well as their by-product, *B. cereus* is not tested. These foods are tested only for total plate count (TPC), coliform, *E. coli*, *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* spp. and *Listeria monocytogenes*. In 2009, the government made a regulation No. HK.00. 06.1.52. 4011 of 2009 on Maximum

Level of Microbiological and Chemical Contaminants in Foods (Hiroaki, 2013). Though under this legislation, *B. cereus* is also not listed.

Ministry of Health (MOH), Ministry of Agriculture and Rural Development (MARD), as well as the Ministry of Industry and Trade (MIT) are the ministries that are responsible for food safety management in Vietnam (Hiroaki, 2013). The main role for food safety management at the national level falls under the responsibility of the Ministry of Health. As for the Ministry of Agriculture and Rural Development, they work on making policy and managing food safety for the primary production sector. Last but not least, the Ministry of Industry and Trade is responsible for making policy and manage food safety sectors that manufacture products. The government of Vietnam sets the maximum limit of *B. cereus* in powdered nutrition products and special medical-use nutrition products for children up to 12 months old at 5×10^2 CFU/g (Huong & Ward, 2013). The regulation stated that *B. cereus* should not be tested during conformity assessment if the food producers are already taking risk control measures in production (HACCP or GMP). Only if the producer fails to take risk control measures will the standards limit be tested.

Prevention and Control Steps. It is impossible to eliminate *B. cereus* completely from foods since *B. cereus* can form spores and normal cooking temperature is not sufficient to eliminate them (Azanza & Centeno, 2004). Despite that, the bacterial number can be reduced by controlling time and temperature to reduce its potential public health risk.

A common practice in Southeast Asian countries is leaving cooked food at room temperature until the next meal. For example, school children usually bring lunch boxes from home. The food was prepared early in the morning and the kids will eat it only during the recess. Cooking heat will encourage the *B. cereus* to reproduce and leaving the foods at room temperature allows the *B. cereus* to further multiply (Schoeni & Wong, 2005. Reheating the food for a short time does not kill *B. cereus* or neutralizes the toxins it produces. By the time they started eating the food, the population of *B. cereus* might have already exceeded the safety limit. To avoid possible food poisoning, food must be eaten immediately after cooking. The practice of cooling rice and other kinds of food at room temperature must be stopped by bringing an end to the practice of preparing meals several hours before serving.

We should keep our cooked food in the fridge below 4°C within two hours after it is cooked as *B. cereus* has a growth temperature of 4°C to 50°C. Alternatively, the food can be kept warm at 60°C (Sandra et al., 2012).

Furthermore, most food contamination comes from cross-contamination of the raw ingredients, water supply and kitchen utensils. Thus, good food handling procedures need to be practised in the kitchen. At the same time, food handlers must ensure that the water used for preparing

the food is clean. Using contaminated water in the cooking process may also contaminate the food if the microorganisms are not killed during the cooking process.

Bacillus cereus is not only present in raw foods, but also processed foods (Lee et al., 2009). Food with high counts of *B. cereus* poses a health hazard to consumers, so precautions should be taken before consumption. Basic sanitary practices must be enforced throughout the chain of food processing.

Since most outbreaks happen in schools, the school authorities must be stricter when selecting food caterers and kitchen cleanliness needs to be constantly monitored. The most important thing to be done is creating awareness among the public regarding food-borne illness.

CONCLUSIONS

In conclusion, *B. cereus* is commonly detected in foods throughout Southeast Asia, especially in rice. It enters the food cycle likely through cross-contamination from the soil, cooking water and kitchen utensils. The amount of *B. cereus* detected in unhusked, raw and cooked rice counted using the MPN technique were all more than 1000 MPN/g. For other food samples, the levels of contamination ranged from 0.5×10^2 to 1.7×10^3 CFU/g. *Bacillus cereus* is often overlooked as food contaminant in Southeast Asia because of the mild and short duration of illness. Consequently, not many affected persons seek medical treatment which in turn causes food poisoning by *B. cereus* to be underreported.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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