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Bacillus cereus and *Bacillus thuringiensis* in ready-to-eat cooked rice in Malaysia

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Abstract: *Bacillus cereus* (*B. cereus)* isolates are toxigenic and can cause food poisoning. Cooked rice is a potentially hazardous food, especially in tropical countries. The aim of this study was to determine the prevalence of *B. cereus* and *B. thuringiensis* in raw and cooked rice marketed in Selangor, Malaysia. A combination of Most Probable Number - Polymerase Chain Reaction (MPN-PCR) method was used to detect *gyrB* gene in *B. cereus* and *B. thuringiensis*. Five local varieties of raw rice samples were negative for *B. thuringiensis* but all (100%) were positive for *B. cereus*. A total of 115 cooked rice samples (*nasi lemak, nasi briyani, nasi ayam* and *nasi putih*) were studied for the presence of *B. cereus* and *B. thuringiensis*. *Nasi ayam* was found to have the highest prevalence (100%) of *B. cereus* compared to *nasi putih* (76.2%) and *nasi lemak* (70.4%). *Nasi briyani* had the lowest prevalence (50%) of *B. cereus*. The frequencies of *B. thuringiensis* were found to be 10, 30 and 35.2 % in *nasi putih* and *nasi ayam, nasi briyani* and *nasi lemak,* respectively. Occurrence of *B. cereus* and *B. thuringiensis* in the samples ranged from < 3 to 1100 MPN/g in different samples. Maximum number of *B. cereus* was observed in *nasi lemak*, *nasi briyani* and *nasi putih* (> 1100 MPN/g) while *nasi ayam* showed less contamination (460 MPN/g) with *B. cereus* which was significantly different ($P < 0.05$) from others. The number of *B. thuringiensis* in *nasi lemak, nasi briyani, nasi putih* and *nasi ayam* were found to be >1100, 93, 9.2 and 3.6 MPN/g, respectively.

Keywords: *B. cereus, B. thuringiensis*, MPN-PCR, cooked rice, food poisoning

Introduction

Food poisoning is caused with presence of bacteria in food due to improper food preparation or cooking process and exposure of food to temperatures of 30°C. Common food poisonings are usually mild, but deaths due to food poisoning are also reported. Food poisoning occurs within 48 hours after consumption of contaminated food or drink. The symptoms include nausea, vomiting, diarrhea and abdominal pain. Most cases of food poisoning are caused by bacteria, viruses or toxins and chemicals (Drobniewski, 1993).

Milk, dairy products, fatty foods, bread, cakes and seafood can easily be contaminated with *Bacillus* spp. *Bacillus cereus* (*B. cereus*) can cause food poisoning. This bacterium is ubiquitous in nature and can be found in soil or in a variety of dried foods such as grains, legumes, starches and spices as vegetative cells and endospores (Rusul and Yaacob, 1995).

According to Food and Drug Administration of the United Stated, food poisonings due to *B. cereus* group have two different clinical syndromes, diarrheal and emetic (vomiting) syndrome. The emetic type causes vomiting after 0.5–6 h of ingestion (Ehling-Schulz et al., 2005) and diarrhoeal type causes abdominal pain and diarrhoea after 8 to 16 h of consumption. The diarrheal syndrome has been associated with a wide variety of food including meats, milk, vegetables and fish while the emetic syndrome has been generally associated with rice products, starchy foods such as potato, pasta, noodles, spaghetti, pastry and cheese products (Granum and Lund, 1997, Shinagawa 1993).

Based on the report of European Food Safety Agency (2005) 1–33% of food-borne poisonings are caused by *B. cereus*. Bean and Griffin (1990) reported that *B. cereus* was responsible for 58 food-borne outbreaks in the United States from 1973–

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1987, representing approximately 3% of the total outbreaks. About 27,360 cases of food-borne illness reported in 1997 were from *B. cereus* (Mead *et al*., 1999). Michael *et al*., 2006, reported that 571 cases of food-borne poisonings in US (between 1998 to 2002) were due to *B. cereus* contamination. In Korea, *B. cereus* was responsible for 15 food-borne outbreaks (392 patients) accounting 5.5% of the total outbreaks in 2009 (Chang *et al*,. 2011).

B. cereus is the cause of "Fried Rice Syndrome" as endospores of this bacterium can survive in improperly cooked rice and this problem is exacerbated when foods are stored in ambient temperature. This situation gives enough time for endospores to germinate (Rusul and Yaacob, 1995).

Nowadays, rice is regarded as the most important staple food for human population in the world especially in Africa, Southeast Asia, the Middle East, Latin America and the West Indies (Boyce *et al*., 1996). About 85% consumption and production of total rice in the world are in Asian countries (Shoichi *et al*., 1989). Therefore, it is crucial to assure the safety of rice products, especially when the cooked products are stored prior to consumption. Incidences of *B. cereus* food poisoning due to consumption of improperly handled boiled rice have been reported. In most of the cases the mentioned food-borne outbreaks came from Chinese restaurants or take-away shops which left the boiled rice to dry off at room temperature (Lee *et al*., 1995).

A recent study on microbiological quality of take-away cooked rice in London showed that 10% of the samples were unsatisfactory and 3% of the samples were in unacceptable quality ($> 10^5$ CFU/g) due to presence of *B. cereu*s and/or other *Bacillus* spp. (Little *et al*., 2002).

In Virginia in1993, a regional public health facility received a report of acute gastrointestinal illness. It was reported in a day-care center where 14 out of 48 persons consuming chicken fried rice had shown acute gastrointestinal symptoms. Chicken fried rice was prepared by a local restaurant and the rice had been cooked one day before and cooled at room temperature before refrigeration. In the morning, the rice which was pan-fried in oil with pieces of cooked chicken was delivered to the day-care center at 10:30 a.m. and without refrigeration or reheating was served at noon. In the mentioned outbreak, vegetative form of the microorganism probably multiplied at the restaurant and the day-care centers while the rice was held at room temperature (Todar, 2009).

In Malaysia, the first outbreak of food poisoning due to *B. cereus* was reported by Rampal (1984). The outbreak affected 114 Malay students staying at a hostel of a religious secondary school in Klang. The students consumed fried noodles and the illness was characterized by abdominal pain, nausea, vomiting and giddiness. Every year food poisoning outbreaks from the school canteen are reported in Malaysia. In 2010, 50 % percent of the total reports (311 cases) of food poisoning incidents were from school canteens. The source of food poisoning was contaminated *nasi lemak* (Utusan online, 2011). Johore Weekly Epidemiological Bulletin reported that there had been 1776 cases of food poisoning in Malaysia until July 2011. Food poisoning occurred after the victims consume ready-to-eat cooked rice (such as n*asi lemak* and *nasi briyani*) kept in at room temperature. The symptoms were reported as abdominal pain, diarrhoea, nausea and vomiting (Fatimah *et al*., 2011).

Some of *B. cereus* outbreaks are under reporting as the illness associated with this bacteria limit itself and does not become severe. A recent survey on culture practices for outbreaks of apparent food-borne illness showed that 20% of state public health laboratories do not make *B. cereus* testing routinely available. The survey also found that most of food handlers (in food stalls and restaurants) were unaware that cooked rice was a potentially hazardous food (Todar, 2009).

B. cereus is a facultatively anaerobic, spore-forming and gram-positive bacterium. Differentiation of *B. cereus* from its closely related microorganism, *Bacillus thuringiensis* (*B. thuringiensis*), depends upon the absence of toxin crystals (Yamada *et al*., 1999). Nowadays *B. thuringiensis* is recognized as bacteria with the ability to produce diarhoeal toxin, whereas in the past decades it was used as biocontrol agent due to its ability to kill insect (Oh *et al*., 2011).

B. cereus and *B. thuringiensis* are highly polyphyletic (Guinebretiere *et al*., 2008) and have similarity in genotype and phenotype (Oh *et al*., 2011). Ash *et al*. (1991a; 1991b) compared the members of *B. cereus* groups using 16S rRNA genes and concluded that four members of the *B. cereus* group (*B. cereus*, *B. thuringiensis*, *B. mycoides* and *B. anthracis*) can be considered one species as they are closely related. Yamada *et al*, (1999), detected *B. cereus* and *B. thuringiensis* using gyrase B (*gyrB*) gene in PCR assay.

Currently, polymerase chain reaction (PCR) is a popular tool for rapid detection of pathogens. The Most Probable Number (MPN) method is widely used as a routine analysis technique to enumerate bacteria in foods. This method allows quantitative data to be calculated from incidence results and is effective for both low and high cell counts (Blodgett,

2006). MPN-PCR has been successfully used for quantitative determination of pathogens in foods (Bach *et al*., 2002).

In Malaysia, some studies on biosafety of *B. cereus* have been carried out (e.g. ready to eat cereals, chocolate, honey, milk by Lee *et al*., (2009); noodles, spices, grains and legumes by Rusul and Yaacob in 1995). There has been no study on the occurrence of *B. cereus* and *B. thuringiensis* in rice products in Malaysia. The aim of this study was to isolate *B. cereus* and *B. thuringiensis* from rice and determine their prevalence using a combination of Most Probable Number - Polymerase Chain Reaction (MPN-PCR) method.

Materials and Methods

Sample Collection

A total of 115 cooked rice samples were collected randomly from restaurants and retail food stores in Selangor, Malaysia. The cooked rice samples were as follows: *nasi lemak* (rice cooked in coconut milk), *nasi briyani* (Persian rice), *nasi ayam* (chicken rice) and *nasi putih* (white rice). All samples were transported to the laboratory immediately and analyzed within 24 h of sample collection. Twenty five raw rice samples from 5 local varieties (*Keladi halus wangi, Keladi wangi, Kanowit halus wangi, Lansam halus wangi* and *Bario*) were obtained from Sarawak, Malaysia.

Sample preparation

Samples were analyzed using the standard procedure for detection of *B. cereus* (Rhodehamel and Harmon, 2001) with modifications described by Lee *et al*. (2009). Briefly, 10 g of each sample was placed in a stomacher bag added with 90 ml of Tryptic Soy Broth (TSB; Bacto™, France) and pummeled in a stomacher (Interscience, France) for 60 s followed by incubation at 37°C for 12 h.

MPN- Multiplex PCR

For MPN analysis, 100 fold and 1000 fold dilutions of the stomached fluid were prepared with Tryptic Soy Broth (TSB; Bacto™, France). 0.1 ml portions of each dilutions of the fluid were transferred into three tubes and the tubes were incubated at 37°C for 18 to 24 h. A loopful of culture from each tube was streaked onto Mannitol Egg Yolk Polymyxin Agar Base (MYP; Difco™) added with sterile Polymyxin B Selective Supplement (Difco™, Germany) and sterile Egg-Yolk Tellurite Emulsion 20% (Merck, Germany) which is a specific media for the isolation and identification of the *Bacillus* species.

DNA extraction

Prior to PCR analysis, the content of MPN tubes were preceded to DNA extraction using boil cell method (Lee *et al*., 2009) with slight modifications. A 1 ml portion of each MPN broth was subjected to centrifugation at 12,000 x g for 1 min and the pellet was resuspended in 500 µl of sterile distilled water. The mixture was boiled for 20 min and immediately cooled at -20°C for 10 min before it was centrifuged at 12,000 x g for 5 min. The supernatant was used for detection of *B. cereus* and *B. thuringiensis* using PCR.

Primers and PCR conditions

For detection of *B. cereus* and *B. thuringiensis* the following primer pairs were used. BCJH-F (5' TCATGAAGAGCCTGTGTACG 3') and BCJH-1R (5' CGACGTGTCAATTCACGCGC 3') were used to amplify a 475 bp fragment of *gyrase B* (*gyr*B) gene to detect *B. cereus*. BTJH-1F (5' GCTTACCAGGGAAATTGGCAG 3') and BTJH-R (5' ATCAACGTCGGCGTCGG 3') were used to amplify a 299 bp fragment of *gyrB* gene to detect *B. thuringiensis* (Park et al., 2007).

Multiplex PCR amplification was performed in a 20 µl reaction mixture containing 5.0 µl of 5X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of deoxynucleoside triphosphate mix, 1.0 µM of each primers, 0.2 U/µl *Taq* polymerase and 2.0 µl of DNA template. All PCR reagents were supplied by Promega and the primers were synthesized by Invitrogen. Amplification was performed on a Veriti 96-Well Thermal Cycler (Applied Biosystems, CA, USA) with the following conditions: initial denaturation at 94°C for 3 min for 1 cycle, 35 cycles consisting of denaturation at 94°C for 45 s, annealing at 63°C for 1 minute and elongation at 72°C for 1 min, and a final extension at 72°C for 7 min. PCR products were electrophoresed on a 1.0% agarose gel at 80V for 40 min. A 100 bp DNA molecular ladder (Vivantis Technologies) was included in each gel. The agarose gel was stained with ethidium bromide and visualized under UV light using Gel Documentation System (SynGene).

Data analysis

All measurements were carried out in triplicate. SPSS (v. 19) statistical package was used to determine if there was any significant difference ($P < 0.05$) between prevalence of *B. cereus* and *B. thuringiensis* in cooked rice samples.

Results

Figure 1 shows the result of PCR amplification for detection of *B. cereus* in raw rice samples and Fig. 2 represent the results of multiplex PCR amplification for detection of *B. cereus* (with product size of 475 bp) and *B. thuringiensis* (with product size of 299 bp) in cooked rice samples. Prevalence of *B. cereus* and *B. thuringiensis* in cooked rice samples are summarized in Table 1. The total prevalence of *B. cereus* and *B. thuringiensis* were found to be 73.04% and 24.3% , respectively. Raw rice samples (n = 25) were contaminated only with *B. cereus* and no contamination with *B. thuringiensis* was observed (Table 2 and Fig. 1).

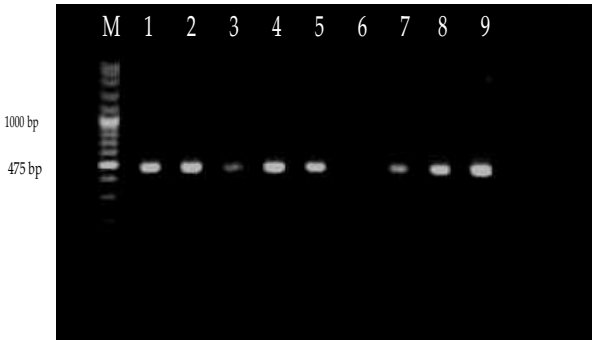

Figure 1. Representative amplification of the gyrB gene for the detection of *B. cereus* (475 bp) in raw rice. Lane M: 100 bp DNA ladder; Lane 1: positive control at 475 bp; Lane 2 – 5 and 7-9 : positive samples; Lane 6: negative control.

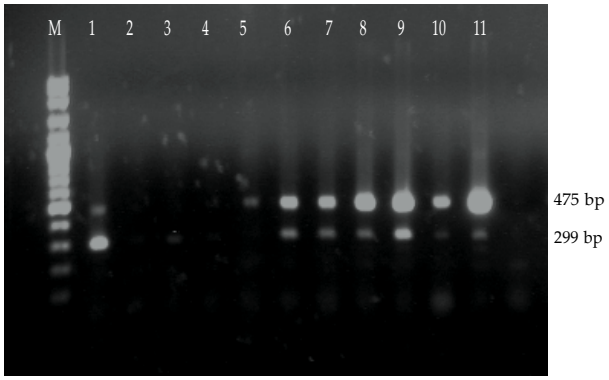

Figure 2. Multiplex PCR products for detection of *B. cereus* and *B. thuringiensis* in cooked rice. Lane M: 100 bp DNA ladder; Lane 1: positive control; Lane 2: negative control; Lane 3 - 11: positive samples.

MPN-PCR results revealed that *nasi ayam* was significantly different ($P < 0.05$) from others in term of *B. cereus* contamination. *Nasi ayam* had the highest contamination level (100%) with *B. cereus,* followed by *nasi putih* (76.2%), *nasi lemak* (70.4%) and *nasi briyani* (50%). Besides, nasi lemak was the most contaminated sample (35.2%) with *B. thuringiensis* followed by *nasi briyani* (30%), *nasi ayam* (10%) and *nasi putih* (10%).

Occurrence of *B. cereus* and *B. thuringiensis* in the samples are listed in Tables 3 and 4. Occurrence of *B. cereus* and *B. thuringiensis* in the samples ranged from < 3 to 1100 MPN/g in different samples. The minimum number of *B. cereus* in samples is 3 MPN/g for *nasi lemak* and *nasi ayam*. *B. cereus* contamination was found to be 3.6 MPN/gr for *nasi putih* and 9 MPN/g for *nasi briyani*. Minimum number of *B. thuringiensis* was found to be 3 MPN/g for *nasi lemak*; followed by 3.6, 9.2 and 19 MPN/g for *nasi ayam, nasi putih* and *nasi briyani*, respectively.

Maximum number of *B. cereus* was observed in *nasi lemak*, *nasi briyani* and *nasi putih* (> 1100 MPN/g) while *nasi ayam* showed less contamination (460 MPN/g) with *B. cereus* which was significantly different ($P < 0.05$) from others (Table 3). Maximum number for *B. thuringiensis* contamination varied in different cooked rice samples. The maximum number of *B. thuringiensis* in *nasi lemak, nasi briyani, nasi putih* and *nasi ayam* were found to be >1100, 93, 9.2 and 3.6 MPN/g, respectively (Table 4).

The minimum numbers of *B. cereus* in raw rice samples were 3, 3.6, 6 and 150 MPN/g for *keladi halus wangi, keladi wangi, kanowit halus wangi, lansam halus wangi* and *Bario,* respectively. The maximum numbers of *B. cereus* were found in *keladi halus wangi* and *lansam halus wangi* (>1100 MPN/g). *Bario, keladi wangi* and *kanowit halus wangi* were contaminated with *B. cereus* as follows: 1100, 53 and 13 MPN/g, respectively. All raw rice samples were negative for *B. thuringiensis* (Tables 3 and 4).

Discussion

As mentioned before, *B. cereus* and *B. thuringiensis* have similarity in genotype and phenotype (Oh *et al.*, 2011). Yamada *et. a.l*, (1999), detected *B. cereus* and *B. thuringiensis* using gyrase B (*gyrB*) gene in PCR assay. In this study the same gene was targeted for detection of *B. cereus* and *B. thuringiensis* and the results were in agreement with their findings. According to the results, nasi ayam showed the highest frequency of *B. cereus* (100%). *Nasi ayam* is steamed rice with chicken soup, added with some spices (such as ginger and garlic). The chicken soup and spices can contribute to the contamination of *nasi ayam* with *B. cereus*, resulting in high contamination level of *B. cereus* (Rusul and Yaacob, 1995; te Giffel *et al*., 1996).

Table 1. Percentage of *B. cereus* and *B. thuringiensis* in Cooked Rice Samples

Sample	Type of sample	n_t	PCR *Bacillus cereus*		PCR *Bacillus thuringiensis*	
			n_p	%	n_p	%
Cooked rice	*Nasi lemak*	54	38	70.4	19	35.2
	Nasi Briyani	20	10	50	6	30
	Nasi Ayam	20	20	100	2	10
	Nasi Putih	21	16	76.2	1	10

Table 2. Percentage of *B. cereus* and *B. thuringiensis* in Raw Rice Samples

Sample	Type of sample	n_t	PCR *Bacillus cereus*		PCR *Bacillus thuringiensis*	
			n_p	%	n_p	%
Raw rice	*Keladi halus wangi*	5	5	100	0	0
	Keladi wangi	5	5	100	0	0
	Kanowit halus wangi	5	5	100	0	0
	Lansam halus wangi	5	5	100	0	0
	Bario	5	5	100	0	0
Total		25	25	100	0	0

n_t= number of total sample , n_p= number of positive sample, %= percentage

Table 3. Occurence and enumeration of *B. cereus* in rice samples using MPN-PCR method

Sample	Type of sample	n_p	MPN/g value			Percentage MPN/g value		
			Min	Med	Max	$< 10^3$	10^3 - 10^4	$> 10^4$
Cooked rice	*Nasi Lemak*	54	3	155	> 1100 [a]	65.8	7.9	26.3
	Nasi Briyani	20	9	53	> 1100 [a]	60	0	40
	Nasi Ayam	20	3	11.1	460 [b]	100	0	0
	Nasi Putih	21	3.6	1100	> 1100 [a]	37.5	12.5	50
Raw Rice	*Keladi halus wangi*	5	3	53	> 1100 [a]	66.7	0	33.3
	Keladi wangi	5	3	16	53 [b]	100	0	0
	Kanowit halus wangi	5	3.6	7.2	13 [c]	100	0	0
	Lansam halus wangi	5	6	26	> 1100[a]	66.7	0	33.3
	Bario	5	150	210	1100 [a]	0	66.7	33.3

[a,b, c] Significant difference ($P < 0.05$) in terms of *B. cereus* contamination

Table 4. Occurence and enumeration of *B. thuringiensis* in rice samples using MPN-PCR method

Sample	Type of sample	n_p	MPN/g value			Percentage MPN/g value		
			Min	Med	Max	$< 10^3$	10^3 - 10^4	$> 10^4$
Cooked rice	*Nasi Lemak*	19	3	23	>1100 [a]	84.2	0	15.8
	Nasi Briyani	6	19	24	93 [b]	100	0	0
	Nasi Ayam	2	3.6	3.6	3.6 [c]	100	0	0
	Nasi Putih	1	9.2	9.2	9.2 [c]	100	0	0
Raw rice	*Keladi halus wangi*	0	< 3	< 3	< 3	100	0	0
	Keladi wangi	0	< 3	< 3	< 3	100	0	0
	Kanowit halus wangi	0	< 3	< 3	< 3	100	0	0
	Lansam halus wangi	0	< 3	< 3	< 3	100	0	0
	Bario	0	< 3	< 3	< 3	100	0	0

[a,b, c] Significant difference ($P < 0.05$) in terms of *B. thuringiensis* contamination

Besides, the *nasi ayam* that is served in restaurants, Chinese coffee shops, school canteen and food stalls is usually cooked in bulk. In Malaysia, *nasi ayam* is commonly served in open trays and is held at room temperature (28-30°C) for several hours. Such conditions allow the germination of *B. cereus* spores.

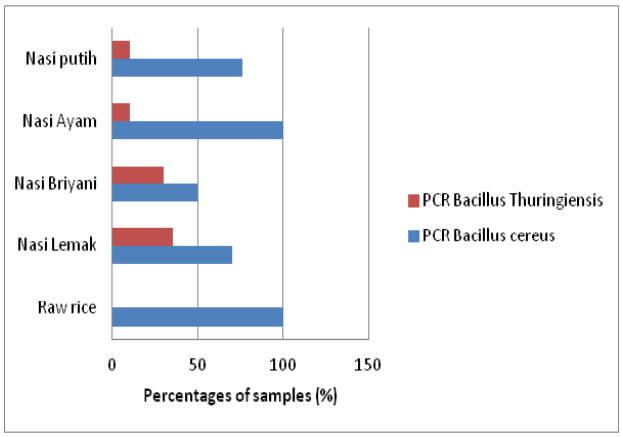

Figure 3. Percentage of *B. cereus* and *B. thuringiensis* in raw and cooked Rice samples.

Nasi lemak is boiled in coconut cream added with some spices. It is traditionally wrapped in banana leaves with cucumber slices, small fried anchovies (*ikan bilis*), roasted peanuts, hardboiled egg, and hot spicy sauce (*sambal*). All mentioned ingredients act as potential sources of *B. cereus* cross contamination. This idea is supported by Rosenquist *et al*. (2005), who found that cucumber is naturally contaminated with *B. cereus*.

Although *nasi putih* is plain rice (without any spices or additional ingredient), it was found to be contaminated with *B. cereus*. Contaminated raw rice which has not been washed /or improperly washed before cooking can result in contamination of cooked rice, as *B. cereus* spores can survive cooking temperature or heat treatment process.

As mentioned before, cooked rice is displayed and served at room temperature that allows the spores of *B. cereus* to grow (*Koo et al., 1998,* Todar, 2009*)*. In tropical countries, such as Malaysia, the scenario is more severe due to high temperature. Other sources for *Bacillus* contamination are dirty hands or cooking stuffs (e.g. knife), handling foods without gloves and flies/insects contamination (Singleton, 2004). Therefore it is recommended to educate food handlers about their responsibilities for food safety and train them on personal hygiene policies and basic practices for safe food handlings. The safe methods for ready-to-eat foods such as cooked rice are: 1) to keep the food at hot temperature (at least at 60°C) 2) or it can be cooked as quickly as possible and then keep at low temperature (< 5°C) for later use. Temperature range of 5 - 60°C will allow *B. cereus* to germinate and produce toxin.

Although 100% of *nasi ayam* samples were positively contaminated with *B. cereus,* none was contaminated more than 10^4 cells which is the unsatisfactory level according to Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods, 2009. According to Grande *et al*. (2006), not every contaminated food can cause food-borne illness as *B. cereus* can only produce toxin in desired conditions/or the bacteria may be found in low numbers not enough for causing symptoms.

It was found that 50% of *nasi putih* and 40% of *nasi briyani* contained more than10^4 cell of *B. cereus*. According to Oh and Cox (2010), *B. cereus* spores can survive boiling and cooking process. If raw rice is contaminated with high level of *B. cereus* spores, the spores will germinate and produce toxin after cooking and during storage at inappropriate temperature. Rice served for breakfast is normally cooked at dawn and displayed in open containers. In such a case the food may easily get contaminated and cause food-borne disease (Daanam *et al.,* 1999).

Results showed that the level of contamination with *B. thuringiensis* was $<10^3$ cell in cooked rice samples (satisfactory according to Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods, 2009). Koo et al. (1998) had a similar observation and stated that typically the bacterial spores exist in low numbers and remain inactive in food.

Ankolekar *et al*. (2009) found 46.6% of the rice samples to be positive for *B. cereus* with the range of 3.6 to 460 CFU/g and 6.1% positive for *B. thuringiensis* with level of 3.6 - 23 CFU/g. It is likely in contrast with present study as it was found that 100% of raw rice samples were positive for *B. cereus* but negative for *B. thuringiensis*. Although in both studies MPN method was used for isolation and enumeration, different methods were used for confirmation of the results. Ankolekar *et al*. (2009) used conventional method while current study used PCR assay.

In addition, it was found that 20% of raw rice samples were contaminated with *B. cereus* with a level of $>10^4$ cells. The finding is in agreement with observations of te-Giffel *et al*. (1996) (reported *B.cereus* in pasta) and Fang *et al*. (2003) (studied *B.cereus* in rice products and ready-to-eat foods). According to Guinebretiere *et al*., 2008, *B. cereus* has the growth temperature of 4°C to 50°C. Unfortunately

the temperature that ready-to-eat foods are usually kept (room temperature) supports the growth of bacteria. On the other hand, interactions between bacteria is an important factor.

All cooked rice samples were contaminated with *B. cereus*. The finding is supported by Oh and Cox, 2010, who reported that in rice-based foods *B. cereus* was the dominant bacterium. The reason is that spores of *B. cereus* have little or no competition with vegetative cells as vegetative cells are killed or suppressed by heating treatment and storage temperature (Granum, 2007). Growth and proliferation of *B. cereus* seemed to be greater than *B. thuringiensis* (Fig. 3) in most of the samples. Eilenberg *et al*. (2000) reported that *B. thuringiensis* is able to growth and proliferate in suitable condition, but multiplication of this bacterium is inhibited by the presence of other pathogens. Nowadays *B. thuringiensis* is recognized as bacteria with the ability to produce diarhoeal toxin, whereas in the past decades it was used as biocontrol agent due to its ability to kill insect. Therefore, it is important to make sure that when *B. thuringiensis* is used as a bioinsecticide it does not produce the diarrheal toxin (Oh *et al*., 2011).

Conclusion

Raw and cooked rice were found to be contaminated with *B. cereus*. The spores of this microorganism will not be destroyed by heat treatment (such as boiling or frying). Keeping foods at ambient temperature allows the spores to germinate and grow fast and subsequently cause food poisoning. Food poisoning generally occurs as a result of poor hygiene and/or food handling practice. As almost all samples were held at room temperature for several hours before consumption, *B. cereus* was detected in all samples. Hence, it is important to educate food handlers about their responsibilities for food safety and train them on personal hygiene policies and basic practices for safe food handlings.

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