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Prevalence and Molecular Detection of Toxigenic Genes of *Bacillus cereus* in Rice-Based Street Foods in Tasikmalaya, Indonesia

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Abstract

Bacillus cereus is a spore-forming foodborne pathogen frequently associated with rice-based foods and capable of causing emetic and diarrheal illnesses through the production of heat-stable and heat-labile toxins. This study investigated the prevalence, contamination levels, and toxigenic gene profiles of *B. cereus* in rice-based street foods. A total of 12 samples, including fried rice, chicken porridge, and *nasi kuning*, were collected from street vendors in Kahuripan Sub-district, Tasikmalaya, Indonesia. Presumptive *B. cereus* was isolated using mannitol yolk polymyxin agar (MYPA) as a selective medium and confirmed biochemically and molecularly by PCR targeting the *gyrB* gene. Toxigenic potential isolate was evaluated by detecting the *nheA*, *hblD*, *cytK*, *entFM*, and *ces* genes. Presumptive *B. cereus* was detected in 91.67% (11/12) of samples, while molecular confirmation identified *gyrB*-positive isolates in 42% (5/12) of samples, originating from fried rice and chicken porridge. No significant association was found between food type and *gyrB* detection ($p > 0.05$). Contamination levels ranged from $< 1.5 \times 10^1$ to 2.95×10^6 CFU g^{-1}. All confirmed isolates harbored at least one toxigenic gene, with *nheA* being the most prevalent (80%), followed by *cytK* (60%), *entFM* (40%), *ces* (40%), and *hblD* (20%). Notably, one of the fried rice isolates carried all 5 toxin-related genes, indicating a high virulence potential. These findings demonstrate that rice-based street foods in Tasikmalaya may pose a significant public health risk due to contamination with toxigenic *B. cereus*. Enhanced hygiene practices, strict temperature control, and molecular surveillance are essential to reduce the risk of foodborne illness associated with street-vended rice products.

Keywords: *Bacillus*; *gyrB*; molecular; rice; toxigenic

INTRODUCTION

Bacillus cereus is a Gram-positive, rod-shaped, motile, facultatively aerobic-anaerobic, spore-forming bacterium and is recognized as a potential cause of food poisoning (Marrollo, 2016). Rice is frequently contaminated with *B. cereus* spores due to practices involved in its cultivation, harvesting, and post-harvest handling, as this microorganism is commonly present in soil (Rodrigo et al., 2021). In China, ready-to-eat rice-based foods have been reported to be contaminated with *B. cereus* in 8.5% of cases (91 out of 1,071 samples) (Chen et al., 2022). In Indonesia, 5% of 120 sushi samples from retail outlets in the Greater Jakarta area (Jabodetabek) were reported to be contaminated with *B. cereus* (Yennie et al., 2022). Other rice-based dishes that have also been

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reported to contain *B. cereus* include fried rice (Tirloni et al., 2019), cooked rice (Albaridi, 2022), *nasi kuning* (Arta, 2016), and *nasi uduk* made from both organic and non-organic rice (Permatasari, 2016).

The presence of *B. cereus* in food products has been widely reported to cause adverse health effects in consumers of contaminated foods. It is recognized as the second most common cause of foodborne outbreaks in Indonesia after *Escherichia coli*, accounting for 19.4% of cases between 2000 and 2015 (Arisanti et al., 2018). In 2020 alone, foodborne outbreaks caused by *B. cereus* constituted 26.67% of reported cases (Apriliansyah et al., 2022).

Contamination of food by *B. cereus* is associated with 2 types of gastrointestinal illnesses, namely emetic syndrome and diarrhea. Emetic syndrome is a food intoxication characterized by nausea and vomiting within 6 to 24 hours, typically occurring around 1.5 hours after consuming the contaminated food (Dietrich et al., 2021). Cereulide, the emetic toxin, is known to be highly resistant to heat, acid, and digestive enzymes, making it difficult to remove or inactivate (Rajkovic et al., 2008). Another illness caused by *B. cereus* is diarrhea, which occurs 8 to 16 hours after ingesting the contaminated food. Diarrheal illness is classified as a toxico-infection, in which enterotoxins are produced by viable bacteria within the human intestine; these toxins are generally sensitive to heat, acid, and proteases (McDowell et al., 2023).

In Tasikmalaya City, consumption of rice-based products such as chicken porridge and fried rice is relatively high, at 0.770 and 0.251 servings per capita per week, respectively (Statistics Indonesia, 2025). Meanwhile, another rice-based product, namely *nasi kuning*, was reported to have caused food poisoning in 2 different areas of Tasikmalaya Regency in 2020 and 2023 (Kristiadi, 2020; Fitrian, 2023). Furthermore, the detection of toxigenic genes provides essential information on the potential health risks caused by *B. cereus* contamination in rice-based products from Tasikmalaya, which may lead to emetic or gastrointestinal disorders, or both. However, information regarding the prevalence and toxigenic genes of *B. cereus* in rice-based food samples in Indonesia, particularly in Tasikmalaya, is still limited, indicating the necessity for further investigation. This study aimed to investigate the prevalence and toxigenic gene potential of *B. cereus* in rice-based street foods from Kahuripan Sub-district, Tasikmalaya, Indonesia.

MATERIALS AND METHOD

Study design

Bacterial isolation was performed using mannitol yolk polymyxin agar (MYPA) as a selective medium. Presumptive *B. cereus* isolates were initially confirmed through biochemical tests, including catalase and motility assays. Molecular confirmation was subsequently carried out by PCR amplification of the *gyrB* gene following DNA extraction. Fisher's exact test was applied to assess the association between food type and the detection of the *gyrB* gene. Isolates confirmed as *B. cereus* (*gyrB*-positive) were further analyzed for 5 major toxigenic genes (*nheA*, *hblD*, *cytK*, *entFM*, and *ces*) associated with foodborne illness.

Sample collection

A total of 12 rice-based food samples were randomly collected from street food vendors located in Kahuripan Sub-district, Tasikmalaya, Indonesia. The samples included fried rice (n = 4), chicken porridge (n = 4), and *nasi kuning* (n = 4). Each sample was aseptically placed in sterile plastic packaging, stored in a cool box at approximately 4 °C, transported to the laboratory, and analyzed within 24 hours in accordance with ISO 6887-1:2017 guidelines.

Isolation and identification of presumptive *B. cereus*

Isolation was conducted following the Bacteriological Analytical Manual (BAM, 2020). Briefly, 25 g of the sample was homogenized with 225 ml of Butterfield's Phosphate Buffer (1:9). A 0.1 ml aliquot was inoculated onto MYPA and incubated at 37 °C for 24 hours. Presumptive colonies were subcultured on nutrient agar slants for further analysis. Biochemical identification included catalase testing using 3% H_2O_2 and motility testing on a semi-solid medium. *B. cereus* ATCC 14579 was used as a positive control.

Molecular confirmation and toxigenic gene detection

Genomic DNA was extracted using the Presto™ Mini gDNA Bacteria Kit. DNA purity was assessed spectrophotometrically (A260/280 = 1.8–2.0). PCR amplification was performed using GoTaq® Green Master Mix under conditions specified in Table 1. The *gyrB* primer was used as

Table 1. The primer sequences and PCR conditions of various target genes

Gene	Primer	Sequence	PCR's product	PCR's condition	Ref
gyrB	gyrB-F gyrB-R	TCATGAAGAGCCTG TGTACG CGACGTGTCAATTC ACGCGC	475 bp	94 °C, 180 sec; 35 cycles (94 °C, 45 sec; 63 °C, 60 sec; 72 °C, 60 sec); 72 °C, 420 sec	Sandra et al. (2012)
ces	ces-F1 ces-R2	TTC CGC TCT CAA TAA ATGGG TCA CAG CAC ATT CCA AATGC	634 bp	95 °C, 180 sec; 35 cycles (94 °C, 60 sec; 52 °C, 60 sec; 72 °C, 60 sec); 72 °C, 300 sec	Kim et al. (2012)
nheA	344S 843A	TAC GCT AAG GAG GGG CA GTT TTT ATT GCT TCA TCG GCT	499 bp	95 °C, 180 sec; 30 cycles (94 °C, 30 sec; 54 °C, 45 sec; 72 °C, 60 sec); 72 °C, 300 sec	Granum et al. (1999)
hblD	hblD-F hblD-R	GTT AGA TAC AGC GAA GCCAC CCG CCA GTT ACA ACAATA	465 bp	95 °C, 600 sec; 35 cycles (95 °C, 30 sec; 52 °C, 30 sec; 72 °C, 30 sec); 72 °C, 600 sec	Zhang et al. (2016)
cytK	cytK-F cytK-R	ATC GGT CAA AAT GCA AAA ACAC ACC CAG TTA CCA GTT CCGAATG	800 bp	95 °C, 600 sec; 35 cycles (95 °C, 30 sec; 52 °C, 30 sec; 72 °C, 30 sec); 72 °C, 600 sec	Zhang et al. (2016)
entFM	entFM-A entFM-B	ATGAAAAAAGTAAT TTGCAGG TTAGTATGCTTTTGT GTAACC	1,269 bp	95 °C, 180 sec; 35 cycles (94 °C, 30 sec; 58 °C, 45 sec; 72 °C, 90 sec); 72 °C, 600 sec	Asano et al. (1997)

a specific primer for detecting the *B. cereus* group, related to its role as a housekeeping gene. The toxigenic genes in *B. cereus* were divided into genes encoding emetic toxins (*ces*) and genes encoding enterotoxins (*nheA, hblD, cytK*, and *entFM*). Amplicons were visualized on a 2% agarose gel following electrophoresis at 85 V for 45 minutes.

Data analysis

Data for the identification and detection of toxigenic genes of *B. cereus* were processed using Microsoft Excel and analyzed using Fisher's exact test.

RESULTS AND DISCUSSION

Prevalence and enumeration of *B. cereus* in rice-based food samples

A total of 11/12 food samples (91.67%), namely 3/4 samples of *nasi kuning* (75%), 4/4 samples of fried rice (100%), and 4/4 samples of chicken porridge (100%), showed presumptive *B. cereus* with their pink colonies appearance and surrounded by a precipitation zone on MYPA (Figure 1). Presumptive *B. cereus* was further identified through biochemical characterization, in which all isolates were positive for catalase. Meanwhile, the results of molecular identification through detection of the *gyrB* gene against all the presumptive *B. cereus* showed that 5/12 (42%) isolates were positive from 5 food samples, including 2 fried rice and 3 chicken porridge (Figure 2). Fisher's exact test indicated that the association between food type and *gyrB* detection was not statistically significant ($p > 0.05$). Furthermore, the contamination levels of *B. cereus* in chicken porridge and fried rice were 8.4×10^2 to 2.95×10^6 CFU g^{-1} and $< 1.5 \times 10^1$ to 3.7×10^5 CFU g^{-1}, respectively (Table 2).

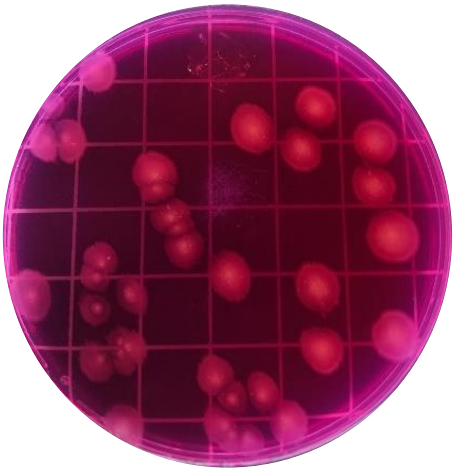

Figure 1. Appearance of presumptive *B. cereus* colonies on MYPA medium

MYPA medium is an ISO-standard and conventional medium used to detect and enumerate presumptive *B. cereus*. Nonetheless, the results of this study are in line with those reported by Fuchs et al. (2022), who found that several non-target organisms were not effectively suppressed by polymyxin B. This is supported by Kabir et al. (2017), who stated that Brilliance medium is better than MYPA. Talahmeh et al. (2020) added that starch-blood-egg yolk-polymyxin B-trimethoprim-ceftazidime (SBYPTC) showed greater selectivity and made differentiation easier by allowing *B. cereus* to grow in separate colonies and inhibiting competing microflora.

Previous investigations have documented the presence of *B. cereus* in various rice-based foods. Saba et al. (2019) reported various prevalence rates of *B. cereus*, namely 60.0% (18/30) in fried rice, 26.7% (8/30) in Waakye, and 23.3% (7/30) in Jollof rice, with bacterial loads exceeding 10^5 CFU ml^{-1}. Similarly, Oyetibo et al. (2022) detected *B. cereus* in 10.6% (5/7) of Ofada rice samples, indicating widespread contamination across different rice preparations.

The *gyrB* gene is one of the existing biomarkers with a high specificity of 96% for analyzing the total members of the *B. cereus* group (Wei et al., 2018). Non-member groups of *B. cereus* (*B. amyloliquefaciens*, *B. subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes*, *Micrococcus luteus*, *Salmonella enterica*, *Staphylococcus aureus*, *Streptococcus pyogenes*) were not amplified through the detection of this target gene (Cruz-Facundo et al., 2023). The *gyrB* gene encodes the B subunit of bacterial DNA gyrase (topoisomerase II), which plays a crucial role in DNA replication, transcription, and recombination by introducing negative supercoils and maintaining DNA topology. It evolves more rapidly than *16S rDNA*, providing higher phylogenetic resolution (Izumi and Suzuki, 2016).

Chicken porridge in Tasikmalaya is commonly served with shredded chicken meat, white pepper, leeks, crackers, and fried soybeans. The presence of *B. cereus* in this dish may result from contamination of raw ingredients such as rice and spices, as its spores are widely found in soil and can survive typical cooking temperatures (Woh and Ng, 2024). Cross-contamination from utensils, cutting boards, or food handlers during preparation can further introduce the bacteria (Komprda et al., 2025). Moreover, improper storage practices, including leaving cooked porridge at room temperature for prolonged periods, allow spores to germinate and produce heat-stable toxins (Navaneethan and Effarizah, 2023; Oliveira et al., 2023).

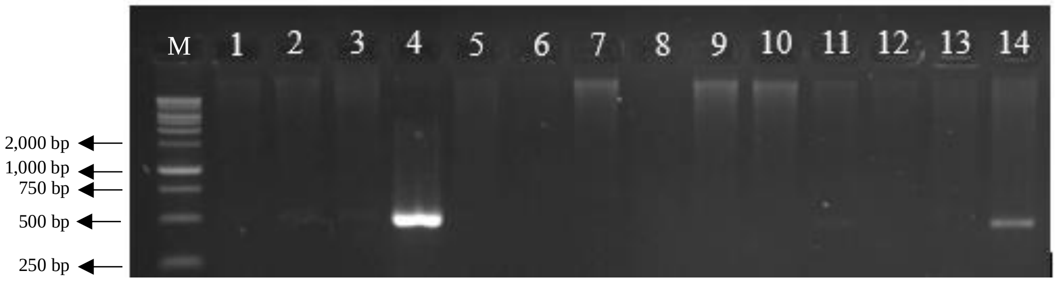

Figure 2. Detection of the *gyrB* gene (475 bp) in various isolates

Note: M = marker; 1 = Fried rice-1a; 2 = Fried rice-1b; 3 = Fried rice-2; 4 = Fried rice-3; 5 = Fried rice-4; 6 = *Nasi kuning*-1; 7 = *Nasi kuning*-3a; 8 = *Nasi kuning*-3b; 9 = *Nasi kuning*-4a; 10 = *Nasi kuning*-4b; 11 = Chicken porridge-1; 12 = Chicken porridge-2; 13 = Chicken porridge-3; 14 = Chicken porridge-4

Table 2. Biochemical characterization, molecular confirmation, and *B. cereus* contamination level in sample(s)

No.	Sample	Isolate code	Biochemical characterization		Molecular confirmation	Contamination level (CFU g^{-1})
			Catalase	Motility	*gyrB* gene	
1	*Nasi kuning*	NSK1	+	+	-	-
2		NSK2	+	+	-	-
3		NSK3	+	+	-	-
4		NSK4	+	+	-	-
5	Fried rice	NSG1	+	+	+	$< 1.5 \times 10^1$
6		NSG2	+	+	-	-
7		NSG3	+	+	+	3.7×10^5
8		NSG4	+	+	-	-
9	Chicken porridge	BUA1	+	+	+	1.92×10^6
10		BUA2	+	+	-	-
11		BUA3	+	+	+	8.4×10^2
12		BUA4	+	+	+	2.95×10^6

Meanwhile, *B. cereus* contamination in fried rice commonly occurs because its spores can withstand cooking temperatures and later germinate if the rice is kept at unsafe temperatures. These spores, which naturally exist in soil and raw grains, may survive boiling and multiply rapidly when cooked rice is stored for prolonged periods at room temperature (Woh and Ng, 2024; Martín-Miguélez et al., 2025). In addition, slow cooling or bulk storage provides ideal conditions for bacterial growth and toxin production, particularly the heat-stable cereulide toxin (Bursová et al., 2024; Mohammadi et al., 2024).

Temperatures ranging from 30 to 37 °C are optimal conditions for both *B. cereus* proliferation and cereulide synthesis. Under these storage temperatures, *B. cereus* populations in cooked rice were reported to increase from approximately 2 to 8 log CFU ml^{-1} within 10 hours, while emetic strains produced the highest concentrations of cereulide, reaching levels of about 3 log ng g^{-1} (Ellouze et al., 2021; da Silva et al., 2022). Furthermore, *B. cereus* detected in fried rice could originate from its toppings, namely chicken (20.0%), vegetables (18.0%), seafood (10.0%), eggs (5.0%), beef (1.0%), spices (28.8%), cooked poultry (32.7%), and fresh-cut vegetables (5.0%) (Perera and Ranasinghe, 2012; Gdoura-Ben Amor et al., 2018). Hence, to prevent microbial growth and toxin formation, staff involved in food production and management must be given appropriate training (Osimani et al., 2018).

Detection of *B. cereus* toxigenic genes

All confirmed *B. cereus* isolates (n = 5) were found to carry at least one toxigenic gene (Table 3). The most frequently detected gene was *nheA* (4/5; 80%), followed by *cytK* (3/5; 60%), *entFM* (2/5; 40%), *ces* (2/5; 40%), and *hblD* (1/5; 20%). Notably, one isolate obtained from fried rice contained all 5 genes, indicating a high toxigenic potential. Other studies have reported a high prevalence of *B. cereus* toxin genes in rice-based and ready-to-eat foods across multiple regions. For example, all rice-based food samples marketed in southern Mexico were positive for the non-hemolytic enterotoxin gene (*nhe*) (Reyes-Roldán et al., 2024). Predominant contamination involved *nheA* (77.9%), followed by *hblA* (64.8%), *ces* (23.2%), and *cytK-1* gene (4.4%) (Jovanovic et al., 2022), while in Poland, 23.4% of ready-to-eat products carried both *nhe* and *cytK*, and 28.6% harbored *nhe* alone.

The predominance of *nheA* is consistent with previous global studies that identify the Nhe complex as the primary enterotoxin responsible for diarrheal symptoms in *B. cereus* infections (Didier et al., 2016; Meng et al., 2025). The simultaneous detection of *cytK* and *entFM* in several isolates further reinforces the pathogenic concern, as both genes are linked to cytotoxicity and epithelial cell damage in the intestinal tract (Fagerlund et al., 2004; Sánchez-Chica et al., 2021). Additionally, the detection of the *ces* gene in 40% of the isolates suggests a notable risk of emetic-type food poisoning, which is frequently

Table 3. Detection results of toxigenic genes in various samples

Sample	Toxigenic gene(s)				
	entFM	*hblD*	*cytK*	*nheA*	*ces*
Fried rice-1	✓	-	✓	✓	-
Fried rice-3	✓	✓	✓	✓	✓
Chicken porridge-1	-	-	✓	-	-
Chicken porridge-3	-	-	-	-	✓
Chicken porridge-4	-	-	✓	✓	-

associated with fried rice and other starch-rich foods (Tuipulotu et al., 2021).

The coexistence of multiple toxin genes within individual isolates is particularly alarming. The isolate harboring all diarrheal and emetic determinants represents a highly virulent profile that is rarely observed in routine food monitoring. Comparable multi-virulent strains have been reported in severe foodborne outbreaks across Asia and Europe (Sastalla et al., 2013; Osimani et al., 2018). Furthermore, the present study demonstrated that specific samples of fried rice and chicken porridge possessed the potential to induce both emetic and diarrheal symptoms, whereas others were associated solely with diarrheal manifestations. These findings underscore the overlooked public health threat of *B. cereus* contamination in Indonesian street food and highlight the urgent need for strengthened molecular surveillance, improved hygiene practices, and targeted food safety education for street vendors to reduce outbreak risks.

CONCLUSIONS

Detection of the *gyrB* gene confirmed the presence of the *B. cereus* group, while bacterial counts in fried rice and chicken porridge reached levels of food safety concern. The widespread occurrence of toxigenic genes, particularly *nheA*, *cytK*, and *ces*, highlights the potential risk of diarrheal and emetic foodborne illness. These results emphasize the need for strict temperature control, hygienic handling, and proper storage of rice-based foods. Further molecular and epidemiological studies are recommended to support effective food safety strategies in Indonesia.

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