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ORIGINAL ARTICLE

Bacillus cereus in Samples of Pre-cooked and Cooked Rice for Sale to the Public in Colima State, Mexico

Bacillus cereus en muestras de arroz pre-cocido y cocido para su venta al público en Colima, México

Alberto Alvarado-Zárate[a], Francisco Vázquez-Roa[a], Daniela Briseño-Lora[a] and Víctor Manuel Cerda-Godínez[a*]

[a] *Instituto Tecnológico de Colima, Av. Tecnológico 1 A.P. 10 y 128, Col. Villa de Álvarez, Código Postal 28976, Villa de Álvarez, Colima, México.*

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[*] *Corresponding author:*
Víctor Manuel Cerda-Godínez
Phone: +52 (312) 312 6393
Electronic mail address:
victorcerda@colima.tecnm.mx

A B S T R A C T

Bacillus cereus is recognized as a pathogen that causes food poisoning. There have been outbreaks associated with the consumption of rice contaminated with this bacterium. It is one of the few pathogens with the ability to spoil food. Outbreaks of diarrheal and emetic syndromes caused by this organism have been widely reported in Mexico, Japan, the United States, the Netherlands, Thailand, Africa, South Korea, the United Kingdom, Belgium, and Finland. In Mexico, there are no data available to evaluate the epidemiological importance of *B. cereus* as a causative agent of enteric diseases, although its possible presence in parboiled and cooked rice could represent a health risk. Thus, the objective of this work was to identify the presence of *B. cereus* in parboiled and cooked rice for sale to the public in Colima, Mexico. The health quality, the quantity of *B. cereus* present and the identification of strains of the *hblA* and *ces* genes, which are involved in diarrheal and emetic syndrome, respectively, were determined. 155 samples of cooked and 32 precooked rice were collected from different establishments in the city of Colima (homes, fixed and street restaurants, inns and supermarkets). Sanitary quality was evaluated by observing the counts of aerobic mesophilic bacteria and total coliform bacteria, which were carried out following the current official methodology. The B. cereus count was performed on MYP agar (mannitol, yolk egg and polymyxin). Finally, the identification of diarrheal or emetic *B. cereus* strains was by amplification of the *hblA* and *ces* genes by PCR. In 87.5 % of the parboiled rice samples and in 23.2 % of the cooked rice samples, high counts of aerobic mesophilic bacteria were obtained (> 10^5 CFU / g), and in 71 % of the total samples analyzed it was demonstrated the presence of viable total coliform bacteria. In 28.1 % of the parboiled rice samples and in 89 % of the cooked rice samples, *B. cereus* was recovered. The *hblA* gene (encoding one of the three BL hemolysin subunits) was amplified in 86 % of *B. cereus* isolates, however, no strains carrying the *ces* gene (encoding cereulide) were identified. All samples of the cooked or parboiled rice samples containing vegetables (corn, potato, green bean, carrot and pea) showed the presence of *B. cereus*, so these ingredients may be related to the presence of the pathogen in cooked rice or precooked. Even in figures far below those considered as a health risk, if it is stored under conditions of temperature abuse, as is often the case in the sampled sites, contaminated rice can represent a risk to the health of the consumer.

Keywords: *Bacillus cereus*, cooked rice, parboiled rice, foodborne illness, food poisoning, diarrheal syndrome, emetic disease.

RESUMEN

Bacillus cereus es reconocido como un agente patógeno causante de intoxicaciones alimentarias. Se han presentado brotes asociados al consumo de arroz contaminado con esta bacteria. Es uno de los pocos patógenos con capacidad de deteriorar los alimentos. Se han informado ampliamente brotes de síndromes diarreicos y eméticos causados por este organismo en México, Japón, Estados Unidos, Países Bajos, Tailandia, África, Corea del Sur, Reino Unido, Bélgica y Finlandia. En México, no existen datos disponibles para evaluar la importancia epidemiológica de *B. cereus* como agente causante de enfermedades entéricas, aunque su posible presencia en arroz precocido y cocido podría representar un riesgo para la salud. Así, el objetivo de este trabajo fue identificar la presencia de *B. cereus* en arroz precocido y cocido para venta al público en Colima, México. Se determinó la calidad sanitaria, la cantidad de *B. cereus* presentes y la identificación de cepas los genes *hblA* y *ces*, que están involucrados en el síndrome diarreico y emético, respectivamente. Se recolectaron de 155 muestras de arroz cocido y 32 precocido de diferentes establecimientos de la ciudad de Colima (hogares, restaurantes fijos y ambulantes, fondas y supermercados). La calidad sanitaria se evaluó observando los recuentos de bacterias mesofílicas aerobias y bacterias coliformes totales, que se realizaron siguiendo la metodología oficial vigente. El recuento de *B. cereus* se realizó en el agar MYP (manitol, yema de huevo y polimixina). Finalmente, la identificación de las cepas de *B. cereus* diarrogénicas o eméticas fue mediante amplificación de los genes *hblA* y *ces* por la técnica de reacción en cadena de la polimerasa. En el 87.5 % de las muestras de arroz precocido y en el 23.2 % de las muestras de arroz cocido se obtuvieron recuentos altos de bacterias mesofílicas aerobias ($>10^5$ UFC/g), y en el 71 % del total de las muestras analizadas se demostró la presencia de bacterias coliformes totales viables. En el 28.1 % de la muestras de arroz precocido y en el 89 % de las muestras de arroz cocido se recuperó *B. cereus*. El gen *hblA* (que codifica una de las tres subunidades de hemolisina BL) se amplificó en el 86 % de *B. cereus* aislados, sin embargo, no se identificaron cepas portadoras del gen *ces* (que codifica la cereulida). Todas las muestras de arroz cocido o precocido que contenían verduras (elote, papa, ejote, zanahoria y chícharo) mostraron presencia de *B. cereus,* por lo que estos ingredientes pueden estar relacionados con la presencia del patógeno en el arroz cocido o precocido. Aun en cifras muy por debajo de las consideradas como un riesgo para la salud, si se almacena en condiciones de abuso de temperatura, como suele ocurrir en los sitios muestreados el arroz contaminado puede representar un riesgo a la salud del consumidor.

Palabras clave: *Bacillus cereus*, arroz cocido, arroz precocido, enfermedad transmisible por alimentos, intoxicación alimentaria, síndrome diarreico, enfermedad emética.

INTRODUCTION

Bacillus cereus is one of the microorganisms involved; it can cause intestinal and extra-intestinal symptoms. Among the intestinal alterations, food poisoning is characterized by diarrhea (due to various enterotoxins, such as nonhemolytic enterotoxin, the hemolysin BL [HBL] complex, cytotoxin K, enterotoxin T, and enterotoxin FM (1, 2) and/or emetic (due to cereulide) syndromes (3, 2). The number of *B. cereus* cells capable of causing disease has been estimated to range between 10^4 and 10^8 cells per g of food (4). Outbreaks of diarrheal and emetic syndromes caused by this organism have been widely reported in Mexico (5), Japan (6), the United States (7), The Netherlands (6), Thailand (8), Africa (9), South Korea (10), the United Kingdom (11, 12), Belgium (13), and Finland (14). Outbreaks caused by *B. cereus* have usually been associated with rice or other grains and vegetables that contain starch in their composition (15). About 95% of emetic-type food poisoning is due to the consumption of rice, macaroni, cheese, and vanilla pudding (16, 17, 2), whereas outbreaks of the diarrheal type are most often related to spices, fruits, nuts, grains, dairy products, soups, and dry foods, although vegetables have also been involved (18, 19). The increase in the consumption of foods with a mild thermal treatment or that are minimally processed and refrigerated has favored the increase of spore-forming pathogenic bacteria, including some strains of psychrotrophic *B. cereus* (12, 20). Similarly, pasteurization of the final container eliminates the risks arising from vegetative pathogenic bacteria but is insufficient for the removal of spores, including those of *B. cereus* (13). In Mexico, there are no data available to assess the epidemiologic importance of *B. cereus* as the causative agent of enteric diseases, even though its possible presence in pre-cooked and cooked rice could represent a health risk.

Purpose of the study

The objective of this work was to determine the sanitary quality and the presence of *Bacillus cereus* strains that carry the *hblA* and *ces* gene, which are involved in diarrhea and emetic syndrome, respectively, in parboiled and cooked rice for sale to the public in Colima. Mexico.

MATERIALS AND METHODS

Sampling

155 samples of cooked rice and 32 precooked rice were collected from different place in the state of Colima, Mexico (table 1). The total samples were completed on

four sampling dates in a period from March to September of the year 2019.

Cooked rice was defined as food ready to eat, in its version with or without added vegetables (corn, potato, green bean, carrot and pea), "white" or "red (with tomato puree)". And, pre-cooked or parboiled rice was defined as the preparation that is used as an ingredient to prepare cooked rice or other foods, usually requiring a terminal heat treatment that is usually sublethal on *B. cereus* spores.

Only three samples of parboiled rice contained added vegetables.

Table 1. Distribution of samples by type and date of sampling

Tabla 1. Distribución de muestras por tipo y fecha de muestreo

	Sampling date				
Rice type	**March**	**May**	**July**	**September**	
- Parboiled rice without vegetables	5	6	7	11	**32**
- Parboiled rice added with vegetables	0	1	0	2	
- Cooked rice without vegetables	2	5	11	8	**155**
- Cooked rice added with vegetables	18	28	48	35	

The sample consisted of collecting approximately 100 g of rice, which was deposited in sterile Whirl-Pak™, Nasco™ bags and transported in a cooler (at 4-8 ºC) to the laboratory for analysis in a period not exceeding 2 hours.

Determination of sanitary quality

Sanitary quality was evaluated by observing the counts of aerobic mesophilic bacteria and total coliform bacteria, which were carried out following the official methodology (NOM-092-SSA1-1994: Method for aerobic bacteria count in plate; and NOM-112-SSA1-1994: Determination of Coliform Bacteria).

With base to this, 10 g of the sample to be analyzed in a sterile plastic bag. Was added a 90 mL volume of peptone diluent (Becton™ Dickinson de México) brought to a temperature similar to the sample. I know mixed in Stomacher 400 (Lab System™) for 1 min at normal speed, until obtaining a complete suspension and homogeneous.

The large particles settle, and the amount was transferred desired by taking from the layers upper suspension. They were made from the dilutions corresponding decimals, shaking in a Vortex™ (Scientific™ Inc.).

***B. cereus* count**

The count of *B. cereus* was carried out on MYP agar (mannitol, yolk egg and polymyxin) (Becton™, Dickinson™) by technique surface extension and the plates were incubated at 30 °C /24-48 h (Incubator Termolab™), agree as suggested by the FDA (2012) (21).

Identification of *hblA* and *ces* genes in *B. cereus* strains by PCR

Genomic DNA was obtained by using the Wizard Genomic DNA purification kit (Promega™), following the manufacturer's specifications. For PCR, a reaction mixture was prepared with 14.8 µl of sterile distilled water, 2.5 µl of 10x buffer (supplied with Taq polymerase), 1.3 µl of 50 mM $MgCl_2$, 0.2 µl of deoxynucleoside triphosphates (10 mM) (Invitrogen™), 2 µl of each primer, 1 U of Taq DNA polymerase (Invitrogen™), and 2 µl of a solution containing about 100 ng of DNA. Amplifications were performed on a MultiGene gradient thermal cycler, using primers for the *hblA* (9) and *ces* (22) genes.

For *hblA* amplification, the following conditions were used: 5 min at 94 ºC, 30 cycles of 45 s at 94 ºC, 45 s at 51 ºC, and 1 min at 72 ºC, followed by one cycle of 5 min at 72 ºC (25).

For *ces* amplification, the following conditions were used: 5 min at 94 ºC, 30 cycles of 45 s at 94 ºC, 45 s at 55 ºC, and 45 s at 72 ºC, followed by one cycle of 5 min at 72 ºC (25). A total of 6 µl of PCR product was then loaded onto an agarose gel containing ethidium bromide and visualized under UV light. DNA from the reference strains was used as positive controls; *B. cereus* ATCC

14579 was used for the *hblA* gene, and for the *ces* gene, the NCTC 11143 strain was used.

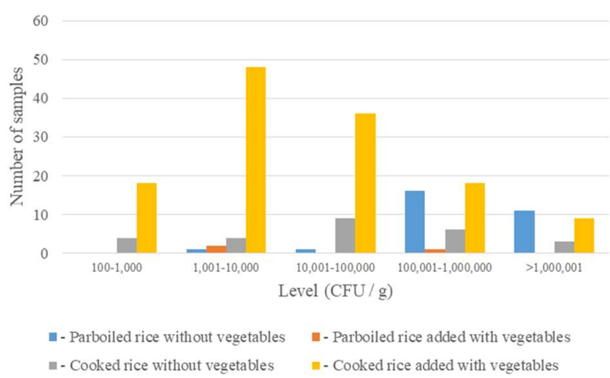
RESULTS AND DISCUSSION

The presence of a high number of mesophilic aerobic bacteria that grow well at or near body temperature means that conditions may have been favorable for the multiplication of pathogenic microorganisms of human or animal origin (23).

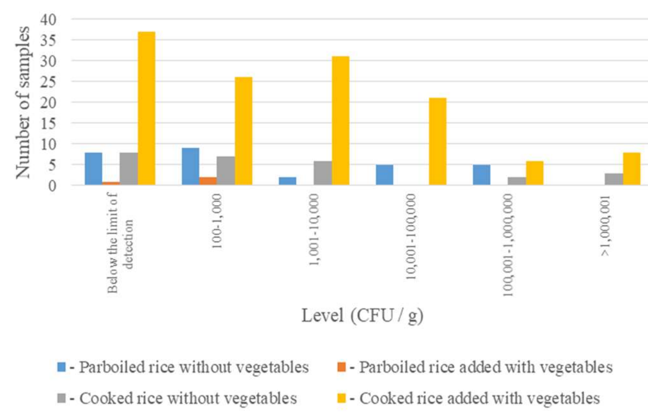
Coliform bacteria are frequently used as a bacterial indicator of the sanitary quality of food and water (24). They are a group of bacteria closely related to soil (sowing), water and the intestinal tract of animals, they have been used as indicators of unsanitary conditions in the production of food and beverages for more than a century. Today, the coliform count is a frequent hygienic indicator in various food and beverage industries. The best-known Coliform, *Escherichia coli*, is a common resident of the intestines of warm-blooded animals, but it can also be found in the natural environment and transmitted to food manufacturing facilities, as well as sources of drinking water.

Rather than looking for a specific species, the coliform test offers a broader view of the organisms in ingredients, finished products, and the overall production environment. In this way, coliforms serve as "indicator organisms", and evidence for their presence in general rather than an individual genus or species may mean that unsanitary conditions exist that can harbor pathogenic organisms. If a sample area is under control, the number of these indicator organisms would be contained in relatively small amounts.

After analyzing the rice samples, it was observed that in 87.5 % of the parboiled rice samples and in 23.2 % of the cooked rice samples, high counts of aerobic mesophilic bacteria were obtained (> 10^5 CFU / g), and in 71 % of the total samples analyzed it was demonstrated the presence of viable total coliform bacteria (figure 1.).



a



b

Figure 1. Distribution of mesophilic aerobic bacteria (a) and coliform bacteria (b) in parboiled and cooked rice
Figura 1. Distribución de bacterias mesófilas aerobias (a) y bacterias coliformes (b) en arroz precocido y cocido

In 28.1 % of the parboiled rice samples and in 89 % of the cooked rice samples, *B. cereus* was recovered (table 2).

Table 2. Frequency* of *B. cereus* positivity in cooked or parboiled rice samples
Tabla 2. Frecuencia* de positividad de *B. cereus* en muestras de arroz cocido o precocido

Rice type	*B. cereus*	
	Negative**	**Positive**
- Parboiled rice without vegetables	23	6
- Parboiled rice added with vegetables	0	3
- Cooked rice without vegetables	17	9
- Cooked rice added with vegetables	0	129

* number of samples; ** Not detected

All samples of the cooked or parboiled rice samples containing vegetables (corn, potato, green bean, carrot and pea) showed the presence of *B. cereus*, so these ingredients may be related to the presence of the pathogen in cooked rice or precooked.

Flores-Urban et al. (2014) reported that *B. cereus* was identified in 57% of the 100 analyzed samples of different vegetables (32% of the broccoli samples, 44% of the carrot samples, 84% of the lettuce samples and 68% of coriander samples). Contamination in these vegetables can be present from their origin or during their processing (peeling and / or cutting). Also the process of pregnant in plastic containers under non-sterile conditions could favor the presence of *B. cereus* in the food. In addition to the fact that changes in eating patterns have been adopted, such as the preference for minimally processed fresh foods and increased prevalence of food prepared outside the home, have contributed to the increased incidence of foodborne illness (26).

At least one strain of *B. cereus* was recovered for each positive sample. 162 strains were evaluated by PCR to determine the *hblA* and *ces* genes. The *hblA* gene (encoding one of the three BL hemolysin subunits) was amplified in 86 % of *B. cereus* isolates, however, no strains carrying the *ces* gene (encoding cereulide) were identified.

CONCLUSION

The inclusion of vegetables as ingredients in the preparation of ready-to-eat or parboiled rice favors the presence of *B. cereus*, which is why it is recommended to improve food preparation practices and if dispensed at the outlet. Because even in figures far below those considered as a health risk, if it is stored in conditions of temperature abuse, as is often the case in the sampled sites, contaminated rice can represent a risk to the health of the consumer.

Conflict of interest:
The authors declare that they have no conflict of interest.

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