Bacillus cereus sensu lato

Subjects: Microbiology

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The potential presence of spore-forming bacteria related to the Bacillus cereus group in Mexican chili powder elaborated from Capsicum annuum L. is of commercial and clinical interest, because chili powder is an essential spice in the Mexican diet and in diets around the globe.

Keywords: Bacillus cereus sensu lato ; tRNACys-PCR ; Chili powder

1. Introduction

Within the Solanaceae family, pepper (*Capsicum annuum* L.) is one of the most economical and agriculturally important plants cultivated all over the world [1][2]. After China, with 17.5 million tons, Mexico is the second largest producer of fresh pepper with 2.7 million tons at 150,000 hectares cultivated annually [2]. Its importance is based on its nutritional content, diverse bioactive compounds, pungency, aroma, and health benefits for the consumers [1][3]. Additionally, diverse genetic lines of *Capsicum annuum* L. have been developed to produce carotenoids with high commercial value in powdered form as spices or as colorants on agro-food, cosmetics, and products from pharma industries [1][2][3][4][5].

In Mexico, chili powder is used as a spice in diverse seasoned foods, fast food, beverages, snacks, fruits, grains, regional spicy candies, and diverse sauces. Thus, chili powder is an important spice in the Mexican diet [2][6]. However, chili powder is produced from sun-dried peppers, and this production process increases the risk of microbial contamination of the product [2]. In some cases, industrial dryers are used to reduce drying time [2]; however, retention of important traits in chili powder is dependent on the drying procedure. For example, color deterioration in chili powder is greatly influenced by moisture content, storage, temperature, atmospheric conditions, and light [1][7]. Thus, drying chili at high temperatures can reduce the volatile compounds, nutrients, and color content in chili powder. All of these conditions during chili powder production are favorable for microbial contamination and demand a comprehensive microbiological risk assessment, with a focus on potential pathogens.

Among the approaches used for the characterization of microbial load in chili powder from various countries are the determination of total aerobic mesophilic bacteria, aerobic spore-forming bacteria, *B. cereus* detection, and determination of members of the Enterobacteriaceae, yeast, and molds [8][9][10][11][12][13][14][15]. Other studies have been conducted for pathogenic species detection, such as *Bacillus cereus*, *Salmonella* spp., *Clostridium perfringens,* or *Escherichia coli* in paprika powder [8][9][10][11][12][13][14][15]. A documented outbreak of human salmonellosis was traced to paprika powdered potato chips as the main vehicle of transmission in Germany when paprika powder imported from South America was used to flavor the product [16]. It is noteworthy that a molecular approach using 16S rRNA gene sequencing of bacteria, isolated from paprika powder, produced in different countries, identified spore-forming bacteria, facilitating the association of a particular species with its geographical origin. This study was limited, however, by the number of bacterial isolates that were examined [12].

In other studies, several *B. cereus sensu lato (s.l.)* strains have been identified as opportunistic pathogens in chili powder, paprika, and other spices of different geographical origins, and several different toxins have been associated with these strains, viz. cereulide, cytotoxin K, hemolysin BL (HBL), and non-hemolytic enterotoxin (NHE) [14][15]. Toxins from these bacteria have been associated with the diarrheal type of *B. cereus* food poisoning, which is typically characterized by abdominal pain and watery diarrhea [17][18][19][20][21]. In addition to these toxigenic characteristics, *B. cereus s.l.* strains are naturally resistant to penicillin and other β-lactam antibiotics because of their content of β-lactamases [13][14][15][22][23], and some studies reveal that resistance may be extended to other commonly used antibiotics, such as chloramphenicol, gentamicin, imipenem, erythromycin, tetracycline, and the trimethoprim/sulfamethoxazole combination [13][14][15][23]. With these genetic characteristics present in *B. cereus s.l.*, its detection is essential in controlling the spread of potential pathogens present on Mexican chili powder.

2. SMB and B. cereus s.l. Determination

The content of spore-forming mesophilic bacteria (SMB) and presumptive *B. cereus s.l*. counts from four chili powder samples were determined, as described in Methodology. As shown in **Table 1**, sample B showed the lowest SMB content with 8.0×10^2 cfu/g, followed by sample A with 1.73×10^3 cfu/g, while sample C and D counts were 2.92×10^5 and 3.24×10^5 cfu/g, respectively. These results document the presence of spore-forming bacteria in the tested chili powder samples, validating the need for the next step, detecting the presence of *B. cereus s.l*. The literature indicates that *B. cereus s.l*. is typically resistant to β-lactam antibiotics [13][14][15][22][23]. Accordingly, we used various concentrations of ampicillin in the culture medium during selection with two purposes, first to eliminate ampicillin-susceptible bacteria and second, to facilitate detection and isolation of ampicillin-resistant *B. cereus s.l*. **Table 1** shows the presumptive *B. cereus s.l*. counts according with the phenotypical characteristics mentioned in Methodology. The lowest presumptive *B. cereus s.l.* counts were observed in sample B, with the limit of detection (LOD) of 100 cfu/g in absence of ampicillin, while in the presence of different concentrations of ampicillin, no presumptive *B. cereus s.l*. could be detected (LOD = 100 cfu/g). Sample A had 200 cfu/g in the absence of ampicillin, while in the presence of ampicillin (5 to 50 µg/mL), 100 cfu/g was detected. At 75 and 100 µg/mL of ampicillin, no presumptive *B. cereus s.l*. could be detected (LOD = 100 cfu/g). In samples C and D, the counts were 600 and 700 cfu/g, respectively, in the absence of ampicillin. In the presence of ampicillin, presumptive *B. cereus s.l*. in sample C could be detected at a concentration of 5 to 25 µg/mL of ampicillin, with an average of 580 cfu/g. In Sample D, presumptive *B. cereus s.l*. counts averaged 470 cfu/g at concentrations of 5 to 75 µg/mL of ampicillin. From these results, a total of 30 presumptive ampicillin-resistant *B. cereus s.l*. colonies from all chili powder samples were selected, and the properties described above were confirmed in the same culture medium.

Table 1. Spore-forming mesophilic bacteria (SMB) and presumptive *B. cereus s.l*. counts in Mexican chili powder samples.

Sample	SMB [a]	BC [b] (0)	BC (5)	BC (10)	BC (15)	BC (20)	BC (25)	BC (50)	BC (75)	BC (100)
A	1.73×10^3	200	100	100	100	100	100	100	˂100	˂100
B	8.0×10^2	100	˂100	˂100	˂100	˂100	˂100	˂100	˂100	˂100
C	2.92×10^5	600	633	600	666	566	433	˂100	˂100	˂100
D	3.24×10^5	700	600	433	633	533	533	333	233	˂100

[a] *Spore-forming mesophilic bacteria (SMB) counts.* [b] *Presumptive Bacillus cereus s.l. (BC) counts. To select colonies with ampicillin-resistance, the medium was supplemented at concentrations of 5, 10, 15, 20, 25, 50, 75, and 100 µg/ mL of ampicillin, indicated by numbers in parentheses. The theoretical limit of detection (LOD) was therefore 100 cfu/g. Numbers below this limit (˂100 cfu/g) in our results mean that bacterial growth was not detected in these conditions.*

3. Phylogenetic Analysis

For the confirmation of the identities of these ampicillin-resistant presumptive *B. cereus s.l*. colonies, we used the $tRNA^{Cys}$-PCR strategy previously published [24]. In **Figure 1**, we show that the $tRNA^{Cys}$ gene in *B. cereus s.l*. is part of cluster of 15 to 17 tRNA genes localized downstream of a ribosomal RNA operon. For the diagnostic and phylogenetic analysis we used the specific DNA region between $tRNA^{Cys}$ and *yebC*/*pmpR*-like gene (which encodes a probable transcriptional regulatory protein) located downstream of a gene sequence of unknown function (DUF gene), indicated in **Figure 1**. The phylogenetic analysis show that this DNA region is specific for *B. anthracis*, *B. cereus s.s.*, *B thuringiensis*, *B. toyonensis,* and *B. wiedmannii* related species, while *B. mycoides* and *B. cytotoxicus* show a different gene organization. Therefore, our PCR test is a suitable approach for verification of the identities of the chili powder isolates.

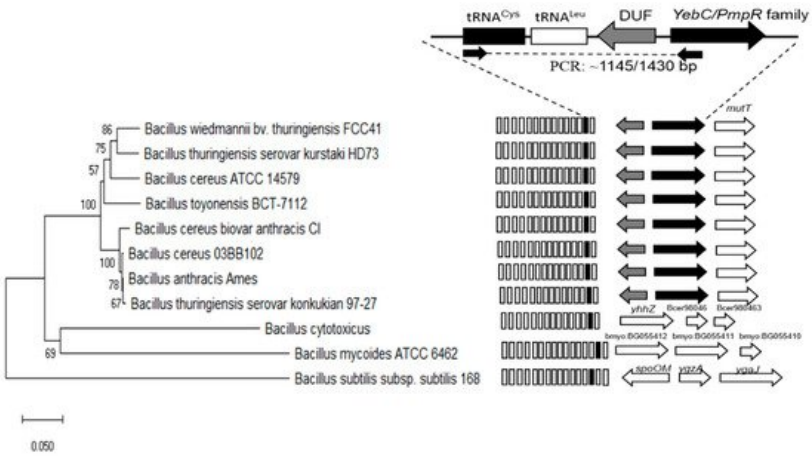

Figure 1. Schematic representation of tRNA^{Cys}-PCR strategy for the *B. cereus* group. The cluster of 15 to 17 tRNA genes and the orientation of three specific genes located downstream of tRNA^{Leu} is shown. The primers indicated by the arrows were predicted to yield products of 1145 and 1430 bp, respectively (see Methodology). Phylogenetic analysis from these regions were obtained in MEGA X, using the neighbor-joining method. *B. subtilis* subsp. *subtilis* 168 was designated as the outgroup taxon.

From a total of 30 presumptive ampicillin-resistant *B. cereus s.l.* colonies selected (6 colonies from sample A, 3 colonies from sample B, 4 colonies from sample C, and 17 colonies from sample D), 25 (83 %) were positive for the tRNA^{Cys}-PCR product (data not shown), when the primers 1517 and 1518 were used as the first option (~1145 bp PCR product). A negative result for tRNA^{Cys}-PCR was obtained for five colonies from sample D, and could be due to a small nucleotide variation in the *yebC*/*pmpR*-like gene region, from which the 1518 primer was designed. Therefore, a new primer, designated 1520, was designed, beginning 285 nucleotides downstream of the DNA region, targeted by the 1518 primer. The tRNA^{Cys}-PCR results (~1430 bp PCR product) were positive (data not shown) with this last primer designed for the rest of presumptive ampicillin-resistant *B. cereus s.l*. colonies (17%).

Our phylogenetic analysis confirmed the results obtained by tRNA^{Cys}-PCR; the presumptive ampicillin-resistant *B. cereus s.l*. colonies are members of the *B. cereus* group. Clustering of the tRNA^{Cys}- *yebC*/*pmpR* genes sequences revealed four major species groups (**Figure 2**). The species isolated from chili powder samples were clustered in Groups I, II, and IV, with the majority of isolates related phylogenetically to *B. cereus* and *B. thuringiensis,* and the isolate SA10-1 closely related to *B. toyonensis* strains from various environmental sources.

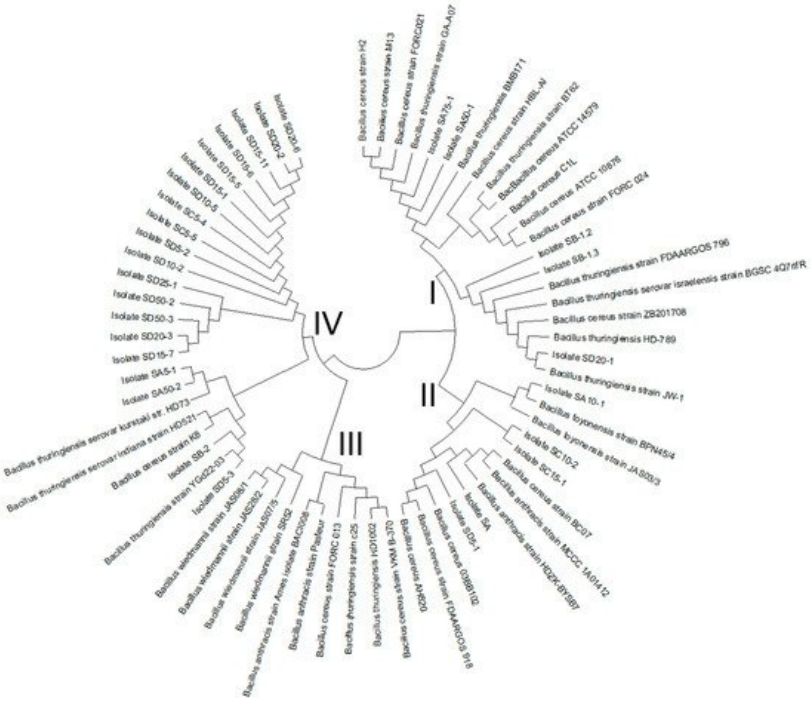

Figure 2. Phylogenetic analysis for tRNA^{Cys}-*yebC*/*pmpR* region. The sequences of the isolates obtained from chili powder samples were compared with sequences of representative strains of *B. cereus* group. The isolated colonies obtained clustered within groups I, II, and IV, respectively (see **Table 2** for correspondence of each isolate).

Table 2. Toxigenic and antibiotics characteristics for *B. cereus s.l* isolates from Mexican chili powder samples. Presence (+) or Absence (−) for toxin production and toxin genes.

Strain	Chili Powder Sample	Toxin Production		Toxin Genes		Antibiotics		Susceptible Increased Exposure
		Hbl	Nhe	*hblC*	*nheA*	Resistance	Susceptible	
***B. cereus* ATCC 10876**	−	+	+	+	+	AMP, CAB, CFT, CTX, DCX, PEN, SXT, CDM	AMK, CHL, CIP, GEN, NET, NTF, NFX, TET, VCM	ERY
SA	**A**	−	+	+	+	AMP, CAB, CFT, CTX, DCX, ERY, PEN, SXT, TET	AMK, CHL, CIP, CDM, GEN, NET, NTF, NFX, VCM	
SA5-1	**A**	+	+	−	−	AMP, CAB, CFT, CTX, DCX, PEN, SXT, CHL, TET	AMK, CIP, GEN, NET, NTF, NFX, VCM	ERY, CDM
SA10-1	**A**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, ERY, PEN, SXT	AMK, CHL, CIP, CDM, GEN, NET, NTF, NFX, TET, VCM	
SA50-1	**A**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, PEN, TET, SXT	AMK, CHL, CIP, ERY, GEN, NET, NTF, NFX, VCM	CDM
SA50-2	**A**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, PEN, TET, SXT, CHL	AMK, ERY, GEN, NET, NTF, NFX, VCM	CIP, CDM
SA75-1	**A**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, NTF, PEN, TET, SXT	AMK, CHL, CIP, CDM, ERY, GEN, NET, NFX, VCM	
SB-2	**B**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, ERY, PEN, SXT, TET, CDM	AMK, CHL, NET, NTF, NFX, VCM	CIP, GEN
SB-1.2	**B**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, ERY, PEN, SXT, TET, CDM	AMK, CHL, NET, NTF, NFX, VCM	CIP, GEN
SB-1.3	**B**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, ERY, PEN, SXT, TET, CDM	AMK, CHL, NET, NTF, NFX, VCM	CIP, GEN
SC5-4	**C**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CDM	AMK, CHL, CIP, NET, NTF, NFX, VCM	GEN
SC5-5	**C**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CDM	AMK, CHL, CIP, NET, NTF, NFX, VCM	GEN
SC10-2	**C**	−	+	+	+	AMP, CAB, CFT, CTX, DCX, ERY, PEN, SXT, TET, CDM	AMK, CHL, NET, NTF, NFX, VCM	CIP, GEN
SC15-1	**C**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, ERY, PEN, SXT, CDM	AMK, CHL, CIP, GEN, NET, NTF, NFX, TET, VCM	
SD5-1	**D**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CDM	AMK, CHL, CIP, NET, NTF, NFX, VCM	GEN
SD5-2	**D**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CHL, CDM	AMK, CIP, NET, NTF, NFX, VCM	GEN
SD5-3	**D**	+	+	+	+	AMP, CAB, CFT, CTX, DCX, PEN, SXT, CHL, TET, CDM	AMK, CIP, NET, NTF, NFX, VCM	GEN, ERY

SD10-2	**D**	**+**	**+**	**+**	**+**	**AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CHL, CDM**	**AMK, CIP, GEN, NET, NTF, NFX, VCM**	
SD10-5	**D**	**+**	**+**	**+**	**+**	**AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CHL, CDM**	**AMK, GEN, NET, NTF, NFX, VCM**	**CIP**
SD15-1	**D**	**+**	**+**	**+**	**+**	**AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CHL**	**AMK, CIP, CDM, GEN, NET, NTF, NFX, VCM**	
SD15-5	**D**	**+**	**+**	**+**	**+**	**AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CHL, CDM**	**AMK, CIP, GEN, NET, NTF, NFX, VCM**	
SD15-6	**D**	**+**	**+**	**+**	**+**	**AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CDM**	**AMK, CHL, CIP, NET, NTF, NFX, VCM**	**GEN**
SD15-7	**D**	**+**	**+**	**+**	**+**	**AMK, AMP, CAB, CFT, CTX, DCX, ERY, NET, PEN, TET, SXT, CHL, GEN, CDM**	**CIP, NTF, NFX, VCM**	
SD15-11	**D**	**+**	**+**	**+**	**+**	**AMK, AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CDM**	**CHL, GEN, NET, NTF, NFX, VCM**	**CIP**
SD20-1	**D**	**−**	**+**	**+**	**+**	**AMP, CAB, CFT, CTX, DCX, PEN, TET, SXT, CDM**	**AMK, CHL, CIP, GEN, NET, NTF, NFX, VCM**	**ERY**
SD20-2	**D**	**+**	**+**	**+**	**+**	**AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CHL, CDM**	**AMK, NET, NTF, NFX, VCM, GEN**	**CIP**
SD20-3	**D**	**+**	**+**	**+**	**+**	**AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CHL, CDM**	**AMK, NET, NTF, NFX, VCM**	**CIP, GEN**
SD20-6	**D**	**+**	**+**	**+**	**+**	**AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT**	**AMK, CHL, CIP, GEN, NET, NTF, NFX, VCM**	**CDM**
SD25-1	**D**	**+**	**+**	**+**	**+**	**AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, GEN, CDM**	**AMK, CHL, NET, NTF, NFX, VCM**	**CIP,**
SD50-2	**D**	**+**	**+**	**+**	**+**	**AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CHL, CDM**	**AMK, CIP, NET, NTF, NFX, VCM**	**GEN**
SD50-3	**D**	**+**	**+**	**+**	**+**	**AMP, CAB, CFT, CTX, DCX, ERY, PEN, TET, SXT, CHL, CDM**	**AMK, CIP, NET, NTF, NFX, VCM, GEN**	

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