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RAPID TESTING OF FOOD MATRICES FOR *BACILLUS CEREUS* ENTEROTOXINS

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ABSTRACT

Nine different food products frequently associated with *Bacillus cereus* outbreaks were chosen as representative matrices to be evaluated with end-point polymerase chain reaction (PCR), enzyme linked immunosorbent assay, lateral flow device and mass spectrometry for detection of enterotoxins associated with human illness. Testing was performed on food portions inoculated with a bacterial strain and incubated at 30°C for either 5 h or 24 h. A screening end-point multiplex PCR targeting enterotoxin genes including the emetic toxin and three diarrheal toxins, hemolytic hemolysin BL (Hbl), nonhemolytic enterotoxin (Nhe), and cytolysin K. Commercially available kits were used to determine the presence/absence of Nhe and Hbl. Finally; a quantitative analysis using mass spectrometry was performed for the detection of the emetic toxin. Definitive results were available after a five hour pre-enrichment in five food products. The following strategy would allow for more efficient testing of surveillance or environmental samples as well as more rapid response time during a foodborne outbreak.

PRACTICAL APPLICATIONS

The application of a strategy for processing and analyzing food products for *Bacillus cereus* and the enterotoxins associated with foodborne illness was explored. Employment of such a strategy will decrease time spent processing negative samples allowing more time for analysis of potentially positive food products.

INTRODUCTION

Bacillus cereus is ranked 10th among foodborne pathogens associated with human illness, accounting for an average of 63,400 cases per year in the U.S.A.; however, due to the short duration and nonspecificity of symptoms, cases are likely to be underreported (Scallan *et al.* 2011). *B. cereus* is a gram-positive spore-forming rod found naturally in soil, with ubiquitous and resistant spores that are able to survive the pasteurization process (Schoeni and Wong 2005). Dairy products are especially susceptible to spoilage by *B. cereus*, although the bacteria and spores are also often detected in rice, pasta, dehydrated foods, meats and vegetables, making it a major concern in food spoilage prevention.

Food poisoning associated with *B. cereus* can be due to infection or intoxication. Infection occurs upon ingestion of spores that germinate in the small intestine and produce diarrheal enterotoxins (Kramer and Gilbert 1989; Granum and Lund 1997; Lund and Granum 1997; Riemann 2006). Three diarrheal enterotoxins regularly detected in foodborne outbreaks are nonhemolytic enterotoxin (Nhe), hemolysin BL (Hbl), and cytolysin K (CytK) (Riemann 2006). Two of the diarrheal enterotoxins, Nhe and Hbl, are tripartite proteins that assemble on the target cell surface to establish pore formation and cellular destruction (Granum *et al.* 1999; Sastalla *et al.* 2013). The protein components of the tripartite proteins are not interchangeable (Sastalla *et al.* 2013). The third diarrheal enterotoxin, CytK is a single protein and was implicated in three fatal cases of necrotic enteritis

(Lund *et al.* 2000). Immunoassays are available and generally used for the detection of Nhe and Hbl in foodborne outbreak investigations, but no commercially prepared assays are available for the detection of CytK.

Intoxication is caused by cereulide produced by *B. cereus* spores after germinating in food that has not been stored properly. Ingestion of the pre-formed toxin typically begets nausea and vomiting. The cereulide is resistant to acid, heat and digestive enzymes. The cyclic dodecadepsipeptide acts as an ionophore disrupting oxidative phosphorylation in the mitochondria and has been associated with several cases of liver failure (Mahler *et al.* 1997; Dierick *et al.* 2005; Posfay-Barbe *et al.* 2008).

Typical toxin detection methods rely on enzyme linked immunosorbent assays (ELISA), but raising an antibody to cereulide has yet to be successful. Initial detection methods were dependent upon oral challenge of primates until investigators noted that culture filtrates from *B. cereus* strains produced vacuoles in HEp-2 cells (Hughes *et al.* 1998). Various methods using different cells lines and boar spermatozoa were evaluated, but results were subjective and sometimes inconclusive (Szabo *et al.* 1991; Agata *et al.* 1994; Hughes *et al.* 1998; Jaaskelainen *et al.* 2003). The genetic locus encoding cereulide biosynthetase (*ces*) in the emetic *B. cereus* strains has been identified and sequenced (Ehling-Schulz *et al.* 2004; Horwood *et al.* 2004; Dommel *et al.* 2010) which has been a key factor for the development of molecular assays used for strain characterization and food testing (Ehling-Schulz *et al.* 2006; Fricker *et al.* 2007; Ueda *et al.* 2013; Hariram and Labbe 2015). However, the most accurate quantitative method available for cereulide detection in contaminated food products is high performance liquid chromatography connected to mass spectrometry (LC/MS) (Biesta-Peters *et al.* 2010; International Organization for Standardization 2014).

An average of 6 days is required for food processing and setting up the routine culture methods including MPN, plating and bacterial identification. Immunoassays are evaluated once the organism has been confirmed as *B. cereus*; however, immunoassays detect only two of the enterotoxins associated with *B. cereus* food poisoning. The use of molecular methods is one way to predict the presence or absence of an enterotoxin gene target. Researchers foresee the use of molecular methods allowing for the detection of the gene targets associated with *B. cereus* food poisoning will be more important than strain determination (Ehling-Schulz and Messelhauser 2013). Gene profiling methods targeting Nhe, Hbl, CytK and *ces* have been used to characterize *B. cereus* isolates (Ehling-Schulz *et al.* 2006; Fricker *et al.* 2007; Tallent *et al.* 2015), but have not been used to evaluate a variety of food matrices in conjunction with other assays. We analyzed nine food products using culture, multiplex end-point polymerase chain reaction (PCR), immunoassays and mass-spectrometric

analysis. The food products were evaluated at two different time points in an effort to determine if sample processing and time to results could be expedited. Our proposed strategy would require a minimum of 2 days instead of the current methods that require a minimum of 5 days. The initial food processing step would be unchanged, but an aliquot of the homogenate would be used for DNA extraction. A serial dilution would be prepared and aliquots plated and incubated. PCR analysis of the DNA extract would guide further studies for enterotoxin testing.

Mention of trade names or commercial products in the paper is solely for the purpose of providing scientific information and does not imply recommendation or endorsement by the U. S. Food and Drug Administration.

MATERIALS AND METHODS

Model Food Products

Milk, canned beef vegetable soup, parboiled rice and dehydrated food products (including beef gravy mix, infant formula, mashed potatoes, chocolate dietary supplement drink mix, whey powder and pancake mix) were purchased from local markets. Dehydrated products were rehydrated with Butterfield's Phosphate Buffered Dilution Water prior to inoculation and incubation. Each food product was evaluated for natural contamination prior to spiking the food with one of the test strains. Both before and after spiking food products with selected bacterial strains, each food product was diluted 1:10 in dilution water and aliquots were added to pre-enrichment broth and plated on nutrient agar as outlined in the *Bacteriological Analytical Manual* (BAM) (United States: FDA 2009) to assess the food for possible natural contamination and growth of the selected strain. The matrix controls and products inoculated with a bacterial strain were evaluated after 5 h and 24 h incubation at 30°C using the PCR assay, immunoassays and LC/MS.

Bacterial Strains and Culture Conditions

The five strains used in this study were FDA strains isolated from food or from clinical samples associated with foodborne illness. One *Staphylococcal aureus* (FDA963-3) and one *B. licheniformis* (FDA1383) were used as exclusivity strains and three *B. cereus* strains (FDA4227A, FDATJL16 and FDA905-9), with different enterotoxigenic profiles, were chosen as inclusivity strains (Table 1). All strains had been preserved in nutrient broth with glycerol and maintained at -80°C. Each strain was retrieved from storage, transferred twice to tryptic soy agar (TSA) (Difco, Franklin Lakes, NJ) and incubated overnight at 30°C. One colony from each strain was transferred to a brain heart infusion broth (pH 7.4) containing 0.1% glucose (BHIG) (Difco, Franklin Lakes,

TABLE 1. SHOWN ARE BASELINE POLYMERASE CHAIN REACTION (PCR) RESULTS AND IMMUNOASSAY RESULTS FOR THE FOUR *BACILLUS* SPP. AND THE *STAPHYLOCOCCUS AUREUS* STRAIN USED IN THIS STUDY TO INOCULATE FOOD SAMPLES

Designation	Strain	PCR		LFD		BDE-NheA/NheB	LFD-NheB	PCRces	PCR-CytK
		RNA	Hbl	Hbl	(L ₂)				
FDA4227A	<i>Bacillus cereus</i>	Pos	Neg	Neg	Pos	Pos	Pos	Pos	Neg
FDATJL16	<i>Bacillus cereus</i>	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Pos
FDA905-9	<i>Bacillus cereus</i>	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Pos
FDA1383	<i>Bacillus licheniformis</i>	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg
FDA963-3	<i>Staphylococcus aureus</i>	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg

Gene targets: Universal rRNA; Hbl, hemolysin BL; Nhe, Non-hemolytic enterotoxin; ces, cereulide biosynthetase; CytK, Cytolysin K; Immunoassays: LFD lateral flow device; BDE, *Bacillus* Diarrhoeal Enterotoxin Visual Immunoassay; pos, positive; neg, negative.

NJ), incubated overnight at 30°C, and the cells were pelleted at 4000 x g for 20 min. The supernatant was discarded and the cells were resuspended in 10 mL of Butterfield's Dilution Water. Enumeration of the resuspended cells averaged 1 x 10⁸ cfu/mL.

Food portions (50 g) were inoculated with 2 mL of resuspended cells. After the inoculated food portions were incubated 30°C for 5 h, 10 g from each were transferred to sterile containers, and the remaining food mixtures were returned to the incubator until the following morning. Each 10 g portion was tested in duplicate and one food portion of each matrix was only inoculated with 2 mL of Butterfield's Dilution Water and used as a control.

DNA Extraction and PCR

DNA was extracted from each food portion using the Power Soil DNA isolation kit (MoBio, Carlsbad, CO) following the manufacturer's instructions. Our multiplex PCR assays included gene targets for Nhe, Hbl, CytK and the cereulide synthetase (*ces*) gene and were performed using primers defined in another study (Ehling-Schulz *et al.* 2006) with universal primers for rRNA to detect PCR inhibitors. PCR assays were performed and used under conditions described previously (Tallent *et al.* 2015).

Immunoassays

The first assay used was the *Bacillus* Diarrhoeal Enterotoxin Visual Immunoassay (BDE) (3M Tecra, St. Paul, MN), a double-sandwich enzyme ELISA kit with detection limits of 2–5 ng/mL (Beecher and Wong 1994) commonly used to detect Nhe during foodborne outbreak investigations. The polyclonal antibodies react with NheA and NheB. The second immunoassay, Duopath Cereus Enterotoxins (EMD Millipore, Darmstadt, Germany) is a gold-labeled immunosorbent assay lateral flow device (LFD) using monoclonal antibodies to detect both NheB and the L₂ component of Hbl, with detection limits of 6 ng/mL and 20 ng/mL, respectively, (Krause *et al.* 2010). Both assays were performed per manufacturer's instructions, except food portions were not

provided enrichment with casein hydrolysate yeast extract broth with 1% glucose (CGY), which is usually recommended when using Duopath. BDE and PCR assays were performed in duplicate, but the LFD was used for either the 5 or 24 h sample from the food inoculated with *B. cereus* and the negative matrix control for the same time, due to limited supply. The LFD was not used to test the expected negative samples such as food inoculated with *S. aureus* or if the *B. cereus* inoculated food showed negative BDE results at 5 h. The negative food matrix was tested with the appropriate 5 h or 24 h food sample.

Mass Spectrometry Standards

Stock analytical standard solutions of synthetic cereulide and isotopically labeled cereulide (Bauer *et al.* 2010), ¹³C₆-Cereulide (Chiralix, Nijmegen, Netherlands), were prepared at 100 µg/mL in methanol. The stock solutions were surplus that had been prepared for laboratories that participated in the validation of the standard (International Organization for Standardization 2014). The stock solutions had been diluted by stepwise 10-fold dilutions in methanol or acetonitrile (Optima Grade, Thermo-Fisher Scientific, San Jose, CA) to yield 10,000, 1000, 100 and 10 ng/mL solutions (Hagblom *et al.* 2002; Biesta-Peters *et al.* 2010; International Organization for Standardization 2014). The standard curve solutions were prepared from a working stock (10 ng/mL) solution for cereulide and isotopically labeled cereulide with acetonitrile, as described (International Organization for Standardization 2014).

Food Extracts

Food portions collected for LC/MS analyses were frozen at –20°C and batch processed. The LC/MS was used for all food samples with Butterfield's Dilution Water as a negative matrix control, *B. cereus* strain FDA4227A as the emetic toxin control and *B. cereus* strain FDATJL16 as the negative control. Duplicate samples were processed dispensing 2.5 g into a conical tube with 500 µL of (100 ng/mL) isotopically labeled cereulide as an internal standard. The tubes were mixed using a vortex mixer and allowed to equilibrate for 30 min. After

equilibration, 29.5 mL of acetonitrile was added to each sample and all samples were placed on a horizontal shaker, shaken vigorously for one hour, and then centrifuged for 10 min at 1500 x g. The supernatants were filtered using 0.20 μm polytetrafluoroethylene (PTFE) syringe filters and dispensed into LC autosampler vials.

LC/MS Analysis

Food extracts were analyzed for cereulide using an Acquity UPLC (Waters Corp., Milford, MA) connected to a 4,000 QTRAP (Sciex, Framingham, MA). A Waters BEH C18 column (2.1 x 100 mm, 1.7 μm , 100 \AA ; Torrance, CA) was used with a 15 min isocratic LC method using 90% acetonitrile with 0.1% formic acid and 10% 10 mM ammonium formate in water with 0.1% formic acid at a flow rate of 0.3 mL/min, 50°C column temperature, and injection volume of 5 μL . The precursor ion for cereulide is m/z 1170.7 with a quantitative ion of m/z 314.4 and qualitative ion m/z 499.4; likewise, the isotopically labeled cereulide precursor ion is m/z 1176.7 and is the quantitative ion is m/z 172.2. The concentration of cereulide in the samples is quantified by normalizing to the isotopically labeled standard and using the calibration standard curve.

RESULTS AND DISCUSSION

Expected assay results were based upon gene profiles established for each inclusivity and exclusivity strain prior to use in the study. Immunoassays and all four PCR targets were negative using the two exclusivity strains, *S. aureus* and *B. licheniformis*. The PCR profiles of *B. cereus* strains included one emetic toxin strain and two strains positive for Hbl and CytK. All three *B. cereus* strains tested positive for Nhe (Table 1). The PCR and immunoassay results were expected to be negative if the initial food product pre-enrichment or agar culture was negative or the food had been inoculated with *B. licheniformis* or *S. aureus*. Foods inoculated with *B. cereus* group strains were expected to be positive for gene targets as projected by the initial strain analysis (Table 1).

The PCR assay sensitivity was evaluated by inoculation of *B. cereus* emetic strain to BHIG and incubated on an orbital shaker at 30°C for 5 h. The inoculum was mixed and 10-fold serial dilutions were prepared for DNA extraction and enumeration. PCR amplification estimation was equivalent to <1 cfu/mL compared to enumeration of log 4.3 cfu/mL.

A total of nine food commodities yielded 707 results with 636 (90%) showing the expected results and 71 (10%) showing unexpected results, that is, positive PCR results with negative immunoassay results (Fig. 1). Five of the food products (mashed potatoes, rice, milk, pancake mix and chocolate dietary supplement drink mix) yielded conclusive results after five hours of incubation.

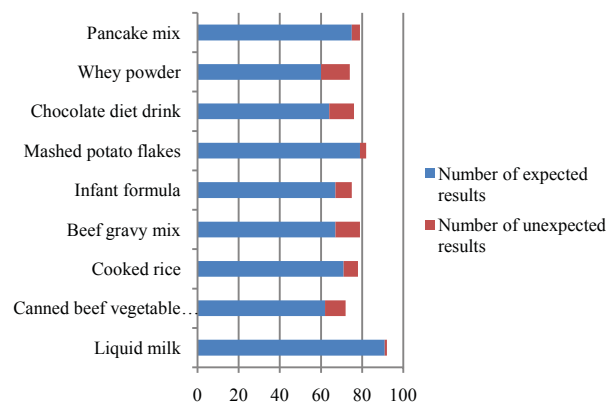


FIG. 1. NUMBER OF EXPECTED AND UNEXPECTED RESULTS BASED UPON INITIAL BASELINE RESULTS OF STRAIN ANALYSIS

The blue bars represent the number of expected results and the red bars represent the number of unexpected results.

Immunoassays, PCR, and mass spectrometric results of the matrix control and food samples spiked with *S. aureus* or *B. licheniformis* were negative unless the food sample was naturally contaminated with a *B. cereus* group strain. Four of the products used in this study (mashed potatoes, chocolate dietary drink mix, whey powder and pancake mix) were naturally contaminated with *B. cereus* group strains. Additionally, the cultures from the gravy mix grew an unidentified gram-negative rod and *S. warneri* was identified from the beef vegetable soup (Table 2).

Analysis of the food matrix controls and the two exclusivity strains showed all negative results after 5 h of incubation, but the four products naturally contaminated with a *B. cereus* group strain showed positive PCR and immunoassay results. The matrix control from the mashed potatoes was positive at 24 h for CytK and the *S. aureus* portion was positive for CytK. Cultured isolates from the mashed potatoes were also positive for CytK. The PCR results of the matrix control and the strain cultured from the chocolate dietary supplement drink mix cultures were positive for Nhe, Hbl and *ces*, but the LC/MS results of the matrix control were negative for cereulide. The *B. licheniformis* inoculated portion was PCR positive for Nhe and Hbl. The whey powder matrix control and two exclusivity strains were PCR positive for Nhe and both the matrix control and *B. licheniformis* inoculated portions were Nhe positive on the BDE immunoassay. The pre-enrichment culture was PCR positive for Nhe and Hbl which was verified using the LFD assay of the matrix control portion. The pancake mix matrix control and isolates cultured from the mix were PCR positive for Nhe and CytK. The immunoassays were positive for Nhe. The bacterial strains isolated from the beef gravy mix and beef vegetable soup were PCR negative for the *Bacillus* gene targets.

The *ces* positive *B. cereus* strain (FDA4227A) used as an inclusivity strain was originally isolated during a foodborne

TABLE 2. SHOWN ARE THE PCR AND ASSAY RESULTS OF MATRIX CONTROLS, ENRICHMENT BROTHS AND STRAINS ISOLATED FROM THE NATURALLY CONTAMINATED FOOD COMMODITIES

Food	Matrix control or contaminant	PCR-RNA	PCR-Hbl	LFD Hbl (L2)	PCR-Nhe	BDE-NheA/NheB	LFD-NheB	PCR-ces	PCR- CytK	MS: cereulide
Gravy mix	matrix control	Neg	Neg	n/t	Neg	n/t	n/t	Neg	Neg	Neg
Gravy mix	GNR	Pos	Neg	n/t	Neg	n/t	n/t	Neg	Neg	n/t
Soup	matrix control	Neg	Neg	n/t	Neg	n/t	n/t	Neg	Neg	Neg
Soup	GPC	Pos	Neg	n/t	Neg	n/t	n/t	Neg	Neg	n/t
Potatoes	matrix control	Pos	Neg	neg	Neg	Neg	neg	Neg	Pos	neg
Potatoes	from broth	Pos	Neg	n/t	Neg	Neg	n/t	Neg	Pos	n/t
Potatoes	<i>Bacillus</i> sp	Pos	Neg	n/t	Neg	n/t	n/t	Neg	Neg	n/t
Potatoes	<i>Bacillus</i> sp	Pos	Neg	n/t	Neg	n/t	n/t	Neg	Pos	n/t
Pancake mix	matrix control	Pos	Neg	neg	Pos	Pos	pos	Neg	Pos	Neg
Pancake mix	from broth	Pos	Neg	n/t	Pos	Pos	n/t	Neg	Pos	n/t
Pancake mix	<i>Bacillus</i> sp	Pos	Neg	n/t	Pos	Pos	n/t	Neg	Pos	n/t
Diet drink mix	matrix control	Pos	Neg	pos	Pos	Pos	pos	Pos	Neg	Neg
Diet drink mix	CGY broth	Pos	Pos	pos	Pos	Pos	pos	Neg	Pos	n/t
Diet drink mix	<i>Bacillus</i> sp	Pos	Neg	n/t	Pos	Pos	n/t	Pos	Neg	n/t
Whey powder	matrix control	Pos	Neg	pos	Pos	Pos	pos	Neg	Neg	Neg
Whey powder	from broth	Pos	pos	n/t	Pos	Pos	n/t	Neg	Neg	n/t

Gene targets: Universal rRNA RNA; Hbl, hemolysin BL; Nhe, Non-hemolytic ENTEROTOXIN; ces, cereulide biosynthetase; CytK, Cytolysin K. Immunoassays: LFD lateral flow device; BDE, *Bacillus* Diarrhoeal Enterotoxin Visual Immunoassay; pos, positive; neg, negative; n/t, not tested.

outbreak investigation. The limit of quantification (LOQ) for the emetic toxin in all foods tested was 0.082 ng/mL that corresponds to 0.98 ug/kg in the food samples tested. The LC/MS cereulide results (Fig. 2) were positive after 5 h of

incubation for the beef gravy, mashed potatoes, milk, rice, and pancake mix, while the infant formula, chocolate dietary supplement drink mix, vegetable beef soup, and whey powder were negative. All samples tested positive for cereulide at

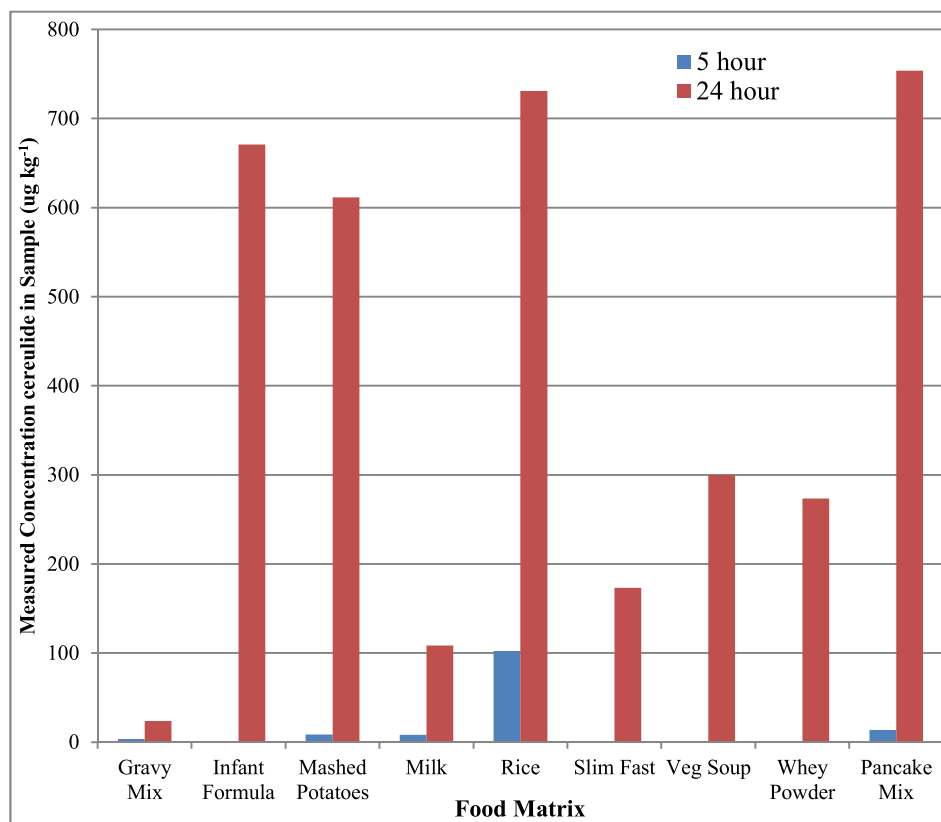


FIG. 2. RESULTS SHOWN ARE FROM DUPLICATE SAMPLE TESTING OF FOOD PRODUCTS INOCULATED WITH A CEREULIDE PRODUCING STRAIN OF *BACILLUS CEREUS* (FDA4227-A) The blue bars indicate cereulide test results following 5 h of incubation and the red bars indicate cereulide test results following 24 h of incubation in ug/kg.

24 h. The PCR results for the food samples inoculated with this strain were positive for the *Nhe* and *ces* gene targets at both times tested with the exception of the whey powder which was negative at 5 h. The BDE assay was negative at 5 h for the beef gravy, beef vegetable soup and whey powder. The beef vegetable soup and the rice samples were negative at 24 h using the BDE immunoassay. The LFD assay was negative for the beef gravy at 5 h and the rice at 24 h.

Two strains used as inclusivity strains in this study were positive on PCR for *Nhe*, *Hbl* and *CytK*. One strain originally isolated from infant formula (FDA905-9) when incubated with rice and tested at 24 h demonstrated the expected PCR results, but surprisingly the BDE and LFD were negative for *Nhe* and *Hbl*. The immunoassay results of the food samples inoculated with the same strain after five hours were negative for the beef gravy, infant formula and beef vegetable soup. Additionally, the PCR for the *Hbl* target was negative for the same samples. The PCR results for the *Nhe* gene were negative for the infant formula and beef vegetable soup. The PCR results for the *CytK* gene were negative for the milk and beef vegetable soup. The second strain positive for the same three gene targets was originally cultured from dry soup mix (FDATJL16). The PCR for both time points showed expected results except the beef gravy mix was negative for *Nhe* and *Hbl*. The BDE results for the beef gravy, chocolate dietary drink mix, beef vegetable soup, and whey powder were negative at five hours and also at 24 h for the rice and beef vegetable soup. The LFD assay was negative for the gravy at five hours and the rice was negative for *Nhe* at 24 h, but positive for *Hbl*.

Food samples were inoculated with bacterial strains and placed at 30°C to mimic time and temperature abuse. Overall the assays at 5 h were less conclusive than at 24 h. For example, analysis of the food inoculated with the inclusivity strains produced expected PCR results in 84% of the five hour assays and 96% of the 24 h assays. The Tecra and the LFD detected diarrheal enterotoxin 72% and 89%, respectively. Finally, LC/MS detected the emetic toxin at 5 h in 44% of samples tested and 100% at 24 h.

Some of the food matrices including beef gravy mix, chocolate dietary supplement drink mix, beef vegetable soup, and whey powder were positive for indigenous flora that may have inhibited the growth of the test strains added to the food. Alternatively, the negative results after the 5 h incubation could be due to a component of the food matrix that delayed both bacterial growth and toxin production. One component that is known to delay growth and enterotoxin production in foods naturally contaminated with *B. cereus* is nisin. The bacteriocin is used in many foods to inhibit bacterial growth, but is not required to be listed as an ingredient (Delves-Broughton *et al.* 1996; Beuchat *et al.* 1997). Similarly, other preservatives (Ceuppens *et al.* 2011) may also have had an inhibitory effect in the short-term incubation,

but these effects diminish as the preservatives degrade over time, hence the results are often positive at 24 h. Evaluation using an intermediate incubation time will be considered in future studies along with growth curves in order to more closely predict the exponential growth phase in an effort to detect the diarrheal enterotoxins and the stationary phase to detect the emetic toxin. Future studies will include the analysis of different food types especially more varieties of the complex food matrices used in this study.

CONCLUSIONS

Traditional methods require a minimum of 5 days to prepare serial dilutions of the food homogenate, culture the dilutions, identify *B. cereus* and determine if the positively identified strains are toxigenic. The study aim was to decrease the analytical time required for the investigation of contaminated food products. A workflow strategy that would streamline *B. cereus* testing would begin with molecular testing of food sample extracts along with enumeration studies followed by performance of toxin testing if the PCR results are positive or if *B. cereus* is isolated. Execution of a triage approach is both cost saving and time saving since products with both negative molecular testing and negative enumeration studies will not be further analyzed and positive molecular tests can target analysis to determine if a functional toxin is present.

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CONFLICT OF INTEREST

No conflict of interest declared.

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