Detection of Emetic Toxin Genes in *Bacillus cereus* isolated Different types of Foods

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Abstract:

The study was carry out from Jul. to Dec. 2010 in aimed to detect the presence of emetic toxin gene in isolates of *Bacillus cereus* isolated from different types of foods from local market at Al-Nasiriya city. Specific PCR primer was used for amplification of the genes. The results showed that 25(41.6%) of 60 *B. cereus* isolates were carred for emetic gene. The higher percentage was 50% found among the isolates from cooked rice and fishes.

الخلاصة:

أجريت الدراسة الحالية للفترة من تموز لغاية كانون الأول 2010 وكانت تهدف الى الكشف عن وجود الجين المسيطر على انتاج الذيفان المقيء من عزلات *Bacillus cereus* المعزولة من عينات مختلفة من الأغذية جمعت من السوق المحلية لمدينة الناصرية. أستخدم باديء نوعي لتقنية تفاعل السلسلة المتضاعف.

أظهرت النتائج ان 25(41،6%) من أصل 60 عزلة كانت تحمل الجين المقيء ، وان أعلى نسبة لحمل الجين كانت بين الجراثيم المعزولة من عينات الرز المطبوخ وعينات السمك.

Introduction:

Bacillus cereus is a common environmental bacterium that contaminates raw materials of food. Otherwise than *Salmonella* and *Campylobacter*, *Bacillus cereus* is a heat resistant bacterium, capable of surviving most cooking procedures due to the production of highly thermo resistant spores. The food involved has usually been heat treated and surviving spores are the source of the food poisoning. The heat treatment induces germination of the spore and the vegetative cells then produce toxins.(1)

Two distinct types of food poisoning i.e. diarrhea and emesis caused by two different types of toxins(2,3) and also fatal meningitis(4). This organism is also responsible for spoilage of different food products(5). As *B. cereus* is a spore former organism, there is a risk of its transmission through heat-treated and processed food products. Some isolates of *B. cereus* can grow at refrigerated temperature(6, 7) and spore can survive at

high temperature. This organism is known to resist even pasteurization process of milk(8). In India, presence of this organism has been reported in various food products including fish(9).

Bacilus cereus is an important food borne pathogen, many strains of *B. cereus* cause food poisoning and other infections. Two principal types of food poisoning caused by *B. cereus*, emetic and diarrheal, have been described. The emetic type is effected by a small cyclic heat-stable peptide, which cause vomiting a few hours after ingestion. Diarrheal types are attributed to enterotoxins, a group of heat-labile proteins causing abdominal pain and diarrhea after incubation for 8 to 16 h and vegetative growth of the bacteria in the intestine (1,2,3).

The causative agent of *B. cereus* emetic food poisoning is a ring structured dodecadepsipeptide 1.2 kDa in size, first identified by Agata *et al.(6)*. Cereulide consists of only three repeats of 2 amino acids and 2 hydroxy acids: D-O-leu-D-Ala-L-O-Val-L-Val.

The enterotoxins are proteins causing cytotoxicity, fluid accumulation in the ligated ileal loop of experimental animals, and dermonecrosis and are lethal for mice (3,4). Two protein complexes from *B. cereus* strains, hemolysin BL (HBL) and non-hemolytic enterotoxin (NHE), and an enterotoxic protein, enterotoxin T (bc-D-ENT), with these characteristics have been characterized (7)

Since (entero-)toxins are the compounds that cause disease and since *B. cereus* may be part of the transient human flora, detection of *B. cereus* by microbiological methods does not suffice to determine definitively its involvement in food borne disease. Therefore, further characterization to elucidate the presence of or the potential to form (entero-)toxins is of great importance(8).

A wide range of foods has been implicated in the emetic-type syndrome including soups,cooked rice, cooked meat, poultry and vegetables, and dessert dishes and sauces, but *B. cereus* has not been isolated in large numbers from fecal specimens .In most of the incidents, large numbers of *B. cereus* have been isolated from remnants of cooked rice, clinical specimens or both(9)

Materials and methods:

Food samples: A total of 150 samples were collected from local shops at Al-Nasiriya city, 30 samples from each of fishes, meat, dairy products ,cooked rice and salad . Samples were collected in sterile bags and transferred to the laboratory for the bacterial analysis within two hours.

Reference strain: *B. cereus* NCIM 2106 was used as a standard strain (positive control), other isolates of *E. coli* were used as negative control.

Isolation of *B. cereus*: Food samples were prepared through weight 25 grams, mixed and squashed under sterile conditions and added to 225 ml of nutrient broth and incubated at 37 ° C for 24 hour, then transferred to a selective media polymixin-pyruvate-egg yolk-mannitol-bromocresol purple agar (PEMPA) (9) .Colonies showing lecithinase positive (surrounded by transparent zone) were picked up and purified . Further confirmation was carried out for morphological and biochemical reactions(10)

Preparation of the isolates for PCR: A modified procedure was done (11).Template of PCR was prepared by boiling method. Overnight growth of bacterial culture in BHI broth was centrifuged at 7000 x g for 10 min at 4°C. Bacterial pellet was washed once in normal saline and suspended in 150 µl of autoclaved millipore (Milli-Q, France) purified water with vortexing. The bacterial suspension was kept on boiling water bath for 10 min and frozen immediately at -70°C. After freezing, the lysate was thawed and centrifuged at 4000 x g to pellet the debris. Five µl of the supernatant was used as template in the PCR reaction.

Polymerase chain reaction: Specific DNA Primer constructed by Fricker (12) was used with PCR kit (**Promega- USA)**as in table 1. PCR was performed with a standard protocol Sambrook *et al.*, [(12)]. Each reaction was carried out by using a 20-ml mixture containing 2 ml of buffer (supplied with *Taq* polymerase), 20 pmol of primer, each deoxynucleoside triphosphate at a concentration of 2 mM, 2.5 U of *Taq* polymerase (ATGC Biotechnologie, Noisy-le-Grand, France), and 200 µg of genomic DNA.

Table (1): primer set sequences used for emetic-gene amplification

No.	size of amplification products (bp)	Oligonucleotide sequence of Primer(5c'e-3')	The direction of the initiator	Location within gene
1	176 bp	CACGCCGAAAGTGATTATACCAA	Forward	8743-8765
2	176 bp	CACGATAAAACCACTGAGATAGTG	Reverse	8895-8918

Steps	Temperature °C	Time / sec.	Number of cycles
DNA denaturation	95	30	1

Annealing	60	30	30
Elongation	72	45	30
Final extension	72	7min.	1

Table(2): Program steps for PCR amplification

PCR products were analyzed using 1.5% agarose gel electrophoresis in 0.5 X Tris boric ethylenediamine tetraacetic acid sodium salt (TEB) containing 0.5µg ethidium bromide buffer. A 100 bp molecular DNA marker (USA) was used to approximate molecular weight of PCR products. After migrated of amplified DNA on the gel electrophoresis, DNA bands were tested by UV transiluminator.

Results:

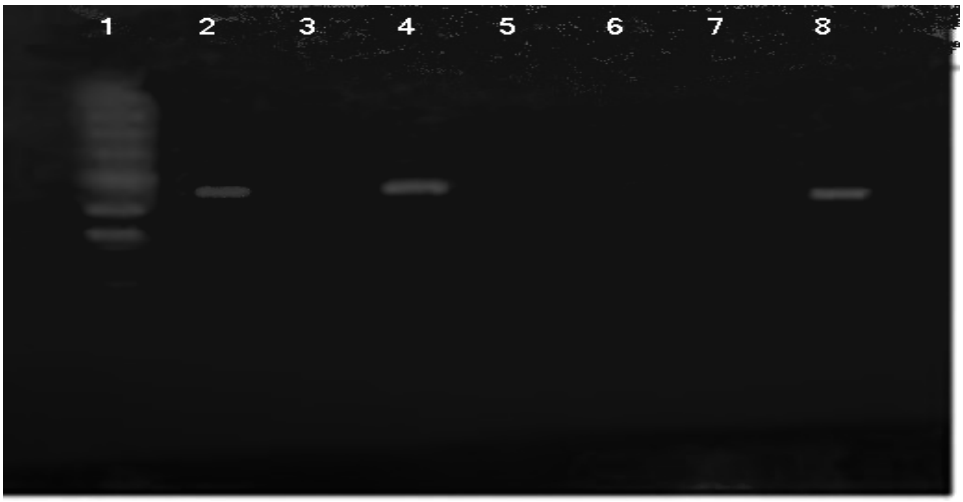
A total of 60 isolates of *Bacillus cereus* were isolated from 150 samples of foods , which were collected from local shops and restaurants at Al-Nasiriya city. 18 isolates from cooking rice, 12 from salad,11 from meat, 10 from fish and 9 from dairy products .

All the isolates were appeared viability for exotoxins production through the production of licithinase on a selective media and haemolysin on blood agar.

The results of PCR amplification for the template DNA of isolates showed that 25(41.6 %) of 60 isolates submitted to the present study were carrying emetic gene (ces) of *B. cereus* . The higher percentage was 50% from isolates of each of cooked rice and fishes ,while the lower percentage from isolates of meat (27.3 %), (Table,3) (Fig.1).

Table (3). Percentages of emetic genes detection .

sample	No. of examined isolates	Emetic gene presence	
		Number	Percentage%
Cooked rice	18	9	50
Salad	12	5	41.6
Meat	11	3	27.3
Fishes	10	5	50
Dairy products	9	3	33.3
Total	60	25	41.6



(Fig. 1). Electrophoresis of PCR products for emetic gene of *B.cereus* on 1.5% agarose gel . Lane 1, DNA marker. Lane 2 , positive control (Ref, strain of *B, cereus*). Lane 3, negative control of *E. coli*. Lane 4,8, positive isolates . Lane 5, 6 , negative isolates.

Discussion:

Diagnosis of *B. cereus* linked to the emetic type of food poisoning was hampered by the lack of genetic information on the toxin genes. Only recently were the genes encoding the enzymatic machinery required for biosynthesis of the emetic toxin cereulide identified and characterized (14, 15). A rapid and prompt method for detection of presence of enterotoxigenic *B. cereus* in food is important to ensure that food products are safe for consumption. The conventional method of differentiation of enterotoxigenic and non-enterotoxigenic strains of *B. cereus* by RPLA test takes long time to yield result. But, PCR is considered as a rapid and reliable method for the detection of presence of specific organism even when the organism is present in low number and the sample is contaminated with some other organisms(16)

The objectives of the study were to find the occurrence of emetic toxin gene in *B. cereus* isolated from different types of foods in local markets of Al-Nasiriya city using specific primers for PCR technique.

The results showed that 25% of the isolates were positive for emetic gene in addition to the reference strain, The higher percentage was 50% from isolates of each of cooked rice and fishes.The results were agreement with study by Fricker *et al*.(13).

Recently, it has been shown that the cereulide synthetase gene cluster is located on a 208-kb megaplasmid that has high homology to the plasmids pXO1 of *Bacillus anthracis* and pBc10987 of *B. cereus* ATCC 10987 (14) .

The presence of the toxin synthetase gene is required for producing cereulide. However, its presence does not show whether the bacterium actually produces or if the food contains cereulide in concentrations sufficient to cause disease. (5)

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