PHYLOGENIC TREE OF *Bacillus cereus* ISOLATED FROM FOOD SOURCES AND MOLECULAR DETECTION OF THEIR VIRULENCE GENES *(Sph and pi-plc)*

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(Received18 April 2019 , Accepted 28April 2019)

Keywords : *Bacillus cereus*, Virulence gene, *sph*, *pi-plc*.

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ABSTRACT

Bacillus cereus strains were previously isolated from different food source were used in this study. The DNA of all isolates was extracted by genomic DNA purification kit and the *16S rDNA* gene was detected in all isolates using universal primers**.** DNA sequencing of the isolates showed high percentage of homology with that strains previously registered at GenBank. Phylogenic tree of the results showed that the local isolates are closely related to those strains previously registered from different countries at the percentage ranged between 99-100%. The *Sph* and *pi-plc* genes were detected in all of the isolates 70/70 (100 %). There were no significant differences ($P > 0.05$) in the presence of those genes in the samples.

INTRODUCTION

Bacillus cereus is mainly known for causing food poisoning and severe nongastrointestinal tract infections. The intestinal and non intestinal pathogenicity of this microorganism is mainly due to the synergistic effects of a number of virulence products that promote intestinal cell destruction and/or resistance to the host immune system. The various

substances produced by *B. cereus* are mainly enterotoxins, hemolysins, phospholipases and emetic toxin, whose activity may overlap in causing human disease. In addition to food poisoning, *Bacillus cereus* is known for causing severe eye infections, but it is also an opportunistic human pathogen, causing different local and systemic infections. In these cases, there is still poor recognition of the various mechanisms ruled by *B. cereus* in the pathogenesis of the human diseases. In early stationary phase, *B. cereus* produces several toxins, such as degradation enzymes, cytotoxic factors etc., which act in a synergistic way (1). Phospholipase and Sphingomyelinase were known to be toxic, but now they are demonstrated to be nontoxic, and some of the hemolysins associated with them are marginally toxic (2). Sphingomyelinase is heat stable metallo-enzyme (Mg^{+2}) with a size of 34 kDa and hydrolyses sphingomyelin (3). It is active through hot – cold incubation (37 °C to 4 °C). It is synthesized as a 333 amino acids and has 306 amino acids in mature protein (4).

The study was aimed for the difference among *Bacillus cereus* isolates which cause food poisoning and determinates the virulence genes in these isolates.

MATERIALS AND METHODS

Identification of *Bacillus cereus* isolates

Bacillus cereus isolates from different food source were previously described (5). The DNA of all isolates was extracted by genomic DNA purification kit and the *16S rDNA* gene was detected in all isolates using universal primers described by (6). The oligonucleotide primers which were used have 1541 bp and their sequence are : F: ATTTGATCCTGGCTTAG and R: AAGGAGGTGATCCAGCC.

DNA Sequencing : The PCR products of 16s rDNA were sent for DNA sequencing to the Macrogen Company, South Korea. Basic local Alignment search tool (blast) sequence analysis were performed by blast algorithmic tool for the sequenced results at https://blast.ncbi.nlm.nih.gov/Blast.cgi. Phylogenic tree were generated using Mega Blast 10 computer software.

Detection of *Sph gene and pi-plc* genes by PCR

These genes were detected by PCR. The primers used in this study were *Pi-PLC* described by Richard and Sherry (7), F: AGT ATG GGG AAT GAC, and R: ACA ATT TTC CCA

CGA. The second gene was *SPh* described by Hsieh *et. al.,* (8), F: CGTGCCGATTTAATTGGGGC and R: AATGTTTTAAACAT GGATGCG. PCR mixture contained 12.5 µl of PCR green master mix (Promeg), 1 µl of each primer (BioNeer) and 5 µl of template DNA in a total volume of 25 µl with nuclease free water. For *pi-plc* gene, the cycling conditions were initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation step at 94°C for 1 min., annealing step at 50°C for 1 min, extension step at 72°C for 1min, and final extension step at 72°C for 8 min. For *sph gene*, the cycling conditions were initial denaturation at 94°C for 5min, followed by 35 cycles of denaturation step at 94°C for 20 sec., annealing step at 56°C for 20 sec, extension step at 72°C for 20 sec., and final extension step at 72°C for 8 min. PCR products were detected in 1.5 % agarose gel stained with ethidium bromide (0.5µg/ml), viewed by U.V. transilluminator and photographed.

RESULTS

DNA sequencing and Phylogenic tree

DNA sequencing of the isolates showed high percentage of homology with that strains previously registered at GenBank. Phylogenic tree of the results showed that the local isolates are closely related to those strains previously registered from different countries at the percentage ranged between 99-100% (Figure 1). Local isolates also showed two groups with 100% homology and 99% homology (Figure 2).

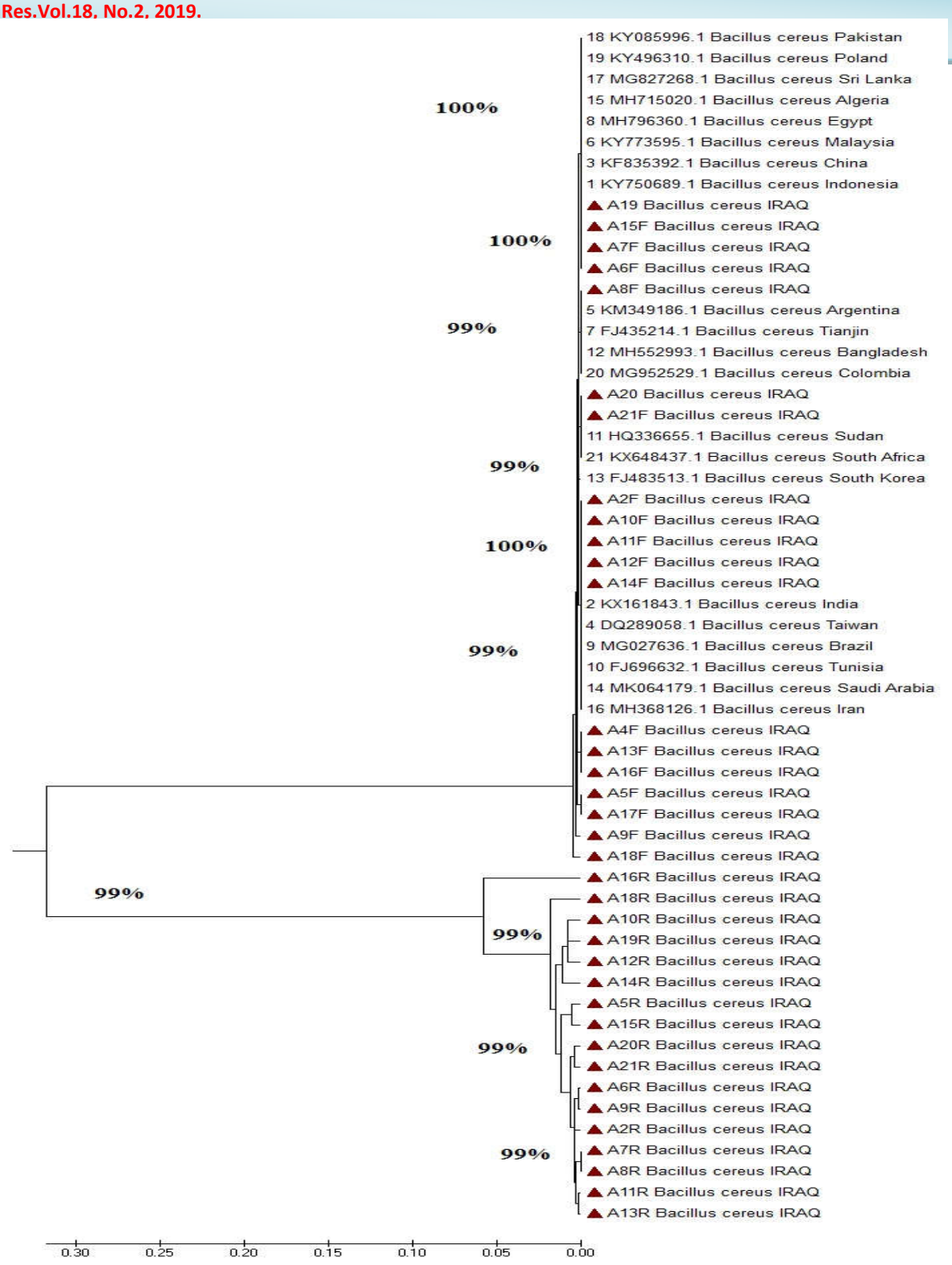

Figure 1: Phylogenic tree of local isolates of *Bacillus cereus* in comparison with previously register isolates

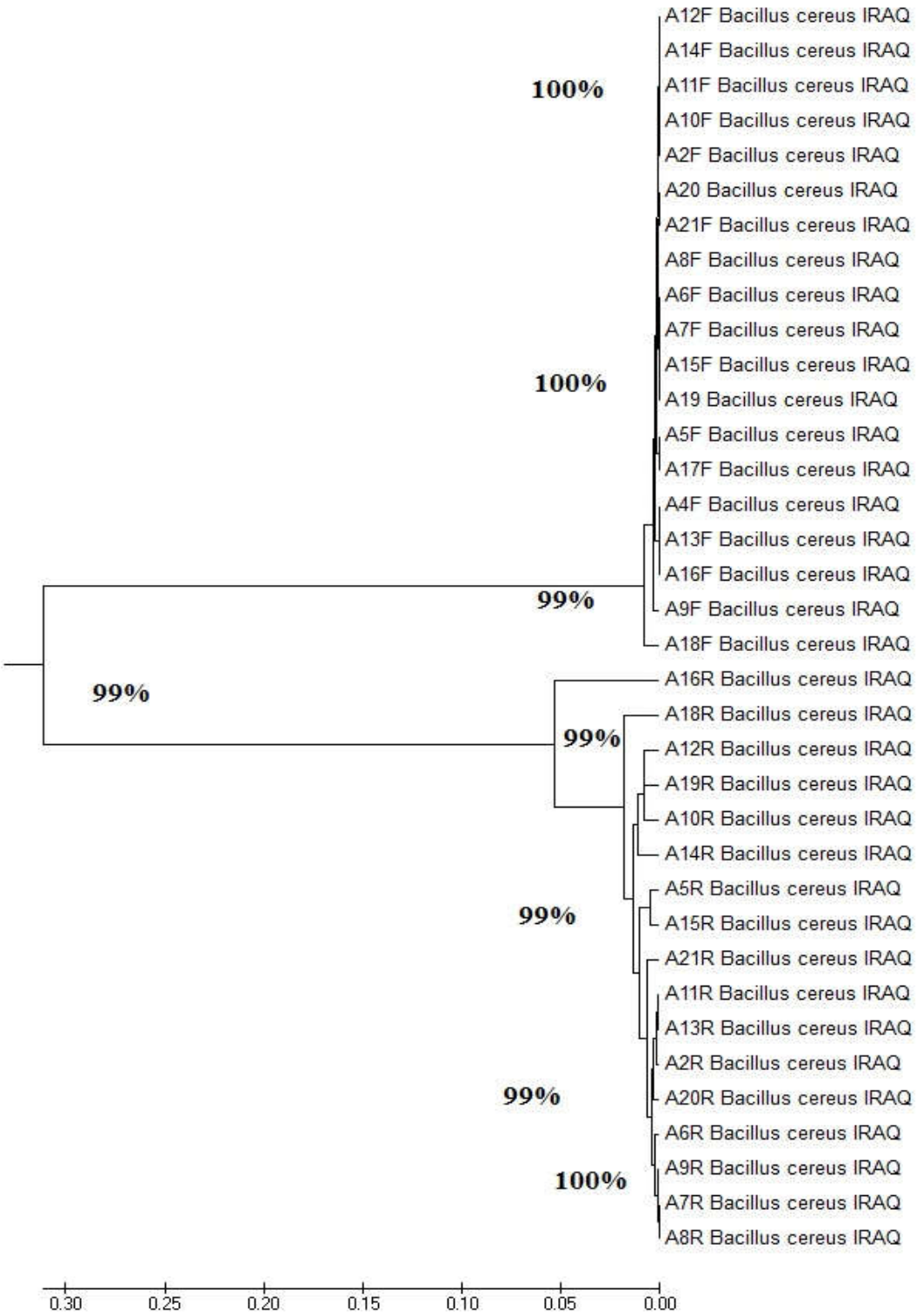

Figure 2: Phylogenic tree of local isolates of *Bacillus cereus*

Molecular detection of *Sph* and *pi-plc gens*

The *Sph* and *pi-plc* gene were detected in all of the isolates 70/70 (100 %). There were no significant differences (P > 0.05) in the presence of the gene in the samples (Table 1, Figure 3 and 4).

Table 1: Detection of *Sph* gene in *Bacillus cereus* isolates

Samples	Isolates	Positive Sph	Positive for *pi-plc*
Cream	6	6(100)	6(100)
Beef meat	6	6(100)	6(100)
Frozen beef meat	5	5(100)	5(100)
Burger	7	7(100)	7(100)
Cooked rice	5	5(100)	5(100)
Uncooked rice	10	10(100)	10(100)
Milk	11	11(100)	11(100)
Yogurt	10	10(100)	10(100)
Soft cheese	5	5(100)	5(100)
Curls cheese	5	5(100)	5(100)
Total	70	70(100)	70(100)

P > 0.05

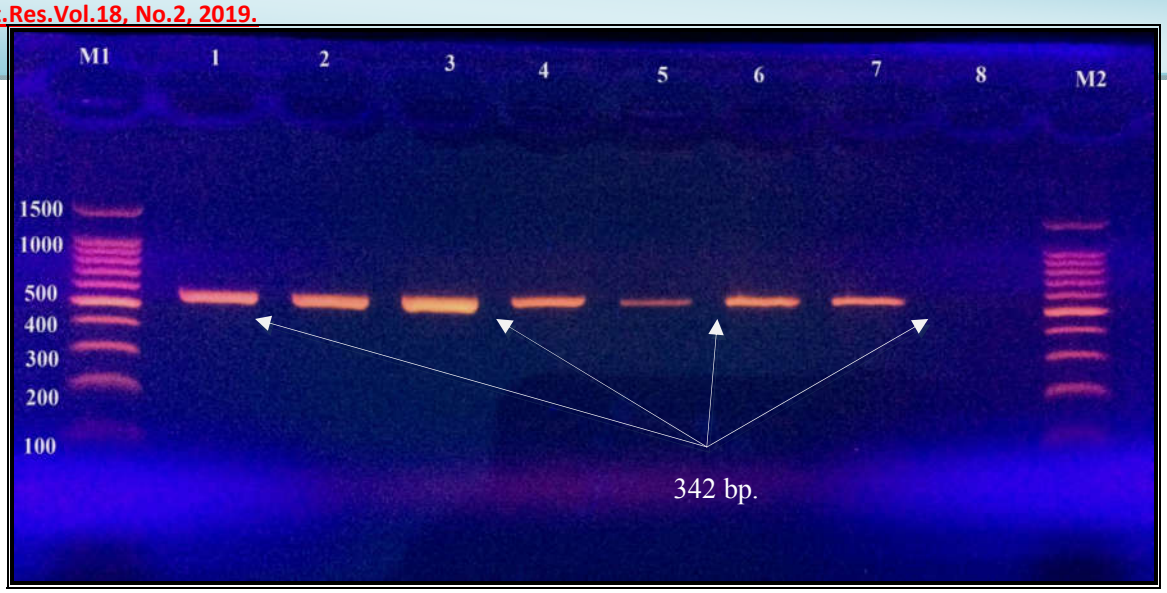

Figure 3: Molecular detection of *Sph* gene in *Bacillus cereus* isolates

Lane M1 = molecular marker; Lane 1- 7 = positive for *Bacillus cereus* isolates approximately 342 bp; Lane 8 = negative control

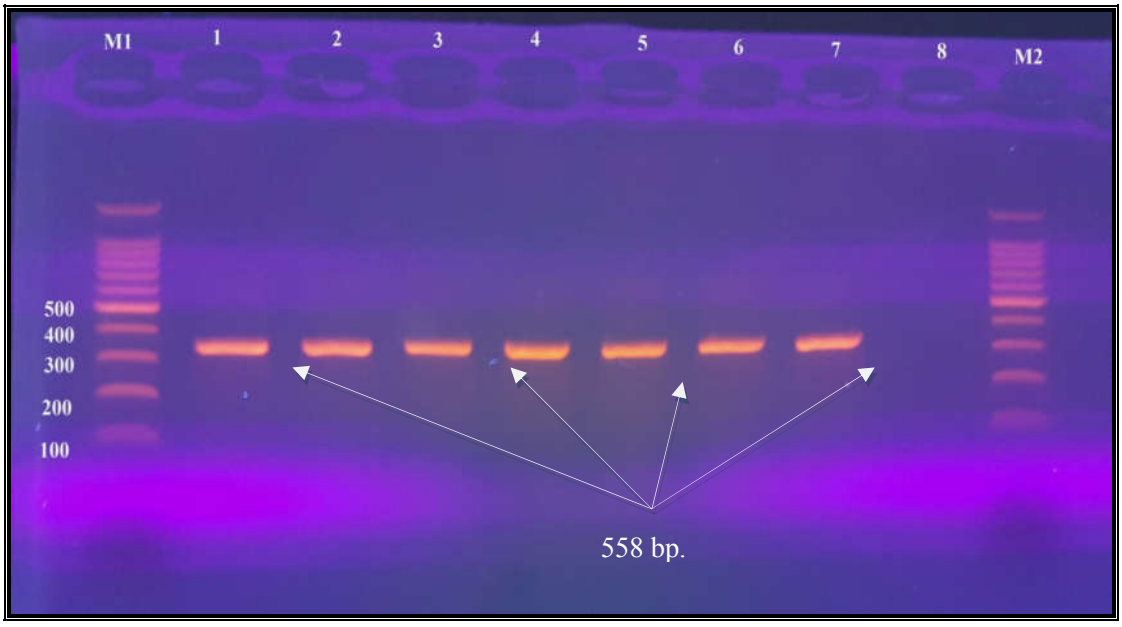

Figure 4: Molecular detection of *pi-plc* gene in *Bacillus cereus* isolates

Lanes M1, M2 = molecular marker; Lane 1-7 = positive for *pi-plc* gene; Lane 8 = negative control.

DISCUSSION

The DNA of all isolates was extracted by genomic DNA purification kit and the *16S rDNA* gene was detected in all isolates (100 %) and distributed in cream, beef meat, frozen beef meat, burger, cooked rice, minced rice samoles (5) . All isolates are compared with GenBank existing data and showed a ≥ 99% homology. The results showed that there are a several change at different loci. Phylogenic tree of the results showed that the local isolates are closely related to those strains previously registered from different countries at the percentage ranged between 99-100%. Local isolates also showed two groups with 100% homology and 99% homology. Bacillus cereus were commonly found in food samples in study area (9, 10, 11).

Enzymes such as phosphatidylinositol-specific phospholipase C (Piplc), and sphingomyelinase (Sph) are other potential virulence factors related to the pathogenicity of *B. cereus* (12; 13; 14). In this study, the *pi-PLC* and *Sph* genes were found in all of the isolates. This was agreed with Horii *et. al.,* (15). Previous studies (9, 10, 11) on emetic genes, enterotoxin genes and toxin genes in *B. Cereus* showed variation in presence of these genes in contrast with present study. We concluded that *B. Cereus* Iraqi isolates are quite similar to those previously registered in GenBank.

رسم الشجرة الوراثية لجراثيم الباسيللس سيرس المعزولة من مصادر غذائية مع التحري الجزيئي لامتلاكها لجينات الضراوه sph , pi-plc

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الخلاصة

تم دراسة سلالات جرثومة الباسيللس سيرس والمعزولة سابقا من المصادر الغذائية. زتم استخلاص الدنا من العزلات الجرثومية باستخدام عدة خاصة بذلك وشخص الجين ١٦س الرايبوسومي في جميع العزلات باستخدام البادئات الخاصة لذلك. اجري تتابع القواعد النتروجينيه وقورن مع العزلات المسجله سابقا في بنك الجيناتوتبين وجود تشابه عالي مع تلك العزلات. نتائج الشجرة الوراثية بينت بان العزلات المحلية قريبة جدا من تلك العزلات المسجلة مسبقا من دول مختلفة بنسبة

٩٩-١٠٠%. كما بينت الدراسة تواجد جينات الضراوة في كل العزلات ٧٠/٧٠ (١٠٠%). ولم يكن هناك اختلاف معنوي بين العزلات في جميع العينات.

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