Molecular Identification of Bacillus Sp. Isolated from Iraqi Soil and Efficiency of Antibiotics Production

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Abstract

The present study aims to isolating and identification local bacterial isolates producing antimicrobials from some of the soil samples of Thi-Qar governorate and detecting the presence of 16SrRNA gene by using DNA sequence technique. This study included collected 25 soil samples from different areas of Thi-Qar governorate. A series of dilutions was done for each sample and was cultured on the Nutrient Agar Medium. The molecular study was carried out by extracting DNA from all isolated by using a simplified method requiring less effort and simpler and less expensive materials. The presence of 16SrRNA gene was detected by using special primers and all isolates were given a positive result of this gene All isolates were tested for their ability to produce antimicrobial materials by taking tablets of isolates and streaking them on Muller Hinton Agar that cultured with one of pathogenic bacteria, which included *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi* and *Staphylococcus aureus* that identified by biochemical tests and VITEK 2 system.

Keywords: Bacillus, Antibiotics, 16SrRNA, Phylogenetic tree

1. Introduction

The genus Bacillus sp. Of the Gram-positive genera, it takes the form of a rod, live antenna, endospores, and this genus possesses many physiological abilities that allow it to live in multiple environments (Al-Allaf, 2011). This genus was first described by researcher Ferdinand Cohn in 1872 as positive bacilli (Banoun, 2015). Species of this genus are found in diverse environments such as soil, mud, dust, aquatic environments, plants, food, and digestive tracts of insects and animals. Species of this genus were isolated from plants and animals as natural flora or as pathogens.

Soil is the main habitat for the species of this genus as it is found on the roots or aerial parts of plants and has a role in helping plants to obtain nutrients while protecting them from disease and helping them produce plant hormones (Lyngwi and Joshi, 2014). Some microorganisms are characterized by their ability to produce secondary metabolites of different structures, as the effectiveness of these substances is regulated by nutrients, growth rate, enzyme activity and incubation period. These organisms produce these substances to help them compete with other organisms in their natural environment and adapt to the various changes that occur. In that environment (Al-saraireh et al., 2015)

The term "antibiotic" is derived from the term "antibiosis" which means "against life" (Bisht et al., 2011), and this term was used by researcher Selman Waksman in 1947 to describe the organic compounds produced by microorganisms that inhibit the growth of other organisms such as bacteria and fungi. Several studies have confirmed the ability of some Bacillus species to produce antimicrobial compounds that are effective against bacteria and fungi (Baruzzi et al., 2011).

Antibiotics can be divided according to their spectrum of activity into broad-spectrum antibiotics, which affect both gram-positive and gram-negative bacteria, and narrow-spectrum antibiotics, which affect a specific group of gram-positive or gram-negative bacteria (Lihan et al., 2014). The antibiotics produced by species of this genus can be classified according to the source of their formation into ribosomally synthesized peptide antibiotics known as bacteriocins, enzymatically synthesized peptide antibiotics, and non-peptide antibiotics (Torome, 2015). Antimicrobial compounds have been used for a variety of purposes, such as delaying spoilage by plant pathogens in agriculture and extending product shelf life in the food industry (Zhao and Kuipers, 2016).

2. Materials and Methods

Sample Collection

Twenty-five soil samples were collected from five different regions, then six decimal dilutions were conducted for each sample, and 1 ml of each dilution was placed on the nutrient acre medium for the purpose of initial isolation if the isolates were chosen based on the phenotypic shape of the colonies, their strength, color and size.

Diagnosis

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The isolates were diagnosed using Gram stain microscopically, and the presence of internal spores was detected using malachite green stain, as the diagnosis was made by VITK 2

Molecular Study

The genetic material of bacterial isolations was extracted using a modified method of boiling, the isolates growth on the of Nutrient Agar Media at a temperature of 37 and for 24 hours. Put 50 µl of ddH2O in Eppendorf tube size 1.5 ml. Then bacterial growth was transferred to the tube at a rate of two to three times. Place the tube in the water bath for 5 minutes at a temperature of 100 C°. Discard the mixture with the cooled centrifuge at 5000 RPM per minute for 5 minutes and then mix the sediment until the sample is homogeneous.

Detection of 16SrRNA Gene

1492F and 27R primer selected to determine the 16SrRNA gene, then the PCR technique was performed

Table (1): Primers used in the study to detect the presence of the 16SrRNA gene

NO.	Primers	Sequencing	No. of BPs	Concentration	Size
1	1492	TACGGYTACCTTGTTACGACTT	22	15 Pmol	1500 bp
2	27	AGAGTTTGATCMTGGCTCAG	20	15 Pmol	1500 bp

Purification of PCR products

Mega Quick-Spin Kite is used to purification of PCR products.

Send Samples

Eight samples were sent in accordance with the company's requirements, which included pure PCR products to the sample and a size of 20 µl and the F and R Primers prepared with a concentration of 17 Pmolfor each one.

Test the ability of isolates to produce antibiotics

To test the ability of bacteria to produce antibiotics, the Agar Disk Diffusion Method was used (Atsede, 2011)

3. Results

All isolates are positive bacteria and by using Malachite Green dye, it was confirmed the presence of spores inside the vegetative cells, taking different locations, which appeared in green.

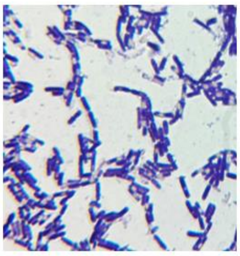

Figure 1: Gram stain to *Bacillus* sp.

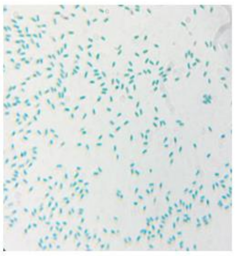

Figure 2: Endospore stain to *Bacillus* sp.

Detection of 16SrRNA

The results of the amplification of the 16SrRNA gene by PCR technology for the bacteria under study and using the front prefix 1492 and the rear initiator showed 27 positive results for this gene, which is located at the base pair 1500 bps as shown in the figure (3).

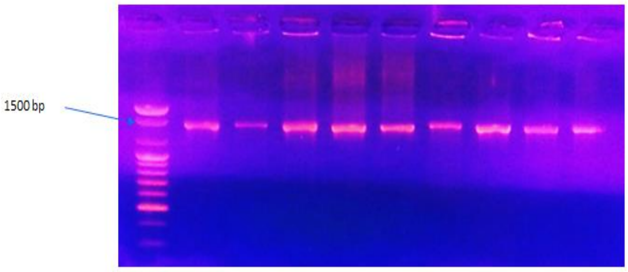

Figure 3: Electrophoresis of DNA extracted from Bacillus sp. after 60 minutes in agarose at a concentration of 1% under 100 V

Purification of PCR products

The purification process was carried out using the Mega quick-spine PCR purification diagnostic kit and as instructed by the manufacturer and shown in figure (4).

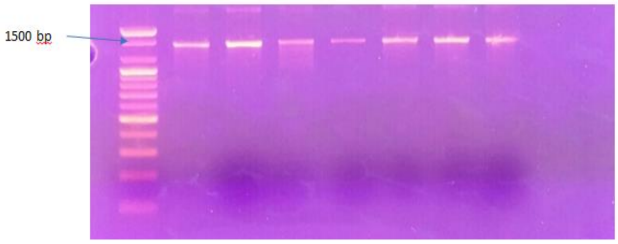

Figure 4: Electrophoresis of (16SrRNA) PCR products extracted from Bacillus sp. after 60 minutes in agarose at a concentration of 1% under 100 V

Compare the results with NCBI

The results of the samples after compared with NCBI showed the presence of three new bacterial strains that were recorded in NCBI and given special codes and the evolutionary tree of the three isolations was draw by Mega 6 programs.

Table (2): Registered new isolates in NCBI

Range of Nucleotide	Country	Location	Substation	Identification	Species
848	Iraq	MF373423	*Bacillus cereus* NM-1/Thi-Qar	86 %	*Bacillus cereus* a11
1413	Iraq	MF423696	*Bacillus thuringiensis* Bac-th/noor-1	98 %	*Bacillus thuringiensis* DB32
1452	Iraq	MF417393	*Bacillus cereus* Bac-cer/Nor-2	97 %	*Bacillus cereus* F5-1-35

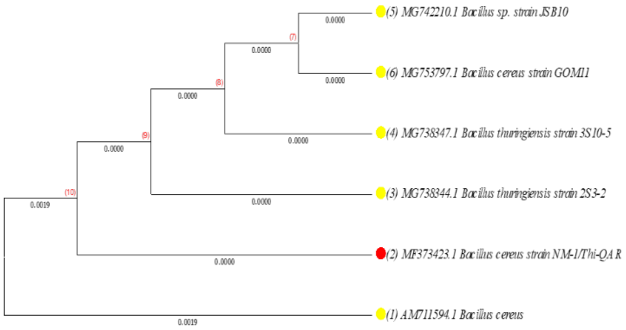

Figure 5: Phylogenetic tree of the isolate Bacillus cereus NM-1 Thi-Qar using Mega 6 software

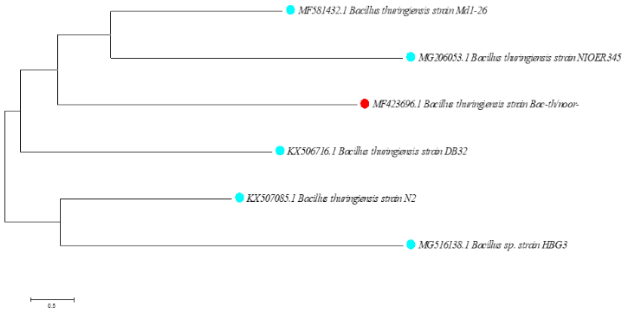

Figure 6: Phylogenetic tree of the isolate Bacillus thuringiensis Bac-th/noor-1 using Mega 6 software.

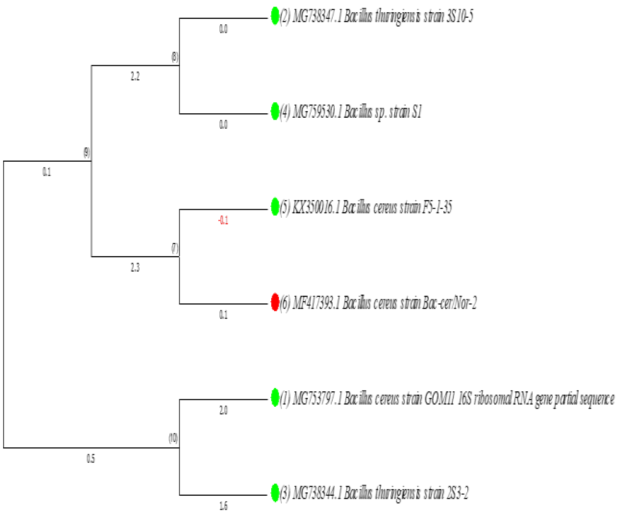

Figure 7: Phylogenetic tree of the isolate Bacillus thuringiensis Bac-cer/Nor-2 using Mega 6 software

Test the ability of isolates to produce antibiotics

The ability of isolated bacteria to produce antibiotics against pathological bacterial species under study was tested and the results were as shown in table.

Table (3): Test the ability of isolates to produce antibiotics

P. mirabilis	S. aureas	K. pneumonia	S. typhi	P. aeruginosa	Isolate
-	+	-	-	+	Bacillus cereus NM-1/Thi-Qar
+	+	+	+	+	Bacillus thuringiensis Bac-th/noor-1
-	+	-	-	+	Bacillus cereus Bac-cer/Nor-2

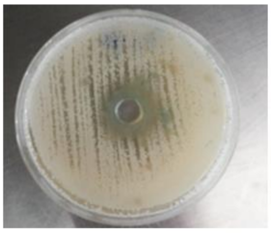

Figure 8: Antimicrobial activity of *Bacillus thuringiensis* Bac-th/noor-1 against *Staphylococcus aureus*

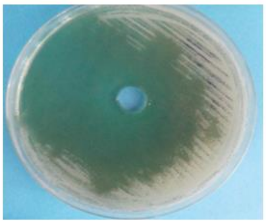

Figure 9: Antimicrobial activity of *Bacillus cereus* NM-1/Thi-Qar against *Pseudomonas aeruginosa*

4. Discussion

In the last decade, the tendency to use DNA sequencing technology has increased, due to the ability of this technology to diagnose bacterial species and genera very quickly, much better than following traditional methods of diagnosis, as the gene used in this technology to diagnose bacteria consists of many nucleotides containing specialized regions that allow by diagnosing bacterial species using special primers that amplify this gene (Jenkis et al., 2012).

The current results showed that all isolates were positive for this gene, and this result agreed with the researcher Nazia (2012) who indicated that the gene size is 1500 bp. The current study reached to obtain 3 new isolates after matching them with that found in NCBI, as the first isolate was diagnosed as Bacillus cereus 16SrRNA with a diagnosis rate of 86%, and it was recorded in NCBI (Bacillus cereus NM-1/Thi-Qar) and given the code MF373423, the second isolate diagnosed Bacillus thuringiensis DB32 with a diagnostic rate of 98% and recorded (Bacillus thuringiensis Bac-th/noor-1) and given the code MF423696, the third isolate diagnosed Bacillus cereus F5-1-35 with a diagnosis rate of 97%, and it was recorded (Bacillus cereus Bac-cer/Nor-2) and given the code MF417393.

All isolates were tested for their ability to produce antimicrobial activity using the method (Atsede, 2011), and the result was obtaining some isolates capable of secreting these substances, such as Bacillus cereus NM-1/Thi-Qar, Bacillus thuringiensis Bac-th/noor-1 and Bacillus cereus Bac-cer / Nor-2 , the result is consistent with the findings of Abo-Shadi et al., (2009) that all the microorganisms isolated from the soil are considered a good source of secondary antioxidants.

5. Conclusions

1- Living organisms, including bacteria, are exposed to many different conditions that lead to mutations in the genetic material, as the Bacillus was distinguished by its exposure to genetic mutations that led to the emergence of 3 new bacteria.

2- Bacillus bacteria were characterized by their ability to produce metabolites with the ability to inhibit the pathogenic bacteria under study.

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