Study Of Isolating The Mitochondrial DNA And The S28 Gene Of Periplaneta Americana In Two Areas Of Samarra City And Making A Genetic Variation For It.

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Abstract

The study was carried out at the end of 2019 and until mid-2020 aimed at identifying the genetic variation between the types of P. americana cockroach isolated from two areas in the city of Samarra, the district of Al-Mutassim and the center of Samarra and the work of comparing it with the international species.

Ten samples of adult P. americana cockroaches were isolated DNA also the analysis of the S28 gene to make the sequence of nitrogenous bases and compare with the global species registered in the Global Genebank.

The results of the current study showed that the best area for the extract of DNA is the mitochondria from the chest area, and the results of the S28 gene amplification showed that there is a percentage of 99% convergence between the species found in the city of Samarra, and also convergence with the global species registered in the Global Genebank, especially with samples from Iran and the United States of America, by 99%.

Introduction

The cockroach, one of the most common pests in city environments, is closely related to food that carries and spreads bacteria that are highly resistant to antimicrobials and can act as a vector for many microorganisms that affect public health. (Islam *et al.*, 2016).

The accurate classification of the cockroach is the cornerstone in developing management strategies for harmful species, so traditional methods of identifying the American cockroach are *P. americana* and other cockroaches based on morphological characteristics were problematic because of their very similar outward appearance, there is a high degree of similarity between adults and cockroaches. (Evangelista, 2014). The difficulty in distinguishing between the sexes due to the similarity in the genital organs is, therefore, necessary to apply a rapid and effective molecular identification method such as using the mitogenome (mitochondrial genes) to complement the morphological classification of *P. americana* mitogenome is characterized by maternal inheritance, reproducibility, and rapid development (Cameron, 2014)(Mustafa, & AL-Samarraie,2020)

It is the study of the genetic variation of the *P. americana* **Target** between two regions in the city of Samarra in central Iraq.

Materials and working methods -1

Breeding cockroach

Cockroaches were collecting from two areas of the city of Samarra, which is the Al-Mu'tasim sub-district and the city center of Samarra. And 300 cockroaches were collected by hand traps. Insects were bred and multiplied in plastic containers with dimensions of 25 x 40 cm. Inside it, wooden

structures put to suit cockroach life have been tunneled and covered with a light cloth. (Atiokeng Tatang, 2017).

And placed inside a room in a dark place with the provision of appropriate conditions of temperature ranging between 25-35 Celsius and relative humidity 65-70% Note that the replacement of water and food every ten days to avoid rot. (Abdaseed, 2017).

DNA Extraction

It was conducted extraction method according to G-spin tissue extract Kit protocol from a Korean company.

Buffers Used In DNA Extraction

Table (1) of the solutions to extract the DNA

the ingredients	the size
Buffer CL	**25 µL**
Buffer BL	**25 µL**
Proteinase K	**22mg x 1 vial**
Buffer CE	**µL 20**
RNase A (Lyophilized powder)	**vial 3mg x 1**
Absolute Ethanol	**µL 200**
Buffer WA	**40 µL**
Buffer WB	**10 µL**

(PCR) Polymerase Chain Reaction

Primers used in the reaction

Use a specialized primer for the gene ITS1 (Kumar et al., 2007) and consists of the following sequences: (Table 2) illustrates the specialized primers.

Dissolve the prepared primer in distilled water devoid of DNA digestive enzymes to obtain a final concentration of 100 pmol / µl. This solution is storage. The solution is prepared daily use by dilution of 10 pmol / µl of the storage solution in 90 ml of distilled water to become the final volume. 100 ml according to the instructions of the supplied company Integrated DNA Technologies company, Canada)).

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'- GAG AGT TMA ASA GTACGT GAA AC- 3 '	52.3	44	720 base pair
Reverse	5'- TCG GAR GGAACC AGC TAC TA - 3 '	56	42	

Table (2)) Primers for a gene S28)

The chain reaction mixture PCR / Master

The polymerase chain reaction was carried out using GoTaq® Hot Start Green Master Mix kit Materials in Schedule (3)

Includes contents in the table (3)

Material	Concetration
i-Taq DNA Polymerase	5U / µl
DNTPs	2.5mM
Reaction buffer (10X)	1x
Gel loading buffer	1x

Table (4) the contents of your interaction combination for the diagnosis of the gene S28

Material	Volume
Taq PCR PreMix	5µl
Forward primer	1µl
Reverse primer	1µl
DNA	1-1.5µl
Distill water	16.5 l
Final volume	**25µl**

Table (5) Optimum conditions for detection of the S28 gene

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min.	1 cycle
2-	Denaturation -2	95°C	45 sec	35 cycle
3-	Annealing	58°C	45 sec	
4-	Extension -1	72°C	45 sec	
5-	Extension -2	72°C	7 min.	1 cycle

The sequence of nitrogenous bases

Polymerase chain reaction (PCR) products were separated on 2% Agarose gel electrophoresis and visualized by exposure to highly violating light (302 nm) after ethidium bromide or red-spot staining. The genetic sequencing was performed by the National Instrumentation Center for Environmental Management (nicem). online at (http://nicem.snu.ac.kr/main/?en_skin=index.html). Biotechnology Laboratory, the device is DNA Sequencer 3730XL, Applied Biosystem). Homology research was conducted using the Basic Local Alignment Search Tool (BLAST) software available at the National Center for Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov) and the BioEdit program. (NCBI) online at (http://www.ncbi.nlm.nih.gov) and the BioEdit program.

Results and Discussion -2

2-1- Molecular genetic study

2-1-1- DNA extraction

The results of the DNA samples extracted from the cockroaches adults, and these collected from the city of Samarra - Iraq, using the prepared kit from the Korean company and measuring the concentration and purity of the DNA, showed that the concentration ranges from (45 - 60 nanogram/microliter) and high purity in the amount (1.8-1.6). The purity is ideal for making the repeat sequence of samples, as shown in Figure (3-1). Researchers explained that obtaining the best DNA from the chest area of an American cockroach insect because some parts do not give good quality of DNA such as legs and wings as well as the abdominal rings because they are full of inhibitors of the extraction process (Helmersson, 2013) Mustafa, et al.,2020).

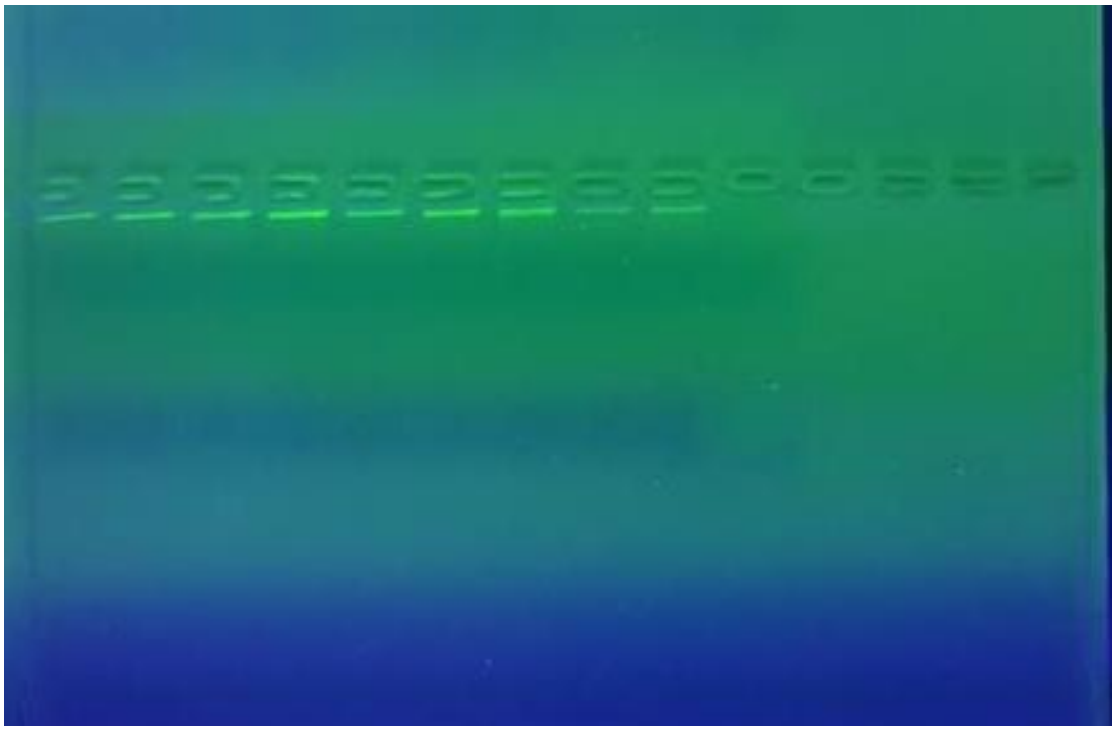

Figure (3-1) shows the DNA samples extracted from the whole American cockroach group and its samples from two areas of the city of Samarra in Iraq. And the stage on the agarose gel with a concentration of 1.5% and a voltage difference of 7 volts / cm2 for a period of half an hour, after the gel was dyed using the healthy red dye Red safe stain, then photographed with a UV machine.

P. americana adults were used in the current study, where DNA from the mitochondria was used to identify samples of unknown origin. (Hebert *et al.*, 2003). The use of the fast way to use DNA deoxyribonucleic (DNA) as a tool complementary to describe and distinguish species, to solve the problems of classification, and the concept of a piece of DNA specific, it is limited to the examination of a single gene or a piece of genes, this means that the molecular information available was also specific. (Helmersson, 2013) (Mohammed, et al,2020) .

2- S28 gene amplification

The results of the analysis of the S28 gene isolated from mitochondrial DNA showed that the bundles resulting from the migration process and using the PCR technique, and according to the appropriate conditions to amplify the S28 gene were of a molecular weight of 650 base pairs, and this is identical to most of the results of the previous molecular studies studied.

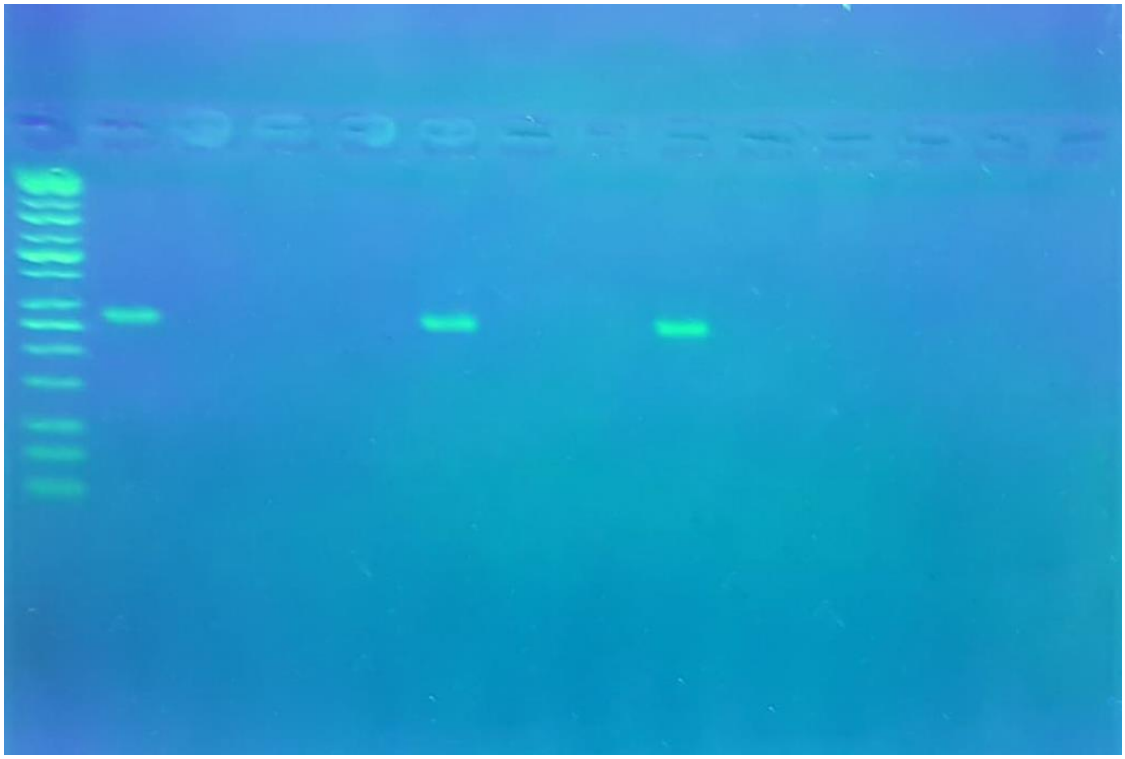

Figure (2-3) shows the results of amplifying the S28 gene from the DNA found in mitochondria of adult cockroaches grouped from two regions of Samarra city in central Iraq and relayed on a .51% agarose gel with a voltage difference of 7 volts / cm2 and for half an hour, dye the gel using A red safe nuclear coloring dye using an ultraviolet ray machine.

As the researcher found Farmani *et al.* (2019) AL-Samarraie,*et al* (2019) during his study of the *P. americana* cockroach located in the city of Urmia in Iran that the size of the ITS1 gene segment is 550 base pair, knowing that the researcher had used other methods with the two methods used in the study, while the results of the researchers Cevahi and Duzlv (2019) showed that the size of the amplification piece 384 is a base pair that amplifies a region of the mt-COI gene of the cockroach species found in the city of Kayseri where it converges with other cockroach species by 99% in Turkey.

No. Of sample	Type of substitution	Location	Nucleotide	Sequence ID	Score	Identities	Source
1	Transvertion	38	T> G	ID:KF899831.1	627	99%	Periplaneta americana
	Transvertion	45	C> G				
5	Transvertion	382	G> T	ID:AF321248.1	1163	99%	Periplaneta americana
	Transvertion	390	C> G				
	Transition	400	G> A				
	Transvertion	407	A> T				
	Transition	431	T> C.				

3- Follow the nitrogenous bases.

After the gene amplification process S28 using the repeat reaction technique, the nucleotide sequences were determined for two samples of the *P. americana* cockroach adult collected; The PCR products were sent to a Korean company, Macrogen.

Using the website of the Global Genebank Organization NCBI and then enter the sub-window Blast nucleotide, and by comparing it with the global types registered in NCBI, The samples studied in Iraq matched by 78 - 99% with the samples registered in the NCBI genebank. The sample No. (1) the collection from Samarra city center matched with the NCBI World Bank and within The global number ID: AF321248.1 with the isolated Iranian sample from four cities in Iran at an amount of 99% and within Score 228. 0 and Value Expect - E 0, corresponding to the phenotypic classification.

Note that Score represents a measure that reflects the degree of similarity between the sequences under study and the sequences of the genetic data bank. The greater the amount of similarity, the greater the amount of value. If it is a measure of the statistical significance of the similarity, if the score falls below 50 points, then this means that there is no similarity mentioned.

As for the value Expectation value, A value that gives an estimate of the number of times expected to obtain the same similarity by pure chance. The lower the E. Value indicates an increase in high similarity between the sequences, which gives greater confidence that these sequences correspond to the sequence in the World Bank.

And since it was valuable E.Value is very close to zero. The sample showed heterogeneities within the nucleotides represented by the presence of inversion heterogeneities in the nucleotide transversion number 1 and the substitution heterogeneities number 1 (G> A; G> A).

While the results of sample No. (2), which were collected from the Al-Mu'tasim area, were shown and registered in the Global Genebank under the serial number ID: KF899831.1 A 99% match rate with the US sample of serial number ID: AF321248.1 and that the match rate is due to the presence of variances in the sequence of nitrogenous bases if they are obtained and Score 0 and 0 E. value 0 - with 4 inversion covariance and 2 substitution covariance; While the phenotypic diagnosis of the aforementioned type showed a match according to the classification keys adopted in the study.

Conclusions:

Sample No. (1) matches the Iranian samples, and sample No. (2) matches the sample of the United States of America.

Recommendations: Conducting a study of other genes for the studied samples, and conducting studies of samples with samples from neighboring countries of Iraq.

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