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DETECTION OF RECA GENE IN *DEINOCOCCUS RADIODURANS* BACTERIA BY USING THE PCR TECHNIQUEN

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ABSTRACT

This study used 200 Different samples including, 100 soil samples collected from Basrah and Ramadi governorate in to areas already exposed to radiation as a result of wars; 100 swab samples, which were collected from two hospitals in Baghdad (Kamal al-Samarrai and Radiation and Nuclear Medicine Hospital) from the operating rooms, equipment and medical instruments after ultraviolet exposure as a means of fogging. Molecular diagnosis was conducted by isolation of DNA content and detection of 16 rRNA gene by using a *Deinococcus* specific hemi-nested primer, then detection of *RecA* gene using polymerase chain reaction (PCR). The results revealed (100% isolates) from both Bsraha and Ramadi soil samples. Results agree with the routine assays carried out the *Deinococcus Radiodurans* isolate from Kamal al-Samarrai Hospital and Radiation and Nuclear Medicine Hospital) showed that all of them carry multiple different isolates but they included *Deinococcus Radiodurans* isolate between (30-10 %) from the 100 swab samples. One of the aims of this study was to adopt an accurate diagnostic method to detect *Deinococcus Radiodurans* by its genetic material contents through extracting DNA and gel electrophoresis of the PCR product for the specific gene. Detection of radiation-resistance gene represented by a confirmatory test was carried out for the selected isolates using the Hemi-nested polymerase chain reaction technique for further characterization up to the species level by the amplification of (***Eub27F*** and ***Eub1107R***) genes .These are specific genes that encode the universal eubacterial characteristic which is unique to DNA damage agents. The result of (***Eub27F*** and ***Eub1107R***) genes were used as a template for the *Deinococcus Radiodurans* specific primer (**Deino202F**) gene then detection of (**RecA**) gene.

All the positive isolates by the routine tests were found to be positive for the molecular tests: Presence of (***Eub27F*** and ***Eub1107R***) genes as their agarose gel revealed with a molecular size about (1100 bp), (**Deino202F**) gene with a molecular size about (900bp) and (**RecA**) gene with molecular size about (254bp).

KEYWORDS: *Deinococcus Radiodurans* specific hemi-nested primer, ***Eub27F*** and ***Eub1107R.***

INTRODUCTION

Deinococcus radiodurans was first discovered in 1956 in a can of ground meat that had been treated with large doses of radiation to remove all hazardous bacteria from the product.[1] Since its discovery, it has been deemed the toughest bacterium in the world. Not only can it withstand and repair DNA damages after extreme amounts of ionizing and UV radiation, but it can survive drought conditions and grow in nutrient poor environments.[2],[3] Its specific abilities make it one of the most interesting bacteria in science today because studying and understanding its mechanisms can lead to nuclear waste pick up and medical uses associated with cancer Radiation tolerant species, retains multiple copies of genes as 'back-up' in the event of radiation damage to DNA. *D. radiodurans* is a gram positive, berry-shaped bacterium.[4]

[5]When grown in cultures, smooth colonies range in color from red to pink *D. radiodurans* ability to withstand extreme amounts of radiation has allowed it to survive in unusual, sterile places, such as a can of meat that had been sterilized using gamma radiation and medical instrument.[6] Because it is not dependent on water , it can survive up to six weeks without it.[7] This radiation resistance belong to RecA protein so that we are generally detected on *RecA* gene using molecular assay with *Deinococcus Radioduranse* specific primer by hemi-nested PCR amplification.[8]

MATERIALS AND METHODS

One hundred samples have been isolated from a variety of sources including different environments, rich in organic nutrients, such as soil, sometimes even from harsh environments like areas exposed to radiation (Rumady and Basrah), room dust and medical instruments Samples were collected from Baghdad hospitals (Radiation and Atomic Medicine and Kamal al-Samarrai).

Identification of bacteria

The radiation resistant isolate was done according to the Bergey's Manual. Bacteria were identified according to.[9], [10], [11]

Morphological and Microscopical characteristics

A. Morphological characteristics

The irradiated soil sample was suspended in 4.5 ml of saline (0.85 % NaCl), vortexed thoroughly and appropriate dilutions were plated on TGY agar plates and incubated for 4-5 days at 30 °C. Morphologically distinct colonies were purified and maintained on TGY plates.[12]

B. Microscopic study by Gram's staining method

Gram's staining was performed as per procedures described by[10] to determine the size, shape and arrangement of bacteria.

Biochemical Tests

Biochemical characterization of the radiation resistant isolates was done according to the Bergey's Manual.[9]

Bacterial DNA extraction and purification

This procedure was carried out by using commercially available DNA extraction and purification kit (i-genomic BYF DNA Extraction Mini Kit) .The procedure was explained in detail in the user's manual.

Bioinformatics tools used for the designing of *RecA* gene for Deinococcus specific primer

The *recA* gene sequences of genus Deinococcus as listed in the NCBI nucleotide Database (http://www.ncbi.nlm.nih.gov/nucleotide/) were the sequence used to blast primer. The primers used in this study are given in Table number (1).

Table(1): Oligonucleotide primer sequences used for PCR amplification

Primer	Sequence (5'-3')	Reference
Eub27F	AGAGTTTGATCCTGGCTCCAG-3-5	[13]
Eub1107R	5-GCTCG TTGCGGGACTTAACC-3	[13]
Deino202F	5-GGGTTGCGTTCCATCAGC-3	[14]
RecA	F :5- CCCCAGGACTGAGCTTTACC-3 R :5- AGGCCTTTTCGATCTGGCTC-3	This study

PCR amplifications of target sequence

The amplification mixture contains a refrigerator of Promega Company. Lyophilized primers were dissolved in a free DNase/RNase water to give a final concentration of (10 pmol/µl). All tubes were centrifuged in a microcentrifuge for 10 seconds. The PCR tubes were transferred to the thermalcycler to start the amplification reaction according to a specific program for each primer.

Amplifications conditions

PCR reaction was optimized with the following parameter

PCR amplification of 16S rRNA gene using universal primers

Universal eubacterial 16S rRNA PCR primers, Eub27F and Eub1107R (Table 2) size (1100bp), obtained from, were used for amplification of 16S rRNA gene either from pure cultures or from soil community DNA. PCR was carried out in 100 µl reaction mixture consisting of:

Table: (2): Mixture of PCR reaction.(Eub27F, Eub1107R gene)

Chemicals/Reagent	Volume (µl)
Master Mix	50
F-Primer	2.5
R-Primer	2.5
DMSO	1
D.W	34
DNA sample	10
Final volume	100 µl

Table (3): PCR program of Eub27F, Eub1107R gene

No.	Steps	Temperature	Time	No. Of cycles
1	Denaturation 1	95C°	4min	1 cycle
2	Denaturation 2	94C°	40sec	35cycles
3	Annealing	65C°	1min	
4	Extension 1	72C°	1min	
5	Extension 2	72C°	10min	1 cycle
6	Holding	15 C°	5min	• cycle

PCR amplification of 16S rRNA gene using *Deinococcus* specific hemi-nested primer

16Sr RNA gene amplicon obtained as above (SectionA) was used as template for hemi-nested PCR using forward *Deinococcus* specific primer Deino202F (Table 4), (numbering corresponding to the 202-222 *D. indicus* 16S rDNA gene sequence) and 1107R as the reverse primer size (900bp). Following additives were added to the PCR mixture individually or in combinations mentioned at a final concentration as given:

Table: (4): Mixture of PCR reaction (specific hemi-nested primer)

Chemicals/Reagent	Volume (µl)
Master Mix	25
F-Primer	1.25
R-Primer	1.25
D.W	17
DMSO	0.5
DNA sample	5
Final volume	50 µl

Table (5): program for hemi-nested PCR

No.	Steps	Temperature	Time	No. Of cycles
1	Denaturation 1	95C°	5min	1 cycle
2	Denaturation 2	94C°	45sec	35cycles
3	Annealing	58C°	30sec	
4	Extension 1	72C°	1min	
5	Extension 2	72C°	10min	1 cycle
6	Holding	15 C°	5min	• cycle

Detection of *RecA* gene designing of *Deinococcus* specific primer **size(245bp)** (Table 6)

Table: (6): Mixture of PCR reaction(*RecA* gene)

Chemicals/Reagent	Volume (µl)
Master Mix	25
F-Primer	1.25
R-Primer	1.25
D.W	17
DMSO	0.5
DNA sample	5
Final volume	50 µl

Table (7): PCR program of *RecA* gene

No.	Steps	Temperature	Time	No. Of cycles
1	Denaturation 1	94C°	4min	1 cycle
2	Denaturation 2	94C°	30sec	35cycles
3	Annealing	57C°	1min	
4	Extension 1	72C°	1min	
5	Extension 2	72C°	5min	1 cycle
6	Holding	15 C°	5min	1 cycle

RESULTS AND DISCUSSION

Result of Polymerase Chain Reaction (PCR) amplified regions showed a molecular weight of 1100 bp; represent the universal region of the 16sRNA gene amplified by E1107 R and Dino 202F. the second PCR amplification was showed a molecular band of 900bp represent the

specific region 16sRNA gene for *Deinococcus Radiodurans* using the primers Eub27F and Eub1107R according to(kim *et al.*, 2002), DNA ladder (100-1000) was used and the gel was photographed by a digital camera (See Figures (1) and (2)).

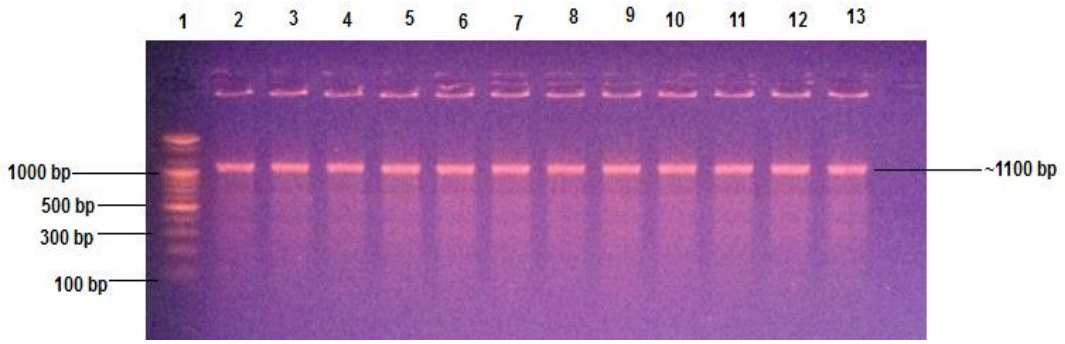

Figure (1): lane 1: 100-bp DNA marker; Lane 2 – 13: PCR amplification of eubacterial 16S rRNA using universal primers (1100bp) were separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide at 60 volts/cm for 1 hour. Photographed under UV light.

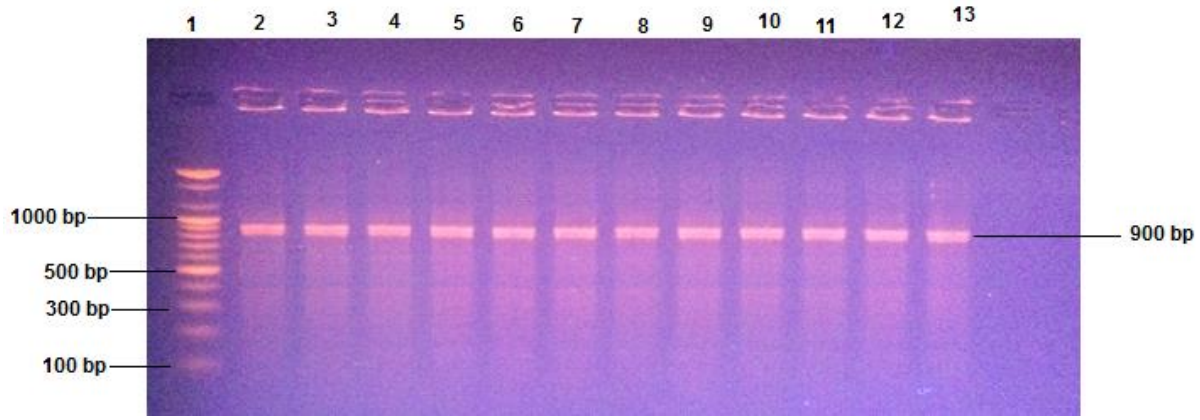

Figure (2): lane 1: 100-bp DNA marker; Lane 2 – 13: Heminested PCR amplification of deinococcal 16S rRNA (900bp) were separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide at 60 volts/cm for 1 hour. Photographed under UV light.

The use of 16S rRNA gene sequences to study bacterial identification and taxonomy has been by far the most common housekeeping genetic marker used for a number of reasons. These reasons include (i) its presence in almost all bacteria, often existing as a multigene family, or operons; (ii) the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution); and (iii) the 16S rRNA gene (1,500 bp) is large enough for informatics purposes.

The strains of Deinococcus radiodurans formed a well delineated subclade in the 16S rRNA gene tree, a taxon that was supported by all of the tree-making algorithms and by a 100 % bootstrap value, which will be essential to the studies work on genotyping using 16sRNA.[15]

Classification of *Deinococcus radiodurans* has been difficult. It is not a member of the archaebacteria because it contains peptidoglycan in its cell wall, yet it contains genes that are not typical of other eubacteria, because of the unique cell wall and radiation resistance, it appeared that *D. radiodurans* and its related species belonged in aphyla of their own.

Upon the development of 16S rRNA technology, it was determined that the phyla *Thermus* and *Deinococcus* were related. Even though members of the phyla *Thermus* are radiation sensitive thermophiles and members of the phyla *Deinococcus* are radiation resistant mesophiles, it has become apparent that each lineage evolved from a common ancestor. When examining *D. radiodurans* and *Thermus thermophilus*, there is a great deal of homology between their genome.[16]

On the basis of phylogenetic and taxonomic data, another genus, the genus *Deinobacter*, was described and added to the family *Deinococcaceae*.

Although the chemotaxonomic and phylogenetic data indicated that *Deinobacter grandis* and the members of the genus *Deinococcus* are closely related, a new genus was created on the basis of the rod-shaped morphology of the new isolate.[17]

The phylogenetic relatedness of the genera *Deinococcus* and *Thermus* was subsequently demonstrated by comparing 16s rRNA catalogs,[18] and the significance of this relationship was further investigated by analyzing nearly complete 16s rRNA sequences. At this time, only one complete 16s rRNA sequence has been determined for the deinococci. This sequence is derived from *Deinococcus radiodurans* UWO 298, which is the type strain of the species, and has recently been used to design oligonucleotide probes which reportedly are strain, species, and genus specific.[19] In order to determine the degree of phylogenetic diversity within and between the genera *Deinococcus* and *Deinobacter*, the 16s ribosomal RNA (rDNA) sequences of the type strains of the six species of these genera were determined by using the universal primer **(Eub1107 R)**. So our results of the first primer (**Eub1107 R-Eub27 F**) consider a primary screening to find the genus that represents the deinococcus.

The members of the genus *Deinococcus* radioduranse are extensively studied because of their exemplary radiation resistance. Both ionizing and non-ionizing rays are routinely employed to select upon the radiation resistant deinococcal population and isolate them from the majority of radiation sensitive population. There are only few studies on the development of molecular tools for the rapid detection and identification of *Deinococci Raduranse* from a mixed population without causing the bias of radiation enrichment. a specific two-step hemi-nested PCR for the rapid detection of deinococci from environmental samples by using specific primer **(Deino 202F)**. The method is sensitive and specific to detect deinococci without radiation exposure of the sample. The new protocol was successfully employed to detect deinococci species from several soil samples, same result has been obtained when it used to detect mixed sample from different geographical regions of India.[20]

It has been shown previously that the RecA protein of *Deinococcus Radiodurans* plays a unique role in the repair of DNA damage in this highly DNA damage resistant organism. Result of Polymerase Chain Reaction (PCR) amplified regions showed a molecular weight of 245 bp; represent the *RecA* gene amplified by RecA R and RecA F see figure (3). This primer was designed on NCBI database.

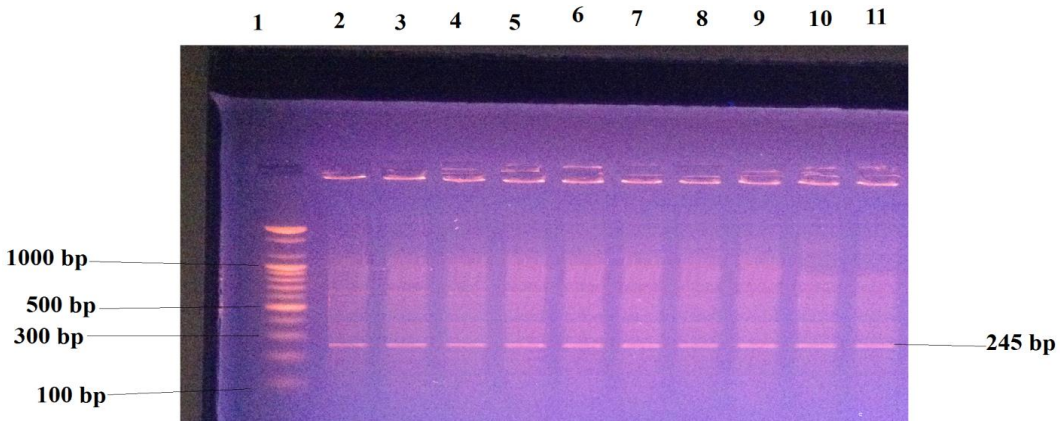

Figure (3) lane 1: 100-bp DNA marker; Lane 2 – 11: PCR amplification of *RecA* gene (245 bp) were separated by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide at 80 volts/cm for 1 hour. Photographed under UV light.

Deinococcus radiodurans is remarkable for its extraordinary resistance to ionizing and UV irradiation and many other agents that damage DNA. This organism can repair >100 double-strand breaks per chromosome induced by ionizing radiation without lethality or mutagenesis.

According to,[21] we have previously observed that expression of *Deinococcus Radiodurans RecA* in *Escherichia coli* appears lethal. In this research, the gene *RecA* was taken from the *Deinococcus Radiodurana* and cloning the specific *RecA* gene in *E.coli* then treated with different radiation level . Result showed that the transformer *E.coli* had higher resistance to Y and UV radiation than the original strain after treated with 150 Gy Y radiation, so here we are mention the roles of *RecA* gene in radiation resistance.

The results have shown that all samples have positive PCR results. This means that *Deinococcus Radiodurans* is present in the Iraqi environment dramatically as a result of remnants of war during the previous years that led to the accumulation of high levels of radiation constituting an environment suitable for the growth of bacteria that have been isolated and to make sure that the primers designed are used in this research are specialized for gene *RecA* returns to *Deinococcus Radiodurans*.

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