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ADOPTION OF CYTOCHROME B AS A MEANS TO IDENTIFY THE SOURCE OF DNA USING POLYMERASE CHAIN REACTION

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ABSTRACT

The present study was aimed to determine the type of biological material of unknown origin through the cytochrome b gene besides PCR, and this is important for samples for forensic medicine lawsuits involved in the investigation Criminal, as the forensic task to resolve the issues in routine casework, sometimes it is necessary to identify the exact species of the biological material. Samples, which included several isolates of *Pseudomonas aeruginosa* and blood of different animal sources included (rabbits, chickens, sheep), human blood and corpses tissue. It was detected amplification of the gene coding for the types of product covered by the study, where *P.aeruginosa* gave the package, which appeared the size of 460 base pairs. As was detected amplification of the gene coding for the following kind of product (Sheep, chickens, rabbits), where the given package size 331, 227, 740 base pairs respectively. The product amplification of the gene coding for the type of human gave specialist pack size of 580 base pairs for each of the blood and corpses tissue samples.

KEYWORDS: polymerase chain reaction, cytochrome b, *Pseudomonas aeruginosa*, corpses tissue.

INTRODUCTION

The polymerase chain reaction is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.[1] PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications.[2] This technique rapidly become one of the most widely used techniques in molecular biology because it is quick, inexpensive and simple. PCR could be definition as in

vitro method for replicate determining sequence from DNA using short nucleotide sequence (primer) had random or specific sequence, deoxy nucleosides triphosphate and polymerase, at which in few hours the small fragment of DNA could be replicate for one billion.[1,3]

The study of Parson (2000) and other studies proposed the identification of vertebrate species by nucleotide sequence analysis of the cytochrome b (*cyt b*) gene.[4] The *cyt b* gene is one of the 37 genes within the circular mitochondrial genome.[5,6] This gene is ideal for species identification as it shows limited variability, within and much greater variation between species, cytochrome b gene is excellent for phylogenetic work because the rate of evolutions in the silent positions which do not change the amino acid sequence of the translated protein is relatively fast.[7] Also contains large regions of interspecies sequence diversity with little or no intraspecific variation, as well as several regions that are conserved, allowing for short fragments along its entire length to be amplified using trans-vertebrate primers.[8]

Aims of Study to determine the type of biological material of unknown origin through the cytochrome b gene sequence analysis by using PCR technique and this is important for samples with mixture DNA or samples of human, animals or bacterial that arrive to lab for forensic medicine lawsuits involved in the investigation Criminal, as the forensic task to resolve the issues.

MATERIAL AND METHODS

Sampling

Samples for genetic analysis were taken from different species included Six isolates of bacteria *Pseudomonas aeruginosa* and blood samples from different animals including (5 Chicken, 5 Rabbits, 5 Sheep) also 5 healthy human blood, in addition to 6 tissue samples taken from corpses. Bacterial samples that collected from hospitals in Baghdad Governorate (Central Teaching Hospital of Pediatric, Al Kindy Teaching Hospital). Clinical specimens from the patients admitted the hospital included urine, wounds, blood and lung fluid. Blood; Blood samples (chicken, Sheep) were taken collaboration with Office of Agricultural Rese, while Rabbit Blood samples taken from Al- Mustansiriyah University department of Biology (Animal House), in addition to human blood taken from healthy donors. Approximately (2-2.5) ml of blood was taken by sterile syringe and place in EDTA tube kept frozen at -20°C until processed further for DNA extraction. Tissue samples were taken from corpses (Forensic Medicine institute) stored in normal saline at -20°C until DNA extraction.

Identification of bacterial isolates

Bacterial isolates were identified using standard microbiological methods primary identification by biochemical tests including Catalase test, Oxidase test and blood hemolysis, after isolation by culture, the API biochemical identification system was used for identification.[9] Then identified via the VITEK® 2 Compact system (Bio Merieux, France), in accordance to the manufacturer's instructions.

DNA Extraction

DNA was extracted from 36 sample including (bacteria, blood, tissue) using Relia Prep™ Blood gDNA Miniprep System extraction kit (Promega) for blood and Wizard® Genomic DNA Purification Kit (Promega) for bacteria and tissue following the manufacturer's instructions.

Purity of the extracted DNA was assessed by calculating the OD260/OD280 nm ratios using nanodrop (Quawell Q5000- USA).

DNA quality by running the total DNA in 1% agarose gel electrophoresis with ethidium bromide-stained agarose gel and under Ultraviolet light, Photographs were documented through a Videodocumantetion system.[10]

Primer Design

Table 1: Primers used in this study.

Specific species primer	Primer sequence (5´- 3´)		PCR product size (bp)
pseudomonas aeruginosa	F	GGCTGGATCATTCGCTACAT	460
	R	CGTTCTCGTCCTTCTTCTTCTT	
Human	F	GACACCTGCGTTTGGTTTATG	580
	R	AGCAATTCCTCTCTGGGTTTAG	
Rabbit	F	CACTCACACACACACCATACA	740
	R	CCCATTCTCAGGCTACTTTCTC	
Sheep*	F	GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA	331
	R	CTATGAATGCTGTGGCTATTGTCGCA	
Chicken*	F	GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA	227
	R	AAGATACAGATGAAGAAGAATGAGGCG	

Cytochrome b gene sequences of the Human, Rabbit and *Pseudomonas* were obtained from Gen Bank (http://www.ncbi.nlm.nih.gov/gene) provided in the public domain by the National Center for Biotechnology Selected sequences of cytochrome b gene were aligned using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST) to achieved similar sequence

among sub species or strains, conserved sequences were analyzes then primers have designed with use of Primer Blast (http://www.ncbi.nlm.nih.gov/BLAST) software. The primers were described in Table 1.

*Numbering is according to.[11]

PCR amplification

The amplification was carried out by using Green Master mix Kit (Promega, USA). Each PCR reaction was carried out in a total volume of 20µl, it composed from 2µl of DNA templet, 1µl of each primers, 10µl green master mix, then the volume was completed to 20µl, PCR program that apply in the thermocyler in Table 2. Successful amplified multiplex PCR product was analysed by agarose gel electrophoresis of 5µl amplified DNA sample was transferred into the wells in 1% agarose gel 1.20hr/100v, one well put 5µl DNA ladder mixed with 1µl of loading buffer. DNA ladder (100bp) was used to assess PCR product size. UV transilluminater was used for the observation of PCR products and were visualized by UV light. Photographed using a digital camera.[15]

Table 2: PCR program that apply in the thermocyler

1 cycle of: Predenaturation at 95°C for 5 minutes
35 cycles of: Denaturation 94°C for 30 second Annealing 60°C for 30 second Extension 72°C for 30 second
1 cycle of: Final extension 72°C for 7 minutes
Hold samples at 4°C (time ∞).

RESULTS AND DISCUSSION

In this study the DNA concentration that extracted from diffrent samples ranged between (178.0-196.0) ng/ul, While the purity (1.8-2.0), in addition using electrophoresis technique to ensure quality of DNA comparing to DNA ladder (Promega) as shown in Figure 1.

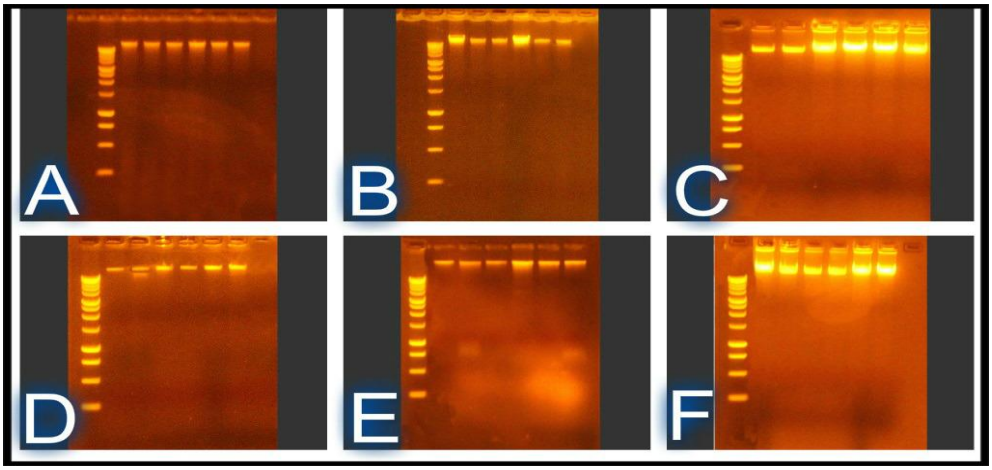

Figure 1: Gel electrophoresis of DNA (100V/80 min.) in 1% agarose, showing the presence of DNA, visualized under UV after staining with Ethidium Bromide.

A: Electrophoresis of the extracted DNA from *Pseudomonas aeruginosa*.
B: Electrophoresis of the extracted DNA from human blood.
C: Electrophoresis of the extracted DNA from corpses.
D: Electrophoresis of the extracted DNA from rabbit blood.
E: Electrophoresis of the extracted DNA from chicken blood.
F: Electrophoresis of the extracted DNA from sheep blood.

The mixing of all DNA samples (5µl of each sample) equals 5.00 micro-grams and amplified of cytochrome b by using primes dedicated to the study, the result 460, 227, 740, 331 and 580 bp for *P. aeruginosa*, chicken, rabbit, sheep and human respectively (Figure 2).

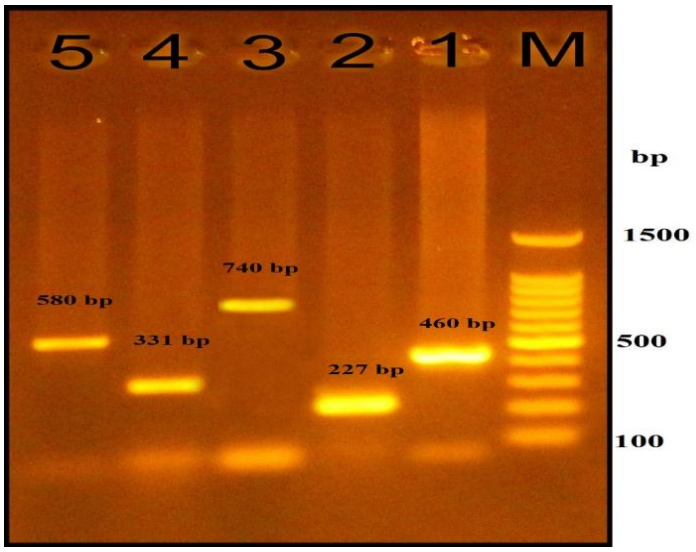

Figure 2: Agarose gel electrophoresis of PCR products amplified cytochrome b for all samples.

- M: DNA ladder (100bp).
- 1: cytochrome b amplified with *P. aeruginosa* species specific primer.
- 2: cytochrome b amplified with chicken species specific primer.
- 3: cytochrome b amplified with rabbit species specific primer.
- 4: cytochrome b amplified with sheep species specific primer.
- 5: cytochrome b amplified with human species specific primer.

A typical PCR reaction is performed in a thermal cycler and involves an initial DNA denaturation, followed by a number of cycles of denaturation, primer annealing and product extension. A final DNA extension step completes the reaction, conventionally PCR consist of (30-40) cycle and 3 steps according to.[12,13]

The results of the PCR products show expected different specific amplified PCR products sizes of 460, 580, 740, 331 and 227 bps for *Cytb* gene of *pseudomonas aeruginosa*, Human, Rabbit, Sheep and Chicken respectively (Figure 2) Detection of gene amplification by polymerase chain reaction Method.[14] Results of the present study demonstrate of the spices specific primers could successfully amplify the cytochrome b gene from different samples, the newly designed primer set successfully amplified the expected DNA fragments in all 24 blood samples for each of the human, rabbit, chicken, sheep. In addition to six samples of *P. aeruginosa* isolates as well as six sample of corpses tissue, results previously agreed with (Satish *et al*, 2009) the successfully discriminated of spices through cytochrome b gene based PCR using the cooked meat of sheep and chicken When used primers designer to the gene cytochrome b get the band with molecular weight 331 and 227 bp.[14] This was confirmed (Ali *et al*, 2014) using meat samples of eight animal specimens (chicken, duck, turkey, goat, sheep, cattle, buffalo and pig) when used primers designer to the gene cytochrome b.[15] This explains the results we have obtained the size of the segment depends on the primer design and its association with DNA template depending on the area bounded by the forward primer and reverse primer.[16] The all results of band multiplying different in the value of molecular weights according to primer design which depends on the type of the gene who designs the primer.

PCR analysis of such samples may show positive results for a violation due to its high sensitivity.[17] Even though contamination was unintentional and at a very low level, therefore, a precaution should be exercised when interpreting the results of species

identification by PCR and analysis of multiple samples should be taken from each lot for an objective evaluation.[18]

CONCLUSIONS

In conclusion, this study suggests an accurate analytical technique for *Pseudomonas aeruginosa*, human, rabbit, sheep and chicken identification, based on PCR analysis of the *Cytb* gene of mitochondrial DNA for enforcement of labeling regulations. This technique is useful and feasible to differentiate species present in mixed sample especially in the forensic medicine. Therefore; it can be suggested as a useful laboratory tool for species identification.

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