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Detection of *Staphylococcal* Species Diversity within Human Skin Microbiome Using a Simple PCR-SSCP Technique

Lubab Aqeel[a] **Rabab Omran**[b]

[a]*Anatomy and Histology Department, College of Veterinary medicine, Al-Qassim Green University, Babel, Iraq.*

[b]*Biology Department, College of Science, University of Babylon, Babel, Iraq.*

lubaalwaeli@gmail.com *omranaljelawi@gmail.com*

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Abstract

Objective.The aim of this study was to detect the diversity of *Staphylococcus* species of a healthy human skin using a simple technique PCR-SSCP.

Methods: Blood samples, saliva, and skin swaps samples werer collected from 50 persons from Hilla City - Iraq. The genomic DNA was extracted from these samples using the Bacteria Genomic DNA Kit. The cocentrations and purity of DNA extract estimated by Nano Drop spectrophotometer. Polymerase chain reaction – single strand conformational polymorphism (PCR–SSCP) technique was performed to detect the diversity between Staphylococcal species in the human skin microbiom using a specific primer of 16SrRNA gene.

Results: The PCR results, indicated that the Staphylococcal species were found within the skin community, but it's not infected blood and mouth of test healthy individuals. SSCP-heteroduplex patterns of PCR products appeared the presence Staphylococcal species diversity within skin microbiome of test healthy individuals.

Conclusion: In spite of the PCR-SSCP, heteroduplex method was simple and cheap and appeared the diversity between Staphylococcalspecies in the human skin microbiome, but it's not diagnosed the bacterial strains. So these results required to confirm by DNA sequencing technique.

1-Introduction

The previous, studiesreported that the human genome, the essence of human biology has been associatedto the human microbiome which represented as a second genome. Our bodies composed of human cells (10%) and the human microbiome which formed about 90%[1]. The human microbiome is an important source of the genetic diversity, illness-causing factors, immunity modifier, have also influenced or modulated the metabolism and alters drug interfaces [2], [3]. Our bodies are harboring microorganisms in skin, gut, and mouth. Currently the human skin microbiome is used as a forensic evidence to discriminate, among persons in a crime scene. The bacterial species diversity of human skin microbiome can be identified depending

on the 16S rRNA encoding gene. Bacterial species in the skin having a diverse and unique composition between individuals. Staffs and investigators in the control of criminal evidence thought that a bacterial fingerprint achieved from surfaces including computer keyboards supports forensic individual identification in case of evidence insufficient [4]. Recently, next-generation sequencing was used to investigate, the bacterial community on objects and fingertips to match the object to the individual. The human microbiome, was identified using high-throughput techniques, including culture-independent identification methods consist of advances in genomic technologies, particularly fingerprinting techniques depending on the separation of 16S rRNA genes using denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism analyses, subsequent next-generation DNA sequencing technology. [5],[6],[4].The physiological state of the skin site is a strong factor for colonizing bacteria, which related to moist, dry, and oily micro-environments [2],[3]. Overall, bacterial diversity is minim in an oily sites (such as the face and back),and maximum in the dry showing sites such as the arms and legs. The intrapersonal variation in a microbial public relationship and structure between symmetric skin sites is lower than interpersonal variation, as determined by 16S rRNA gene sequencing [7],[2],[3].

In present of study, detect the diversity of *Staphylococcus* of a healthy human skin using simple technique alike PCR-SSCP as pro-fingerprinting of the human microbiome.

2-Materials and Methods

1-2 Sampling and Bacterial DNA Extraction

About 50 human samples were collected from different body sites of healthy persons, including blood samples, mouth (saliva) and arm skin swaps to detect the presence of *Staphylococcus spp.* as causative agents for any infections or as a normal flora. These samples were transported to the Laboratory of Biotechnology and Genetic Engineering, Biology Department, College of Science, the University of Babylon in Iraq. Genomic DNA was extracted from these samples using the Bacteria Genomic DNA Kit (Geneaid, Taiwan).

2-2 Estimation DNA Concentrations and Purity

The concentrations and purity of DNA extracts were estimated by a NanoDrop spectrophotometer (OPTIZEN POP - Korea). The purified DNA extracts were stored at -20 ℃ until used.

3-2 Detectionof Staphylococcalspeciesby PCR Technique

PCR was used to detect Staphylococcal species using specific primers for the regions of the 16S rRNA genes that are conserved among staphylococci and single by comparison to other eubacterial species. The forward primer:

5'-CCTATAAGACTGGGATAACTTCGGG-3' and the reverse:

5'-CTTTGAGTTTCAACCTTGCGGTCG-3' and the amplified fragment was 791bp [8] . PCR was carried out in the reaction mixture containing 1 µl from each forward and reverse primers, 12.5 µl of Green Master Mix, 3 µl of genomic DNA (50 ng) and the volume of the reaction was completed up to 20 µl by adding 2.5 µl of the Nuclease-free water. Amplification was carried out in a TRIO Thermal Cycler (Biometra, Germany) programmed for 5 min pre-denaturation at 94°C; 35 cycles, 30sec at 94°C (DNA denaturation), 1 min at 60°C (annealing) and 1min at 72°C (extended); and a final extension of 5 min**.** PCR products were electrophoresed using gel electrophoresis (Cleaver Scientific – UK) in 2% agarose at 70 V for 90 min and visualized by ethidium bromide. Photos were taken using a gel documentation system(Cleaver Scientific –UK).

4-2 SSCP-heteroduplex pattern analysis

The amplified fragments are subsequently suitable for downstream Single strand conformation polymorphism (SSCP) tests. The PCR-SSCP method was performed using 8% polyacrylamide gel (PAG) containing 7% glycerol and 1× TBE. The gel was prepared and poured into the medium-casting tray (20 × 20 × 0.1.; H×W×T). For each 10µl amplified fragment, 10µl of 2X SSCP loading dye was added to a microfuge tube. The contents were quietly mixed and placed into a 95°C water bath for 7 min and then on ice for 5 min. Then, the samples were loaded into 8% SSCP gel. The SSCP loading dye composed of (25ml total volume): 23.75 ml of 99% formamide, 1.25 ml of 1% xylene cyanol solution and 10 mg of bromophenol blue. The samples migrated under electricpower 200 V (7.5V/cm), 100 mA for 160 min using vertical gel electrophoresis (Cleaver Scientific – UK). After that gels visualized by ethidium bromide. Photos were taken using gel documentation system (EBOXCX – UK).

3- Statistical Analysis

Statistical analysis was carried out using SPSS version 16,P value ($P \leq 0.05$) was considered statistically significant.

4- Results and Discussion

The genomic DNA wasextracted from the blood, saliva and skin samples as a first step to isolate the genomic DNA of bacteria, then the 16S rRNA encoding gene was amplified using specific primers for Staphylococcal species but not other eubacteria.

The Table (1) showed the concentrations and purity of extracting DNA.

The quality and quantity of genomic DNA,extracted from samples are key feature most facilities consider when choosing a protocol. Measuring ultraviolet light absorbance using spectrophotometry at different wave lengths (230 nm, 240 nm, 260 nm, and 280 nm) is an initial quick and efficient way of determining purity and concentration of nucleic acid samples. The concentration is usually calculated from DNA absorbance reading at 260 nm using the Beer-Lambert law.The purity of nucleic acid samples is assessed in a 260/280 absorbance

ratio, and values in the range of 1.8–2.0 are generally considered acceptable. The 260/230 absorbance ratios between 2.0 and 2.2 are also considered to be adequate as a secondary measure of the purity of DNA [9].

The figure (1) revealed the gel electrophoresis of a single band (791bp) of amplified DNA fragments of the 16S rDNA gene of Staphylococcal species in skin samples, but the other samples (blood and saliva) appeared negative results for the bacterial detections.

These results indicated that the Staphylococcal species were found within the skin community, but it's not infected blood and mouth of test healthy individuals.

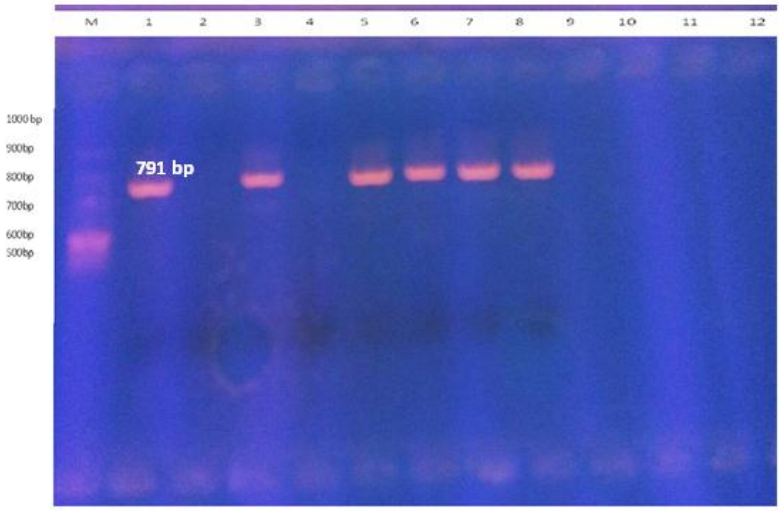

Fig.1: PCR pattern of 16S rDNA (791bp) of *Staphylococcal* speciesin different human samples

Electrophoresis in 2% Agarose at 70 volts for 90 min. M: Ladder marker 100-1000bp; Lanes: 1,3,5-8 for skin samples; Lanes: 2&4 for blood samples and 9-12 for saliva samples.

Everyone has a large number of microorganisms. Human microbiome refers to the sum of microorganisms in the human body. Human identification is important in the forensic science and will continue to do-so. However,trace evidence such as inadequate quality and low DNA copy counts cannot be detected with the rigor necessary to prosecute crimes, including those involving violence [4].

The offender can take steps to reduce the contamination of the crime scene like blood, semen, and fingerprints, which can complicate the detection of the offender. Bacterial DNA is a new method in forensic science. Bacterial DNA is more resistant to environmental factors than human DNA and thus can persist longer on the surface than human DNA. The bacterial DNA configuration is influenced by the surrounding environment [2],[3] and the individual's microbiome.

It is conceivable that different bacterial patterns can distinguish individuals with gender, age, and different lifestyle, data can also be used to build a suspect's image [7],[10],[4].

Some researchers hypothesized that the profile could be possible by analyzing the DNA pattern of the skin bacteria. To assess this, they created differences between individuals and conducted a study to identify individuals. The skin bacteria left on the surfaces that were touched were identified using the next generation of sequencing, which is a bioinformatics technique that uses molecular and arithmetic approaches to generate and analyze DNA sequences.

The next generation of sequencing used to analyze bacterial DNA to distinguish different bacterial manifestations among individuals in a way that has the forensic value, such as analyzed bacterial signatures left by different individuals on surfaces including fingertips, computer keyboard and knob using pyrosequencing based on 16S rRNA gene [4].

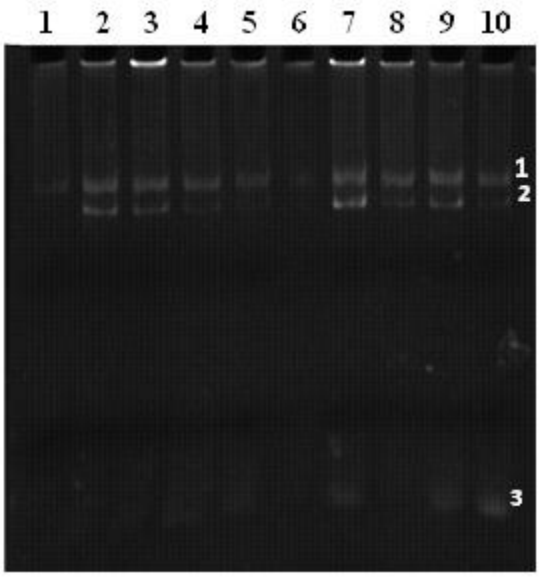

Fig. 2: SSCP-heteroduplex pattern analysis of 16S rDNA (791bp) of *Staphylococcal species*.

Lanes 1and6 two bands; Lanes 2-5 and 7-10 three-bands (heteroduplex). Electrophoresis conditions: 8% polyacrylamide, power applied: 200V (7.5V/cm) – 100mA, for 120 min. The DNA bands stained using ethidium bromide.

To analyze the PCRproducts of *Staphylococcal s*pecies,the SSCP-heteroduplex patterns were observed by ethidium bromide staining (Fig. 2). Bands of double-stranded DNAs were detected at the bottom of the acrylamide gel (band 3). The mobilities of single-stranded and heteroduplex DNAs (band 1and band 2) were lower than those of double-stranded DNAs.

A PCR product from single straining showed two bands compatible to two single-stranded DNAs derived from double-stranded DNA (Fig. 2, lane 1 and6). When the PCR products that were amplified from the mixtures containing genomic DNAs of *Staphylococcal* species more than one strain looked different banding shapes caused by the mixtures of strains were observed. The heteroduplex bands do not represent a single strain, but a mixture of two strains. Although the heteroduplex bands can be discerned by their stained color, they may form complex banding patterns in the analysis of natural people.

SSCP technique was primarily developed for examining point mutations in human DNA [11]. It since has been extended for studying the variability of plant pathogens, including viruses, nematodes, and fungi [12],[13] . It also was used as a paired technique with species-specific PCR in the development of genetic markers for rapid identification of different species of microorganisms.

5- Conclusion

In spite of, the PCR- SSCP heteroduplex method was simple and cheap and appeared the diversity between Staphylococcalspecies in human skin microbiome, but it's not diagnosed the bacterial strains. So these results required to confirm by DNA sequencing technique.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

7- References

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الخلاصة

الهدف من الدراسة هو الكشف عن انواع بكتريا الستافيلوكوكس من عينات الجلد في الانسان وذلك بواسطة استخدام تقنية SSCP-PCR وكانت العينات المسحوبه من الدم والجلد واللعاب حيث جمعت من ٥٠ شخص من مناطق مختلف من بابل.

تم استخلاص الدنا بواسطة استخدام كت البكتريا للدنا وتم قياس التراكيز والنقاوة باستخدام جهاز النانو دروب ومن ثم استخدمت تقنية SSCP-PCR للكشف عن وجود طفرة بين العينات البكتيريه باستخدام برايمر ١٦ SrNA جين.

الكلمات الدالة: انواع الستافيلوكوس، بكتريا جلد الانسان، Pcr-sscp.