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Gel Electrophoresis and Polymerase Chain Reaction (PCR) Amplification of DNA extracted from *Staphylococcus aureus*

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Abstract
Deoxyribonucleic acid (DNA) itself is a chain of nucleotides. Under most conditions, DNA is double-stranded, consisting of two such nucleotide chains that wind around each other in the famous shape known as the double helix. Staphylococcus aureus is a versatile and virulent pathogen in humans, who serve as natural reservoirs for this pathogen. DNA-based methods for detecting foodborne bacterial pathogens usually result from extracting DNA from samples without enrichment. Physiological and mechanical barriers to the isolation of DNA from complex organic material may occur. This experiment aimed to visualize and amplify the extracted DNA from Staphylococcus aureus using Polymerase chain reaction (PCR) and gel electrophoresis. Agarose gel electrophoresis is routinely used to separate proteins, DNA or RNA. The objective of the PCR is to produce a large amount of DNA in a test tube, starting from only a trace amount. The experiment was conducted at the biotechnology laboratory of (Devkota et al. (2012) at West Visayas State University, La Paz, Iloilo City, on May 9, 2019. The agarose gel electrophoresis result indicated a successful amplification of the desired DNA fragment during the Polymerase Chain Reaction (PCR) with gene primer sizes of 1.5 to 2.0bp.

Keywords: DNA extraction, Gel Electrophoresis, Polymerase Chain Reaction, *Staphylococcus aureus*

1. INTRODUCTION

Deoxyribonucleic acid (DNA) is a chain of nucleotides. Under most conditions, DNA is double-stranded, consisting of two nucleotide chains that wind around each other in the famous double helix shape.

Staphylococcus aureus is a versatile and virulent pathogen in humans, who serve as natural reservoirs for it. DNA-based methods for detecting foodborne bacterial pathogens usually involve extracting DNA from samples without enrichment. Physiological and mechanical barriers to the isolation of DNA from complex organic material may occur (Chapaval et al., 2008). s

The Polymerase chain reaction (PCR) DNA amplification method is robust and sensitive (Lee et al., 1992). PCR aims to produce a large amount of DNA in a test tube, starting from only a trace amount. The true power of PCR is its ability to amplify the precise sequence of DNA of interest. PCR amplification includes three main steps: Denaturation, annealing, and extension. These steps combine to form one "cycle" of PCR, and a complete PCR amplification undergoes 40 cycles.

Agarose gel electrophoresis is routinely used to separate proteins, DNA or RNA. Nucleic acid molecules are size separated by an electric field where negatively charged molecules migrate toward (Yılmaz et al., 2012) an anode (positive) pole. The molecular weight determines the migration flow, where small-weight molecules migrate faster than larger ones (Sambrook & Russel, 2001). In addition to size separation, nucleic acid fractionation using agarose gel electrophoresis can be an initial step for further purification of a band of interest. Extension of the technique includes excising the desired "band" from a stained gel viewed with a UV transilluminator (Yılmaz et al., 2012).

This experiment aimed to visualize using gel electrophoresis and amplify the isolated DNA from *Staphylococcus aureus* using Polymerase chain reaction (PCR).

The DNA extracted from *S. aureus* was analyzed based on its reaction in agarose gel electrophoresis and Polymerase Chain Reaction. The concentration and purity of the extracted DNA (Lara et al., 2018) were interpreted based on the data obtained from the spectrophotometric quantification.

2. REVIEW OF RELATED LITERATURE

The study by R. P. Hearn and K. E. Arblaster (2010) emphasizes the significance of DNA extraction as a practical and engaging method for students to explore DNA. It highlights the advantages of students using their DNA, fostering a sense of ownership and enthusiasm. The research presents a cost-effective and straightforward DNA extraction and visualization protocol, utilizing buccal mucosal epithelia as a convenient and noninvasive cell source. The study employs seven critical criteria for evaluating the protocol: safety, DNA yield, quality, cost-effectiveness, user-friendliness, reliability, and time efficiency. Through separate investigations on each stage of the protocol and the adaptation of a fast-boiling approach, the study identifies optimal conditions for cell harvest, lysis, purification, and precipitation. A validation study conducted in a classroom setting showcases the performance of the optimized protocol, which features the use of Lucozade Hydro Active Fitness Water for mouthwash, a specific lysis buffer, and precipitation with sodium chloride and ethanol. The protocol demonstrates enhanced DNA yield, cost efficiency, and simplicity, utilizing easily accessible equipment and reagents.

DNA extraction and polymerase chain reaction (PCR) (Gupta, 2019) are cornerstone techniques in molecular biology. This extensive review examines a range of physical and chemical methodologies utilized for DNA extraction to obtain high-quality DNA with significant yield. PCR plays a vital role in amplifying DNA, making it an indispensable tool in molecular research. The review delves into the foundational principle of PCR and its various adaptations, offering insight into their applications and nuances. This review, authored by Nalini Gupta in 2019, consolidates essential insights from previous DNA extraction and PCR studies, elucidating their pivotal roles and contributions to advancing molecular biology research.

The rapid and precise isolation of high-purity microbial genomic DNA is indispensable for genome analysis. This review scrutinizes a study comparing a one-hour microwave-based procedure with enzymatic and boiling methods for extracting genomic DNA from Gram-negative and Gram-positive bacteria. The microwave pre-heating methodology exhibited notable results, yielding high DNA concentrations and purity levels for MRSA and ESBL strains (80.1 and 91.1 µg/mL; OD260/280, 1.82 and 1.70, respectively). The quality of the DNA was further affirmed through PCR detection of mecA and CTX-M genes.

The study's findings support the efficacy of the microwave-based extraction method as a rapid, efficient, and cost-effective approach applicable to a spectrum of bacteria types. Authored by Omar B. Ahmed, Atif H. Asghar, and Mogahid M. Elhassan in 2014, this review underscores the significance of optimized DNA extraction techniques in enabling swift and reliable genomic analysis across diverse microbial strains.

DNA molecules are visualized under UV light following staining with a suitable dye. This review, authored by Pei Yun Lee, John Costumbrado, Chih-Yuan Hsu, and Yong Hoon Kim in 2012, underscores the significance of agarose gel electrophoresis in facilitating precise and efficient DNA fragment analysis.

Agarose gel electrophoresis is a pivotal method for segregating DNA fragments with diverse sizes, spanning from 100 base pairs to 25 kilobases. Derived from seaweed genera Gelidium and Gracilaria, agarose comprises recurring agarobiose subunits of L- and D-galactose. When agarose polymers associate non-covalently, they construct a network of bundles that determine the gel's molecular sieving properties upon gelation. This technique has revolutionized DNA separation, surpassing sucrose density gradient centrifugation, offering only an approximate size estimation. During agarose gel electrophoresis, DNA is introduced into pre-cast wells within the gel and subjected to an electric current.

DNA molecules' negatively charged phosphate backbone makes them migrate towards the positively charged anode in the electric field. Due to DNA's uniform mass/charge ratio, molecules are segregated by size within the agarose gel in a manner where the migration distance is inversely proportional to the logarithm of their molecular weight. The prevailing model for DNA movement within an agarose gel is termed 'biased reptation', where the leading edge advances and drags the rest of the molecule. Several factors influence the migration rate of DNA, including the molecule's size, agarose concentration, DNA conformation, applied voltage, ethidium bromide presence, agarose type, and electrophoresis buffer.

3. MATERIALS AND METHODS

Materials used in Gel Electrophoresis and Polymerase Chain Reaction (PCR) Amplification with DNA extracted from Staphylococcus aureus include specific reagents and equipment tailored for each technique. Here is a list of common materials used for each process:

2.1. Materials for Gel Electrophoresis:

- Agarose powder prepares the agarose gel matrix to separate DNA fragments.
- Buffer solution: Provides the appropriate pH and ionic strength for the gel and running buffer.
- DNA samples: Extracted DNA from Staphylococcus aureus for analysis.
- Loading dye: A tracking dye mixed with DNA samples for visualization during electrophoresis.
- DNA size markers: Known DNA fragments of predefined sizes for estimating the size of unknown DNA fragments.
- Ethidium bromide or alternative DNA stain: Visualising DNA bands under UV light.
- Electrophoresis chamber: Holds the agarose gel for the separation of DNA fragments.
- Power supply: Provides the electric current necessary for DNA migration through the gel.

2.2. Gel documentation system: Captures images of the separated DNA bands for analysis.

- UV transilluminator: Used to visualize ethidium bromide-stained DNA bands on the agarose gel.

2.3. Materials for PCR Amplification:

- DNA template: Extracted DNA from Staphylococcus aureus to be amplified.
- Primers: Short DNA sequences that bind to specific regions, flanking the target DNA sequence.
- DNA polymerase: Enzyme synthesizing new DNA strands based on the template DNA.

- Nucleotides (dNTPs): Building blocks for DNA synthesis (dATP, dCTP, dGTP, dTTP).
- Buffer solution: Provides optimal conditions for DNA polymerase activity during PCR.
- Magnesium chloride (MgCl2): Essential cofactor for DNA polymerase activity.
- Thermal cycler: Equipment that controls PCR's heating and cooling cycles.
- PCR tubes/strips: Containers for holding reaction mixtures during PCR.
- PCR reaction mix: Mixture containing DNA template, primers, DNA polymerase, dNTPs, and buffer.
- PCR tube rack: Holds PCR tubes or strips during the PCR process.
- PCR tube opener: Tool for conveniently opening and closing PCR tubes or strips.

PCR is used to amplify specific DNA regions, including those extracted from Staphylococcus aureus. The amplified DNA can then be analyzed, quantified, or used for further downstream applications. Gel Electrophoresis is often used after PCR to separate and visualize the amplified DNA fragments based on size and charge.

Therefore, the sequence of steps usually involves PCR amplification of the extracted DNA from Staphylococcus aureus first, followed by Gel Electrophoresis to analyze and visualize the amplified DNA fragments.

In a Polymerase Chain Reaction (PCR) amplification process with 40 cycles, the three main steps of Denaturation, annealing, and extension are repeated in each cycle to amplify the target DNA region exponentially. Here is how these steps are included in a typical 40-cycle PCR process:

2.3.1. Denaturation:

In the first cycle, the DNA sample is denatured by heating to separate the double-stranded DNA into single strands. The high temperature causes the hydrogen bonds between the (LeHotan, 2013) complementary base pairs to break, resulting in two single DNA strands. This step typically lasts 30 seconds to 1 minute in each cycle.

2.3.2. Annealing:

After Denaturation, the temperature is lowered to allow the primers to anneal to the single-stranded DNA template (Alsaedi, 2017). The primers are short DNA sequences that bind to specific regions, flanking the target DNA sequence. Annealing typically occurs at around 55-65°C and lasts 30 seconds to 1 minute in each cycle.

2.3.3. Extension:

Once the primers are annealed, the temperature is raised to activate the DNA polymerase enzyme for extension. The DNA polymerase synthesizes a new complementary DNA strand by adding nucleotides to the primer-bound template strand. Extension typically occurs at a temperature around 72°C and lasts about 1-2 minutes, depending on the length of the target DNA region.

2.3.4. Cycling:

After the first Denaturation, annealing, and extension cycle is completed, the process is repeated for 40 cycles. Each cycle doubles the amount of DNA present, exponentially amplifying the target DNA region. With each cycle, the number of DNA copies increases rapidly, reaching a high level of amplification by the end of the 40 cycles.

The pure genomic DNA of Staphylococcus aureus from culture was harvested and centrifuged in a microcentrifuge tube. After which, a 100 µL Elution Buffer BE was added, and the cells were resuspended. Then, the cell suspension was placed into the NucleoSpin® Bead Tube Type B. 40 µL Buffer MG was added. Then 10 µL Liquid Proteinase K was added, and the tube was closed. The NucleoSpin® Bead Tube

was agitated on a swing mill or similar device. NucleoSpin® Bead Tube 30 s at 10,000 rpm and was centrifuged. To adjust the binding conditions of the cells, 600 µL Buffer MG was added and mixed—centrifuge for 30 s at 10,000 rpm. The supernatant (~500-600 µL) was transferred onto the NucleoSpin® Microbial DNA Column; it was placed in a 2 ml Collection Tube and centrifuged for 30 s at 10,000 rpm. The collection tube was discarded with flow-through. Put the column into a fresh Collection Tube with 2 ml. On the first wash, 500 µL Buffer BW was added. It was centrifuged for 30 s at 10,000 rpm. Discarded flow-through and place the column back into the Collection Tube. On the second wash, 500 µL Buffer B5 was added to the column and centrifuged for 30 s at 10,000 rpm. Discarded flow-through and place the column back into the Collection Tube. To dry the Silica membrane, the cells were centrifuged for 30 s at 10,000 rpm. Pure DNA was highly eluted by placing the NucleoSpin® Microbial DNA Column into a 1.5 mL nuclease-free tube, and 100 µL Buffer BE was added to the column. It was incubated at room temperature for 1 min and centrifuged for 30 s at 10,000 rpm.

Here are the phases of Gel Electrophoresis:

1. **Preparation Phase:**

Prepare an agarose gel by mixing agarose powder with a buffer solution and heating until dissolved. Pour the agarose solution into a gel tray and insert a comb to create wells for loading DNA samples.

2. **Loading Phase:**

Mix the extracted DNA from Staphylococcus aureus with a loading dye for better visualization. Using a micropipette, load the DNA samples and DNA size markers into the wells.

3. **Running Phase:**

Submerge the gel tray in an electrophoresis chamber filled with buffer solution. Apply an electric current to the gel, causing DNA fragments to migrate based on size and charge.

4. **Visualization Phase:**

Stain the DNA fragments with a fluorescent dye like ethidium bromide. Visualize the separated DNA bands under UV light and capture an image using a gel documentation system (Pai et al., 2017).

5. **Analysis Phase:**

Analyze the gel image to determine the sizes and quantities of DNA fragments present in the sample. Compare the DNA bands with the size markers to estimate the size of the DNA fragments.

Genomic DNA was analyzed using a spectrophotometer. It was amplified through PCR for 1 hour. After this, gel *agarose* wells were loaded with DNA samples and visualized using the UV transilluminator. Nucleic acid molecules are size separated by an electric field, where negatively charged molecules migrate toward an anode (positive) pole. The molecular weight determines the migration flow, where small-weight molecules migrate faster than larger ones.

4. RESULTS AND DISCUSSION

Figure 1 shows the spectrophotometric analysis of the extracted DNA from S. aureus. The analysis showed a pure DNA sample based on the spectrophotometric standard ratio, which ranges from 1.8 to 2.0, indicating that pure DNA is present in the sample.

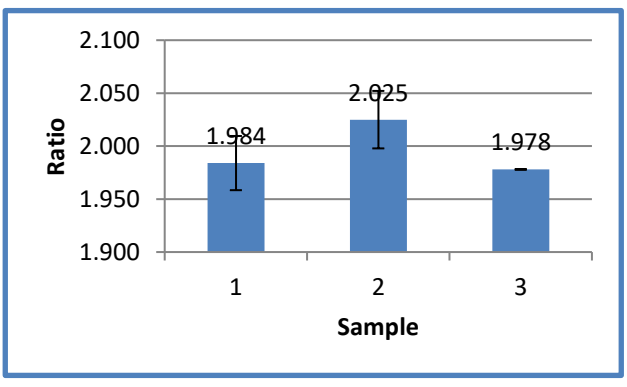

Figure 1. *Spectrophotometric results of the extracted DNA from S. aureus.*

Figure 2 shows the visualization of the PCR product from the genomic DNA samples alongside a 1kb hyperladder. Bands formed indicate that fragments of DNA were present. The figure shows that DNA fragments in wells 2-7 have the same fragment length, while those in wells 10, 11, 13, 14, and 16-20 have almost the same length.

Figure 2. *Gel visualization of PCR samples of genomic DNA compared to BiolIne.*

Compared to the Bioline DNA standard, the bands show that the extracted DNA fragments have been successfully amplified, as the band lies within the 1.5bp-1.6bp range, the standard range to determine whether the PCR successfully amplified the desired DNA fragment. The DNA that was loaded in well seven shows varied fragments. The DNA was not amplified successfully in well 13 because air bubbles formed, and the gel was broken.

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