Spectrophotometric Quantitation, Gel Electrophoresis Visualization And PCR Amplification Of Gdna Eluted From Staphylococcus Aureus: A Model For Student Researchers

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Abstract: This paper aimed to model purification, amplification and visualization a microbial genomic DNA (gDNA) eluted from Staphylococcus aureus prepared overnight at 37˚C and to compare the amplified gDNA with the standard DNA ladder 1 kb as standardized procedure for student research. The purity of the gDNA was also determined using spectrophotometric analysis with the A260/A280 ratio of 2.0 as the highest and 1.9 as the lowest which indicated the gDNA eluted was within the purity range. The genomic DNA with 4 replicates (A1, A2, A3 and A4) were amplified through polymerase chain reaction (PCR) machine for 1 hour using PCR master mix of 5 µL PCR buffer, 1 µL forward primer, 1 µL reverse primer, 12.8 µL SnpH2O and 0.2 µL Taq polymerase. The amplified DNA as well as the gDNA quality were then determined by loading the EtBr-stained gel agarose wells with DNA samples including the DNA ladder 1 kb as positive control using 1.5 g agarose gel and was photographed using transmitted UV light and polaroid film. Results showed the PCR product indicated successful clones of the gDNA with gene primer sizes of 1500 bp to 1517 bp.

Index Terms: gDNA, Polymerase Chain Reaction, Gel Electrophoresis, Spectrophotometry, Staphylococcus aureus, elution, buffer

1 INTRODUCTION

Staphylococcus aureus was discovered in the 1880s and is classified as a low gram-positive, non-spore forming bacteria [1] and facultative anaerobe [3] that has the ability to grow at temperatures from 25°C to 43°C and at pH levels of 4.8 to 9.4 [2]. This bacterial species mainly colonizes the membranes and skin of endotherms, and infections can include benign skin lesions to systemic illnesses such as endocarditis and osteomyelitis that are considered life-threatening [3]. In 2005, the Center for Disease Control and Prevention estimated that there were 31.8 cultures confirmed invasive methicillin resistant S. aureus (MRSA) infections in the U.S. per 100,000 individuals, which amounted to 94,360 cases [4]. The mode of transmission is primarily direct contact, usually skin-to-skin contact with a colonized or infected individual. Contact with contaminated objects or surfaces may also play a role [5-8]. S. aureus is capable of producing variety of toxins and is able to acquire resistance to various antibiotics [2]. Today, more than 60% of S. aureus isolates are resistant to methicillin and some strains have developed resistance to more than twenty antimicrobial treatments [9]. Acquisition of resistance in S. aureus commonly results from either gene mutations leading to drug target modifications or reduction of drug efficacy, as well as acquisition of a resistance genes from other organisms by some form of horizontal genetic exchange [10]. The genome of S. aureus is approximately 2.8 Mbp with a G+C content of 33% [2, 9, 10].

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This paper describes how gDNA from S. aureus can be purified, visualized and amplified using molecular techniques such as spectrophotometry, gel electrophoresis and polymerase chain reaction for research and other laboratory experiments that require standard protocol for student research.

2 MATERIALS AND METHODS

A 1.5 mL pure genomic DNA (gDNA) was harvested from a prepared overnight culture of Staphylococcus aureus incubated at 37˚C temperature and was centrifuged using a microcentrifuge tube to disrupt the cells using 100 µL elution buffer BE. Samples were then lysed in a NucleoSpin Bead Tube Type B by adding 40 µL buffer MG and 10 µL liquid proteinase K. The sample was then agitated in an optimal duration of 30 Hz in 12 minutes and was centrifuged for 30 s at 11,000 x g to clean the lid of the NucleoSpin Tube. The DNA binding condition was adjusted by adding 600 µL buffer MG and mixed them using vortex in 3 seconds. In order to clean the lid and sediment glass beads and all debris, the sample was centrifuged for 30 s at 11, 000 x g. The supernatant (~500-600 µL) was transferred onto the NucleoSpin Microbial DNA Column, placed in a 2 mL collection tube for the binding DNA stage. The silica membrane was washed twice using the 500 µL buffer BW for 30 s at 11, 000 x g. Flow- through was discarded and column was placed back into the collection tube. After the silica membrane was dried and residual wash buffer was removed, the highly pure DNA was eluted. This was done by placing the NucleoSpin Microbial DNA Column into a 1.5 mL nuclease-free tube added with 100 µL buffer BE onto the column incubated at room temperature for 1 min and centrifuged for 30 s at 11, 000 x g. The gDNA purity was analyzed using spectrophotometer at 260nm/280nm ratio. The genomic DNA with 4 replicates (A1, A2, A3 and A4) were amplified through polymerase chain reaction (PCR) machine for 1 hour using PCR master mix of 5 µL PCR buffer, 1 µL forward primer, 1 µL reverse primer, 12.8 µL SnpH2O and 0.2

µL Taq polymerase. While PCR was going on, a 1.5 g of agarose gel was prepared for gel electrophoresis. The amplified DNA quality was then determined by loading the stained gel agarose wells with DNA samples including the DNA ladder 1 kb as positive control and photographed using transmitted UV light and polaroid film.

3 RESULTS AND DISCUSSION

Spectrophotometric analysis. Figure 1 shows the absorbance ratios of the gDNA from four replicates expressed as optical density at 260nm/280nm used to assess DNA quality. Replicate 1 has the highest A260/A280 ratio of 2.0 while Replicate 4 has the lowest A260/280 ratio of
1.97. The ratios indicated that the gDNA samples eluted from S. aureus were free from the possible contamination of molecules such as RNA or protein since it is within the purity range of 1.8 to 2.0.

260nm	280nm	Mean	SD
1.951	2.017	1.984	0.026
2.011	2.039	2.025	0.027
2.000	1.956	1.978	0.000

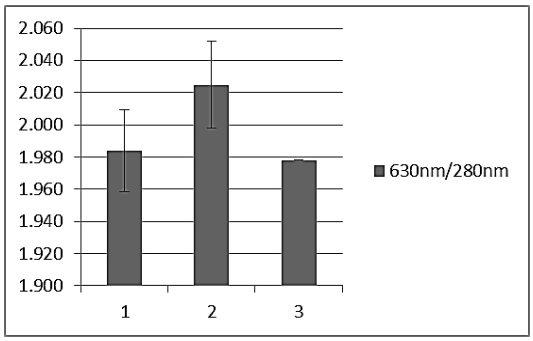

Fig. 1: Absorbance levels of *g*DNA from *S. aureus* at 260nm/280nm ratio using spectrophotometric analysis

Agarose gel electrophoresis. Figure 2 shows the gel visualization of the PCR product from the gDNA samples ran in 1.5% agarose gel alongside with 1kb DNA ladder (gene marker) as the positive control (well 7). The sizes were compared to the Bioline 1kb hyperladder DNA size standard.
The maximum gDNA in all cases was between 1500 bp to 1517 bp. In well 13, no DNA fragment was visualized.

Fig. 2: (a) Gel visualization of PCR samples of *g*DNA compared to (b) Bioline 1kb DNA standard. Lanes 1, 2, 3 and 4 are *g*DNA, lane 7 is gene marker 1kb ladder

The PCR products labeled 8 to 18 (except well 13) have an estimated 1500 to 1517 base pairs (bp) which indicated a successful amplification the gDNA. The failure of DNA visualization in well 13 may be due to a couple of reasons: probably well may be broken, gel is disturbed during removal of the comb so DNA was unable to move out of the well, or probably air bubbles were formed during the casting of the gel. The remaining gDNA bands migrated ideally which indicated the DNA was extracted properly and the gel preparation is pretty good. The images were captured under the UV transilluminator to show the effect of EtBr on the gel electrophoresis results. A quality electrophoresis gel must have good and sharp bands, minimum primer dimers, beautifully separated DNA ladder, and no traces of other DNA in the gel. For a good quality result of gel electrophoresis, reusing the gel and the buffer is not recommended or use only twice if necessary. Prepare buffer freshly every time as well as the electrophoresis tank, preserve DNA and DNA ladders properly in the cold chain, and use high quality chemicals.

5 CONCLUSION

S. aureus is an important species of facultative anaerobes whose genomic DNA analysis have many implications in the field of microbiology and medicine. Standardized molecular techniques such as spectrophotometry, gel electrophoresis and polymerase chain reaction can be used to analyze gDNA of S. aureus for potential research among students and young professionals.

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