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A Novel Method of Extraction and Purification of Bacterial DNA for PCR Detection of MecA, 16SrRNA, VanB

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Abstract: Bacteria are the main pest of nosocomial infections in healthcare facilities around the world. The aim of this study is to modify and minimize the amount of time, while still having no influence on the extraction's purity of DNA the method of extracting the DNA molecule and compare it with other methods that take longer to extract and purify DNA. This study examined two processes for isolating DNA from Gram-positive bacterial species: a boiling method and an enzymatic method that took one hour. In addition, the collected DNA was subjected to spectrophotometry, agarose gel electrophoresis, and PCR for both quantitative and qualitative examination. The results presented in this work show that boiling method pretreatment facilitates efficient cell lysis and DNA extraction, leading to increased yields of isolated bacterial DNA. In addition, DNA suitability for PCR-based identification of mecA,16SrRNA, VanB to Gram positive S. aureus strains was analyzed. Our findings demonstrated that the DNA generated by this simple approach is low-cost, quick, and safe, and that the process may be utilized in PCR methods on a wide range of gram-positive and gram negative species, as well as in laboratories without the necessary materials, tools, or technology..

Keywords: PCR, Extraction Bacterial, Gram positive, MecA, 16SrRNA, VanB.

Introduction

Bacterial control has grown in popularity Antimicrobial-resistant microbes have emerged. Extended spectrum beta-lactamase (ESBL) and Methicillin-resistant S. aureus (MRSA) are two examples (Cotton et al., 2008; Najm et al., 2022). Bacteria are the main pest of nosocomial infections in healthcare facilities around the world (Pesquero et al., 2012).

Where bacterial genomic DNA carries all of the genetic data required by cells of bacteria to function properly. Where molecules of DNA are big molecules, they're big molecules, and in the majority of bacteria, they're arranged into a single circular chromosome. Therefore, quick isolation of genomic DNA from human cells and organisms is necessary for DNA analysis techniques like PCR, gene sequencing, and fingerprinting (Chaitanya et al., 2019; Creager, 2020).

Availability of current DNA extraction is from methods important for obtaining microbiological studies. There have been many reported DNA extraction and as well as methods for purification Initially, it was researched with varying degrees of success (Omar et al., 2014).

In general, three major phases are involved in the extraction of microbial genomic DNA: destruction of cells, DNA extraction (Li et al., 2020). Genomic DNA is typically extracted using a particular solution and purified using phenol/chloroform extraction next to isopropanol or ethanol precipitation (Stojan et al., 2023). While DNA is formed in almost all living things, substances such as RNA, proteins, and their relatives. These variations should be considered when developing or selecting a method of cell lysis (Fleige et al., 2006; Wilson et al., 1990).

Several methods for extracting DNA from bacterial cells are currently in use, comprising enzymatic, chemotherapy, or thermoregulatory the process of biological lysis degradation of the cell wall with particles or a mix of the aforementioned foregoing (Aaliya et al., 2021; Boltje et al., 2009). Because of its excellent sensitivity for detecting specific target sequences, PCR is likely to become a regular diagnostic tool. However, because standard methods for preparing DNA or RNA from tissues or cells appear to be too difficult for regular assays, novel methods for extracting DNA from bacteria are described and their application to PCR is appraised (Gupta, 2019). In this study, to modify and minimize the time the method of extracting the DNA molecule and compare it with other methods that take longer to extract and purify DNA. This study examined two processes for isolating DNA from Gram-positive bacterial species: a boiling method and an enzymatic method that took one hour. In addition, the collected DNA was subjected to spectrophotometry, agarose gel electrophoresis, and PCR for both quantitative and qualitative examination..

Methods

Samples young strains of S. aureus were these strains were obtained from a range of clinical samples and were used in this study. of any site in cancer patients are submitted to the clinical bacteriology laboratories in many hospitals (Central Children's Hospital Teaching and Oncology Hospital in AL- Basra City, South Iraq). They were diagnostic by Vitek 2 and biochemical tests, when extracting DNA, samples were incubated at 37 °C on selective media mannitol salt agar.

According to the World Medical Organizations' Helsinki Declaration "Ethical Principles for Scientific Medical Research with Human Participation" (as adjusted in 2000), in addition to " All participants in this study, both adults and children, were informed of the Iraqi republication rules for clinical practice and accepted to participate " (Accepted of the Iraqi Ministry of Health dated February 13, 2023 No. 116).

2.1. DNA extraction methods:

In this technique, 5-7 pure colonies of overnight growth microorganisms taken by disposable lob were employed. The colonies of bacteria were put in an Eppendorf tube using 100 microliters of highly pure water with nuclease levels below the limit, mixing gently and boiled in a water bath at 100 °C for 10 minutes then add 800 microliters of highly pure water and mixing gently, and then centrifuged for ten minutes at 12000 rpm, after centrifugation and by micropipette transfer the

supernatant to a new Eppendorf tube and keep it in freezing at -20°C, until usage as a template for PCR amplification. Where the PCR was performed using 10 microliters of the supernatant.

2.2. Analysis of DNA concentration and purity:

The produce and purity of DNA The spectrophotometric approach (BIO-RAD Smart Spec 3000; USA) was utilized to estimate the yield and purity of DNA. To this end, protein impurities were verified at 280 nm and DNA absorbance was measured at 260 nm (µg DNA/g sample; 1 A 260 = 50 µg/mL DNA) (Samuel et al., 2003).

2.3. The 16S rRNA and other gene detection:

The gene encoding 16S rRNA and other genes were amplified using the primers listed in Table 1 on a Thermal Cycler (Application of Biosystems, USA). The PCR master mix contained 12 µL of Dream Taq Green master mix (Thermo Fisher Scientific, USA), 1.5 µL of each primer (10 µM), and 10 µL of DNA template, (without adding nuclease-free water). Cycling conditions were one denaturation cycle for 5 minutes at 95°C and 30 amplified cycles for denaturation (1 minute at 94°C), annealing (45 minutes at 55°), extended (1 minute at 72°C), and termination (5 minutes at 72°C), as per the manufacturer's recommendations (Hoque et al., 2022).

Electrophoresis (Bio-Rad, Bio-Rad Laboratories Pte. Ltd., Singapore) at 80 V for 30 minutes was used to observe the amplification products on 2% agarose gel (Thermo Fisher Scientific, Massachusetts, USA).

The PCR products were separated electrophoretically on a 1.5% agarose gel with DNA safe dye and observed with a UV transilluminator. A Gene Ruler TM 100 bp DNA Ladder was employed. The PCR products were separated electrophoretically on a 1.5% agarose gel with DNA-safe dye and observed with a UV transilluminator. As a molecular weight marker, the Gene Ruler TM 100 bp DNA Ladder was employed.

2.4. The mixture of PCR reaction:

Amplification of DNA was carried out in a final volume of 23µl containing the following as mentioned Table1.

Table 1: Components of the PCR Reaction Mixture.

No	Contents of mixture	Volume
1	Master mix	**10µl**
2	Forward primer	**1.5µl**
3	Reverse primer	**1.5µl**
4	DNA template	**10µl**
Total volume		**23µl**

Table 2: The primer's sequences and optimal conditions for PCR.

Gene	Primer sequence (5´- 3´)	Size of product bp	Conditions	References
16S rRNA	F5´-AGAGTTTGATCCTGGCTCAG- 3´ R5´-GGTTACCTTGTTACGACTT-3´	1500	Step1:95°C, 1min. Step2:94°C, 30 sec. Step3:55°C, 40sec. Step4:72°C,1.5min. Step5: 72°C, 5min.	Lin *et al.*, (2008) [14]
mecA	F5´-AAA ATC GAT GGT AAA GGT TGG C- 3 R5´-AGT TCT GCA GTA CCG GAT TTG C- 3´	532	Step1: 94°C, 5min. Step 2: 94°C,30sec. Step 3:63°C, 30sec. Step 4;72°C, 3min. Step 5:72°C,10min.	Johnson and Stell, (2000) [15]
vanB	F5´-GTA GGC TGC GAT ATT CAA AGC-3´ F5´-GCC GAC AAT CAA ATC ATC CTC-3´	300	Step1:94°C. 3 min. Step2: 94°C, 1min. Step3:61°C , 30sec. Step4:72°C, 3min. Step5: 72°C ,7 min.	Yamamoto *et al.*, (1995) [16]

Results and Discussion

In this study, we examined the yield and purity of DNA extracted by boiling and enzymatic lysis over the course of an hour. Furthermore, the suitability of DNA for PCR-based detection of *vancB*, *16SrRNA*, and *mecA* from strains of Gram-positive *S. aureus* was analyzed.

The findings of this study indicate that the pre-treatment with the boiling approach increases the yields of isolated bacterial DNA by facilitating effective cell lysis and DNA extraction. As a result, compared to the enzymatic method and other techniques, boiling approaches produce larger amounts of DNA extracted from various strains (Table 3).

Table 3. Concentration and purity of bacterial S,aureus by two DNA extraction methods.

Microorganism	DNA Concentration µg/ml		A260/A280	
	Boiling	Enzymatic	Boiling	Enzymatic
S. aureus	82.0	60.8	1.61	1.75
(Gram-positive)	81.5	60.2	1.63	1.76

	81.1	60.3	1.62	1.73
Mean ± S.D	81.3 + 0.2	60.4 ± 0.26	1.62 ± 0.01	1.74 ± 0.01

The boiling process was improved in this work for the fast extraction and purification of microbial genomic DNA for PCR analysis. The extraction of DNA from bacteria took around 40 minutes. In this investigation, no alcohol was utilized as a precipitant for concentrating; instead, nucleic acids were separated using a high-speed centrifuge. The volume of DNA extracted in this method is very large, about (0.8 ml), and this quantity is sufficient to carry out all molecular experiments. When not being used, purified DNA should be kept at -20°C or -70°C without essentials like TE buffer. Figure 1 demonstrates that a single, distinct band of DNA extracted using the technique utilized in this study emerges on an agarose gel, showing that it had not been destroyed. Figure 1 presents PCR amplification of the 16srRNA, mecA and VancB genes for S. aureus. Thus, the utilization of low-cost facilities and the lack of harmful substances might improve the efficiency and speed of this process. Our findings demonstrated that the DNA generated by this simple approach is low-cost, quick, and safe, and that the process may be utilized in PCR-based methods on a wide range of gram-positive and gram-negative species, as well as in laboratories without the necessary materials, tools, or technology. The boiling technique may also be entirely competitive with the traditional phenol chloroform technique.

We compared this approache to DNA extraction from Gram-positive and Gram-negative methods. Figure 1 shows how DNA extracted using the boiling method in this work shows up as a distinct single band on the agarose gel, indicating that the DNA was not destroyed.

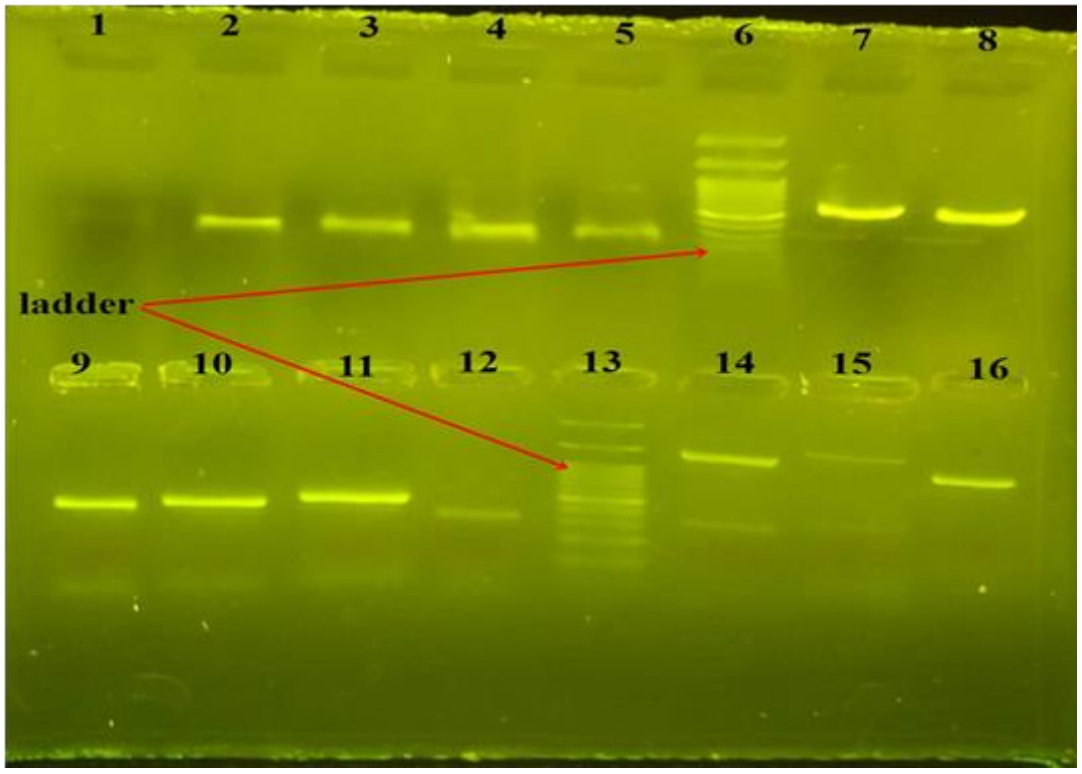

Figure 1: Ethidium bromide agarose gel electrophoresis for amplification 16SrRNA specific gene in S. aureus was identified via PCR detection of 1500 bp (80 volt, 1hrs and 30 min). Lane 6,13: DNA ladder. Lanes: 14,15,16 represent the identified 16SrRNA gene. Lane :7,8,9,10 represent the identified mecA, 532bp.lane :1,2,3,4 represent the identified VancB ,300 bp.

Table 4. Summary of extraction methods

№	Extraction protocol name	purity	Manualor automated	Chemistry/ mechanis	Specialized equipment	Kits / supplies required	yield	References
1	Modified Boiling method	1.70 A260/A280	manual	Boiling water	None	All reagents are made in house	0.88	Current study
2	Enzymatic method	mean A260/A280 = 1.83	manual	Boiling water	None	Enzymatic kit	0.88	Current study

This paper is a modest contribution to the ongoing discussions about the development of DNA analytical methods such as PCR, cloning, hybridization, sequencing, and fingerprinting has made it possible to analyze bacterial genomes in great detail. Numerous protocols have been developed and documented for the separation and purification of DNA from different kinds of bacteria. DNA isolation is typically a multi-step process that includes lysis of the cell using lytic enzymes and/or detergents, extraction of the DNA using organic solvents, and precipitation of alcohol to recover the DNA(Dairawan et al., 2020; Lewis, 2023). A few of these techniques take a lot of time and are not particularly effective. Therefore, a rapid, easy-to-use, effective, and economical technique for extracting bacterial DNA is therefore required. The yield and purity of the extracted DNA are of great importance for further investigation, including PCR-based diagnostics of infectious pathogens.

In comparison to many commercial kits, several reports showed that direct boiling is helpful for DNA extraction. The cost of preparing molecular samples would also be greatly lowered and its efficiency would increase. The enhanced boiling process offers several advantages, including the elimination of the need for toxic chemicals such as phenol and specialized enzymes. As a result, it is quick, simple, and inexpensive, and it may be used to isolate high-yield analytical grade DNA from gram-positive and gram-negative bacteria(Glenn, 2011). The author (Durgadevi A., 2018) there is a previous study on a modified method using fewer chemicals and techniques leading to PCR and the sequencing of 16S rRNA genes quality Isolation of DNA from Goat Rumen Digesta (Ali et al., 2021; Aphale et al., 2018; James et al., 2021). Following that, population DNA extracted using the boiling process approach could provide standard PCR amplification with general bacterial primers and specific bacterial 16S rRNA gene primers(Ahmed et al., 2017), demonstrating its suitability for a wide range of biological applications. In addition to, DNA amplification of the target bacteria was performed using a final volume containing 23 µl, after optimizing the boiling process in this work for rapid extraction and purification of microbial genomic DNA for PCR analysis.

For the extraction of DNA for molecular methods, several previous techniques were either followed or preceded by enzymatic or detergent treatment. Several companies made use of earlier concepts and developed commercial kits that may be used to extract DNA from various biological sources (Merk et al., 2006). Despite offering more straightforward DNA extraction techniques, such kits increased the cost of investigations requiring DNA extraction. This study examined less complicated techniques for extracting DNA in order to yield samples of bacterial DNA. The aim was of this study is to modify and reduce the amount of time and substances required while maintaining the quality of the extracted DNA the method of extracting the DNA molecule and compare it with other methods that take longer to extract and purify DNA. However, in this investigation, no alcohol was utilized as a precipitant for concentrating; instead, nucleic acids were separated using a high-speed

centrifuge. The volume of DNA extracted in this method is very large, about (0.8 ml), and this quantity is sufficient to carry out all molecular experiments.

The methods depended only on heat, with no reagents added. Boiling was used to heat bacterial material (suspended in distilled water with no other ingredients) for extraction of DNA from bacteria took where around 40 minutes. It was demonstrated that these methods produced sufficient DNA molecules to carry out further molecular biology experiments.

Conclusion

The techniques (heating of bacteria) discussed here are, in summary, reasonably simple, affordable, effective, and quick ways to extract DNA from bacteria for use directly in molecular operations. Summing up the results, it can be concluded that our findings demonstrated that the DNA generated by this simple approach is low-cost, quick, and safe. The process may be utilized in PCR-based methods on various gram-positive and gram-negative species. Furthermore, this research was concerned with compared to other, more time-consuming methods of DNA extraction and purification, they deliver excellent results. The study results could convince others to try the procedure on various biological materials, such as fungi and all types of bacteria.

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