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Quality improvement of the DNA extracted by boiling method in gram negative bacteria

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Abstract: Several methods of Deoxyribonucleic acid (DNA) extraction have been applied to extract bacterial DNA. The amount and the quality of the DNA obtained for each one of those methods are variable. The study aimed to evaluate bacterial DNA extraction using conventional boiling method followed by alcohol precipitation**.** DNA extraction from Gram negative bacilli was extracted and precipitated using boiling method with further precipitation by ethanol. The extraction procedure performed using the boiling method resulted in high DNA yields for both *E. coli* and *K. pneumoniae* bacteria in (199.7 and 285.7µg/ml, respectively) which was close to control method (229.3 and 440.3µg/ml). It was concluded that after alcohol precipitation boiling procedure was easy, cost-effective, and applicable for high-yield quality of DNA in Gram-negative bacteria.

Key words: DNA; boiling method; extraction; Gram negative

Introduction

Extraction and purification are essential steps to determine DNA size, shape and function. They are used to detect bacteria and viruses as well as diagnosing disease and genetic disorders. Several methods (chemical or physical) of DNA extraction have been applied in the scientific research of molecular bacteriology [1]. Despite the wide variety of these methods, there are some similarities among them. The amount and the quality of the DNA obtained through each of the common extraction methods are variable. But generally, they aim to separate DNA present in the nucleus of the cell from other cellular components. Some of them are too laborious, others are time-consuming while in most them, the obtained results is not satisfying (chopped DNA) for routine diagnostic or identification work [1, 2]. The boiling procedure which involves thermal lysis is rapid, simple, and effective than standard methods for bacterial DNA isolation [3, 4]. The process requires three times centrifugation to collect the cells, to eliminate the cell debris after the boiling procedure to pellet the total precipitated DNA [5.6]. Ethanol precipitation is a commonly used technique for concentrating and de-salting DNA preparations in aqueous solution [7]. After precipitation, the nucleic acids can then be separated from the rest of the solution by centrifugation. The study aimed to evaluate bacterial DNA extraction using conventional boiling method followed by alcohol precipitation.

Material and Methods

Extended spectrum beta lactamase (ESBL)-producing bacilli (express the TEM gene) namely *Escherichia coli* (*E. coli*) and *Klebsiella pneumonia* (*K. pneumoniae*) were obtained and cultured on blood agar base (Oxoid). An aliquot of 1000µL of cell suspension containing 10^7 cells/mL from each of *Escherichia coli* (*E. coli*) and *K. pneumoniae* was transferred to microtubes and incubated. Cell suspensions were centrifuged at 4,500 rpm for 5 min at 4°C, and the pellets obtained were used for DNA extraction by boiling method with a modification. The collected material was placed into a tube containing 50 µL nuclease-free water, then subjected to boiling at 100°C for five minutes. The mixture was centrifuged at 3000g for 10 minutes. The DNA-containing upper aqueous phase was transferred into a separate 2 ml Eppendorf tube and 0.7 volumes of cold absolute ethanol was added. The aqueous phase was recovered by centrifugation for 20 min, and genomic DNA was precipitated by ethanol [8]. The pellet was washed in cold 70% ethanol then after a further centrifugation step the ethanol was removed, and the nucleic acid pellet was allowed to dry before being resuspended in aqueous TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The conventional phenol-chloroform DNA extraction, according to the protocol used by Ahmed et al [2], from the same overnight broth cultures was used as the control. The quality of the extracted DNA (absence of degradation) was estimated based on the size of the DNA fragments or relative position of the DNA smears in 1.5% agarose gels. Total extracted DNA was quantified using Qubit Fluorometer 2.0. A

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master-mix prepared from 199 microliter buffer plus 1 microliter from Qubit High Sensitivity reagent, once the mixture prepared, 198 microliter dispensed in each tube, then 2 microliter from DNA has been added to the mixture then vortexed for 2 seconds and incubated in dark for 2 minutes, reads taken by Qubit Fluorometer 2.0 as in table 1. All ESBL isolates were screened for bla TEM gene by PCR with specific primers (TEMF ATGAGTATTCAACATTTCCGTG, TEMR TTACCAATGCTTAATCAGTGAG) amplified at 840-bp fragment[9]. The thermal cycling conditions were: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 51°C for 30 s and extension at 72°C for 1 min, and final extension at 72°C for 10 min. DNA bands were viewed under UVP BioDoct It Imaging System after staining with ethidium bromide.

Table 1: Concentration of bacterial DNA isolated by the boiling method.

Sample	*E. coli*		*K. pneumonia*	
	Boiling method ng\mL	Control method ng\mL	Boiling method ng\mL	Control method ng\mL
Reading 1	206	239	288	404
Reading 2	195	223	287	455
Reading 3	198	226	282	462
Means	199.7±4.643	229.3±6.944	285.7±2.624	440.3±25.850

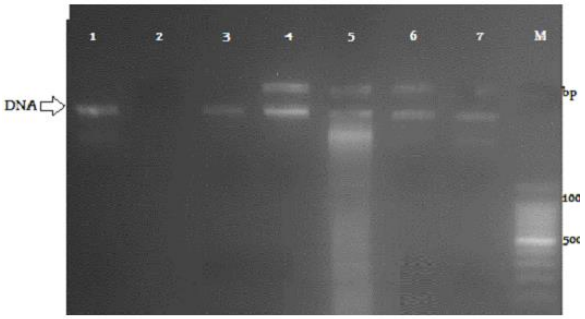

Figure 1: Purified bacterial DNA analyzed by electrophoresis in 1.5% agarose gel. Lanes 1: positive control. Lanes 2: negative control; Lanes 3-7: bacterial DNA; lane M: 100-bp DNA ladder.

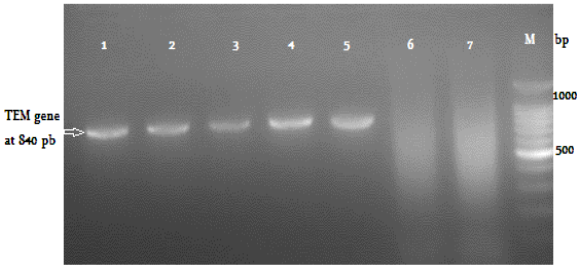

Figure 2: PCR detection of TEM in genomic DNA of ESBL Gram-negative strains analyzed by electrophoresis in 1.5% agarose gel. Lane1: positive control; lanes 2-5: 840-bp fragment of the TEM gene; Lanes 6&7: negative control; lane M: 100-bp DNA ladder.

Results and Discussion

In this study, the boiling method was modified for the rapid extraction and pure bacterial genomic DNA for the purposes of PCR analysis. The lasted time to extract DNA from bacteria was roughly estimated in 30 min. Alcohol precipitation is commonly used for concentrating, desalting, and recovering nucleic acids. Precipitation was mediated by the addition of ethanol. Purified DNA should be stored at –20°C or –70°C under slightly basic conditions (e.g., Tris×Cl, pH 8.0 or TE buffer) because acidic conditions can cause hydrolysis of DNA [10]. Table 1 shows that the extraction procedure performed using the boiling method resulted in high DNA isolation yields for both *E. coli* and *K. pneumoniae* bacteria in samples (199.7 and 285.7µg/ml, respectively) which is close to control method (229.3 and 440.3µg/ml). Figure 1 shows that DNA extracted by the method used in this study appears as a clear single band in the agarose gel, which indicates that it was not degraded. Figure 2 presents PCR amplification of the ESBL TEM gene. Thus, efficiency and speed of this method could be enhanced with the use of inexpensive facilities and the absence of toxic chemicals. In the present study, the classic phenol extraction was used as a control (standard) method to prepare DNA for amplification because this method has been proved to yield more positive PCR results for Gram-negative bacteria [11]. Our results showed that the DNA produced by this simple method is of low cost, fast and safe and the protocol can be used in PCR-based techniques on a wide range of Gram negative organisms, and in laboratories lacking supplies, equipment and technology. In addition, the boiling method could be completely competitive with the classical phenol chloroform method. Many reports suggested that direct boiling is useful for DNA extraction than many commercial kits [12]. It would also significantly reduce the cost and improve the efficiency of the sample preparation for metagenomics studying of human oral and gut microbiome diversity [12, 13]. The improved boiling method has many advantages, such as dispensing without use of hazardous chemicals like phenol and specific enzymes. Thus, it is fast, easy, cheap and can be applied for high-yield isolation of analytical-quality DNA from Gram-negative bacteria.

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