World Journal of
Experimental Biosciences

Volume 4, Number 1: 62-65
ISSN: 2313-3937
2016

Research article

Evaluation three methods of the extraction and purification of bacterial DNA of Gram positive and Gram negative bacteria

Hannaa Farhan Abbas[1]*, Ibtisam Hammood Naser AL Musawi[2]

ABSTRACT

Three methods were evaluated to choose the rapid and simple method of DNA extraction that is suitable for PCR detection. Ten isolates of Gram positive and Gram negative bacteria (*Bacillus subtilis, Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes, S. pneumonia: Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, S. typhimurium and Proteus mirabilis*). Gram positive bacteria were more resistant to cellular lyses due to high concentration of peptidoglycan within bacterial cell wall. The qualitative assessment of bacterial DNA revealed that DNA extraction with QuickExtract™ Bacterial DNA Extraction Kit method produced the highest DNA purity and highest DNA yield as compared with the other two methods. In conclusion, QuickExtract™ Bacterial DNA extraction kit method is technically simpler and more rapid than Wizard Genomic DNA purification kit and phenol- chloroform method which was more slow and low purity of yielded DNA. QuickExtract™ Bacterial DNA extraction kit is the best method for extracting genomic DNA from Gram positive and Gram negative bacteria, providing single-tube system. No toxic organic solvents, suitable for high-throughput applications, ready Lyse™ Lysozyme supplied and long DNA generated.

Keywords: DNA extraction, Gram positive bacteria, Gram-negative bacteria

Citation: Abbas HF, AL Musawi IHN. (2016) Evaluation three methods of the extraction and purification of bacterial DNA of Gram positive and Gram negative bacteria. *World J Exp Biosci* **4**: 62- 65.

Received February 3, 2016; Accepted February 18, 2016; Published March 4, 2016.

INTRODUCTION

The DNA extraction is one of the most commonly used procedures in genetics, molecular biology, and biochemistry. The isolation of prokaryotic nucleic acid is much less work-intensive than those described for plants and animals. Most Bacteria have cell walls that can be easily broken through and lysed for the isolation of DNA and RNA. Ideally the material used should be grown from pure culture. Depending on the type of organism used for genetic material, either agar or liquid cultures can yield similar results, as long as there is no contamination in the process. Extra care must be taken when using nutrient rich media, since most airborne bacteria can grow in this as well. This DNA can be used for restriction digests, Southern and Northern blot analysis, genomic library construction, and PCR [1]. Usually, two factors have to be particularly considered during the extraction procedure. The first is to

*Correspondence: Abbas.HF.hannaa_f1975@yahoo.com.
Department of Biology, College of Science, Al- Mustansiriyah University, Baghdad, Iraq.
Full list of author information is available at the end of the article.

maximize the DNA yield. The second is to ensure that the extracted DNA is amenable to several enzymatic treatments like PCR amplification [2]. In other words, the greatest challenge is the extraction of high-quality PCR-compatible DNA from the intestinal microflora. Several methods have been evaluated for bacterial cell wall lysis and DNA extraction using detergents, proteolytic enzymes, lysozyme, mechanical disruption, temperature changes alone or in various combinations, DNA stool mini- kit, *etc*. Although the DNA stool mini-kit method is convenient, rapid and highly efficient, it is not widely applied on account of its high cost [3,4]. The aim of an extraction procedure is to obtain a high quality and high yield of DNA from the samples. The extracted DNA should contain the least amount of proteins, RNA, or any PCR inhibitors. Removing those inhibitors is one of the key factors for a successful PCR [5,6]. Because of The isolation and purification of DNA is a key step for most protocols in molecular biology studies and all recombinant DNA techniques [7]. Several DNA extraction methods were widely used to isolate DNA from bacteria including phenol extraction but they often involve multiple, time consuming steps including the handling of toxic chemicals [8].

Various commercially available DNA extraction kits and systems are becoming increasingly popular because of their ease of use, limited labor, and ability to consistently produce high-quality DNA. Because of proprietary considerations of the manufacturer, the composition of some components in these kits is not revealed to the user [1]. Direct-extraction methods provide a simple approach in which bacteria are incubated with reagent(s), usually at elevated temperature, to produce a PCR-ready extract without further processing (other than addition of neutralizer and/or centrifugation to remove solids).These direct methods are fast, but DNA recovery can vary widely between bacterial genera and it is usually necessary to dilute the extract to avoid PCR inhibition [9,10].

From the extraction methods already published for various bacteria, we choose and compared three methods for extracting of DNA from Gram positive and Gram-negative bacteria. Phenol–chloroform method, QuickExtract™ Bacterial DNA Extraction Kit and Wizard Genomic DNA purification Kit methods were used in current study. The aim of this study is to choose the simplest and the most accurate and the easier method from the three extraction method which gives the largest amount of DNA and the highest purity of DNA that will be suitable for PCR technique.

MATERIALS AND METHODS

Bacterial isolates and growth conditions

Ten Bacterial isolates of Gram positive (*Bacillus subtilis*, *Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes and S. pneumoniae*) and Gram negative (*Escherichia coli*, *Klebsiella pneumoniae, Salmonella typhi, S. typhimurium and Proteus mirabilis*) bacteria were used in this study. Bacteria were grown overnight in Tryptone Soya Broth (TSB; Oxoid, Basingstoke, UK) and then washed three times in phosphate buffered saline (PBS; NaCl 8 g/l, KCl 0.2 g/l, Na_2HPO_4 1.15 g/l, KH_2PO_4 0.2 g/l). Bacteria were re-suspended in TSB to OD 660 nm.

DNA extraction

Three methods were used to extract and purify total bacterial DNA, the phenol–chloroform method, Wizard Genomic DNA purification Kit method and by QuickExtract™ Bacterial DNA Extraction Kit method.

Phenol–chloroform method

Pellet of bacteria was dissolved in 467 µl TE buffer, followed by addition of 30 µl of 10% SDS and 3 µl (20 mg/ml proteinase K). After incubation for 1 h at 37°C, 50 µl phenol: chloroform: isoamyl alcohol (1:25:24) was added and mixed by gentle inversion. Aqueous phase decanted into a new tube and; 0.1 ml of 3 M sodium acetate and 0.6 ml of isopropanol was added to it. The mixture was swirled slowly until DNA precipitated which was spooled with Pasteur pipette. DNA was dried and washed by dipping end of pipette into 1 ml of 70% ethanol for 30 s before dissolving in 150 µl TE buffer [11].

Wizard Genomic DNA purification Kit

The wizard genomic DNA purification kit (Promega / USA) was used according to the manufacturer's instruction.

QuickExtract™ Bacterial DNA Extraction Kit

0.5 ml of 10^8 bacterial suspensions was centrifuged at 1,700 x g (5,000 rpm) in a microcentrifuge for 3 min to pellet the cells. The bacterial cell pellet washed once with 0.5 ml of sterile water, then re-centrifuged at 1,700 x g (5,000 rpm) for 3 min. Carefully, the supernatant removed and discarded. Hundred microliter of QuickExtract Bacterial DNA Extraction Solution (Epicentre /USA) was Added to the cell pellet. Then 1 µl of Ready-Lyse Lysozyme solution added to each tube and mixed gently by inversion, made certain that both the bacteria and the Ready-Lyse Lysozyme are dispersed in solution. Incubated the suspension at room temperature for 15 minute (If the solution was not clear, we waited an additional hour at room temperature). Observed the lysis periodically; digestion can be extended to several hours if necessary. The sample was heated at 80°C for 2 min to kill any remaining viable bacteria.

Measurement of DNA concentration and purity

The yield and purity of DNA were determined by spectrophotometric method (BIO-RAD Smart Spec 3000; USA); for this purpose, DNA absorbance was measured at 260 nm (µg DNA/g sample; 1 *A* 260 = 50 µg/mL DNA) and protein impurities were checked at 280 nm [12]. The yield and purity of each DNA extraction method was statistically analyzed by excel 2003.

Detection The quality of total bacterial DNA

For each method tested, the presence and quality of the extracted genomic DNA from all the ten tested bacterial isolates were analyzed (run) into 1% agarose gel containing ethidium bromide at room temperature. Ten microliters of the DNA extracted by each method was added into the gel for 30 min at 150 V. The DNA purity and concentration according to the following equations: DNA purity, absorbance at 260 nm / absorbance at 280 nm. DNA yield (µg), DNA concentration (µg/µl) * total sample volume (ml).

RESULTS

Qualitative and quantitative assessment of DNA

Gram-positive bacteria were more resistant to cellular lyses due to the high concentration of peptidoglycan within the cell wall. The results obtained revealed that DNA extraction with QuickExtract™ Bacterial DNA Extraction Kit method produced the highest DNA purity and highest DNA yield when compared with other two methods (**Table 1**). Depending on the DNA extraction method used, DNA yield varied significantly.

Table 2. DNA yields and purity obtained by three DNA extraction methods

Isolates	Phenol–chloroform		Wizard Kit		QuickExtract Kit	
	Yield	purity	Yield	purity	Yield	purity
Bacillus subtilis	586.80	1.55	899.22	1.50	1956.11	1.80
Staphylococcus aureus	1110.54	1. 07	790.21	1.00	1745.03	1.54
S. epidermidis	1031.76	1.32	597.54	1.63	1950.06	1.90
Streptococcus pyogenes	983.60	1.25	977.98	1.01	1679.20	1.94
S. pneumonia	1005.42	1.21	764.81	1.93	1788.31	1.93
Escherichia coli	1722.25	1.56	895.20	1.64	1698.03	1.4
Klebsiella pneumonia	899.17	1.28	951.03	1.22	1977.52	1.33
Salmonella typhi	1328.01	1.60	1055.65	1.09	1850.11	1.97
S. typhimurium	1801.12	1.38	981.98	1.43	1870.51	1.02
Proteus mirabilis	966.76	1.07	988.95	1.01	1716.92	1.22

The extracted DNA by using the three methods was observed for degradation by 1% agarose gel electrophoresis. All DNA extracted by QuickExtract™ Bacterial DNA Extraction Kit, produced sharp bands, whereas the bands produced by the other two methods were not sharp and appeared with smear (**Fig 1, a, b** and **c**).

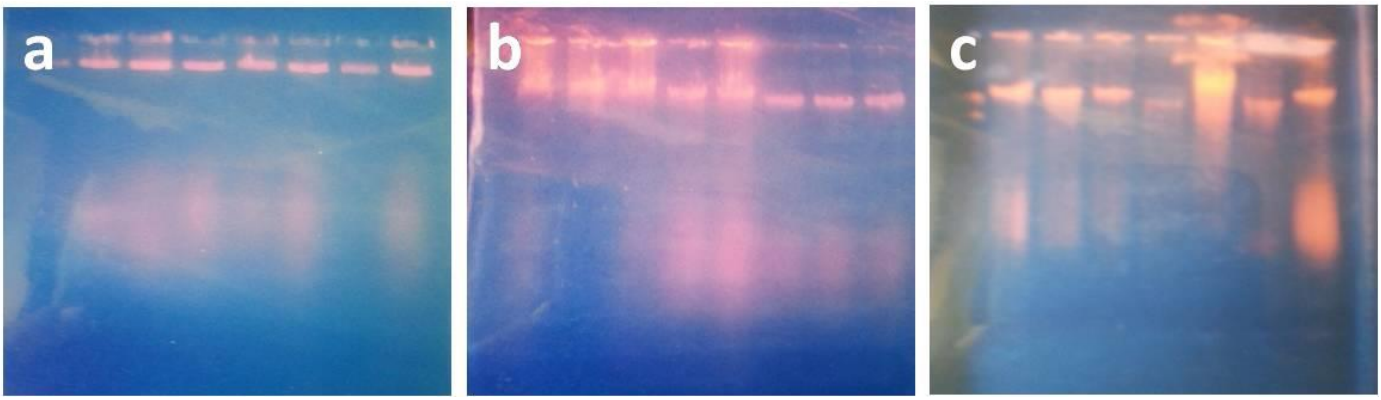

Fig 1. Electrophoresis graphs of bacterial DNA extracted from eight isolates by using a, QuickExtract™ Bacterial DNA Extraction Kit; b, Wizard Genomic DNA purification Kit; c, Phenol–chloroform method. The isolates from right to left: *Bacillus subtilis, Staphylococcus aureus, S. epidermidis, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumonia, Salmonella typhi* and *Proteus mirabilis.*

DISCUSSION

Bacterial lysis is the key to obtain bacterial DNA. Gram-positive bacteria were much more resistant to cellular lyses due to the high concentration of peptidoglycan within the cell wall. These results are consistent with the results of Sambrook and Russell [17]. In previous studies on DNA extraction from the bacteria, researchers used the traditional DNA extraction process that involved SDS, proteinase K digestion and other by phenol chloroform extraction method. In phenol–chloroform method bacterial cells are disrupted by initial treatment with the enzyme, egg-white lysozyme, which hydrolyzes the peptidoglycan that makes up the structural skeleton of the bacterial cell wall. The resultant cell walls are unable to withstand osmotic shock. Thus, the bacteria lyse in the hypotonic environment. The detergent, sodium dodecyl sulfate, (SDS, sodium dodecyl sulfate) then completes lysis by disrupting residual bacterial membranes. SDS also reduces harmful enzymatic activities (nucleases) by its ability to denature proteins. The chelating agents, citrate and EDTA (ethylene-diamine tetraacetic acid), also inhibit nucleases by removing divalent cations required for nuclease activity. This experiment employs a variety of fractionation methods to purify the bacterial DNA. Perchlorate ion is used to dissociate proteins from DNA. Chloroform–isoamyl alcohol is used to denature and precipitate proteins by lowering the dielectric constant of the aqueous medium [9,10,13].

Acknowledgment

This research was supported under Department of biology, College of Science, Al- Mustansiriyah University and Research and Training Forensic DNA Centre , AL Nahrain University, Baghdad, Iraq. The authors thank all members of laboratory of pathological analyses in al- kindy hospital for assistance with the collection some of bacterial isolates.

Conflict of interest

The authors declare that they have no conflict of interests.

REFERENCE

1. **Michele KN**, **Phaedra D**, **Mary E**, **David K**, **Aloysius P**, **et al.** (2002). DNA Isolation Procedures. Methods and Tools in Biosciences and Medicine Techniques in molecular systematics and evolution, ed. by Rob DeSalle *et al*. Birkhäuser Verlag Basel/Switzerland.
2. **Spaniolas S**, **Tsachaki M**, **Bennett MJ** and **Tucker GA**.(2008) Evaluation of DNA extraction methods from green and roasted coffee beans. *Food Control* **3**: 257-262.
3. **Aldous WK**, **Pounder JI**, **Cloud JL**, **Woods GL**. (2005) Comparison of six methods of extracting Mycobacterium tuberculosis DNA from processed sputum for testing by quantitative real-time PCR. *J Clin Microbiol* **43**: 2471-2473.
4. **Buck GE**, **O'Hara LC**, **Summersgill JT**. (1992) Rapid, simple method for treating clinical specimens containing Mycobacterium tuberculosis to remove DNA for polymerase chain reaction. *J Clin Microbiol* **30**: 1331-1334.
5. **Sidransky D**, **Tokino T**, **Hamilton SR**, **Kinzler KW**, **Levin B, et al.** (1992) Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. *Science* **256**: 102-105.
6. **Deuter R**, **Pietsch S**, **Hertel S**, **Muller O**. (1995) A method for preparation of fecal DNA suitable for PCR. *Nucleic Acids Res* **23**:3800-3801.
7. **Sambrook J**, **Fritsch EF**, **Maniatis T**. (1989) Molecular Cloning: A laboratory Manual (2nd Edition). Cold Spring Harbor Laboratory, New York.
8. **Ausbel FM**, **Brent R**, **Kingston RE**, **Moore DD**, **Seidman JG**, et al. (1995) Current Protocols in Molecular Biology. John Wiley and Sons, 2.4.1.
9. **Mandel M**, **Marmur J**. (1968) Use of ultraviolet absorbance-temperature profiles for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B**:195.
10. **Marmur J**. (1961) A procedure for the isolation of desoxyribonucleic acid from microorganisms. *J Mol Biol* 3:208.
11. **Parayre S**, **Falentin H**, **Madec MN**, **Sivieri K**, **Le Dizes AS**, **Sohier D**, **Lortal S.** (2007) DNA extraction method and optimization of PCR-temporal temperature gel electrophoresis to identify the predominant high and low GC-content bacteria from dairy products. *J Microbiol Methods* **69**:431–441.
12. **Samuel M**, **Lu M**, **Pachuk CJ**, **Sat i shchandran C**. (2003) A spectrophotometric method to quantify linear DNA. *Anal Biochem* **313**: 301-306.
13. **Puglisi JD**, **Tinoco I**. (1989) Absorbance melting curves of RNA. *Methods Enzymol* **180**:304.
14. **Khan IU**, **Yadav JS**. (2004) Development of a single-tube, cell lysisbased,genus-specific PCR method for rapid identification of mycobacteria: optimization of cell lysis, PCR primers and conditions, and restriction pattern analysis. *J Clin Microbiol* **42**: 453-457.
15. **Tan H**, **Wang J**, **Zhao ZK**. (2007) Purification and refolding optimization of recombinant bovine enterokinase light chain overexpressed in Escherichia coli. *Protein Expr Purif* **56**:40-47.
16. **Ki JS**, **Chang KB**, **Roh HJ**, **Lee BY**, **Yoon JY**, **Jang GY**. (2007) Direct DNA isolation from solid biological sources without pretreatments with proteinase-K and/or homogenization through automated DNA extraction. *J Biosci Bioeng* **103**: 242-246.
17. **Sambrook J**, **Russell D**. (2001) Molecular cloning: A laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
18. **Dauphin LA**, **Stephens KW**, **Eufinger SC**, **Bowen MD**. (2010) Comparison of five commercial DNA extraction kits for the recovery of Yersinia pestis DNA from bacterial suspensions and spiked environmental samples. *J Appl Microbiol* 108: 163–172.
19. **Heller LC**, **Davis CR**, **Peak KK**, **Wingfield D**, **Cannons AC, et al.** (2003) Comparison of methods for DNA isolation from food samples for detection of shiga toxin-producing Escherichia coli by real-time PCR. *Appl Environ Microbiol* **69**: 1844–1846.
20. **Elizaquível P**, **Aznar R**. (2008) Comparison of four commercial DNA extraction kits for PCR detection of *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7 and *Staphylococcus aureus* in fresh, minimally processed vegetables. *J Food Prot* **71**: 2110–2114.

Author affiliation

1. *Department of Biology, College of Science, Al-Mustansiriyah University, Baghdad, Iraq.*
2. *Research and Training Forensic DNA Centre, AL Nahrain University, Baghdad, Iraq.*