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Comparison of three DNA extraction methods for polymerase chain reaction (PCR) analysis of bacterial genomic DNA

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Rapid isolation of high-purity microbial genomic DNA is necessary for genome analysis. In this study, the authors compared a one-hour procedure using a microwave with enzymatic and boiling methods of genomic DNA extraction from Gram-negative and Gram-positive bacteria. High DNA concentration and purity were observed for both MRSA and ESBL strains (80.1 and 91.1 µg/ml; $OD_{260/280}$, 1.82 and 1.70, respectively) when the extraction protocol included microwave pre-heating. DNA quality was further confirmed by PCR detection of *mecA* and *CTX-M*. In conclusion, the microwave-based procedure was rapid, efficient, cost-effective, and applicable for both Gram-positive and Gram-negative bacteria.

Key words: DNA, MRSA, ESBL, gene, enzymatic, boiling, microwave.

INTRODUCTION

Surveillance of bacteria has become an important interest since the emergence of antimicrobial-resistant organisms. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) and extended spectrum beta lactamase (ESBL) bacteria are a leading cause of nosocomial infections in health care facilities in many countries. Bacterial genomic DNA encodes all genetic information necessary for functioning of bacterial cells. The DNA molecules are large and, in most bacteria, are organized into single circular chromosomes. Rapid isolation of genomic DNA from microorganisms is essential for DNA analyses such as PCR, gene cloning, sequencing, and fingerprinting (Cheng and Jiang, 2006). The availability of effective DNA extraction methods is essential for microbiology studies. Several DNA isolation and purification strategies have previously been investigated with variable rates of success (Lakay et al., 2007; Menon and Nagendra, 2001; Orsini and Romano-Spica, 2001).

In general, isolation of bacterial genomic DNA involves three main steps: cell disruption, DNA extraction, and DNA purification. Genomic DNA is usually extracted with a special extraction buffer and is further purified by phenol/chloroform extraction followed by isopropanol or ethanol precipitation (Fredricks and Relman, 1998). While DNA composition is more or less universal in all species, contaminants such as RNA and proteins and their relative amounts differ considerably. These variations should be taken into consideration while developing or selecting a cell lysis method (Moore et al., 2004).

Many strategies currently used for the extraction of DNA from bacterial cells, such as enzymatic, chemical or thermal lysis, mechanical disruption of the cell wall by beads or sonication, or a combination of the above (Rantakokko and Jalava, 2002; Reischl et al., 2000; Tongeren et al., 2011). Variation in the lysing efficiency and DNA purity can fundamentally affect successful DNA analysis by

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methods such as PCR. Each cell disruption approach has specific advantages and disadvantages. Chemical methods use detergents to solubilize cell membranes. Commonly used detergents are SDS, Triton X-100, and CTAB (Ausbel et al., 1998). The disadvantage of detergent-based cell lysis is that detergents often contaminate DNA samples and inhibit further manipulations. Enzymes attacking the components of cell surface or cytosol are often added to the detergent-based lysis buffers. However, these enzymes lack efficacy against most Gram-positive bacteria: lysozyme is not active for staphylococci and most of the bacilli strains, while lysostaphin is active only for staphylococci. Proteinase K is very efficient for Gram-negative bacteria (Bollet et al., 1991). Heat is also applied to enhance lysis of bacterial cells. Sonication, grinding in liquid nitrogen, shredding with rigid spheres or beads, and application of mechanical stress such as filtration have been used for difficult-to-lyse samples prior to or in conjunction with lysis solutions (Shahriar et al., 2011). These methods still have disadvantages, which include laborious manipulations such as four to six changes of microcentrifuge tubes, multiple stages of incubition, precipitation, elution, washing and drying, or requirement of special equipment. The DNA yield and purity are often poor due to the multi-step manipulations; therefore, an easy, rapid, and efficient method for DNA extraction that can be used on a routine basis needs to be developed. Microwave irradiation has proven to be useful in extraction of DNA from different species (Muller et al., 1998; Goodwin and Lee, 1993; Bollet et al., 1991). Microwave irradiation is presumed to act by exposing DNA normally protected by cellular structures.

In this study, we compared three methods of DNA isolation from Gram-negative and Gram-positive bacterial species: a one-hour procedure using an ordinary microwave and enzymatic and boiling methods. Quantitative and qualitative analysis of extracted DNA was performed by spectrophotometry, agarose gel electrophoresis, and PCR.

MATERIALS AND METHODS

Methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative extended spectrum beta lactamase (ESBL)-producing bacilli were obtained from the microbiology laboratory at the Department of Environmental and Health Research, the Custodian of the Two Holy Mosques Institute for Hajj and Umrah, Umm Al-Qura University, Makkah, Saudi Arabia**.** Gram-positive MRSA express the *mecA* gene and Gram-negative ESBL producers express the *CTX-M* gene.

Strains were grown on chocolate agar slants. Bacteria were collected and pelleted using a microcentrifuge and were cultured in nutrient broth for 24 h at 37°C with agitation at 100 rpm. Cell suspensions were centrifuged at 4,500 rpm for 5 min at 4°C, and the pellets obtained were used for DNA extraction by the three methods. Three test tubes containing cell pellets (approximately 0.1 g each) were used for DNA extraction. In the microwave lysis method, cell pellets were washed with 1 ml of TE (10 mM Tris, pH 8, 10 mM EDTA) and were resuspended in 100 µl of TE. After addition of 50 µl of 10% SDS the mixture was incubated for 30 min at 65°C. The lysates were centrifuged and supernatants were removed. The microtubes were then placed in a microwave oven (with specifications; LG grill, model No. MG-604AZ, input 220v-50/bHz, microwave 1350 w, RF output 900 w,2,4500 MH) and heated twice for 1 min at 900 W or three times for 1 min at 750 W. The pellets were dissolved in 200 µl of TE and were extracted with an equal volume of phenol/chloroform/ isoamyl alcohol (25:24:1) for 15 min. The aqueous phase was recovered by centrifugation for 20 min and precipitated with ethanol (Bollet et al., 1991).

In the enzymatic method, cell pellets were resuspended in 564 µl of TE buffer, 10 µg of crystalline lysozyme was added and mixed thoroughly, and the samples were incubated for 10 to 60 min at 37°C. Then, 6 µl of proteinase K (10 mg/ml) was added followed by 30 µl of SDS (10 to 20%), and the samples were mixed thoroughly and incubated at 37°C until the suspension became relatively clear and viscous. Next, 100 µl of 5M NaCl was added, and the samples were incubated at 65°C for 2 min, followed by addition of 80 µl of 65°C-preheated CTAB/NaCl solution and incubation at 65°C for 10 min. The suspension was extracted with an equal volume (approximately 800 µl) of phenol/chloroform/isoamyl alcohol (25:24:1) and centrifuged at 15,000 × *g* for 5 min. The DNA-containing upper aqueous phase was transferred into a separate 2 ml Eppendorf tube and 0.7 volumes (approximately 560 µl) of isopropanol were added. The aqueous phase was recovered by centrifugation for 20 min, and genomic DNA was precipitated by ethanol (Moore et al 2004). In the boiling lysis method, cell pellets were resuspended in 0.1 ml of molecular biology-grade water and were centrifuged at 15,000 × *g* for 10 min. Pellets were resuspended in 40 µl of water, subjected to boiling at 100°C in a water bath for 10 min, cooled on ice, centrifuged at 15,000 × *g* for 10 s, and stored at -20°C (Queipo-Ortuño et al*.,* 2008).

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% agarose gel electrophoresis for 35 min at 90 V using 5 x TBE running buffer (4.84 g/L Tris, 0.37 g/L EDTA, pH 8). Gels were stained with ethidium bromide (2 g/ml) and DNA bands were viewed under UVP BioDoct-IT digital imaging system.

To determine DNA concentration, total extracted DNA was quantified using UV/VIS spectroscopy at 260 nm using an equation: dsDNA concentration = 50 µg/mL × OD_{260} (Sambrook et al., 1989). DNA purity was assessed by calculation of $A_{260/280}$ ratio after measuring absorbance at 280 nm.

All MRSA isolates were screened for the presence of the *mecA* resistance gene by PCR using specific primers MECA P4 (5'-TCCAGATTACAACTTCACCAGG-3') and MECA P7 (5'-CCACTTCATATCTTGTAACG-3') which resulted in amplification of the 162-bp fragment (Kader et al., 2011). The thermal cycling conditions were as follows: an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 30 s and extension at 72°C for 1 min, with final extension at 72°C for 5 min. All ESBL isolates were screened for *bla CTX-M* gene by PCR with specific primers CTX-MF (5'-TTTGCGATGTGCAGTACCAGTAA-3') and CTX-MR (5'-CGATATCGTTGGTGGTGCCATA-3') which resulted in amplification of the 544-bp fragment (Ahmed et al., 2013). 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.

RESULTS

We compared three different approaches to DNA extraction from Gram-positive and Gram-negative methods. Figures 1, 2 represent only results derived from the micro-

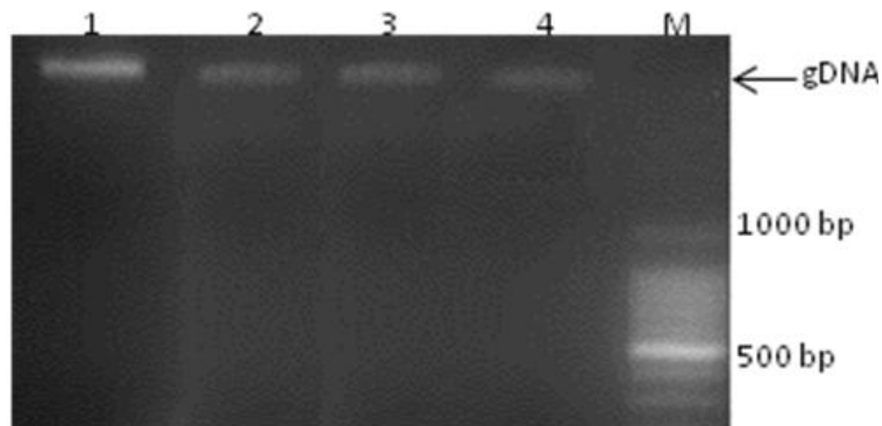

Figure 1. Purified bacterial DNA analyzed by electrophoresis in 1.5% agarose gel. Lanes 1: positive control. Lanes 2, 3 and 4: bacterial DNA; lane M: 100-bp DNA ladder.

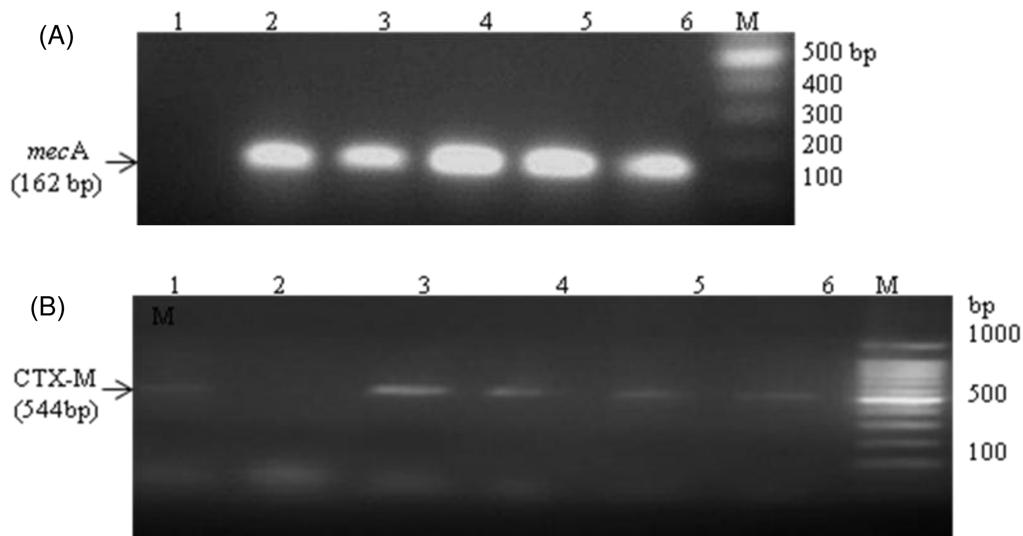

Figure 2. (A): PCR detection of *mec*A in genomic DNA of Gram-positive MRSA strains analyzed by electrophoresis in 1% agarose gel. Lane1: negative control; lane 2: positive control; lanes 3, 4, 5, and 6: 162-bp *mec*A gene fragment of the MRSA strains; lane M: 100-bp DNA ladder. (**B):** PCR detection of the *CTX-M* gene in genomic DNA samples of Gram-negative ESBL bacteria analyzed by electrophoresis in 1% agarose gel. Lane1: positive control; lane 2: negative control; lanes 3, 4, 5, and 6: 544-bp fragment of the *CTX-M* gene; lane M: 100-bp DNA ladder.

microwave based method other methods are not shown. Table 1 shows that the extraction procedure performed using the microwave method resulted in high DNA isolation yields for both Gram-positive and Gram-negative bacteria (80.1 and 91.1 µg/ml, respectively). In addition A260/A280 ratios suggest that the quality of isolated DNA was acceptable for further analytical applications (1.82 and 1.70, respectively).

Figure 1 shows bacterial genomic DNA from ESBL Gram-negative (lane 3, 4, and 5) and MRSA Gram-positive strains (lane 6 and 7) analyzed by agarose gel electrophoresis. The quality of the isolated DNA was further confirmed by PCR analysis. Figure 2 presents PCR amplification of the MRSA mecA gene and ESBL CTX-M gene, respectively.

DISCUSSION

The advances in DNA analytical techniques, including PCR, cloning, hybridization, sequencing, and fingerprinting, have enabled comprehensive analysis of the bacterial genomes. Several protocols of isolation and purification of DNA from various types of bacteria have been developed and described. In general, DNA isolation is a multi-step proce-

Table 1. Concentration and purity of bacterial MRSA and ESBL DNA isolated by three DNA extraction methods.

Microorganism	DNA Concentration μg/ml			A260/A280		
	Boiling	Enzymatic	microwave	Boiling	enzymatic	Microwave
	71.0	61.8	80.3	1.21	1.75	1.81
MRSA (Gram-positive)	71.5	62.2	79.9	1.23	1.76	1.82
	71.1	62.3	80.1	1.22	1.80	1.83
Mean ± S.D.	71.2 ± 0.26	62.1 ± 0.26	80.1 + 0.2	1.22 ± 0.01	1.77 ± 0.03	1.82 ± 0.01
	65.4	55.6	91.4	1.16	1.56	1.68
ESBL (Gram-negative)	65.7	55.2	91.0	1.15	1.54	1.72
	65.4	55.7	91.2	1.20	1.55	1.70
Mean ± S.D	65.5 ± 0.17	55.5 + 0.26	91.2±0.2	1.17 ± 0.03	1.55 ± 0.01	1.70 ± 0.02

dure involving cell lysis by treatment with lytic enzymes and/or detergents, DNA extraction with organic solvents, and DNA recovery by alcohol precipitation (Moore et al., 2004). Some of these methods are time consuming and not very efficient. The yield and purity of the extracted DNA is essential for subsequent analysis including PCR-based diagnostics of infectious pathogens; therefore a rapid, easy-to-use, efficient, and cost-effective method for bacterial DNA isolation is necessary.

In this study we compared a one-hour procedure for DNA extraction using an ordinary microwave oven with two other procedures-sample boiling and enzymatic lysis-in terms of DNA yield and purity. In addition, DNA suitability for PCR-based detection of *mecA* from Gram-positive MRSA and *CTX-M* from Gram-negative ESBL strains was analyzed. The boiling procedure developed by Reischl et al. involves thermal lysis and has been used for total DNA extraction from *S. aureus* (Reischl et al., 2000). However, the cell wall of Gram-positive bacteria, which contains a peptidoglycan layer, is more robust compared to that of Gram-negative bacteria. Although the boiling method is more rapid, simple, and effective than standard methods for bacterial DNA isolation (Queipo-Ortuño et al., 2008), its yields are too low for subsequent analytical procedures.

The results presented in this study show that microwave pre-treatment facilitates efficient cell lysis and DNA extraction, leading to increased yields of isolated bacterial DNA (Table 1). Figure 1 shows that DNA extracted by the methods used in this study appears as a clear single band in the agarose gel, which indicates that it was not degraded. The yields of DNA extracted from different strains using enzymatic and boiling approaches are lower than that obtained after microwave pre-heating (Table 1), which is consistent with the previous results (Bollet et al., 1991). One probable explanation is that commercially available enzymes can be contaminated with microbial DNA. In addition, these enzymes often require special storage conditions such as refrigeration (Tongeren et al., 2011). The other reason may be variations in susceptibility to cleavage with proteinase K underlined by differences in the protein structure of cell wall among bacterial species (Moore et al., 2004). Thus, Gram-positive bacteria are more resistant to proteinase K than the Gram-negative species (Bollet et al., 1991). Previously the microwave lysis method followed by direct PCR has been found to be less time consuming, 5 hours, as compared to 9 hours by conventional technique (Menon and Nagendra 2001). Also Reyes-Escogido et al., 2010 established a Chelex 100-Microwave method for the purification of bacterial genomic DNA (gDNA) in less than 20 min which was useful for multiple purposes.

These results showed that DNA extraction using microwave pre-heating was more successful than the enzymetic and boiling methods. The DNA isolated from both Gram-positive and Gram-negative bacteria using the microwave pre-heating was more in quantity and was of better quality compared to that obtained by other methods. From these results, it can therefore be concluded that the microwave-based procedure, which is easy, rapid, and cost-effective, can be applied for high-yield isolation of analytical-quality DNA from both Gram-positive and Gram-negative bacteria.

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