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EXPERIMENTAL ARTICLES

A Robust Universal Method for Extraction of Genomic DNA from Bacterial Species[1]

Sina Atashpaz[a, b, 2], Sajjad Khani[a,b,2], Abolfazl Barzegari[a], Jaleh Barar[a,b], Sepideh Zununi Vahed[c], Reza Azarbaijani[d], and Yadollah Omidi[a,b, 3]

[a] *Research Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran*

[b] *School of Advanced Biomedical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran*

[c] *Faculty of Biology, Tarbiat Moallem Azarbayjan University, Tabriz, Iran*

[d] *Department of Molecular Biology, Iranian Biological Research Center (IBRC), Karaj, Iran*

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Abstract—The intactness of DNA is the keystone of genome-based clinical investigations, where rapid molecular detection of life-threatening bacteria is largely dependent on the isolation of high-quality DNA. Various protocols have been so far developed for genomic DNA isolation from bacteria, most of which have been claimed to be reproducible with relatively good yields of high-quality DNA. Nonetheless, they are not fully applicable to various types of bacteria, their processing cost is relatively high, and some toxic reagents are used. The routine protocols for DNA extraction appear to be sensitive to species diversity, and may fail to produce high-quality DNA from different species. Such protocols remain time-consuming and tedious, thus to resolve some of these impediments, we report development of a very simple, rapid, and high-throughput protocol for extracting of high-quality DNA from different bacterial species. Based upon our protocol, interfering phenolic compounds were removed from extraction using polyvinylpyrrolidone (PVP) and RNA contamination was precipitated using LiCl. The UV spectrophotometry and gel electrophoresis analysis resulted in high A_{260}/A_{280} ratio (>1.8) with high intactness of DNA. Subsequent evaluations were performed using some quality-dependent techniques (e.g., RAPD marker and restriction digestions). The isolated DNA from 9 different bacterial species confirmed the accuracy of this protocol which requires no enzymatic processing and accordingly its low-cost making it an appropriate method for large-scale DNA isolation from various bacterial species.

Key words: bacteria, genomic DNA extraction, RAPD.

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Genomic DNA/RNA isolation is the first and the most important requirement in carrying out molecular biology techniques such as PCR, restriction enzyme analysis, Southern hybridization, genomic DNA library construction [1], mutation detection or linkage analysis [2, 3], as well as DNA microarray gene expression profiling [4]. Furthermore, bacterial fingerprinting methods such as random amplification of polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), repetitive extragenic palindromic (REP) and enterobacterial repetitive intergenic consensus (ERIC) are the most dominant ways for the evaluation of genetic variations [5–9]. All these techniques require a reasonable amount of DNA with good enough quality, fidelity and concentration [10]. Therefore, an efficient procedure for DNA extraction is required, combining simplicity with high yields of pure DNA suitable for molecular biology studies.

Most of the routinely used protocols for preparation of bacterial chromosomal DNA are primarily based on two strategies including either cetyl trimethyl ammonium bromide (CTAB) or lysozyme/detergent-based method followed by improvement of the procedure by removal of protein and RNA contamination [11, 12]. However, many of gram-positive bacteria, such as *Staphylococcus aureus*, are resistant or weakly susceptible to lysozyme due to their cell wall structure [1]. Further, substances such as polysaccharides, glycoproteins, and lipopolysaccharides were reported to interfere with the extraction process through co-precipitating with DNA, thus imposing inadvertent extraction problems [1]. Accordingly, problems such as poor yields and interference with restriction enzymes digestion have been reported to be associated with some standard DNA extraction protocols [13, 14].

[1] The article is published in the original.
[2] Equal contribution of Sina Atashpaz and Sajjad Khani.
[3] Corresponding author; e-mail: yomidi@tbzmed.ac.ir

In general, many existing protocols are laborious, time-consuming, and, moreover, restricted to certain types of bacteria [15]. Furthermore, due to chemotypic heterogeneity and different characteristics of the extracellular material of different bacterial species, development of an optimal DNA extraction procedure with a single protocol appears to be required for attaining such goals [14, 16]. Thus, an ideal protocol needs to be a rapid, efficient and safe procedure that should be applicable to a wide range of bacteria. To deal with these problems, we aimed to optimize a method to provide an effective extraction of genomic DNA from different types of bacteria with different cell wall structure (i.e. gram-negative and gram-positive).

MATERIAL AND METHODS

Bacterial Strains and Culturing Condition

Bacterial isolates representing 9 different types of species (differing in cell wall structure and extracellular material) were obtained from the Persian Type Culture Collection (PTCC), Tehran-Iran. Reference strains of Staphylococcus aureus (PTCC 1112), *Staphylococcus epidermidis* (PTCC 1435), *Enterococcus faecalis* (PTCC 1319), *Bacillus subtilis* (PTCC 1720), *Rhizobium radiobacter* (PTCC 1654), and *Micrococcus luteus* (PTCC 1109) were used. We also used some bacteria which were isolated from soil (*Rhodococcus* sp. and *Streptomyces* sp.) and yogurt samples (*Lactobacillus* sp).

Extraction Procedure

General DNA extraction kit with some modifications was exploited for isolation of various bacterial chromosomal DNA [17–19].

The extraction process was carried out as follows:

(1) Selected colonies of bacteria (i.e., 2–3 colonies) were ground into a fine powder in liquid nitrogen using precooled mortar and pestle. In order to extract DNA from liquid culture, the bacterial pellet obtained by centrifugation was freezed using liquid nitrogen in a 1.5 ml Eppendorff tube and then transferred to the mortar.

(2) The lysing buffer (2% CTAB (Merck, Hohenbrunn, Germany), 100 mM Tris–HCl (Merck, Darmstadt, Germany), 1.4 M NaCl (Merck, Darmstadt, Germany), 1% PVP (AppliChem, Darmstadt, Germany), 20 mM disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA; Merck, Darmstadt, Germany), 0.2% LiCl (Merck, Darmstadt, Germany). The pH was adjusted at ~8 for the solution used before autoclaving). Then 800 μl of solution was added to the sample, mixed vigorously and then transferred to a 1.5 ml centrifuge tube.

(3) The sample was incubated at 65°C (30 min for gram-negative bacteria; 2 h for gram-positive bacteria). The sample was gently shacked every 10 min.

(4) The sample was centrifuged at 6160 *g* (10000 rpm) for 5 min at 4°C.

(5) The supernatant was transferred into a new tube and an equal volume of chloroform-lsoamilalchol (Merck, Darmstadt, Germany) (24 : 1 vol/vol) was added. Then the tube was gently flipped several times.

(6) The sample was centrifuged at 8870.4 *g* (12000 rpm) for 8 min at 4°C. The centrifugation speed (*g*) should be adjusted to the designated value to avoid shearing stress and obtain intact DNA. The upper phase was then transferred into a new tube.

(7) In order to increase the yield of extracted DNA, 100 μl of 5 M sodium acetate (Merck, Darmstadt, Germany) was added into the tube and mixed by gentle flipping.

(8) Equivalent volume of cold (–20°C) isopropanol (Merck, Darmstadt, Germany) was added to precipitate the DNA, then the sample was stored at –20°C for 30 min. Isopropanol should be added dropwise and mixed gently, since rapid addition may cause DNA fragmentation.

(9) The sample was precipitated at 12073.6 *g* (14000 rpm) for 10 min at 4°C.

(10) For the first washing step, the supernatant was removed and 500 μl of 96% ethanol (Merck, Darmstadt, Germany) (4°C) was added to the sample which was then centrifuged at 8870.4 *g* (12000 rpm) for 5 min.

(11) For the second washing step, the supernatant was removed and 500 μl of 70% ethanol (4°C) was added to the sample which was then centrifuged at 8870.4 *g* (12000 rpm) for 5 min.

(12) The supernatant was discarded and the pellet was dried at room temperature. Note: higher centrifugation speeds or excessive drying period may decrease the water solubility of the pellet.

(13) The pellet was dissolved in 50 μl TE buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0), (Merck, Darmstadt, Germany)] and DNA solution was stored at –20°C.

Quantity and Quality Assessment of the Extracted DNA

Quantity and quality of extracted DNA. The extracted DNA was quantified using a NanoDrop 1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, United States). The A_{260}/A_{280} absorbance ratio was used to determine undesired contaminations.

To evaluate the quality and intactness of the extracted DNA, gel electrophoresis was used. The extracted DNA (5 μl) was loaded on 1% agarose gel (Invitrogen, California, United States), which contained ethidium bromide (1 μg/ml) for DNA staining. For image acquisitions, a G:Box™ gel documentation system (Syngene, Cambridge, United Kingdom) was used.

RAPD analysis. Analysis of RAPD was carried out in order to test the intactness/fidelity of the genomic

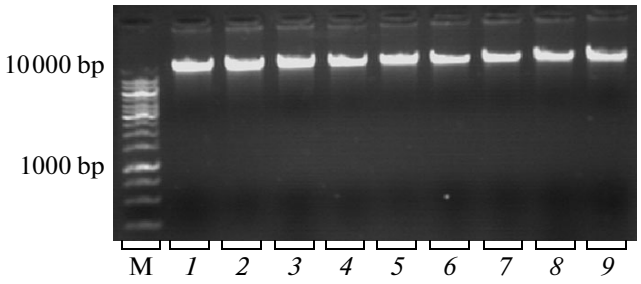

Fig. 1. Electrophoretic analysis of total DNA on 1% agaros gel. Lanes *1–9* represent *Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis, Streptomyces* sp., *Enterococcus faecalis, Micrococcus luteus, Rhodococcus* sp., *Rhizobium radiobacter* and *Lactobacillus* sp., respectively. M = 1 kb Plus DNA Ladder (GeneRuler^{TM}, Fermentase, GmbH, Germany).

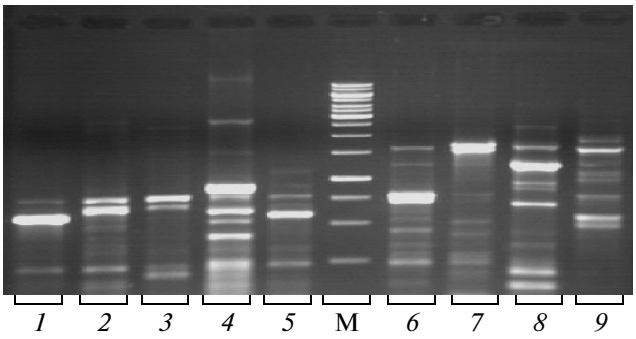

Fig. 2. PCR amplification of DNA extracted from 9 species of bacteria using random primer P17; about 50 ng of DNA was used for the PCR reaction. Amplified PCR products were electrophoresed on an agarose gel (1.5%). Lanes *1* to *9* were ordered as on Fig. 1 (GeneRuler^{TM}, Fermentase, GmbH, Germany).

DNA and to determine the possible inhibitory materials which may interfere with the reaction. PCR reaction was performed in a 25 μl volume, consisting of: 12.5 μl PCR Master Kit (2×), (Cinagen Inc., Tehran, Iran), 0.4 μM; primer set as P17: 5'-CCTGGGCTTG-3' (Cinagen Inc., Tehran, Iran) and 50 ng/μl genomic DNA. The amplification program consisted of one initial denaturation at 94°C for 5 min followed by 40 cycles of 60 s at 94°C for denaturation, 60 s at 40°C for primer annealing, 60 s at 72°C for extension and DNA synthesis and final extension at 72°C for 10 min. The products were separated on 1.5% agarose gel containing ethidium bromide (1 μg/ml), then images were taken using a G:Box™ gel documentation system (Syngene, Cambridge, United Kingdom).

Digestion with restriction enzymes. The restriction enzyme digestion methodology was used to assess the purity, quality and intactness of the extracted DNA. Each reaction was carried out in a total volume of 20 μl containing the following: 1 μg DNA, 4 μl of 10× Tango buffer (Fermentas, GmbH, Germany) and 0.5 μl of restriction enzyme, *Eco*R I (10 u/μl) (Fermentas, GmbH, Germany) incubated at 37°C for 4 h. The products were separated on 1.5% agarose gel containing ethidium bromide (1 1 μg/ml), and images were obtained using G:Box system.

Bacterial species and results of absorbance ratio measured by NanoDrop 1000 Spectrophotometer

Species	Yield (ng/μl)	$A_{260/280}$	$A_{260/230}$
S. aureus	652.4	2.00	1.98
B. subtilis	246.2	1.92	1.63
S. epidermidis	432.2	1.82	1.33
Streptomyces sp.	131	1.85	1.21
E. faecalis	570.9	1.94	1.99
Rhodococcus sp.	132.7	1.87	1.20
M. luteus	90	1.95	1.40
R. radiobacter	96	1.80	1.31
Lactobacillus sp.	138.6	1.73	1.20

RESULTS

The extracted genomic DNA from 9 different species of bacteria was found to be intact by agarose gel electrophoresis even though it was exposed to several steps of extraction/purification (Fig. 1).

The spectrophotometric analysis for A_{260}/A_{280} resulted in average about 1.90. This indicates that the extracted DNA was free from protein contamination (table).

Furthermore, in order to check the efficiency and reliability of the method, the RAPD technique was applied and the PCR product yielded a clear band pattern and adequate intensity (Fig. 2). Although many of the PCR-based techniques do not necessarily require high-quality DNA, pure DNA is recommended for PCR-based assays such as RAPD, which is susceptible to artifactual polymorphism [7, 20].

Extracted DNA obtained using this protocol was directly used for quality assessment with restriction digestion and the result for digestion with *Eco*RI showed that the isolated DNA was very pure with high quality for enzymatic reaction, in which a minimal inhibitory effect of the extraction process was observed (Fig. 3).

DISCUSSION

Many protocols have been developed for extraction of bacterial genomic DNA; however, only a few of them provide for optimal DNA isolation from widespread types of bacteria. Having applied some classical modifications in the currently used protocols, we aimed to optimize such protocols to achieve an easy-

to-handle and less toxic (yet highly efficient) extraction system. This new DNA isolation method may provide a platform for biotechnological investigations where the intactness and quality of the DNA appears to be a crucial requirement.

In this study, selection of bacteria was performed based upon the structural complexity of cell wall, amount of extracellular material (*Lactobacillus* sp., *S. aureus*, *B. subtilis*, and *Streptomyces* sp.), economical importance (some species of *Streptomyces* and *Lactobacillus*, as well as *B. subtilis*), and bacterial significance in clinical (*S. aureus, S. epidermidis, E. faecalis* and *M. luteus*) and biotechnological researches (*B. subtilis, Streptomyces* sp., and *R. radiobacter*). It is now well documented that gram-positive bacteria possess an intricate cell wall architecture, together with the extensive concentration of peptidoglycan within their cell wall [3, 13, 16]; the extraction of the DNA from these bacteria appears therefore to be challenging and problematic. Hence, we examined this protocol for chromosomal DNA extraction in various bacteria including gram-positive as well as one gram-negative one (i.e., *R. radiobacter*) to assess its effectiveness, reproducibility and comprehensiveness as a universal system.

This protocol requires a single step for lysing the bacterial cells, which is then followed by straightforward isolation of DNA with chloroform–isoamyl alcohol and ethanol for precipitation and purification of the genomic DNA. In order to eliminate RNA from the extracts, LiCl was used in the lysing buffer to selectively precipitate the large molecules of RNA [21, 22]. This selective precipitation is more advantageous than RNase treatment, in which the RNA is enzymatically degraded into smaller fragments, but not removed from the extracts [21, 22]. Furthermore, in this method we do not utilize β-mercaptoethanol that may be toxic to some extent [23]. CTAB was used as an effective detergent which separates the polysaccharides from DNA during extraction [11, 23]. The function of EDTA is to bind DNA thus protecting it from degradation [2, 24]. To eliminate polysaccharides and secondary metabolites during precipitation, potassium acetate in combination with isopropanol were utilized [25, 26]. In addition, high concentrations of PVP were employed to promote the precipitation of phenolic compounds through interaction with them. PVP can protect DNA against the oxidative activity of secondary metabolites and DNase enzymes in lysed bacteria [24, 27].

Considering all these issues, our protocol seems to be a comprehensive, relatively simple and rapid method that is an efficient approach with high yields of pure DNA in comparison with other existing methods. Moreover, our studies revealed (see Fig. 1 and table) that application of this protocol can provide a robust podium for the preparative isolation of high-quality and pure genomic DNA from any types of bacteria in a relatively time-efficient manner. Since this protocol provides high-quality genomic DNA for PCR amplification (Fig. 2), restriction enzyme digestion (Fig. 3) and presumably some other related molecular biology studies, it may be used for a range of techniques that require high-quality genomic DNA. Besides, this method requires no enzymatic processes; hence its low cost makes this protocol very attractive for the large-scale isolation of genomic DNA.

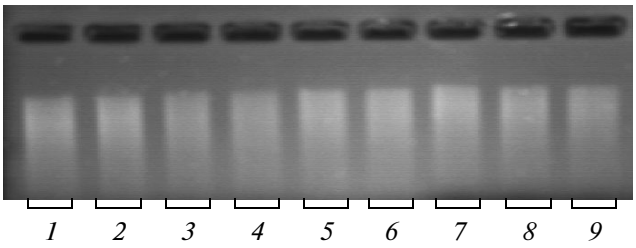

Fig. 3. Results of DNA digestion on 1.5% agarose gel with *Eco*RI restriction enzyme. Lanes *1* to *9* were ordered as on Fig. 1.

In general, the intactness of DNA is the prime cornerstone of genomic investigation in the era of omics technologies. In particular, rapid molecular detection of life-threatening and clinically important bacteria appears to be largely dependent on development of an efficient DNA isolation method. With trivial differences, our protocol yielded high-quality intact DNA from various bacterial species implying its accuracy and robustness. Thus, upon our examinations and previous results, we propose that this present protocol could serve as a universal competent method for isolation of genomic DNA from a variety of samples (e.g., plants, fungi, animal and microorganisms) even though they contain different types of extracellular materials as well as dissimilar cellular envelops architecture.

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