

Full Length Research Paper

Rapid DNA extraction of bacterial genome using laundry detergents and assessment of the efficiency of DNA in downstream process using polymerase chain reaction

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Genomic DNA extraction from bacterial cells involves processes normally performed in most biological laboratories. Therefore, various methods have been offered, manually and kit, but these methods may be time consuming and costly. In this paper, genomic DNA extraction of *Pseudomonas aeruginosa* was investigated using some laundry detergent brands available in Iran. Afterwards, efficiency of the detergents was compared with manually standard methods and kits. To evaluate the efficiency of the genomic DNA in the processes in which DNA is used as a template, the polymerase chain reaction (PCR) tests and enzyme digestion of PCR product were used. The results show that the detergents could be used to extract genomic DNA. Among the brands studied, five-enzyme Taj and three-enzyme Saftlan had the best performance compared to standard methods.

Key words: Bacterial genome, DNA extraction, laundry powder, detergent.

INTRODUCTION

Genomic DNA extraction from bacterial cells with high purity and concentration is of common processes in molecular research and clinical laboratories and various methods have been presented for this purpose. In all these methods, using chemical compounds or physical methods to lysis the cell is the first stage of bacterial genome extraction. Therefore, in the most common methods the chemical compounds of Tris, HCl, NaCl, SDS, and EDTA are used to lysis Gram-positive bacteria and lysozyme with sucrose and proteinase K are used to lysis Gram-negative bacteria (Syn and Swarup, 2000; Lee et al., 2003; Cheng and Jiang, 2006; Park, 2007; Herbon et al., 2009). Nowadays, many biologic companies have offered different kits to expedite and facilitate the process, but in all these methods the cost increases somewhat due to the necessity in usage of

specific materials. On the other hand, it should be noted that laboratory processes are mostly empirical that have been standardized based on the best results, hence it is possible to achieve similar or even better results than standard methods using alternative and simpler methods with lower cost (Drabek and Petrek, 2002; Garsia-spalveda et al., 2010).

The application of laundry detergents is an alternative methods used by some researchers to extract genomic DNA (Bahl and Pfenninger, 1996; Drabek and Petrek, 2002). Detergents can influence the bacterial cell wall membrane based on the chemical compounds and enzymatic activities, and cause release of cell genomic content without deleterious effects on the genome. Being fast, low cost and available are advantages of detergents, but the important point in using them is that various brands with different chemical compounds and enzymatic activities according to the amount of chemical compounds and number of enzymes (5, 3 and without enzyme), may show different results and sometimes compounds in the powders themselves prevent obtaining

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Table 1. Primers for amplification of genes from *P. aeruginosa* isolates.

Primer	Nucleotide sequence (5' to 3')	Length product	Restriction enzyme	Restriction fragment
Las/I-F	ATGATCGTACAAATTGGTCGG	600 bp	EcoRI	400 - 200 bp
Las/I-R	GTCATGAAACCGCCATG			

proper results in the next molecular processes such as PCR (Neumann et al., 1992; Bahl and Pfenninger, 1996;).

In this study, in order to evaluate and compare the performance of different laundry powder brands in Iran, some of the most well-known brands including Taj, Saftlan, Darya and Pak were selected to extract genomic DNA of Gram-negative bacteria *Pseudomonas aeruginosa*, a major cause of nosocomial infections in the world (Montero et al., 2010; Moor and Flaws, 2011). Afterwards, PCR tests were used to confirm the results and along with trial processes in which genomic DNA is used as template.

MATERIALS AND METHODS

Use of laundry powder

In this study, five-enzyme Taj brand (containing amylase, protease, lipase, mannanase and cellulase), three-enzyme Saftlan brand (containing cellulase, mannanase and lipase) and Darya and Pak brands without enzyme were used.

Bacterial samples

In this study, standard strain of *P. aeruginosa* PTCC 1310 and 50 *P. aeruginosa* isolated from different clinical specimens that were approved in laboratory by cultural and biochemical methods were investigated.

Genomic DNA extraction using laundry powder

In order to evaluate the efficiency of the mentioned brands, the dilutions of the powders were prepared in sterile water to the amount of 5, 10, 20, 40 and 80 mg/L (Nasiri et al., 2005). To extract genomic DNA using the powder, 1 ml of a 24-h culture containing the *P. aeruginosa* were transferred to a microtube and then adding 700 µL of desired dilution each microtube and without any incubation was mixed by vortexing for 1 min. After centrifugation at 11,000 rpm for 3 min, the supernatant containing the intracellular substances was transferred to a new microtube. To remove soluble proteins, 3 M sodium acetate was added to the same volume of isolate and the microtube was manually shaken for 1 min. In order to completely remove the soluble proteins, the solution was centrifuged at 14,000 rpm for 20 min. The supernatant solution lacking cellular proteins was transferred to a new microtube. Furthermore, 2.5 ml of pure ethanol was added to the solution to precipitate genomic DNA and after a minute of hand shaking, it was centrifuged at 14,000 rpm for 3 min. After the removal of the supernatant, 700 ml of ethanol (70%) was added to the sediment genomic DNA and centrifuged at 14,000 rpm for 10 min, then the supernatant was removed and DNA dried. Finally, 100 µL sterile distilled water was added to the sediment and incubated at 37°C for

1 h to dissolve the extracted genome in the water. It is notable that RNase can be used to remove RNA.

Genomic DNA extraction using manual and kit methods as control samples

Manual genomic DNA extraction of *P. aeruginosa* was performed using phenol chloroform method. To extract genomic DNA by a kit, the product of the BIONEER Company (Germany made) was used and the results of these two methods were compared with extracted genome by laundry powder.

Extraction analysis

The amount of A260/A280 was detected by nanodrop to determine the purity and concentration of extracted DNA. Also, the genome was investigated on 0.8% agarose gel to evaluate the integrity of DNA and accuracy of extraction.

Extraction approval based on genomic DNA

PCR reaction was performed to determine the existence of inhibitor and interference during the process. For this purpose, amplification of Las/I gene from *P. aeruginosa* was considered. This gene is one of the main constituent genes of quorum-sensing system in *P. aeruginosa* producing an enzyme that can synthesize an autoinducer of this system called C12-AHL (Schaber et al., 2004). Table 1 shows the primers used during PCR reaction. The final volume of PCR reaction mixture was 30 µL. The reaction mixture consisted of 15 µL 2X master mix (Ampliqon III, Denmark) containing 1.5 mM MgCl₂, 1 µL template, 20 pmol of R and F primers and double distilled sterile water to final volume of 30µL.

The PCR was performed with a thermocycler (Eppendorf) under the following cycling conditions; 95°C for 5 min, then 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 30 s followed by final extension at 72°C for 10 min. PCR products were subjected to electrophoresis on 1.5% agarose gel, then stained with ethidium bromide and products were visualized using gel documentation. Moreover, enzyme digestion of PCR product (RFLP) with EcoRI was performed to confirm PCR product.

RESULTS

DNA extraction analysis by determination of the A260/A280

After several repeat, concentration and purity of the extracted genomic DNA using different dilutions of laundry powder brands was evaluated using Nanodrop (Thermo, Swiss) and the results are shown in Table 2.

Table 2. Performance results of different dilutions of the brands tested based on purity factor and concentration of extracted DNA.

Concentration (ng/ μ L)	Purity factor ¹ (A260/280)	Dilution (mg/L)	Brand name
537.9	1.3 \pm 0.16*		
249.4	0.016 \pm 1.85	10	
284.4	0.016 \pm 2.12	20	Taj
402.8	0.016 \pm 2.08	40	
5	0.016 \pm 0.56	80	
208.2	0.017 \pm 1.1	5	
301.3	0.017 \pm 1.5	10	
201	0.017 \pm 1.94	20	Saftlan
300.7	0.017 \pm 1.78	40	
21.1	0.017 \pm 1	80	
178.5	0.015 \pm 0.92	5	
191.1	0.015 \pm 1.2	10	
250.6	0.015 \pm 1.64	20	Darya
203.7	0.015 \pm 2.03	40	
12.4	0.015 \pm 0.62	80	
302.3	0.015 \pm 1.12	5	
195.2	0.015 \pm 1.05	10	
101.1	0.015 \pm 2.04	20	Pak
182	0.015 \pm 1.95	40	
48	0.015 \pm 0.85	80	

¹Values given are the average of three replications. *Represents the standard deviation (0.01) for purity factor.

The results indicate that 10, 20 and 40 mg/L dilutions of Taj brand powder and 40 mg/L dilution of Saftlan, Pak and Darya brand powders, considering purity factor and DNA concentration have the best separation and purification efficiency.

DNA extraction analysis by gel electrophoresis

Figure 1 shows the best results that confirmed integrity and safety of DNA extracted by different dilutions of laundry powders using gel electrophoresis technique on 1% agarose gel. According to these results, extracted DNA using 40 mg/L Saftlan and Taj brand powders had the best integrity and perfection with no smear. This genome was used as a template DNA for PCR and RFLP tests.

PCR results

The results of PCR products amplified from extracted

DNA using 40 mg/L of Saftlan and Taj laundry powders showed that gene amplification of Las/I was successful (Figure 2). Moreover, no PCR inhibition and smear was observed using materials during the extraction.

RFLP results

The results of PCR products that were amplified from extracted DNA using Saftlan and Taj laundry powders were well digested by EcoRI and 200 and 400 bp bands indicated accuracy of PCR process (Figure 2).

DISCUSSION

Previous studies that were often on DNA extraction of eukaryotic cells (such as human blood cells, goat blood cells, etc.) showed that laundry powders could be utilized for DNA extraction of blood cells with high purity and quality (Neumann et al., 1992; Bahl and Pfenninger, 1996; Syn and Swarup, 2000; Drabek and Petrek, 2002;

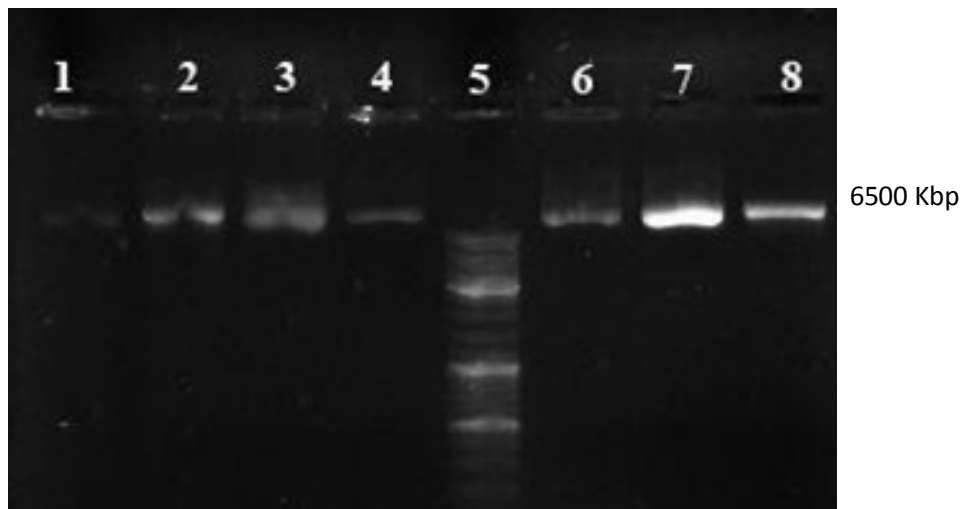


Figure 1. Analysis of extracted genomic DNA from *P. aeruginosa* on 0.8% agarose gel. Lane 1, Extracted DNA manually; lane 2, 20 mg/L Taj; lane 3, 40 mg/L Darya; lane 4, 40 mg/L Pak; lane 5 is DNA size marker (100 bp DNA ladder, SM#333); lane 6, 40 mg/L Softlan; lane 7, 40 mg/L Taj and lane 8, extracted DNA using kit.

Lee et al., 2003; Park, 2007). No study have been done regarding DNA extraction of bacteria using laundry powders, and often different methods such as phenol-chloroform (manual method), boiling and commercial kits have been performed, with each of these methods having advantages and disadvantages. In this study, different laundry powder brands which are common in Iran for the first time were used to extract the genome of a major causal agent of nosocomial infections in the world, *P. aeruginosa* (Moor and Flaws, 2011).

Moreover, as reported by Nasiri et al. (2005) and Kumar et al. (2006), different dilutions of detergents used to extract genomic DNA were useful, although according to the amount of dilution and brands type, purity of the DNA varies, which should be noted during DNA extraction. Unlike Nasiri and colleagues study that reported no influence on purity and integrity of DNA during extraction by using different laundry powder brands and no significant difference between results (Nasiri et al., 2005), this study showed that on average the purest DNA was obtained by 10, 20 and 40 mg/L dilutions of Taj powder brand and with regard to DNA concentration and purity factor, all studied brands showed the best result in 40 mg/L dilution. More also, 20 and 10 mg/L dilutions of Taj brand showed better efficiency compared to other brands in the same dilution. Differences in the two studies are due to differences in method used and the type of cells extracted during DNA extraction. Unlike Cheng and colleagues' study that reported that the phenol usage for bacterial lysis instead of chemical compounds and chloroform for removal of excess compounds were suitable methods for the genome extraction of the bacteria, the results in this study showed that the use of laundry powder for DNA extraction from bacteria

without phenol and chloroform (in manual method) is more appropriate and cost effective. Since phenol saturation is difficult and more people show allergy to it, most producers of commercial kits are trying to eliminate this compound, and also laundry powders are immoderately available (Syn and Swarup, 2000; Lee et al., 2003; Cheng and Jiang, 2006; Park, 2007; Herbon et al., 2009).

This study shows that genomic DNA extracted using 40 mg/L dilution of Taj brand have the best quality that is even comparable to DNA extraction using kit (lane 8 in Figure 1) and the manual method (lane 1 in Figure 1). However, the results of different dilutions and other brands are considerable. Like other studies (Pusch, 1997; Nasiri et al., 2005; Cheng and Jiang, 2006; Kumar et al., 2006) the results of this study showed that the purity and concentration of genomic DNA extracted using different brands may be variable, which is possibly due to the different type and ratio of the base material constituents of the powder and the type of the enzymes in them. The results of PCR and enzyme digestion of PCR product amplified using genomic DNA extracted by 40 mg/L dilution of Taj and Saftlan brands, as an example, indicated that these processes using genomic DNA extracted by laundry detergent can be performed remarkably and this method usage has no effect on the downstream processing in which the extracted DNA was used as a template. Detergents used in this study are synthetic and consist of various compounds; active anionic (alkyl sulfates ROSO₃Na) as main compound, sodium poly phosphate as an eliminator of ions existing in water and regulator of solution buffer, carboxymethyl cellulose as an organic separator for lipid suspension are the most important of them. Due to high osmotic

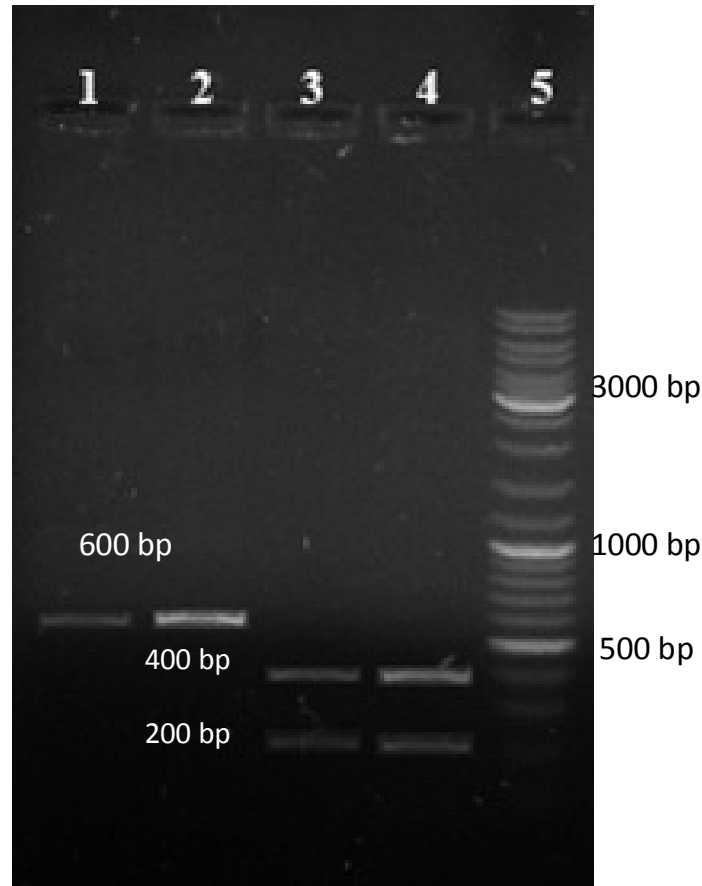


Figure 2. Analysis of PCR product digestion results on 2% agarose gel. Lane 1, PCR product of genomic DNA extracted by 40 mg/L softlan, lane 2, PCR product of genomic DNA extracted by 40 mg/L Taj; lane 3, PCR product digestion using EcoRI (Softlan brand); lane 4, PCR product digestion using EcoRI (Taj brand); lane 5 is DNA size marker (100 bp DNA ladder, SM#333).

conditions in detergent-containing solution, cell lysis would be achieved by treatments with this solution (Bajpai and Tyagi, 2007). Moreover, enzymes and separator organic compounds existence leads to denaturation of cell proteins that can be isolated from the DNA by adding a concentrated salt solution. Alkyl sulfates, due to its positive charge, might affect the isolation of molecules like DNA that is negatively charged (Park, 2007; Bajpai and Tyagi, 2007).

Finally, in consistence with the report of Nasiri et al. (2005), it was revealed that it is possible to extract genome using individual ingredients of laundry powders since the use of these chemical compounds separately is not cost effective. More also, genome extraction using laundry powder has advantages including:

1. The stages of DNA extraction are less; hence genome extraction can be performed in less time.
2. It is inexpensive since the use of phenol, chloroform, RNase, SDS, lysozyme and proteinase K are not

necessary.

3. In this method, DNA extraction product compared to other methods such as phenol and chloroform has higher purity and quality.

4. Since phenol and chloroform are not used, it also a safe method for the user.

According to these results therefore, this method can be used as a rapid and inexpensive alternative to standard DNA extraction methods in molecular biology laboratories.

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