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Microbial DNA Extraction from Mangrove Sediment by Different Methods

[1]M. Amopour Bahnamiry, [1]H. Motamedi, [2]S. sfouri and [1]A. Hedayatkhah

[1]Department of Biology, Faculty of science, Shahid Chamran University, Ahvaz, Iran
[2]Faculty of Engineering, Persian Gulf University, Bousher, Iran

Abstract: Since DNA extraction is an important step of metagenomic studies, eight different DNA extraction methods were designed. They were usedsodium dodecyl sulfate, proteinase K, Lysozyme and sonication in different combinations. The sea sediment was subjected to these eight methods and the yield was analyzed via spectrophotometry and gel electrophoresiswhich depicted the contribution of each treatment method in the extraction procedureof extracted DNA. Results revealed that although applying SDS only resulted in lowest amount of extracted DNA, enhancing this method with sonication lead to the highest yield in extraction. Moreover, applying lysozyme and SDS, also enhancing this method by adding a sonication step resulted in desirable results. Furthermore, developing other methods by adding a sonication displayed the same trend. An important reason for increase in the amount of extracted DNA in enhanced methods with sonication would be better cell lysis by applying this step in extraction method.

Key words: DNA Extraction · Lysozyme · Proteinase K · Sonication · SDS

INTRODUCTION

During recent decades molecular methods have been applied progressively to investigate the microbial biodiversity. Many of these methods are genome based approaches, thus it is important to obtain DNA or RNA in good quality and quantity [1].

In gnome base studies some technique are PCR dependent while the other is PCR independents, however one of the most important steps in all of them is genome extraction particularly DNA extraction. Greater amounts of extracted DNA can provide much different genetic pattern for further examinations and as a result will have significant effect on biodiversity studies [2, 3]. For evidence, many previous researches showed that the microbial diversity of the studied samples is depended on the quality and quantity of extracted DNA from those samples [4, 5]. This getsmore important when the case sample will be soil or sediment samples particularly when the DNA extraction is done without a pre-enrichment process for microbial communities of samples. In other words the extracted DNA will directly utilize for further analyses.

There is various procedures for DNA extraction from sediments, some of them applied physical treatments such as ultrasonic waves and some other applying chemical, biological or a combination of treatments like treatment with SDS, enzymes or both of them [6-8]. One of the most serious issues in DNA extraction is the association of DNA with unwanted materials particularly enzymes inhibitors such as humic acid which is an important and the most general obstacle for DNA extraction from soil and sediments. This component gave light yellow to brown color to extracted DNA solution. The Mg^{++} ions of PCR buffer can gather by this material, consequently the Taq enzyme activity in PCR process will interfered by this material. Thus, removal of this substance is very important that can be done by applying chemical treatments or other methods [7-9].

SThis study is aimed to investigate the effects of different extraction treatments and optimize the extraction process in order to achieve a simple, effective and inexpensive method for DNA extraction from sea sediments.

MATERIALS AND METHODS

Sampling: Sampling was performed at geographical address of N 27° 46' and E 52° 65' in Nay-Band National Sea Park of Asalayoeh region in Iran.

Corresponding Author: Mostafa Amopour Bahnamiry, Department of Biology, Faculty of science, Shahid Chamran University, Ahvaz, Iran.

The samples were taken by a cylindrical tube (8 cm diameter, 100 cm height) from a region of one m^2 area. From each sampling spot, 3 samples were taken and the sediments of 20 cm of the sampling cylinder center then mixed as the main sample of the spot. The rhizosphere samples were taken from the superficial sediments of mangrove's roots and the sediment samples taken from 50-100 m away from the trees growth line. Immediately after sampling, the samples were transferred to ice bags container. For long keeping all samples were stored at-22°C [10].

Pretreatment of Sediments: First, in order to remove the PCR inhibitor materials such as humic acid, there was a pretreatment on sediment samples. The pretreatment was done based on Doung*etal* optimized method (2004):

Five grams of sediments mixed with 5 ml 0.1M phosphate buffer [pH 6.6] and was vortexing for 2 min. Then the mix was supplemented with 3.5 ml aluminum solution and vortexing for 2 min again. Finally, 2.5 ml NaOH [1M] was added [11].

DNA Extraction: After pretreatment process, different physical, chemical and biological treatments were performed for DNA extraction. Eight methods were designed for optimization. All methods were tested in triplicate mode.

Method A-Treatment with SDS: The sediments mix from pretreatment process were blended with 2.5 ml DNA extraction buffer (100 mMTris-HCl [pH8.0], 100 mM sodium EDTA [pH8.0], 100 mM sodium phosphate [pH8.0], 1.5 M NaCl, 1% Cetyltrimethyl ammonium bromide(CTAB)) and vortexing for 30 second, then horizontally shook at 150 rpm for 30 minute at 37°C. Afterwards, it was supplemented with 1.5 ml 20% SDS and incubated in a 65°C water bath for 2h. Every 20 min the sample was gently shook by end-over-end inversion for 10-20 sec. The supernatant was taken after centrifugation at 5000 g for 5 min. The aqueous phase was recovered by gently mixing with chloroform-isoamyl alcohol solution (24:1) and then precipitated with 0.6 volume cold isopropanol for 3 hours at-20°C. The sediments were collected by centrifugation at 16000 g (15 min, 4°C). The sediment was directed to 200 µl cold 70% ethanol for 15 min on ice bath and then the DNA pellet obtained by centrifugation at 16000 g (15 min, 4°C). These samples were dried at room temperature. Following, the dried DNA samples were supplemented with 200 µl sterile deionized water (Milli-Q water) and transfer to-22°C for long term storing [7]. In this method the sea sediments just treated with 20% SDS solution without any supplemented enzyme.

MethodB-Treatment with SDS and Proteinase k: The procedure was almost the same as method A. however, before adding SDS, proteinase K enzyme was added at 20 mg per ml and horizontally shook for 30 min at 37°C. It is important to add proteinase K before SDS due to this fact that SDS is capable of enzyme deactivation. Further steps were the same as method A.

MethodC-Treatment with SDS and Lysozyme: The procedure was the same as method B, but the applied enzymewas lysozyme that was added at 5 mg per ml and horizontally shook for 90 min at 37°C.

MethodD-Treatment with SDS and Proteinase K: The procedure was combination of method B and method C. After initial step, lysozyme was added at 5 mg per ml for 90 min at 37°C. Afterwards, proteinase K was added at 20 mg per ml for 30 min at 37°C. Further steps were the same as method A.

Methods E-H: These methods were the same as methods A to D, respectively, but after the treatment procedure, there was a 10 min sonication which was done in 2 minutes cycles and cold temperature.

Analyzing the Quality of Genome: The amount of 5 µl of genome extracted solution was load on 0.7% agarose gel electrophoresis at 90 voltages for 50 min. Also, All samples were directed to spectrophotometry at 260 nm to estimate DNA density. DNA yields depicted the contribution of each treatment method in the extraction procedure.

RESULTS AND DISCUSSION

Optimization of DNA Concentration for PCR Reaction: The best density of DNA for PCR reaction was opted based on quality and quantity of PCR yield for each concentration. The result revealed that applying 10 ng of extracted DNA samples was the best concentration in this case, also lower and higher density than 10 nm resulted in reduction in the yield of PCR.

DNA Extraction: After applying eight different methods for DNA extraction, the concentration of extracted DNA from each method was calculated by spectrophotometry and the results is shown in Table 1. The test was done in triplicate mode, therefore the results is the average of three samples.

Table 1: Results of DNA extraction for one gram sediment from eight designed methods

Method	Special condition	Yield (µg/g)
Method A	20 % SDS	23.0
Method B	20 mg/ml Proteinase K » 20%SDS	26.3
Method C	5 mg/ml lysozyme » 20%SDS »	33.3
Method D	5 mg/ml lysozyme » 20 mg/ml Proteinase K » 20%SDS	28.3
Method E	20 % SDS » 10 min sonication	44.0
Method F	20 mg/ml Proteinase K » 20%SDS » 10 min sonication	34.0
Method G	5 mg/ml lysozyme » 20%SDS » 10 min sonication	39.3
Method H	5 mg/ml lysozyme » 20 mg/ml Proteinase K » 20%SDS » 10 min sonication	36.0

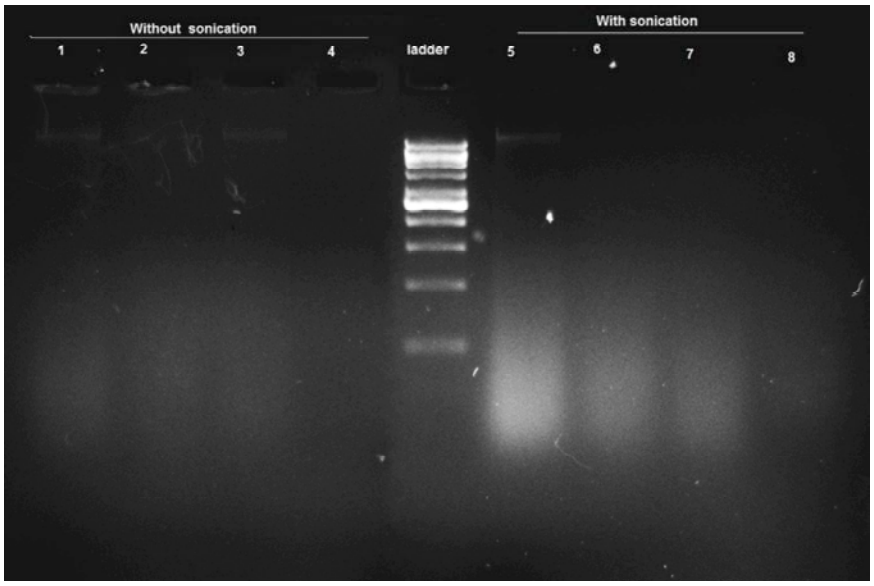

Fig. 1: The gel electrophoresis of extracted DNA from 8 method. 1 to 8 are represented A to H methods respectively.

SDS is an effective non-ionic detergent that can solubilize either of proteins and lipids even in cell membrane. Thus, this substance is capable of breaking down the cell membranes, so that the cell content include the genome will exposed. The results of DNA extraction methods revealed that for method A which was done via 20% SDS there was the yield of 23 µg per gram sediment. This is the lowest productivity among all eight approaches. However, when the method modified by adding a sonication step (method E) it resulted to 44 µg DNA from the same amount of sediments. Sonication is a cell lysing method by itself that in some studies on DNA extraction, this method showed highest productivity in comparison with other applied methods [12, 13]. Therefore, it can be concluded that applying both of SDS and sonication on sediments showed a synergy effect that resulted in a better result which in this study was the best method in the matter of DNA productivity. This trend is displayed in Fig. 1.

Method B, was done by applying proteinase K enzyme and then SDS and resulted in 26.3 µg/g DNA. It is approximately 14% higher than method A. Since proteinase K can degrade the membrane proteins, utilizing it with SDS lead to more efficacious lysis in cells. When lysozyme was used instead of proteinase K, the results enhanced to 33.3 µg/g DNA (method C). Lysozyme is a significant penetrating and cell lysing enzyme. The results of this study revealed that utilizing this enzyme instead of proteinase K is more effective for DNA extraction even when both of them were used (method D). However, it is important to mention this fact that applying lysozyme will result in better cell breaking; on the other hand it may affect the quantity of DNA extraction [14].

For method D although the result was better that method A and B with the yield of 28.3 µg/g DNA, but it was not as successful as method C in the case of obtained DNA. The reason may be owing to the feature of lysozyme that mentioned before.

Methods E to H were done the same as A to D, respectively, but there was just an additional step of sonication. The results showed that applying just this step resulted in higher productivity in all methods. To clarify in the case of SDS treatment (method A), enhancing the procedure with 10 min sonication resulted in 91% improvement in DNA extracting yield. Thus, it is crystal clear that sonication associate SDS and lead to higher efficiency in cell breaking. The same pattern was observed for the other three methods (B,C.D) and their enhanced methods (F,G,H). For method B with 26.3 µg/g extracted DNA, there was 30% increase in amount of extracted DNA for enhanced method F. While it was only 18 % growth in DNA extraction yields for method C with 33.3 µg/g DNA to the enhanced method G with 39.3 g/g DNA. The lower enhancement could be due to this fact that lysozyme is a very effective cell breaking agent by itself; therefore, sonication did not show significant impact on the cell lysing process. However sonication shows higher synergic effect with proteinase K which can be due to this fact that proteinase K is not as successful as lysozyme in the matter of cell lysing. In the case of method D with 28.3% yield, it improved with 27% in productivity by enhancing the method as the method H with 36.0 µg/g extracted DNA.

Bourrain *et al.* [12] applied different DNA extraction method include lysozyme with SDS, sonication in a water bath and thermal shock. They reported that lysozyme with SDS was the most effective step in the cell lysis procedures. Moreover, Luna *et al*. [2] reported that the highest amount of extracted DNA was obtained with the SDSlysis protocol. Jiang *et al*. [13] reported that extracting procedures have different results based on the type of cell. Appling SDS, lysozyme, sonication and freeze-thaw extract archaeal or bacterial DNA but failed for fungi cells. However, combination of SDS, lysozyme and vigorous shaking successfully released fungal DNA.

CONCLUSION

DNA extraction is an essential step in many metagenomic studies. Different lysis protocol was designed by SDS, lysozyme, proteinase K and sonication. The highest yield was achieved when SDS was continued with a further sonication steps. Although, the results revealed that SDS with lysozyme is also a significant method for DNA extraction.

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