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New modified protocol of DNA extraction Comparison with other extraction methods for polymerase chain reaction analysisof gnomic DNA from Cyanophyceae isolates

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ABSTRACT

The isolation of DNA from biological samples is a crucial step in the process of DNA-based molecular biological assays. In this study, the authors compared three procedures using a phenol-chloroform-isoamyl, Wizard® Genomic DNA Purification Kit and modification boiling methods of genomic DNA extraction from filament and colonial cyanophytceae genus. High DNA concentration and purity were observed for both *Anabaena fertellisima.*and *Gloeocapsa indicus* isolates (190.4and 130.3µg/ml; OD260/280, 2.04 and 1.89, respectively) when the extraction protocol included modification boiling methods. The comparison with other extraction protocols clearly indicates that this optimized method allows the recovery of a larger amount of DNA. Furthermore, the extracted DNA presents a high molecular weight, a reduced degradation and an excellent overall quality. It can be directly used for molecular biology purposes such as PCR, and clone library construction.

KEYWORDS: DNA extraction, *Anabaena fertellisima*, *Gleocapsa indicus*, modified boiling, Polymerase Chain Reaction (PCR).

INTRODUCTION

While DNA isolation seems to be a routine procedure for most organisms including viruses, bacteria, fungi, parasites, insects, mammals and plants [1], it is, for various reasons, a rather difficult one when performed on cyanobacteria [2]. The common problems encountered in DNA isolation from cyanobacteria mainly range from cell lysis efficiency [2,3], to purification issues [4]. Eventhough several methods for extracting cyanobacterial DNA have already been reported in literature [5], their respective efficiencies can greatly vary from one species to another [2].

The isolation of DNA from biological samples is a crucial step in the process of DNA-based molecular biological assays. Whether the DNA is extracted from a plant or animal tissue or from a bacterium, the product obtained has to be pure or free from contaminants (proteins, carbohydrates) to be used in numerous applications in molecular biology including PCR, genotyping, DNA sequencing, etc.

A wide variety of protocols are found in the literature to extract and purify genomic DNA from different tissues. All protocols start with cell lysis as the first step, followed by deproteination and precipitation of DNA. The most commonly used method is the phenol /chloroform extraction, which is tedious and time-consuming

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[6]. The other extraction methods include salting out DNA extraction [7] and the guanidium isothiocyanate DNA extraction method [8]. There are many different and versatile commercial kits suitable for genomic DNA extractions from QIAamp, Puregene and Dynabeads [9].

The aim of this study was to develop a simple and rapid method to extract DNA from cyanobacterial which is useful for any routine molecular biological assay. The method used in this study to extract and purify genomic DNA is the boiling methods of genomic DNA extraction method [10] with modifications, comparing with two others methods.

MATERIALS AND METHODS

Collection ,Isolation and Purification of Algae:

The cyanobacterial samples were collected from Al-Wathba station located in the center of Baghdad. This station located on longitude 20°44'45.58"E and latitude 20°33'33.55"N and are isolated by streak plate method [11],BG-11with minerals nutrient solution solidified by 2% agar-agar and autoclaved, after sterilization with 45-50 C˚ was poured in petri-dishes and left to solidify. Then the surface of each plate was inoculated with 1 ml of sampled water, the inoculum distributed with a sterile spreader or streaking using a sterile loop. The inoculated plates were kept in a cooled illuminated incubator with about 200 µE/m²/s light intensity and 26± 2 C˚ for 7- 10 days. Aggregated colonies were observed on the surface of plates. Part from these colonies was stroke on other plates. Each subculture was examined intervally, this method was repeated till a unialgal culture or cultures have been gained [11]. A small part of unialgal culture (which was microscopically confirmed as unialgal culture) was transferred into BG-11with minerals nutrient solution within a 250 ml sterile flask and incubated for 2-3 weeks according to method of [12] to get appropriate growth. In order to sustain the viability of the unialgal growth, these cultures should be renewed every two weeks by sub culturing into another BG-11with minerals nutrient solution.

Obtained algal isolates were identified with help of classical algal classification references [13,14]. Algae were grown on BG-11with minerals agar slants collected and pelleted by using centrifuge (5000 rpm/10 min) to obtain heavy growth, 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.

DNA extraction methods:

A- phenol-chloroform-Isoamyl method:

Algal growth heavy growth, Placed in 1.5ml tube containing 200 µl lysis buffer (200 mMTris-HCL, mM25 EDTA, 0.5 % SDS, 250mM NaCl) and crushed with conical grinder, incubated for 20 min at 100˚C, added 150µl of sodium acetate (3.0 M), kept at -20 ˚C for 10 min. centrifuged at 12000 rpm for 10 min, supernatant was extracted once with phenol-chloroform-Isoamyl alcohol (25:24:1) and subsequently with chloroform, then centrifuged at 12000 rpm for 10 min. DNA was precipitated with equal volume of isopropanol and centrifuged at 12000 rpm for 10 min.Pelt DNA washed with 200 µl of 70% ethanol, centrifuged at 12000 rpm for 10 min, dried and suspended in 50 µl TE buffer (10 mMTris-HCl, 1 mM EDTA, pH= 7.4)[15].

B- The Wizard® Genomic DNA Purification Kit:

The extraction was performed according to the instruction of the manufacturer. The Wizard® Genomic DNA Purification Kit is designed for isolation of DNA from white blood cells [16], tissue culture cells and animal tissue, plant tissue, yeast, and Gram positive and Gram negative bacteria.

C- Modified Rapid boiling:

as followings this procedure of DNA extraction was accomplished according to rapid boiling method [10] with modifications were done by as:

Algal growth (about 14 days growing cells) was concentrated by centrifuge(5000 rpm/10 min.) to obtain heavy growth, placed in 1.5 ml eppendrouff tube containing 300 microliter TE buffer(10 mMtris-hcl,pH=8 and 1 mM EDTA)vortex for few secondsplaced tubes in 80˚C water bath for 20 min. Centrifugation for five min. at 5000rpmsupernatant was transferred to new sterilized eppendrouff tube and store in ice until use(if need you can precipitate DNA by adding equivalent amount of cool isopropanole and wash by 70% ethanol and dissolve DNA with 50 microliter of TE buffer)

Estimation of the DNA Concentration and Purity:

Nanodrop instrument was used for Estimation of DNA concentration and purity, one microleter of each DNA samples was applied in nanodrop in order to measuring optical density (OD) at wave length 260nm and 280nm. an OD of one corresponds to about 50 µg/ml for double strand DNA. The final concentration of DNA was calculated according to the formula which mentioned bellow [17]:

DNA concentration µg/ml= O.D 260nm 50 dilution factor

The purity was calculated according to formula:
DNA purity = O.D 260nm / O.D 280nm
The ratio used to detect nucleic acid contamination with protein.

Conventional PCR Technique:

PCR technique was applied on seven cyanobacterial isolates (*Anabaena fertellisima.*, *Gloeocapsa indicus*) which were isolated from the station of study.

Primers Selection and Preparation:

The aminotransferase (AMT) domain which is located on the modules *mcy*E of the microcystin synthetase enzyme complex was chosen as the target sequence for the set of primers (HEPF/ HEPR) because of its essential function in the synthesis of all microcystins and nodularins[18]. It was possible to amplify a 472 bp PCR product from the AMT domain of all tested hepatotoxic species.

Primers were provided in lyophilized form, dissolved in sterile distilled water to give a final concentration of 100 mol/ µl as recommended by provider and stored in a deep freezer until used in PCR amplification. The primer sequence used in this listed in Table (1).

Table 1: The primers sequence used in conventional PCR (Jungblut and Neilan, 2006).

Primer name	Sequence 5´⟶3´	Length	GC %	PCR products
HEPF	TTTGGGGTTAACTTTTTTGGGCATAGTC	28	44	472bp
HEPR	AATTCTTGAGGCTGTAAATCGGGTTT	26	48	

Table 2: The program used in the thermocyler PCR.

Stage	Temperature (time) HEPF/ HEPR	
Initial denaturation	95˚C (2min)	
Denaturation	95˚C (90sec)	35 cycles
Annealing	44˚C (40sec)	
Extension	72˚C (1min)	
Final extension	72˚C (5min)	

Determination of PCR Specificity:

PCR specificity was determined by using negative control, DNA template which used as negative control was extracted from chlorophyta *Zygnema sp*. by using protocols as described in [19].

Results:

We compared three different approaches to DNA extraction from cyanobacterial methods. Figures 1 represent only results derived from the three methods for the detection of cyclic peptide hepatotoxin genes by using HEPF and HEPR primers was developed to identify potentially microcystin or nodularin in two genus of cyanobacteria. Table 1 shows that the extraction procedure methods resulted in high DNA isolation yields for *Anabaena fertellisima* and *Gloeocapsa indicus*(190.4±0.05and103.1±0.9µg/ml, respectively) when modified boiling method used. In addition A260/A280 ratios suggest that the quality of isolated DNA was acceptable tothe same method (modified boiling method) for *Anabaena fertellisima*. and *Gloeocapsa indicus.* (2.04±0.15and 1.91±0.005, respectively).

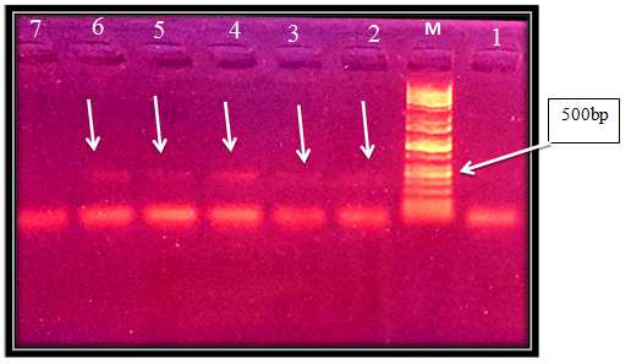

Fig. 1: Gel electrophoresis of amplified *mycE*(472bp) in cyanobacterial isolates. Agarose (1.5%), 5 V/cm for 1.5hrs, stained with ethidum bromide and visualized on a UV transilluminator.

Lane 1. Negative controle *Zygnema sp.*, Lane M.100 bp DNA ladder, Lane 2-3.*A. fertellisima.*and *G. indicus* by a phenol-chloroform-isoamyl method respectively. Lane4-5.*A.fertellisima*and *G. indicus.* by a

modified rapid boiling method respectively. Lane 6-7.*A. fertellisima* and *G. indicus* by a DNA Purification Kit respectively.

Table 1: Concentration and purity of Cyanobacterial *A. fertellisima*and *G. indicus* DNA isolated by three DNA extraction methods.

Microorganism	DNA Concentration µg/ml			A260/A280(Purity)		
	phenol	Kit	Boiling	phenol	Kit	Boiling
A. fertellisima	181.4	32.01	190.4	1.409	1.69	2.03
	181	32	190.5	1.41	1.65	2.2
	181.5	31.6	190.5	1.42	1.77	1.927
Mean ± S.D.	181.29±0.26	31.8± 0.2	190.4±0.05	1.41±0.006	1.68±0.02	2.04±0.15
G. indicus	165.31	17.51	103.1	1.41	1.66	1.9
	165.4	17.5	104.0	1.43	1.7	1.9
	165	17.6	103.0	1.39	1.7	1.87
Mean ± S.D.	165.2 ± 0.2	17.5± 0.05	103.3±0.5	1.41±0.02	1.68±0.02	1.89±0.01

Chemical modified boiling showed the best results with respect to cell lysis and DNA purity. Although all three chemical methods tested had some beneficial effects, the methods differed with respect to time consumption, DNA quality and quantity recovered.

Discussion:

The advances in DNA analytical techniques, including PCR, cloning, hybridization, and sequencing, have enabled comprehensive analysis of the cyanobacterial genomes. Several protocols of isolation and purification of DNA from various types of cyanobacteria have been developed and described. In general, DNA isolation is a multi-step procedure involving cell lysis by treatment with lytic enzymes and/or detergents, DNA extraction with organic solvents, and DNA recovery by alcohol precipitation [20]. 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 toxigenic cyanobacteria; therefore a rapid, easy-to-use, efficient, and cost-effective method for cyanobacterial DNA isolation is necessary.

In this study we compared a procedure for DNA extraction using an ordinary modified rapid boiling with two other procedures- a phenol-chloroform-Isoamyland The Wizard® Genomic DNA Purification Kit -in terms of DNA yield and purity. In addition, DNA suitability for the detection of cyclic peptide hepatotoxin genes by using HEPF and HEPR primers was developed to identify potentially microcystin or nodularin in two genus of cyanobacteria .In this papers we used the boiling procedure developed by [10]. This procedure involves thermal lysis and has been used for total DNA extraction from bacterial with our modified by reduce the temperature of water bath to 80 ˚C instated of 100˚C and increase the time to 20min. instated of 10 min and use TE buffer instead of D.W.

Usually, two factors have to be particularly considered during the extraction procedure. The first is to maximize the DNA yield. The second is to ensure that the extracted DNA is amenable to several enzymatic treatments like PCR amplification [21].

Standard phenol-chloroform extraction procedure has been shown to produce quantitative results [22]. However, it is also considered to be time intensive regarding a large number of field samples and particularly for early warning monitoring a more time efficient and easy-to-use technique for DNA extraction would be required[23].

Chemical methods use detergents to solubilize cell membranes. Commonly used detergents are SDS, Triton X-100, and CTAB [24]. The disadvantage of detergent-based cell lysis is that detergents often contaminate DNA samples and inhibit further manipulations. These methods still have disadvantages, which include laborious manipulations such as four to six changes of microcentrifuge tubes, multiple stages of incubation, precipitation, elution, washing and drying, or requirement of special equipment. The DNA yield and purity are often poor due to the multistep manipulations; therefore, an easy, rapid, and efficient method for DNA extraction that can be used on a routine basis needs to be developed [25].

In general, when only enzymatic methods were used, bad results were obtained, such as with the Wizard® Genomic DNA Purification Kit [26]. Cyanobacteria proved to be very difficult to disrupt only by enzymatic treatment ,with its complex multi-layered cell wall [27] and specific ultrastructures such as the polysaccharidic sheath surrounding the cells [28].

another 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 [29]. thus, this procedure may be modified to get higher cyanobacterial DNA yields.

Certainly represents obstacles for the enzymatic degradation. In contrast, a purely mechanical lysis such as the Fast prep method [30] was powerful enough to induce cell lysis, without any enzymatic help.

Mechanical lysis is indeed known to allow the extraction of nucleic acids from a wide variety of organisms for which lysis can be otherwise difficult, such as plants [31,32] or fungi [33], However, the resulting DNA is strongly sheared by this mechanical lysis, and does not allow to obtain intact high molecular weight DNA.

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The combination of a soft mechanical treatment with chemical lysis appeared as the best alternative for efficient cyanobacteria cell lysis. Such a combination had been reported to be successful with *Microcystis aeruginosa* samples [2].

The use of a mechanical lysis such as the vibration by vortex method developed by [34]. proved to be more appropriate. This method was developed to isolate nucleic acids from plant-fungus complexes, which present similar difficulties as the one encountered with cyanobacteria (i.e. high production of polysaccharides, few high molecular weight DNA).

The DNA extracted with the optimized protocol presents a high molecular weight, a reduced degradation and an excellent overall quality. The developed procedure is fast and reproducible. It does not require the utilization of toxic compounds such as phenol, which could lead to the production of hazardous waste. Finally, it is suitable for molecular biology techniques such as polymerase chain reaction.

Conclusion:

These results showed that DNA extraction using rapid modified boiling was more successful than the Kit and phenol-chloroform-Isoamyl methods. The DNA isolated from both cyanobacteria using the rapid modified boiling 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 rapid modified boiling procedure, which is easy, rapid, and cost-effective, can be applied for high-yield isolation of analytical-quality DNA from cyanobacteria.

REFERENCES

[1] Liu, D., 2009. Handbook of nucleic acid purification. CRC Press, Boca Raton, USA.
[2] Fiore, M.F., D.H. Moon, S.M. Tsai, H. Lee, J.T. Trevors, 2000. Miniprep DNA isolation from unicellular and filamentous cyanobacteria. J. Microbiol. Methods, 39: 159-169.
[3] Billi, D., M.G. Caiola, L. Paolozzi, P. Ghelardini, 1998. A method for DNA extraction from the desert cyanobacteriumChroococcidiopsis and its application to identification of ftsZ. Appl. Environ. Microbiol., 64: 4053-4056.
[4] Porter, R.D., 1988. DNA transformation. Methods Enzymol., 167: 703-712.
[5] Kallas, T., M.C. Rebière, R. Rippka, N. Tandeau de Marsac, 1983. The structural nif genes of the cyanobacteria Gloeothece sp. and Calothrix sp. share homology with those of Anabaena sp., but the Gloeothece genes have a different arrangement. J. Bacteriol., 155: 427-431.
[6] Köchl, S., H. Niederstätter and W. Parson, 2005. DNA extraction and quantitation of forensic samples using the phenol-chloroform method and real-time PCR. In: A. Carracedo, (Ed) Methods in molecular biology, Vol. 297: forensic DNA typing protocols. Humana, Totowa, pp: 13-29.
[7] Rotureau, B., G. Gego and B. Carme, 2005. Trypanosomatid protozoa: A simplified DNA isolation procedure. Experimental Parasitology, 111(3): 207-209.
[8] Kotlowski, R., A. Martin, A. Ablordey, K. Chemlal, P. Fonteyne and F. Portaels, 2004. One-tube cell lysis and DNA extraction procedure for PCR-based detection of *Mycobacterium ulceran*sin aquatic insects, mollusks and fish. Journal of Medical Microbiology, 53: 927-933.
[9] Cler, L., D. Bu, C. Lewis and D. Euhus, 2006. A comparison of five methods for extracting DNA from paucicellular clinical samples. Molecular and cellular probes, 20: 191-196.
[10] Ruppe, E., S. Hem, S. Lath, V. Gautier, F. Ariey, J.L. Sarthou, D. Monchy and G. Alert, 2009. CTX-M βLactamase in Eschirechia coli from community-acquired Urinary tract infections,Cambodia. J.Emerg. Infect. Dis., 15(5): 741-8.
[11] Stein, J.R., 1973. Handbook of Phycological Methods: Culture Methods and Growth Measurements. Cambridge University Press. Cambridge.
[12] Jawad, A.L., 1982. Interactions Between Cyanobacteria and OtherMicroorganisms Ph.D. Thesis to Liverpool University, pp: 123-125.
[13] Prescott, G.W., 1978. How to Know the Freshwater Algae. Wm. C.Brown, Dubuque, IA.
[14] Desikachary, T.V., 1959. Cyanophyta. Indian Council of AgriculturalResearch, New Delhi, pp: 686.
[15] Matehkolaei, A.R., K. Makimura, M.R. Shidfar, F. Zaini, M.R. Eshraghian, N. Jalaizand, S.N. Sisakht, L. Hosseinpour and H. Merhendi, 2012. Use of single-enzyme PCR-restriction digestion barcode targeting the internal transcribed spacers (ITS rDNA) to identify Dermatophyte Species. Iranian J. Publ. Health, 41(3): 82-94.
[16] Engene, N., S. Gunasekera, W.H. Gerwick, V.J. Paul, 2013. Phylogenetic interference Reveal a Large Externt of Novel Biodiversity in Chemically Rich tropical Marine Cyanobacteria,Appl Environ Microbiol., 79(6): 1882-1888.
[17] Sambrook, J. and D. Russela, 2001. molecular Cloning: Laboratory manual.3 rd. ed. Cold spring harbor, New York. USA.

[18] Jungblut, A.D. and B.A. Neilan, 2006. Molecular Identification andEvolution of the Cyclic Peptide Hepatotoxins, Microcystin and Nodularin, Synthetase Genes in Three Orders of Cyanobacteria. Arch. Microbiol., 185: 107-114.
[19] Doyle, J.J., J.L. Doyle, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull*; 19: 11-15.
[20] Moore, E., A. Arnscheidt, A. Kruger, C. Strompl and M. Mau, 2004. Simplified protocols for the preparation of genomic DNA from bacterial cultures. Molecular Microbial Ecology Manual, Second Edition, 101: 3-18.
[21] Spaniolas, S., M. Tsachaki, M.J. Bennett and G.A. Tucker, 2008. Evaluation of DNA extraction methods from green and roasted coffee beans. Food Control., 3: 257-262.
[22] Kurmayer, R. and T. Kutzenberger, 2003. Application of realtimePCR for quantification of microcystin genotypes in apopulation of the toxic cyanobacteriumMicrocystis sp.Appl Environ Microbiol., 69: 6723-6730.
[23] Schober, E. and R. Kurmayer, 2006. Evaluation of different DNA sampling techniques for the application of the real-time PCR method for the quantification of cyanobacteria in water.Applied Microbiology, 42: 412-417.
[24] Ausbel, M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman and K. Struhl, 1998. Current protocols in Molecular Biology. Wiley and Sons, New York, USA. pp: 561-563.
[25] Ahmed, B.O., A.H. Asghar and M.M. Elhassan, 2014. Comparison of three DNA extraction methods for polymerase chain reaction (PCR) analysis of bacterial genomic DNA, African Journal of Microbiology Research, 8(6): 598-602.
[26] Ausbel, M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman and K. Struhl, 1998. Current protocols in Molecular Biology. Wiley and Sons, New York, USA. pp: 561-563.
[27] Ciferri, O., 1983. Spirulina, the edible microorganism. Microbiol. Rev., 47: 551-578.
[28] Hoiczyk, E. and A. Hansel, 2000. Cyanobacterial cell walls: news from an unusualprokaryotic envelope. J. Bacteriol., 182: 1191-1199.
[29] Tongeren, S.P., J.E. Degener and H.J.M. Harmsen, 2011. Comparison of three rapid and easy bacterial DNA extraction methods for use with quantitative real-time PCR. Eur. J. Clin. Microbiol. Infect. Dis., 30 (9): 1053-1061.
[30] Feurer, C., F. Irlinger, H.E. Spinnler, P. Glaser and T. Vallaeys, 2004. Assessment of the rind microbial diversity in a farmhouse-produced vs a pasteurized industriallyproduced soft red-smear cheese using both cultivation and rDNA-based methods.J. Appl. Microbiol., 97: 546-556.
[31] Haymes, K.M., I.A. Ibrahim, S. Mischke, D.L. Scott and J.A. Saunders, 2004. Rapid isolation of DNA from chocolate and date palm tree crops. J. Agric. Food Chem., 52: 5456-5462.
[32] Roberts, A.V., 2007. The use of bead beating to prepare suspensions of nuclei for flowcytometry from fresh leaves, herbarium leaves, petals and pollen. Cytometry A 71: 1039-1044.
[33] Borneman, J. and R.J. Hartin, 2000. PCR primers that amplify fungal rRNA genes fromenvironmental samples. Appl. Environ. Microbiol., 66: 4356-4360.
[34] Chachaty, E. and P. Saulnier, 2000."The Nucleic Acid Protocol Handbook: Bacterial DNA Extraction for Polymerase Chain Reaction and Pulsed-Field Gel Electrophoresis", Humana Press Inc., Totowa, NJ,