Journal of Tropical Resources and Sustainable Science

journal homepage: jtrss.org

New Method for Isolation of DNA from River Sediment Microbes of Sungai Kelantan, Malaysia

Noratikah Kamal Baharin[1], Noorkhalilie Che Abd Aziz[1], Faisal Mohamad[2], Suganthi Appalasamy[1,*]

[1]Faculty of Earth Science, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia.
[2]School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia.

Received 12 April 2017
Accepted 7 May 2017
Online 24 November 2017

Keywords:
DNA extraction; Microbes; River sediment; Sungai Kelantan

☒*Corresponding author:
Dr. Suganthi Appalasamy
Faculty of Earth Science,
Universiti Malaysia Kelantan,
Malaysia.
Email: suganthi.a@umk.edu.my

Abstract

Extraction of pure genomic DNA from microbes isolated from river sediments often hindered by co-isolated carryovers from the reagents used which reduces the yield and quality of DNA. Existing methods were lengthy, expensive and does not result in high yield of genomic DNA in short time. This study describes a rapid and inexpensive DNA extraction method that involved minimal purification step without phenol or other carcinogenic reagents. The microbial DNA extraction steps also does not require the use of special laboratory equipment. This method uses Cetyltrimethylammonium bromide (CTAB) followed by ethanol based purification steps and within one hour the extraction of microbial DNA could be completed. The extracted genomic DNA from microbes isolated from river sediment of Sungai Kelantan, Malaysia using the method described in this study is of quality suitable for other downstream researches such as metagenomics sequencing.

1. Introduction

Situated in northeastern of Peninsular Malaysia, Kelantan's longest river is the Kelantan River or commonly known as Sungai Kelantan. Feeding more than 180 streams and draining at a catchment area of 11,900 square kilometer, Sungai Kelantan is considered as a lowland stream flowing towards the river mouth (Ibbitt *et al*., 2002; Ahmad *et al*., 2009). The river flows through major towns in Kelantan; Kuala Krai, Tanah Merah, Pasir Mas and Kota Bharu (Kelantan state capital) before flowing into the South China Sea (Ambak and Zakaria, 2010). With the recent flood catastrophe that almost covered the nearby cities of Sungai Kelantan, the level of pathogenic contamination in the Sungai Kelantan remains unknown. Formed by the combination of Galas River (Sungai Galas) and Lebir River (Sungai Lebir) situated near Kuala Krai, Sungai Kelantan plays a prominent role in the lives of local people, eg. in domestic use, as a transportation mode, agriculture and plant irrigation, and small scale fishing activities (Ambak and Zakaria, 2010).

Flooding is usually associated with an increased risk of infection and changes in the water microbial community. The movement of water from the nearby industries into the river during and after flood could be the contributing factor to the accumulation of various heavy metal and other pollutants in Sungai Kelantan water. Changes in the accumulation of these materials on the bottom of river that forms the sediment layer of Sungai Kelantan could also change the dynamic of microbe and its abundance based on the carbon source availability (Donderski & Wilk, 2001; Sigee, 2004; Black, 2008; Okafor, 2011). Hence, metagenome analysis of the total microbes in river sediment must be first conducted to determine the microbial diversity of Sungai Kelantan post flood. The documented microbial community in Sungai Kelantan could also be used to identify novel bacteria and the enzymes that can be applied in pharmaceutical industries other than contributing the information to plan for river mitigation by the local agencies.

Investigation on river sediment microbe involves rapid isolation of DNA from the sampled sediment. Good DNA isolation method warranted for isolated DNA to be in high purity and quantity. Downstream steps in molecular biology such as metagenomics analysis of microbial DNA, detection of mutation and other high end processes requires the starting material ie DNA to be in good quality that falls in the range of 1.8 – 2.0 ratio for A260/280 spectrophotometer analysis (Nishiguchi *et al*., 2002). Partially degraded DNA or presence of other contaminant can be characterized by presence of smearing during Agarose Gel Electrophoresis (AGE) whereas fragmented DNA is indicated by presence of multiple bands during AGE. A good isolation protocol for DNA extraction will produce a single band at the highest point

eISSN Number: 2462-2389

during AGE (Sambrook & Russell, 2001). However, most protocols for DNA isolation from sediment microbe previously reported by Ekwanzala *et al*. (2017), Jie *et al*. (2016), Mason *et al*. (2014), Hilyard *et al*. (2008) were using commercial kits or hazardous chemicals such as phenol and liquid nitrogen in the extraction method (Tsai & Olson, 1991) or involve a lengthy procedure (up to 7 hours) for a few samples (Mao *et al*., 2013). Adding to that, DNA extraction with limited equipment and chemicals is nearly impossible with commercial DNA isolation kit and protocols. Thus, an easy, rapid and effective method for DNA isolation is needed and hence this study reports a simple, inexpensive yet robust protocol for river sediment DNA isolation.

2. Materials and Methods

2.1. River Sediment Sampling in Sungai Kelantan, Kelantan

River sediments sample were collected in Sungai Kelantan using 3 meter clean PVC pipe. About 250 g of river sediment were collected at three meters from the river bank at four different locations along Sungai Kelantan. Each location was separated by distance of 100 m. The collected sediment samples were kept in clean zip lock bags and stored in 4 °C in cold box. The zip lock bags containing the river sediments were then transferred to freezer at -10 °C till next step.

2.2. DNA Extraction of River Sediment Microbe

The chilled river sediment collected from four locations were transferred to a clean 1000 ml beaker. The sediment were then vacuum-filtered through Whatman filter paper no. 1. The filtrate from the river sediment were then again vacuum-filtered with Whatman filter paper 0.25 µm. The filter paper then transferred to 2.0 ml microcentrifuge tubes with 500 µL of Tris-EDTA (TE) (Sigma-Aldrich, St. Louis, Missouri, USA) buffer (pH 8). The filter paper together with the buffer were mixed by repeated pipetting. Cetyltrimethylammonium bromide [CTAB (Sigma-Aldrich, St. Louis, Missouri, USA)] buffer with amount 500 µL was added into mixture of solution of TE and filter paper. Fifty µL of Proteinase K (Sigma-Aldrich, St. Louis, Missouri, USA) (20mg/ml) was added into solution. About 10 µL lysozyme (Sigma-Aldrich, St. Louis, Missouri, USA) (100 mg/ml) was added and the tubes were slowly inverted. The tube then was rested in ice for 10 minutes. The solution then was incubated in water incubator at 65 °C for 10 min. The filter paper was removed and about 500 µL chloroform (analytical grade) (Sigma-Aldrich, St. Louis, Missouri, USA) was added and the solution was mixed thoroughly by repeated inversion. The tubes containing the buffer mixture were incubated at room temperature (24 °C) for 10 minutes. The mixtures were then centrifuged at 14,000 rpm for five minutes and 250 µL of supernatant (aqueous) layer was transferred to a new 2.0 ml microcentrifuge tube. The step was repeated with chloroform (equal volume) when there was presence of white protein layer. Approximately 500 µL of cold absolute ethanol (analytical grade) (Sigma-Aldrich, St. Louis, Missouri, USA) was added and mixed gently with the aqueous layer until the DNA precipitates. The mixture then was centrifuged for 15 minutes at 14,000 rpm and absolute ethanol was removed by pipetting. The remaining salts from DNA extraction was washed away by using one mL of 70% ethanol. The pellet was centrifuged at 10 000 rpm for two minutes, and the remaining ethanol was discarded. The white pellet that was formed at the bottom of the microcentrifuge was air dried for 20 minutes in laminar flow. The pellet of extracted DNA then was resuspended in 50 µL of sterile TE buffer (pH 8.0) and was kept at -20 °C in freezer.

2.3. Agarose Gel Electrophoresis (AGE) of Extracted River Sediment Microbial DNA

The extracted soil sediment microbial DNA from the four different locations along Sungai Kelantan were electrophoresed using 1% agarose gel (Sigma-Aldrich, St. Louis, Missouri, USA) and run at 80 volt for 30 minutes. The agarose gel with the microbial DNA was stained with three µL of Ethidium bromide (10 mg/mL) in dark room of Microbiology Laboratory, Universiti Malaysia Kelantan, Jeli Campus. The stained DNA was viewed and documented using Gel Doc XR System (Bio- Rad Laboratories, Berkeley, USA).

2.4. Quality and Quantity Analysis of Soil Sediment Microbial DNA

One microliter of extracted microbial DNA was used to assess the quality and quantity of the extracted DNA at A260 and A280 using NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, EUA).

3. Results and Discussion

Obtaining high quality DNA is the most crucial step in molecular biology studies, especially when dealing with environmental samples that could be laden with pollutants. In laboratories with minimal set up and equipment, usage of expensive commercial kit or hazardous chemicals for extraction of DNA is not preferred. A simple protocol with easy to find bench top chemicals and equipment must be formulated and hence the method reported in this study proven to be effective in isolating high quality DNA using minimal equipment and chemicals.

DNA isolation involves a few basic steps ie. cell lysis, disruption of cellular structure to produce lysate, inactivation of cellular enzymes followed by separation of desired soluble DNA from cell debris and other insoluble materials. Finally, the separated DNA is then purified to

eISSN Number: 2462-2389

be free of soluble proteins and other nucleic acids (Rice, 2016). According to Clarke (2009), CTAB buffer function to remove the membrane lipids and promote cell lysis. CTAB extraction buffer is a commonly used reagent for DNA isolation of a wide range of organisms (Ops Diagnostic, 2016). This study's main objective was to isolate total genomic DNA from all the microbes in the river sediment for metagenome analysis, hence the CTAB buffer and the protocol used proved to be rapid and efficient in accomplishing the isolation of DNA (Table 1). The whole protocol took less than an hour to accomplish.

Table 1: The DNA concentration (ng/µl) and quality (A260/A280 ratio) of Sungai Kelantan sediment microbes reading from Nanodrop spectrophotometry to analyse the purity and efficiency of the reported new protocol.

River sediment microbial DNA	DNA concentration (ng/ µl)	A260/A280 (O)
Sample sediment A	2.512	2.04
Sampke sediment B	1.682	2.07
Sample sediment C	7.266	1.98
Sample sediment D	11.126	1.82

Table 1 show the quantity of total genomic DNA of sediment microbe isolated from Sungai Kelantan. The quantity of DNA in one microliter is in range of 1.682 to 11.126 ng/ul, hence in 50 ul of TE buffer, the DNA concentration is in range of 84.1 to 556.3 ng. The A260/A280 ratios for the DNA isolated from river sediment microbe were in the range of 1.82 to 2.07, which falls in the pure DNA category. The obtained DNA quality and concentration obtained in this study using the reported protocol indicated successful isolation of DNA that was in the range required for metagenomics sequencing of 16sRNA for microbial samples (McCafferty *et al*., 2013). The concentration of isolated DNA using the protocol in this study is comparable to a number of commercial extraction kits previously reported by Burbach *et al.* (2015) who stated the isolated DNA concentration in that study to be in the range of 1.5 to 47.9 ng/mg sample. Ratio of absorbance at 260 nm to 280 nm is one of the common method in estimating concentration and purity of a solution (Manchester, 1995; Theresa, 1999). However, Adam (2003) argues that DNA is not the only molecule that absorbs UV light at 260 nm easily, RNA and aromatic protein also absorbs light at 280 nm which could contribute greatly to the absorbance ratio of 260 nm:280 nm. Hence, to overcome this, Agarose Gel Electrophoresis (AGE) of DNA was done to reaffirm the purity of the isolated genomic DNA from sediment microbe. As different nucleic acid molecules migrate differently in horizontal agarose gel electrophoresis system when supplied with electric power, based on molecule size with the standard DNA, the presence of contaminating protein and other nucleic acid such as RNA could be detected on AGE (Glasel, 1997). Figure 1 shows the result for AGE of isolated genomic DNA for sediment microbe from Sungai Kelantan. The presence of single band at the highest position on agarose gel indicate the success of the new isolation protocol in DNA extraction from sediment microbes. There were no smear or other band present in the agarose gel which reaffirms the DNA purity calculation using A260/28 ratio (Wang & Rossman, 1995).

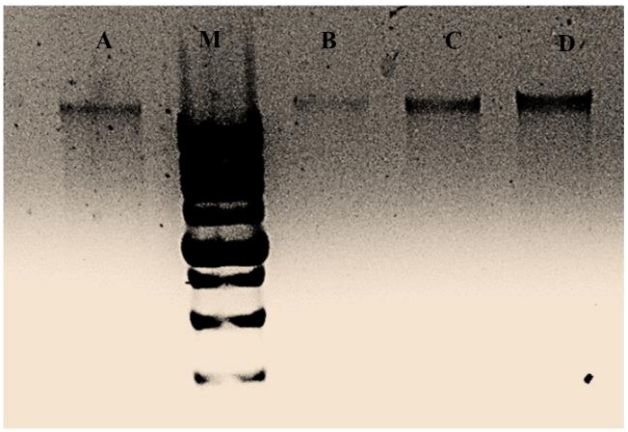

Figure 1: Agarose gel electrophoresis (1%) of total river sediment microbial DNA from Sungai Kelantan extracted using CTAB plus Proteinase K and lysozyme. Microbial DNA from river sediment from four different location along Sungai Kelantan (A, B, C, D) and 100 bp ladder (M) ran for 30 minutes at 80 volt power were then stained under EtBr for viewing under UV Gel Doc system (BioRad Laboratories, Berkeley, USA).

As metagenomics analysis requires high quality DNA free of contaminating proteins, cationic detergent Cetyltrimethylammounium bromide (CTAB) is effective in eliminating polysaccharides and polyphenols (Ops Diagnostic, 2016). In previous study by Rajagopal *et al*. (2014), CTAB was used for preparation of competent bacteria cells (*E. coli* and *Bifidobacterium sp*.) and yeast (*Kluyveromyces lactis*). According to Rice (2016), by adding the proteinase K into the sample, the DNA as well as other cellular protein may be degraded and hence TE buffer was added to prevent DNA from degraded.

According to Fatima *et al*. (2014), various parameters such as incomplete cell lysis and DNA degradation may influence the effectiveness of DNA extraction procedure. This was because the low yield of DNA would result in less diversified pool of templates (Hwang *et al*., 2012). According to Hwang *et al*. (2012), the resistance to cell lysis treatments could be influenced by the difference of cell wall component of bacteria. However, this study required isolation of total genomic DNA of microbes found in the river sediment of Sungai Kelantan which consists of both Gram negative and positive bacteria. Therefore, to improve the cell lysis, lysozyme was added into the treatment to damage the bacteria cell wall and proteinase K to digest the protein and eliminate the contamination (Van Oss, 1989; Theresa, 1999; Rice, 2016).

eISSN Number: 2462-2389

4. Conclusion

The DNA extraction protocol discussed in this study was successful in isolating high quality and quantity of total genomic DNA from river sediment microbe using CTAB plus Proteinase K and lysozyme buffer. The isolated DNA from river sediment of Sungai Kelantan could be used for subsequent metagenomics sequencing and analysis in future.

Acknowledgement

The authors would like to thank Ministry of Higher Education for Fundamental Research Grant Scheme (FRGS/1/2015/SG05/UMK/02/1) and Universiti Malaysia Kelantan, Jeli Campus for the facilities and cooperation from laboratory assistant who contributed the ideas and supports in order to finish this paper.

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eISSN Number: 2462-2389