ประสิทธิภาพของวิธีการสกัดดีเอ็นเอในสาหร่ายสีเขียวขนาดเล็ก
An Efficiency of DNA Extraction Methods for Green Microalgae

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บทคัดย่อ

ปัจจุบันการศึกษาสาหร่ายในระดับชีวโมเลกุลได้รับความสนใจและมีจำนวนเพิ่มมากขึ้น ซึ่งงานวิจัยทางชีวโมเลกุลนี้ต้องการดีเอ็นเอทั้งเชิงปริมาณและคุณภาพ วิธีการสกัดดีเอ็นเอจากสาหร่ายที่ใช้ในปัจจุบันมีด้วยกันหลายวิธี แต่ละวิธีมีข้อจำกัดแตกต่างกันไป เช่น ใช้เวลานาน มีค่าใช้จ่ายสูง และไม่สามารถประยุกต์ใช้ได้กับสาหร่ายทุกชนิด งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพของวิธีสกัดดีเอ็นเอต่างๆ เมื่อประยุกต์ใช้กับสาหร่ายขนาดเล็ก โดยทดลองสกัดดีเอ็นเอจากสาหร่ายสีเขียวทั้งสิ้น 5 สกุล รวม 30 ตัวอย่างด้วยวิธี SDS (Sodium dodecyl sulfate), CTAB (Cetyltrimethylammonium bromide), DTAB (Dodecyltrimethylammonium bromide), Triton x-100 และ Chelex -100 ตรวจวัดปริมาณและความบริสุทธิ์ของดีเอ็นเอที่สกัดได้โดยการวัดค่าการดูดกลืนแสงด้วยสเปกโตรโฟโตมิเตอร์ และใช้เป็นชิ้นดีเอ็นเอแม่แบบสำหรับเพิ่มจำนวนยีน 18s *rDNA* พบว่าการสกัดดีเอ็นเอด้วยวิธี SDS ให้ปริมาณดีเอ็นเอมากที่สุดและดีเอ็นเอที่สกัดได้มีความบริสุทธิ์สูงสุด รองลงมาคือ การสกัดโดยวิธี CTAB การสกัดดีเอ็นเอด้วยวิธี SDS, CTAB และ DTAB ให้ปริมาณและคุณภาพของดีเอ็นเอเพียงพอในการนำไปเพิ่มปริมาณดีเอ็นเอด้วยวิธีพีซีอาร์บริเวณยีน 18s *rDNA* ดีเอ็นเอของสาหร่ายที่สกัดได้จากวิธี SDS, CTAB และ DTAB จำนวน 24, 16 และ 5 ตัวอย่างตามลำดับ ยังสามารถนำไปตัดด้วยเอนไซม์ตัดจำเพาะ *Mse*I ต่อไปได้ ส่วนการสกัดดีเอ็นเอด้วยวิธี Triton x-100 และวิธี Chelex -100 ไม่เหมาะสมในการนำมาประยุกต์ใช้สกัดดีเอ็นเอจากสาหร่ายสีเขียวขนาดเล็ก ฉะนั้นวิธี SDS เป็นวิธีการที่มีประสิทธิภาพที่สุดในวิธีทั้งหมดที่นำมาใช้สกัดดีเอ็นเอจากสาหร่ายสีเขียวขนาดเล็กในการศึกษาครั้งนี้

คำสำคัญ: การสกัดดีเอ็นเอ, สาหร่ายขนาดเล็ก, ประสิทธิภาพ, ดีเอ็นเอ

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ABSTRACT

Nowadays, the molecular study in microalgae is getting more attention and has been continuously reported. Further study in the molecular research work requires both quantitative and qualitative of extracted DNA. There are several methods for DNA extraction applied for microalgae. Each method has different limitations, such as time consuming and high cost. Additionally, some cannot be well applied for all microalgal species. Therefore, this research aimed to study an efficiency of various DNA extraction methods applied to microalgae. DNA samples were extracted from 5 genera of class Chlorophyceae with a total of 30 samples of microalgae using SDS (Sodium dodecyl sulfate), CTAB (Cetyltrimethylammonium bromide), DTAB (Dodecyltrimethylammonium bromide), Triton x-100 and Chelex-100 methods. The quality and quantity of DNA was determined by absorbance measurement using spectrophotometer and used as DNA template for DNA quality evaluation using 18s-*rDNA* gene amplification. The results showed that DNA extraction by SDS method gave the highest amount and the highest purity of extracted DNA, followed by CTAB extraction method. The SDS, CTAB and DTAB method provided a sufficient quantity and quality of extracted DNA, which can be further used as sample for 18s rDNA gene production via PCR (polymerase chain reaction) technique. Extracted DNA of microalgae by SDS, CTAB and DTAB method from 24, 16 and 5 samples respectively can be further cut by restriction enzyme *Mse*I. As the DNA extraction using the Triton x-100 and Chelex-100 methods is not appropriate to apply for DNA extraction from green microalgae, the SDS method is the most efficient method for green microalgal DNA extraction used in this study.

Key words: DNA extraction, microalgae, efficiency, DNA

INTRODUCTION

Microalgae are important photosynthesizing organisms on Earth. They are a primary producer in food chain and have been utilized as source of food for human and animal (Martin and Alexander, 2018). They are considered as an important biomass for industrial and medical perspectives (Michael, 2018). Microalgae have also been used for biorefinery and feedstock for biofuel production (Philip *et al.*, 2011). Currently, various molecular studies have been reported for microalgae including DNA barcoding (Beom-Ho *et al.*, 2014), microsatellite library construction (John *et al*., 2010) and whole genome sequencing (Crysten and Sabeeha, 2019). In molecular research work, a good quality and quantity of DNA are a prerequisite for further process in the study. There are several standard methods for DNA extraction such as SDS (Sodium dodecyl sulfate) method (Marmur, 1961; Takashi *et al*., 2012), CTAB (cetyltrimethylammonium bromide) method (Doyle and Doyle, 1990), high salt low pH method (Guillemaut and Drouard, 1992), Triton x-100 method (Tomasz *et al.*, 2017), Chelex -100 method (Utkarsha *et al.*, 2018), salt method (Aljanabi and Martinez, 1997) and NaOH method (Hill-Ambroz *et al.*, 2002). These protocols provide different quality and quantity of extracted DNA. In addition, all those protocols show different result of DNA extraction, which were vary according to microalgal species. Variety of DNA extraction methods have been used in various of algae, for example, using CTAB method for brown algae (Phillips *et al.*, 2001) and using SDS method for seaweed and green algae such as *Chlamydomonas reinhardii* (Su and Gibor,1988; Newman *et al.*, 1990). Previously researches of algal DNA extraction reported that lysis buffer in different DNA extraction method should be taken into account and concerned due to the variation of intracellular and secondary compounds on algal cell wall (Doyle and Doyle, 1990). Cell wall of some algae comprises of algaenans, while other contains

dinosporins and glycoprotein (Doyle and Doyle, 1990; Lodhi *et al.*, 1994), which are difficult to lyse. This difficulty of algal DNA extraction is well-known and continually reported. (Lucy *et al*., 2012; Tear *et al.*, 2013; Maneeruttanarungroj and Incharoensakdi, 2016) Furthermore, algae always develop unique cell wall composition adapted to their inhabiting area, such as mucilage sheaths containing a compounds that tolerant to cell lysis (Barsanti *et al*., 2001; Popper *et al*., 2014). Due to those reasons, optimization of DNA extraction method for variety uses in algae are challenging.

One of the common uses for DNA extraction is Doyle and Doyle method (Doyle and Doyle, 1987), which comprises of CTAB, a cell lysis solution that will complex with protein and polysaccharide and result in elimination of those compounds on cell wall (Semagn *et al*., 2006). In addition, chelating resin Chelex -100 method (Walsh *et al*., 1991) is also wildly used in extraction of *Chlamydomonas* because it is simple, rapid and low cost. Moreover, Chelex -100 has been proven that it is good extraction method for PCR (Polymerase Chain Reaction) amplification efficacy (Walsh *et al*., 1991; Ward, 1992; Cao *et al*., 2009). This method has been used to extract *E. coli* bacteria by boiling in just one step which is sufficient to obtain high quality DNA. However, this protocol is unable to use with all organisms (Kwon *et al*., 2010) and still needs additional steps. To choose the right DNA extraction methods, species characteristics as component in cell wall and on cell surface must be well considered. The majority cell wall components in algae are lipid, pectin, cellulose and secondary metabolites (Zoe *et al.*, 2014). The hardest of DNA extraction of *Chlorella vulgaris* was reported resulting from hardness of cell wall and small cell size (Friedl, 1995). Success in development the extraction method was also reported using DTAB (dodecyltrimethylammonium bromide) co-assisted with cell wall digestion method by MiniBeadBeater (Biospec Products, Bartlesville, OK, USA), followed by chloroform extraction and lastly ethanol precipitation (Marvin and Karen, 2004). In conclusion, there is the difficulty of DNA extraction in eukaryotic microalgae as a result from different cell wall structure. Moreover, the contaminants in the extracted DNA can inhibit the action of enzymes, rendering the DNA useless for downstream applications.

Therefore, the purpose of this study is to examine an efficiency of various DNA extraction methods including SDS (Sodium dodecyl sulfate), CTAB (Cetyltrimethylammonium bromide), DTAB (Dodecyltrimethylammonium bromide), Triton x-100 and Chelex-100 methods for DNA extraction of 30 green microalgal samples.

MATERIALS AND METHODS

Microalgal samples used in this study

A total of 30 algal samples from 5 genera of class Chlorophyceae (Fig. 1) was used in this study. The samples composed of 6 isolates of genus *Chlorella*, *Chlorococcum*, *Coelastrum*, *Haematococcus* and *Scenedesmus*. All algal samples are provided by Algae Excellent Center, Thailand Institute of Scientific and Technological Research (TISTR) (Table 1).

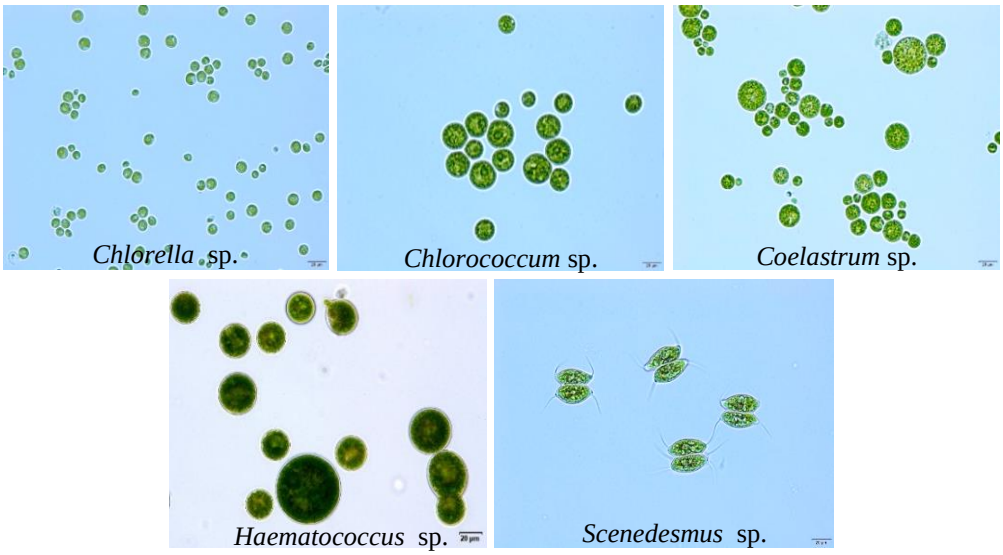


Figure 1 Five genera of microalgae used for DNA extraction

Microalgae culture

Microalgae were purified using streak plate method on BG-11 agar with 300 µg/ml Ampicillin (Mokhzanni *et al.*, 2016). The isolated algal colony was picked up and cultured in BG-11 medium (Stanier *et al.*, 1971). The cultures were incubated on rotary shaker at 120 rpm at 28°C with continuous 60 µE m^{-2} s^{-1} LED light for 21 days. The cultured algae were centrifuged at 12,000 rpm for 10 minutes in 2 ml microfuge tube. Microalgal cells were collected and washed twice using distilled water. Cells were stored at -40°C for 24 hours before DNA extraction in the next step.

Genomic DNA extraction

Microalgal cells (100 mg) were added into 2 ml microfuge tube. Extraction buffer (100 nM Tris-HCl pH 8.0, 100 nM EDTA pH 8.0, 1.5 M NaCl) amounting to 300 microliters (µl) was added for resuspension. Cell suspension was ground in cold mortar and pestle (-40^{o}C) for 10 minutes, followed by addition of 300 µl extraction buffer and mixed well for 30 seconds. Five microfuge tubes, containing ground algal cells in extraction buffer, were prepared for comparison of 5 DNA extraction methods.

Table 1 Microalgal samples used in this study

Sample number	Isolate	Scientific name	Sample location
1	TISTR 8262	*Chlorella* sp.	Bangkok
2	TISTR 8263	*Chlorella* sp.	Bangkok
3	TISTR 8264	*Chlorella* sp.	Pathum Thani
4	TISTR 8411	*Chlorella* sp.	Nakhon Pathom
5	TISTR 8432	*Chlorella* sp.	Pathum Thani
6	TISTR 8580	*Chlorella vulgalis*	Nonthaburi
7	TISTR 8412	*Chlorococcum humicola*	Bangkok
8	TISTR 8461	*Chlorococcum humicola*	Nonthaburi
9	TISTR 8973	*Chlorococcum humicola*	Nakhon Pathom
10	TISTR 8481	*Chlorococcum infusionum*	Nonthaburi
11	TISTR 8268	*Chlorococcum* sp.	Nonthaburi
12	TISTR 8509	*Chlorococcum* sp.	Nakhon Pathom
13	TISTR 8604	*Coelastrum astroideum*	Bangkok
14	TISTR 8477	*Coelastrum microporum*	Nonthaburi
15	TISTR 8566	*Coelastrum morus*	Nakhon Ratchasima
16	TISTR 8501	*Coelastrum reticulatum*	Nakhon Pathom

Table 1 (Continued)

Sample number	Isolate	Scientific name	Sample location
17	TISTR 8452	*Coelastrum* sp.	Bangkok
18	TISTR 8725	*Coelastrum sphearicum*	Pathum Thani
19	TISTR 8647	*Haematococcus lacustris*	Japan
20	TISTR 8467	*Haematococcus* sp.	Nonthaburi
21	TISTR 8478	*Haematococcus* sp.	Nonthaburi
22	TISTR 8611	*Haematococcus* sp.	Nonthaburi
23	TISTR 8805	*Haematococcus* sp.	Nakhon Ratchasima
24	TISTR 8809	*Haematococcus* sp.	Nakhon Ratchasima
25	TISTR 8457	*Scenedesmus acuminatus*	Bangkok
26	TISTR 8433	*Scenedesmus acutiformis*	Pathum Thani
27	TISTR 8540	*Scenedesmus acutus*	Bangkok
28	TISTR 8444	*Scenedesmus armatus*	Bangkok
29	TISTR 8498	*Scenedesmus praetervisus*	Nakhon Pathom
30	TISTR 8440	*Scenedesmus quadricauda*	Bangkok

TISTR = Thailand Institute of Scientific and Technological Research

Three hundred µl solution of 20% SDS (Method 1) (Doyle and Doyle, 1987), 10% CTAB (Method 2) (Marvin and Karen, 2004), 10% DTAB (Method 3) (Marvin and Karen, 2004), 10 % Triton x-100 (Method 4) (Tomasz *et al*., 2017) and 10% Chelex -100 (Chelating Ion Exchange Resin) (Method 5) (Walsh *et al*., 1991) was added in each microfuge tube, containing ground algal cells in extraction buffer, then mixed well for 30 seconds and incubated at 65^{o}C for 5 minutes. The mixture was centrifuged at 12,000 rpm for 10 minutes. Then, the supernatant was transferred to a new microfuge tube and 1 volume of phenol: chloroform: isoamyl alcohol (25:24:1) which equals the transferred supernatant was added. The mixture was mixed well and centrifuged at 12,000 rpm for 10 minutes. The supernatant was transferred to a new microfuge tube and 1 volume of cold 90% ethanol was added to precipitate DNA. The tube was centrifuged at 12,000 rpm for 10 minutes. The supernatant was then discarded. One ml of 70% ethanol was added to wash the DNA pellet and the tube was centrifuged for at 12,000 rpm 10 minutes. The ethanol was then discarded. The tube was dried for 10 min in the laminar flow and 30 µl of 1xTE (10 mM Tris-1 mM EDTA) buffer was added to dissolve DNA.

DNA quality evaluation using 18s *rDNA* gene amplification and digestion by *Mse*I restriction enzyme

Genomic DNAs of 30 algal samples were used as DNA template for DNA quality evaluation using 18s *rDNA* gene amplification. The 18s *rDNA* gene region was amplified via PCR using 18s forward primer (5'-GTCAGAGGTGAAATTCTTGGATTTA-3') / reverse primer (5'- AGGGCAGGGAC GTAATCAACG-3') and *Taq* DNA polymerase (Apsalagen, Bangkok, Thailand), following the reaction: (1) 94°C for 5 min, (2) 94 °C for 30 sec, (3) 53 °C for 45 sec, (4) 72 °C for 40 sec, 35 cycles of (2)-(4) and 72 °C for 2 min. For the isolated DNA quality evaluation, 1 µg of genomic DNA of all 30 algal samples were completely digested by *Mse*I restriction enzyme at 37^{o}C for 60 min. PCR and digestion products were electrophoresed in 1% (w/v) agarose gel.

RESULTS AND DISCUSSION

DNA quantity and quality evaluation by spectrophotometry and gel electrophoresis

Five DNA extraction methods including SDS (Sodium dodecyl sulfate), CTAB (Cetyltrimethylammonium bromide), DTAB (Dodecyltrimethylammonium bromide), Triton x-100 and Chelex-100 methods were adapted to extract genomic DNA of 30 samples from five algal genera, including 6 isolates of genus *Chlorella*, *Chlorococcum*, *Coelastrum*, *Haematococcus* and *Scenedesmus*. Genomic

DNA quality and quantity evaluation was performed using spectrophotometry at OD_{260} and OD_{280} nm and gel electrophoresis. The concentration of DNA and the ratio of absorbance, A, at 260 and 280 nm were calculated in all 30 samples (Table 2). The ratio of absorbance within 1.8-2.0 can be considered as a good quality DNA sample (Wang *et al*., 2011).

Based on the ratio of $A_{260/280}$ of the genomic DNA sample, method 1 (SDS) gave the highest DNA quality, followed by method 2 (CTAB). The ratio of $A_{260/280}$ of 30 DNA samples obtained from SDS method varied from 1.78 to 2.05, with an average of 1.93 and only 6 out of 30 algal samples showed less value than 1.8-2.0. The ratio of $A_{260/280}$ of 30 DNA samples obtained from CTAB method varied from 1.65 to 1.99, with an average of 1.83 and 12 out of 30 algal samples showed smaller value than 1.8-2.0 (Table 2). The ratio of $A_{260/280}$ of all 30 samples obtained from method 3 (DTAB), 4 (Triton x-100) 5 (Chelex -100) was smaller value than a range of 1.8-2.0, indicating poor quality DNA samples (Table 2). The result from the ratio of $A_{260/280}$ was consistent with the result from gel electrophoresis. Figure 2 indicated examples of very poor quality degraded DNAs, shown as smear DNA bands (lane 4 and 5), of *Coelastrum reticulatum* (TISTR 8501) obtained from Triton x-100 and Chelex-100 methods. DNAs of these 2 methods cannot be further used in the next part of the experiment on PCR amplification of 18s *rDNA* region and *Mse*I restriction enzyme digestion. That the SDS method gave the best result in DNA quality and quantity is due to a principal of this method that using a high concentration of SDS for cell lysis.

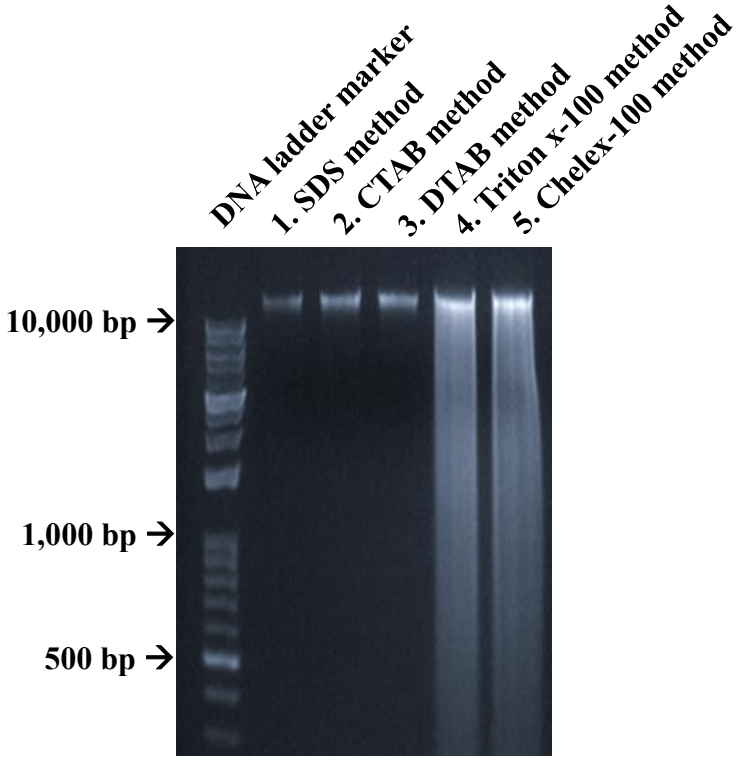


Figure 2 Agarose gel electrophoresis of *Coelastrum reticulatum* (TISTR 8501) genomic DNAs isolated using SDS, CTAB, DTAB, Triton x-100 and Chelex-100 methods.

The results from the ratio of $A_{260/280}$ of all 30 algal DNAs, extracted using DTAB, Triton x-100 and Chelex-100 methods, were lower than 1.8 (Table 2), indicating poor quality DNA samples. This result indicated that the contaminants in DNAs affected the calculation of DNA quantity in these samples. DNAs of 30

algal samples obtained from SDS and CTAB methods were in good quality based on the ratio of $A_{260/280}$ and gel electrophoresis. The concentration of DNA of 30 algal samples, obtained from SDS method varied from 250.0 to 1,059.0 ng/µl, with an average of 435.8 ng/µl, while the concentration of DNAs obtained from CTAB method varied from 190.0 to 740.0 ng/µl, with an average of 448.7 ng/µl (Table 2). The low quality of the extracted DNA can inhibit the action of enzymes and cause a series problem for downstream molecular applications, for example library construction and marker development (Sirakov, 2016).

The presence of contaminants in the DNA solution may result in degradation of DNA on long term storage and inhibition of the polymerase chain reaction. In order to remove impurities, additional steps should be employed. Improvement of DNA quality has been reported using ammonium acetate to precipitate proteins and a sodium acetate-isopropanol mixture to pellet out DNA before being washed with ethanol (Utkarsha *et al*., 2018). The degraded DNA obtained from Triton x-100 and Chelex-100 methods might be come from the cell wall breaking process is too harsh. The method should be improved by decreasing the grinding time and the concentration of Triton x-100 and Chelex-100 and adding EDTA (ethylenediaminetetraacetic acid) for preventing DNA degradation (Shivji *et al*., 1992). A lysis buffer mixture, for example SDS together with Chelex-100, can also be added to increase the capacity to inhibit polyphenols and polysaccharide. These secondary metabolite compounds can interrupt not only polymerase activity, but also the reaction of DNA digestion (Doyle and Doyle, 1990.; Lodhi *et al*., 1994). Normally, the genomic DNA, contaminated with polysaccharide, always causes a thickening pellet after precipitation with alcohol.

Table 2 Quantity and quality of genomic DNA extracted from five methods.

Sample	Method 1 (SDS)				Method 2 (CTAB)				Method 3 (DTAB)				Method 4 (Triton x-100)		Method 5 (Chelex - 100)	
	Quantity (ng/µl)	$A_{260/280}$	18s	*Mse*I	Quantity (ng/µl)	$A_{260/280}$	18s	*Mse*I	Quantity (ng/µl)	$A_{260/280}$	18s	*Mse*I	Quantity (ng/µl)	$A_{260/280}$	Quantity (ng/µl)	$A_{260/280}$
1	312	1.81	+	+	422	1.69	+	-	609	1.23	+	-	780	1.33	690	1.56
2	334	1.92	+	+	345	1.77	+	-	490	1.34	+	-	650	1.23	770	1.61
3	500	1.98	+	+	533	1.95	+	+	780	1.25	+	-	800	0.98	680	1.78
4	456	1.99	+	+	677	1.81	+	-	445	1.56	+	-	608	1.35	898	1.54
5	359	1.95	+	+	576	1.78	+	-	354	1.55	+	-	590	1.12	685	1.55
6	290	1.87	+	-	350	1.67	+	+	633	1.79	+	+	589	0.94	783	1.60
7	422	1.88	+	+	558	1.81	+	-	450	1.76	+	+	787	1.33	560	1.68
8	335	1.96	+	+	520	1.88	+	+	340	1.75	+	+	675	1.10	670	1.67
9	899	2.02	+	+	609	1.92	+	-	452	1.24	+	-	902	1.03	854	1.71
10	559	2.00	+	-	360	1.78	+	+	980	1.18	+	-	1,005	1.29	1,025	1.56
11	355	1.89	+	+	450	1.87	+	+	656	1.15	+	-	604	1.14	507	1.66
12	320	1.98	+	+	568	1.98	+	-	465	1.12	+	-	599	0.84	675	1.67
13	312	1.79	+	-	443	1.79	+	-	335	1.79	+	+	550	1.26	775	1.69
14	250	1.97	+	+	190	1.69	+	+	380	1.16	+	-	469	1.22	760	1.73
15	299	1.96	+	+	365	1.65	+	-	450	1.11	+	-	554	1.17	674	1.65
16	1,059	1.99	+	+	740	1.98	+	+	645	1.18	+	-	756	1.25	801	1.72
17	423	1.78	+	+	346	1.90	+	-	544	1.22	+	-	443	1.05	599	1.77
18	298	1.90	+	-	309	1.92	+	+	354	1.24	+	+	409	1.28	552	1.79
19	309	2.04	+	+	400	1.99	+	+	509	1.22	+	-	688	1.23	754	1.65
20	445	1.84	+	+	467	1.66	+	-	514	1.15	+	-	597	1.11	610	1.66
21	397	1.88	+	+	367	1.75	+	+	435	1.16	+	-	498	1.16	554	1.69
22	599	1.99	+	-	458	1.76	+	-	466	1.18	+	-	670	1.07	790	1.71
23	633	2.05	+	-	490	1.83	+	+	399	1.19	+	-	567	1.09	800	1.69
24	403	1.92	+	+	455	1.83	+	-	505	1.17	+	-	680	0.99	818	1.62
25	434	1.95	+	+	399	1.85	+	-	400	1.25	+	-	560	1.17	432	1.65
26	386	1.88	+	+	345	1.93	+	+	408	1.23	+	-	630	1.14	556	1.67
27	443	1.84	+	+	409	1.99	+	+	508	1.22	+	-	798	1.20	803	1.60
28	388	1.97	+	+	400	1.94	+	+	489	1.20	+	-	530	1.22	460	1.64
29	400	2.02	+	+	406	1.85	+	+	398	1.29	+	-	607	1.02	597	1.72
30	455	1.93	+	+	503	1.68	+	+	515	1.31	+	-	620	1.21	743	1.78
Average/Range	435.80 250-1,059	1.93 1.78-2.05	+=30 - = 0	+=24 - = 6	448.67 190-740	1.83 1.65-1.99	+=30 - = 0	+=16 - =14	496.93 335-980	1.31 1.11-1.79	+=30 - = 0	+ = 5 - =25	640.5 409-1,005	1.15 0.84-1.35	695.83 432-1,025	1.67 1.54-1.79

Sample 1-6 = *Chlorella* sp. Sample 7-12 = *Chlorococcum* sp. Sample 13-18 = *Coelastrum* sp. Sample 19-24 = *Haematococcus* sp. Sample 25-30 = *Scenedesmus* sp.

$A_{260/280}$ = Absorbance ratio at 260 and 280 nm

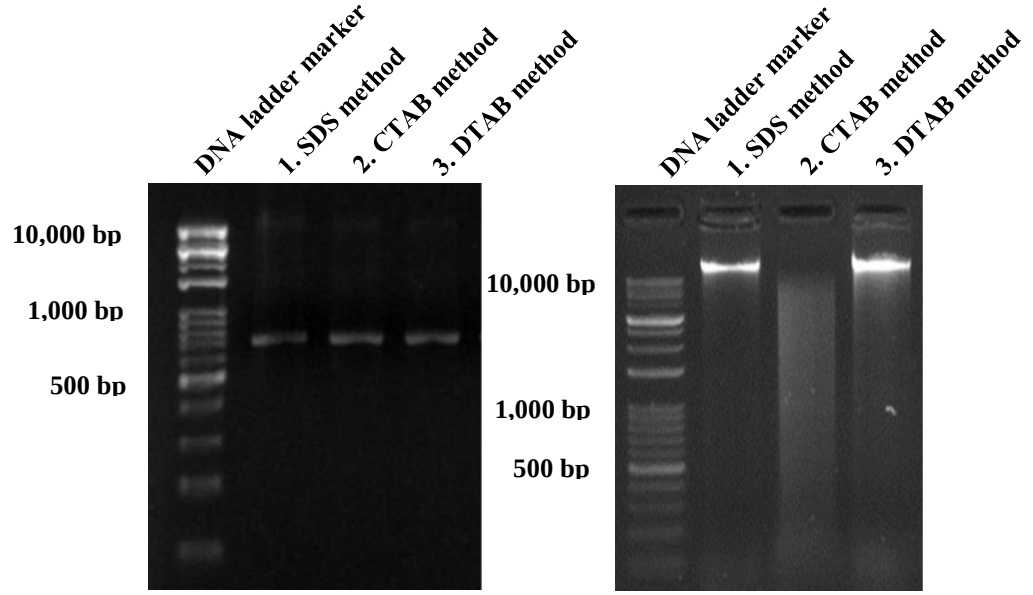
+ = DNA can be used in PCR to amplify 18s *rDNA* gene region/ can be digested with *Mse*I restriction enzyme

- = DNA can not be digested with *Mse*I restriction enzyme

DNA quality evaluation by 18s *rDNA* gene amplification and digestion with *Mse*I restriction enzyme

Genomic DNAs of 30 algal samples that were isolated using SDS, CTAB and DTAB methods were further used as DNA template in PCR for 18s *rDNA* gene amplification. The result showed that the 18s *rDNA* region can be amplified from all 30 algal DNAs, isolated using all 3 methods. Figure 3A was an example of gel electrophoresis of PCR products obtained from 18s *rDNA* gene amplification of *Chlorococcum infusionum* (TISTR 8481) DNAs, isolated using 3 methods. PCR is an efficient technique to amplify DNA from a small amount of DNA template and DNA with some degree of contaminants. This can be seen by the amplification of 30 samples of low-quality algal DNAs obtained from DTAB extraction method.

Genomic DNAs of 30 algal samples, extracted using SDS, CTAB and DTAB methods were digested by *Mse*I restriction enzyme. The result indicates that 24 of 30, 16 of 30 and 5 of 30 DNA samples, isolated using SDS, CTAB and DTAB methods, respectively can be digested by *M*seI restriction enzyme (Table 2). The result confirmed that SDS method is the best DNA extraction method for microalgae. However, this method cannot apply to all algae species. Different cell wall structures and components among algal species result in contaminants after the extraction process and may interrupt *M*seI activity (Lodhi *et al*., 1994; Sangwan *et al*., 2000; Pirttila *et al*., 2001; Muhammad *et al*., 2012; Sevindik *et al*.,2016). DNAs obtained from SDS method of *Chlorella vulgalis* and *Coelastrum sphearicum* cannot be digested by *Mse*I, while DNAs obtained from CTAB and DTAB methods can be cut into small fragments. DNAs obtained from only CTAB method of *Chlorococcus infusionum* and *Haematococcus* sp., sample number 23 (Table 1), can be cut by *Mse*I, as well as DNAs obtained from only DTAB method of *Coelastrum astroideum*. Figure 3B showed an example of gel electrophoresis of *Mse*I digestion products of *Chlorococcum infusionum* (TISTR 8481) DNAs, extracted using the 3 methods.



(A) PCR/18s **(B)** Digestion/*Mse*I

Figure 3 Agarose gel electrophoresis of (A) PCR products at 18s *rDNA* region and (B) digestion products by *Mse*I restriction enzyme of *Chlorococcum infusionum* (TISTR 8481) genomic DNA, isolated using SDS, CTAB and DTAB methods.

CONCLUSION

Among 5 DNA isolation methods including SDS, CTAB, DTAB, Triton x-100 and Chelex-100 methods, the SDS method results to the highest quality and quantity of DNA, followed by the CTAB method. These two methods show the advantages of being simple, rapid, inexpensive and requiring a small amount of algal tissue. The isolated DNA appears sufficiently pure and enough quantity for further application in molecular such as restriction endonuclease digestion and amplification using the polymerase chain reaction. The SDS method shows the general applicability for isolation of DNA from diverse species of microalgae studied. Having considered the results mentioned above, the SDS method is recommended for green microalgal DNA extraction used in this study.

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