



A simple and inexpensive physical lysis method for DNA and RNA extraction from freshwater microalgae

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Abstract

In this work, a simple and inexpensive physical lysis method using a cordless drill fitted with a plastic pellet pestle and 150 mg of sterile sea sand was established for the extraction of DNA from six strains of freshwater microalgae. This lysis method was also tested for RNA extraction from two microalgal strains. Lysis duration between 15 and 120 s using the cetyltrimethyl ammonium bromide (CTAB) buffer significantly increased the yield of DNA from four microalgal strains (*Monoraphidium griffithii* NS16, *Scenedesmus* sp. NS6, *Scenedesmus* sp. DPBC1 and *Acutodesmus* sp. DPBB10) compared to control. It was also found that grinding was not required to obtain DNA from two strains of microalgae (*Choricystis* sp. NPA14 and *Chlamydomonas* sp. BM3). The average DNA yield obtained using this lysis method was between 62.5 and 78.9 ng/mg for *M. griffithii* NS16, 42.2–247.0 ng/mg for *Scenedesmus* sp. NS6, 70.2–110.9 ng/mg for *Scenedesmus* sp. DPBC1 and 142.8–164.8 ng/mg for *Acutodesmus* sp. DPBB10. DNA obtained using this method was sufficiently pure for PCR amplification. Extraction of total RNA from *M. griffithii* NS16 and *Mychonastes* sp. NPD7 using this lysis method yielded high-quality RNA suitable for RT-PCR. This lysis method is simple, cheap and would enable rapid nucleic acid extraction from freshwater microalgae without requiring costly materials and equipment such as liquid nitrogen or beadbeaters, and would facilitate molecular studies on microalgae in general.

Keywords Microalgae · Nucleic acid · DNA extraction · RNA extraction · Sea sand · Physical lysis

Introduction

In recent years, microalgae have gained much interest and were exploited for the production of biodiesel, polyunsaturated fatty acids and commercially important pigments and metabolites (Koller et al. 2014). An important step in bio-prospecting for microalgae is species identification and traditionally, identification of microalgae has relied on microscopy. This method is time consuming and morphological characters can vary from species to species depending on

environmental factors, making identification difficult (Hejazi et al. 2010).

Recently, molecular methods have been widely used in phylogenetic studies of microalgae, utilizing genes such as the ribosomal genes (18S, 5.8S and 28S), its transcribed spacers (ITS1 and ITS2) (Hejazi et al. 2010) and as well as plastidal genes (Lee et al. 2013). These molecular methods often require a DNA extraction and purification step. Although protocols for direct PCR (dPCR) amplification have been established for a number of microalgae (Tear et al. 2013; Radha et al. 2013), this method may not work across all species of microalgae, as well as for molecular methods which require intact, good quality DNA or RNA such as AFLP-PCR (Donaldson et al. 1998), Southern blotting (Pratheesh et al. 2014) and real-time qPCR (Hou et al. 2010).

Optimization of nucleic acid extraction methods is favorable because different species of algae often may not allow optimal nucleic acid production from a particular extraction method (Simonelli et al. 2009; Eland et al. 2012), which could be due to high levels of polyphenols,

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polysaccharides (Xu et al. 2004) or a large range of recalcitrant cell wall structures (Tear et al. 2013; Eland et al. 2012). Strains which possess recalcitrant cell wall structures often require mechanical disruption methods such as freezing, grinding, beadbeating and high-pressure homogenization to release intracellular DNA. These equipments and reagents may not be readily available in the laboratory, especially in small laboratory settings.

Commonly used lysis methods for nucleic acid extraction from microalgae involve freezing and thawing in liquid nitrogen and beadbeating (Yuan et al. 2015; Eland et al. 2012; Fawley and Fawley 2004). Although unconventional cell lysis methods for nucleic acid extraction such as those employing sea sand have been reported for other organisms such as date palm (Arif et al. 2010), fungi (Wang and Chang 2003) and seaweed (Chan et al. 2004), studies on the use of such lysis methods on microalgae is limited and yet to be tested on a wide selection of microalgal strains. In this study, a simple and inexpensive physical lysis method utilizing a cordless drill, plastic pellet pestle and sterilized sea sand for the extraction of DNA from six strains and RNA from two strains of freshwater microalgae is described.

Materials and methods

Culture and maintenance of microalgal strains

Single colonies of locally isolated freshwater microalgal strains were used to inoculate 5 mL of Bold's Basal Medium (BBM), scaled up to 100 mL and maintained as starter cultures in 250-mL Erlenmeyer flasks. Flasks were agitated every 2–3 days. Starter cultures were tested for sterility by periodically plating onto nutrient agar. The microalgal strains used in this study, accession numbers and GPS coordinates are summarized in Table 1.

Initiation of larger scale culture and preparation of cells for nucleic acid extraction

Starter cultures were used to inoculate 2000 mL of BBM at an initial cell density of 2×10^5 cells mL⁻¹ in 3-L Erlenmeyer flasks. Flasks were sealed with sterile cotton wool and parafilm. Aeration was provided via a commercial aquarium pump and autoclaved silicon tubing at a flow rate of 200–300 mL min⁻¹ through a 0.22- μ M cellulose acetate filter (Sartorius). Cultures were grown under fluorescent lighting at ~ 2000 lx at 26 ± 2 °C and manually agitated every 1–2 days to prevent settling of cells. Cells were grown for 13–39 days and were harvested by centrifugation at 6000 rpm for 3 min in 50-ml falcon tubes, and washed once with MiliQ water. Approximately 100 mg (wet weight) of cells was stored at -20 °C in 1.5-mL microcentrifuge tubes until further use. For RNA extraction, cells were harvested using RNase-free falcon tubes and washed once with Milli-Q water treated with 0.05% (v/v) diethylpyrocarbonate (DEPC). Approximately 100 mg (wet weight) of cells in RNase-free microcentrifuge tubes was immediately used for RNA extraction.

Nucleic acid extraction procedure

Genomic DNA was extracted from frozen cells using the cetyltrimethyl ammonium bromide (CTAB) method with minor modifications. In brief, 150 mg of sterile sea sand (ca. 0.1–0.3 mm) was added to frozen cells and lysed by grinding with a conical pellet pestle (7–10 cm long) (Cat.#: MGR113, BioBasic) attached to a cordless drill (Skil 2212) at maximum speed (1000 rpm) in 500 μ L of CTAB extraction buffer containing 100 mM Tris–Cl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4M NaCl, 1% PVP and 2% CTAB. Next, the mixture was incubated in a 65 °C water bath for 1 h and inverted five to ten times every 15 min to mix. RNA was removed by adding 3 μ L of RNase A (10 mg mL⁻¹) followed by incubation for 15 min at 37 °C. After that, DNA was purified by a single

Table 1 Microalgal strains used in this study

Microalgal strain	Genbank accession	Source	GPS coordinates
Monoraphidium griffithii NS16 ^a	KP162147.1	Freshwater lake	2.8336N, 101.7636E
Chlamydomonas sp. BM3	KT720479.1	Freshwater lake	2.8919N, 101.7919E
Scenedesmus sp. NS6	KT720478.1	Freshwater lake	2.8336N, 101.7636E
Scenedesmus sp. DPBC1	KF680451.1	Freshwater pond	2.8092N, 101.7633E
Choricystis sp. NPA14	MG012356	Freshwater pond	2.8174N, 101.7686E
Acutodesmus sp. DPBB10	KY315602	Freshwater pond	2.8092N, 101.7633E
Mychonastes sp. NPD7 ^b	KP202155	Freshwater pond	2.8174N, 101.7686E

^aUsed for both DNA and RNA extraction

^bUsed for RNA extraction only

chloroform:isoamyl alcohol (24:1) extraction, followed by precipitation with 1 vol of isopropyl alcohol and washing with 70% (v/v) ethanol. The DNA pellet was air dried in a laminar flow cabinet and resuspended in 50 μ L of TE buffer (10 mM Tris–Cl, 1 mM EDTA) (pH 8.0). DNA was separated on 0.8% (w/v) agarose containing 1X RedSafe® (Intron Biotech) and visualized under a UV transilluminator. DNA concentration and purity were determined spectrophotometrically (NanoPhotometer P300, Implen).

Total RNA was extracted using the CTAB–LiCl method with minor modifications. All reagents and tubes used for RNA extraction were treated with 0.05% (v/v) DEPC. Solutions containing Tris or EDTA were prepared using DEPC water under RNase-free conditions. In brief, cells were lysed by grinding with 150 mg of DEPC-treated sea sand for 60–90 s in 500 μ L of CTAB extraction buffer containing 3% (v/v) β -mercaptoethanol on a -20 °C chiller rack (ViPlus chiller, Vivantis). After that, RNA was purified with an equal volume of phenol:chloroform (25:24) (pH 5.2) (Amresco) followed by extraction with chloroform:isoamyl alcohol (24:1). RNA was selectively precipitated with 2.7M LiCl overnight and further purified by standard ethanol precipitation. RNA was stored as a precipitate in ethanol at -20 °C, until further use. Prior to electrophoresis, the RNA pellet was recovered and dissolved in 50 μ L of DEPC water. The “Bleach Gel” method containing 1% (v/v) Clorox® as described by Aranda et al. (2012) was used to separate the RNA. Gel images of DNA were captured using a smartphone camera (Alcatel One Touch Idol X + 6043D), while for RNA, gel images were captured using a compact camera (Panasonic Lumix FH8).

Preliminary experiment and optimization of physical lysis parameters

A preliminary experiment was conducted to test the suitability of different lysis methods for each microalgal strain. Lysis methods tested in the preliminary experiment were inversion (control), pipetting (60 s), vortexing with 150 mg of sea sand (60 s) and grinding with 150 mg of sea sand (60 s). The simplest lysis method which produced the highest DNA yield for each strain was used in the optimization study. Parameters for the optimization study are outlined briefly in Table 2. Lysis parameters for both preliminary and optimization experiments were tested in independent replications ($n = 5$ –11). Data were tested for homogeneity of variance and normality using Levene’s test and Shapiro–Wilk test followed by one-way ANOVA. Statistical significance was contrasted using the Tukey–Kramer method at 95% confidence level.

Results and discussion

In many strains of microalgae, a recalcitrant cell wall containing complex polysaccharides, algaenans or silica is known to be a major barrier to nucleic acid extraction, hence requiring certain forms of physical lysis to facilitate nucleic acid extraction (Banerjee et al. 2002; Fawley and Fawley 2004; Eland et al. 2012; Yuan et al. 2015). Physical lysis methods previously described for nucleic acid isolation from microalgae, such as freezing and thawing in liquid nitrogen and beadbeating (Fawley and Fawley 2004; Kim et al. 2012; Eland et al. 2012) as well as grinding in liquid nitrogen with a mortar and pestle (Huo et al. 2017), involve specialized equipment and supplies including glass/zirconia beads and liquid nitrogen which may not be readily available in small laboratory settings.

Strains with a more delicate cell wall such as *Chlorella* and *Chlamydomonas*, on the other hand, may simply be lysed, at least partially, by incubation in the extraction buffer itself (Lin et al. 2010; Yuan et al. 2015). For such strains, the use of harsh mechanical methods such as beadbeating to achieve extensive cell lysis may result in DNA fragmentation (Yuan et al. 2015). However, Yuan et al. (2015) demonstrated that this setback could be circumvented, by extended incubation in lysis buffer for 3 days, followed by beadbeating of the remaining cells after centrifugation. In our study, we noticed that incubation in CTAB buffer itself is adequate to release a decent amount of DNA from *Chlamydomonas* sp. BM3 and *Choricystis* sp. NPA14 (Figs. 1, 2) as well as from *Chlorella sorokiniana* NS5 (Genbank acc: KM502980.1) (data not shown). Vortexing or grinding with sand did not further increase the amount of DNA obtained from these strains in the preliminary study ($p > 0.05$) (Fig. 2). However, grinding significantly increased the yield of DNA by 1.5-fold, 20-fold, 2.8-fold and 9.2-fold from *M. griffithii* NS16 ($p = 0.026$), *Scenedesmus* sp. NS6, *Scenedesmus* sp. DPBC1 and *Acutodesmus* sp. DPBB10 ($p < 0.001$), respectively, compared to control (Figs. 1, 2; Supplementary Table 1). Therefore, grinding was used in the subsequent optimization study for the abovementioned strains.

In the optimization study, the highest DNA yield for *M. griffithii* NS16 was obtained by grinding for 120 s (78.58 ng/mg), which is approx 2.5-fold higher than control (22.1 ng/mg) ($p < 0.001$). However, there is no significant difference in DNA yield between 120 s and 60 s ($p = 0.217$). Therefore, 60 s of grinding would be sufficient for this strain (Fig. 3; Table 2).

It is interesting to observe that the time at which cells were harvested, presumably at different growth phases, has an effect on the efficacy of cell lysis for *Scenedesmus* sp. NS6 and *Scenedesmus* sp. DPBC1. Cells that were

Table 2 The effect of lysis duration on the yield and purity of DNA from different microalgal strains in the optimization study

Microalgal strain	Lysis method/duration	DNA yield (ng/mg \pm SE)	Purity ($A_{260/280} \pm$ SE)	Purity ($A_{260/230} \pm$ SE)		
<i>Monoraphidium</i> sp. NS16	Inversion	22.21 \pm 3.62	1.71 \pm 0.05	0.83 \pm 0.13		
	Grinding (30 s)	39.53 \pm 7.62	1.83 \pm 0.02	1.15 \pm 0.17		
	Grinding (60 s)	62.51 \pm 5.39	1.88 \pm 0.02	1.31 \pm 0.16		
	Grinding (120 s)	78.58 \pm 5.21	1.89 \pm 0.02	1.42 \pm 0.12		
<i>Scenedesmus</i> sp. NS6	(Early harvest)	Inversion	15.33 \pm 2.12	1.57 \pm 0.17	1.99 \pm 0.49	
		Grinding (30 s)	164.12 \pm 15.48	1.98 \pm 0.01	2.31 \pm 0.03	
		Grinding (60 s)	217.46 \pm 26.35	1.99 \pm 0.01	2.30 \pm 0.01	
		Grinding (120 s)	247.01 \pm 11.83	2.19 \pm 0.15	2.43 \pm 0.09	
	(Late harvest)	Inversion	7.16 \pm 0.83	1.85 \pm 0.06	2.01 \pm 0.17	
		Grinding (30 s)	42.19 \pm 4.98	1.94 \pm 0.02	2.06 \pm 0.06	
		Grinding (60 s)	76.55 \pm 10.85	1.99 \pm 0.05	2.07 \pm 0.10	
		Grinding (120 s)	71.75 \pm 11.93	1.95 \pm 0.01	2.07 \pm 0.04	
<i>Scenedesmus</i> sp. DPBC1	(Early harvest)	Inversion	73.10 \pm 7.02	1.97 \pm 0.01	2.17 \pm 0.02	
		Pipetting (30 \times)	110.95 \pm 5.18	1.97 \pm 0.01	2.22 \pm 0.02	
	(Late harvest)	Inversion	16.85 \pm 8.69	1.61 \pm 0.21	1.00 \pm 0.18	
		Grinding (30 s)	70.24 \pm 9.08	1.88 \pm 0.01	1.39 \pm 0.04	
		Grinding (60 s)	74.94 \pm 6.09	1.86 \pm 0.01	1.37 \pm 0.03	
		Grinding (90 s)	71.34 \pm 7.75	1.87 \pm 0.01	1.42 \pm 0.06	
		<i>Acutodesmus</i> sp. DPBB10	Inversion	21.91 \pm 4.81	1.88 \pm 0.02	0.91 \pm 0.10
			Grinding (15 s)	142.80 \pm 15.97	1.94 \pm 0.01	1.84 \pm 0.08
Grinding (30 s)	158.53 \pm 20.52		1.90 \pm 0.03	1.81 \pm 0.10		
Grinding (60 s)	164.75 \pm 30.89		1.90 \pm 0.02	1.90 \pm 0.09		
Pipetting (30 \times)	330.39 \pm 31.22		1.99 \pm 0.01	2.57 \pm 0.10		
<i>Choricystis</i> sp. NPA14	Inversion		699.83 \pm 90.60	1.89 \pm 0.004	2.09 \pm 0.06	
	Pipetting (30 \times)	699.49 \pm 73.82	1.87 \pm 0.03	2.00 \pm 0.08		

harvested early (22 days for NS6 and 13 days DPBC1) produced more DNA than cells that were harvested at later stages of growth (36 days for NS6 and 23 days for DPBC1) (Fig. 3; Table 2). For NS6, when cells were harvested 14 days earlier, grinding for 30 s produced nearly threefold more DNA (164.12 ng/mg) compared to cells that were harvested late (42.19 ng/mg), and extending the grinding duration to 120 s produced approximately 2.4-fold more DNA (247.01 ng/mg) compared to cells that were harvested late (71.75 ng/mg). When compared to cells that were harvested late, early harvest with grinding produced roughly two- to threefold more DNA for *Scenedesmus* sp. NS6.

However, for *Scenedesmus* sp. DPBC1, when cells were harvested early, physical lysis was no longer a prerequisite to obtain decent amounts of DNA compared to *Scenedesmus* sp. NS6. As depicted in Fig. 3, inversion alone produced nearly threefold more DNA (73.1 ng/mg) for cells harvested early compared to cells harvested late (16.85 ng/mg), and this amount is comparable to DNA obtained by grinding of old cells for 30–90 s (70–75 ng/mg). The addition of slightly

more shear forces (pipetting for 30 \times) to cells harvested early produced approximately 50% more DNA per mg of cells, while for older cells, pipetting or even vortexing with sand has no significant effect ($p > 0.05$) (Fig. 2). For *Scenedesmus* sp. DPBC1 harvested late, 30 s of grinding would suffice to produce reasonable amounts of DNA, as there is no significant difference ($p > 0.05$) between 30, 60 and 90 s of grinding (Fig. 3; Table 2). In contrast, pipetting, vortexing and grinding had no significant effect ($p > 0.05$) on the yield of DNA from *Chlamydomonas* sp. BM3 and *Choricystis* sp. NPA14 (Fig. 3; Table 2), suggesting that either the degree of cell lysis was nearly complete for all lysis conditions presented here, or a more effective cell disruption method such as grinding in LiN2 or beadbeating is probably required to further lyse the cells.

The difference in ease of extraction of DNA from both *Scenedesmus* strains in our study could likely be due to variations in cell wall composition brought about by changes in culture conditions, such as decreased light penetration, nitrogen depletion and pH shift as the culture age. Studies on the cell wall composition of microalgae, such as

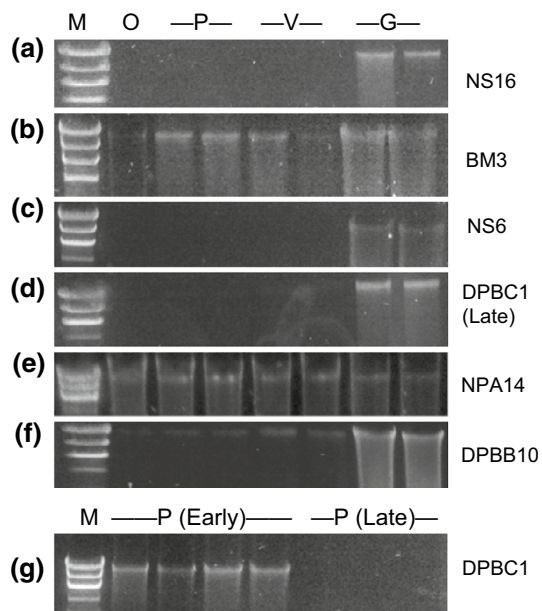


Fig. 1 Agarose gel electrophoresis of genomic DNA extracted from different strains of microalgae in the preliminary study. **a** *M. griffithii* NS16; **b** *Chlamydomonas* sp. BM3; **c** *Scenedesmus* sp. NS6; **d** *Scenedesmus* sp. DPBC1 (late harvest); **e** *Choricystis* sp. NPA14; **f** *Acutodesmus* sp. DPBB10 and **g** *Scenedesmus* sp. DPBC1 (early/late harvest). M: λ HindIII marker, O: inversion, P: pipette for 60 s, V: vortex for 60 s, G: grinding for 60 s

Chlorella and *Chlamydomonas*, noted high variability in cell wall composition, even in replicate cultures (Gerken et al. 2013), while depletion of nutrients such as nitrogen was reported to increase cell wall thickness and cell size in *Chlamydomonas* (Van Donk et al. 1997), *Nannochloropsis* (Yap et al. 2016; Jeong et al. 2017) and *Chlorococcum* (Yap et al. 2016).

We are uncertain if the phase at which cells were harvested had a similar effect towards DNA yield for the remaining strains, including DPBB10 (*Acutodesmus* sp.) which belongs to the same family as *Scenedesmus*, although we presume that it is very likely. Harvesting of microalgae cells in the early stages of growth, e.g., log phase, may probably be the better choice for downstream applications requiring intact DNA, given that DNA does not change over different phases of growth, unlike RNA whose composition varies depending on environmental factors. For RNA extractions, grinding may or may not be necessary depending on the growth phase/time at which RNA/gene expression is to be studied. For *Acutodesmus* sp. DPBB10, the highest yield of DNA was obtained by grinding for 60 s (164.75 ng/mg), which is approximately 6.5-fold higher than control (21.9 ng/mg) ($p < 0.001$). However, there is no significant difference between 15, 30, and 60 s of grinding ($p > 0.05$), thus 15 s of grinding would suffice to obtain a reasonable amount of DNA from this strain.

Overall, the DNA obtained in this study is of reasonable integrity, with minimal smearing for all strains (Figs. 1, 4). The $A_{260/280}$ values obtained in this study range from 1.57 to 2.19 (Table 2), which indicates minimal contamination with contaminants that absorb at 280 nm such as proteins. On the other hand, $A_{260/230}$ values range from 0.83 to 2.57. Some strains (NS6, DPBC1, BM3 and NPA14) appear to produce better $A_{260/230}$ values (Table 2), and apparently the time at which cells were harvested also seems to influence the $A_{260/230}$ values. This is noticeable for DPBC1, where early harvesting produced desirable $A_{260/230}$ values (2.17–2.22) but late harvesting did not (1.00–1.42). The accumulation of storage products such as carbohydrates as growth declines at the late-log/stationary phase is likely the reason for the low $A_{260/230}$ values (Van Donk et al. 1997). This phenomenon could also be strain dependent as the $A_{260/230}$ values for *Scenedesmus* sp. NS6 were similar regardless of the harvesting period (Table 2). Although a number of strains (NS16, DPBB10, DPBC1) did not produce desirable $A_{260/230}$ values (Table 2), this did not pose a problem for PCR, as amplification could be carried out even at high (undiluted) concentrations of template DNA (≥ 150 ng) for all strains used in this study (data not shown), although polysaccharides are known PCR inhibitors (Sipahioglu et al. 2006).

Physical lysis methods previously described for RNA isolation from microalgae usually involve beadbeating with glass/zirconia beads coupled with freezing and thawing in liquid nitrogen (Kim et al. 2012). To assess the usefulness/possibility of our lysis method for RNA extraction, we tested this lysis method on two strains of microalgae, *M. griffithii* NS16 and *Mychonastes* sp. NPD7. Immediately after harvesting, cells were ground for 60–90 s in DEPC-treated sea sand in a -20 °C chiller rack (ViPlus chiller, Vivantis) to minimize possible degradation of RNA. High-quality RNA was obtained from both strains, as indicated by the intensity ratio of the 28S and 18S rRNA and the presence of smaller RNA species (Fig. 5). To further evaluate RNA quality, reverse transcription was performed using gene-specific primers spanning the intron–exon junctions of the 18S rRNA gene. The absence of intron-containing amplification products indicated that the RNA from both strains was pure and sufficiently free from genomic DNA contamination (data not shown). The precise location of intron–exon junctions of the 18 s rRNA gene for both *M. griffithii* NS16 and *Mychonastes* sp. NPD7 can be found in Genbank under the accession numbers KP162147 and KP202155, respectively.

To our knowledge, the use of sea sand to facilitate nucleic acid extraction is limited to only a handful of organisms, namely seaweed (*Gracilaria changii*) (total RNA, mortar and pestle with liquid nitrogen) (Chan et al. 2004), date palm leaves (mortar and pestle) (Arif et al. 2010) and fungi (*Pythium myriotylum*) (vortexing) (Wang and Chang 2003). The effectiveness of sea sand used in this study could

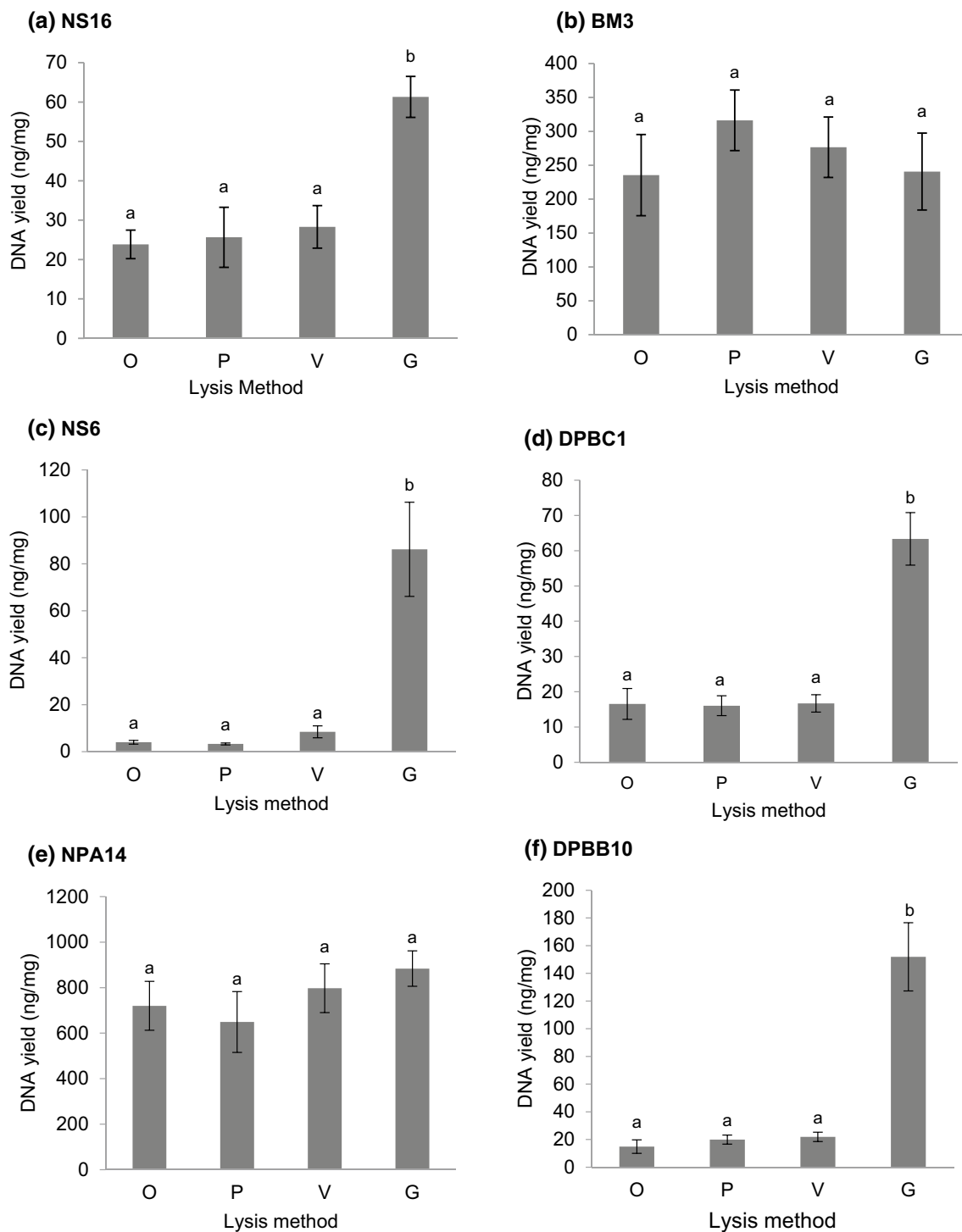


Fig. 2 Effect of lysis method on the yield of DNA from different strains of microalgae in the preliminary study. Error bars correspond to standard error of the mean. Different letters indicate values are significantly different ($p < 0.05$) (Tukey–Kramer). **a** *M. griffithii* NS16

($n_h = 6.7$), **b** *Chlamydomonas* sp. BM3 ($n=5$), **c** *Scenedesmus* sp. NS6 ($n_h = 6.9$), **d** *Scenedesmus* sp. DPBC1 ($n_h = 11$), **e** *Choricystis* sp. NPA14 ($n=5$), **f** *Acutodesmus* sp. DPBB10 ($n_h = 8.5$). O: inversion, P: pipette for 60 s, V: vortex for 60 s, G: grinding for 60 s

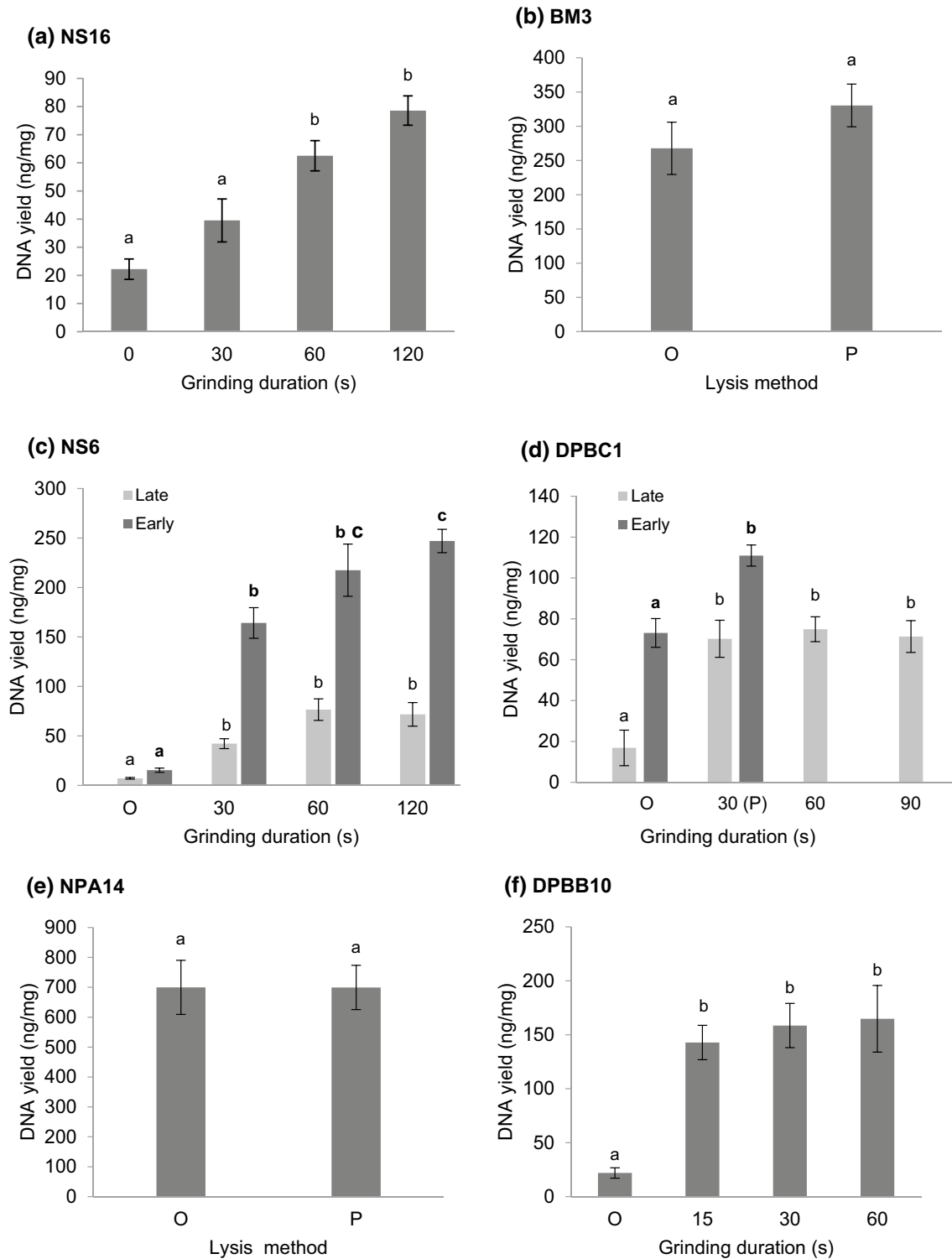


Fig. 3 Effect of grinding duration and lysis method on the yield of DNA from different strains of microalgae in the optimization study. Error bars correspond to standard error of the mean. Different letters indicate values are significantly different ($p < 0.05$) (Tukey–Kramer/*t* test). **a** *M. griffithii* NS16 ($n_h = 6.7$), **b** *Chlamydomonas* sp. BM3

($n = 5$), **c** *Scenedesmus* sp. NS6 (late/early harvest) ($n_h = 5.3$), **d** *Scenedesmus* sp. DPBC1 (late/early harvest) ($n_h = 5.8$), **e** *Choricystis* sp. NPA14 ($n = 5$), **f** *Acutodesmus* sp. DPBB10 ($n_h = 7.0$). O: inversion, P: pipette for 30×

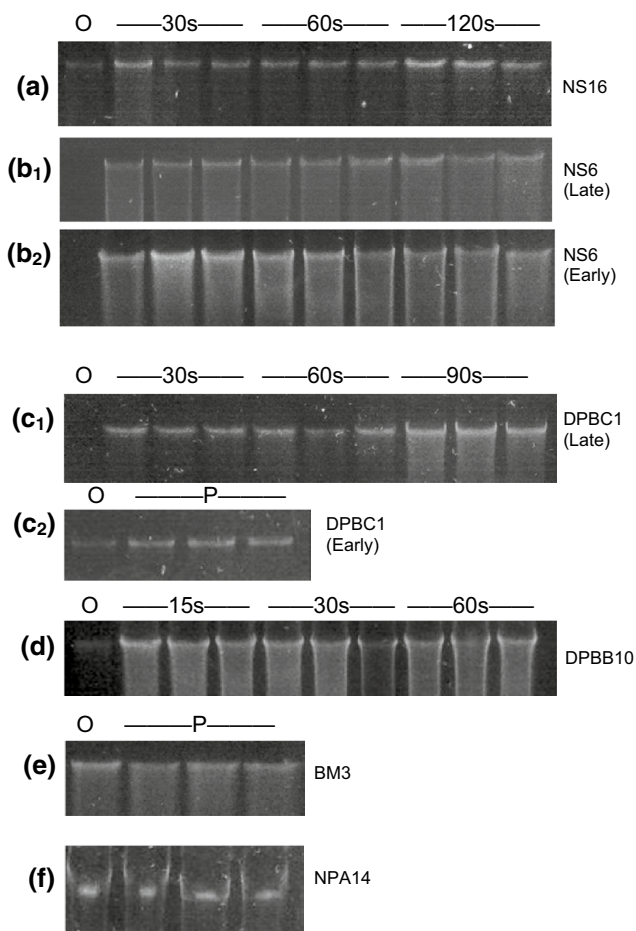


Fig. 4 Agarose gel electrophoresis of genomic DNA extracted from different strains of microalgae in the optimization study. **a** *M. griffithii* NS16; **b**_{1–2} *Scenedesmus* sp. NS6 (late/early harvest); **c**_{1–2} *Scenedesmus* sp. DPBC1 (late/early harvest); **d** *Acutodesmus* sp. DPBB10; **e** *Chlamydomonas* sp. BM3; **f** *Choricystis* sp. NPA14. O: inversion, P: pipette for 30×

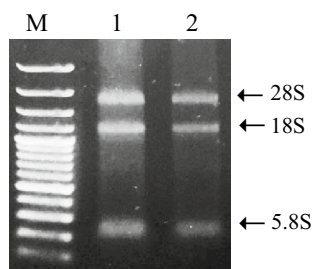


Fig. 5 Agarose gel electrophoresis of total RNA from *M. griffithii* NS16 (Lane 1) and *Mychonastes* sp. NPD7 (Lane 2) extracted using the CTAB–LiCl method with grinding for 60–90 s. M: 100bp+ ladder

be attributed to its small size (ca. 0.1–0.3 mm), which is similar to glass beads used in beadbeating of microalgae (0.1–0.6 mm) (Kim et al. 2012) and coupled with the rapid rotating and compressing action of the pestle.

The lysis method described here is cheap, where the only equipment required is a cordless drill (Skil 2212) which can be purchased at local hardware stores for approximately MYR 180 (USD 42.6) and plastic pellet pestles. If a pellet pestle is not readily available, an easily obtainable alternative would be 1-mL micropipette tips which have been flame-blunted to snugly fit the bottom of the microcentrifuge tube. We found that the performance of flame-blunted pipette tips was comparable to pestles (data not shown), although the initial volume of buffer used during grinding should be less than 500 μ L, ideally 200–300 μ L to prevent spilling over. If desired, pellet pestles can be washed, sterilized with 70% ethanol and reused several times (~ten times) before wearing out from friction.

Conclusion

The physical lysis method presented here is simple and does not require expensive equipments and reagents such as beadbeaters, homogenizers and liquid nitrogen for DNA and RNA extraction from microalgae. We believe that this lysis procedure could be used in combination with commercially available extraction kits and shorter nucleic acid extraction protocols to further facilitate cost-effective and efficient DNA and RNA extraction from microalgae.

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Author contributions WY—conceptualized the idea, designed the experiments, supervised the work and drafted the manuscript; RAK—assisted in WY in drafting of the manuscript and analysis of data; LML—conducted all the DNA extractions; BK—assisted WY in drafting part of the manuscript; YSL—conducted part of the preliminary experiment and assisted in formatting of the manuscript; HYC—conducted the RNA extractions, RT-PCR and identification of two of the microalgal strains (*M. griffithii* NS16 and *Mychonastes* sp. NPD7). All authors reviewed the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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