Inclusion of 2-Mercaptoethanol in Lysis Buffer Could Interfere with Isolation of High Molecular Weight DNA from Freshwater Microalgae

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Abstract 2-mercaptoethanol (2-ME), alongside polyvinylpyrrolidone is commonly used in plant DNA extractions to deal with polyphenols, which could interfere with extraction and downstream applications. 2-ME is also commonly used to denature proteins and nucleases, especially RNases. On the contrary, we found that the presence of 2-ME in lysis buffer interfered with DNA extraction from 12 strains of freshwater microalgae, resulting in DNA with poor integrity. We also found that the TNES-urea buffer, commonly used for preservation and DNA extraction from fish, appears as effective as the SDS and CTAB buffer for some microalgae strains. Results from our study suggests that the inclusion of 2-ME in DNA extraction protocols may be detrimental for isolation of good quality DNA from freshwater microalgae, and therefore recommend eliminating it or testing varying concentrations of 2-ME when developing species-specific extraction protocols for microalgae.

Keywords 2-Mercaptoethanol · DNA extraction · Microalgae · Lysis buffer · TNES-urea

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Bioprospecting for microalgae strains with biotechnological potential is currently an ongoing endeavor. Molecular methods such as PCR amplification of ribosomal genes have become a common procedure for identification of microalgae. Other molecular methods such as AFLP, RFLP and southern blotting are also commonly used in phyco-logical studies [1–3]. Isolation of intact, high molecular weight DNA is often a prerequisite for these molecular methods.

Reports on DNA extraction from microalgae often employ commercially available kits [4, 5], although traditional methods are still frequently used, especially in laboratories with financial constraints and for large number of extractions. Traditional methods such as the cetyltrimethyl ammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) method and its modifications, often include chemicals such as polyvinylpyrrolidone (PVP), dithioerythritol (DTT) and 2-mercaptoethanol (2-ME) to deal with contaminants such as polyphenols, which may interfere with extraction and downstream applications. PVP works by absorbing polyphenols through formation of hydrogen bonds, whereas 2-ME prevents oxidation and polymerization of polyphenols [6]. 2-ME is also used to denature proteins, and is often used in RNA extraction to inhibit RNase activity [7].

In a preliminary study conducted on *Chlorella sorokiniana* NS5, we found that high molecular weight DNA with good integrity could only be obtained using the TNES-urea buffer. This buffer is commonly used for preservation and DNA extraction from fish, mollusk and animal tissue [8, 9]. We shortly discovered that excluding 2-ME from the standard SDS/CTAB lysis buffer yielded DNA of good integrity from *C. sorokiniana*. We find this phenomenon intriguing, as 2-ME, acting as an antioxidant and nuclease inhibitor should not interfere with DNA

extraction. To further confirm our findings, we subsequently tested the effects of 2-ME on 11 different microalgae strains, as presented here in this study.

Axenic cultures of microalgae were used to inoculate 1.5 L of Bold's Basal Medium (BBM) in 2.5 L Erlenmeyer flasks as described [10]. The accession numbers and GPS coordinates of microalgae strains used in this study is presented in Table 1. Approximately 100 mg (wet weight) of cells were harvested from log phase cultures by centrifugation. The cell pellets were washed once with MiliQ water and stored at -20°C . Cells were lysed in 0.5 mL of CTAB, SDS and TNES-urea buffer, with or without 2-ME (Cat.# M3148, Sigma). Individual components of the CTAB, SDS and TNES-urea buffer are listed in Table 2. Next, the mixture was incubated at 65°C for 1 h with mixing. RNA was removed by adding 3 μL of RNase A (10 mg mL^{-1}). After that, DNA was purified by a single chloroform:isoamyl alcohol (24:1) extraction, followed by precipitation with 1 vol of isopropanol and washing with 70% (v/v) ethanol. The DNA pellet was air dried and reconstituted in 50 μL TE buffer (pH 8.0). DNA was separated on 0.8% (w/v) agarose containing 1X RedSafe[®] (Intron Biotech). Images were captured using a smartphone camera under a UV transluminator.

We found that the presence of 2-ME in the lysis buffer resulted in poorer DNA integrity and/or yield for all 12 strains of microalgae (Fig. 1). This negative effect is very prominent in some strains (BMAC5 and DPAD5), in which the presence of 2-ME resulted in fully degraded DNA (Fig. 1h, i). For other strains (DPAA16 and DPBC1), this negative effect was subtle (Fig. 1b–j). The negative effects of 2-ME is also evident when added to TNES-urea buffer (Fig. 1k, l). The TNES-urea buffer, originally formulated by Asahida et al. [8] is generally used for preservation and DNA extraction from animal tissue. In some microalgae

Table 2 Individual components of the CTAB, SDS and TNES-urea buffer

Component	Final concentration		
	SDS	CTAB	TNES-urea
Tris–Cl (pH 8.0)	100 mM	100 mM	10 mM
EDTA (pH 8.0)	50 mM	20 mM	10 mM
NaCl	500 mM	1.4 M	125 mM
SDS	2% (w/v)	–	0.5% (w/v)
CTAB	–	2% (w/v)	–
Urea ^a	–	–	4 M
2-ME ^a	1% (v/v)	0.2% (v/v)	–
Polyvinpyrrolidone (PVP) ^a	–	1% (w/v)	–

^aAdded just before use

strains such as NS5, DPAD5 and DPAA16, the TNES-urea buffer appears to be as effective as SDS or CTAB buffer (without 2-ME) (Fig. 1), despite having a lower concentration of Tris, EDTA, NaCl and SDS (Table 2). To our knowledge, there are no reports on the use of TNES-urea buffer for DNA extraction from microalgae.

A brief summary of various microalgae DNA extraction methods, which may or may not include 2-ME (0.1–2%) is presented in the supplementary table (Table S1). We are unsure if the observations from our study apply only to our microalgae strains, as we have not found any reports specifically addressing this phenomenon. Although a number of reports do include 2-ME in their lysis buffer, especially as part of the CTAB buffer, these reports have mainly focused on PCR amplification for strain identification or phylogenetic studies [11, 12]. Thus, gel images of genomic DNA are often not provided. While a number of reports do provide gel images of genomic DNA, many of

Table 1 Accession numbers and GPS coordinates of microalgae strains used in this study

Microalgae strain	Genbank accession	GPS coordinates
<i>Monoraphidium griffithii</i> NS16	KP162147.1	2.8336N, 101.7636E
<i>Chlamydomonas</i> sp. BM3	KT720479.1	2.8919N, 101.7919E
<i>Scenedesmus</i> sp. NS6	KT720478.1	2.8336N, 101.7636E
<i>Scenedesmus</i> sp. DPBC1	KF680451.1	2.8092N, 101.7633E
<i>Choricystis</i> sp. NPA14	MG012356	2.8174N, 101.7686E
<i>Acutodesmus</i> sp. DPBB10	KY315602	2.8092N, 101.7633E
<i>Chlorella sorokiniana</i> NS5	KM502980.1	2.8336N, 101.7636E
<i>M. minutum</i> DPAD5	KY315601	2.8092N, 101.7633E
<i>Scenedesmus</i> sp. BMAC5	KY315600	2.8919N, 101.7919E
<i>Scenedesmus</i> sp. NS9-2	MF780733	2.8336N, 101.7636E
<i>M. litorale</i> DPBA5 ^a	–	2.8092N, 101.7633E
DPAA16 (unidentified)	–	2.8092N, 101.7633E

^aTentative identification based on cell morphology

Fig. 1 Agarose gel electrophoresis of genomic DNA extracted from different strains of microalgae with or without 2-ME. **a** *Chlamydomonas* sp. BM3, **b** *Scenedesmus* sp. DPBC1, **c** *Choricystis* sp. NPA14, **d** *Scenedesmus* sp. NS6, **e** *M. griffithii* NS16, **f** *Acutodesmus* sp. DPBB10, **g** *Chlorella sorokiniana* NS5, **h** *M. minutum* DPAD5,

i *Scenedesmus* sp. BMAC5, **j** *M. litorale* DPBA5, **k** *Scenedesmus* sp. NS9-2 and **l** DPAA16 (unidentified). M: λ HindIII marker/100 bp ladder, SDS: SDS extraction buffer, CTAB CTAB extraction buffer, TNES TNES-urea buffer, + β /– β with/without 2-ME

which indicate good quality DNA (Table S1), no comparison was made between the absence and presence of 2-ME. Hence, we are unable to reliably draw conclusions.

Findings from other research that is most relevant to ours, was from a study conducted by Jagielsky et al. [13] on the pathogenic microalgae *Prototheca wickerhamii*. A comparison was made between four traditional methods and two commercial kits. 2-ME was only present in the kit's lysis buffer (as per manufacturer's protocol). They found that good quality DNA was only obtained for SDS + Triton-x buffer (glass beads) and CTAB buffer (LiN₂), both which do not contain 2-ME. Although not discussed in their report, we think that 2-ME is likely the culprit for the failure of both kits to produce DNA. Failure to obtain reasonable amounts of DNA for the remaining methods could be attributed to inefficient cell lysis (Table S1).

In contrast, good quality DNA was obtained from *C. vulgaris* and *C. minutissima* with the DTAB method and 20 mM (0.12% v/v) 2-ME, although no comparison was made with extractions without 2-ME [14]. However, results from our study indicated otherwise for *C. sorokiniana* (Fig. 1g). We think that this may be due to biochemical differences between species, differences in lysis buffer composition (e.g. CTAB vs DTAB) or the higher concentration of 2-ME used in our study (0.2 vs 0.12%). This is further reflected in strains such as DPAA16, where the effects of 2-ME is obvious for TNES-urea buffer, but not for SDS (Fig. 1i).

We suspect that there might be interactions between 2-ME and certain metabolites released from the algal cells during lysis (which may be strain/species dependant), as well as with components in the lysis buffer, leading to the production of reactants which may cause DNA degradation. Metabolites such as neocarzinostatin (NCS), a peptide from *Streptomyces carzinostaticus*, was reported to cause extensive DNA degradation in the presence of 2-ME. It was proposed that 2-ME converts NCS to an unstable active form which degrades DNA, by reduction of disulphite bridges [15]. Similar findings has been reported for bleomycin, a glycopeptide from *S. verticillus* [16].

Further investigation is warranted to determine metabolites specific to microalgae and its interactions with 2-ME, which may lead to DNA degradation. The effects of varying concentrations of 2-ME as well as individual components in the lysis buffer will be tested in future experiments. Based on the findings from our study, we suggest eliminating or testing different concentrations of 2-ME when developing or optimizing DNA extraction methods for new microalgae strains, as 2-ME could likely interfere with isolation of good quality DNA from microalgae.

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Authors' Contributions W.Yee—conceptualized the idea, designed the experiments, supervised the work and drafted the manuscript; J.N.Kumar and P.D.Muthusamy—assisted in W.Yee in drafting of the manuscript. All authors reviewed the manuscript.

Compliance with Ethical Standards

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

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