Genes Genet. Syst. (2022) **97**, p. 247–256

Optimization of nucleic acid extraction and amplification of a thiamine biosynthesis gene fragment from selected Malaysian seaweeds

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(Received 12 July 2022, accepted 4 August 2022; J-STAGE Advance published date: 11 January 2023)

Obtaining high-quality nucleic acid extracted from seaweeds is notoriously difficult due to contamination with polysaccharides and polyphenolic compounds after cell disruption. Specific methods need to be employed for RNA isolation in different seaweed species, and therefore studies of the thiamine biosynthesis pathway have been limited. Two selected Malaysian species which are highly abundant and underutilized, namely *Gracilaria* sp. and *Padina* sp., representing the red and brown seaweeds, respectively, were collected to develop optimized total RNA extraction methods. Prior to that, DNA was extracted, and amplification of the 18S rRNA gene and the THIC gene (encoding the first enzyme in the pyrimidine branch of the thiamine biosynthesis pathway) from the DNA template was successful in *Gracilaria* sp. only. RNA was then extracted from both seaweeds using three different existing methods, with some modifications, using cetyltrimethylammonium bromide, guanidine thiocyanate and sodium dodecyl sulphate. Methods I and III proved to be efficient for *Padina* sp. and *Gracilaria* sp., respectively, for the extraction of highly purified RNA, with A_{260}/A_{280} values of 2.0 and 1.8. However, amplification of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase and the THIC gene was successful in only *Gracilaria* sp. cDNA derived from extracted RNA. Further modifications are required to improve the exploitation of nucleic acids from brown seaweeds, which has been proven to be difficult. This work should pave the way for molecular studies of seaweeds generally and for the elucidation, specifically, of the thiamine biosynthesis pathway.

Key words: DNA extraction, seaweed, thiamine, vitamin B1

INTRODUCTION

Seaweeds are plant-like organisms that can be found growing abundantly in Malaysia, especially on the west coast of Peninsular Malaysia and East Malaysia. The most diverse genera of Rhodophyta and Phaeophyta found in Port Dickson are *Gracilaria* (red seaweed) and *Padina* (brown seaweed), respectively (Asmida et al., 2017). In Malaysia, *Gracilaria* sp. is an indigenous agarophyte seaweed (Phang, 1994) that has good agar properties and can adapt to harsh mangrove conditions (Phang et al., 1996). It is cultivated worldwide for commercial food-

Edited by Koji Murai
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DOI: https://doi.org/10.1266/ggs.22-00088

grade agar to overcome the lack of *Gelidium* (Bixler and Porse, 2011). Meanwhile, *Padina* sp. is one of the main raw materials used for alginate production in Asian countries and has become important in terms of economy and ecology (Kaladharan and Kaliaperumal, 1999). The metabolites and bioactive compounds of these seaweeds such as polyphenols, alginate, carrageenan and vitamins (Morrow et al., 2011; Machado et al., 2014) have advanced applications in the food, pharmaceuticals (Cao et al., 2016) and textiles industries (Mahmood and Siddique, 2010). These seaweeds are widely eaten and cooked as marine vegetables as they are rich in fibre, minerals, proteins (Norziah and Ching, 2000) and vitamins (A, B, C, E) (Sethi, 2012).

Thiamine (vitamin B1) is essential as a dietary supplement for most animals. Deficiency of thiamine in human may lead to impairment of thiamine pyrophosphate-dependent enzymes involved in central metabolic processes like the TCA cycle and the pentose phosphate pathway (Belanger et al., 1995; Tang et al., 2006; Croft et al., 2007), and is associated with cardiovascular disease (Thornalley et al., 2007), beriberi (Fattal-Valevski, 2011) and neurological and psychiatric disorders (Dhir et al., 2019). Interestingly, the thiamine content in seaweeds has been shown to be comparable to that in other food sources, and seaweeds thus are an excellent option for thiamine (Sánchez-Machado et al., 2004; MacArtain et al., 2007). These studies have noted that the thiamine content in seaweed is higher than that reported in cooked vegetables (cauliflower, cabbage) and adequate for recommended daily intakes. Recent data have suggested that boosting thiamine content can increase resistance to stresses not only in humans but also in plants (Tian et al., 2016). The expression of thiamine biosynthesis genes in oil palm (*Elaeis guineensis*) reportedly contributes to biotic and abiotic stress resistance (Yusof et al., 2015; Abidin et al., 2016; Yee et al., 2016; Kamarudin et al., 2017a, 2017b; Rahman et al., 2017).

The lack of research on the identification of genes coding for enzymes involved in the thiamine biosynthesis pathway may be because a nucleic acid extraction method for seaweeds remains unestablished. Extraction of high-quality nucleic acids is one of the crucial steps prior to mRNA isolation and other molecular biological experiments. Therefore, the extracted nucleic acids must be free from any contamination by polysaccharides, polyphenols, proteins and lipids (Buckingham, 2019). However, as seaweeds contain cellulose, polysaccharides, sulphated fucans and phenolic compounds (Vreeland et al., 1998; Michel et al., 2010), the isolation of RNA has been problematic due to its similarity in structure and reaction with other compounds (Chan et al., 2004; Generalić Mekinić et al., 2019). These compounds usually co-precipitate with nucleic acids during the extraction process (Wang et al., 2008). In addition, RNA should be isolated carefully as it has a very short half-life and easily degrades after being extracted from cells (Buckingham, 2019).

For this reason, specific methods are required for successful RNA isolation from different seaweed species and for the same species grown in different environments (Hong et al., 1997; Gehrig et al., 2000). Although the best method for RNA extraction from brown seaweeds uses cetyltrimethylammonium bromide (CTAB) extraction buffer and selective concentration by lithium chloride precipitation (Pearson et al., 2006), the composition of polysaccharides and polyphenolics varies in each seaweed species. Moreover, localization or the origin of the seaweeds may also affect the RNA extraction efficiency due to different environmental conditions that yield variable seaweed composition. This project was carried out to optimize the DNA and RNA extraction method for *Gracilaria* sp. and *Padina* sp., and hence to amplify a fragment of the phosphomethylpyrimidine synthase (THIC) gene, which is one of the key enzymes in the thiamine biosynthesis pathway.

RESULTS AND DISCUSSION

Collection of seaweed The seaweeds used in this study, *Gracilaria* sp. and *Padina* sp., were collected on the basis of their abundance in the selected area (Fig. 1). Phaeophyta are found growing in the mid-tidal zone with high light intensity, while Rhodophyta prefer subtidal areas with low light intensity (Asmida et al., 2017). According to Othman et al. (2018), changes in species richness are related to the frequency of sampling, water physicochemical parameters (temperature and pH) and symbiotic associations. However, the abundance of seaweed species in Malaysia has yet to be fully described.

Optimization of DNA extraction procedure DNA was extracted from collected seaweed species using a Qiagen Plant Mini Kit with a slight modification of the supplier's protocol. Modification is required as seaweeds are known to contain viscous polysaccharides, which can make the extraction process complicated and time-consuming. Steps were taken to optimize DNA extraction in which samples were ground until a white powder appeared. After buffer AP1 was added to 0.5 g sample in a 1.5-ml microcentrifuge tube, the mixture was vortexed for a few seconds until the powder was evenly dispersed. Instead of breaking down the RNA present in the samples using enzymes, which may be inhibited by the viscous polysaccharides, this study utilizes heat which will disrupt the RNA and may further lyse the polysaccharides. The samples were incubated in a water bath at 65 °C for 10 min, during which they were inverted every 2 min.

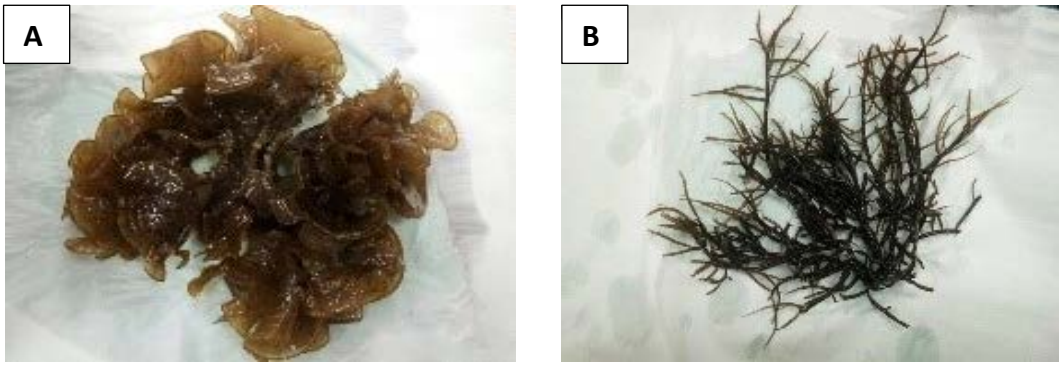

Fig. 1. Seaweed species collected in Port Dickson: *Gracilaria* sp. (A) and *Padina* sp. (B).

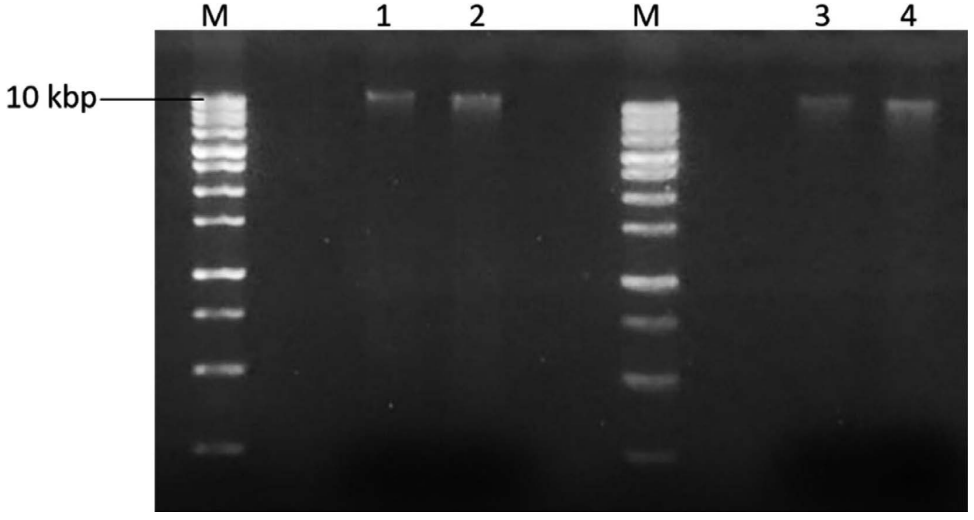

Fig. 2. Gel electrophoresis of extracted DNA from *Gracilaria* sp. and *Padina* sp. Lanes M represent a DNA size marker (1 Kb DNA Ladder, Bioline). Lanes 1 and 2 represent DNA extracted from *Gracilaria* sp. while lanes 3 and 4 represent DNA extracted from *Padina* sp.

Analysis of nucleic acid integrity by gel electrophoresis Extracted DNA was visualized as bands that appeared on gel electrophoresis (Fig. 2). Extracted DNA was analysed further by determining the purity and concentration of nucleic acid using a NanoDrop Spectrophotometer. Carrying out DNA quantification and quality analyses is crucial before proceeding with polymerase chain reaction (PCR). The ratios A_{260}/A_{280} and A_{260}/A_{230} were calculated to measure the freedom of nucleic acids from protein contamination and to determine the content of organic contaminants, respectively (Logemann et al., 1987; Manickavelu et al., 2007). As summarized in Table 1, DNA concentrations of *Gracilaria* sp. and *Padina* sp. were 240 and 243 µg/µl, which is adequate for PCR reaction. In addition, the 260/280 nm absorbance ratios of 2.11 and 2.12 for *Gracilaria* sp. and *Padina* sp., respectively, showed that the purity of the extracted DNA was high, without proteins and phenols. Before proceeding with amplification of transcripts of the thiamine biosynthesis gene THIC, molecular identification of each species had to be conducted. As shown in Fig. 3, 18S rRNA gene fragments were successfully amplified from *Gracilaria* sp. (900 bp). THIC is the first enzyme in the pyrimidine branch of the thiamine biosynthesis pathway. Amplification of THIC from *Gracilaria* sp. genomic DNA (390 bp) is shown in Fig. 4 with a range of annealing temperatures from 53 to 57 °C.

Table 1. DNA concentrations obtained from the optimized extraction method

Seaweed	Concentration (µg/µl)	$(A_{260}-A_{320})/(A_{230}-A_{320})$	$(A_{260}-A_{320})/(A_{280}-A_{320})$
Gracilaria sp.	240.22	2.16	2.11
Padina sp.	242.51	1.6	2.12

Comparison of RNA extraction methods In this study, three different extraction methods were tested to find the most suitable method for RNA extraction from selected Malaysian seaweeds. The presence of polyphe-

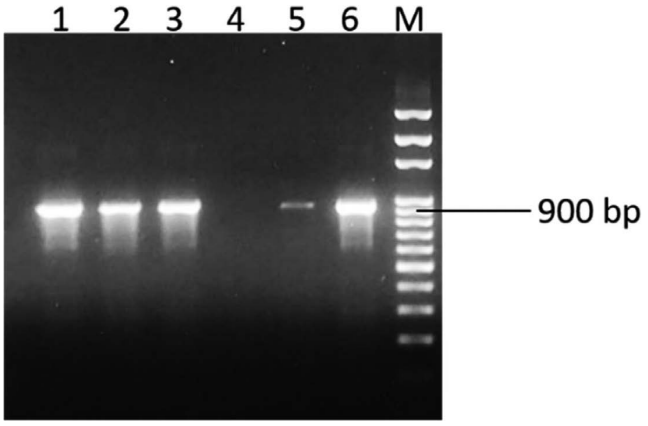

Fig. 3. Gel electrophoresis image shows amplification of an 18S rRNA gene fragment by PCR from *Gracilaria* sp. (900 bp). Lane M represents a 100-bp DNA ladder (Bioline). Lanes 1, 2 and 3 (replicate 1) and lanes 4, 5 and 6 (replicate 2) represent the annealing temperatures of 53, 55 and 57 °C, respectively, for *Gracilaria* sp. DNA.

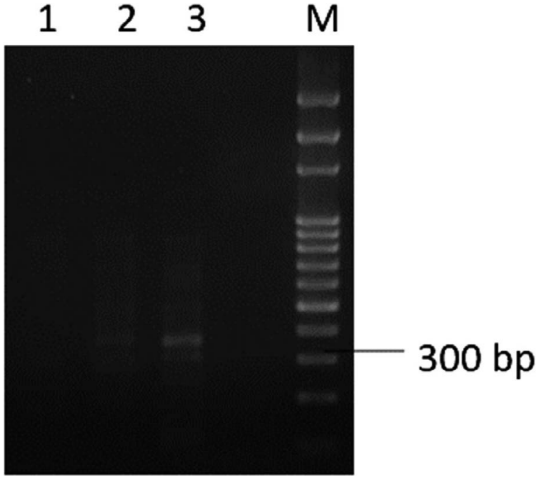

Fig. 4. Gel image shows gradient PCR for amplification of the THIC gene fragment (300 bp) from *Gracilaria* sp. Lane M represents a 100-bp DNA ladder. Lanes 1, 2 and 3 represent the annealing temperatures 53, 55 and 57 °C with *Gracilaria* sp. DNA.

nolic compounds and polysaccharides in seaweeds had been proven to affect the quality and quantity of RNA extracted from seaweeds (Saunders, 1993). For this reason, the stability of the ionic strength of the extraction buffer used is important to ensure that nucleic acid extraction and cell disruption are effective. Low ionic strength allows the polysaccharides to bind and precipitate with RNA. In contrast, cell disruption becomes more difficult due to decreasing protein solubility if the ionic strength is too high.

In method I, and in agreement with previous studies (Murray and Thompson, 1980; Coyer et al., 1995), CTAB gave a high-quality result for RNA extraction in brown seaweed and removed the contaminants by solubilizing the plant cell wall and lipid membranes and by denaturing proteins. The addition of dithiothreitol (DTT, 50 mM) to the extraction buffer is important to ensure that the nucleic acid is fully solubilized and that nuclease activity is inhibited by disrupting disulphide bond formation (Pearson et al., 2006). DTT acts as an enzyme-stabilizing and reducing agent by preventing oxidation reactions in the extraction buffer (Apt et al., 1995; Netto and Stadtman, 1996; Getz et al., 1999). Thus, it disrupts membranes and releases nucleic acid. The use of NaCl along with CTAB and lithium chloride as precipitation agent in the extraction buffer can give the best result in protein denaturation and nucleic acid co-precipitation. However, only RNA from *Padina* sp. was extracted with a good result when we used this method.

For method II, guanidine thiocyanate, sodium citrate and sarkosyl (N-lauroyl sarcosine or sarcosine) were used to denature protein molecules and polysaccharides in order to release the nucleic acid. β-Mercaptoethanol and sarkosyl are widely used for nucleic acid extraction from seaweeds (Hong et al., 1995; Phillips et al., 2001). Guanidine thiocyanate is a chaotropic agent in the extraction buffer that acts as a denaturing agent towards protein molecules. The use of an extraction buffer containing guanidine salt gives the best result for RNA extraction from plants (Falcão et al., 2008). Absolute ethanol was used for RNA precipitation because RNA is insoluble in alcohol. Proteins come out of solution after the addition of alcohol and centrifugation. A white precipitate forms immediately after the addition of ethanol if the RNA concentration in the sample is sufficiently high. However, the quality of RNA obtained from both *Gracilaria* sp. and *Padina* sp. extracted using this method was inadequately efficient as the ratios of A_{260}/A_{280} readings in *Gracilaria* sp. and in *Padina* sp. were 1.7 and 1.6, respectively (Table 2). The ratio for extracted total RNA should be between 1.8 and 2.2 to ensure that the RNA is pure. Alginate and cellulose in the cell walls may also affect the quality of RNA extracted. In addition, higher phenolic and polysaccharide compounds in the tissues also affect the quality of RNA obtained.

Method III utilizes sodium dodecyl sulphate (SDS) to extract nucleic acids from seaweeds. SDS is a chaotropic agent, like guanidinium thiocyanate, and is involved in destroying hydrogen bonds in water structure (Farrell, 1993). It dissolves membranes, disrupts protein and nucleic acid interaction, and inactivates ribonuclease (Pawlowski et al., 1994; Matthews et al., 2000). Once solubilized, protein is completely denatured after the addition of phenol–chloroform and becomes insoluble in aqueous solution (Rio et al., 2010). Phenol–chloroform plays a crucial role in promoting cell lysis, and DNA and protein separation, while β-mercaptoethanol acts as an antioxidant that aids RNase and oxidation protection. Cell disruption occurs when the hydrophobic

effects decrease after the destruction of the water structure and this leads to the unfolding and dissociation of protein molecules (Jousson et al., 1998). Meanwhile, any enzymatic activity requiring divalent cations as cofactors is inhibited and ionic strength suppressed by EDTA, which is used as an extraction buffer and inhibits RNase activity by binding to Mg^{2+} and Ca^{2+} ions. SDS is widely used for RNA extraction from other species of red algae, namely *Carpopeltis*, *Rhodymenia*, *Rhabdonia* (Saunders, 1993) and *Gracilaria chilensis* (Meneses and Santelices, 1999). In this method, isopropanol and NaCl were used to precipitate the RNA from the solution. The ionic strength needed for RNA precipitation was contributed by NaCl because this neutral salt has monovalent ions. In the present work, RNA from both red and brown seaweeds was successfully extracted using method III, with A_{260}/A_{280} absorbance ratios of 1.81 and 1.78, respectively (Table 2). In addition, SDS solubilization and phenol extraction are routinely used to deproteinize RNA in biological material. The integrity of the nucleic acid in both *Gracilaria* sp. and *Padina* sp. was examined on agarose gels, and Fig. 5 shows the bands obtained from both seaweeds using all three methods. The 18S and 28S ribosomal RNA bands show that the RNA is intact.

Table 2. RNA concentrations obtained from three optimized extraction methods

Seaweed	Method	Concentration (ng/μl)	A_{260}/A_{280}
Gracilaria sp.	Method I	55.72	1.700
	Method II	32.50	1.700
	Method III	134.00	1.811
Padina sp.	Method I	296.69	1.998
	Method II	165.26	1.647
	Method III	272.09	1.777

RNA quantification and quality assessment The determination of total RNA concentration is important before the conversion of RNA to cDNA. The conversion to cDNA requires an appropriate standardized amount of total RNA to ensure a standard concentration of cDNA used for PCR. Besides that, the purity of RNA is important because impurities will disturb the amplification reaction, and this will lead to false results. In this study, the concentration and purity of total RNA were determined spectrophotometrically by measuring absorbance at 260 and 280 nm. The quantity and quality of extracted RNA are shown in Table 2. The ratios of A_{260}/A_{280} for method III in *Gracilaria* sp. and method I in *Padina* sp. were 1.8 and 2.0, which indicates that the RNA was highly purified. The values obtained indicate that RNA samples were separated excellently from phenolics, proteins, polysaccharides and other compounds using these methods. Although method III could also be considered suitable to extract pure RNA from *Padina* sp., the quality of RNA may be low. Thus, it can be concluded that method III is more suitable to extract pure RNA from *Gracilaria* sp. while method I is optimal for *Padina* sp.

Amplification of GAPDH and THIC gene transcripts The transcripts of two genes of interest, GAPDH

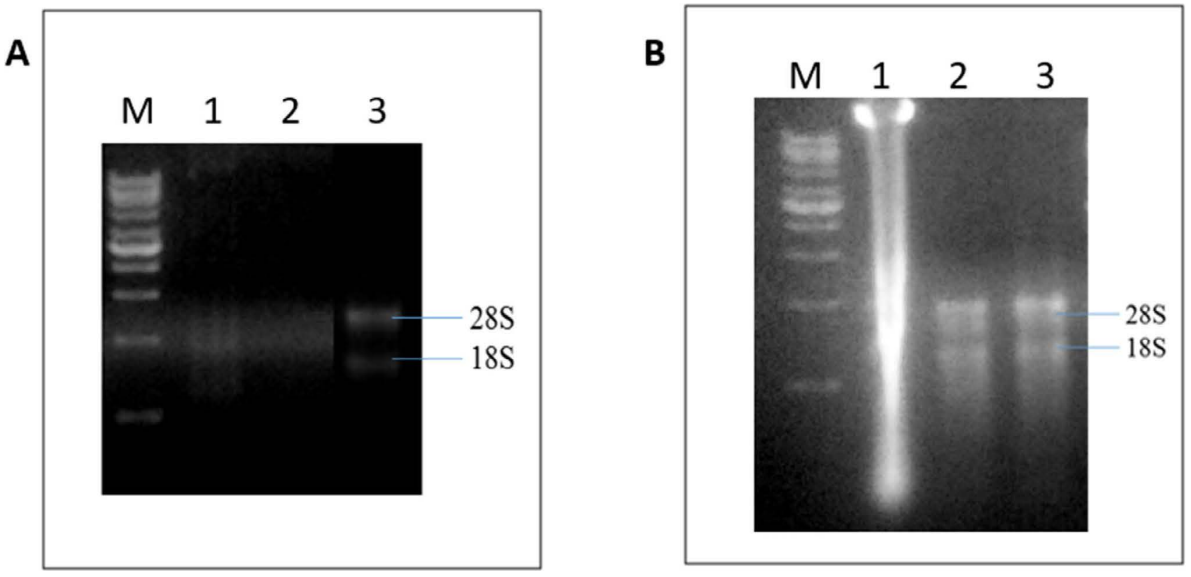

Fig. 5. Total RNA extraction by three different methods for *Gracilaria* sp. (A) and *Padina* sp. (B). Double bands indicate two ribosomal RNA subunits, 18S and 28S. Lane M represents a DNA size marker (1 Kb DNA Ladder). Lanes 1, 2 and 3 represent the RNA extracted using method II, method I and method III, respectively.

and THIC, were amplified after the conversion of RNA to cDNA. GAPDH is considered as a housekeeping gene because it is involved in the maintenance of basic cellular function and expressed in all cells of an organism under any given condition, while THIC is a gene encoding the enzyme phosphomethylpyrimidine synthase. We attempted to amplify fragments of these two genes from both seaweeds; however, they were only successfully amplified from *Gracilaria* sp. The optimum annealing temperature for each primer designed was determined using gradient PCR (Figs. 6 and 7). The annealing temperatures used for PCR ranged from 50 °C to 60 °C. Fig-

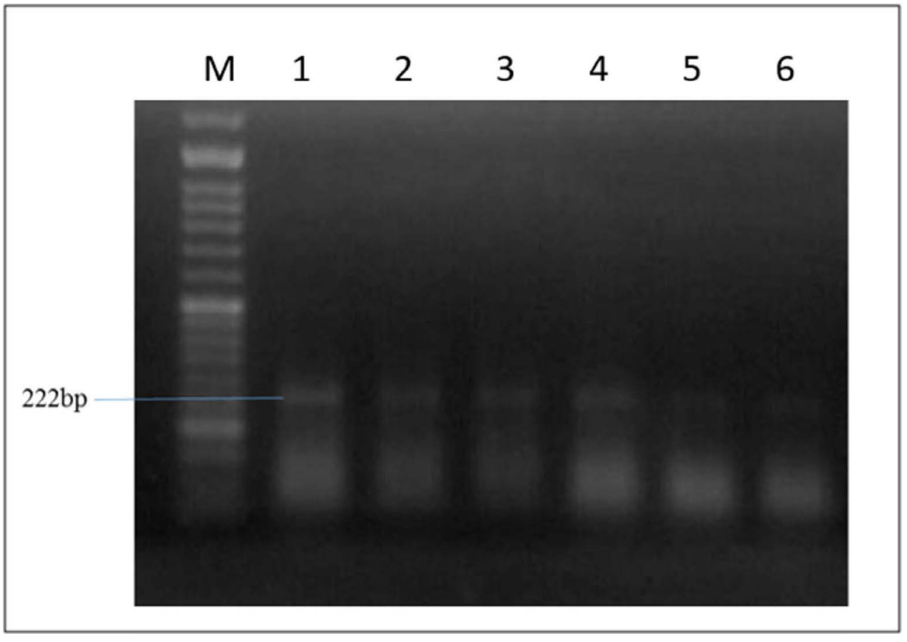

Fig. 6. Gel image shows gradient PCR for GAPDH gene fragment amplification from *Gracilaria* sp. Lane M represents a DNA size marker (100-bp DNA ladder). Lanes 1, 2, 3, 4, 5 and 6 represent the annealing temperatures of 50.0, 51.9, 53.8, 56.1, 58.0 and 60.0 °C, respectively.

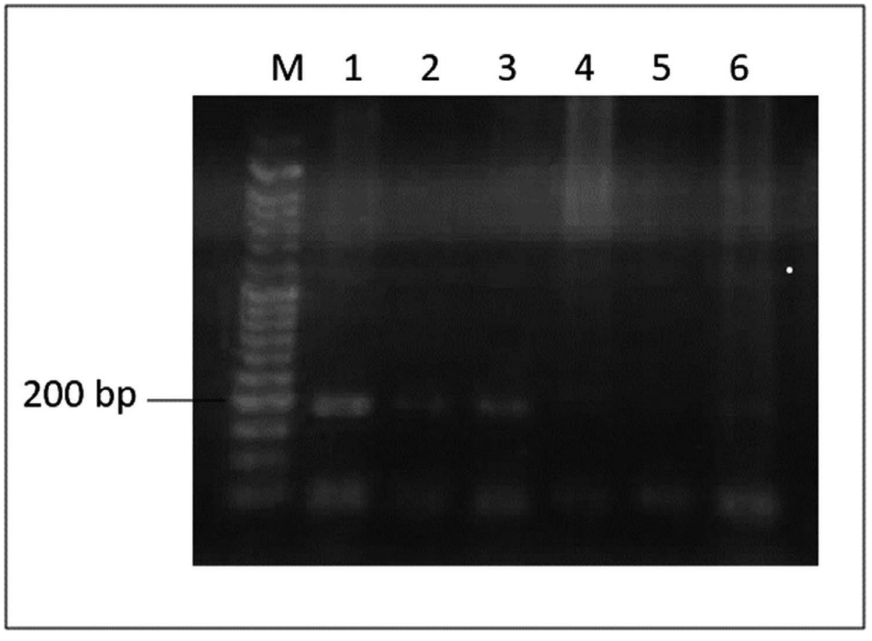

Fig. 7. Gel image shows gradient PCR for THIC gene fragment amplification from *Gracilaria* sp. Lane M represents a DNA size marker (50 bp DNA ladder, Bioline). Lanes 1, 2, 3, 4, 5 and 6 represent the annealing temperatures of 50.0, 51.9, 53.8, 56.1, 58.0 and 60.0 °C, respectively.

ures 6 and 7 show that the amplification of both GAPDH and THIC gene fragments from *Gracilaria* sp. was successful at 50 °C.

MATERIALS AND METHODS

Sampling *Gracilaria* sp. and *Padina* sp. were collected at Tanjung Tuan and Tanjung Biru at Teluk Kemang, Port Dickson, Malaysia. Collected samples were cleaned using tap water to remove sand, debris and epiphytes, and immediately frozen in liquid nitrogen before being kept at –80 °C. The collection, cleaning and storage of the samples were carried out within the same day to avoid RNA degradation.

DNA extraction Total DNA was extracted using a Qiagen DNeasy Plant Mini Kit (Germany) with some modifications. Each seaweed sample was ground in liquid nitrogen to a fine white powder using a pre-chilled sterile pestle and mortar. The powdered sample (0.5 g) was transferred to a 2-ml microcentrifuge tube, and 400 µl of AP1 buffer was added. The mixture was vortexed for a complete mix and incubated at 65 °C for 10 min. The tube was inverted every 2 min of incubation time. The following procedures were as recommended by the manufacturer. The extract was stored at –20 °C until further use.

RNA extraction Three methods were selected and modified to be used in this study. Method I is adopted from Sim et al. (2013), method II from Falcão et al. (2008) and method III from Li and Trick (2005). The components of each extraction buffer used in all methods are listed in Table 3.

Table 3. Extraction buffer components

Method	Buffer components
Method I	100 mM Tris-HCl (pH 8.0)
	2 M NaCl
	20 mM EDTA
	2% CTAB (w/v)
	50 mM DTT
Method II	6.5 M guanidine thiocyanate
	100 mM Tris-HCl (pH 8.0)
	0.1 M sodium citrate (pH 5.5)
	0.1 M β-mercaptoethanol
	2% sarkosyl
Method III	100 mM Tris-HCl (pH 8.0)
	1.5% SDS
	150 mM EDTA
	50 µl β-mercaptoethanol

Method I: Approximately 0.5 g of finely ground frozen seaweed tissue was added to 1 ml of CTAB extraction buffer (100 mM Tris-HCl (pH 8.0), 2 M NaCl, 20 mM EDTA and 2% CTAB (w/v)) and 50 mM DTT. Six silica beads were added and the suspension was vortexed for 5 min, mixed vigorously after the addition of isoamyl alcohol:chloroform (24:1), and centrifuged for 5 min at 12,000 rpm. One millilitre of supernatant was transferred to a new tube and 1 ml of absolute ethanol was added and mixed. The mixture was incubated at –20 °C for 1 h and then centrifuged at 12,000 rpm for 10 min, after which the supernatant was discarded. The RNA pellet was recovered by centrifugation, washed with ice-cold 75% ethanol, and air-dried for 10 min. It was then dissolved in 30 µl of autoclaved distilled water and stored at –80 °C until further use.

Method II: Approximately 0.5 g of ground frozen seaweed tissue was added to 1 ml of extraction buffer (6.5 M guanidine thiocyanate, 100 mM Tris-HCl (pH 8.0), 0.1 M sodium citrate (pH 5.5), 0.1 M β-mercaptoethanol and 2% sarkosyl). Six silica beads were added and the suspension was vortexed for 3 min and then incubated on ice for 5 min. Isoamyl alcohol:chloroform (1 ml) was added to the mixture, which was shaken for 15 s and then centrifuged at 12,000 rpm at 4 °C for 5 min. One millilitre of the aqueous phase was transferred to a new tube and 1 ml of absolute ethanol was added. The extracted RNA was allowed to precipitate at –20 °C for 1 h. It was then centrifuged at 12,000 rpm at 4 °C for 5 min, washed with 800 µl of 75% of ethanol (v/v), and air-dried for 10 min. A total of 30 µl autoclaved distilled water was used to dissolve the RNA pellet, and the preparation was stored at –80 °C until further use.

Method III: Approximately 0.5 g of ground frozen seaweed tissue was added to 800 µl of extraction buffer (100 mM Tris-HCl (pH 8.0), 1.5% SDS, 150 mM EDTA) and 50 µl of β-mercaptoethanol. Six silica beads were added and the suspension was vortexed for 10 min, after which 500 µl of phenol:chloroform was added and vortexed for 3 min. The mixture was centrifuged for 5 min at 13,000 rpm. A total of 600 µl of supernatant was

Table 4. Primers designed for genes of interest amplification

Description	Primer name	Sequence (5′–3′)
18S rRNA (Rhodophyta)	18s_R_F	ATTAGAGTGTTCAAAGCAGGC
	18s_R_R	TCAGTTCATCTAGCTCTCCC
18S rRNA (Phaeophyta)	18s_B_F	CATTCTTCGGTTGCGTGTCG
	18s_B_R	CATTCAATCGGTAGGTGCGA
THIC	SeaweedFTHIC	CATTGAACAAGCCGAGCAGG
	SeaweedRTHIC	TCCTCGCGAAACAATCCCAG
GAPDH	SeaweedFGAPDH	CATTGAACAAGCCGAGCAGG
	SeaweedRGAPDH	GGGGTGGGCACACGCAGAGC

transferred to a new tube, and 600 µl of isopropanol and 500 µl of 1.5 M NaCl were added. The mixture was then mixed carefully by inversion and incubated for 15 min on ice, centrifuged for 10 min at 13,000 rpm, and the supernatant was removed. The RNA pellet was washed with 500 µl of 75% ethanol and then centrifuged at 13,000 rpm for 10 min. The supernatant was discarded, and the pellet was air-dried for 10 min and dissolved in 30 µl of autoclaved distilled water. The RNA was stored at −80 °C until further use.

All extracted RNA was assessed for its quality and quantity using a NanoDrop spectrophotometer ND-2000 (Implen, Germany). The quality of the RNA was assessed using the formula below:

1) *Nucleic acid purity* $(A_{260}/A_{280}) = (A_{260} - A_{320}) / (A_{280} - A_{320})$
2) *Nucleic acid purity* $(A_{260}/A_{230}) = (A_{260} - A_{320}) / (A_{230} - A_{320})$

In order to improve the accuracy of the nucleic acid determination, all absorbance values were corrected against turbidity to detect other possible contaminants by deducting the A_{320} values.

Amplification of gene transcripts of interest Data mining of the nucleotide and amino acid sequences of the 18S rRNA and THIC genes was performed for different seaweed and microalga species for primer design. Table 4 shows the list of primers used for the amplification of gene fragments in this study.

PCR amplification 18S rRNA and THIC gene fragment sequences from DNA templates were amplified by PCR using exTEN 2X PCR Master Mix (1st BASE, Singapore) and a T100 thermal cycler (Bio-Rad, USA). The reaction conditions were: an initial denaturation at 95 °C for 4 min 30 s, followed by 34 cycles of denaturation (95 °C, 30 s), annealing (50–63 °C, 30 s) and extension (72 °C, 1 min), and a final extension (72 °C, 5 min). The final product was stored at –20 °C. Amplicon sizes were determined by agarose gel electrophoresis.

For the conversion of RNA to cDNA, a Tetro cDNA Synthesis Kit (Bioline, USA) was used for RT-PCR. The RT-PCR mixture was incubated in a thermocycler for 30 min at 45 °C, followed by 5 min at 85 °C, and hold at 4 °C. The mixture was then put on ice. The cDNA formed was then subjected to PCR using a MyTaq PCR Kit (Bioline). Amplification of the gene of interest was conducted using a thermocycler (Biometra, Germany). Cycling conditions of the PCR were: an initial denaturation for 1 min at 95 °C, followed by 30 cycles of denaturation for 45 s at 95 °C, gradient annealing at 56–61 °C (or the optimized temperature of the primer) for 45 s and extension for 1 min at 72 °C. A final extension step was set for 5 min at 72 °C and then hold at 4 °C. The PCR product was kept at –20 °C until further analysis.

CONCLUSIONS

Despite the challenges in nucleic acid extraction, DNA and RNA of good quality and integrity were successfully extracted from both *Gracilaria* sp. (red seaweed) and *Padina* sp. (brown seaweed) with minor modifications of previously published methods. For RNA extraction, we suggest that method I is the most suitable for *Padina* sp., while method III is preferable for *Gracilaria* sp. However, amplification of the THIC gene from extracted DNA was only successful for *Gracilaria* sp. For the amplification of GAPDH and THIC genes from cDNA derived from RNA extracted from both seaweeds, gene fragments were again only successfully amplified from *Gracilaria* sp. The inability to amplify gene fragments from *Padina* sp. may be due to the specificity of the primers used as well as the quality of the extracted nucleic acids. Our findings represent a preliminary study towards exploration of Malaysian seaweed, as many aspects of our procedures can be improved. Gene fragments of major enzymes, including THIC, and also of other enzymes involved in thiamine biosynthesis can be amplified by designing new species-specific primers. This will be a good starting platform for further studies on the genes involved in the biosynthesis of useful compounds produced by local Malaysian seaweed species including *Gracilaria* sp. and *Padina* sp. Further studies on other thiamine biosynthesis genes such as thiazole synthase, thiamine diphosphate kinase and thiamine monophosphatase will improve our understanding of the thiamine biosynthesis pathway and the regulation of thiamine production in seaweeds. In the future, the amount of thiamine and its intermediates in selected seaweed species should be quantified together with the gene expression of the biosynthesis pathway to obtain an understanding of its regulation and thiamine accumulation. This study should provide a basis for the elucidation of the thiamine biosynthesis pathway in Malaysian seaweed species so that these marine plants can be utilized to their full potential.

This project was supported by Skim Geran Penyelidikan Fundamental (FRGS), Kementerian Pendidikan Tinggi Malaysia (5524589); Geran Putra IPS Universiti Putra Malaysia (9511100 & 9515900); Science Fund Grant Ministry of Science, Technology and Innovation (MOSTI), Malaysian Government (5450799); Higher Institution Centre of Excellence (HICOE) Research Grant (Innovative Vaccines and Therapeutics against Fish Diseases) (Project No. 6369100); and SATREPS (JICA-JST): COSMOS-MOHE G4-B Research Grant (Microalgae for Sustainable Aquaculture Health: Microalgae Vaccine Delivery System) (Project No. 6300866).

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