SEQUENCE ANALYSIS OF 18s DNA OF *Melosira* sp., *Dunaliella* sp., *Isochrysis* sp. AND *Porphyridium* sp.

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ABSTRACT

Phytoplankton has high level of biodiversity. In previous years phytoplankton was identified by their morphological characters. However, their morphology might change in different environments. These difficulties can be overcome by comparing their 18S rDNA sequences. This research is aimed to verify the identity of *Melosira* sp., *Dunaliella* sp., *Isochrysis* sp. and *Porphyridium* sp. Here, PCR method was used to amplify 18s DNA sequences. Three primer pairs were used, i.e. 18S-F and 18S-R; 501F and 1700R; 18S-2F and 18S-2R. PCR products were sequenced. MEGA5 was used to make phylogenetic tree. Genus verification for *Isochrysis* sp., *Dunaliella* sp. and *Melosira* sp. were conducted successfully using Blast and phylogenetic tree. 18s DNA sequence of *Porphyridium* sp. shows an interesting result and needs further verification.

Keywords: Phytoplankton, *Melosira* sp., *Dunaliella* sp., *Isochrysis* sp., *Porphyridium* sp.

INTRODUCTION

Phenotypic based phytoplanton identification can be quite challenging since the algae phenotype can vary depending on the nutritional and environmental condition. Moreover, it is quite often that the phenotypic based classification do not reflect the phylogeny relationship. Various attempts have been made to change the classification so that the new classification reflects phylogeny relationship better (Woese et al., 1990; Cavalier-Smith, 1998). In order to have a classification that reflects phylogeny, efforts have been made to compare certain DNA sequences. DNA sequences that are often used to determine the phylogenetic relationship of an organism is a gene encoding 18S rRNA of eukaryotic organisms and genes encoding 16S rRNA for prokaryotic organisms (Rivas et al., 2004). Identification techniques using gene encoding 18S rRNA has been frequently performed in the molecular classification of phytoplankton (Suutari et al., 1990; Krienitz et al., 2001; Brouard et al., 2008).

MATERIALS AND METHODS

Materials

Microalgae culture (*Melosira* sp., *Dunaliella* sp., *Isochrysis* sp., *Porphyridium* sp.), vitamin B12, DNA kit (Puregene), isopropanol, ethanol 72%, oligonucleotide primers, agarose (Invitrogen), loading buffer (New England Biolabs), 1kb DNA ladder (New England Biolabs), tris acetate EDTA (Vivantis), sterile water, sterile seawater, taq polymerase (Kappa2G Fast), PCR mastermix, NPK, HCl, and NaOH.
Phytoplankton culturing

Microalgae were cultured in sterile water with 30 ppm salinity. 0.1% NPK and 0.01 % vitamin B12 were added to the culture. Microalgae were cultured in 1L flask, for 2 weeks, with 16 hours light and 8 hours dark, at 22oC.

DNA isolation

Microalgae culture was centrifuged at 13,000 g for 5 min and the supernatant was discarded. 300 mL lysis buffer is used to resuspend 50 mg pellet. The suspension was incubated for 1 hour at 65oC. 1.5 mL proteinase K was added and the mixture was incubated at 55oC for 1 hour. 1.5 mL Rnase was then added to the mixture and it was incubated at 37oC for 30 min, followed by the second incubation for 1 minute on ice. Then, 100 mL protein precipitation solution was added to the mixture, followed by vortex at max. speed for 20 sec. Sample was centrifuged at 16,000 g for 5 minutes. Supernatant was put into new tube and 300 mL isopropanol was added, followed by a centrifugation for 1 min at 16,000. Pellet was removed and diluted with 100 mL DNA hydration solution, followed by incubation at 65oC for 1 hour. Sample was stored in – 30oC.

Amplification of genes encoding 18S rRNA

18S rRNA gene was amplified using 3 different pairs of oligonucleotide primers. The first pair is 18S-F (5’-ACCTGGTTGATCCTGCCAG-3’) and 18S-R (5’-TGATCCTTGYGCAGGTTAC-3’) (Stay et al., 2001), the second primer pair is 501-F (5’-GGGTCTGGTTTTGAAATGAGG-3’) and 1700-R (5’-CCGAAGTCTTCAACGCACATC-3’), the third primer pair is 18S-2F (5’-TTCAAATTTCTGCCCTATCAACT-3’) and 18S-R (5’-ACTTCACCGGACCATCAAAT-3’).

Sequencing and phylogenetic tree construction

Amplified DNA samples were sequenced by sequencing company, 1st Base, Singapore. The sequences were alligned by ClustalW program (Larkin et al.,2007). Homologue sequences were searched using Blast in NCBI site. Phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis 5 (MEGA5) program (Tamura et al., 2011).

RESULTS AND DISCUSSION

Melosira sp.

DNA sample of Melosira sp. was amplified using 18S-F and 18S-R. Using gel electrophoresis, the sequence is around 2,000 bp and the constructed phylogenetic tree is shown in Figure 1.

The constructed phylogenetic tree showed that the analysed Melosira sp. is in the same cluster with Melosira dubia s0076 strain and Melosira cf. Octogona strain CCMP483 and therefore related to them. Blast analysis and the phylogenetic tree verified the genus, i.e. Melosira. To verify the species, it will require to analyse its Internal Transcribed Spacer (its) sequence.
DNA genome sample of *Dunaliella* sp. was amplified using 18S-F and 18S-R primer pairs. The amplicon is ± 750 bp using gel electrophoresis. The constructed phylogenetic tree is shown in Figure 2.

The constructed phylogenetic tree showed that the analysed *Dunaliella* sp. is closely related to *D. parva*, *D. tertiolecta* strain CCMP 1320, *D. bardawil* strain 12, *Dunaliella* sp. ABRINW-G3, *Dunaliella primolecta* strain UTEX LB 1000. Both Blast analyses and the constructed phylogenetic tree verified the genus of the analysed *Dunaliella* sp. sample. The species of the sample cannot be determined since the amplicon is only partial sequence, it

Figure 2. Multiple sequencing allignment was conducted using clustalW (Larkin *et al.*, 2007). Phylogenetic tree was constructed using MEGA5 software (Tamura *et al.*, 2011). The arrow indicates the analysed *Dunaliella* sp. sample
is ± 750 bp while the length of the gene encoding 18S rRNA is ± 1,800 bp (5). Alternatively, analysing Internal Transcribe Spacer 2 (ITS 2) can assist species determination (Chenet et al., 2001; Schultz et al., 2005; Lattès et al., 2011).

_Isochrysis sp._

DNA genome of _Isochrysis sp._ was amplified using 18S-F and 18S-R primer pair. The size of the amplicon is ± 1,800 bp. The following phenotypic tree was constructed using MEGA5 software (Figure 3).

![Phylogenetic tree of Isochrysis sp.](image)

**Figure 3.** Multiple sequencing alignment was conducted using clustalW (Larkin et al., 2007). The arrow indicates the analysed _Isochrysis sp._ sample

The constructed phylogenetic tree showed that _Isochrysis sp._ has close relation to _Isochrysis galbana_ strain DB, _Isochrysis sp._ zhangjiangensis, _Isochrysis sp._ strain 8701, _Isochrysis sp._ strain CCAP 027/14 dan _Isochrysis galbana_ strain AL. Both of Blast analyses and the constructed phylogenetic tree verified the genus of the analysed _Isochrysis sp._ sample.

_Porphyridium sp._

DNA sample of _Porphyridium sp._ was amplified using 18S-F and 18S-R. Using gel electrophoresis, the amplicon’s sequence is ± 2,000 bp and the constructed phylogenetic tree is shown in Figure 4

![Phylogenetic tree of Porphyridium sp.](image)

Blast analysis and the constructed philogenetic tree showed that the analysed _Porphyridium sp._ sample is closely related to _Tetraselmis sp._ and it is not in close relation to _Porphyridium aeruginum, Porphyridium purpureum_, dan _Porphyridium sordidum_. _Tetraselmis sp._ belongs to the phylum Chlorophyta and has similar phenotype to class Cholorophyceae and Prasinophyceae (Norris & Pearson, 1975), while _Porphyridium sp._ belongs to the phylum Rhodophyta (Schultz et al., 2005) that has different phenotype to
phylum Chlorophyta. Therefore, it is necessary to amplify the gene encoding the 18s rRNA of the analysed *Porphyridium* sp. sample with different primer pair to obtain longer sequence for further verification needs to be done. As an alternative, a comparison of rbcL gene sequences can be conducted to verify the sample’s identity.

Figure 4. Multiple sequencing allignment was conducted using clustalW (Larkin et al., 2007). Phylogenetic tree was constructed using MEGA5 software (Tamura et al., 2011). The arrow indicates the analysed *Porphyridium* sp. sample

**REFERENCES**


