

## 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 phytoplankton 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 (6). 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 22°C.

### 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 65°C. 1.5 mL proteinase K was added and the mixture was incubated at 55°C for 1 hour. 1.5 mL Rnase was then added to the mixture and it was incubated at 37°C 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 65°C for 1 hour. Sample was stored in – 30°C.

### 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'-TGATCCTTCYGCAGGTTAC-3') (Stay et al., 2001), the second primer pair is 501-F (5'-GGGTCTGGTTTTGAAATGAGG-3') and 1700-R (5'-CCGAAGTCTTCACCAGCACATC-3'), the third primer pair is 18S-2F (5'-TTCAAATTTCTGCCCTATCAACTT-3') and 18S-R (5'-ACTTCACCGGACCATCCAAT-3').

### Sequencing and phylogenetic tree construction

Amplified DNA samples were sequenced by sequencing company, 1<sup>st</sup> Base, Singapore. The sequences were aligned 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.

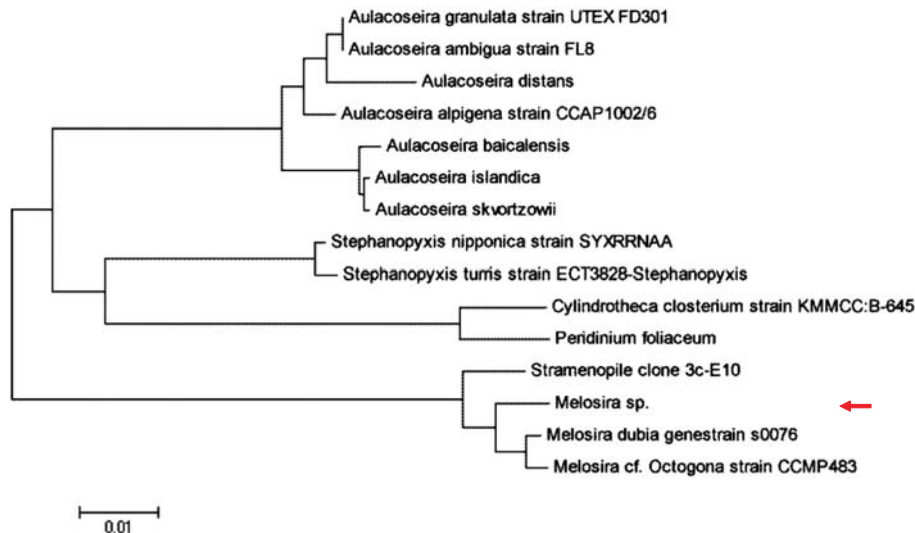


Figure 1. Multiple sequencing alignment was conducted using clustalW (Larkin *et al.*,2007). Phylogenetic tree was constructed using MEGA5 software (Tamura *et al.*, 2011). The arrow indicates the analysed *Melosira* sp. sample

***Dunaliella* sp.**

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. ABRIINW-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

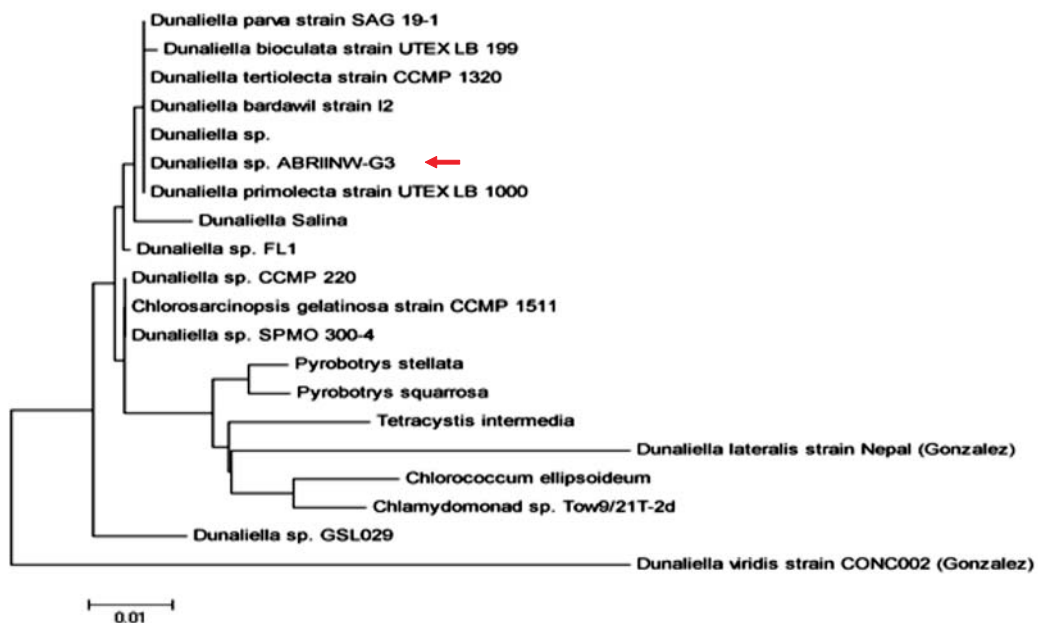


Figure 2. Multiple sequencing alignment was conducted using clustalW (Larkin *et al.*,2007). Phylogenetic tree was constructed using MEGA5 software (Tamura *et al.*, 2011). The arrow indicates the analysed *Dunaliella* sp. sample

is  $\pm 750$  bp while the length of the gene encoding 18S rRNA is  $\pm 1,800$  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  $\pm 1,800$  bp. The following phenotypic tree was constructed using MEGA5 software (Figure 3).

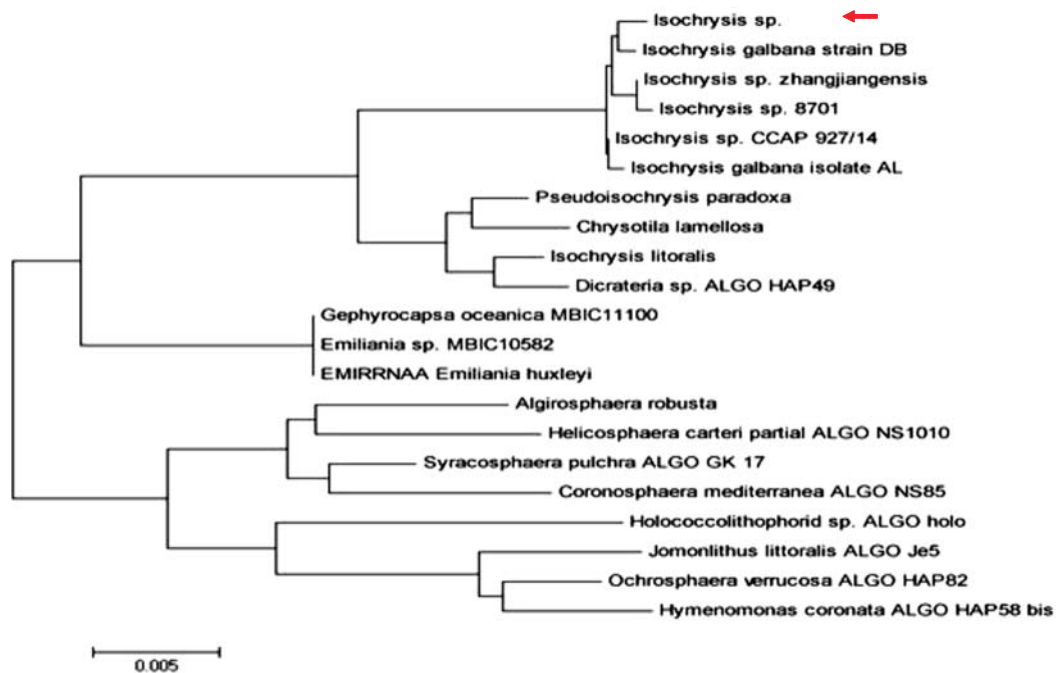


Figure 3. Multiple sequencing alignment was conducted using clustalW (Larkin *et al.*, 2007). The arrow indicates the analysed *Isochrysis* sp. sample

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  $\pm 2,000$  bp and the constructed phylogenetic tree is shown in Figure 4

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.

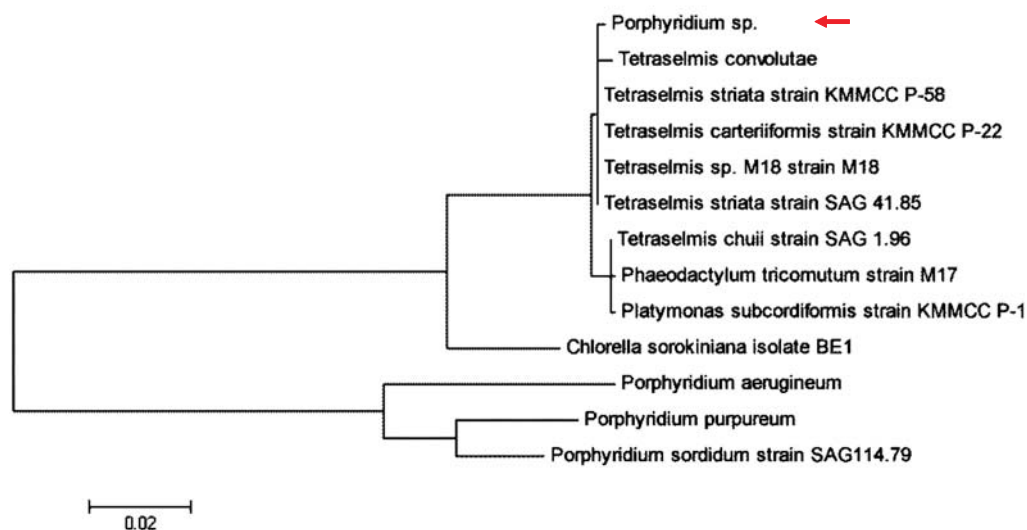


Figure 4. Multiple sequencing alignment was conducted using clustalW (Larkin *et al.*, 2007). Phylogenetic tree was constructed using MEGA5 software (Tamura *et al.*, 2011). The arrow indicates the analysed *Porphyridium* sp. sample

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