Observation, Isolation and Characterization of Microalgal Red Tide Agent Dinoflagellates *Prorocentrum* sp.

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Abstrak

Pengamatan, Isolasi dan Karakterisasi Agen Mikroalga Red Tide Dinoflagellata Prorocentrum sp.

Spesies Dinophyte mempunyai habitat dari kutub, perairan tropis, tetapi semakin berlimpah di perairan tropis atau hangat. Dinophyte diduga sebagai penyebab terjadinya "red tide" sehingga nampak berwarna kuning kemerahan di laut ketika malam hari disebabkan aktivitas bioluminescence. Penelitian menggunakan Dinophyte yang diisolasi dari pantai dan sampel air yang diperoleh dari di Prefektur Iwate, Jepang. Tujuan dari penelitian adalah untuk mengetahui taksonomi terutama ciri – ciri dari Dynophyte yang menyebabkan blooming. Spesies ini memiliki karakter yang spesifik berbentuk oval (panjang 20-30 µm dan lebar 1-20 µm), kloroplas berwarna kuning, nukleus yang besar, dua flagel yang berbeda, yang salah satunya disebut flagellum transfer, tidak memiliki selaput tengah yang, ornamen sel yang indah "theca"dengan tulang belakang. Berdasarkan hasil squensing pada 18 S rDNA, Dinophyte mempunyai kesamaan dengan strain Prorocentrum MBIC11147 (100%), Di masa yang akan datang penelitian Prorocentrum sp. bisa digunakan sebagai model squensing, perilaku mikroalga red tide

Kata kunci: alga, Dinophyte, karakterisasi, isolation, Prorocentrum, red tide

Abstract

Dinophyte species inhabit from polar, temperate to tropical waters, but tend to be more abundant in tropical or warm waters. The Dinophytes is suspected as one of the genera causing red tide in the sea with their yellow-redish colour that make the sea glows in the night because of their bioluminescence activity. In this work, the Dinophyte was isolated from offshore, and water sample collected in Iwate Prefecture, Japan. Purposes of the studies were for understanding the taxonomic features in particular of the dinophytes that usually occur in blooming areas. The species has specific characters, such as oval shape (20-30 µm long and 1-20 µm wide), yellow chloroplast, large nucleus, possesses two different flagellas which one of them is specific called transfer flagellum, no middle furrow and beautiful ornament cell covering (theca) with spine. Based on the partial sequencing of the 18 S rDNA, the Dinophyte is precisely same as the reference strain Prorocentrum MBIC11147 (100%), which was determined as Prorocentrum sp. In the future, this study could be uses as model of sequel behavior of the microalgal red tide.

Keywords: algae, Dinophyte, characterization, isolation, Prorocentrum, red tide

Introduction

Microalgae were recognized by their general characters of the pigment or color of organisms, storage of chemical products, photosynthesis ability, and cell walls (Bellinger and Sigee, 2013). Furthermore, they are classified based on the presence or absence of the flagellate cells, reserve polysaccharides and cell wall constituent. Base on those characters, life cycle and the endosymbiotic theory in the evolution process, the algae divided into 11 divisions (Cyanophyta, Prochlorophyta, Glaucophyta, Rhodophyta, Heterokontophyta, Haptophyta, Cryptophyta, Dinophyta, Euglenophyta, Chlorarachniophyta and Chlorophyta). Taxonomically, positions of the algae are in the Regnum Kingdom of eubacteria and eukaryote with one division of Cyanobacteria belong to the prokaryote and the other 10 divisions belongs to the eukaryote (Van De Hoek *et al.,* 1995).

The eukaryote Dinophytes division is consisting only one class, the Dinophyceae with 12 orders of Gymnodiniales, Gloeodiniales,

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Thoracospaerales, Phytiodiniales, Dinotrichales, Dinamoebidales, Noctilucales, Blastodiniales, Syndiniales, Peridinales, Dynophysiales and Prorocentrales. Most of the Dinophytes are living in the marine area which has major characters such as unicellular flagellate (only a few are coccoid or filamentous) and the flagellate cells have two dissimilar flagella (transfers flagellum and longitudinal flagellum). Most of them have transfer furrow, chloroplast with colors of brown, green, blue or red, although some of them do not have chloroplast called obligate heterotrophic, chlorophyll a and c, eyespot, a trichocysts on the surface of cell, there is armor cell cover (theca), possess a starch as a carbon storage, haplontic life cycle and the DNA is localized in small nodules scattered throughout the chloroplast (Taylor, 1987).

The Dinophytes have specific movement, gliding along forward and backward. In the water column, they move up and down, which is suspected as a response to the light. In the day they migrate to the surface of water and in the night they move down to several meters deep. On the contrary, some report says the movements of Dinophytes is not depend on the light response, as found in one of the species Ceratium which moves up and down in the water column for several days in complete darkness (Van *et al.,* 1995; Taylor, 1987).

It is assumed that movement of Dinophytes because of the response to temperature. This freedom of movement causing the dinophytes spread widely in the waters, especially marine area. Almost 90% of them are living in the sea. In fact, Dinophytes is abundance in the tropic and sub-tropic areas, but sometime they are also abundance in temperate area especially in the late spring and summer.

Dinophytes is suspected as agent of the sea red tide which is caused large number of enable fish killed and also the mortality other marine living organisms by poison of the toxin as byproduct of algal blooming. Actually, the algal blooming consist several type of algae, the Dynophytes occurred after the decline of diatom bloom and following by the toxic algae of the division heterokontophyte (Bartnicki and Valiyaveetil, 2008).

The red tide was widely known and studied, but there are still missing links on how to control it. Many factors are assumed to involve for instance, the deprivation of oxygen, physical factor (wind, wave, tidal current, temperature, pH and *etc.*), nitrification, nutrient and toxin formation (Taylor, 1974). Therefore, studying the behavior of algae and their life cycle are important in order to know their regulation in the waters column for controlling the sea bloom.. The aim of the study is to isolate and characterized the Dynophytes collected from Kamashi bay, Iwate, Japan.

Materials and Methods

Sampling

Microalgae were collected from the offshore waters, using planktonet 20 µm pore size in Kamaishi bay, Iwate Prefecture Japan at the end of autumn. Planktonet was washed 1-2 times before collecting the waters. The water samples were taken several times in order to obtain dense plankton. The sample were enrich in the selected medium of IMK-sea water in the plastic ice cream cup. One liter Daigo IMK (Nihon Pharmaceutical Co, Ltd) -sea water media contain 200 mg $NaNO_3$, 1.4 mg Na_2HPO_4, 5 mg K_2HPO_4, 2.68 NH_4Cl, 5.2 mg Fe-EDTA, 0.332 mg Mn-EDTA, 37.2 mg Na_2-EDTA, 0.0023 mg $ZnSO_4$-$7H_2O$, 0.014 mg $CoSO_4$-$7H_2O$, 0.00073 mg Na_2MoO_4-H_2O, 0.0025 mg $CuSO_4$-$5H_2O$, 0.0017 mg H_2SeO_3, 0.2 mg Thiamin HCl, 0.0015 mg Biotin, 0.0015 mg Vitamin B12, and 0.018 mg $MnCl_2$-$4H_2O$.

Isolation of algae by capillary micro-pipetting

Apparatus for capillary micro-pipetting procedures were prepared, including elongated Pasteur pipette with specific hole size and length, silicon slant, three hole slide glass, microscope and flat bottom 24-well-plates. The silicon slant was connected with the base of Pasteur pipette. The IMK-sea water medium was poured into 24-well-plates and three of the holes of the glass side. One droplet of sample was added into the first hole of glass slide. The presence and diversity of algae was observed under A light microscope. The target isolation Dinophytes was determined and sucked by the sharp tipped Pasteur pipette. The captured algae were blew into second hole of slide glass and observed under microscope. The single celled of Dinophyte was then sucked and blew into the third hole for wash. After sucking one more time, the single cell was blew and finally cultivated into one of the hole of 24 wells plate contain the media. The observation was done every day. Developing cells from a single Dinophyte were selected for collection and further experiments.

Microalgal cultures

The single cell of Prorocentrum was cultivated at IMK medium and gradually transferred into bigger scale of volume (10 mL-500 mL). The behavior and growth was observed from 500 mL culture series (triple replication) with cultivation time during 10 days. The growth was measured by cell multiplication in turbidity of 600 nm wavelength in

the spectrophotometer absorbance, everyday (Shimadzu UV Pharma Spec 1700, UV-Visible spectrophotometer). Reproduction process and cell movement was observed under inverted microscope. Storage food was conducted by determination of lipid, protein and total carbohydrate with methods of Bligh and Dryer (1959), Bradford assay (1976) and the phenol-sulphuric acid assay (Taylor, 1995), respectively.

Microalgal characters

Microalgae characters was carried out by serial cultivation which is necessary for observing the growth, cell shape, feature cells, chloroplast, structure cell, storage food, movement, and reproduction methods. Most of the activity was observed under the microscopes as described below; Light microscope. Microscope (Nikon, Tokyo, Japan), connected to a camera, was used for isolation and morphological observation. For documentation, 100 times magnification was used with immersion oil.

Inverted microscope. Inverted microscope (Olympus CKX41 SF, Japan) was used for observing the movement, flagella, cell structures and cell shape. Electron microscope. Scanning electron microscope Shimadzu JEOL , Tokyo, Japan, was used for cell architecture observations. A four step preparation was performed; fixation, dehydration, dying, and coating, following the standard method. Two ml culture of isolated Prorocentrum was apply with mounting shape in the filter paper and dried up in the room temperature and washed several times with purified water, after that dried up again under tungsten lamp in the room temperature. The dry filter paper containing cell was placed in the specific round aluminum probe with carbon tape and coating with ion Pt/Pd in the ion sputter chamber. The sample is then observed in the Scanning electron microscope.

Molecular analyses

The determination and identification of the species of the strain was conducted by molecular approach. The 18S rDNA method for identifying the strain was performed following Sekiguchi's protocol (2003). DNA extraction was done using Fast DNA-kit (Applied Biosystem). Ten ml culture was prepared, centrifuged and the cell pellet was collected. The cell pellet was applied for DNA extraction. The electrophoresis was performed in 1% Agarose gel with λHind III for marker and running for 30-40 min. The primer used was the general primers for nuclear –encoded 18S rDNA sequences (Two primers, 5'-TACCTGGTTGATCCTGCCAGTA-3' and 5'-ATTACCGCGGCTGCTGGCACC-3'). The PCR was conditioned for 30 cycles (94°C for 1 min, 55°C for 1 min, and 72°C for 2 min) using Progene C type. Purification of PCR product (1800 bp) was done using the QIAquick Gel Extraction Kit Protocol (Qiagens). The purified DNA product were reacted with sequencing reaction of cycle sequencing kit (BigDye® Terminator V3.1) and applied in the sequencer (Perkin-Elmer, Foster).

Result and Discusion

Morphological observation trough light microscope shows that cells of Dinophyte is approximately oval, with the anterior narrower than the posterior, thus often egg-shaped . The cell is laterally compressed and composed of two valves with a small cluster of eight periflagellar platelets. The right valve has an indentation where the periflagellar area is found. The valves have numerous pores, except in the central area, where a large pyrenoid is present beneath the valves. There is a row of marginal pores at the valve periphery. The valves are smooth, except for the pores, which is might be trichocysts. The nucleus is located in the posterior of the cell, and the species is photosynthetic, with a number of small brown chloroplasts. The cells varies considerably in size as well as shape: 25-58µm in length; 22-49 µm wide.

A phenotypic observations were resulting (1) the algal motility showed it is moving gliding with pulling flagella which are consist two flagella with one flagellum longer (transverse) than the other and have such as pointing spine, (2). The cell shape is ovoid, asymetrically, and have size more than 10 µm, (3). Chloroplast color is yellow-brown, and more than three (many), (4). There is a eyespot or stigma with yellow-red color at outside of the chloroplast, (5). The organel for ejectile structure is a tricocyst, and (6). the nucleous is large and conspicuous, called dinokaryon (Figure 1, 2 and 3).

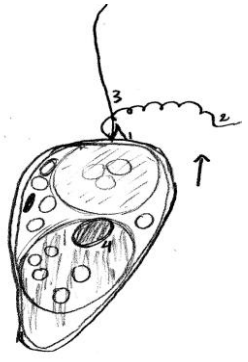

Figure 1. Ilustration of evaluated Prorocentrum under light microscope observation. *Notes:* 1; spine, 2; transfer, 3; flagella, 4; chloroplast.The Prorocentrum moved forward (arrow direction).

Figure 2. Light microscopy image of evaluated Prorocentrum.Notes: 1; small platelets, 2; yellow chloroplast, 3; spine (silica), 4; nucleus; Picture: magnified 100x, scale bar; 10 µm

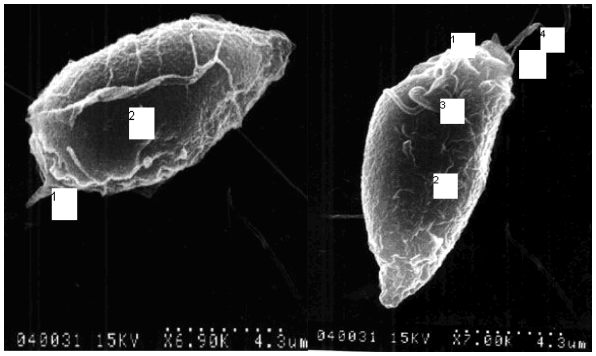

Figure 3. Scanning electron microscope (SEM) image of observed Prorocentrum sp. Notes: 1; pine, 2; cell cover, 3; transfer, 4; flagella

Storage product was dominated by starch, small amount protein and lipid (Figure 5). The starch content in the cell is around 50%, protein around 20% and lipid around 35% base on the dry weight cell, respectively.

Molecular analysis showed that the organism belong to the dynophyte which exactly description is *Prorocentrum* sp. Which is closed to Prorocentrum mican Eichdenberg as described in the phylogeny tree (Figure 8). It's come from the electrophoresis result which was exhibiting only single band mean the culture is single cell (Figure 6). The single band was cut and amplify using PCR method and the DNA assembly was analyses by NJ plot ways (Figure 7.).

The single cell of Prorocentrum was cultivated in sequences volume media starting from 10 mL into 500 mL. The growth in the IMK medium was very fast with duration cultivation within 3-5

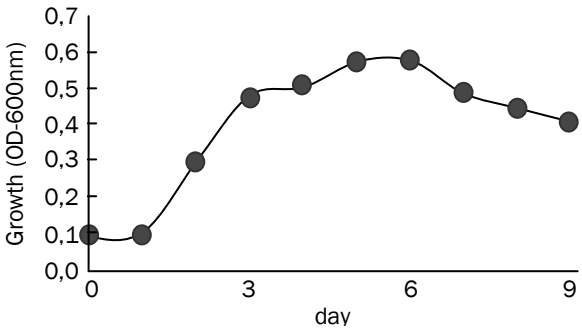

Figure 4. Growth Profile of *Prorocentrum* sp.

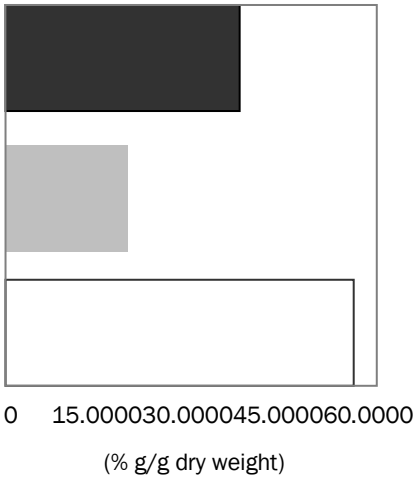

Figure 5. Storage Food Profile of Prorocentrum sp.

days has already achieve the stationary phase (Figure 4). Reproduction type during observation was through asexual reproduction.

Currently, Iwate Prefecture is one of the sources of fisheries Industry in Japan. The geographical data showed that Iwate faces the Pacific Ocean to the east with sheer, rocky cliffs along most of the shoreline interrupted by a few sandy beaches (Louis, 2002). In the past Iwate has been famous for its mineral wealth especially in the form of gold, iron, coal and sulfur but these are no longer produced. There is still an abundance of hot water for onsen or hot springs, which is the basis of a thriving industry. The forests of the prefecture are another valuable resource. Before World War II the forests were mainly composed of beech but since then there has been a huge swing towards the production of faster growing Japanese cedar. Recently, though, there has been a push to restore the original beech forests in some areas for fisheries industry. Iwate's industry is concentrated around Morioka and specializes in semiconductor and communications manufacturing. As of March 2011, the prefecture produced 3.9% of Japan's beef and 14.4% of broiler chickens. In 2009, 866 tons of

dolphins and whales were harvested off the coast of Iwate, accounting for more than half of Japan's total catch of 1,404 tons (Kyodo news, 2011). However, those fisheries yield often destroyed by suddenly dead of the fish causing by seabloom, therefore, the study of the behavior of one of the seabloom agent will be useful for predicting the fish harvesting or fishing, even how to avoid the bloom.

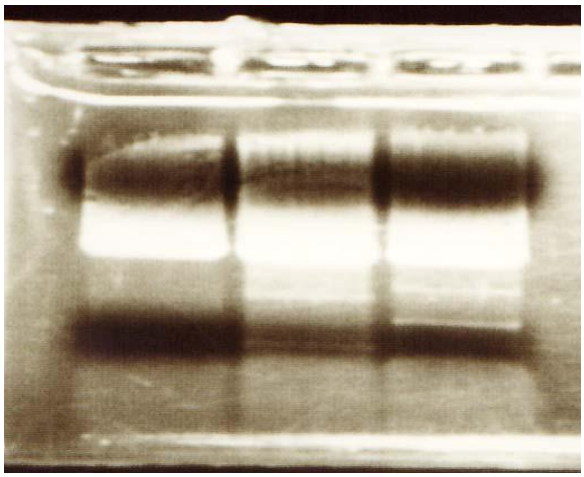

Figure 6. Electrophoresis result image of obtained band from extract Prorocentrum's DNA (Single Major Band)

Base on the growth observation of one of algal agents seabloom showed that Prorocentrum growing very fast or thriving in the suitable media, only three days needed for achieve stationary phase, and then stay at the state for 4-10 days before going to decline phase (Figure 1.). This result imply that the agent of red tide grow very fast in the suitable condition of nutrient. There are some other researches results that showed the waterbloom are going very fast because of the sufficient nutrient and raising of the water temperature, the effect of global warming. In common at temperate regions, only species from the genus Dinophysis are held responsible for shellfish poisoning that suspected some relation to the different ecological strategies of the two genera, namely the planktonic nature of Dinophysis versus the benthic/epiphytic nature of toxic Prorocentrum species. Further study showed that the threat of global warming has trigger the growth of benthic toxic microalgae in southern temperates waters, such as Prorocentrum. One of the strain Prorocentrum I066-01 is presented a mean growth rate of 0.49 divisions d(-1), not common in temperate strains, and only comparable with tropical strains. The parent toxins found were okadaic acid (OA) and dinophysistoxin-1 (DTX1). The major diol esters were D8- and D9- congeners of both OA and DTX1 (Vale *et al.,* 2009; Nagahama *et al*., 2011; Paerl and Paul, 2012).

Observation on the Prorocentrum cell composition showed that the cell consist about average 56% of carbohydrate, 13% of protein, 9% of lipid and others 22% base on dry weight (Figure 2). A Prorocentrum has wide range of cell composition, those proximates analyses result showed that prorocentrum maybe good agent for source of carbohydrates that can be used for energy substrate, such as for alcohol fermentation.

The identification and determination of microalgae

The capillary micro-pipetting technique is a conventional technique which depends on skill and knowledges of microalgal taxa. To isolate the Dinophytes using this technique is easy because Dinoflagellates are visible in big size and colorful. Their large size around 20-60 µm in length and 5-30 µm in width, specific movement with the flagella pulling the body and the transfer for drive the direction, yellowish color of chloroplast and large nucleus called dynokaryon are easily determined and compared to the other microalgal divisions. As the sampling time was taken at nearly winter, the collected Dinophytes are less diverse, because the Dinophytes favor warm waters. During our sampling we obtained only Prorocentrum and Gymnodinium species.

According to the theory of evolution of the division dinophyte, Prorocentrum is generated from Gymnodinium which is already lost the polygonal plates and the middle furrow (Hoek et al, 1995). Generally the order Prorocentrales has characters the armoured cell cover like watch-glass, has no transfer and longitudinal furrow, two of different flagella arise in the anterior end, have chloroplast, swimming forward and move spirally (Taylor, 1980), as shown in the Figure 2.

Morphological observation by light microscope showed the isolated strain (No. 6) has characters such as ovoid shape with specific movement, posses the dinokaryon (large nucleus), yellow chloroplast with a 3-layer envelope of the chloroplast membrane, 2 flagella (long flagellum and transfers flagellum), tinny platelets on the surface cell near the base of cell, yellow-red dark eye spot, granule near the chloroplast which is maybe the cellulose and the spine in the apical of cell (Figure 1). The strain movement was forward, gliding spirally using the flagella to pull up and roll the body. When the strain was cultivated in the tube reactor without shaking, some of the cells were settling down in the bottom but some of them moving up and down in the water column.

The scanning electron microscope observation showed the ovoid shape of the isolated

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[GENETYX-MAC : Nucleotide Sequence Homology Data ]
Reference strain MBIC 11147
1st Nucleotide Sequence
  File Name       : C.18S
  Sequence Size   : 1795

2nd Nucleotide Sequence
  File Name       : C_e.18S
  Sequence Size   : 1795
  Unit Size to Compare = 1
  Pick up Location    = 1

Note: S, sample; R, reference MBIC1147
[100.0% / 1795 bp]    OPT.Score : <   7180 >

S1' TACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT
**********************************************************
R1" TACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCT

S61' CAGTATAAGCTTCTATACGGCGAAACTGCGAATGGCTCATTAAAACAGTTATAGTTTATT
**********************************************************
R61" CAGTATAAGCTTCTATACGGCGAAACTGCGAATGGCTCATTAAAACAGTTATAGTTTATT

S121' TGATGGTCATTCTTTACATGGATAACTGTGCTAATTGTAGAGCTAATACATGCGCCCAAA
**********************************************************
R121" TGATGGTCATTCTTTACATGGATAACTGTGCTAATTGTAGAGCTAATACATGCGCCCAAA

S181' CCCGACTTCGAGGAAGGGTTGTGTTTATTAGTTACAGAACCAACCCAGGCTTCGCCTGGT
**********************************************************
R181" CCCGACTTCGAGGAAGGGTTGTGTTTATTAGTTACAGAACCAACCCAGGCTTCGCCTGGT

S241' CTTGTGGTGATTCATAATAACCGAACGAATCGCATGGCTTCGCTGGCGATGAATCATTCA
**********************************************************
R241" CTTGTGGTGATTCATAATAACCGAACGAATCGCATGGCTTCGCTGGCGATGAATCATTCA

S301' AGTTTCTGACCTATCAGCTTCCGACGGTAGGGTATTGGCCTACCGTGGCAATGACGGGTA
**********************************************************
R301" AGTTTCTGACCTATCAGCTTCCGACGGTAGGGTATTGGCCTACCGTGGCAATGACGGGTA

S361' ACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGG
**********************************************************
R361" ACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGG

S421' AAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAAGAAATAAC
**********************************************************
R421" AAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACAAGAAATAAC

S481' AATACAGGGCATATCTGTCTTGTAATTGGAATGAGTAGAATTTAAATCCCTTTACGAGTA
**********************************************************
R481" AATACAGGGCATATCTGTCTTGTAATTGGAATGAGTAGAATTTAAATCCCTTTACGAGTA

S541' CCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATA
**********************************************************
R541" CCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATA

S601' TTAAAGTTGTTGCGGTTAAAAAGCTCGTAGTTGGATTTCTGCCGAGGACGACCGGTCCGC
**********************************************************
R601" TTAAAGTTGTTGCGGTTAAAAAGCTCGTAGTTGGATTTCTGCCGAGGACGACCGGTCCGC
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S661' CCTCTGGGTGAGTATCTGGCTCGGCCTGGGCATCTTCTTGGAGAACGTAGCTGCACTTGA
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R661" CCTCTGGGTGAGTATCTGGCTCGGCCTGGGCATCTTCTTGGAGAACGTAGCTGCACTTGA

S721' CTGTGTGGTGCGGTATCCAGGACTTTTACTTTGAGGAAATTAGAGTGTTTCAAGCAGGCT
****
R721" CTGTGTGGTGCGGTATCCAGGACTTTTACTTTGAGGAAATTAGAGTGTTTCAAGCAGGCT
S781' TTCGCCTTGAATACATTAGCATGGAATAATAAGATAGGACCTCGGTTCTATTTTGTTGGT
****
R781" TTCGCCTTGAATACATTAGCATGGAATAATAAGATAGGACCTCGGTTCTATTTTGTTGGT

S841' TTCTAGAGCTGAGGTAATGATTAATAGGGATAGTTGGGGGCATTCGTATTTAACTGTCAG
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R841" TTCTAGAGCTGAGGTAATGATTAATAGGGATAGTTGGGGGCATTCGTATTTAACTGTCAG

S901' AGGTGAAATTCTTGGATTTGTTAAAGACGGACTACTGCGAAAGCATTTGCCAAGGATGTT
****
R901" AGGTGAAATTCTTGGATTTGTTAAAGACGGACTACTGCGAAAGCATTTGCCAAGGATGTT

S961' TTCATTGATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCCTAGTCTTA
****
R961" TTCATTGATCAAGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCCTAGTCTTA

S1021' ACCATAAACCATGCCGACTAGAGATTGGAGGTCGTTATCTATACGACTCCTTCAGCACCT
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R1021" ACCATAAACCATGCCGACTAGAGATTGGAGGTCGTTATCTATACGACTCCTTCAGCACCT

1081' TATGAGAAATCAAAGTCTTTGGGTTCCGGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAA
****
1081" TATGAGAAATCAAAGTCTTTGGGTTCCGGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAA

1141' GGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGG
****
1141" GGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATTTGACTCAACACGG

1201' GGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGATTGATAGCTCTTTCTTGATTC
****
1201" GGAAACTTACCAGGTCCAGACATAGTAAGGATTGACAGATTGATAGCTCTTTCTTGATTC

1261' TATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGT
****
1261" TATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGT

1321' TAACGAACGAGACCTTAACCTGCTAAATAGTTACACGTAACTTCGGTTACGTGGGCAACT
****
1321" TAACGAACGAGACCTTAACCTGCTAAATAGTTACACGTAACTTCGGTTACGTGGGCAACT

1381' TCTTAGAGGGACTTTGCGTGTCTAACGCAAGGAAGTTTGAGGCAATAACAGGTCTGTGAT
****
1381" TCTTAGAGGGACTTTGCGTGTCTAACGCAAGGAAGTTTGAGGCAATAACAGGTCTGTGAT

1441' GCCCTTAGATGTTCTGGGCTGCACGCGCGCTACACTGATGCGTTCAACGAGTTTATGACC
****
1441" GCCCTTAGATGTTCTGGGCTGCACGCGCGCTACACTGATGCGTTCAACGAGTTTATGACC

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1501' TTGCCTGGAAAGGTTGGGTAATCTTTTTAAATCGCATCGTGATGGGGATAGATTATTGCA
**********************************************************
1501" TTGCCTGGAAAGGTTGGGTAATCTTTTTAAATCGCATCGTGATGGGGATAGATTATTGCA

1561' ATTATTAATCTTCAACGAGGAATTCCTAGTAAGCGCGAGTCATCAGCTCGTGCTGATTAC
**********************************************************
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1621' GTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAGTGATCCGGTGAATAA
**********************************************************
1621" GTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATTGAGTGATCCGGTGAATAA

1681' TTCGGACTGCAGCAGTGTTCAGTTCCTGAACGCTGCAGCGGAAAGTTTAGTGAACCTTAT
**********************************************************
1681" TTCGGACTGCAGCAGTGTTCAGTTCCTGAACGCTGCAGCGGAAAGTTTAGTGAACCTTAT

1741' CACTTAGAGGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG
**********************************************************
1741" CACTTAGAGGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGG
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Figure 7. 18S rDNA sequences of evaluated Prorocentrum compared with MBIC 11147

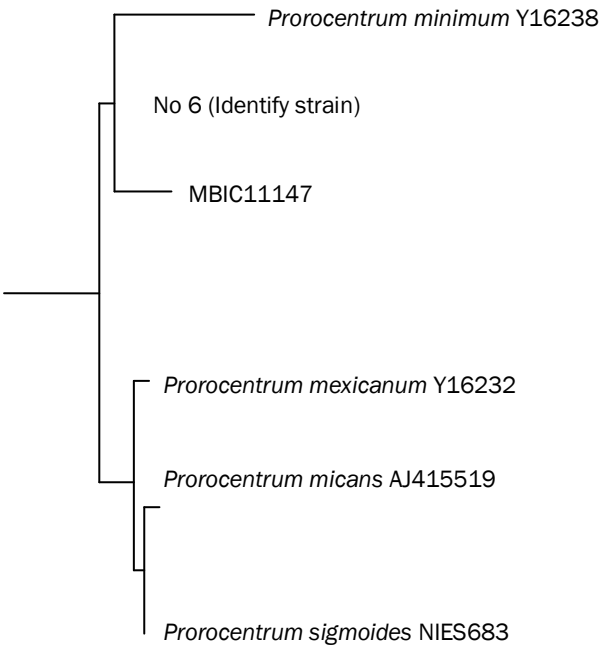

Figure 8. Phylogenic tree of observed Prorocentrum (No.6) base on NJ-analyzes

strain was clearly viewed. The surface cell architecture was ornamented with one intact plate theca with the spine and flagella in the anterior of cell (Figure 2). The theca of isolated strain that actually contain the silica was not smooth but there was some wave-line, rough and rigid. The intact silica cell cover should be the identity of Dynophyte that differentiate it from the Diatomae division, which also has silica cover that is separated from the cell body.To confirm the microscopy observation, molecular approach was performed. The deoxy nucleic acid (DNA) was extracted and purified. The electrophoresis image resulted was shown in Figure 3 (one major band). Base on the observation that isolated strain has real nucleus and cell wall that is mean eukaryote microorganism; the procedures and

primers for 18S rDNA was applied. After replication the DNA content by polymerase chain reaction (PCR), the DNA sequencing was done. When the DNA sequences were analyzed and adjusted by sequences consensus with Marine Biotechnology Institute (MBI) Gene Library, Kamaishi Japan, the evaluated isolate was 100% same with strain MBI culture No. 11147, *Prorocentrum* sp (Figure 4).

The construction of phylogenic tree showed in the Figure 5. The isolated strain (No.6) significantly resembled with strain MBIC 11147, which is close to P. minimum Y16238, P. mexicanum Y16232, P. micans AJ415519 and P. sigmoides NIES683.

Conclusion

Phenotypic observation, molecular identification and an evaluation of the cell property leading to the conclusion it seem the isolated strain was determined as *Prorocentrum* sp. and 100% same with strain MBIC 11147 collection. The propose taxon is:

Division : Dynophyta
Class : Dynophyceae
Order : Prorocentrales
Family : Prorocentraceae
Genus : Prorocentrum
Species : *Prorocentrum* sp.

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