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Diurnal Modification of a Red-Tide Causing Organism, *Chattonella antiqua* (Raphidophyceae) from Korea

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Blooms of *Chattonella* species are normally during summer in inland seas with high nutrients from the land and inflowing water. These blooms cause mass fish kills worldwide. We isolated a *Chattonella* strain from the south coast of Korea and identified it as *C. antiqua*. It is known that the morphological changes of phytoplankton correspond to the diurnal vertical migrations that follow an intrinsic biological clock and a nutrient acquisition mechanism during the day and night. In electron micrographs, *C. antiqua* clearly showed a radial distribution of lipid bodies in subcellular regions and plastids composed in which thylakoid layers were perpendicular to the surface. A single pyrenoid was present in each plastid and it was found at the end of the plastid towards the center of the cell. Throughout the day, plastids of *C. antiqua* cells appeared as an expanded net-like recticulum. During the night, however, the plastids changed their shape and contracted toward the cell periphery. The electron density of pyrenoids was increased in cells harvested during the night.

Key Words: *Chattonella*, chloromonads, red-tide, Raphidophyceae, ultrastructure

INTRODUCTION

Raphidophycean flagellates or chloromonads are unicellular biflagellate algae lacking cell walls and eyespots. Their two flagella arise near the apex of the cell: one extends anterior with tubular flagella hairs and the other lacks appendages and is directly towards the cell posterior (Graham and Wilcox 1999; Heywood 1980, 1990).

This group of flagellates has been variously classified as the Chloromonadales (Smith 1950), Chloromonadineae (Fritsch 1935), Chloromonadophyceae (Fott 1968; Klein and Cronquist 1967), or Chloromonadophyta (Loeblich and Fine 1977). The assemblage includes the three freshwater genera *Gonyostomum*, *Merotricha* and *Vacuolaria*, and five the marine genera *Chattonella*, *Fibrocapsa*, *Haramonas*, *Heterosigm* and *Olisthodiscus* (Christensen 1964; Mostaert *et al.* 1998).

The genus Chattonella includes seven species: *C. antiqua*, *C. globosa*, *C. marina*, *C. minima*, *C. ovata*, *C. subsalsa* and *C. verruculosa*. Among these species, *C. antiqua* and *C. marina* are typical red tide flagellates and have been known to cause mass fish kills in North America, New Zealand, Australia, and Japan (Hallegraeff *et al.* 1998; Honjo 1994; Munday and Hallegraeff 1998).

In Korea, there have been few reports of mass fish kills caused by *Chattonella* species (Lee *et al.* 2005): however, the incidence of *Chattonella* red tide has recently increased on the south and west coasts. For example, since the first occurrence of *Chattonella* species (cell densities of 200-1,100 cells / mL) in Jindong Bay in April 1983 (Park *et al.* 1987), *Chattonella* blooms have occurred in Jangheng in August to September of 2005 and in Taean and Boryung in 2006. *Chattonella* has been reported only rerely on the south and west coasts of Korea (Jangheung, Koheung, Tongyoung, and Wando) (Kim 2005; NFRDI 2006).

Most studies on *Chattonella* species have been conducted with Japanese cultures (Hara and Chihara 1982, 1985; 1987; Hara *et al.* 1985, 1994; Inouye *et al.* 1992).

These studies examined a variety of different biological features including nutrient supply and growth physiology (Nakamura *et al.* 1983), descriptive morphology (Imai 2000), phylogeny (Bowers *et al.* 2002), distribution (Lee *et al.* 2000), and fish-killing mechanisms (Kim *et al.* 2000). However, taxonomic and physiological aspects of Korean *Chattonella* species are poorly understood.

Therefore, primary studies including morphology and

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ultrastructure based on local isolates of *Chattonella* are required (Bourdelais *et al*. 2002; Dahl *et al*. 2004; Hallegreff *et al.* 1998; Imai *et al.* 1998; Khan *et al.* 1998; Naustvoll *et al.* 2002; Ono and Takano 1980).

Chattonella is a single-celled organism in which motility involves two flagella. The absence of a cell wall is associated with extensive variation in cell shape that includes ovoid, teardrop or cylindrical forms. Some cells have a jugular at the cell anterior, whereas others have a perpendicular sulcus. Spherical plastids are colored yellow-brown or yellow-green and distributed in the cell periphery with a highly regular arrangement. Lipid bodies and mucocysts which reflect light are located close to the cell membrane (Heywood 1980, 1990).

Because morphological characteristics such as organelles vary by environment, investigations of the cell ultrastructure would be useful to understand the source of the physiological difference and/or the spectrum of the environmental change in single celled organisms including phytoplanktons (Seo and Fritz 2000a; Zhou and Fritz 1994). For example, the ability of phytoplankton cells to produce plastid morphologies that correspond to different conditions allows them to optimize physiological processes such as photosynthesis, nitrogen reduction, and toxin production (Zhou and Fritz 1994).

This study examined the taxonomy comment of a Korean isolate of *Chattonella* based on the rDNA sequence comparison with *C. antiqua* from Japan. In addition, this study describes the diurnal morphological changes in a Korean isolate of *C. antiqua.*

MATERIAL AND METHODS

Cultures

Chattonella antiqua (NF-F-CAN1) was isolated from Jangheung, Chonnam, Korea during a bloom in early August, 2003. Two Japanese isolates, *C. antiqua* (OC-B5) and *C. marina* (MS3P) were previously isolated from Seto Inland Sea in Japan during the summer of 1985 and were kindly provided by Dr. Yamaguchi of the National Research Institute of Fisheries and Environment of Inland Sea Fisheries Research Agency (Japan).

Cells were grown in f/2 medium without silica prepared in artificial seawater (Guillard and Ryther 1962; Guillard 1975), with each culture containing a volume of 1L. Cells were incubated in chambers (set for a 12:12 h LD cycle) under 22 ± 1°C and 1,200 Lux of light intensity provided by cool-white 40 W fluorescent lamps. Media and culture dishes were replaced biweekly.

Isolation of genomic DNA

Cells were harvested during the exponential phase by centrifugation. Approximately 100 μL of pellet were suspended in 400 μL of extraction buffer, supplied in the DNA extraction kit (DNeasy plant Mini Kit, QIAGEN) and DNA was extracted following the manufacturer's protocol.

PCR and sequencing

PCR primers based on conserved sequences among phytoplankton species were designed and synthesized. For amplifying the 18S rRNA gene, a forward primer, 18SF (5'-CAC CTG GTT GAT CCT GCC AGT AG-3') and a reverse primer, 18SR (5'-GTT CAG CCT TGC GAC CAT ACT CC-3') were adopted from Ki *et al.* (2004). For the amplifications from the partial sequence of the 18S rRNA gene to the partial sequence of the 28S rRNA gene, a forward primer, SSUCOF_SEO (5'-TTC CGT TAA CGA ACG AGA CC-3') and a reverse primer, LSU2COR_SEO (5'-AGT ATC GCT ACG AGC CTC CA-3') were utilized.

PCR amplifications were carried out with a thermocycler (BIOMETRA 96 Well PCR system, Whatman Biometra, Germany). PCR assays contained a 50 μL mixture. Thermocycling used the following regime: an initial 5min at 95°C was followed by successive treatments of 35 cycles of 95°C for 20 sec, 52°C for 30 sec, and 72°C for 60 sec. After the completion of the cycles, extension was facilitated at 72°C for 10min. Two microliters of PCR product were loaded onto a 1.2% agarose gel in 1 x TAE buffer along with 1 μL of loading buffer. The agarose gel was premixed with ethidium bromide and photographed (Changeable UV Transilluminator DUT-260, Core BioSystem).

Following the PCR product purification (Accuprep PCRpurification kit, Bioneer, Korea), all PCR products were sequenced on the Perkin-Elmer Applied Biosystems (ABI) 377A DNA sequencer using an ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer), according to the manufacture's protocol. Sequences data were aligned and compared manually (Multiple sequence Alignment by CLUSTALW).

Light and fluorescent microscopy

Live cells, or cells fixed with 0.5% glutaraldehyde solution (final concentration in 0.2 M phosphate buffer, pH 7.2) were examined using light and fluorescence microscopy observations. Cells were observed under dif-

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CAK             TCTAGTATAAA-CGACTCTATACTGTGAAACTGCGAATGGCTCATTA-TATCAGTTATAG
CAJ             ------------------------------------------------------------
CMJ             --A-------CTTT-T-ACTA-T-T----C-A-ACGGG-TCGT--CAC---AT---G-CC

CAK             TTTATTTGATAGTACCTACTACTTGGATAAC-CGTA-GTAATTCTAGAGCTAATACATGC
CAJ             ------------------------------------------------------------
CMJ             C-GTA--TG-G-C----GGGCG-A-CTCT-CAT---T-G--CCCCCTCTTC--GGT--CA

CAK             ATCAACTCCCAACTG-CTTCGGCGGACGGGACGTATTTATTAGATGGAAACCA--ATGCC
CAJ             ------------------------------------------------------------
CMJ             TG-TC-GGGT-----G-C--C-T--TA--T--TG-GCCGA--AT-CT---GT-TA----T

CAK             GA-GTTAACTCTCGGGTTTTG--TGGTGAATCATAGTAACTGTGCGAATCGTATGCCTTT
CAJ             ------------------------------------------------------------
CMJ             A-CA-C-G----T-T---G--GACACCCG--A-A-C-CT-----TATCG--G--TTGA--

CAK             GGCGACGATGGTTCATTCAAGTTTCTGCCCTATCAGCTTCGGAT----GGTAGGGTATTG
CAJ             ------------------------------------------------------------
CMJ             TT---T--A-AACT-G-T-GC--CACC--GCGCAGA-CAA----CTGAAAC--AT-C---

CAK             GCCTACCATGGCTTTAACGGGTAACGGAGAATTGGGGTTCGATTCCGGAGAGGGAGCCTG
CAJ             ------------------------------------------------------------
CMJ             -G-C---C---TCA-G-T-AACGTGA--A---CC-TTCCACT-----A---ATATA----

CAK             AGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGTAAATTACCCAATCCTGACACA
CAJ             ------------------------------------------------------------
CMJ             --G-TA--G--TTC-GTG-GG-AG--AT-T--TTA---AG-TA-TTGT----AGC-TG-G

CAK             GGGAGGTAGTGACAATAAATAACAATGCTAGGCTTTTAAAGTCCGGCAATTGGAATGAGA
CAJ             ------------------------------------------------------------
CMJ             TCA------CCT-TT-CCCGCGTG--ATAC-ACCAC-C--ACA-ACT-TAGT---AAGCC

CAK             ACAATTTAAATCCCTTATCGAGGAACCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGG
CAJ             ------------------------------------------------------------
CMJ             C---GA-T--AGT--G--T-GT-GT----CAA-ACT-G------C--TT--AGAG--TAC

CAK             TAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGAT
CAJ             ------------------------------------------------------------
CMJ             ---G--T--AAGA-C--TTTG-ACC-G-TC-CTA-AT--G-CG-G-AT-T----AAAC-C

CAK             TTC-TGGTGGGAGTGATCGGTCGGCTTCGCAAGGA-GTCTGCACCTGTATCTTCTCTT--
CAJ             ------------------------------------------------------------
CMJ             -A-ACAC-CTC-ACA-CAAAATATG-CTAAG----C-CG----A-----ATG----AATG

CAK             --GCCATCCTTGGGGAAG-----GCGCTTCT---TGTAT-TCGCTTA--CGGGTTGCGAT
CAJ             ------------------------------------------------------------
CMJ             GA----CT----CC-G--TATAA-TAG--A-AAGC---CA--A--G-GT-ATAC-ATAG-

CAK             TCTCCCCATCTTTTAC-TGTGAAAAAATTAGAGTGTTCAAAGCAGGCTTAGGCCGTTGAA
CAJ             ------------------------------------------------------------
CMJ             ----T-A-C-CG-C--A-A--GAAAAGCGTAG-CC-GTT-TCT-CAACC-AA-AAC-AT-

CAK             TACATTAGCA--TGGAATAATGAGATAGGGCCTTGGTGGTTTTCTATTTTGTTGG-----
CAJ             ------------------------------------------------------------
CMJ             -TG-----T-AAC-A-T----TCACC--A-AA-C-AG-T---AAG--GACC--A-ACATA

CAK             TTTGCACGCCAAGGTAATGATTAAA--------------
CAJ             ---------------------------------------
CMJ             --CTATTATGCGTCT---CG-C--CATTCTAGAAGCTAC
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Fig. 1. Alignment of three rDNA sequences with 18SF primer from *Chattonella antiqua* of both Korean (CAK) and Japanese strains (CAJ) and from *C. marina* of Japanese strain (CMJ). Dash (-) denotes a base identical to sequence located in the top line of each alignment set. The SSU rDNA sequence of Korean strain (CAK) is completely identical to that of Japanese strain (CAJ).

ferential interference contrast (DIC) optics using an Eclipse-E600 microscope (Nikon, Japan) equipped with excitation filter (330-380 nm) and barrier filter (420 nm) Images were captured using the Laser Optik System (Jenoptik, Jena, Germany).

For observations of cells were stained with the DNA-specific fluorochrome 4', 6'-diamidino-2-phenylindole (DAPI, Molecular Probes Inc., Oregon). For DAPI staining, cells were harvested by centrifugation and washed three times with 0.1 M phosphate buffer (pH 7.4). Cells were then submerged in 10 X volume of 100% methanol for 1 hr and were subsequently washed three times with the same buffer. Cells were then stained with an aqueous solution of DAPI (10 μg / mL in final concentration) for 1 hr in the dark.

Transmission Electron Microscopy (TEM)

For TEM, cells were harvested by centrifugation and fixed at 4°C for 2 h in a mixture solution of 1% glutaraldehyde and 1% osmium tetroxide (final concentration in the culture medium). Samples were dehydrated in a graded ethanol series, infiltrated and embedded in

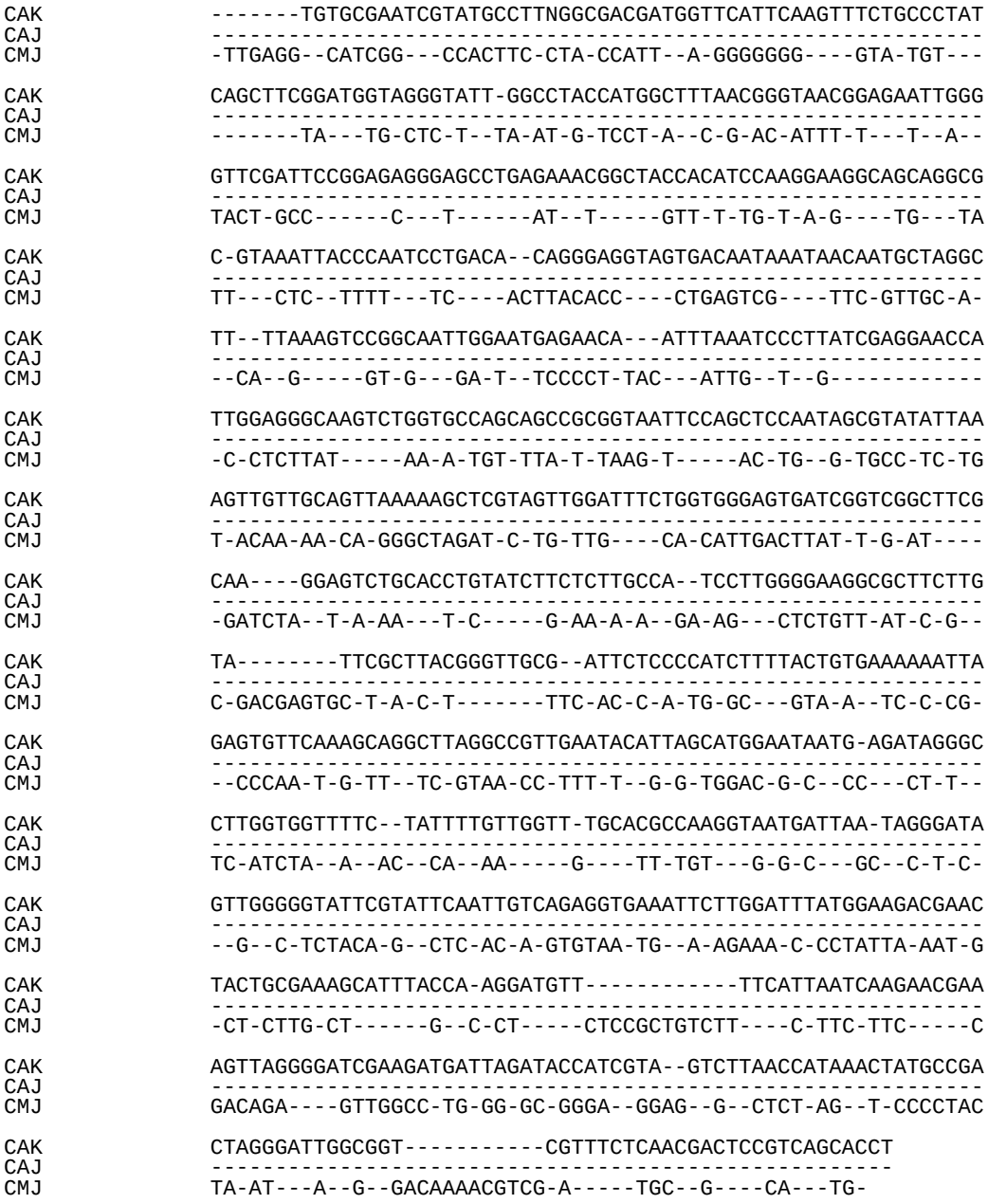

Fig. 2. Alignment of three rDNA sequences with 18SR primer from *Chattonella antiqua* of both Korean (CAK) and Japanese strains (CAJ) and from *C. marina* of Japanese strain (CMJ). Dash (-) denotes a base identical to sequence located in the top line of each alignment set. The SSU rDNA sequence of Korean strain (CAK) is completely identical to that of Japanese strain (CAJ).

L.R. White resin (Electron Microscopy Sciences, Pennsylvania). Samples were sectioned with a glass knife and post-stained with 2% ethanolic uranyl acetate for 20 min and 2% lead citrate for 10 min. Thin sections (50 nm thickness) were examined at 80 kV under a JEM1200EXII TEM (JEOL, Japan). The length and the width of plastids from electron micrographs of transverse sections was measured and calculated to get a relative ratio (length / width) on the purpose of a comparison between the day and the night cells.

Observation of diurnal migration

To analyze the diurnal vertical migration of the cells in the laboratory, 3.2 L of a stationary culture (400 cells / mL) was divided into 4 sets of 50 mL borosilicate graduated cylinders (total 64 cylinders, each having 50 mL of culture). Cultures were entrained for 24 h in a chamber (set for continuous light) at 22 ± 1°C and 1,200 Lux provided by cool-white 40 W fluorescent lamps. Beginning the next day and for the following three days

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CAK             ----------------GGGTATGCTACGCATTGCCTTTTCTACTTCTTAGAGGGACTTTC
CAJ             ------------------------------------------------------------
CMJ             ----------------C-C-----T-----------------------------------

CAK             GGTGACTAACCGAAGGAAGTTGGGGGCAATAACAGGTCTGTGATGCCCTTAGATGTCCTG
CAJ             ------------------------------------------------------------
CMJ             ------------------------------------------------------------

CAK             GGCTGCACGCGCGCTACACTGATGCATGCAACGAGTAATGACCTTGGCCGGAAGGCCTGG
CAJ             ------------------------------------------------------------
CMJ             ------------------------------------------------------------

CAK             GTAATCTTTTGAACGTGCATCGTGATAGGGATAGATTATTGCAATTATTAATCTTGAACG
CAJ             ------------------------------------------------------------
CMJ             ------------------------------------------------------------

CAK             AGGAATTCCTAGTAAACGCGAGTCATCAGCTCGCATTGATTACGTCCCTGCCCTTTGTAC
CAJ             ------------------------------------------------------------
CMJ             ------------------------------------------------------------

CAK             ACACCGCCCGTCGCACCTACCGATTGAATGATTCGGTGAAAATCTCGGACTATGGCTTGA
CAJ             ------------------------------------------------------------
CMJ             ------------------------------------------------------------

CAK             AACTTTATTGTGACTTGCCGTTAGGAAGTTATTTAAACCTCATCATTTAGAGGAAGGTGA
CAJ             ------------------------------------------------------------
CMJ             ------------------------------------------------------------

CAK             AGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCACACCGATCCTAA
CAJ             ------------------------------------------------------------
CMJ             ------------------------------------------------------------

CAK             ACGGGATCCGTCCCATCGTGAACTTGTTTCCGGGCCTTGGCCCGTTGACAGCTTTTACAC
CAJ             ------------------------------------------------------------
CMJ             ------------------------------------------------------------

CAK             CCATCCAACCAAACCTCAAAACCAAACATTTTGACCCTACCAACCCATTCGACTGAACCG
CAJ             ------------------------------------------------------------
CMJ             ------------------------------------------------------------

CAK             TAACGGTTGCTTCTCTGGAAGCAATCGGCGATTTAAACAAATCATACGACTTTCAGCAGC
CAJ             ------------------------------------------------------------
CMJ             ------------------------------------------------------------

CAK             GGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGCGATACGTAATGCGAATT
CAJ             ------------------------------------------------------------
CMJ             ------------------------------------------------------------

CAK             GCAGAGTCCAGCGAGTCATCAAATGTTCGAACGCACCTGGCACTTCCGGGATATTCCTGG
CAJ             ------------------------------------------------------------
CMJ             ------------------------------------------------------------

CAK             GAGTATGCTTGTTAGAGTGTCTGTTGGACCATCTCCCTTGTTCCTTCGGGAAGAAGTGGC
CAJ             ------------------------------------------------------------
CMJ             ----------------------------------------------------A-------

CAK             GGTAGTTGCCGTACA---
CAJ             ------------------
CMJ             ------------------
```

Fig. 3. Alignment of three rDNA sequences with SSUCOF_SEO primer from *Chattonella antiqua* of both Korean (CAK) and Japanese strains (CAJ) and from *C. marina* of Japanese strain (CMJ). Dash (-) denotes a base identical to sequence located in the top line of each alignment set. The SSU rDNA sequence of Korean strain (CAK) is completely identical to that of Japanese strain (CAJ).

10 mL of culture from each of five strata in a cylinder were harvested in sequence every 3 h and the number of cells in each stratum was counted.

RESULTS

Identification of Korean isolates

The rDNA sequence analysis revealed that our cultured material was identical to *C. antiqua* from Japan (Figs 1 to 4). Therefore we referred to the Korean isolate as *C. antiqua* in this paper. The sequence data showed a large phylogenic gap between *C. antiqua* and *C. marina*.

Ultrastructure

Two different stages of *Chattonella* cells were found: a round temporary cyst stage and a teardrop shaped motile stage with the elongated end at the cell posterior (Fig. 5). The nucleus in the motile stage was also teardrop shaped with the elongated end pointed towards the cell anterior (Fig. 6). Transverse sections of *Chattonella* cells showed electron-dense lipid bodies and radial distributed plastids with distinct thylakoids (Fig. 7). Plastids

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CAK             ------------AGATTAGCCATGCATGTCTAGTATAAACGACTCTATACTGTGAAACTG
CAJ             ------------------------------------------------------------

CAK             CGAATGGCTCATTATATCAGTTATAGTTTATTTGATAGTACCTACTACTTGGATAACCGT
CAJ             ------------------------------------------------------------

CAK             AGTAATTCTAGAGCTAATACATGCATCAACTCCCAACTGCTTCGGCGGACGGGACGTATT
CAJ             ------------------------------------------------------------

CAK             TATTAGATGGAAACCAATGCCGAGTTAACTCTCGGGTTTTGTGGTGAATCATAGTAACTG
CAJ             ------------------------------------------------------------

CAK             TGCGAATCGTATGCCTTTGGCGACGATGGTTCATTCAAGTTTCTGCCCTATCAGCTTCGG
CAJ             ------------------------------------------------------------

CAK             ATGGTAGGGTATTGGCCTACCATGGCTTTAACGGGTAACGGAGAATTGGGGTTCGATTCC
CAJ             ------------------------------------------------------------

CAK             GGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGTAAATTAC
CAJ             ------------------------------------------------------------

CAK             CCAATCCTGACACAGGGAGGTAGTGACAATAAATAACAATGCTAGGCTTTTAAAGTCCGG
CAJ             ------------------------------------------------------------

CAK             CAATTGGAATGAGAACAATTTAAATCCCTTATCGAGGAACCATTGGAGGGCAAGTCTGGT
CAJ             ------------------------------------------------------------

CAK             GCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAA
CAJ             ------------------------------------------------------------

CAK             GCTCGTAGTTGGATTTCTGGTGGGAGTGATCGGTCGGCTTCGCAAGGAGTCTGCACCTGT
CAJ             ------------------------------------------------------------

CAK             ATCTTCTCTTGCCATCCTTGGGGAAGGCGCTTCTTGTATTCGCTTACGGGTTGCGATTCT
CAJ             ------------------------------------------------------------

CAK             CCCCATCTTTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCTTAGGCCGTTGAATACA
CAJ             ------------------------------------------------------------

CAK             TTAGCATGGAATAATGAGATAGGGCCTTGGTGGTTTTCTATTTTGTTGGTTTGCACGCCA
CAJ             ------------------------------------------------------------

CAK             AGGTAATGATTAAA
CAJ             --------------
```

Fig. 4. Alignment of two rDNA sequences with LSU2COR_SEO primer from *Chattonella antiqua* of both Korean (CAK) and Japanese strains (CAJ). Dash (-) denotes a base identical to sequence located in the top line of each alignment set. The SSU rDNA sequence of Korean strain (CAK) is completely identical to that of Japanese strain (CAJ).

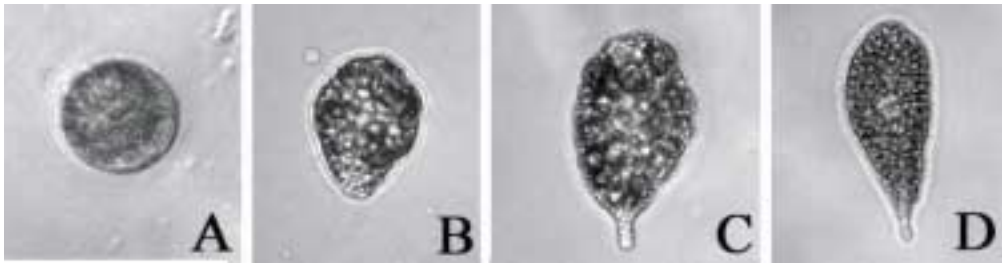

Fig. 5. Morphological variation of *Chattonella antiqua* (NF-F-CAN1): a cyst (A) and motile cells (B-D).

occupied the region between the cell membrane and the layer of cytoplasm surrounding the nucleus. Some plastids extended in contact with the cell membrane or other cell organelles; membrane connections between organelles were not evident.

Daytime cells had an overall net-like arrangement of plastids with the intervening space showing numerous large vacuoles: the relative ratio of plastids was 1.50 ± 0.3 in the daytime cells. A few plastids extended to the center and few others were close to the cell membrane (Fig. 8). Each plastid had a distinct pyrenoid located at the end of the plastid facing the cell interior (arrow in Fig. 8B).

Chattonella cells harvested during the night showed a different distribution of organelles (Fig. 9). Accordingly, plastids were contracted and arrayed in the cell periphery (Fig. 10). The relative ratio of plastids (length / width) was 1.38 ± 0.1 in night cells. Thylakoids were tightly

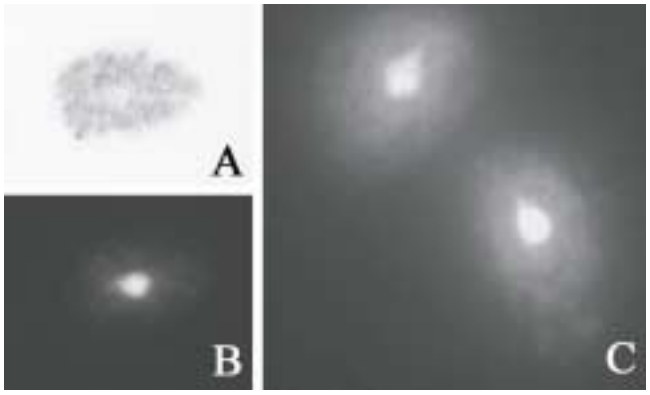

Fig. 6. Water drop shaped nucleus of *Chattonella antiqua* (NF-F-CAN1).

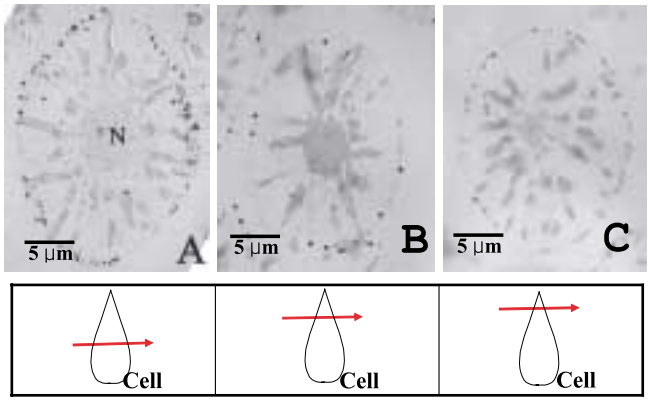

Fig. 7. Electron micrographs of the day phase cells of *Chattonella antiqua* (NF-F-CAN1). Note nucleus(N) in the middle of the cell(A, B and C).

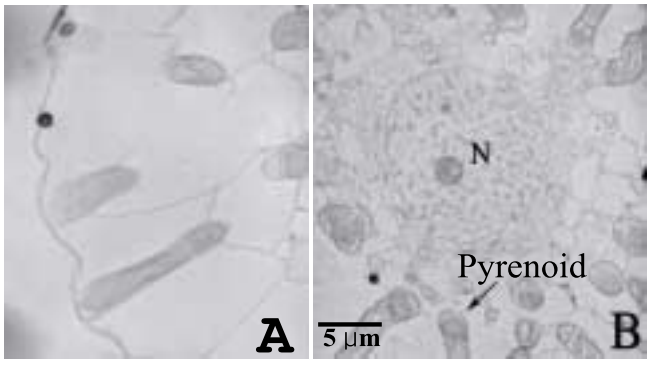

Fig. 8. Electron micrographs of the day phase cells of *Chattonella antiqua* (NF-F-CAN1). Note the position of plastids and pyrenoids (arrow) in the cytoplasm and nucleus (N) in the middle of the cell.

stacked perpendicularly inside of the plastid (Fig. 11C).

In longitudinal section, it was apparent that thylakoids were arranged approximately parallel to the longitudinal axis of the plastids (arrow in Fig. 11C). Electron density of the nighttime plastids (arrow in Fig. 9A) was higher

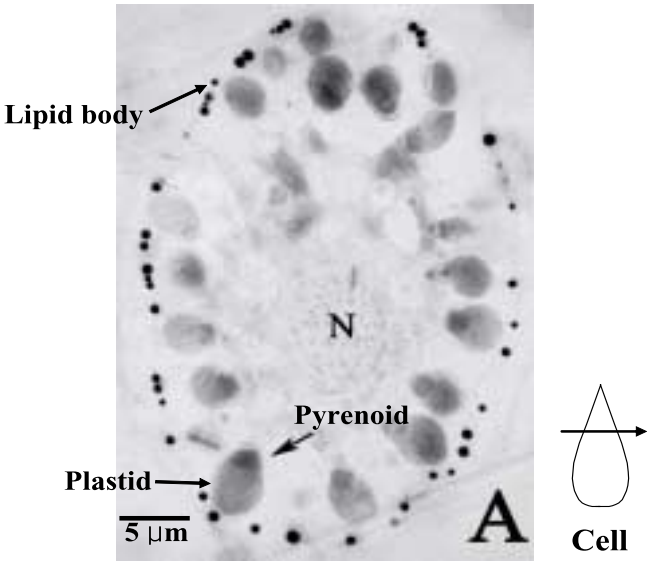

Fig. 9. Electron micrographs of night phase cells of *Chattonella antiqua* (NF-F-CAN1). Note the position of the nucleus (N) and pyrenoids (arrow).

than that of the daytime plastids (arrow in Fig. 8B). There were no conspicuous differences in the distribution of lipid bodies between the day and the night (Fig. 8A and Fig 10).

Diurnal vertical distribution

The largest proportion of cells occurred in the surface stratum of the graduated cylinder (Fig. 12). Fewer cells were at lower strata of the cylinder. Cells near the surface showed a distinct diurnal vertical migration, while cells in the lower strata showed a more or less number during the experimental period. From a trough-to-trough calculation of cell density in the uppermost stratum, the intrinsic circadian time of *C. antiqua* was determined to be 21.0 ± 1 h.

DISCUSSION

In chloromonads, plastids are usually numerous, 2-5 μm long, discoid and parietal. Plastids have a ring shaped nucleoid, chlorophylls a and c, and several xanthophylls. A girdle band in the plastid is absent in *Chattonella* species, but covers the entire organelle in *Gonyostomum* and *Vacuolaria*. (Heywood 1980).

Just as in some dinoflagellates (Seo and Fritz 2000b) the plastid of *Chattonella* is a variable organelle that responds to changing environmental conditions. For example, the change in coloration from green to yellowish-green to yellow, presumably based on the differential accumulation of carotenoids is dependent on changing

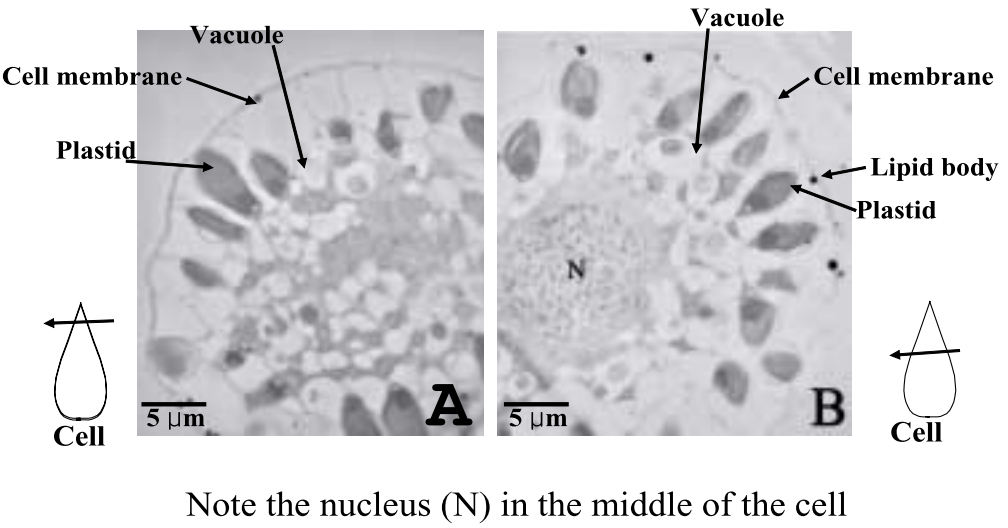

Fig. 10. Electron micrographs of the night phase cells of *Chattonella antiqua* (NF-F-CAN1). Note plastids are contracted toward the cell periphery (A and B).

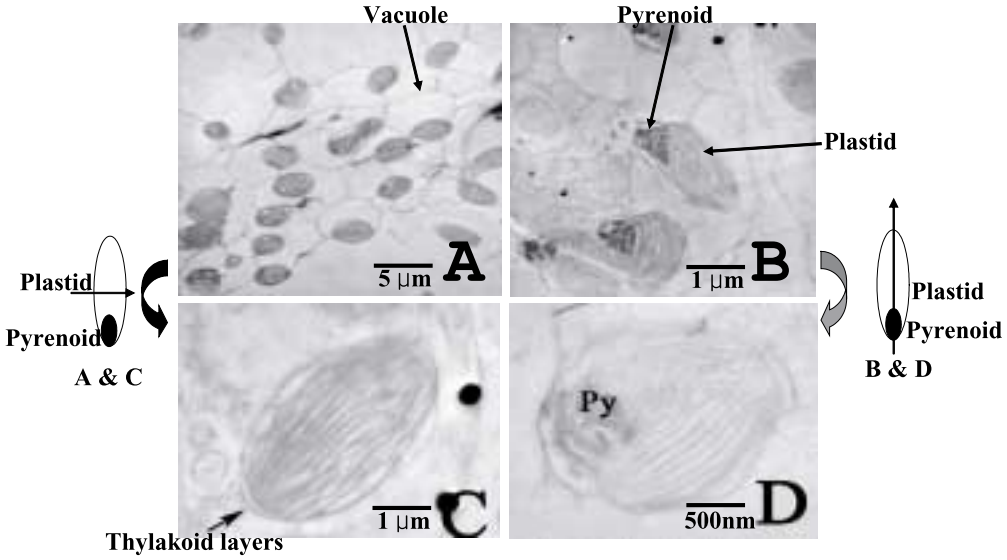

Fig. 11. Electron micrographs of the night phase cells of *Chattonella antiqua* (NF-F-CAN1). Note plastids filled parallel running thylakoids (A and arrow in C), and inward side of plastids was filled with pyrenoid (B and D).

culture conditions, including irradiation and nutrient supply (Holland and Enjumet 1956, Subrahmanyan 1954). Like other phytoplankton, plastids of chloromonads show radial distribution in cell periphery and expend to close to the cell membrane and/or to the nucleus (Graham and Wilcox 1999, Heywood 1980, 1990, Hovasse 1945), while this is not always the case (Mignot 1976).

Plastid movement and ultrastructure are closely linked to the photosynthesis in phytoplanktons (Herman and Sweeney 1975; Rensing *et al.* 1980; Seo and Fritz 2002; Sweedney 1981). Morphological change of plastids in Raphidophycean species has been reported by few previous studies. For example, plastids of *Gonyostomum semen* were arrayed radial in cytoplasm (Hovasse 1945). In the case of *C. subsala*, the position of plastids was changed by light, (Subrahmanyan 1954). It showed that the cell itself changed the position of organelles to maximize cellular activity by its intrinsic diurnal clock.

Corresponding to the previous observations, cylindrical shaped plastids of *C. antiqua* cells were vertical to the cell surface line always, although the position in cell periphery and shape of plastids was in response to the time of harvesting cells. We also found that ultrastruc-

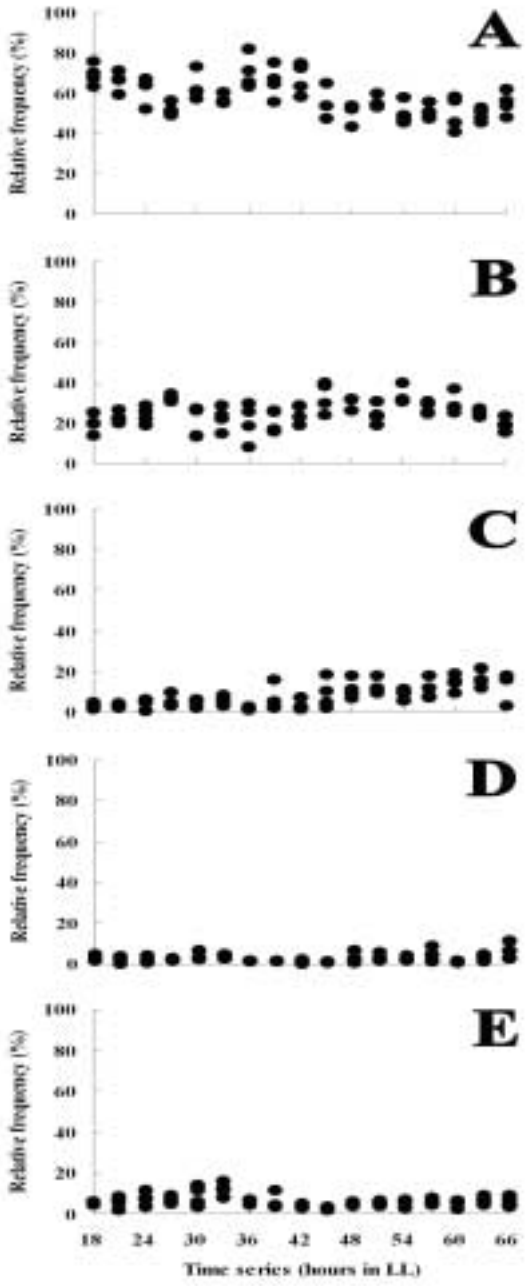

Fig. 12. Vertical distribution of *Chattonella antiqua* (NF-F-CAN1) under continuous light (A: top, B: mid-top, C: middle, D: mid-bottom, E: bottom).

ture of radial distributed plastid is changed diurnally.

Pyrenoids are important metabolic regions in photosynthetic cells, but appear to be present only in the marine genus *Chattonella* among chloromonads where they occur at the pole of the plastids (Heywood 1980). From the studies on euglenoids, dinoflagellates, and chlorophytes, it appears that pyrenoids contain the enzyme, Rubulose-1,5- bisphosphate carboxylase/oxidase essential for the carbon fixation reactions associated with the dark reaction of photosynthesis (Nagy-Toth *et al.* 1991; Nassoury *et al.* 2001; Osafune *et al.* 1990; Palmer 1995). Pyrenoids can change their morphology or even their occurrence depending upon the cell's life history and available carbon sources (Nagy-Toth *et al.* 1991; Osafune *et al.* 1990). Because the photosynthetic system of chlorophyll containing organisms and the key enzyme in this process are vulnerable to incorporating O_2 rather than CO_2, the pyrenoids have been suggested as the site most likely to spatially separate the oxygen sensitive RuBISCO from the oxygen evolving light reactions (Jenks and Gibbs 2000; Morse *et al.* 1995; Nassoury *et al.* 2001). We observed that Pyrenoids of *C. antiqua* showed a different electron density in the day and the night. More intensive studies may reveal the alternation of pyrenoids in *C. antiqua* following to the time of day.

Vertical migration is a common feature in phytoplankton species (Eppley *et al.* 1968; Heiskanen 1995; Walker and Pitcher 1991). In general, the vertical distribution of phytoplankton species is closely related to nutritional requirements and light intensity (Eggersdorfer and Hader 1991; MacIntyre *et al.* 1997). Proposed advantages of vertical migration include nutrient uptake and escape from predation by zooplankton (Cullen and Horrigan 1981; Hays 1995; Villareal *et al.* 1999; Watanabe *et al.* 1995). Phytoplanktons undergo vertical migration by dropping to depths at night to reach deeper waters with higher nutrient levels. Cells then migrate upward during the day by regulating buoyancy, in order to maximize photosynthesis (Lieberman *et al.* 1994; Qi *et al.* 1997).

Our experiments with *Chattonella antiqua* from Korea have failed to observe 'vertical migration' in all cells. We saw some cells at the surface of the experimental graduated cylinders. Most cells were buoyant on the middle stratum, and 10-20% of cells were at the bottom. The so-called 'once in a generation' migration hypothesis would explain the stratified distribution of *C. antiqua* during our experiment. According to the hypothesis, phytoplankton cells sink to the bottom in a particular phase of the life history (Ballek and Swift 1986; Rivkin *et al.* 1984; Seo and Fritz 2000a). Mature cells are buoyant and stay near the water surface. Following division cells lose their buoyancy and begin to sink.

It is possible to connect the requirement of nutrient set by intrinsic biological clock and the life history together (Villareal and Lipschultz 1995). Biological clocks are found in the physiological processes of living beings, including plants, animals, fungi and cyanobacteria (Aschoff 1965; Ditty *et al.* 2003). Biological clocks may be defined by three characteristics (Dunlap *et al.* 2003;

Dvornyk *et al*. 2003; Koukkari and Southern 2006; Refinetti 2006; Takahashi and Zatz 1982). First, the clock persists in constant conditions (for example constant dark) with a constant period. Second, the period of the biological clock can be reset by exposure to external cues such as a light or dark pulse, while the intensity is an important factor in the degree to which the clock is reset. Third, the intrinsic clock is temperature compensated, meaning that it proceeds at the same rate within a range of temperatures.

In phytoplankton, for example, the non-motile dinoflagellate *Pyrocystis noctiluca* shows different internal nitrogen contents depending on the life history phase of the cell (Ballek and Swift 1986; Bhovichitra and Swift 1977). Most cells, about 80-90% of a population, were found in the surface water (Sukhanova and Rudyakov 1973), and their nitrogen content was high at 2.2 ± 0.4 μM. When the nitrogen content dropped under a threshold (0.8 ± 0.3 μM), the cell sank to the bottom (Balleck and Swift 1986; Bhovichitra and Swift 1977; Villareal and Lipschultz 1995).

Our study showed that *Chattonella antiqua* isolated from Korea changes cellular organelles depending on the time of the day, obviously related to the intrinsic biological clock controlling photosynthesis and metabolic reactions. If it is consistent with the example of other phytoplankton, the diurnal changes in *C. antiqua* are likely to be an adaptation to nutrient availability and stage in life history.

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