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First record of a marine microalgal species, *Chlorella gloriosa* (Trebouxiophyceae) isolated from the Dokdo Islands, Korea

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Abstract: *Chlorella gloriosa* (Chlorellaceae, Trebouxiophyceae) was isolated from seawater off the coast of the Dokdo Islands in Korea. An axenic culture was established using the streak-plate method on f/2 agar media supplemented with antibiotics, allowing identification of the isolate by morphological, molecular, and physiological analyses. The morphological characteristics observed by light and electron microscopy revealed typical morphologies of *C. gloriosa* species. The molecular phylogenetic inference drawn from the small-subunit 18S rRNA sequence verified that the microalgal strain belongs to *C. gloriosa*. Additionally, gas chromatography-mass spectrometry analysis showed that the isolate was rich in nutritionally important omega-3 and -6 polyunsaturated fatty acids and high-performance liquid chromatography analysis revealed that the high-value antioxidants lutein and violaxanthin were biosynthesized as accessory pigments by this microalga, with arabinose, galactose, and glucose as the major monosaccharides. Therefore, in this study, a Korean marine *C. gloriosa* species was discovered, characterized, and described, and subsequently added to the national culture collection.

Keywords: *Chlorella gloriosa*, Dokdo Islands, first record, Korean marine microalga

INTRODUCTION

Chlorella (Chlorophyta, Trebouxiophyceae, Chlorellales) was first isolated in culture and described by Beijerinck (1890), subsequently becoming among the most extensively studied and widely cultivated microalgae. The genus is cosmopolitan and commonly found in freshwater and soil, although there have been some identified marine species. There are currently 37 taxonomically accepted species in genus *Chlorella* (Guiry and Guiry 2019), which comprises small, globular cells (~2−10 μm) containing a single chloroplast with a pyrenoid (Bock *et al.* 2011). *Chlorella* cells reproduce asexually by mitosis, forming daughter cells within the parental cell. When these daughter cells are matured into autospores, the parental cell wall ruptures, and the daughter cells are released (Yamamoto *et al.* 2004).

Chlorella salina was originally isolated in culture from an oyster breeding tank at Conway in North Wales by Butcher (1952) and shared morphological similarities with *Chlorella vulgaris* Beijerinck, despite a thinner cell wall and different shape of the chromatophore. Because it was a marine species, it was distinguished from *C. vulgaris* Beijerinck and subsequently renamed *C. gloriosa* Molinari & Calvo-Pérez in honor of Professor Gloria Chacón Roldán de Popovici by Calvo-Pérez Rodó and Molinari-Novoa (2015). A China-originating *C. salina* strain KMMCC-79 (Bae and Hur 2011) was maintained at the Korea Marine Microalgae Culture Center (KMMCC); however, there have been no

Table 1. Sampling site

Depth (m)	Temperature (°C)	Salinity (PSU)	Latitude	Longitude
14.0	18.0	37.5	37°14′25.75″N	131°52′15.07″E

PSU: practical salinity unit.

reports of Korean *C. salina* strains.

In this study, we isolated and identified a unicellular microalga, *C. gloriosa* strain MM0063, from seawater off the coast of Dokdo Islands, Dokdo-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, Korea. This report provides information on the first record of this species in Korea and its morphological, molecular, and chemotaxonomic features.

MATERIALS AND METHODS

1. Sample collection and isolation

Seawater samples were collected in sterile 50-mL conical tubes in July 2018, off the Dokdo Islands, Dokdo-ri, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do, Korea (Table 1). Samples were immediately transported to the laboratory, and 1 mL of each sample was spread onto f/2 agar (Guillard and Ryther 1962; Guillard 1975) containing the broad-spectrum antibiotic imipenem (Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 100 μg mL^{-1} in order to prevent bacterial growth (Hong *et al.* 2015). The plates were incubated in a culture room at 18°C under cool fluorescent light (~40 μmole m^{-2} s^{-1}) in a light/dark cycle of 14 h/10 h until green microalgal colonies formed. A single colony was streaked on a fresh f/2 agar plate supplemented with imipenem (20 μg mL^{-1}), with this step repeated until a pure culture was produced.

2. Morphological identification

An axenic culture was autotrophically grown in f/2 medium for 4 weeks at 18°C with shaking at 160 rpm on an orbital shaker (SH30; Fine PCR, Gunpo, Korea). Live cells were examined at 1,000× magnification under a Nikon Eclipse Ni light microscope (Tokyo, Japan). For field emission scanning electron microscopy (FE-SEM), 10-mL aliquots of cultures at ~2×10^6 cells mL^{-1} were fixed for 10 min in osmium tetroxide (OsO_4; Electron Microscopy Sciences, Hatfield, PA, USA) at a final concentration of 1% (v/v). The fixed cells were collected on 3-μm pore size, polycarbonate membrane filters (Whatman, Kent, UK) and washed three times with 50% filtered seawater diluted with distilled water to remove residual salts. The membranes with attached cells were then dehydrated in an ethanol series (10, 30, 50, 70, 90, and 100% ethanol, followed by two changes in 100% ethanol; Merck, Darmstadt, Germany) and immediately dried using an automated critical point dryer (EM CPD300; Leica, Wetzlar, Germany). The dried filters were mounted on an aluminum stub (Electron Microscopy Sciences) using copper conductive doubled-side tape (Ted Pella, Redding, CA, USA) and coated with gold in an ion sputter (MC1000; Hitachi, Tokyo, Japan). Cells and surface morphologies were observed by FE-SEM (S-4800, Hitachi, Tokyo, Japan). For transmission electron microscopy (TEM), cells were transferred to a 10-mL tube and fixed in 2.5% (v/v) glutaraldehyde (final concentration) for 1.5 h, after which the contents of the tube were placed in a 10-mL centrifuge tube and concentrated at 1,610 × *g* for 10 min in a Vision Centrifuge VS-5500 (Vision, Bucheon, Korea). The resulting pellet was subsequently transferred to a 1.5-mL tube and rinsed in 0.2 M sodium cacodylate buffer (Electron Microscopy Sciences) at pH 7.4. After several rinses, cells were post-fixed for 90 min in 1% (w/v) OsO_4 in deionized H_2O and embedded in agar. Dehydration was performed in a graded ethanol series (50, 60, 70, 80, 90, and 100% ethanol, followed by two changes in 100% ethanol), and the material was embedded in Spurr's resin (Electron Microscopy Sciences). Sections were prepared on an EM UC7 ultramicrotome (Leica, Wetzlar, Germany) and stained with 3% (w/v) aqueous uranyl acetate (Electron Microscopy Sciences), followed by lead citrate (Electron Microscopy Sciences). The sections were visualized on a JEOL-1010 TEM (JEOL, Tokyo, Japan) at a voltage of 100 kV.

3. Molecular identification

For molecular analysis, genomic DNA was extracted using a DNeasy Plant mini kit (Qiagen, Hilden, Germany) and purified with a Wizard DNA Clean-Up system (Promega, Madison, WI, USA) to remove polymerase chain reaction (PCR) inhibitors. The universal forward primer NS1 (White *et al.* 1990) and newly designed reverse primer in this study, Chlorella_gloriosa_1768R (5′-TGA TCC TTC TGC AGG TTC ACC-3′), were used to amplify the 18S rRNA sequence. The universal primer set ITS1/ITS4 described by White *et al.* (1990) was employed to amplify the internal transcribed spacer (ITS) region. The D1−D2 region of the large-subunit rRNA gene was amplified using the NL1/NL4 primers (O'Donnell 1993). Additionally, the regions of ribulose bisphosphate carboxylase/oxygenase (RuBisCO; *rbc*L) and plastid elongation factor (*tuf*A) were amplified with

Table 2. Results from BLAST searches using the ITS, 18S and 28S rRNA sequences, and the *rbc*L and *tuf*A genes of strain MM0063

Marker gene	Accession No.	Length (bp)	Closest match (GenBank accession No.)	Overlap (%)	Sequence similarity (%)
18S rRNA	MN513335	1,768	*Chlorella gloriosa* SAG 8.86 (KM020042)	100	99.72
ITS	MK182466	649	*Chlorocystis* sp. IOAC690S (KC137973)	100	99.54
28S rRNA	MN513337	601	*Desmochloris halophila* UTEX 2073 (HE610118)	100	93.19
*rbc*L	MN519695	1,382	*Halochlorococcum* sp. NIES-1838 (MG721795)	100	90.53
*tuf*A	MN519696	896	*Halochlorococcum* sp. NIES-1838 (MG721820)	100	85.38

primer sets *rbc*L 7F/*rbc*L 1391R (Verbruggen *et al.* 2009) and *tuf*A F/*tuf*A R (Fama *et al.* 2002; Vieira *et al.* 2016), respectively. PCR products were purified with a DokDo-Prep PCR purification kit (Elpis-Biotech, Daejeon, Korea), and purified PCR products were cloned into a pGEM-T Easy Vector System I (Promega) and sequenced at Macrogen (Daejeon, Korea). Phylogenetic analysis was performed with the 18S rRNA sequence of strain MM0063 using the software package MEGA ver. 7.0 (Kumar *et al.* 2016), and closely related sequences were downloaded from the National Center for Biotechnology Information (NCBI) database, manually trimmed, and aligned with MEGA software using the ClustalW tool. The best-fit nucleotide-substitution model (Kimura 2-parameter + Gamma distributed with Invariant sites, K2 + G + I) was selected using MEGA 7.0 based on Bayesian information criterion. This model was used to build a maximum likelihood (ML) phylogenetic tree with 1,000 bootstrap replicates. *Pseudendoclonium* spp. were used as an outgroup. DNA sequences obtained in this study were deposited in NCBI under accession numbers MK182466, MN513335, MN513337, MN519695, and MN519696 (Table 2).

4. Gas Chromatography/Mass Spectrometry (GC/MS) analysis

For biochemical analyses, the isolate was autotrophically grown in f/2 medium for 4 weeks at 18°C with shaking at 160 rpm on an SH30 orbital shaker (Fine PCR), and cells were harvested by centrifugation at 2,063 × *g* (1580R; Labogene, Daejeon, Korea). Samples were freeze-dried and pulverized to enhance extraction efficiency. Lipid extraction was performed using a modified Bligh-Dyer method developed by Breuer *et al.* (2013). The fatty acid methyl ester (FAME) composition was analyzed using a 7890A gas chromatograph equipped with a 5975C mass-selective detector (Agilent, Santa Clara, CA, USA), with GC runs performed on a DB-FFAP column (30 m, 250 μm ID, 0.25-μm film thickness; Agilent). The initial oven temperature of the gas chromatograph was 50°C and maintained for 1 min. The temperature was increased to 200°C at a rate of 10°C min^{-1} for 30 min, followed by subsequent increases to 240°C at a rate of 10°C min^{-1}, where it was held for 20 min. The injection volume was 1 μL with a split ratio of 20 : 1. Helium was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. The MS parameters were as follows: injector and source temperatures were 250°C and 230°C, respectively, and an electron-impact mode at an acceleration voltage of 70 eV was used for sample ionization, with an acquisition range from 50 m z^{-1} to 550 m z^{-1}. Compound identification was performed by matching the mass spectra with those in the Wiley/NBS libraries, with searches with a match value > 90% considered valid.

Table 3. Gradient profile and mobile-phase composition

Time (min)	Solvent A (%)	Solvent B (%)
0	100.0	0.0
20	61.8	38.2
22	25.0	75.0
33	20.0	80.0
36	10.0	90.0
37	0.0	100.0
40	0.0	100.0
42	100	0

5. High Performance Liquid Chromatography (HPLC) analysis

Pigment extraction was performed using the method developed by Zapata *et al.* (2000). Briefly, 1 mg of freeze-dried biomass was extracted in 90% HPLC-grade acetone (Daejung, Siheung, Korea) and filtered through a Whatman polytetrafluoroethylene (PTFE) syringe filter with a pore size of 0.2 μm (Whatman, Florham Park, NJ, USA). Samples were mixed with HPLC-grade H_2O (Daejung) to avoid peak distortion (Zapata and Garrido 1991) by adding 40 μL of HPLC-grade H_2O to 200 μL of each sample extract immediately before injection. Samples were then analyzed on an Agilent 1260 Infinity HPLC system (Agilent, Waldbronn, Germany) equipped with a Discovery C18 column

(25 cm × 4.6 mm, 5 μm; Supelco, Bellefonte, PA, USA) at 33°C. Pigment profiles were detected and quantified by absorbance at 440 nm. The mobile-phase gradient was programmed as described by Sanz *et al.* (2015) at a constant flow rate of 1 mL min^{-1} and comprised a mixture of methanol : 225 mM ammonium acetate (82 : 18, v : v) as solvent A and ethanol as solvent B (Table 3). HPLC-grade methanol was purchased from Daejung, and HPLC-grade ammonium acetate was obtained from Fluka (Sigma-Aldrich), respectively. Pigment standards [β-carotene, chlorophyll (Chl) *a*, Chl *b*, lutein, and violaxanthin] were purchased from Sigma-Aldrich.

6. Elemental analysis

For elemental analysis, freeze-dried biomass samples were sieved through ASTM No. 230 mesh (opening: 63 μm) for ultimate analysis. To determine carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) contents, we used a Flash 2000 elemental analyzer (Thermo Fisher Scientific, Milan, Italy). Ultimate analysis was performed in duplicate, and gross calorific values (GCVs) were estimated using the following equation developed by Friedl *et al.* (2005): $GCV = 3.55C^2 - 232C - 2{,}230H + 51.2C \times H + 131N + 20{,}600$ (MJ kg^{-1}). Protein content was calculated from the N content derived from ultimate analysis by using a conversion factor (× 6.25; Mariotti *et al.* 2008).

7. Carbohydrate analysis

For monosaccharide analysis, 50 mg of freeze-dried biomass samples were hydrolyzed in 2.5 mL 2 N sulfuric acid (Sigma-Aldrich) at 94°C for 3 h. When the reaction tubes were cooled to room temperature, a drop of 40% calcium carbonate (Sigma-Aldrich) was added to the hydrolysates. Samples were filtered through a 0.2-μm PTFE filter (Whatman) and analyzed on a Prominence Modular HPLC system (Shimadzu, Kyoto, Japan) with a Sugar-Pak I column (10 μm, 6.5 mm × 300 mm; Waters, Milford, MA, USA), with a mobile phase of 0.01 M ethylenediaminetetraacetic acid calcium disodium salt (Sigma-Aldrich) at a flow rate of 0.5 mL min^{-1}. The oven temperature was maintained at 90°C, and the injection volume was 20 μL. All monosaccharide standards (arabinose, fructose, fucose, galactose, glucose, lactose, maltose, mannitol, mannose, rhamnose, ribose, sorbitol, sucrose, and xylose) were obtained from Sigma-Aldrich. Monosaccharide contents in mg g^{-1} dry weight (DW) biomass were quantified by calculating the total peak areas of each monosaccharide derived from a calibration curve.

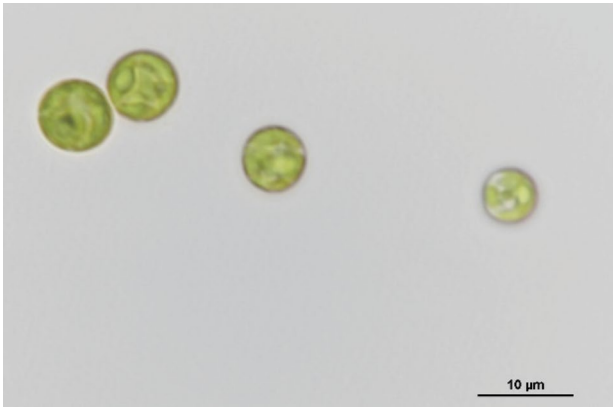

Fig. 1. Light microscopy of *Chlorella gloriosa* MM0063.

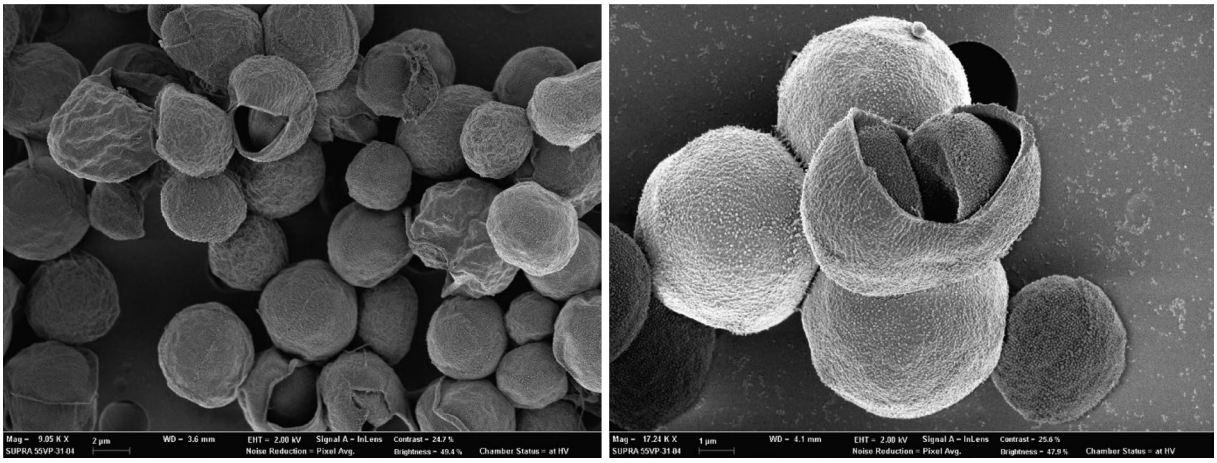

Fig. 2. FE-SEM images of *Chlorella gloriosa* MM0063.

RESULTS

The cells were non-motile and round in shape, with a diameter of ~4 μm to ~7 μm (Figs. 1, 3). Cells were surrounded by a thin and smooth cell wall, and a prominent cup-shaped chloroplast and pyrenoid were present (Figs. 1, 3). Additionally, we observed asexual propagation by the successive division of a mother cell into daughter cells and bursting of the parent wall (Figs. 2, 3). Overall, strain MM0063 showed typical morphology of the species *C. gloriosa.*

Molecular characterization inferred from sequence analyses of the 18S rRNA sequence also showed that the isolate was closely related to *C. gloriosa* species (Table 2, Fig. 4). Therefore, this marine microalga was identified as *C. gloriosa* MM0063 and deposited at the National Marine Biodiversity Institute of Korea (MABIK) and Korean Collection for Type Cultures (KCTC) under the accession numbers MABIK-LPS-0119 and KCTC13751BP, respectively. Additionally, we analyzed the ITS region, 28S rRNA sequence, and *rbc*L and *tuf*A genes, but no conclusive evidence of its taxonomic position was found based on BLAST searches using the NCBI database (Table 2).

The FAME profile of *C. gloriosa* MM0063 is reported in Table 4. The major cellular fatty acids of the isolate were $C_{16:0}$ (22.5% ± 0.4%), $C_{18:2}$ n-6 (10.6% ± 0.6%), and $C_{18:3}$ n-3 (30.6% ± 0.2%). Strain MM0063 was rich in polyunsaturated fatty acids (PUFAs), and trace amounts of alkane ($C_{15}H_{32}$, 1.1% ± 0.1%), saturated fatty acids (SFAs; $C_{14:0}$, 2.7% ± 0.1%; $C_{15:0}$, 1.0% ± 0.3%; and $C_{18:0}$, 1.0% ± 0.3%), and unsaturated fatty acids ($C_{16:1}$ n-7, 0.4% ± 0.0%; $C_{16:2}$ n-4, 1.4% ± 0.1%; $C_{16:3}$ n-3, 5.4% ± 0.5%; $C_{16:4}$ n-3, 7.1% ± 0.4%; and $C_{18:1}$ n-9, 2.2% ± 0.4%) were detected.

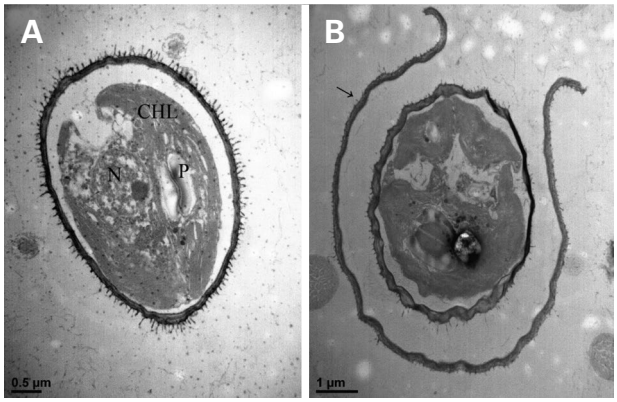

Fig. 3. (A) TEM images of *Chlorella gloriosa* MM0063. CHL: chloroplast; N: nucleus; P: pyrenoid. (B) The arrow indicates the ruptured parental cell wall.

The pigment profile of *C. gloriosa* MM0063 is summarized in Table 5. The major pigments of the isolate were Chl *a* (43.6% ± 0.4%), Chl *b* (11.3% ± 0.3%), and lutein (27.0% ± 0.3%), and the minor carotenoids of the isolate were violaxanthin (2.4% ± 0.4%) and β-carotene (5.2% ± 0.7%). Other minor pigment peaks were not identified.

The elemental composition of strain MM0063 biomass is presented in Table 6. The GCV and protein content based on ultimate analysis were 13.8 MJ kg^{-1} and 23.0%, respectively.

The carbohydrate composition of the isolate is presented in Table 7. Its major monosaccharides were glucose (48.8%, 124.1 mg g^{-1} DW), galactose (28.6%, 75.1 mg g^{-1} DW), and arabinose (11.7%, 27.8 mg g^{-1} DW), and a small portion of minor sugars (fructose 7.3%, mannitol 1.4%, and sorbitol 2.1%) were detected.

DISCUSSION

Light and electron microscopy observations suggested that the isolate shared typical morphological characteristics with *C. gloriosa*, including the presence of a smooth layer in the cell wall and a pyrenoid in the chloroplast (Figs. 1, 3). Additionally, binary fission into daughter cells and ruptured parent cells were observed by both light and electron microscopy (Figs. 2, 3). These features agree with the original description of *C. salina* by Butcher (1952) and those of the recently accepted *C. gloriosa* strain by Calvo-Pérez Rodó *et al.* (2015). Moreover, our phylogenetic inferences and genomic analyses confirmed that strain MM0063 belongs to *C. gloriosa*. The sequencing of organelle genomes for *C. gloriosa* MM0063 and *C. gloriosa* SAG 8.86. (= CCAP 6005/8) should be conducted in the near future, because comparative studies and phylogenetic analyses will provide important information on the evolution of both the microalgae and their organelles, such as mitochondria and chloroplasts. These kinds of phylogenomics studies have recently allowed insight into the evolution of Trebouxiophyceae (Martínez-Alberola *et al.* 2019). Other molecular markers, such as the ITS, 28S rRNA sequence, and the *rbc*L and *tuf*A genes, were unable to provide appropriate information concerning taxonomic positions due to the lack of sequence information available in public databases. Currently, *C. gloriosa* SAG 8.86 (GenBank accession No. KM020042) is the only available sequence of *C. gloriosa* species; therefore, our results provide useful data for future taxonomic studies of *C. gloriosa* and its close relatives.

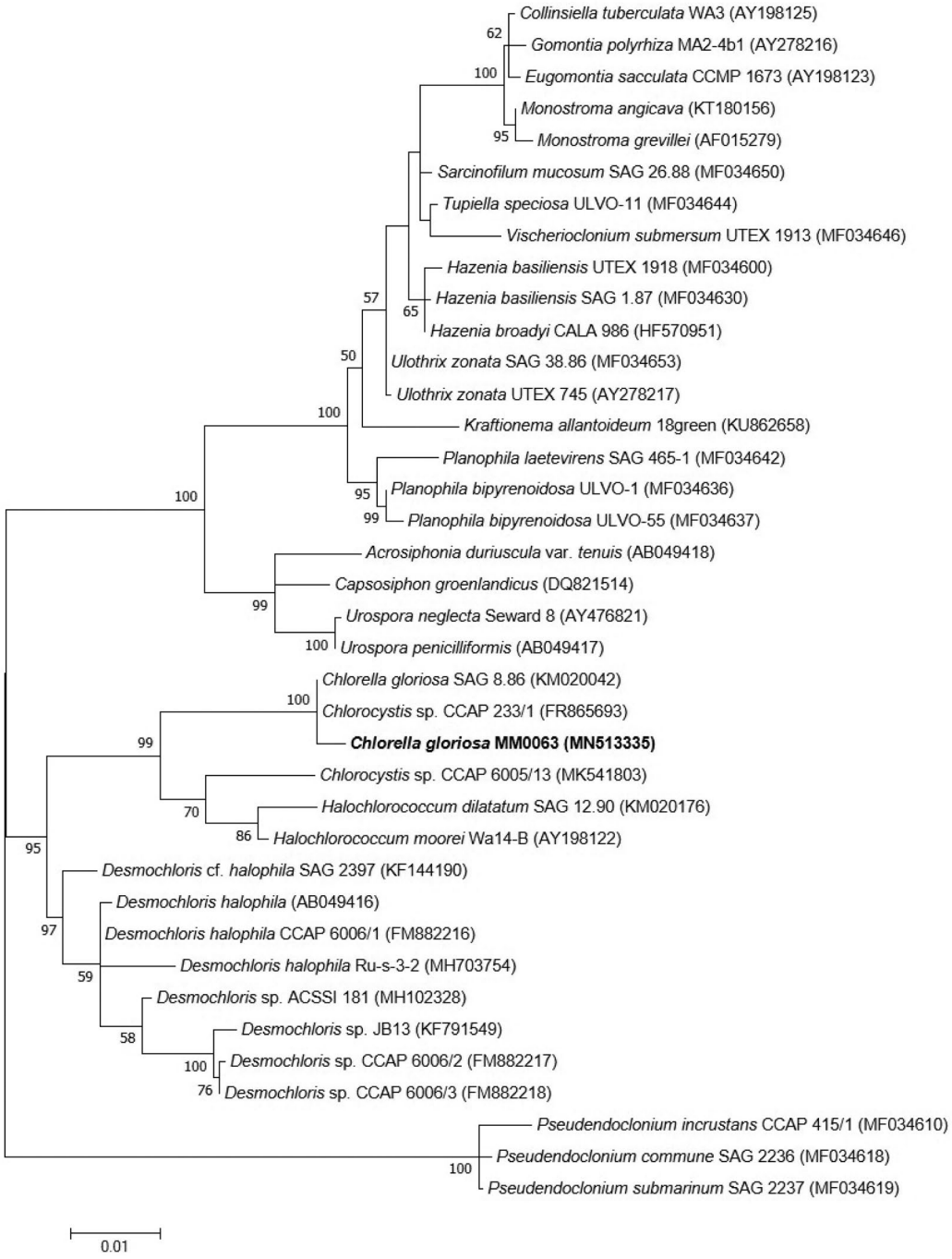

Fig. 4. The phylogenetic relationship between strain MM0063 and its closely related species inferred from the 18S rRNA sequence. The tree was generated by the ML method using 1,000 bootstrap replicates. The scale bar represents a 1% difference in nucleotide sequences.

Analysis of the cellular fatty acid composition of strain MM0063 revealed a richness in $C_{16:0}$ (22.5%) SFAs and $C_{18:2}$ n-6 (10.6%) and $C_{18:3}$ n-3 (30.6%) PUFAs. Previous studies report that essential omega-3 and -6 PUFAs exert a variety of beneficial health effects (Mehta *et al.* 2009). Omega-3 PUFAs are mainly derived from marine sources, such as fish oils, and a variety of commercial products are available worldwide. Given global climate change and overfishing issues, as well as increasing environmental pollution, including heavy metals and radioactive materials in the

Table 4. Lipid profile of strain MM0063

Component	Content (%)[a]	Note
Pentadecane ($C_{15}H_{32}$)	1.1±0.1	Alkane (minor)
4-Methylbenzaldehyde (C_8H_8O)	0.1±0.0	
Myristic acid ($C_{14:0}$)	2.7±0.1	
Pentadecanoic acid ($C_{15:0}$)	1.0±0.3	
Palmitic acid ($C_{16:0}$)	22.5±0.4	SFA (major)
Palmitoleic acid ($C_{16:1}$ n-7)	0.4±0.0	
Hexadecadienoic acid ($C_{16:2}$ n-4)	1.4±0.1	
Roughanic acid ($C_{16:3}$ n-3)	5.4±0.5	
Hexadecatetraenoic acid ($C_{16:4}$ n-3)	7.1±0.4	
Stearic acid ($C_{18:0}$)	1.0±0.3	
Oleic acid ($C_{18:1}$ n-9)	2.2±0.4	
Linoleic acid ($C_{18:2}$ n-6)	10.6±0.6	Omega-6 PFUA (major)
α-Linolenic acid ($C_{18:3}$ n-3)	30.6±0.2	Omega-3 PFUA (major)

[a] Values represent the average±standard deviation of three independent experiments.

Table 5. Pigment profile of strain MM0063

Peak number	Retention time	Compound	Content (%)[a]
1	16.5	–	3.9±0.2
2	19.5	Violaxanthin	2.4±0.4
3	25.2	Lutein	27.0±0.3
4	26.0	–	6.7±0.4
5	28.7	Chl *b*	11.3±0.3
6	31.9	Chl *a*	43.6±0.4
7	40.9	β-carotene	5.2±0.7

[a] Values represent the average±standard deviation of three independent experiments.

Table 6. Ultimate analysis results of *C. gloriosa* MM0063

Elemental composition	Ultimate analysis (wt%)[a]
C	31.9±0.2
H	5.7±0.1
N	3.7±0.0
S	2.0±0.1

[a] Values represent the average±standard deviation of three independent experiments.

Table 7. Monosaccharide composition of strain MM0063

Compound	Content	
	%	mg g^{-1} DW
Arabinose	11.7	27.8
Fructose	7.3	19.0
Galactose	28.6	75.1
Glucose	48.8	124.1
Mannitol	1.4	3.5
Sorbitol	2.1	5.6

ocean, the sustainability of marine fish as a safe resource of omega-3 PUFAs is in question (Kang 2011; Jeromson *et al.* 2015). Therefore, this marine microalga might be useful as a clean and sustainable omega-3 alternative to fish-based oil.

Pigment analysis showed that the isolate is able to biosynthesize a high concentration of lutein, a carotenoid with antioxidant properties and extensively used in the food, pharmaceutical, and cosmetics industries. Lutein has recently received increased attention because of its beneficial effects on eye health (Buscemi *et al.* 2018). Commercial sources are mainly extracted from marigold flowers; however, the lutein content in *Tagetes erecta* is very low, and the extraction process requires several steps that result in low yields (Ausich 1997; Piccaglia *et al.* 1998; Del Campo *et al.* 2007; Vechpanich and Shotipruk 2011). Additionally, the carotenoid violaxanthin is biosynthesized by the isolate as a minor pigment component, with this compound exhibiting anti-inflammatory effects and widely used as a food colorant (Soontornchaiboon *et al.* 2012; Raposo *et al.* 2015; Torregrosa-Crespo *et al.* 2018). Therefore, *C. gloriosa* MM0063 biomass mass-cultivated under controlled conditions could serve as alternative sources of lutein and violaxanthin.

Quantitative and qualitative analyses of monosaccharides in microalgae are essential steps for the optimal utilization of microalgal biomass (Ortiz-Tena *et al.* 2016). Carbohydrate analysis revealed that the major monosaccharides of the isolate include glucose, galactose, and arabinose. The arabinose content of strain MM0063 (27.8 mg g^{-1} DW) was higher than that of sugarcane bagasse (~11.0–17.0 mg g^{-1} DW), which is a typical feedstock for arabinose production (Templeton *et al.* 2012). Additionally, arabinose reportedly exerts beneficial effects on health (Seri *et al.* 1996; Liu *et al.* 2013) and was approved for use as a safe food additive (i.e., a low-

calorie sweetener) by the United States Food and Drug Administration. Moreover, arabinose is a legal food additive in China and Japan (Hu *et al.* 2018), and arabinose-containing functional foods are popularly marketed as beneficial for pre-diabetic and diabetic patients in these countries. Therefore, this isolate is potentially useful as an alternative to current terrestrial plant-based sources. These findings contribute to a better understanding of the diversity of microalgal monosaccharides.

The physiological properties reported here offer insight into chemotaxonomic markers, as well as morphological and molecular characteristics, of the isolated strain. Moreover, the results support future improvements in the production of high-value products through evaluation of the effects of various culture conditions, such as adding additional carbon sources and modifying media components, on stain MM0063.

In this study, we provided the first record of *C. gloriosa* in Korea. This marine microalga could serve as a promising candidate for further phylogenetic and evolutionary studies, as well as a potential biological resource to produce biochemicals of commercial interest in industrial fields. Since a large number of domestic Trebouxiophyceae still remain unknown (Kim *et al.* 2018), further efforts should be made in order to explore the diversity of Trebouxiophyceae in Korea.

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