J. Microbiol. Biotechnol. (2015), 25(2), 238–246
http://dx.doi.org/10.4014/jmb.1410.10032

jmb

Morphological, Molecular, and Biochemical Characterization of Astaxanthin-Producing Green Microalga *Haematococcus* sp. KORDI03 (Haematococcaceae, Chlorophyta) Isolated from Korea

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Received: October 15, 2014
Revised: November 4, 2014
Accepted: November 4, 2014

First published online
November 10, 2014

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pISSN 1017-7825, eISSN 1738-8872

A unicellular red microalga was isolated from environmental freshwater in Korea, and its morphological, molecular, and biochemical properties were characterized. Morphological analysis revealed that the isolate was a unicellular biflagellated green microalga that formed a non-motile, thick-walled palmelloid or red aplanospore. To determine the taxonomical position of the isolate, its 18S rRNA and *rbcL* genes were sequenced and phylogenetic analysis was performed. We found that the isolate was clustered together with other related *Haematococcus* strains showing differences in the *rbcL* gene. Therefore, the isolated microalga was classified into the genus *Haematococcus*, and finally designated *Haematococcus* sp. KORDI03. The microalga could be cultivated in various culture media under a broad range of pH and temperature conditions. Compositions of the microalgal cellular components were analyzed, and its protein, carbohydrate, and lipid compositions were estimated to be 21.1 ± 0.2%, 48.8 ± 1.8%, and 22.2 ± 0.9%, respectively. In addition, D-glucose and D-mannose were the dominant monosaccharides in the isolate, and its amino acids were composed mainly of aspartic acid, glutamic acid, alanine, and leucine. Moreover, several polyunsaturated fatty acids accounted for about 80% of the total fatty acids in *Haematococcus* sp. KORDI03, and the astaxanthin content in the red aplanospores was estimated to be 1.8% of the dry cell weight. To the best of our knowledge, this is the first report of an *Haematococcus* sp. isolated from Korea, which may be used for bioresource production in the microalgal industry.

Keywords: *Haematococcus* sp. KORDI03, astaxanthin, bioresource production, phylogenetic analysis

Introduction

The genus *Haematococcus* described by Flotow (1844) is a freshwater unicellular microalga belonging to the class Chlorophyceae [10, 36]. It is widely distributed worldwide, and its natural habitats are characterized by their unstable temporary conditions, such as small rock pools, water holes, and other small natural (or artificial) water bodies, including birdbaths [10, 25]. To date, a total of seven species have been taxonomically classified in the genus *Haematococcus* at AlgaeBase (http://www.algaebase.org/), and *H. pluvialis* Flotow has been thoroughly investigated among these species. This microalga tends to accumulate the valuable carotenoid pigment, which is known as astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′dione), that can protect against high light intensity and oxygen radicals [28, 38]. However, to the best of our knowledge, there have been no reports on the isolation of *Haematococcus* in Korea.

Astaxanthin is one of the most valuable ketocarotenoids owing to its biological function as a vitamin A precursor [16] and as a more efficient antioxidant than β-carotene and vitamin E [24]. Although the majority of this carotenoid is synthetically derived, it is generally used as a food coloring agent or natural feed additive in aquaculture and poultry, and for medicinal and cosmetic applications owing to its efficient antioxidant capacity [28]. Astaxanthin is produced by several microalgae and other microorganisms, such as *Haematococcus* [38], *Chlorella zofungiensis* [4], the red yeast *Phaffia rhodozyma* [6, 32], and the marine bacterium *Agrobacterium aurantiacum* [15, 37]. The content of astaxanthin reported in these microorganisms was between 0.04% and 2.7% of the dry cell weight [6, 11, 17, 21, 32], and the green microalga *Haematococcus* is known as the richest source of natural astaxanthin production among these microalgae [28].

Since 2008, we have screened several microalgae strains with advantageous properties for the production of biodiesel and high-value products. In this study, we identified and characterized a new strain of *Haematococcus* (herein designated *Haematococcus* sp. KORDI03), which was isolated at the Korea Institute of Ocean Science & Technology (KIOST, Ansan, Korea). The morphological and biological properties of the isolated microalga were investigated, and its taxonomical position was analyzed based on the molecular characteristics. Furthermore, the composition of the cellular components (including astaxanthin) in the isolate was determined, showing that this isolate is a strong candidate for the production of high-value products.

Materials and Methods

Microalgal Isolation and Culture Conditions

In April 2009, pale-reddish standing water was observed by the naked eye on the rooftop surface of a building (longitude 126°83′E, latitude 37°29′N) at the KIOST, and it gradually turned dark red. Sparsely aggregated red microalgal populations were observed in the standing water by light microscopy; therefore, the water (average temperature 23.8°C, pH 7.5) was sampled in May 2009. From the collected water sample, red microalgal cells were isolated using micropipettes, and serially diluted and incubated in 96-well plates at 25°C under 12 h light/12 h dark cycles at 100 μmol photons/m^2/s in BG-11 medium [1]. The uniagal wells were then streaked on BG-11 agar plates and cultured under the same conditions mentioned above, and single colonies were repeatedly subcultured until a pure isolate was obtained. The growth of the isolated microalga was examined on Tris-acetate phosphate (TAP) medium [22], Bold's basal medium (BBM) [30], and BG-11 medium under the same culture conditions described above. The growth characteristics of the microalgal isolate was determined at a range of salinity, pH, and temperature conditions, with salinity varying from 0 to 50 psu (adjusted using NaCl), pH ranging from 3 to 11 (adjusted using NaOH or HCl), and temperature ranging from 10°C to 35°C under the same conditions as mentioned above, excluding the variants.

Microscopy

The isolated microalga was examined under a light microscope (LM, Eclipse 80i; Nikon Co.). Images were obtained using a camera (DXM 1200C; Nikon Co.), and the cellular size was calculated with an image analyzer (NIS-Elements BR 3.0; Nikon Co.). For scanning electron microscopy (SEM) analysis, the microalgal cells were fixed in 2.5% glutaraldehyde, post-fixed in 2% osmium tetroxide, dehydrated in an ethanol series (30.0%, 50.0%, 70.0%, 80.0%, 90.0%, 95.0%, and 100%), dried with 100% hexamethyldisilazane, and then gold-coated for 90 sec in a Sputter Coater (SCD050; BAL-TEC). Coated samples were examined by field emission SEM (AURIGA; Carl Zeiss). For ultrastructural analysis, the cells were fixed, post-fixed, stained with 0.5% uranyl acetate, dehydrated as described above, transited with 100% propylene oxide, and embedded in Spurr's resin [34]. The samples were sectioned using an ultra-microtome (EM UC7; Leica Microsystems), stained with 2% uranyl acetate and lead citrate, and examined using transmission electron microscopy (TEM; JEM1010; JEOL Ltd.).

Molecular Identification and Phylogenetic Analysis

Genomic DNA was extracted using a DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's instructions. Amplification of the 18S rRNA gene was performed with primers SR1 [20] and 18SC2M (5′-GCA GGT TCA CCT ACG GAR ACC-3′), which were slightly modified based on the 18SC2 primer [3]. PCR conditions were as follows: initial denaturation at 94°C for 5 min, 30 cycles of 94°C for 40 sec, 50°C for 40 sec, and 72°C for 2 min, and a final extension at 72°C for 10 min. To amplify the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene from the isolate, primers ChlorRbclF (5′-GCN GGT GTW AAA GAY TAY GG-3′) and ChlorRbclR (5′-TAC CAC CWG AAG CWA CHG GCA-3′) were designed based on the conserved regions of the known sequences from Chlorophyta, and PCR was performed with the following steps: pre-denaturation at 95°C for 3 min, followed by amplification for 30 cycles at 95°C for 30 sec, 52°C for 60 sec, and 72°C for 90 sec, and a final extension at 72°C for 7 min. The amplified approximately 1 kb PCR product was cloned into the pCR2.1-TOPO vector using the TOPO TA Cloning kit (Invitrogen) and sequenced at Macrogen Inc. (Korea). Sequencing of the middle regions in the amplified 18S rRNA fragments was performed using the 18SJMID primer (5′-CAA TAG CGT ATA TTT AAG TTG-3′).

The obtained 18S rRNA and *rbcL* sequences were compared with the sequences of related taxa obtained from the GenBank

database at the National Center for Biotechnology Information using a BLASTN search (http://www.ncbi.nlm.nih.gov/BLAST). The sequences were aligned with other sequences using MEGA 4.0, and nucleotide differences and genetic distances among the sequences were estimated. The phylogenetic trees were reconstructed using the maximum-likelihood method by the PhyML3.0 program [18] with the K2P nucleotide substitution model, and using the Bayesian method by the Mrbayes program [23] with the 4 by 4 codon models. For the maximum-likelihood analysis, the transition/transversion ratio, proportion of invariable sites, and gamma distribution parameters were optimized with four substitution categories, and bootstrapping values were calculated from 1,000 replicates. For the Bayesian method, the number of generations was 1,000,000, and the chains were sampled every 100th generation to calculate the posterior probability of each node after burning the first 25% of the chains.

Biochemical Component Analysis

The proportion of major components (ash, carbohydrate, lipid, moisture, protein) in lyophilized microalgal cells was determined according to the Association of Official Analytical Chemists (AOAC) [2]. Crude lipid and protein content were determined using the Soxhlet Method (AOAC Official Method 920.39) and the Kjeldhal Method (AOAC Official Method 976.05), respectively. The ash content was determined by weighing samples before and after heating in a furnace at 550°C, the moisture content was determined by weighing the sample before and after 24 h in a dry oven at 105°C, and the carbohydrate content was determined by the difference. Amino acid profiles in the isolate were analyzed by the amino acid analyzer (Sykam S433; Sykam) using the ninhydrin method, and the monosaccharide composition was determined using high-performance anion-exchange chromatography coupled with a pulsed amperometric detection system (HPAEC-PAD; Dionex Corp.). Lipids in the isolate were extracted three times with a mixture of dichloromethane and methanol (1:1 (v/v)), as described by Bligh and Dyer [5]. Internal standard (*n*-nonadecanoic acid; Sigma-Aldrich Co.) was added to the samples, and the remaining solvent was dried under the presence of N_2 gas. The extracted lipids were subjected to alkaline hydrolysis using 0.5 N KOH with heating at 70°C for 30 min. A mixture of hexane and diethyl ether (9:1 (v/v)) was added three times in the samples, after which alcohol and sterol were removed. The fatty acids were extracted with a mixture of hexane and diethyl ether (9:1 (v/v)), and were converted into fatty acid methyl ester (FAME) using BF3-MeOH with heating at 70°C for 30 min. The FAME was isolated three times with a mixture of hexane:diethyl ether (9:1). The double-bond position of monounsaturated fatty acids (MUFAs) in the samples was determined by treating a subsample of FAMEs with dimethyl disulfide [31]. The fatty acids were quantified using gas chromatography (GC; Agilent 7890A; Agilent Technologies) equipped with a flame ionization detector with a ZB-5MS column (60 m × 0.32 mm × 0.25 μm; Phenomenex), with helium as the carrier gas. The samples were injected in splitless mode at an initial oven temperature of 50°C, an injector temperature of 250°C, and a detector temperature of 320°C. The oven temperature was then ramped at 10°C/min to 120°C and 4°C/min to a final temperature of 300°C. Individual fatty acids were identified using a gas chromatography-mass spectrometry detector (GC-MSD; Agilent 7890A GC-Agilent 5975C MSD; Agilent Technologies) operating at 70 eV with a mass range acquisition of 50−700 amu. The column and its setting conditions for GC-MSD were similar to those of GC-FID. Fatty acids were named by shorthand IUPAC nomenclature of A:B (n-x), where A is the number of carbons in individual fatty acids, B is the number of double bonds, and X of n-X is the position of the first double bond numbered from the terminal methyl end (-CH_3) of the fatty acid.

Astaxanthin Analysis

Authentic astaxanthin standard isolated from *H. pluvialis* was purchased from Wako Pure Chemical Industries, Ltd. (Japan) and used in this study. For the extraction of astaxanthin from our isolate, 200 mg of the lyophilized microalgal cells in the aplanospore stage was homogenized using a tissue homogenizer, and the total pigments were extracted in 10 ml of acetone. After 1 min of sonication, the mixture of microalgal cells and solvent was separated by centrifugation at 3,000 rpm for 10 min, and the supernatant containing pigments was collected. The extraction procedure was repeated at least three times until the cell debris was almost colorless. All of the above processes were conducted in the dark.

Analysis was performed on the YL9100 HPLC system (Young Lin Co.) equipped with an YL9110 pump and YL9160 photodiode array (PDA) detector. Astaxanthin was analyzed with an YMC C30 (YMC, Schermbeck) analytical column (5 mm, 250 × 4.6 mm I.D.). The mobile phase was an isocratic mixture of acetone and water (84:16 (v/v)) for the first 21 min, followed by a 4 min linear gradient to 97:3 acetone:water (v/v) for the remaining 50 min run. The flow rate was maintained at 1 ml/min. The detection wavelength of the PDA detector was set between 190 and 700 nm, and the chromatographic peaks were measured at a wavelength of 480 nm to facilitate astaxanthin detection [14].

Nucleotide Sequence Accession Numbers and Strain Deposition

Partial sequences of the 18S rRNA and *rbcL* genes of *Haematococcus* sp. KORDI03 were deposited in GenBank under the accession numbers FJ877140 and GU395291, respectively. A living culture of *Haematococcus* sp. KORDI03 was deposited in the Korean Collection for Type Cultures (KCTC) at the Korean Research Institute of Bioscience and Biotechnology under the accession number KCTC 12348BP.

Results and Discussion

Cell Morphology and Ultrastructure

Morphological characteristics of the isolated microalga

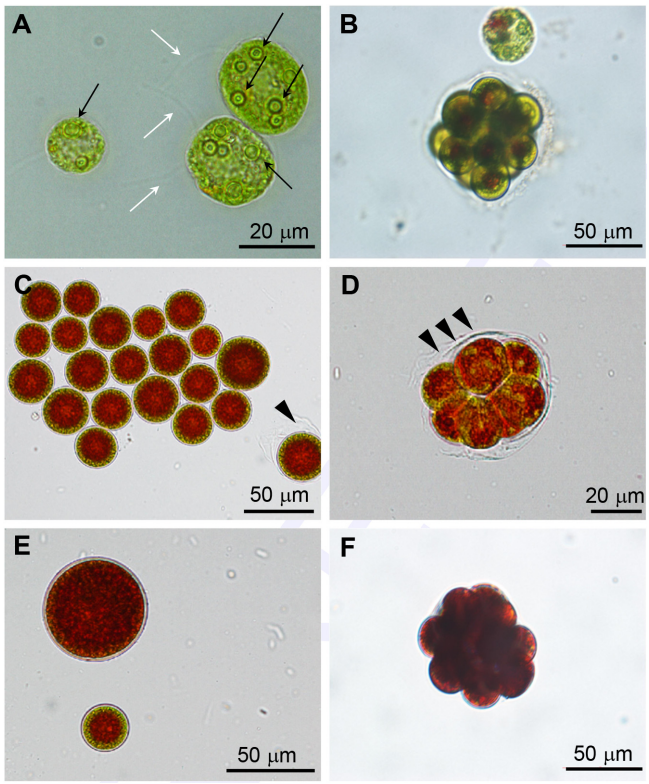

Fig. 1. Light micrographs of *Haematococcus* sp. KORDI03 in an axenic culture.

The biflagellate (white arrow) vegetative cells were ovoid to ellipsoidal with broadly rounded ends, and several pyrenoids occupied the whole cell's periphery (black arrow) (**A**). Asexual reproduction of non-motile palmelloid cells with some astaxanthin accumulation (**B**). Astaxanthin accumulated towards the center of intermediate cells (**C**). The intermediate cells divided by asexual reproduction (**D**). Note that the cell wall was surrounded by gelatinous extracellular matrix (arrowheads). Large red aplanospores (≥50 μm) (**E**) and asexual reproduction of aplanospore (**F**) were observed.

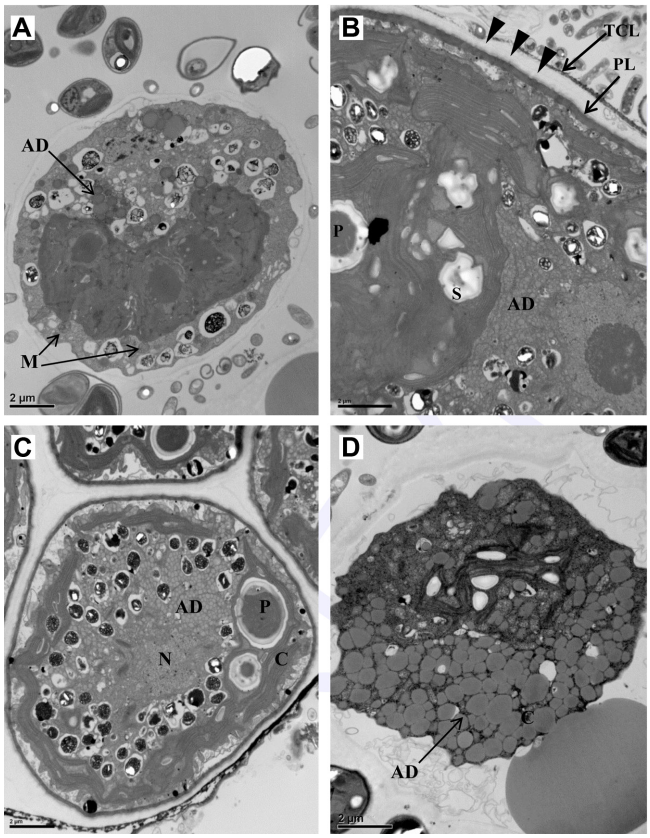

Fig. 2. Transmission electron micrographs of sectioned *Haematococcus* sp. KORDI03 cells.

General ultrastructure of the palmelloid cell (**A**) and high-magnification view of its cellular organelles (**B**). Note that the cell wall is surrounded by gelatinous extracellular matrix (arrowheads) between the intermediate tripartite crystalline layer and plasmalemma. General ultrastructure of intermediate cells in the asexual reproduction stage (**C**). Astaxanthin-containing lipid droplets accumulate towards the center of intermediate cells. General ultrastructure of red aplanospore showing large oil droplets (**D**). Some oil droplets are fused and extruded. C, Chloroplast; P, Pyrenoid; M, Mitochondria; G, Golgi apparatus; S, Starch granule; AD, Astaxanthin-containing lipid droplet; N, Nucleus; TCL, Intermediate tripartite crystalline layer; PL, Plasmalemma.

were investigated based on the LM and SEM analyses. From the axenic microalgal culture, motile-flagellated cells (vegetative cells, Fig. 1A), non-motile intermediate cells (palmelloid with some astaxanthin accumulation, Fig. 1B), and red cyst cells (aplanospores, Figs. 1C–1F) were observed. During the laboratory culture, the flagellated cells were changed to cells in the non-motile palmelloid stage within relatively short periods (1–2 days), and finally developed into the aplanospore stage, which formed crimson-colored red cysts. The biflagellate vegetative cells were ovoid to ellipsoidal with broadly rounded ends, and were 24.9 ± 4.2 μm in length and 23.0 ± 5.2 μm in diameter (Fig. 1A). Cells of the palmelloid stage reproduced asexually by cell division and formed 8 to 32 zoospores (Fig. 1B), which frequently developed into thick-walled red cysts after culturing for more than 10 days (Figs. 1D and 1F). Both non-motile green or red spherical palmelloid cells were 30.9 ± 10.2 μm in diameter on average, and relatively large red cyst cells (≥50 μm) were occasionally observed (Fig. 1E). The thick-walled red cyst cells were surrounded by a gelatinous extracellular matrix (Figs. 1C and 1D).

Based on ultrastructural analysis using TEM, several cellular organs such as the nucleus, chloroplast, pyrenoid, mitochondria, starch granule, and astaxanthin-containing lipid droplets were observed in the *Haematococcus* isolate (Figs. 2A–2D). Protoplasts could separate from the cell

wall (to which it was connected by thin branched or unbranched cytoplasmic strands), and the space between the cell wall and protoplast filled with watery mucilage or gelatinous surrounding wall materials (Fig. 2A). The nucleus was typically found in the center of the cell, and mitochondria and thylakoids were observed on the edge of the cell (Figs. 2A and 2B). Several small starch granules were located between the thylakoids, and the pyrenoid was surrounded by large starch granules that were generally observed during the dividing stages (Figs. 2C and 2D). During asexual reproduction, peripheral formation of the astaxanthin-containing lipid droplets was gradually observed in intermediate cells in the palmelloid stage, and these droplets were densely dispersed throughout cyst cells in the aplanospore stage (Figs. 2A and 2B).

Molecular Identification and Phylogenetic Analysis

The obtained 18S rRNA (1,748 bp) and *rbcL* (1,102 bp) genes of the isolate were compared against the GenBank database. The sequenced 18S rRNA gene was highly similar to those derived from the genus *Haematococcus*. According to the BLASTN search against 18S rRNA, the

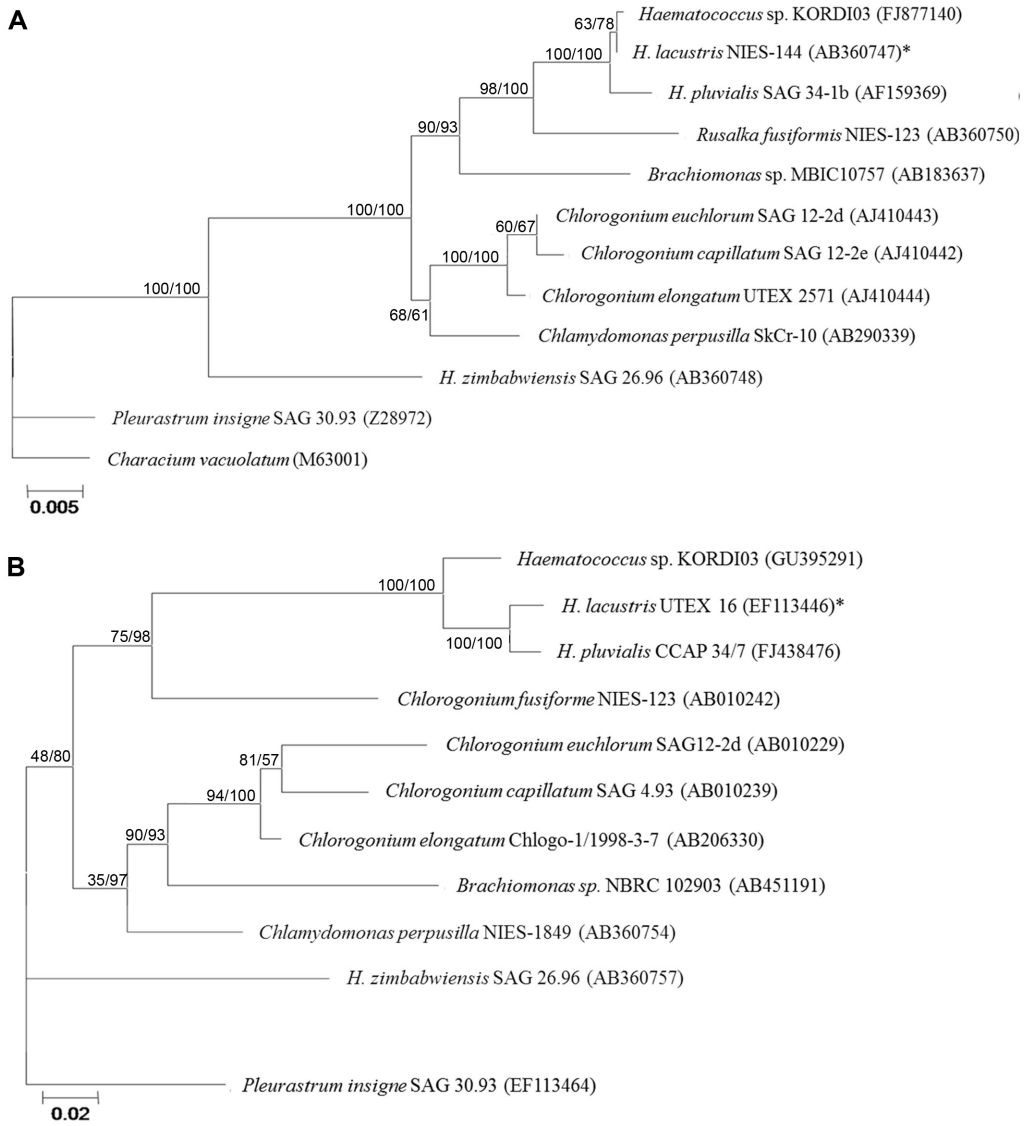

Fig. 3. Maximum clade credibility trees reconstructed based on the K2P model of nucleotide substitution using a dataset of 12 18S rRNA sequences (**A**) and 11 *rbcL* sequences (**B**) derived from the genus *Haematococcus* and other similar microalgal strains.
Values at the branches indicate maximum-likelihood bootstrap (1,000 replicates)/Bayesian inference posterior probability. The scale bar represents 0.005 and 0.02 nucleotide substitutions per site, respectively. **H. lacustris* has been synonymized with *H. pluvialis*.

most similar species were *H. lacustris* NIES-144 (GenBank Accession No. AB360747), which had been synonymized with *H. pluvialis* [19] and showed 99.9% sequence identity, followed by *H. pluvialis* SAG 34-1b (GenBank Accession No. AF159369) with 99.6% identity, and *H. zimbabwiensis* SAG 26.96 (GenBank Accession No. AB360748) with 95.6% identity. In addition, the *rbcL* gene was compared with other sequences derived from the genus *Haematococcus* in GenBank, and was most similar to *H. lacustris* (synonymized with *H. pluvialis*) UTEX 16 (GenBank Accession No. EF113446) with 96.2% sequence identity, followed by *H. pluvialis* CCAP 34/7 (GenBank Accession No. FJ438476) with 96.0%, and *H. zimbabwiensis* SAG 26.96 (GenBank Accession No. AB360757) with 85.8% identity among the matched *Haematococcus* strains that had more than 80% query coverage.

To determine the precise phylogenetic position of this isolate in the genus *Haematococcus*, detailed phylogenetic analyses were conducted using a dataset of 12 18S rRNA and 11 *rbcL* gene sequences derived from *Haematococcus* and other relatives of the phylum Chlorophyta. The resultant tree using the 18S rRNA genes suggested that the newly isolated microalga was closely related to other *Haematococcus* species, including *H. pluvialis* and *H. lacustris* (synonymized with *H. pluvialis*) (Fig. 3A). Therefore, the isolate was taxonomically classified into the genus *Haematococcus*, and was designated *Haematococcus* sp. KORDI03. However, *H. zimbabwiensis* clearly formed distinct lineages from other related *Haematococcus* species in our phylogenetic analysis, suggesting that the genus *Haematococcus* is polyphyletic as previously reported [9, 10, 13, 29, 33]. Similar results were obtained based on the phylogenetic trees inferred using *rbcL* genes (Fig. 3B). Phylogenetic analysis of the *rbcL* genes clustered *Haematococcus* sp. KORDI03 with *H. pluvialis* and *H. lacustris* (synonymized with *H. pluvialis*) strains, but the isolate formed a separate lineage showing divergence from these species, and supported its uniqueness.

Growth Characteristics

Haematococcus sp. KORDI03 could be cultivated on BG-11, BBM, and TAP medium without significant differences. However, regardless of its culture media, our isolate predominantly remained as red cyst cells. When the old cultures were inoculated into fresh media, the newly produced flagellated cells transformed into non-motile palmelloid cells within only 1–2 days. These physiological characteristics were similar to the previous report of *H. pluvialis*, which were mostly in the palmelloid stage [25]. In this study, *Haematococcus* sp. KORDI03 could survive under a broad range of pH conditions (pH 3–12), and its growth significantly improved at pH 5–9. The isolate could also be grown under a wide range of temperatures (15–30°C), with maximum growth at 20°C. However, it retained viability at temperatures as low as 10°C. Moreover, the isolate could survive under sub-saline conditions (up to 10 psu), but was not tolerant to saline conditions.

Biochemical Composition and Pigment Analysis

To evaluate the potential of *Haematococcus* sp. KORDI03 as a novel bioresource, the proportion of crude cellular components (protein, carbohydrate, ash, lipid, moisture) was determined. From the lyophilized red cyst cells, the content of ash, carbohydrate, lipid, moisture, and protein was estimated to be 4.9 ± 0.8%, 48.8 ± 1.8%, 22.2 ± 0.9%,

Table 1. Comparison of cellular components in lyophilized *Haematococcus* sp. KORDI03 and *H. pluvialis*.

Contents (%)	Protein	Lipid	Carbohydrate	Ash	Moisture	Reference
Haematococcus sp. KORDI03	21.1 ± 0.2	22.2 ± 0.9	48.8 ± 1.8	4.9 ± 0.8	3.1 ± 0.1	This study
H. pluvialis	23.6	13.8	38.0	17.7	6.0	[27]

Table 2. Comparison of amino acid content (mg/100 mg) and composition (%) in *Haematococcus* sp. KORDI03.

	Asp	Thr	Ser	Glu	Gly	Ala	Cys2	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	NH_4	Arg	Pro	Total
Contents (mg/100 mg)	1.18	0.56	0.59	1.37	0.63	0.12	-[a]	0.75	-	0.49	1.11	0.27	0.49	0.58	0.58	0.24	-	-	10.02
Composition (%)	11.8	5.6	5.9	13.7	6.3	11.8	-	7.5	-	4.9	11.1	2.7	4.9	5.8	5.8	2.4	-	-	100.0

Asp: aspartic-acid; Thr: threonine; Ser: serine; Glu: glutamic acid; Gly: glycine; Ala: alanine; Cys2: cystine-cystine dimer; Val: valine; Met: methionine; Ile: isoleucine; Leu: leucine; Tyr: tyrosine; Phe: phenylalanine; His: histidine; Lys: lysine; NH_4: ammonia; Arg: arginine; Pro: proline.

[a]Not detected.

3.1 ± 0.1%, and 21.1 ± 0.2%, respectively. The overall composition of crude cellular components in *Haematococcus* sp. KORDI03 was similar to that reported in *H. pluvialis* [27], except for the relatively high accumulation of crude lipids in our isolate (Table 1). Furthermore, the composition of amino acids, monosaccharides, and fatty acids in *Haematococcus* sp. KORDI03 was examined. The total amino acid content in our isolate was estimated to be 10.02 mg/100 mg, composed of 46.0% essential amino acids. The general amino acid profile of *Haematococcus* sp. KORDI03 was similar to *H. pluvialis* [27], and glutamic acid, aspartic acid, alanine, and leucine were dominantly detected from both *Haematococcus* strains. However, different from *H. pluvialis*, several amino acids such as arginine, cysteine, methionine, and proline were not detected in our isolate (Table 2). The total monosaccharide content was estimated to be 20.92 mg/100 mg, and D-glucose (46.0%) and D-mannose (40.9%) were the dominant monosaccharides in *Haematococcus* sp. KORDI03 (Table 3). These results were similar to a previous report [8] showing that the dominant amino acid and monosaccharide in Chlorophyceae were glutamic acid and glucose, respectively.

The fatty acid composition in the microalga was further examined and compared with that reported from *H. pluvialis* [27]. The lipids in *Haematococcus* sp. KORDI03 and *H. pluvialis* contained mainly hexadecanoic and octadecanoic fatty acids, and only traces of other fatty acids. However, the profile of fatty acids in *Haematococcus* sp. KORDI03 clearly differed from *H. pluvialis* based in its detailed composition (Table 4). The content of total FAMEs in *Haematococcus* sp. KORDI03 was estimated to be 40.8 mg/g, which included saturated fatty acids (SFAs, 15.0%), MUFAs (6.0%), and an extremely high amount of polyunsaturated fatty acids (PUFAs, 79.0%). The major fatty acids in our isolate were C16:0 (13.7%), C18:2ω6 (24.9%), and C18:3ω3 (39.7%); the

Table 3. Comparison of monosaccharide content (mg/100 mg) and composition (%) in *Haematococcus* sp. KORDI03.

Monosaccharides	*Haematococcus* sp. KORDI03	
	Content (mg/100 mg)	Composition (%)
L(-) Fucose	0.66	3.2
L(-) Rhamnose	0.36	1.7
D(+) Arabinose	0.24	1.1
D(+) Galactose	0.82	3.9
D(+) Glucose	9.62	46.0
D(+) Mannose	8.55	40.9
D(+) Xylose	0.67	3.2
Total	20.92	100.0

Table 4. Comparison of fatty acid composition (%) in *Haematococcus* sp. KORDI03 and *H. pluvialis*[a].

Fatty acids	*Haematococcus* sp. KORDI03	*H. pluvialis*
C12:0	-[b]	0.1
C14:0	0.1	0.5
C15:0	0.1	-
C16:0	13.7	29.0
C16:1	0.5	0.6
C16:1ω9	(0.5)	-
C16:2	0.4	-
C16:2ω6	(0.4)	-
C16:3	3.5	-
C16:3ω3	(3.3)	-
C16:3ω6	(0.2)	-
C16:4	3.3	-
C16:4ω3	(3.3)	-
C17:0	-	0.2
C17:1	-	1.3
C18:0	0.7	2.1
C18:1	4.9	25.9
C18:1ω9	(4.9)	-
C18:2	24.9	20.8
C18:2ω6	(24.9)	-
C18:3	39.7	12.8
C18:3ω3	(38.0)	-
C18:3ω6	(1.7)	(1.6)
C18:4	5.8	1.4
C18:4ω3	(5.8)	-
C20:0	-	0.6
C20:1	0.5	0.3
C20:1ω9	(0.5)	-
C20:2	-	1.2
C20:3	-	0.5
C20:4	0.9	1.4
C20:4ω6	(0.9)	-
C20:5	0.6	0.6
C20:5ω3	(0.6)	-
C22:0	-	0.4
C24:0	0.3	0.2
C24:1	0.1	-
C24:1ω9	(0.1)	-
Σ SFAs	15.0	33.2
Σ MUFAs	6.0	28.1
Σ PUFAs	79.1	38.7
Total	100.0	100.0

[a][27].

[b]Not detected.

minor components were C16:3ω3 (3.5%), C16:4ω3 (3.3%), C18:1ω9 (4.9%), and C18:4ω3 (5.8%). On the other hand, *H. pluvialis* contained higher concentrations of SFAs (33.2%) and MUFAs (28.1%), including mainly C16:0 (29.0%) and C18:1 (25.9%), respectively. In recent years, *Haematococcus* species have been proposed as potential microalgal feedstock owing to their lipid content and fatty acid composition [12]. In the same manner, *Haematococcus* sp. KORDI03 may be used for biodiesel production owing to its enclosing MUFA and PUFA classes and a relatively low composition of SFAs. To date, several unsaturated fatty acids such as C18:1ω9, C18:2ω6, and C18:3ω3 have been used for industrial biodiesel production [26], and *Haematococcus* sp. KORDI03 contained considerable amounts of unsaturated fatty acids, including 18:1ω9 (4.9%), 18:2ω6 (24.9%), and 18:3ω3 (38.0%). With regard to PUFAs in *Haematococcus* sp. KORDI03, the maximum degree of unsaturation of the chains in major fatty acids was 3, and the length was intermediate with a maximum of 18 carbons. Moreover, only 15.0% of SFAs were detected from the total fatty acids of *Haematococcus* sp. KORDI03, those which can affect the quality of produced biodiesel owing to its unsuitable viscosities and poor cold-flow properties [35].

In general, the genus *Haematococcus* is known to have astaxanthin concentrations ranging from 1.0% to 3.0% of the dry cell weight, and has gained industrial acceptance as a "concentrated" form of natural astaxanthin [28]. In this study, the astaxanthin content of *Haematococcus* sp. KORDI03 was estimated to be 1.8% of the dry cell weight, indicating that the isolate can be also considered as a "concentrated" form of natural astaxanthin, similar to other industrially utilized *Haematococcus* strains. Under certain stress conditions, such as nutrient deprivation, increased salinity, high irradiance, and exposure to high temperature, the astaxanthin accumulation of *Haematococcus* can reach up to 4% of the dry cell weight in laboratory cultures [7]. Further studies are currently in progress to investigate the optimal conditions of *Haematococcus* sp. KORDI03 for increase of the productivity of microalgal biomass and the accumulation of astaxanthin.

Acknowledgments

This study was supported by a research grant from the Marine Biotechnology Program funded by the Ministry of Oceans and Fisheries of Korean Government (PM57770), and was partially supported by a research grant funded by the Korea Institute of Ocean Science & Technology (PE99213).

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