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Characterization of MABIK Microalgae with Biotechnological Potentials

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Abstract This article emphasized the physiological characteristics of the selected marine microalgal strains obtained from the culture collection of the National Marine Biodiversity Institute of Korea (MABIK). Therefore, in this study, 13 different marine microalgal strains belonging to the phylum Chlorophyta were analyzed for the composition of fatty acids, elements, photosynthetic pigments, and monosaccharides, as well as the lipid and protein contents. The results presented that the primary fatty acids were palmitic ($C_{16:0}$), palmitoleic ($C_{16:1}$ n-7), stearic ($C_{18:0}$), oleic ($C_{18:1}$ n-9), linoleic ($C_{18:2}$ n-6), and α-linolenic (ALA, $C_{18:3}$ n-3) acid in the evaluated microalgae. The lipid contents of heterotrophically grown strains ranged from 15.1% to 20.4%. The calorific values of the strains were between 17.4 MJ kg^{-1} and 21.3 MJ kg^{-1}. The major monosaccharides were galactose, glucose, and mannose, while the primary photosynthetic pigments were chlorophyll-a (Chl*a*), chlorophyll-b (Chl*b*), and lutein, respectively. Based on the results, the microalgal strains showed high potentials in the use of microalgae-based technologies to produce biochemicals, food, and renewable fuels as they are rich in sustainable sources of high-value bio-compounds, such as antioxidants, carbohydrates, and fatty acids.

Keywords : Biotechnological potentials, MABIK, Marine microalgae, Korea

Introduction

Since the Nagoya Protocol came into effect in 2014, each nation has been competing for obtaining beneficial bioresources and building diverse biobanks. In recognition of the current trends, the Ministry of Oceans and Fisheries of Korea (MOF) announced the 1st Administrative Master Plan for Marine Bioresources for the next 5 years [1]. Its main goal is to promote the development of exploitation and management strategies

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for sustainable utilization of Korean maritime bioresources and economic prosperity of the nation.

In particular, marine microalgae were appointed as one of the 4 priority target marine bioresources in the Implementation Plan in 2019 [2] for the MOF's 1st Master Plan due to their potentials for a wide range of profitable bioindustry [3, 4, 5]. The most frequently applied fields of marine microalgae include human and animal nutrition, biofuel production, carbon dioxide (CO_2) fixation, cosmetics, nutrient removal from wastewater, and pharmaceuticals. A few microalgae species including *Chlorella* have been used as human and animal food for over 6 decades. They have been used in aquaculture as food additives for bivalve mollusks and fishmeal. It was reported that their nutritional compositions such as carbohydrates, proteins, vitamins, lipids, antioxidants, and other trace elements are suitable for aquaculture feeds [6]. Also, microalgae have become an attractive candidate for biofuel production because they show higher photosynthetic efficiency and a greater oil yield than terrestrial sources [7, 8]. Microalgal lipids can be converted into biodiesel via transesterification, carbohydrates into ethanol and H_2, and proteins into biogas by anaerobic digestion [9]. In addition, marine microalgae are also particularly of interest as a source of various cosmeceuticals and nutraceuticals because they can produce a large number of biomolecules such as carotenoinds, chlorophylls, and polyunsaturated fatty acids (PUFAs), which are useful for human and animal health and development [10, 11]. Mycosporine-like amino acids (MMAs) from *Chlamydomonas*, *Chlorella*, and *Dunaliella* are reported to act as natural sunscreens to protect skin from ultraviolet (UV)-induced damage [12, 13, 14]. Microalgal carotenoids such as astaxanthin, β-carotene, canthaxanthin, lutein, and zeaxanthin found in *Dunaliella* are important pigment that have been commonly used in cosmetic applications. Microalgal wastewater treatment is an effective means to remove utrients from contaminated water. Numerous studies have demonstrated the use of wastewater as microalgae culture media, since they can efficiently uptake carbon, nitrogen, and phsophorus from waste water [15, 16, 17, 18, 19]. Greenhouse gas can be reduced through the biological transformation of CO_2 from organic carbon and the atmosphere into microalgal biomass. It was estimated that the production of 1.0 kg of dry microalgae biomass fixes approximately 1.83 kg of atmospheric CO_2 [20].

An applicable list of the prioritized Korean marine microalgae including 34 species with biotechnological potentials was is proposed by our group for successful execution of the MOF's 1st Administrative Master Plan and the Implementation Plan in 2019 [21]. In addition, the MABIK has just launched the Korean Marine Microalgae Biobank (KMMB) in accordance with the Korean Government's key policies. The MABIK has collected and maintained marine microalgal strains from diverse marine environments in Korea since 2016. Among them, microalgal strains belonging to the priority target species list and other candidates species that could be used for bioindustry were selected and tested for their biotechnological potentials. In this study, we determined physiological characteristics of the selected marine microalgae and compared chemotaxonomic analysis results in order to suggest their commercial potentials in various indusutries as well as to exercise our sovereign rights over marine bioresources on Korean territory.

Materials and Methods

Strain selection and biomass harvest

A total of 13 strains representing 5 major families within the phylum Chlorophyta was chosen from the marine microalgae culture collection at MABIK and they were tested for their bioactive and biotechnologically relevant compound production by chemotaxonomic analyses. Information on each strain is presented in Table 1. All the strains were incubated at 20°C with cool fluorescent light (approximately 40 μ mole m^{-1} s^{-1}) in a light : dark cycle (16 : 8 h) and shaking at 160 rpm on an orbital shaker (SH30; Fine

PCR, Gunpo, Korea). All the tested isolates were incubated in Reasoner's 2A medium (R2A, MBcell, Seoul, Korea) except for strains MM0020 and LIMS-PS-1511 which tris-acetate-phosphate medium (TAP, UTEX, Austin, TX, USA) and f/2 medium (AusAqua, Wallaroo, SA, Australia) were used, respectively. Well-grown cells were harvested by centrifugation at 2063 ×*g* (1580R, Labogene, Daejeon, Korea) for further analyses. All the biomass was freeze-dried and pulverized with a mortar and pestle to enhance the extraction efficiency.

Table 1. MABIK strains used in this study.

Taxonomic group (family)	Species	Strain	Origin	Medium
Chlorellaceae	*Auxenochlorella protothecoides*	MM0012	Cheongsan-myeon, Wando-gun, Jeollanam-do, Korea	R2A
	Auxenochlorella protothecoides	MM0013	Odong-dong, Masanhappo-gu, Changwon, Gyeongsangnam-do, Korea	R2A
	Chlorella sorokiniana	MM0034	Gijang-eup, Gijang-gun, Busan, Korea	R2A
	Micractinium sp.	MM0050	Wando-eup, Wando-gun, Jeollanam-do, Korea	R2A
	Micractinium sp.	MM0052	Hwangsan-myeon, Haenam-gun, Jeollanam-do, Korea	R2A
Chlamydomonadaceae	*Chlamydomonas hedleyi*	MM0020	Geojin-eup, Goseong-gun, Gangwon-do, Korea	TAP
	Graesiella emersonii	MM0036	Gijang-eup, Gijang-gun, Busan, Korea	R2A
Dunaliellaceae	*Dunaliella salina*	LIMS-PS-1511	Cheonseong-dong, Gangseo-gu, Busan, Korea	f/2
Scenedesmaceae	*Desmodesmus* sp.	MM0023	Jukwang-myeon, Gosung-gun, Gangwon-do, Korea	R2A
	Desmodesmus sp.	MM0025	Geunnam-myoen, Uljin-gun, Gyeonsangbuk-do, Korea	R2A
	Tetradesmus obliquus	MM0026	Geunnam-myoen, Uljin-gun, Gyeonsangbuk-do, Korea	R2A
	Tetradesmus obliquus	MM0061	Chuksan-myeon, Yeongdeok-gun, Gyeongsangbuk-do, Korea	R2A
Selenastraceae	*Monoraphidium* sp.	MM0030	Jugwang-myeon, Goseong-gun, Gangwon-do, Korea	R2A

Fatty acid analysis and lipid quantification

Lipid extraction was performed as described by Breuer *et al*. [22]. The fatty acid (FA) composition was analyzed using a 7890A gas chromatograph equipped with a 5975C mass selective detector (Agilent Technologies, Santa Clara, CA, USA). Gas chromatography/mass spectrometry (GC/MS) runs were performed on a DB-FFAP column (30 m, 250 μm ID, 0.25 μm film thickness; Agilent Technologies) based on our previous publication [23]. Compound identification was performed by matching the mass spectra with those in the Wiley/NBS libraries. Searches showing a match value higher than 90% were considered valid. In addition, a colorimetric sulfo-phospho-vanillin (SPV) method was used to measure the total lipid content (%) of each microalgae [24]. Statistical analysis was performed using IBM SPSS Statistics 25.0 (IBM Co., Armonk, NY, USA). Lipid content was statistically tested using one-way analysis of variance (ANOVA) and post-hoc Tukey's honestly significant difference (HSD) test. P values < 0.05 were considered statistically significant.

Biomass characterization

The pulverized samples were sieved through ASTM

No. 230 mesh (opening = 63 μm). Ultimate analysis was conducted to determine the carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) contents using a Flash 2000 elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA) in duplicate. Gross calorific value (GCV) was estimated using the following equation developed by Friedl *et al*. [25]: [GCV = $3.55C^2$ – 232C – 2230H + 51.2C × H + 131N + 20600 (MJ kg^{-1})]. Protein content was calculated from the N content in the ultimate analysis by using a conversion factor (× 6.25) [26].

Photosynthetic pigment analysis

Pigment extraction was performed using the method developed by Zapata *et al*. [27]. Briefly, freeze-dried microalgal biomass was extracted in 90% high performance liquid chromatography (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 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 based on the method used in our previous publication [28]. HPLC-grade methanol was purchased from Daejung, and HPLC-grade ammonium acetate was purchased from Fluka (Sigma-Aldrich, St. Louis, MO, USA), respectively. Pigment standards such as β-carotene, chlorophyll (Chl*a*, Chl*b)*, lutein, neoxanthin, and violaxanthin were obtained from Sigma-Aldrich.

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, 40% calcium carbonate (Sigma-Aldrich) was added to the hydrolysates to neutralize the reaction. Then, 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) according to our previous publication [10, 23]. 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.

Results and Discussion

Our research group has previously reported an applicable list of the prioritized Korean marine microalgal species that should be considered in the future research and development (R&D) plans as precautions against the current and yet-to happen Access and Benefit Sharing (ABS) issues at international and national levels [21]. The list served as the cornerstone of the MOF's 1st Administrative Master Plan and the Implementation Plan in 2019 [1, 2]. As listed in Table 1, 4 strains belonging to these priority target species (*A. protothecoides*, *C. hedleyi*, and *D. salina*) according to the Korean Government's Master and Implementation Plans [1, 2] were selected for this study. In addition, 9 strains representing biochemically important Chlorophyta microalgae (*C. sorokiniana*, *Desmodesmus* sp., *G. emersonii*, *Micractinium* sp., *Monoraphidium* sp., and *T. obliquus*) were also included and analyzed for their biotechnological potentials.

Fatty acid composition and lipid content

Lipid profiles of the strains are summarized in Table 2. Major fatty acids include palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$ n-7), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$ n-9), linoleic acid ($C_{18:2}$ n-6), and α-linolenic acid (ALA, $C_{18:3}$ n-3). $C_{16:0}$ was abundant saturated fatty acid (SFA) in all the strains tested. Polyunsaturated fatty acids (PUFAs) such as $C_{18:2}$ n-6 and $C_{18:3}$ n-3 were also present as major fatty acids. The FA profile is generally considered as a chemotaxonomic marker to define microalgal groups of various taxonomic ranks. In this study, the FA profiles of the selected MABIK strains

showed that all the strains were able to biosynthesize $C_{16:0}$ as one of their major fatty acids. $C_{16:0}$ and $C_{18:0}$ are the most common components in marine microalgae [29] and these SFAs could be used as ideal competent sources for biodiesel production due to their high cetane numbers. However, the high harvesting and extraction costs are the main obstacles to be overcome in the microalgae-based biofuel production [30]. It is also recommended that the level of PUFAs in biodiesel should be kept to a minimum [31]. Omega-3 ($C_{18:3}$ n-3) and omega-6 ($C_{18:2}$ n-6) PUFAs were also produced by these marine microalgae strains as their major fatty acids. Numerous studies have reported that these essential PUFAs have beneficiary effects on human health [32, 33]. Omega-3, and omega-6 PUFAs are mainly derived from fish oils, and a great number of commercial products is available all around the world. Due to the current overfishing issues as well as increasing heavy metal accumulation in fish accompanied by ocean pollution, the sustainability of marine fish as a safe resource of omega-3 and omega-6 PUFAs is facing serious doubt [34, 35]. Therefore, these marine microalgal strains seem to be one of the best alternatives to fish-based oil as clean and sustainable PUFA sources.

Lipid contents (Fig. 1, Table 2) of the strains ranged from 15.1% to 20.4% except for strain LIMS-PS-1511 (7.5%). Lipid contents of the tested strains were well over the typical lipid content (10%) of microalgae. Only strain LIMS-PS-1511 had a low lipid content of 7.5% which accounts for autotrophic culture condition in f/2 medium while other strains were heterotrophically cultivated in R2A and TAP media. Further cultivation research is needed to determine optimal growth conditions for the improved lipid production through evaluating the effects of additional carbon sources and modified media components on these MABIK strains.

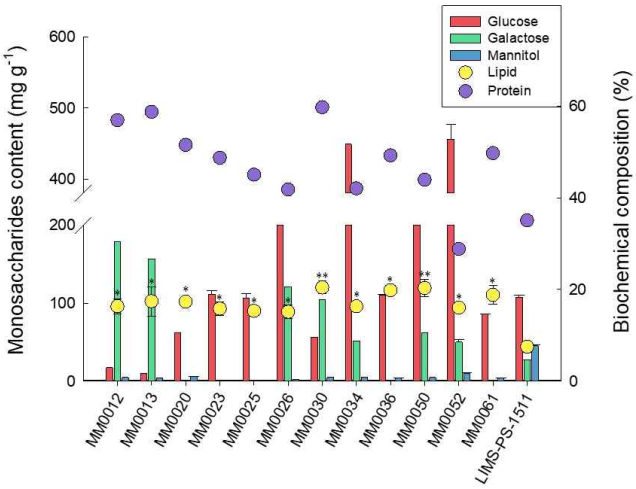

Figure 1. Monosaccharide composition and biomass properties of MABIK strains. * $p < 0.05$, ** $p < 0.01$.

Table 2. Analytical data on major fatty acid composition of lipid of MABIK strains (% total lipid)*.

Fatty acid	MM 0012	MM 0013	MM 0020	MM 0023	MM 0025	MM 0026	MM 0030	MM 0034	MM 0036	MM 0050	MM 0052	MM 0061	LIMS-PS-1511
$C_{16:0}$	7.1	10.3	18.3	21.4	22.4	18.0	18.1	27.7	16.8	22.7	22.9	20.3	19.3
$C_{16:1}$ n-7	1.0	0.0	2.6	3.8	4.9	2.0	1.9	1.2	0.9	2.5	7.1	2.2	0.0
$C_{18:0}$	1.8	1.1	1.2	1.8	0.9	1.3	0.8	1.4	1.7	1.2	0.7	1.0	1.6
$C_{18:1}$ n-9	3.8	5.8	0.0	31.6	28.2	16.4	13.6	5.9	17.6	10.7	7.8	10.3	3.7
$C_{18:2}$ n-6	29.4	30.0	9.8	17.0	9.2	5.2	4.8	9.4	10.7	16.9	13.2	12.1	5.6
$C_{18:3}$ n-3	21.9	29.4	16.4	8.9	11.2	28.3	27.0	23.1	27.2	22.4	24.1	24.2	31.7
Lipid(%)	16.3±1.7	17.4±3.2	17.3±0.9	15.8±1.5	15.3±0.8	15.1±1.5	20.4±1.4	16.3±0.7	19.8±1.1	20.3±1.9	16.0±0.5	18.8±2.0	7.5±1.2

*Values represent the average±standard deviation of three independent experiments and values without ranges are from single analysis.

Biomass characterization of MABIK strains

Ultimate analysis results are shown in Table 3. The GCVs based on ultimate analysis ranged from 17.4 MJ kg^{-1} to 21.3 MJ kg^{-1} (Fig. 1). The GCV of each strain

was calculated in order to understand the potential of microalgal biomass as a biofuel feedstock. The results showed that the GCVs were very similar to those of the terrestrial energy crops (17.0-20.0 MJ kg^{-1}) [36]. Since fine particulate matters have become a national concern in recent years due to air pollution, some of old coal-burning power stations in Korea have converted to biomass-burning stations and many plants are considering this transfomration in the near future. Hence, microalgae pellet made of mass-cultivated microalgae biomass would be an excellent mixed combustion biofuel for these coal power stations.

Protein contents (%) calculated from N content of each strain were around 50% except for strains MM0052 and LIMS-PS-1511. Therefore, most of the marine microalgae biomasses analyzed in this study may serve as an excellent animal feed due to their high protein contents.

Table 3. Composition of MABIK strains (% dry weight)*.

Element (%)	MM 0012	MM 0013	MM 0020	MM 0023	MM 0025	MM 0026	MM 0030	MM 0034	MM 0036	MM 0050	MM 0052	MM 0061	LIMS-PS-1511
C	47.6±0.1	47.3±0.0	48.0±0.0	46.6±0.1	46.4±0.1	47.1±0.2	49.7±0.2	46.0±0.1	47.6±0.2	44.5±0.0	43.4±0.2	47.7±0.1	42.4±0.2
H	6.9±0.1	7.0±0.0	6.9±0.1	7.3±0.1	7.3±0.1	7.2±0.1	7.4±0.0	7.2±0.0	7.1±0.0	7.0±0.0	6.8±0.1	7.4±0.0	6.3±0.1
N	9.1±0.3	9.4±0.1	9.8±0.0	7.8±0.0	7.2±0.0	6.7±0.1	9.5±0.1	6.7±0.1	7.9±0.0	7.0±0.1	4.6±0.0	8.0±0.3	5.6±0.0
S	0.5±0.0	0.5±0.1	0.5±0.0	0.0±0.0	0.0±0.0	0.4±0.0	0.4±0.1	0.4±0.0	0.0±0.0	0.5±0.0	0.0±0.0	0.6±0.0	0.4±0.1
GCV (MJ kg^{-1})	20.1±0.0	20.0±0.0	20.4±0.0	19.5±0.1	19.4±0.0	19.6±0.1	21.3±0.0	19.1±0.0	20.0±0.1	18.5±0.0	17.7±0.1	20.1±0.0	17.4±0.1
Protein (%)	56.9±1.6	58.7±0.5	61.5±0.1	48.7±0.0	45.0±0.1	41.8±0.8	59.7±0.5	42.0±0.7	49.2±0.3	43.9±0.5	28.8±0.2	49.7±2.1	35.0±0.3

*Values represent the average±standard deviation of two independent experiments.

Monosaccharide composition of MABIK strains

Qualitative and quantitative simple sugar analysis results are shown in Table 4. Galactose, glucose, and mannose were the most abundant monosaccharides. Arabinose, maltose, and sorbitol were also biosynthesized as trace monosaccharides. Marine microalgae are also capable of accumulating large quantities of carbohydrates in their cells apart from lipid [37]. In this study, carbohydrate analysis revealed that the major monosaccharides of the strains were galactose, glucose, and mannose. Quantitative and qualitative analyses of monosaccharides in microalgae are essential steps for the maximal utilization of microalgal biomass [38] since residual biomass after extraction of valuable components from microalgae still contains considerable amounts of carbohydrates that can be hydrolyzed into fermentable sugars for bioethanol production via fermentation. Also, only mild pretreatment is required to release the carbohydrates from microalgae biomass for subsequent fermentation process and bioethanol production since they do not contain lignocellulosic compounds unlike plant materials [37]. Furthermore, one of the most abundant form of simple sugars in microalgae is glucose which is a preferred carbon source for *Saccharomyces cerevisiae*. Minor monomers such as arabinose, maltose, mannitol, and sorbitol were also detected in these microalgae. Strain LIMS-PS-1511 produced unknown sugar compound that did not match any of the monosaccharide standards used in this study. Even though only a minute amount of arabinose was biosynthesized by strain MM0052, this monosaccharide was reported to have beneficial effects on human [39, 40] and it was also approved for use as a safe food additive by the United States Food and Drug Administration. Our monomer analysis results would contribute to a better understanding of the diversity of marine microalgae monosaccharides.

Table 4. Sugar composition of MABIK strains*.

Monosaccharide	MM 0012	MM 0013	MM 0020	MM 0023	MM 0025	MM 0026	MM 0030	MM 0034	MM 0036	MM 0050	MM 0052	MM 0061	LIMS-PS-1511
Arabinose (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1±0.1	0.0	0.0
mg g^{-1}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0±1.2	0.0	0.0
Galactose(%)	87.0	87.0	0.0	0.0	0.0	32.6	60.7	9.8	0.0	21.0	9.3±0.7	0.0	3.7±0.0
mg g^{-1}	178.3	156.8	0.0	0.0	0.0	121.0	104.6	51.4	0.0	61.8	50.0±3.5	0.0	26.9±0.5
Glucose (%)	8.7	8.7	51.5±1.3	54.6±2.2	48.5±2.5	65.9	33.7	88.4	57.4±0.2	76.3	87.7±4.0	44.5±0.1	77.4±0.3
mg g^{-1}	17.3	10.3	62.9±1.5	111.6±4.6	106.7±5.6	237.1	56.2	449.0	110.4±0.4	216.8	456.0±20.8	86.0±0.1	107.4±3.2
Maltose (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2±0.3
mg g^{-1}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.6±2.5
Mannitol (%)	2.3	2.3	5.0±0.1	0.0	0.0	0.7	2.9	0.9	2.1±0.1	1.6	0.9±0.2	2.0±0.0	12.3±0.2
mg g^{-1}	4.7	4.1	6.2±0.1	0.0	0.0	2.5	4.9	4.6	4.1±0.1	4.6	10.0±0.4	4.0±0.1	45.2±1.3
Mannose (%)	0.0	0.0	43.5±1.6	45.4±1.8	51.5±2.6	0.0	0.0	0.0	40.6±0.2	0.0	0.0	53.5±0.2	0.0
mg g^{-1}	0.0	0.0	53.2±2.0	92.9±3.7	113.1±5.8	0.0	0.0	0.0	78.0±0.3	0.0	0.0	103.5±0.3	0.0
Sorbitol (%)	2.0	2.0	0.0	0.0	0.0	0.8	2.8	1.0	0.0	1.1	0.0	0.0	0.0
mg g^{-1}	5.5	4.1	0.0	0.0	0.0	2.9	4.8	5.1	0.0	3.3	0.0	0.0	0.0
Unknown	-	-	-	-	-	-	-	-	-	-	-	-	3.3
mg g^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	N.A.

*Values represent the average±standard deviation of three independent experiments and values without ranges are from single analysis.

Pigment composition of MABIK strains

Pigment analysis results are displayed in Table 5. Major photosynthetic pigments of the MABIK strains were Chl*a*, Chl*b*, and lutein (Fig. 2, Table 5). Other accessory pigments such as β-carotene, neoxanthin, and violaxanthin were also detected in most strains. Since color of microalgae is one of the most obvious characteristics, each microalgal phylum possesses its own particular pigment patterns. In this study, Chl*a*, Chl*b*, and lutein are the primary photosynthetic pigments in all the tested strains. Chlorophylls have been widely used as food and pharmaceutical colorants and they are also reported to have health promoting activities [41]. Lutein, another major pigment, is one of the most important carotenoids in food, pharmaceutical, and cosmetics industries. It has recently received a significant attention due to its beneficial effects on human eye health [42]. Currently, the main source of lutein for the market is extracted from the petals of marigold flowers. However, the lutein content in *Tagetes erecta* is very low which resulting in low yields [43, 44, 45, 46]. Therefore, mass-cultivated biomass of these MABIK strains under controlled conditions could serve as alternative sources of lutein.

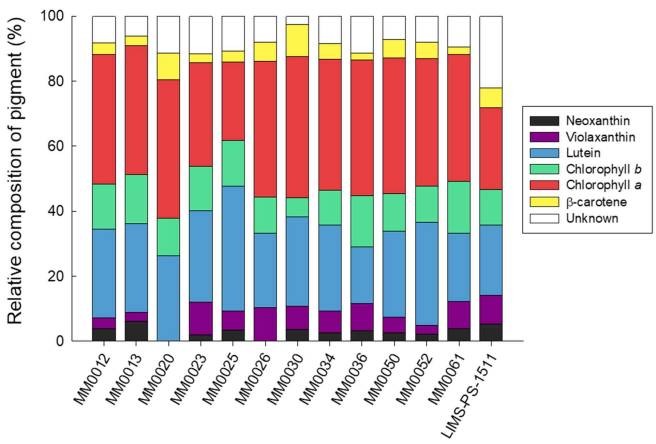

Figure 2. Photosynthetic pigment profiles of MABIK strains.

Table 5. Photosynthetic pigment composition of MABIK strains (% total pigment).

Pigment	MM 0012	MM 0013	MM 0020	MM 0023	MM 0025	MM 0026	MM 0030	MM 0034	MM 0036	MM 0050	MM 0052	MM 0061	LIMS-PS-1511
Neoxanthin	3.8±0.6	6.1±0.3	-	2.0±0.1	3.4±0.1	-	3.6±0.3	2.6±0.5	3.3±0.4	2.6±0.5	2.2±0.2	3.9±0.2	5.2±0.1
Violaxanthin	3.5±0.1	2.8±0.0	-	10.1±0.1	6.0±0.0	10.3±0.6	7.2±0.2	6.7±0.5	8.4±0.2	4.9±0.9	2.8±0.1	8.3±0.1	8.9±0.0
Lutein	27.1±0.6	27.2±0.2	26.2±0.2	28.1±0.2	38.4±0.1	22.8±1.3	27.5±0.2	26.5±0.5	17.3±0.3	26.4±0.4	31.5±0.4	21.1±0.1	21.7±0.1
Chl*b*	13.9±0.0	15.1±0.0	11.6±0.0	13.6±0.0	14.0±0.0	11.2±0.1	5.8±0.4	10.6±0.3	15.8±0.0	11.4±0.2	11.2±0.0	15.8±0.1	10.8±0.1
Chl*a*	39.9±0.3	39.6±0.0	42.7±0.4	31.8±0.0	24.0±0.0	41.8±0.7	43.5±1.3	40.4±0.6	41.6±0.1	41.9±0.6	39.2±0.3	39.0±0.2	25.3±0.1
β-carotene	3.6±0.2	3.1±0.0	8.1±0.0	2.7±0.0	3.5±0.0	5.9±0.3	9.7±1.5	4.7±0.7	2.1±0.0	5.5±0.4	5.0±0.4	2.4±0.0	6.1±0.1
Unknown	8.2	6.0	11.4	11.7	10.6	7.9	2.7	8.5	11.5	7.2	8.1	9.4	22.0

*Values represent the average±standard deviation of three independent experiments.

In this study, marine microalgal strains originated from Korean waters were selected and examined by chemotaxonomic analyses for their industrial potentials. The physiological properties reported in this article offer insight into chemotaxonomic markers of the MABIK strains as well as their commercial importance. Particularly, *Desmodesmus* sp. MM0023, *Desmodesmus* sp. MM0025, *C. sorokiniana* MM0034, *Micractinium* sp. MM0050, and *T. obliquus* MM0061 had over 20% of $C_{16:0}$ SFA so that they could be used as a feedstock for biodiesel production. In addition, *T. obliquus* MM0026, *Monoraphidium* sp. MM0030, and *D. salina* LIMS-PS-1511 had a high omega-3 to omega-6 ratio. Even though both omega-3 and omega-6 PUFAs are essential, an excessive intake of omega-6 is reported to cause negative health outcomes [47,48]. Recently, fish oil in the aquaculture feed has been increasingly replaced by terrestrial vegetable oils that often contain high levels of omega-6 PUFAs and the imbalance between omega-3 and omega-6 levels has deteriorated the nutritional quality of farmed fish [49,50,51]. Hence, these marine microalgae strains could be used for the production of designed aquafeeds for balancing the dietary omega-6 and omega-3 ratios. Also, *A. protothecoides* MM0012, *A. protothecoides* MM0013, and *Monoraphidium* sp. MM0030 had over 50% of protein contents and therefore they could be used as an animal feed. *Desmodesmus* sp. MM0025 and *Micractinium* sp. MM0052 had high lutein contents and thus they have poteintial to replace *T. erecta*. All these information presented here are also available both in the KMMB (https://www.mbris.kr/biobank/microAlgaeBank/microAlgaeBankSearch.do) and Marine Bio Resources Information System (MBRIS, https://www.mbris.kr/pub/main/publicMainPage.do) homepages.

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