Microbiol. Biotechnol. Lett. (2016), 44(3), 400–407
http://dx.doi.org/10.4014/mbl.1512.12008
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Biochemical Composition of a Korean Domestic Microalga *Chlorella vulgaris* KNUA027

Ji Won Hong[1], Oh Hong Kim[2], Seung-Woo Jo[3], Hyeon Kim[2], Mi Rang Jeong[4], Kyung Mok Park[5], Kyoung In Lee[6], and Ho-Sung Yoon[2,3,4,7]*

[1]*Marine Plants Team, National Marine Biodiversity Institute of Korea, Seocheon 33662, Republic of Korea*
[2]*Advanced Bio-resource Research Center,* [3]*Department of Energy Science,* [4]*School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu 41566, Republic of Korea*
[5]*Department of Pharmaceutical Engineering,* [6]*Biotechnology Industrialization Center, Dongshin University, Naju 58245, Republic of Korea*
[7]*Research Institute for Dok-do and Ulleung-do Island, Kyungpook National University, Daegu 41566, Republic of Korea*

Received: December 21, 2015 / Revised: April 24, 2016 / Accepted: April 30, 2016

A unicellular green alga, *Chlorella vulgaris* KNUA027, was isolated from the roots of *Panax ginseng* seedlings and its biotechnological potential was investigated. The results of GC/MS analysis showed that *C. vulgaris* KNUA027 was rich in nutritionally important polyunsaturated fatty acids (PUFAs) such as alpha-linolenic acid ($C_{18:3}$ ω3, 45.8%, 50.8 mg/g) and hexadecatrienoic acid ($C_{16:3}$ ω3, 11.8%, 13.1 mg/g). Therefore, this Korean indigenous microalga may have potential as a source of omega-3 PUFAs. It was also found that the saturated palmitic acid ($C_{16:0}$, 37.1%, 41.2 mg/g), which is suitable for biodiesel production, was one of the major fatty acids produced by strain KNUA027. The proximate analysis showed that the volatile matter content was 88.5%, and the ultimate analysis indicated that the higher heating value was 19.8 MJ/kg. Therefore, the results from this research with *C. vulgaris* KNUA027 may provide the basis for the production of microalgae-based biofuels and biomass feedstock.

Keywords: **Biofuel feedstock, *Chlorella vulgaris*, microalga, PUFA**

Recently, photosynthetic microalgae have gained particular interest as a new source for industrially important biomolecules because they are able to convert carbon dioxide (CO_2) to various types of products such as carbohydrates, lipids, and proteins with minimal growth requirements [6, 29]. In particular, microalgae are now considered as one of the most attractive candidates for biofuel and polyunsaturated fatty acid (PUFA) production due to their higher photosynthetic efficiency and oil yield compared to terrestrial crops [16, 20, 37]. In this study, a Korean indigenous microalga, *Chlorella vulgaris* KNUA027 was isolated and identified, and its potential as biofuel and PUFA feedstock was investigated.

Algal samples growing around the root of *Panax ginseng* seedlings on Petri dish at Sangju Campus, Kyungpook National University (36° 22'N, 128° 08'E) were collected in February 2013. Samples were then inoculated into 100 ml BG-11 medium [30] (Table 1) with meropenem (Yuhan Pharmaceuticals, Korea) at a concentration of 100 μg/ml. The flasks were incubated at 25℃ with shaking at 160 rpm under cool fluorescent light (approximately 70 μmole m^{-2} s^{-1}) until algal growth was apparent. Well-grown algal cultures (1.5 ml) were centrifuged at 3,000 × *g* for 15 min (Centrifuge 5424, Eppendorf, Germany) and resulting pellets were streaked onto BG-11 agar supplemented with meropenem (20 μg/ml). Plates were then incubated in a light:dark cycle (16:8 h) at 25℃ and a single colony was aseptically

***Corresponding author**
Tel: +82-53-950-5348, Fax: +82-53-953-3066
E-mail: hsy@knu.ac.kr

Table 1. Composition of BG-11 medium.

Compound	Amount (g/l)
$NaNO_3$	1.5
$CaCl_2 \cdot 2H_2O$	0.036
Ferric ammonium citrate	0.012
$EDTA \cdot Na_2 \cdot 2H_2O$	0.001
K_2HPO_4	0.04
$MgSO_4 \cdot 7H_2O$	0.075
Na_2CO_3	0.02
Trace metal solution[a]	1 ml/l

[a]H_3BO_3, 2.86 g/l; $MnCl_2 \cdot 4H_2O$, 1.81 g/l; $ZnSO_4 \cdot 7H_2O$, 0.222 g/l; $Na_2MoO_4 \cdot 2H_2O$, 0.39 g/l; $CuSO_2 \cdot 5H_2O$, 0.079 g/l; $Co(NO_3)_2 \cdot 6H_2O$, 0.049 g/l.

transferred to fresh BG-11 plates to obtain an axenic algal culture.

For morphological identification, live cells were harvested, suspended in sterile distilled water, and inspected at ×1,000 magnification on a Nikon Eclipse E100 Biological Microscope (Japan). For molecular analysis, genomic DNA was extracted using a DNeasy Plant Mini kit (Qiagen, Germany). The primer sets NS1/NS8 and ITS1/ITS4 [39] were used to amplify the 18S rRNA gene and internal transcribed spacer (ITS) region, respectively. The D1-D2 region of the large subunit rRNA gene of the isolate was amplified using the NL1 and NL4 primers [27]. The ITS2 rRNA secondary structure of strain KNUA027 was predicted in the ITS2 Database (http://its2.bioapps.biozentrum.uni-wuerzburg.de) [18, 40]. Phylogenetic analysis was performed with the ITS sequence of strain KNUA027 using the software package MEGA ver. 6.0 [38]. Its closely related *Chlorella* sequences were downloaded and aligned in the MEGA software, with the ClustalW tool. The best-fit nucleotide substitution model (T92) was selected using MEGA 6.0 based on the Bayesian information criterion. This model was used to build a maximum likelihood (ML) phylogenetic tree with 1,000 bootstrap replicates. Due to the highly conserved nature of rRNA, the plastid-encoded *psa*A (photosystem I P700 chlorophyll a apoprotein A1) and *psb*A (photosystem II reaction center protein D1) were also sequenced using primer sets, psaA130F-psaA1760R for *psa*A and psbAF1-psbAR2 for *psb*A, respectively [42]. The DNA sequences obtained were submitted to the NCBI database and their accession numbers were listed in Table 2. Also, the strain obtained in this study was deposited in the Korean Collection for Type Cultures (KCTC) under the accession number KCTC 12965BP.

For biomass characterization, the isolate was inoculated into BG-11 medium in triplicate and incubated at 25℃ for 20 days until the culture reached its late exponential phase. Cells were harvested by centrifugation at 3,220 *g* (Centrifuge 5810R, Eppendorf, Germany) and immediately freeze-dried. The lipids were then extracted using a modified version of the Bligh-Dyer method [41]. The fatty acid composition of the cultures was decided by GC/MS (Jeol JMS700 mass spectrometer equipped with an Agilent 6890N GC, Agilent Technologies, USA). Peak identification and compound assignment were performed based on electron impact mass spectrum and the National Institute of Standards and Technology mass spectral libraries [35] were used as reference databases.

The remaining freeze-dried biomass samples were pulverized with a mortar and pestle and sieved through ASTM No. 230 mesh (opening = 63 μm). Ultimate analysis was conducted in order to determine the carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) contents using a Flash 2000 elemental analyzer (Thermo Fisher Scientific, Milan, Italy). Higher heating value (HHV) was estimated by the following equation developed by Friedl *et al.* [10]: [$HHV = 3.55C^2 - 232C - 2{,}230H + 51.2C \times H + 131N + 20{,}600$ (MJ/kg)]. Protein content was calculated

Table 2. BLAST sequence alignment output using 5 different marker genes of *Chlorella vulgaris* KNUA027.

Marker gene	Accession number	Length (bp)	Closest match (GenBank accession number)	Overlap (%)	Sequence similarity (%)	Taxonomic affinity
18S rRNA	KU306723	1,771	*Chlorella vulgaris* CCAP 211/21A (KJ756823)	100	100	*Chlorella vulgaris*
ITS	KU306724	782	*Chlorella vulgaris* CCAP 211/11S (FR865660)	100	99	*Chlorella vulgaris*
28S rRNA	KU306725	517	*Chlorella vulgaris* LS 120 (KC912856)	100	100	*Chlorella vulgaris*
*psa*A	KX066372	1,611	*Chlorella vulgaris* NIES-227 (AB260919)	99	99	*Chlorella vulgaris*
*psb*A	KX066373	998	*Chlorella vulgaris* C-27 (AB001684)	100	99	*Chlorella vulgaris*

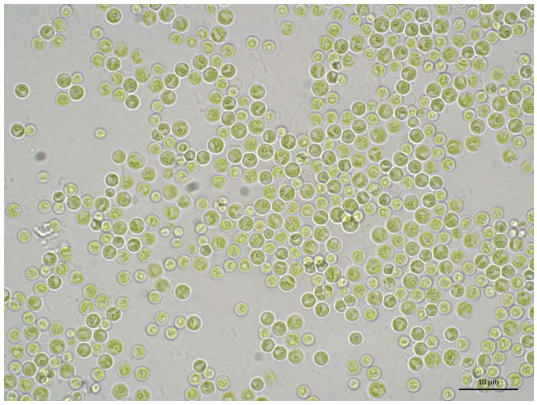

Fig. 1. Ligit microscopy of *Chlorella vulgaris* KNUA027 at ×1,000 magnification on a Nikon Eclipse E100 Biological Microscope (Japan).

from the N content in the ultimate analysis by using the conversion factor (× 6.25) [23]. Proximate analysis was carried out on a DTG-60A thermal analyzer (Shimadzu, Japan). Platinum pans were used to contain 30 mg of α-alumina (α-Al_2O_3) powder (Shimadzu, Japan) as a reference material and approximately 10 mg of each sample, respectively. Nitrogen (> 99.999%, N_2) was supplied as the carrier gas at a rate of 25 ml/min to protect the microalgae powder from oxidation. Samples were heated from 50 to 900℃ at a rate of 10℃/min. Thermogravimetric analysis (TGA) data were analyzed by ta60 Ver. 2.21 software (Shimadzu, Japan).

Strain KNUA027 had common features of the genus *Chlorella*. The algal cells were solitary, non-motile, and

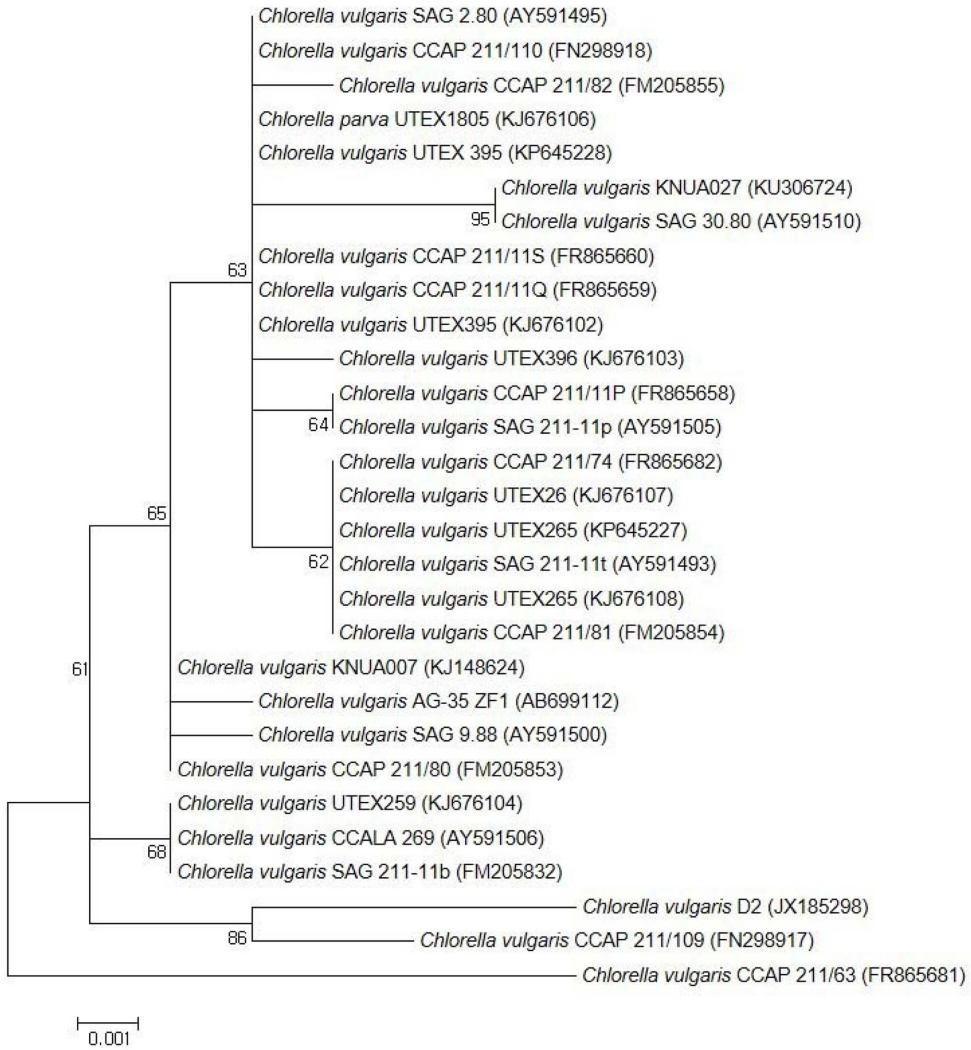

Fig. 2. The phylogenetic relationship of strain KNUA027 and its closely related species inferred from the ITS sequence data.

http://dx.doi.org/10.4014/mbl.1512.12008

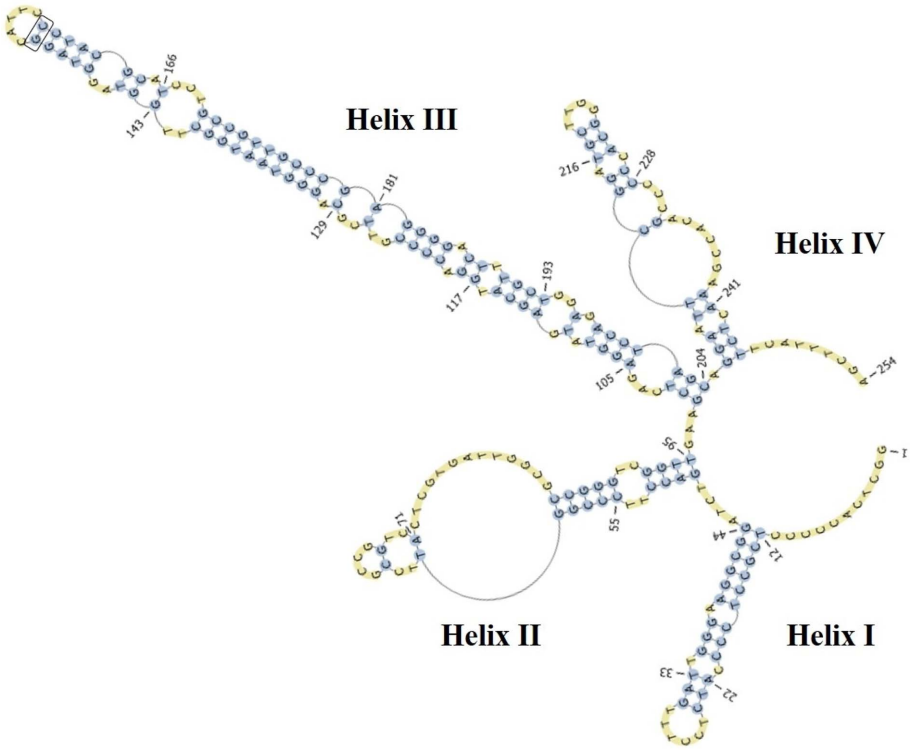

Fig. 3. ITS2 secondary structure for *Chlorella vulgaris* KNUA027. The key CBCs at the tip of helix III are indicated in a box.

round to slightly ellipsoid in shape. The microorganism had a predominant cup-shaped chloroplast and its sizes ranged from approximately 1–3 μm in diameter (Fig. 1). Molecular characterization inferred from sequence analyses of the genes for 18S rRNA, 28S rRNA, the ITS region, *psa*A, and *psb*A showed that the isolate belonged to the *C. vulgaris* group (Fig. 2, Table 1). Furthermore, strain KNUA027 exhibited a G-C pairing on the top of helix III (Fig. 3). These key compensatory base changes (CBCs) in the ITS2 secondary structure also confirmed that strain KNUA027 belonged to the genus *Chlorella* [15]. Therefore, the isolate was identified as *C. vulgaris* strain KNUA027.

The fatty acid profile of *C. vulgaris* KNUA027 is summarized in Table 3. Analysis of the cellular fatty acid composition of strain KNUA027 revealed that α-linolenic acid (ALA, 45.8%, 50.8 mg/g), palmitic acid (37.1%, 41.2 mg/g), and hexadecatrienoic acid (HTA, 11.8%, 13.1 mg/g) were the major fatty acids. Numerous studies have demonstrated that ALA and HTA have many beneficial health effects [25] and various commercial omega-3 products are available worldwide [28]. As omega-3

Table 3. Fatty acid profile of *Chlorella vulgaris* KNUA027.

Component	Content (%)	Yield (mg/g DW)
8-heptadecene ($C_{17}H_{34}$)	1.4	1.6
Heptadecane ($C_{17}H_{36}$)	0.7	0.8
9,12-cis-hexadecadienoic acid ($C_{16:2}$ ω4)	0.6	0.7
Hexadecatrienoic acid ($C_{16:3}$ ω3)	11.8	13.1
Palmitic acid ($C_{16:0}$)	37.1	41.2
Linoleic acid ($C_{18:2}$ ω6)	2.7	3.0
α-Linolenic acid ($C_{18:3}$ ω3)	45.8	50.8

PUFAs are primarily derived from refined fish oils, this isolate may have the potential to be used as an alternative to fish-based sources. The 16-carbon saturated palmitic acid suitable for biodiesel production was also autotrophically biosynthesized by strain KNUA027 as one of the major fatty acids. Recent studies on the biodiesel production by *Chlorella vulgaris* have demonstrated that palmitic acid ($C_{16:0}$), linolenic acid ($C_{18:2}$), linoleic acid ($C_{18:2}$), and oleic acid ($C_{18:1}$) were the main fatty acids regardless of culture medium and *C. vulgaris*

Table 4. Major fatty acids produced by *Chlorella vulgaris* strains from previous studies.

Strain	Medium	Major fatty acid	Reference
UTEX 259	BBM	Oleic acid ($C_{18:1}$, 61.0%), palmitic acid ($C_{16:0}$, 24.6%)	[1]
Not specified	SWM[a]	Linolenic acid ($C_{18:3}$, 22.1%), oleic acid ($C_{18:1}$, 19.6%)	[2]
CCAP 211/11	MBL	Linoleic acid ($C_{18:2}$, 24.6%), palmitic acid ($C_{16:0}$, 23.7%)	[3]
Not specified	BBM	Palmitic acid ($C_{16:0}$, 19.8%), linoleic acid ($C_{18:2}$, 17.7%)	[4]
TISTR 8261[b]	Chu13	Palmitic acid ($C_{16:0}$, 41.4%), oleic acid ($C_{18:1}$, 27.8%)	[7]
Not specified	BG-11	Linolenic acid ($C_{18:3}$, 28.2%), linoleic acid ($C_{18:2}$, 18.2%)	[8]
CCAP 211	BBM	Palmitic acid ($C_{16:0}$, 63.0%), linolenic acid ($C_{18:3}$, 13.0%)	[9]
CCAP 211	BBM	Palmitic acid ($C_{16:0}$, 39.2%), linolenic acid ($C_{18:3}$, 20.4%)	[11]
INETI 58	CM[c]	Palmitic acid ($C_{16:0}$, 25.1%), linolenic acid ($C_{18:3}$, 19.1%)	[12]
Not specified	BG-11	Palmitic acid ($C_{16:0}$, 53.0%), linolenic acid ($C_{18:3}$, 8.9%)	[13]
UTEX 2714	OCM[d]	Palmitic acid ($C_{16:0}$, 32.0%), oleic acid ($C_{18:1}$, 18.0%)	[14]
UTEX 395	BBM	Oleic acid ($C_{18:1}$, 16.8%), linolenic acid ($C_{18:3}$, 9.3%)	[21]
Not specified	N11	Palmitic acid ($C_{16:0}$, 62.4%), stearic acid ($C_{18:0}$, 19.5%)	[22]
Not specified	Conway	Palmitic acid ($C_{16:0}$, 29.1%), linoleic acid ($C_{18:2}$, 24.6%)	[24]
Not specified	MBM	Palmitic acid ($C_{16:0}$, 22.9%), oleic acid ($C_{18:1}$, 21.5%)	[26]
211/11B	BBM	Linoleic acid ($C_{18:2}$, 22.1%), palmitic acid ($C_{16:0}$, 19.8%)	[36]
CCTCC M 209256	SSM[e]	Oleic acid ($C_{18:1}$, 45.4%), palmitoleic acid ($C_{16:1}$, 23.3%)	[43]

[a]Synthetic wastewater medium; [b]Co-culture with *Rhodotorula glutinis*; [c]*Chlorella* medium; [d]Optimized culture medium; [e]Synthetic seawater medium.

strain (Table 4). Likewise, palmitic acid (37.1%) was one of the most abundant fatty acids in strain KNUA027. However, the high content of ALA (45.8%) makes the isolate an interesting candidate for further in-depth study involving omega-3 production. In addition, *C. vulgaris* KNUA027 was reported to produce a trace amount of heptadecane (0.7%, 0.8 mg/g). As heptadecane is a 17-carbon alkane hydrocarbon known as one of the major components of petrodiesel [19], this microalga-derived alkane can be directly used as a biodiesel component without having to convert triglycerides into liquid hydrocarbons.

In proximate analysis by TGA, the moisture content (MC) is determined by the mass loss before 110℃ under N_2 atmosphere, the volatile matter (VM) refers to the mass loss between 110–900℃ under N_2 as a result of thermal decomposition, and the remaining mass represents fixed carbon (FC) and ash [5]. The moisture, VM, and FC and ash contents of strain KNUA027 were 5.0%, 88.5%, and 6.5%, respectively (Fig. 4). The VM is defined as the part of solid fuel that is driven-off as a gas by heating and typical biomass generally has a VM content of up to 80% (crop residue: 63–80%; wood: 72–78%). The VM content of the microalga used in this study was

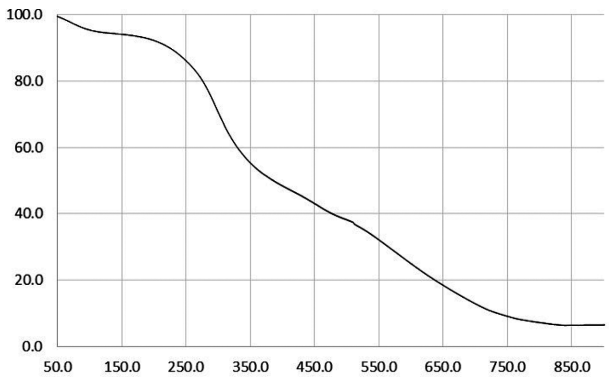

Fig. 4. TGA profile of *Chlorella vulgaris* KNUA027. The mass change in percentage is on the y-axis and temperature (℃) is on the x-axis [5].

higher than the range of wood-based biomass feedstocks. The HHV was also calculated to understand the potential of algal biomass as a biofuel feedstock (Table 5). The results showed that the HHV was within the range of the terrestrial energy crops (17.0–20.0 MJ/kg) [32]. A number of previous studies have been demonstrated on the HHVs of *Chlorella* strains under different autotrophic growth conditions and the biomass samples were characterized in the range of around 20.0–30.0 MJ/kg

Table 5. Sample component and elemental composition of *Chlorella vulgaris* KNUA027.

Component	Proximate analysis (wt%)	Elemental composition	Ultimate analysis (wt%)
MC[a]	5.0	C	47.4
VM[b]	88. 5	H	6.7
FC[c] + Ash	6.5	N	7.3
		S	0.6
		HHV[d] (MJ/kg)	19.8
		Protein	45.7

[a]Moisture content; [b]Volatile matter; [c]Fixed carbon; [d]Higher heating value.

[17, 31, 34]. However, these HHV results cannot be directly compared with our results because of the different culture conditions. Given the higher photosynthetic efficiency and biomass productivity [33], strain KNUA027 holds promise as a potential source for biomass feedstocks over crop plants. As high carbon content is a desirable property for fuel, if the higher concentration of CO_2 in the medium is available, the higher HHV are possible. In addition, the biomass may also serve as an excellent animal feed because of its high protein content (45.7%).

In conclusion, this Korean indigenous microalga, *C. vulgaris* KNUA027 could serve as potential biological resource to produce compounds of biochemical interest. The real potential of the isolate described in this paper should be evaluated through further cultivation studies at molecular, laboratory, and field scales.

Acknowledgments

This work was supported by the Advanced Biomass R&D Center (ABC) of Global Frontier Project funded by the Ministry of Science, ICT and Future Planning (2015M3A6A2065698), Korea, the Freshwater Microalgae–based Bioenergy Research and Development Project from Chilgok-gun, Gyeongsangbuk-do, Korea, the Establishment of Infrastructure for Sustainable Use of Marine Resources (2016M00400), funded by National Marine Biodiversity Institute of Korea (MABIK), and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2016R1A6A1A05011910), Korea.

References

1. Aguoru CU, Okibe PO. 2015. Content and composition of lipid produced by *Chlorella vulgaris* for biodiesel production. *Adv. Life Sci. Technol.* **36**: 96-100.
2. Ahmad F, Khan AU, Yasar A. 2013. The potential of *Chlorella vulgaris* for wastewater treatment and biodiesel production. *Pak. J. Bot.* **45**: 461-465.
3. Al-Iwayzy SH, Yusaf T, Al-Juboori RA. 2014. Biofuels from the fresh water microalgae *Chlorella vulgaris* (FWM-CV) for diesel engines. *Energies* **7**: 1829-1851.
4. Battah M, El-Ayoty Y, Abomohra A-EF, El-Ghany SA, Esmael A. 2013. Optimization of growth and lipid production of the Chlorophyte Microalga *Chlorella vulgaris* as a feedstock for biodiesel production. *World Appl. Sci. J.* **28**: 1536-1543.
5. Bi Z, He BB. 2013. Characterization of microalgae for the purpose of biofuel production. *Biol. Eng. Trans.* **56**: 1529-1539.
6. Borowitzka MA. 2013. High-value products from microalgae—their development and commercialisation. *J. Appl. Phycol.* **25**: 743-756.
7. Cheirsilp B, Suwannarat W, Niyomdecha R. 2011. Mixed culture of oleaginous yeast *Rhodotorula glutinis* and microalga *Chlorella vulgaris* for lipid production from industrial wastes and its use as biodiesel feedstock. *N. Biotechnol.* **28**: 362-368.
8. Chu FF, Chu PN, Cai PJ, Li WW, Lam PK, Zeng RJ. 2013. Phosphorus plays an important role in enhancing biodiesel productivity of *Chlorella vulgaris* under nitrogen deficiency. *Bioresour. Technol.* **134**: 341-346.
9. Converti A, Casazza AA, Ortiz EY, Perego P, Del Borghi M. 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chem. Eng. Process.* **48**: 1146-1151.
10. Friedl A, Padouvas E, Rotter H, Varmuza K. 2005. Prediction of heating values of biomass fuel from elemental composition. *Anal. Chim. Acta* **544**: 191-198.
11. Frumento D, Casazza AA, Al Arni S, Converti A. 2013. Cultivation of *Chlorella vulgaris* in tubular photobioreactors: a lipid source for biodiesel production. *Biochem. Eng. J.* **81**: 120-125.
12. Gouveia L, Oliveira AC. 2009. Microalgae as a raw material for biofuels production. *J. Ind. Microbiol. Biotechnol.* **36**: 269-274.
13. Hamed SR. 2015. Complementary production of biofuels by the green alga *Chlorella vulgaris*. *Int. J. Renew. Energy Res.* **18**: 936-943.
14. Heredia-Arroyo T, Wei W, Ruan R, Hu B. 2011. Mixotrophic cultivation of *Chlorella vulgaris* and its potential application for the oil accumulation from non-sugar materials. *Biomass Bioenergy* **35**: 2245-2253.
15. Hoshina R, Iwataki M, Imamura N. 2010. *Chlorella variabilis* and *Micractinium reisseri* sp. nov. (Chlorellaceae, Trebouxiophyceae): Redescription of the endosymbiotic green algae of *Paramecium bursaria* (Peniculia, Oligohymenophorea) in the 120th year. *Phycol. Res.* **58**: 188-201.
16. Huntley ME, Redalje DG. 2007. CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. *Mitigation Adapt. Strateg. Glob. Chang.* **12**: 573-608.
17. Illman AM, Scragg AH, Shales SW. 2000. Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. *Enzyme Microb. Technol.* **27**: 631-635.

18. Koetschan C, Förster F, Keller A, Schleicher T, Ruderisch B, Schwarz R, *et al*. 2010. The ITS2 Database III—sequences and structures for phylogeny. *Nucleic Acids Res.* **38**: D275-D279.
19. Knothe G. 2010. Biodiesel and renewable diesel: a comparison. *Prog. Energy Combust. Sci.* **36**: 364-373.
20. Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N. 2008. Biofuels from microalgae. *Biotechnol. Prog.* **24**: 815-820.
21. Lohman EJ, Gardner RD, Pedersen T, Peyton BM, Cooksey KE, Gerlach R. 2015. Optimized inorganic carbon regime for enhanced growth and lipid accumulation in *Chlorella vulgaris*. *Biotechnol. Biofuels* **8**: 1.
22. Mallick N, Mandal S, Singh AK, Bishai M, Dash A. 2011. Green microalga *Chlorella vulgaris* as a potential feedstock for biodiesel. *J. Chem. Technol. Biotechnol.* **87**: 137-145.
23. Mariotti F, Tomé D, Mirand PP. 2008. Converting nitrogen into protein—beyond 6.25 and Jones' factors. *Crit. Rev. Food Sci. Nutr.* **48**: 177-184.
24. Marudhupandi T, Gunasundari V, Kumar TT, Tissera KR. 2014. Influence of citrate on *Chlorella vulgaris* for biodiesel production. *Biocatal. Agric. Biotechnol.* **3**: 386-389.
25. Mehta LR, Dworkin RH, Schwid SR. 2009. Polyunsaturated fatty acids and their potential therapeutic role in multiple sclerosis. *Nat. Clin. Pract. Neurol.* **5**: 82-92.
26. Mitra D, van Leeuwen JH, Lamsal B. 2012. Heterotrophic/mixotrophic cultivation of oleaginous *Chlorella vulgaris* on industrial co-products. *Algal Res.* **31**: 40-48.
27. O'Donnell K. 1993. *Fusarium* and its near relatives. pp. 225-233. *In* Reynolds DR, Taylor JW (eds.), The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics. CBA International, Wallingford.
28. Packaged Facts. 2012. The Global Market for EPA/DHA Omega-3 Products. Published online at: http://www.packagedfacts.com/Global-EPA-DHA-7145087/ (accessed on 7 April 2016).
29. Pignolet O, Jubeau S, Vaca-Garcia C, Michaud P. 2013. Highly valuable microalgae: biochemical and topological aspects. *J. Ind. Microbiol. Biotechnol.* **40**: 781-796.
30. Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. 1979. Genetic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* **111**: 1-61.
31. Rizzo AM, Prussi M, Bettucci L, Libelli IM, Chiaramonti D. 2013. Characterization of microalga *Chlorella* as a fuel and its thermogravimetric behavior. *Appl. Energy* **102**: 24-31.
32. Ross AB, Jones JM, Kubacki ML, Bridgeman T. 2008. Classification of macroalgae as fuel and its thermochemical behaviour. *Bioresour. Technol.* **99**: 6494-6504.
33. Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, *et al*. 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenergy Res.* **1**: 20-43.
34. Scragg AH, Illman AM, Carden A, Shales SW. 2002. Growth of microalgae with increased calorific values in a tubular bioreactor. *Biomass Bioenergy* **23**: 67-73.
35. Stein SE, Scott DR. 1994. Optimization and testing of mass spectral library search algorithms for compound identification. *J. Am. Soc. Mass Spectrom.* **5**: 859-866.
36. Stephenson AL, Dennis JS, Howe CJ, Scott SA, Smith AG. 2010. Influence of nitrogen-limitation regime on the production by *Chlorella vulgaris* of lipids for biodiesel feedstocks. *Biofuels* **1**: 47-58.
37. Tabatabaei M, Karimi K, Sárvári Horváth I, Kumar R. 2015. Recent trends in biodiesel production. *Biofuel Res. J.* **7**: 258-267.
38. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**: 2725-2729.
39. White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315-322. *In* Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego.
40. Wolf M, Achtziger M, Schultz J, Dandekar T, Müller T. 2005. Homology modeling revealed more than 20,000 rRNA internal transcribed spacer 2 (ITS2) secondary structures. *RNA* **11**: 1616-1623.
41. Yeo I, Jeong J, Cho Y, Hong J, Yoon H-S, Kim SH, *et al*. 2011. Characterization and comparison of biodiesels made from Korean freshwater algae. *Bull. Korean Chem. Soc.* **32**: 2830-2832.
42. Yoon HS, Hackett JD, Bhattacharya D. 2002. A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. *Proc. Natl. Acad. Sci. U.S.A.* **99**: 11724-11729.
43. Zheng H, Yin J, Gao Z, Huang H, Ji X, Dou C. 2011. Disruption of *Chlorella vulgaris* cells for the release of biodiesel-producing lipids: a comparison of grinding, ultrasonication, bead milling, enzymatic lysis, and microwaves. *Appl. Biochem. Biotechnol.* **164**: 1215-1224.

http://dx.doi.org/10.4014/mbl.1512.12008

국문초록

한국 토착 미세조류 클로렐라 불가리스 KNUA027 균주의 생화학적 조성

홍지원[1], 김오홍[2], 조승우[3], 김현[2], 정미랑[4], 박경목[5], 이경인[6], 윤호성[2,3,4,7]*

[1]*국립해양생물자원관 해양식물팀*
[2]*경북대학교 신바이오소재연구소*
[3]*경북대학교 에너지과학과*
[4]*경북대학교 첨단복합 생명과학인력 양성사업단*
[5]*동신대학교 제약공학과*
[6]*동신대학교 생물자원산업화지원센터*
[7]*경북대학교 울릉도·독도연구소*

인삼 유묘 뿌리 주변에서 자라고 있는 단세포 녹색 조류, 클로렐라 불가리스 KNUA027을 순수분리한 후 본 분리균주의 생물공학적 활용 가능성에 대해 조사를 실시하였다. 가스크로마토그래프/질량분석기를 이용한 분석 결과, 본 균주에는 영양학적으로 중요한 알파 리놀렌산($C_{18:3}$ ω3, 45.8%, 50.8 mg/g) 및 헥사데카트리엔산($C_{16:3}$ ω3, 11.8%, 13.1 mg/g)과 같은 다가불포화지방산이 풍부한 것으로 밝혀졌다. 따라서, 본 국내 토착 미세조류는 잠재적인 오메가-3 다가불포화지방산 원료가 될 수 있다고 사료된다. 또한, 바이오디젤 생산에 적합한 것으로 알려져 있는 팔미트산($C_{16:0}$, 37.1%, 41.2 mg/g) 역시 본 균주에 의해 주요 지방산 성분으로 생합성 되는 것으로 확인되었다. 근사분석 결과 KNUA027 균주의 휘발성물질 함량은 88.5%였으며, 원소분석 결과 고위발열량은 19.8 MJ/kg으로 나타났다. 본 KNUA027 균주를 이용한 연구결과는 미세조류 기반 바이오연료와 바이오매스 생산을 위한 기초자료 역할을 할 수 있을 것으로 기대된다.