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Contributed Paper

Enhancement of Biomass, Lipid and Hydrocarbon Production from Green Microalga, *Botryococcus braunii* AARL G037, by UV-C Induction

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ABSTRACT

Botryococcus braunii, a green microalga, has been acknowledged as a possible source of renewable fuel that holds significant potential. However, it has been associated with a slow growth rate and low productivity. Thus, the aim of this study was to investigate the effects of ultraviolet (UV)-C on *B. braunii* AARL G037 for the purposes of improving biomass, lipid and hydrocarbon production levels. The survival rate was found to be in a range of 0.66-12.54% after being exposed to UV-C radiation for 3-21 minutes. The highest level of biomass production was acquired from UV-C induced isolate no. U18J10 at 2,325.67 mg L^{-1} and was equivalent to 1.32 times higher than the wild type. The highest hydrocarbon and lipid contents were found in *B. braunii* U18N14, which were recorded at 76.03% and 34.00%, respectively. Meanwhile, wild type, *B. braunii* AARL G037 accumulated hydrocarbons and lipids at only 45.81% and 22.70%, respectively. A study on the biodiesel properties revealed that 9-octadecanoic acid, methyl ester (C18:1) was the major fatty acid methyl ester. Moreover, biodiesel properties acquired from *B. braunii* wild type and all UV-C induced isolates were conformed to the EN14214 standard.

Keywords: biodiesel, fatty acid methyl ester, random mutation, renewable fuel

1. INTRODUCTION

Botryococcus braunii is a colony-forming green microalga belonging to the class Trebouxiophyceae. This microalga has a high potential for use in biofuel production due to it produces large quantities of hydrocarbons and lipids. Hydrocarbons from this microalga stored in extracellular matrix surrounding individual cells of the colony and can readily be converted into liquid fuels such as gasoline, diesel and aviation turbine fuel after catalytic cracking or hydrocracking [1, 2]. Additionally, in terms of hydrocarbon production, this microalga is also been known to produce large amounts of lipids that accumulated in

their cell and can be used as a raw material for biodiesel [3]. A significant challenge of employing this microalga for these purposes is its slow growth rate. The specific growth rate of *B. braunii* is rather low, which explains the long period of time needed for cultivation. In terms of biofuel commercial production by *B. braunii*, it is necessary to increase the quantity of biomass along with its levels of hydrocarbon and lipid production.

Physical mutagenesis is an alternative technique that has been used to induce mutagenesis and has been recommended as an effective technique for the improved yields of microalgae strains and to successfully increase productivity. UV radiation, gamma rays or x-rays can be used as the mutagen in this method. As a mutagen, UV radiation is of significant interest due to the fact that it is considered effective, easy to obtain, requires no specialized training to use, is very cheap, causes less pollution and is relatively safe to use. When compare to UV-A (wavelength 315 to 400 nm) or UV-B (wavelength 280 to 315 nm), UV-C (wavelength 100 to 280 nm) has higher energetic radiation and the wavelength at 254 nm deoxyribonucleic acid (DNA) has the strong absorption peak, which made more damage to DNA, causing to induce mutagenesis[4]. In recent decades, there have been several previous studies that have reported positive success when using UV-C radiation to improve the productivity of microalgae [5]. In 2017, Sivaramakrishnan and Incharoensakdi induced *Scenedesmus* sp. with UV-C. It was found that, the mutant had a higher biomass and lipid content than the wild type with an increase from 1.9 to 2.4 g/L and from 40 to 55% of dry cell weight, respectively [6]. *Chlorella vulgaris* was mutated with UV-C. The results revealed that, the UV-715 mutant isolates gave biomass and amounts of hexadecadienoic acid (C16:2) and linolenic acid (C18:3) higher than those in the wild type [7]. Additionally, improvement biomass settling and carotenoids production ware done on *Dunaliella salina* by UV-C mutagenesis. The amount of total carotenoids and beta-carotene of *Dunaliella salina* mutant were doubled in 24 h and cell settled in 15 h [8]. However, UV-C random mutagenesis to improve growth, lipid and hydrocarbon properties of *B. braunii* has never been reported.

Therefore, in an attempt to enhance the productivity of *B. braunii*, we have proposed the use of UV-C as a mutagen for the inducement of mutagenesis in this study. *B. braunii* was either exposed or not exposed to UV-C radiation in order to compare hydrocarbon and lipid content levels, as well as levels of biomass, hydrocarbon and lipid productivity along with its biochemical composition.

2. MATERIALS AND METHODS

2.1 Microalgal Strains and Molecular Characterization

B. braunii AARL G037 was obtained from the culture collection of the Applied Algal Research Laboratory, Department of Biology, Faculty of Science, Chiang Mai University. This algal strain was originally isolated from a fresh water reservoir in Chiang Mai Province, Thailand. *B. braunii* UTEX LB572 was purchased from the culture collection of the University of Texas. Both strains were cultivated in CA medium under constant light at 80 μmol photons m^{-2} s^{-1} and were cultured at 25 °C.

To confirm algal species, genomic DNA was extracted from fresh microalgal biomass using a DNA Mini Kit (Geneaid, Taiwan). The protocol was done following the manufacture's protocol with slight modifications. The 18S rRNA gene was subjected to amplification with the forward primer 63F (52-ACGCTTGTCTCAAAGA

TTA-3') and the reverse primer 1818R (5′-ACGGAAACCTTGTTACGA-3′). PCR was carried out in a thermo-cycler (Bio-Rad/ MJ mini™) using the PCR program with 10 min of initial denaturing at 94 °C, followed by 30 cycles of 94 °C denaturing for 1 min at a temperature of 55 °C annealing for 30 sec, and an extension of 55 °C for 45 sec and 72 °C in the final extension for 5 min. The PCR product was separated on agarose gel and then purified using a GF-1 PCR Clean-up Kit according to the manufacture's protocol (Vivantis, USA). A phylogenetic tree was constructed by employing the maximum likelihood method and 10000 bootstrap replications. Mega7 was used in the evolutionary analysis.

2.2 UV-C Induction Procedure, Screening and Selection of Induced Isolates

B. braunii AARL G037 was used as the wild type microalga for UV-C induction. Each 5 mL aliquot of the wild type specimen was added to petri dish. The plates were placed on a shaker and then shook at 250 rpm, after which they were kept under a UV-C lamp (254 nm, 15W GE lightning). In random mutagenesis trials, UV-C was exposed to the samples in each petri dish for 0-30 min. The induced samples were spread onto solidified CA medium with 1% agar. Then, the plates were incubated at 25 °C under constant light. The survival rate was calculated after 45 days from the time of plating the specimens [9].

UV-C induced colonies that grew fast, formed larger colonies than the wild type and had a survival rate of less than 10% were selected and re-suspended with CA medium. Approximately 2000 isolates were selected by micropipetting method from the suspension to be scaled up. Well-grown induced isolates were selected for the investigation of lipid and hydrocarbon accumulation under a fluorescence microscope using the Nile red staining technique [10]. Some isolates which had a high growth rate and high accumulations of hydrocarbon and lipid in the cells were selected to be sub-cultured until constant growth occurred before comparing the growth rate and biochemical productivity levels with those of the wild type and *B. braunii* UTEX LB572 specimens.

2.3 Determination of Growth and Morphological Analysis

Triplicates of the individual isolates with an initial biomass density of 200 mg L^{-1} were cultivated in 300 mL working volume of liquid CA medium in 500 mL Erlenmeyer flasks. The cultures were operated in photoautotrophic mode, agitated by being shook at 120 rpm under constant light at 80 µmol photons m^{-2} s^{-1} and were cultured at 25 °C for 24 days. Microalgal growth was monitored every 4 days. The dry cell weight (mg L^{-1}) was measured. Maximum specific growth rate (μ_{max}; day^{-1}) and biomass productivity (mg L^{-1} day^{-1}) were calculated. One hundred replicates of the colonies and the cell size were observed under a light microscope and measured using 'Image J 1.50d' software. At the end of cultivation, cells were harvested by filtration and were then freeze-dried.

2.4 Biochemical Composition Analysis

2.4.1 Carbohydrate and protein estimation

Freeze-dried microalgal powder were suspended in 10 mL 6M HCl, 15 mL of distilled water and boiled for 30 min. Then, specimens were quantified for carbohydrate content using the phenol-sulfuric acid method with glucose as a standard ($y = 9.8543x + 0.0314$, $R^2 = 0.9994$) [11].

For protein estimation, freeze-dried

microalgal powder was pretreated by being suspended in 3 mL of 2 M NaOH, boiled for 20 min, cooled down on ice and 1 mL of suspension was then mixed with 2 mL of distilled water before ultrasonication. The crude protein content was estimated by Lawry method using BSA (Bovine Serum Albumin) as standard curve ($y = 0.6789x + 0.0379$, $R^2 = 0.9927$) [12].

2.4.2 Total lipid extraction and hydrocarbon extraction

To determine total lipid content, freeze-dried biomass was extracted by Dichloromethane: Methanol 2:1 (v/v) and lysed using a 10-kHz sonicator [13]. Then, specimens were centrifuged. Extracted solvent was transferred to pre-weight screw cap test tube while re-extracted cell pellets until they appeared colorless. The pool extracted solvent was evaporate to complete dryness under a stream of nitrogen. Total lipid content was measured gravimetrically and was expressed as percentages (%) of dried weight. Total lipid productivity (mg L^{-1} day^{-1}) was then calculated.

Freeze-dried microalgal biomass was extracted by homogenizing the substance with n-hexane and lysed using a 10-kHz sonicator. Then, the samples were stored overnight at room temperature and were then centrifuged. The pellets were re-extracted until they appeared colorless in the supernatant. The supernatant was evaporated to complete dryness under a stream of nitrogen in pre-weighed glass vials. Hydrocarbon content (%) and hydrocarbon productivity (mg L^{-1} day^{-1}) were then measured.

2.4.3 Fatty acid methyl ester (FAME) preparation and analysis

Conversion of the total lipid content to FAME was performed according the method of Anahas and Muralitharan [14]. In brief, microalgal lipid was mixed with saponification reagent and boil 30 min afterwards, methylation reagent was added and heat 20 min under 80 °C. Then, cool at room temperature before extracted FAME with extraction solvent. Finally, alkaline-base wash solution was added and gentle mix. FAME was dissolve in upper organic solvent phase. FAME was measured for the purposes of quantification and identification using GC-MS. The evaluation of biofuel properties from the FAME profiles including cetane number (CN), iodine value (IV), saponification value (SN), degree of saturated (DU), long chain saturated factor (LCSF) or cold filter plugging point (CFPP) were investigated following the method of Talebi *et al* [15].

3. RESULTS AND DISCUSSIONS

3.1 Microalgal Strains and Molecular Characteristics

B. braunii AARL G037 was identified based on its morphological features including cell size, colony size, shape and color. It was found that *B. braunii* isolate AARL G037 displayed cells appearing as yellow-green, sub-spherical in shape, and presented colonies of 67.13±11.06 µm in length and 41.45±5.22 µm in width. Cells were 9.03±0.78 µm in length and 5.66□ 0.66 µm in width. The 18s rRNA gene specific primers were used for the species confirmation of *B. braunii* AARL G037. The results indicated that the 18S rRNA sequences appeared as close relatives to known *B. braunii* specimens. Consequently, this isolated species was confirmed as *B. braunii* (Figure 1).

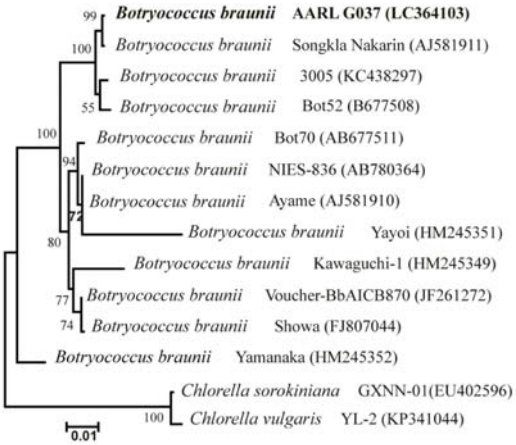

Figure 1. Phylogenetic tree of 18S rRNA gene sequences of *B. braunii* AARL G037 deduced using the maximum likelihood method.

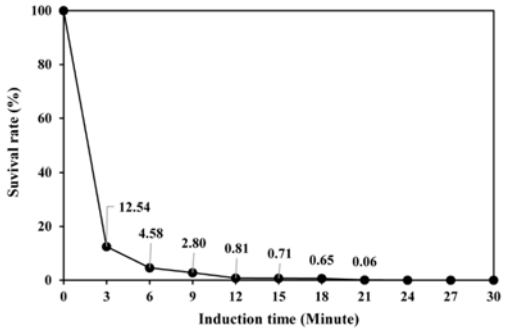

Figure 2. Survival rate of *B. braunii* AARL G037 in the presence of different UV-C induction times.

3.2 UV-C Induction, Screening and Selection from UV-C Induced Isolates

To analyze and optimize the induction time of UV-C for 10% survival rate, wild type *B. braunii* AARL G037 was exposed with different duration times of UV-C (0 - 30 min). As is shown in Figure 2, the survival rate of *B. braunii* AARL G037 deceased with an increase in UVC induction time. The results of our study are similar to those of Sivaramakishnan and Incharoensakdi [6] who stated that the survival rate declined when the duration time of UV-C exposure was increased. Under the UV-C induction of 6 - 21 min, the survival rate showed a level of lower than 10% and reached 0% at induction time over 21 min. We assume and believe that a survival rate of less than 10% is strongly associated with a mutagenic effect. It has long been known that when the survival rate is low or when there is a long duration time of exposure to the mutagen, biochemical structure will likely yield disastrous effects and result in a high mutation rate [9]. In this study, the UV-C induction time of 6 - 21 min resulted in a low survival rate (less than 10%) of *B. braunii* AARL G037 in the optimal inducement range and this was suitable for the improvement of productivity.

In reference to the colonies forming on solidified agar, some induced colonies appeared differently in terms of their characteristics when compared to the wild type, such as with regard to size and color. The large colony forming from the plates that had been exposed to UV-C induction times of between 6 to 21 min were selected for screening in a micro-cuvette combined with the Nile red staining technique. Two rapid growing isolates with high quantitative values of hydrocarbon and lipid contents in their cells (U18N14 and U18J10) were selected for comparison of the relevant properties of the wild type and *B. braunii* UTEX LB572.

3.3 Determination of Growth and Morphological Analysis

The growth curves of the *B. braunii* wild type, two UV-C induced isolates U18N14, U18J10 and *B. braunii* UTEX LB572 are shown in Figure 3. At the end of the cultivation period, the biomass productivity values of U18N14 and U18J10 were 82.85±6.0 and 96.98±5.0 mg L^{-1} day^{-1}, which were greater than those of the wild type (41.85±1.0 mg L^{-1} day^{-1}) 0.98 and 1.32-fold, respectively.

Furthermore, they also generated significantly more biomass than *B. braunii* UTEX LB572 (62.44±2.1 mg L^{-1} day^{-1}) at 0.33 and 0.55-fold, respectively. Observation of the trend of specific growth rate revealed the same results as total biomass production (Table.1).

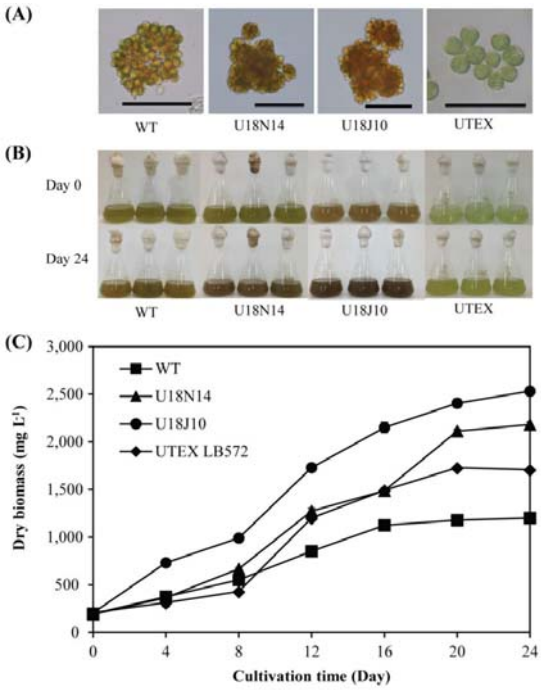

Figure 3. (A) Colony of *B. braunii* AARL G037 wild type, UV-C induced isolates U18N14, U18J10 and *B. braunii* UTEX LB572 under light microscope (scale bar = 50 □M). (B) Appearance of *B. braunii* AARL G037 wild type, UV-C induced isolates U18N14, U18J10 and *B. braunii* UTEX LB572 between day 0 and day 24 of cultivation time. (C) Growth curves of *B. braunii* AARL G037 wild type, UV-C induced isolates U18N14, U18J10 and *B. braunii* UTEX LB572.

Table 1. Growth kinetics of *B. braunii* AARL G037 wild type, two UV-C induced isolates U18N14, U18J10 and *B. braunii* UTEX LB 572 in this study.

Isolate	Biomass production (mg L^{-1})	Biomass productivity (mg L^{-1} day^{-1})	μ_{max} (day^{-1})
WT	1,004.33 ± 20.52[a]	41.85 ± 0.10[a]	0.072 ± 0.000[a]
U18N14	1,988.33 ± 10.53[c]	82.85 ± 0.06[c]	0.110 ± 0.000[c]
U18J10	2,325.67 ± 10.15[d]	96.90 ± 0.05[d]	0.123 ± 0.000[d]
UTEX LB572	1,498.67 ± 40.93[b]	62.44 ± 0.21[b]	0.108 ± 0.001[b]

In each column, different letters indicate significant differences ($p<0.05$) using one-way ANOVA test with post-hoc Tukey HSD test.

In comparison with other reports, Xu *et al.* revealed that the biomass productivity and specific growth rates of *B. braunii* IPE001 cultured in 250 mL Erlenmeyer flasks were 37 mg L^{-1} day^{-1} and 0.077 day^{-1}, respectively [16]. These figures were lower than those of all of the UV-C induced isolates in this experiment but were similar to those of *B. braunii* AARL G037 (WT). Moutel *et al.* cultivated *B. braunii* 755 in Erlenmeyer flasks containing four-fold concentrations of modified CHU13 with 15 mM $NaHCO_3$. The results revealed that microalga yielded biomass productivity at a level of 13 mg L^{-1} day^{-1}, which was lower than the microalgal biomass productivity in this study [17]. On the other hand, Zhang *et al.* cultured *B. braunii* under the mixotrophic mode in a photobioreactor by glucose feeding of 0.5-4 g L^{-1} and a maximum exposure of light intensity at 270 µmol photon m^{-2} s^{-1}. The average value of biomass productivity to glucose within the entire cultivation process was 234 mg L^{-1} day^{-1}. Although, microalgal biomass productivity values of both the induced isolates and the wild type in this research study were lower than in the studies of Zhang *et al* [18]. The biomass productivity of induced isolates could be further improved by optimized culture conditions such as by being cultivated in a photo-bioreactor supplied with CO_2 or culture in mixotrophic mode.

Previous publications that have used UV-C to induce microalgae produced results that were similar to this study. UV-C incremental biomass of *Chlorella* sp. FACHB-1298 was used to generate mutagenesis by UV-C for a study on growth and lipid accumulation. The author has reported that the biomass of UV-C mutant isolates gave higher yields than the wild type beginning at the initial stage of the cultivation process until the end [4]. It could be possible that random UV- C mutagenesis led to mutation resulting in change and this can be ascribed to the fact that UV-C induced isolates enhanced cell growth.

The results of the observation of the morphological features under a microscope are presented in Table 2 and Figure 3 (A). The selected induced isolates gave different phenotypes depending on the individual isolates. The colony sizes of all the induced isolates, but were slightly bigger than its parent wild type. The colonies gave larger but cell size had reduced. It can be observed that, UV-C induced isolates produced rapid biomass and higher specific growth rate which would lead to rapid cell division in their colony. Thus, cell size had reduced and compacted in colony and cause the colony of induced isolates larger than wild type. The results of this study coincided with the findings of previous reports using various radiation dosages of UV-C irradiation on *Chlorella* sp., in which it was found that the size of the microalgae gradually increased when it was exposed to UV-C [8]. Additionally, the size of the mutants *Synechococcus* sp. D1 and D8 appeared smaller than the wild type [19]. The above-mentioned study demonstrates that the selected mutant produced a variety of phenotype characteristics depending on each isolate.

Table 2. Colony and cell size of *B. braunii* AARL G037 wild type, two selected UV-C induced isolates U18N14, U18J10 and *B. braunii* UTEX LB572; (n) are representative of the replicated numbers for measurement and (±) the standard deviations.

Isolate	Colony size (µm)		Cell size (µm)	
	Length (n = 100)	Width (n = 100)	Length (n = 100)	Width (n = 100)
WT	43.16 ± 10.95[b]	36.73 ± 6.76[b]	8.81 ± 1.65[b]	4.48 ± 0.90[b]
U18N14	50.70 ± 10.84[c]	37.95 ± 6.52[b]	7.96 ± 0.86[a]	3.99 ± 0.50[a]
U18J10	51.59 ± 9.19[c]	37.72 ± 6.72[b]	7.98 ± 0.96[a]	3.86 ± 0.51[a]
UTEX LB572	30.38 ± 3.38[a]	21.04 ± 2.61[a]	10.05 ± 1.82[c]	5.81 ± 1.11[c]

In each column, different letters indicate significant differences ($p<0.05$) using one-way ANOVA test with post-hoc Tukey HSD test.

3.4 Biochemical Composition Analysis

3.4.1 Carbohydrate and protein estimation

The protein and carbohydrate contents of different isolates of *B. braunii* in this study are presented in Figure 4. When analyzed, a comparison of the biochemical composition between the wild type and the induced isolates found that the protein content of U18N14 and U18J10 decreased along with the carbohydrate content and these results were less than those of their wild type. Conversely, when examined, a proportional biochemical presence of protein, carbohydrate, lipid and hydrocarbon contents was observed in the microalgal biomass (Figure 4). It was found that carbohydrate and protein values in the mutants decreased along with increasing lipid and hydrocarbon contents, which were clearly higher than in the wild type as is presented in the discussion below. According to previously published literature, microalgae harvest sun light, water, and CO_2 which are assimilated into the reserve storage biochemical components of carbohydrates, lipids, and proteins. Under genetic manipulation by mutagens or stress conditions, microalgae can shift the biochemical pathway toward the biosynthesis of storage lipids [20]. Many previous researchers have successfully improved microalgae strains and observed that the biochemical synthesis pathways were changed. *Synechocystis* PCC 6803 mutant isolates D8, D9 and D10 revealed that after being mutagenized with chemical reagents, the lipid content increased while carbohydrate content decreased [19]. The enzyme that is involved with the fatty acid biosynthetic pathway is acetyl-CoA carboxylase (ACCase). This enzyme is both regulated and a key regulator of the flux of carbon into fatty acid synthase. Srivaramakrishnan and Incharoensakdi enhanced the lipid production of *Scenedesmus* sp. by UV-C mutagenesis. The studies reported that a mutant revealed greater ACCase activity than the wild type [6]. Similarly, *Chlorella* sp., when induced with UV-C, was reported as having a high activity of ACCase [4]. Therefore, further studies are needed to investigate ACCase activity in order to understand and confirm the findings related to the relevant biochemical changes.

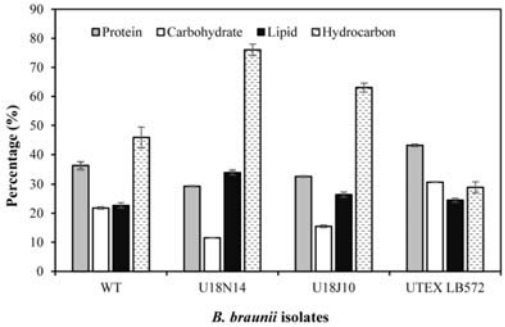

Figure 4. Protein, carbohydrate content from *B. braunii* AARL G037 wild type, two selected UV-C induced isolates U18N14, U18J10 and *B. braunii* UTEX LB572.

3.4.2 Lipid content and productivity

Lipids are primary compounds that are used for the storage of carbon and energy. The content and productivity of lipids are necessary parameters that are used to evaluate biomass. Biomass is suitable as a raw material for biofuel production in much the same way hydrocarbon can be used, as has been discussed above. The figures related to lipid content are presented in Figure 4. Total lipid content of *B. braunii* wild type, induced isolates U18N14, U18J10 and *B. braunii* UTEX LB572 were 22.7±0.92, 34.0±0.75, 26.4±0.90 and 24.5±0.70%, respectively. Induced isolates U18N14 and U18J10 produced lipid content in amounts that were significantly higher than the wild type. Total lipid contents of various strains are shown in Table 3. It was observed that the lipid content of the induced isolates U18N14 and U18J10 were higher than that of *B. braunii* 756 (10.41 - 12.71%) [21], but still less than *B. braunii* IPE001 (46.39 - 46.54%) [22]. In terms of lipid productivity, *B. braunii* AARL G037 (WT) produced 9.50±0.37 mg L^{-1} day^{-1}, while mutant isolates U18N14 and U18J10 produced 28.17±0.64 and 25.61±0.87 mg L^{-1} day^{-1}, respectively, which were greater than those of the wild type by 1.97- and 1.40-fold, respectively. However, these levels of productivity were higher than in the UTEX LB572 by 0.87- and 0.67-fold, respectively. There have been many published studies that have described using ultraviolet mutagenesis as a tool for the promotion of microalgal lipid content, productivity or to generate high performance strains of microalgae. *Tetraselmis suecica* was mutagenized by UV-C combined with fluorescent and microplate reader cell density screening. The results showed that the two improving isolates M5 and M24 had lipid content values at 114 and 123% increases over their wild type [5]. The mutant *Scenedesmus* sp. M22 obtained from UV-C mutagenesis was used to determine the growth rate and lipid content in different H_2O_2 concentrations with the wild type. The investigation showed that *Scenedesmus* sp. M22 accumulated lipid contents in levels that were higher than the wild type [6].

Table 3. Lipid content and productivity values of *B. braunii* AARL G037 wild type, selected UV-C induced isolates U18N14, U18J10, *B. braunii* UTEX LB572 in previous studies.

Strain / isolate	Lipid content (%)	Lipid productivity (mg L^{-1} day^{-1})	Reference
B. braunii AARL G037 (WT)	22.7 ± 0.92[a]	9.50 ± 0.37[a]	This study
B. braunii U18N14	34.0 ± 0.75[c]	28.17 ± 0.64[c]	This study
B. braunii U18J10	26.4 ± 0.90[b]	25.61 ± 0.87[b]	This study
B. braunii UTEX LB572	24.5 ± 0.70[ab]	15.30 ± 0.48[ab]	This study
B. braunii IPE001	46.39-46.50	16.68-20.36	[22]
B. braunii 756	10.41-12.71	-	[21]
B. braunii UTEX LB572	21	5.5	[23]
B. braunii SK	15.8	21.3	[24]
B. braunii TRG	25.1-37-5	24.1-64.5	[24]

- ; no data

The results of lipid content and productivity in this study, in each column, different letters indicate significant differences ($p<0.05$) using one-way ANOVA test with post-hoc Tukey HSD test (n=3).

3.4.3 Hydrocarbon content and productivity

Hexane extracts are generally regarded as crude hydrocarbon fractions. Hydrocarbon content is an important factor in evaluating the utility of the biomass of microalga for hydrocarbon production in terms of commercial biofuel production. The hydrocarbon content of each isolate in this research study is shown in Figure 4. Surprisingly, UV-C induced isolates U18N14 and U18J10 revealed having high amounts of hydrocarbon content at 76.03 ± 1.95 and 63.03 ± 1.54%, respectively, which reflected increases of 30.16 and 17.16 % in hydrocarbon content when compared with the wild type (45.87 ± 3.61%). Additionally, *B. braunii* UTEX LB572 accumulated hydrocarbon content at only 28.90 ± 1.91%. A comparison with previous research work is presented in Table 4. The hydrocarbon content of both mutants U18N14 and U18J10 were higher than 5.7% of *B. braunii* SAG 30.81 [25], 28% of *B. braunii* UTEX LB 572 [26] and 30-39% of the *B. braunii* Showa [27] .

In terms of hydrocarbon productivity, the hydrocarbon productivity was closely related to biomass productivity and hydrocarbon content. The hydrocarbon productivity of the wild type *B. braunii* AARL G037 was 19.19 ± 1.47 mg L^{-1} day^{-1}, which was lower than both of the improved isolates U18N14 and U18J10. Meanwhile, the hydrocarbon productivity of U18N14 and U18J10 were 62.99 ± 1.57 and 61.08 ± 1.52 mg L^{-1} day^{-1}, respectively and were higher than those of the wild type at 2.28 and 2.18-fold. In this study, *B. braunii* UTEX LB572 revealed hydrocarbon productivity at 18.05 ± 1.24 mg L^{-1} day^{-1}, which was less than *B. braunii* AARL G037 (WT) and the two induced isolates U18N14 and U18J10. When levels of hydrocarbon productivity were compared to the findings of other published research studies, the data revealed that the levels of hydrocarbon productivity of WT, U18N14 and U18J10 were greater than of *B. braunii* SAG 30.81 (12.5 mg L^{-1} day^{-1}) [25]. Moutel *et al.* examined *B. braunii* 761 and determined that the microalga yielded 14 mg L^{-1} day^{-1} of hydrocarbon productivity, which was not as much as observed in this study [17]. Under ultrasound conditions, IPE001 generated hydrocarbon productivity at 13.1 mg L^{-1} day^{-1} [22]. A possible explanation therefore is that increased lipid and hydrocarbon content may simultaneous changing of enzymes which involve carbon flux or biosynthesis pathway, and then result in the improvement of the lipid and hydrocarbon content [28]. Moreover, UV-C induction is known to be effective in promoting the growth of *B. braunii* AARL G037 as it can increase the hydrocarbon content that leads to the production of microalga resulting in a high level of hydrocarbon productivity.

So far, it has been acknowledged that these results indicate that UV-C induction is effective in enhancing certain properties of *B. braunii* AARL G037 including growth rate, hydrocarbon content, hydrocarbon productivity, lipid content and lipid productivity. In addition, UV-C mutagenesis would be a suitable choice in generating high performance microalgae among other species.

Table 4. Hydrocarbon content and productivity of *B. braunii* in this study and in other previous reports.

Strain / isolate	Hydrocarbon content (%)	Hydrocarbon productivity (mg L^{-1} day^{-1})	Reference
B. braunii AARL G037 (WT)	45.87 ± 3.61[b]	19.19 ± 1.47[b]	This study
B. braunii U18N14	76.03 ± 1.95[d]	62.99 ± 1.57[d]	This study
B. braunii U18J10	63.03 ± 1.54[c]	61.08 ± 1.52[c]	This study
B. braunii UTEX LB572	28.90 ± 1.91[a]	18.05 ± 1.24[a]	This study
B. braunii UTEX LB572	28	-	[26]
B. braunii SAG 30.81	5.7	12.5	[25]
B. braunii Showa	30-39	-	[27]
B. braunii IPE001	24.3-30.9	8.4 – 13.1	[16]
B. braunii IPE001	40.10-40.37	16.68-20.36	[22]
B. braunii	29.7	71	[18]

- ; no data

The results of hydrocarbon content and productivity in this study, in each column, different letters indicate significant differences ($p<0.05$) using one-way ANOVA test with post-hoc Tukey HSD test (n=3).

3.4.4 Composition of fatty acid methyl ester and biodiesel properties

After conversion of total lipids to FAME, the wild type, U18N14, U18J10 and UTEX LB572 FAME profiles were assessed and are shown in Table 5 and supplementary Figure 1. A comparison of FAME between the wild type and the mutants revealed certain similarities. However, *B. braunii* UTEX LB572 contained various varieties of FAME including Butyric acid (C4:0), Lauric acid (C12:0) or Docosahexaenoic acid (C22:6). All of the microalgal isolates revealed FAME values that were higher than 96.5%. Additionally, Oleic acid (C18:1), hexadecanoic acid or Palmitic acid (C16:0) were the major FAME constituents. Generally, these fatty acids were found to be dominant in microalgal lipids. The dominant FAME in this report was similar to that of the study of *B. braunii* LB 572 which was treated with 34 mM NaCl and revealed that C16:0, C18:1 and C18:2 were the major fatty acids present [29]. In the fatty acid profiles of *B. braunii* KMILT that had been cultivated with various KNO_3 concentration values, the main fatty acids were found to be C16:0 and C18:1 [30]. In *B. braunii*, C18:1 was reported to be a precursor and an intermediate for hydrocarbon production. Nevertheless, dominant lipid compositions from microalgae were likely to influence the main lipid profiles in oily terrestrial plants which are usually used as sources of feed stock for biodiesel production, as is the case with palm, rapeseed and jatropha plants [31].

It has been observed that Oleic acid (C18:1) and Linolenic acid (C18:3) values of induced isolates increased after being treated with UV-C while, Palmitic acid (C16:0), stearic acid (C18:0) and Linoleic acid (C18:2) decreased while, oleic acid (C18:1) and gamma-linolenic acid (C18:3) were slightly increased. Furthermore, both induced isolates revealed a decrease in the composition of saturated fatty acids (SFA). Conversely, there was an increase in monounsaturated fatty acids. These results are similar to those of with Hu *et al.* who mutated *Desmodusmus* sp. with heavy carbon ions and the results showed a slight decrease in the C16:0 content of the mutant [32]. In addition, the results were similar to those published in the report of David *et al*, in which *Tetraselmis suecica* UV-C mutagenized

specimens produced C16:0 and C18:2 in lower values than the wild type. Sarayloo *et al.* reported that, mutated *Chlorella vulgaris* showed decrease in C18:2 and increase C18:1. A reason of changing fatty acid composition may be due to down-regulation of gene encoding "12 fatty acid desaturase which desaturates C18:0 to C18:1. Moreover the overexpression of "12 fatty acid desaturase leads to convers C18:0 to C18:1 [33]. Patel *et al* induced *Synnechocystis* PCC 6803 by chemical mutagen and reported that C16:0 values were reduced, while C18:1 and 18:3 had decreased in value. Additionally, decreased levels of SFA along with higher contents of MUFA were recorded [19]. Unsaturated fatty acids can play an important role in producing biodiesel for use in cold weather. Thus, in this study, there was a considerable increase in the prospect of using fatty acids for biodiesel feed stock.

Table 5. FAME composition (%) of *B. braunii* AARL G037 wild type and selected UV-C induced isolates U18N14, U18J10 and *B. braunii* UTEX LB572.

Carbon number of FAME	WT	U18N14	U18J10	UTEX LB572
Butyric acid (C4:0)				0.40
Lauric acid (C12:0)				0.20
Myristic acid (C14:0)	0.15	1.14	0.13	0.41
Pentadecanoic acid (C15:0)				0.25
Palmitic acid (C16:0)	30.87	28.36	25.60	27.28
Palmitoleic acid (C16:1)	4.88	4.58	8.72	
Margaric acid (C17:0)	0.10	0.53	0.66	
Stearic acid (C18:0)	0.37	0.08	0.12	1.00
Oleic acid (C18:1)	54.65	59.39	59.03	49.10
Linoleic acid (C18:2)	6.30	1.73	1.32	4.03
Gamma-linolenic acid (C18:3)	1.55	2.75	3.31	10.80
Arachidic acid (C20:0)	0.15	0.13	0.11	
Eicosenoic acid (C20:1)				0.80
Arachidonic acid (C20:4)				0.47
Docosahexaenoic acid(C22:6)				3.33
Other	0.98	1.31	1.00	1.93

An evaluation of biodiesel properties and a comparison with the biodiesel EN14214 standard are shown in Table 6. Iodine value (IV) is the quantified total unsaturation or double bond of fatty acids in fuel. In the biodiesel standard EN 14214, a limit IV to 120 g I_2 per 100 g^{-1} of the lipid content was recorded. The wild type and mutants showed 68.64-73.24 g I_2 100 g^{-1} lipid contents. Cetane number (CN) is demonstrative of a time delay in the ignition of fuel for diesel engines. If oil contains high CN, it will mean a shorter ignition time. Wild type and induced isolates revealed CN values in the range of 56.64-57.74. The cold filter plugging point (CFPP) plays an important role in the flow properties of biodiesel during the winter season or at low temperatures. In this study, the wild type and improved isolates presented CFPP values of between -7.90 to -5.73 °C. *B. braunii* UTEX LB572 reported CFPP at -6.34 °C. In addition, cloud point (CP) and pour point (PP) are also indicative of the flow properties at low temperatures. Density (ρ) and viscosity (υ) are two other important factors used in evaluating biodiesel properties. According to the biodiesel standard EN 14214, the density was set at 0.86-0.90 g cm^{-3} and viscosity was set 3.5-5.0 mm^2 s^{-1}. All microalgal isolates that were investigated here exhibited the values of

the biodiesel properties, i.e. IV, CN, CFPP, υ as well as ρ, which conformed to values of the biodiesel standard EN 14214. The quality of biodiesel depend on the proportion of each fatty acid in microalgal lipid, which changes according to in various species and culture conditions [34]. Hence, optimum ratio of all fatty acids composition including saturated, mono-and polyunsaturated fatty acids in microalgae oil were necessary for produced good quality of biodiesel [35].

The biodiesel properties of the *B. braunii* strain in this study were compared to other strains of *B. braunii* and other microalgal species studied in previously published papers. The comparison revealed that each of the biodiesel parameters displayed various results depending on the microalgal species and its lipid profile. Nonetheless, the values of biodiesel quality from almost all of the species of microalgae that are shown in Table 6 are within the range of the biodiesel standard EN14214.

Table 6. Biodiesel properties of *B. braunii* AARL G037 wild type and selected UV-C induced isolates U18N14, U18J10, *B. braunii* UTEX LB572 and other microalgae studied in previous reports.

	SFA	MUFA	PUFA	DU	SV	IV	CN	LCSF	CFPP	CP	PP	APE	BAPE	OS	HHV	υ	ρ	Reference
standard EN14214						<120	>51		<5/-20							3.5- 5.0	0.86-0.90	
standard ASTM D6751-02							>47									1.9- 6.0	0.86-0.90	
standard IS15607							>51		6/18		3/15					2.5- 6.0	0.86-0.90	
B. braunii	9.9	79.6	10.5	99	188	90	55.1							13.8	39.2	4.39	0.86	[36]
B. terbilis	43.2	44.3	12.6	67	184	64	61.7							12.2	37.3	4.13	0.82	
Scenedesmus sp. (WT)	25.3	65.54	9.16	83.86	205.1	77.26	55.52											[6]
Scenedesmus sp. (Mutant)	28.17	68.55	3.28	84.32	205.14	77.68	55.43	3.65										
Scenedesmus obliquus				82.58	195.03	71.62	58.17	5.73 5.08	-5	-4.2								
Chlorella pyrenoidosa				79.85	196.9	69.33	58.42		1.52									[37]
Anabaena sp. MBDU 006	38.35	15.63	42.56	123.48	188.38	117.39	48.85	13.87	-0.49	5.32	-11.39	46.22	74.12		39.94	4.57	0.87	
Nostoc sp. MBDU007	77.4	10.05	9.71	96.82	238.78	30.79	62.22	3.42	27.09	11.25	-1.04	10.7	18.38		39.17	4.03	0.88	[14]
B. braunii AARL G037(WT)	31.64	59.53	7.85	75.23	203.78	69.67	57.41	3.01 2.73	-5.73	9.93	5.39 8.47	70.35	9.70	17.61	39.05	3.83	0.87	
B. braunii U18N14	30.24	63.97	4.48	72.93	203.04	68.64	57.74	3.23	-7.03	9.36	3.95	68.35	7.49	28.91	38.92	3.82	0.86	
B. braunii U18J10	26.62	67.75	4.63	77.01	203.55	73.24	56.64		-7.90		2.38	38.29	8.16	28.06	39.04	3.81	0.87	This study
B. braunii UTEX LB572	29.54	49.90	18.64	87.18	201.35	98.72	51.20		-6.34		3.34	96.85	43.24	10.54	38.62	3.60	0.86	

SFA: Saturated fatty acid (%), MUFA: Mono unsaturated fatty acid (%), PUFA: Poly unsaturated fatty acid (%), DU: Degree of unsaturation, SV: Saponification value (mg g^{-1}), IV: Iodine value (g I_2 100 g^{-1} lipid), CN: Cetane number, LCSF: Long chain saturated factor, CFPP: Cold filter plugging point (°C), CP: Cloud point (°C), PP: pour point (°C), APE: Allylic position equivalent, BAPE: Bis-allylic position equivalent, OS: Oxidation stability (h), HHV: Higher heating value, υ: Kinematic viscosity (mm^2 s^{-1}), ρ: Density (g cm^{-3}).

4. CONCLUSION

UV-C treatment can promote the productivity of *B. braunii*, as has been determined in this study. The use of UV-C induction to enhance the production of hydrocarbons, lipids or biomass seems to be a promising approach in obtaining these useful microalgal metabolites on a large scale in the industrial sector. It is believed that further studies should focus on identifying the optimized conditions for UV-C induced isolates for greater productivity, gene stability or improved gene frequency in the induced isolates.

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