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Mass cultivation of new algae *Tetraselmis straiata* BBRR1 under open raceway ponds for biodiesel and biocrude production

Annakkili Baskara Boopathy[1,2,3], Thanasekaran Jayakumar[3], Muthu Ganasan Rajaram[1], Natarajan Mohan[1], Chinnasamy Senthil[4], Subramanian Nagaraj[1], Ramasamy Rengasamy[1], Manjunath Manubolu[5], Joen-Rong Sheu[3*], Chao-Chien Chang[6,7,8*]

[1]Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai-600 025, Tamil Nadu, India; E-Mail: annakkilibaskar@gmail.com (A.-B.B); raja.ram091@gmail.com (M.-G.R); jnmohan08@gmail.com (N.M); nagalilly@gmail.com (S.N); profrrengasamy@yahoo.com (R.R)

[2]Department of Biochemistry, Caussanel College of Arts and Science, Muthupettai, Ramanathapuram-623523,Tamil Nadu, India

[3]Department of Pharmacology, Graduate Institute of Medical Sciences, Taipei Medical University, Taipei, Taiwan; tjaya_2002@yahoo.co.in (T.J);

[4]Aban Biotechnology division, Thandalam - 602 105, Kanchipuram, Tamil Nadu, India; E-Mail: senthilc@aban.com (C.S); [5]Department of Evolution, Ecology and Organismal Biology, Ohio State University, Ohio-43212, USA; E-Mail: manubolu.1@osu.edu (M.M)

[6]Department of Cardiovascular Center, Cathay General Hospital, 106 Taipei, Taiwan; [7]Department of Pharmacology, School of Medicine, College of Medicine, Taipei Medical University, 106 Taipei, Taiwan; [8]Division of Cardiology, Department of Internal Medicine, School of Medicine, College of Medicine, Fu Jen Catholic University, 242 New Taipei City, Taiwan

[*]**Correspondence to:** Dr. Joen-Rong Sheu, Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, No. 250, Wu-Hsing St., Taipei 11031, Taiwan. Tel: +886-2-27361661 ext. 3199; Fax: +886-2-27390450; E-mail: sheujr@tmu.edu.tw; Dr. Chao-Chien Chang, Department of Cardiology, Cathay General Hospital, No. 280, Sec. 4, Jen-Ai Rd, Taipei 106, Taiwan. Tel: +886-2-27082121-3116; Fax: +886-2-27099408; e-mail: change@seed.net.tw

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Abstract: In the process of modernization and development, a human being always needed energy, which increased the dependency on the available sources of fossil fuel. *Tetraselmis,* a green algal genus belong to the order Chlorodendrales, are described by their strong green coloured chloroplast, flagellated cell bodies, and the occurrence of a pyrenoid within the chloroplast. In this study, four different strains of *Tetraselmis* species were successfully isolated from the saltpans Kovelong, Chennai, Tamil Nadu, India. The isolated strains were cultured in the normal basal medium and their morphological features were subsequently studied. The species of *Tetraselmis straiata* (*T. straiata*) Butcher BBRR1 was confirmed using molecular identification of 18S rRNA gene analysis and its observed systematic position. Among the four different isolates, *T. straiata* Butcher BBRR1 recorded a highest biomass concentration of 0.58 ± 0.021 g L^{-1}, 15% lipids, 19% proteins and 17% carbohydrates when it grown under laboratory condition. Whereas, in open raceway ponds, *T. straiata* BBRR1 produced 0.95 ± 0.06 g L^{-1} biomass, 19% lipids, 28% proteins and 21% carbohydrates in an modified CFTRI I medium. The fatty acids profile of *T. straiata* Butcher BBRR1showed the presence of 33.14 % Palmitic acid, 22.64% 11- Octadecenoic acid and 21.94% Heptadecanoic acid. Since *T. straiata* BBRR1 can be cultivated in open ponds without a major contaminations, this species can be used as novel biomass feedstock to produce biofuels. This study may suggest the potential of *T. straiata* BBRR1 for biofuel production and could compete the energy demand in the future. In addition, this species contains healthful components of carotenoids, lipids and proteins, all these may provide a health benefits beyond basic nutrition.

Keywords: green algae; biomass; fatty acids; *Tetraselmis straiata*; lipids; carotenoids; raceway ponds

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1. Introduction

In the process of modernization and development, human being always needed energy, which increased the dependency on the available fossil fuel sources. The exhaustive use of fossil fuel sources has raised the serious concerns worldwide not only about energy security but also for negative impact on environment. India's growing demand for petroleum-based fuel has created challenges for the country's energy security, as almost 90% of its crude oil requirement is imported from oil producing countries. Algae have been widely used for fuel production because of their high photosynthetic efficiency, high biomass production, and fast growth [1]. Microalgae as biomass has potential chemical composition such as proteins, lipids and carbohydrates [2]. Many algae accumulate substantial amounts of non-polar lipids, mostly in the form of tri acyl glycerol or hydrocarbons, and these levels may reach up to 20-50% of dry cell weight. Algae can also grow in saline, brackish and coastal seawater with slight struggle [3]. Microalgae can provide an alternative biofuel feedstock due to their rapid growth rate, greenhouse gas fixation ability (net zero emission balance) and high production capacity of lipids and do not compete with human and animal food crops. Moreover, they will grow on non-arable land and saline water [4].

Microalgae cultivation is a promising methodology for solving some of the future problems of biomass production (i.e. renewable food, feed and bioenergy production). There is no doubt that in conjunction with conventional growth systems, novel technologies must be developed in order to produce the large-scale sustainable microalgae products [5]. The most conservative scenario contains algae oil from microalgae grown in open ponds on non-arable land filled with salt water [6]. Many algae are exceedingly rich in oil, which can be converted into biodiesel. The oil content of some microalgae exceeds 80% of the dry weight of algae

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biomass [7]. Aquatic biomasses present an easy adaptability to grow in different conditions either in fresh or marine water or in a wide range of pH. This makes the aquatic biomass more adaptive or an enhanced CO_2 fixation to afford a high biomass production. CO_2 is usually bubbled from beneath at rates for optimum uptake by microalgae cells [8]. Microalgae are traditionally considered as good source of fatty acids [3], and the fatty acids in microalgae are suitable for biofuel synthesis [9]. The fatty acid profiles of microalgae has been well established [10,11]. Microalgae have a strong capacity to produce lipids, which can be easily converted to biodiesel.

The most cost effective cultivation system for mass culture production *of Tetraselmis straiata* (*T. straiata)* BBRR1 is to grow in an outdoor open airway pond. However, trials on cultivating *T. straiata* in non-axenic systems often fail due to thriving of other green algal and cyanobacterial contaminants, and hence it relatively slow downs the growth of *T. straiata*. Therefore, methodological or technological breakthrough to control the growth of these contaminating species is awaited to minimize the oil production cost using microalgae. The aim of the present study is to mass cultivate the alga *T. straiata* isolated from the saltpans bodies Kovelong, Chennai, Tamil Nadu, India in the open raceway pond. The growth performance in terms of biomass, pigments, lipids, proteins and carbohydrates were analyzed. Carotenoids are linked with numerous health benefits, such as hindrance of age-related macular degeneration, cataract, some cancers, rheumatoid arthritis, muscular dystrophy and cardiovascular problems. Since *T. straiata* holds substantial amounts of carotenoids, this species may also have health benefits for human beings.

2. Results and Discussion

2.1. Isolation of Tetraselmis spp.

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In the present attempt, water samples collected from the saltpans (60 ppt) Kovelong, Chennai, Tamil Nadu, India, were brought to the laboratory and segregated by using spread plate technique (Figure 1a,b.) on f/2 agar medium. Four different isolates of quardriflagellate alga, *Tetraselmis* were isolated and identified as, *Tetraselmis chuii*, *T. gracilis*, *T. straiata* and *T. tetrathele* based on their morphological features of colour, colony, morphology and cell size [12]. *Tetraselmis* is a euryhaline microalga and commonly present in saltpans and marine environments [13, 14]. The colonies *T. chuii* Butcher is a green four-flagellated alga, ovoid body shape with a distinct curve (cell length: 12-14 µm, width: 9-10 µm). *T. gracilis* (Kylin) Butcher cells are ellipsoid and slightly compressed, with four anterior lobes; pyrenoid large, subbasal, with U-shaped starch shield; median eyespot, large, red - orange. Its cell length: 8-12 µm and width 6-9 µm. *T. striata* Butcher cells showed marked longitudinal rows of granules and posterior eyespot with cell length of 10-21 µm and width 8-17 µm. *T. tetrahele* (G.S.West) Bucher cells compressed, with deep and wide four-lobed apical furrow and its cell length 10-16 µm and width 8 -12 µm. These colonies are bright green and grown in f/2 medium under 30 μEm^{-2} S^{-1} light intensity 12/12 light dark cycle at 24 ± 1°C. For marine and brackish strains, the f/2 medium is the most commonly used medium [15].

2.2. Dry biomass and total lipids in Tetraselmis species

The four different species of microalgae *Tetraselmis* were taken for the growth analysis under laboratory conditions revealed that the isolate of *T. straiata* showed a maximum biomass and lipid content of 0.58 ± 0.021 g L^{-1} (Table 1) and 151 ± 3 mg L^{-1} (Table 2), respectively, and followed by *T. chuii*, *T. tetrahele* and *T. gracilis* (Figure 2a, b). Huerlimann et al. [16] observed that *T. gracilis* showed a maximum biomass of 0.35 g L^{-1}, which was 8.0% less than the test

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alga, *T. straiata* and the specific growth rate of 0.25. Arkronrat et al. [17] reported a maximum and a specific growth rate of 0.16 in *Tetraselmis* sp., which was 36% less than the present alga, *T. straiata*. In the present study, the isolate of *T. straiata* Butcher had maximum lipid content than the other three isolates. Thirty-day-old culture of *T. straiata* Butcher was harvested and analysed the biochemical parameters. *T. straiata* had maximum lipid content of 33.60% dry weight and lipid productivity of 29.14 mg L^{-1} day^{-1} (Table 3). Huerlimann et al. [16] had found only 18.6 mg L^{-1} day^{-1} of lipid productivity on *Tetraselmis* sp., which was 37% less than the present isolate *T. straiata*.

The morphology of alga within in each chemical competition could vary in relation to age and culture conditions [18]. As the morphological heterogeneity of the alga makes the identification difficult, molecular tools like PCR play a vital role in confirming the systematic position of the experimental alga. In the present study, a partial 18S region of the ribosomal RNA gene was isolated and amplified with the specific primers. The sequence was found 99% similarity with *T. straiata* JQ315813 KMMCC 1157 strain. The obtained sequence was compared with the existing sequences in the NCBI database by the BLAST algorithm homology (sequence identity) confirmed with a close relationship of the isolated candidate *T. straiata*. Based on the classical taxonomy as well as molecular taxonomy, the test alga was identified and confirmed as *T. straiata* Butcher BBRR1 (Figure 3). The sequence was submitted in the GenBank, NCBI and the Accession Number is KP317837.

2.3. Phytochemicals, lipids, biomass and fatty acids of T. straiata BBRR1

The isolate *T. straiata* BBRR1 performed well than the rest of three isolates in the laboratory conditions and was scaled up to 150 L in an open pond. It was then transferred to the raceway pond containing 1350 L Modified CFTRI - ABRR I medium. The initial concentrations

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of 0.05 ± 0.004 mg L^{-1} and 0.03 ± 0.002 mg L^{-1} of chlorophyll *a* and b were increased during the growth period and they raised up to 6.13 ± 0.005 mg L^{-1} and 5.23 ± 0.003 mg L^{-1} on 15th and 18th day, respectively. The concentration of chlorophyll *a* and b were recorded maximum on 15th day and reached a plateau thereafter (Figure 4 a, b), although the productivity in terms of biomass continued to increase until day 15. This data indicated that the cells continued to grow resulting on the net increase in the biomass concentration up to day 15. Similarly, the initial concentration of 0.15 ± 0.002 mg L^{-1} of total carotenoid content was increased up to 56.5 ± 0.23 mg L^{-1} on 18th day (Figure 4c). The initial biomass of 0.12 ± 0.01 g L^{-1} was increased up to a maximum of 0.95 ± 0.06 g L^{-1} on Modified CFTRI-ABRR I medium, which was 28% higher than control on 15th day (Figure 5). Based on the total biomass productivity, the lipid production and comparative biochemical analysis was calculated in *T. straiata* BBRR1 cultivated under laboratory and open raceway pond as shown in Tables 3 and 4. Daily biomass productivity for the *T. straiata* BBRR1 cultivated under open raceway pond was observed about 0.063, and 0.050 g L^{-1} d^{-1}, specific growth rates of 0.45 and 0.39, division rates of 0.64 and 0.56 and generation times of 1.55 and 1.79 in the Modified CFTRI-ABRR I medium and f medium (control), respectively (Table 5). The present isolate, *T. straiata* BBRR1 showed an aerial biomass productivity of 8.83 g L^{-1} m^2 d^{-1} in Modified CFTRI-ABRR I medium. Similarly, Fon-Sing and Borowitzka [19] reported that the alga recorded average biomass productivity of 8.3 g L^{-1} m^2 d^{-1} in *Tetraselmis* sp.

The alga had a maximum total lipid of 152 ± 39 mg L^{-1} on 18th day, which was 19% more than control (f medium). Total lipid percentage / ash - free biomass of the sample in Modified CFTRI-ABRR I medium was 27.50%, as against 29.76% in control on 18th day. The organism showed lipid productivity of 8.43 and 7.06 mg L^{-1} d^{-1} when it was grown in Modified CFTRI - ABRR I medium and control medium, respectively. *Tetraselmis* was the most suited for the mass

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cultivation due to fast growth, ease of culture and relatively high lipid content. Rodolfi et al. [11] reported *Tetreselmis* and *Nannochloropsis* showed high lipid productivity and found to be the promising marine species for biomass production.

Nutrients management could change biochemical constituents of algae reported by Hsieh and Wu [20]. During the study period, the lipid content in *T. straiata* BBRR1 increased due to growth limiting factors like nutrient deficiency in the lack of nitrogen and phosphate sources. Pernet et al. [21], Li et al. [22] and Arumugam et al. [23] also reported that the biomass and lipid content of microalgae were affected at different cultivation and nutrients conditions. The culture in the open system was observed periodically under compound microscope, and the end of the study the algal biomass in the pond was harvested and dried. The different parameters such as lipids, proteins, and carbohydrates were similar to the observations made under laboratory conditions and open raceway ponds (Table 4).

The alga *T. straiata* BBRR1 produced 15.2% of lipid content on its dry weight basis under open raceway pond. The total content of lipids varies from 1-85% of the dry weight with values higher than 40% being typically reached under nutrient limitation studied by Regan [24]. The fatty acid composition of hexane extracted FAMEs was analysed by GC-MS. The isolate *T. straiata* BBRR1 revealed the presence of 33.14 % of Palmitic acid, methyl ester, followed by 22.64% 11- Octadecenoic acid, methyl ester, 21.94% Heptadecanoic acid, 16 -methyl ester and minimum of 5.94% Palmitoleic acid, methyl ester, and 3.14% Pentadecanoic acid, methyl ester (Table 6). Similarly, Sharmin et al. [9] found significant amounts of myristic acid (C 14:0), palmitic acid (C 16:0), stearic acid (C 18:0), and palmitoleic acid (C 16:1) in marine microalgae *Skeletonema costatum* available in Bangladesh Coast and they suggested this algae to be used as biodiesel feedstock. Moreover, *T. straiata* BBRR1 contains hexadecatrienoic acid,

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benzenedicarboxylic acid, oleic acid, arachidonic acid, eicosapentaenoic acid and 9-Octadecenoic acid methyl ester.

Based on the results obtained in the present study, the volumetric productivity of biomass were 0.089 g L^{-1} d^{-1} in Modified CFTRI-ABRR I medium. This study also indicates that *T. straiata* BBRR1 has the potential to produce 48.72 dry tons of biomass ha^{-1} $year^{-1}$. In the view of shrinking fossil fuel sources, algae mass cultivation for biofuel production is gaining importance day by day. However, the cost of production of algal biomass needs to be reduced below $200 dry tons to make algae as an attractive biomass feedstock for biofuel production. However, the cost of growth medium used for mass cultivation of *T. straiata* BBRR1 was estimated as $53,000. Similarly, the cost of the cultivation media required to produce 1 ton of algal biomass through open raceway ponds was estimated as $1128 t^{-1}. As the estimated cost of growth medium used for mass cultivation of *T. straiata* BBRR1 was higher than the selling cost of biofuel algae, future research efforts are needed to reduce the nutrients cost through the use of municipal, agricultural and industrial waste streams rich in organic and inorganic nutrients which are renewable in nature.

3. Materials and Methods

3.1. Isolation of Tetraselmis spp.

The algal samples collected from the saltpans of Kovelong, Chennai, Tamil Nadu, India were brought to the laboratory and the colonies were segregated based on their morphological features using compound microscope [12]. A single colony of the four different isolates was picked up with the help of micropipette. After thoroughly washed with sterile f/2 medium for five times, the isolates was subjected to grow in the f/2 agar medium [15]. The culture medium

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was kept at 24 ± 1ºC in thermostatically controlled room, illuminated with cool fluorescent lamps at irradiance of 30 $\mu Em^{-2}s^{-1}$, under 12/12 light dark cycle.

3.2. Laboratory studies

Experiments were conducted in 500 mL Erlenmeyer flasks with 270 mL of sterile f/2 medium and inoculated with 30 mL of optimally grown four different isolates of *T. straiata* separately. The experimental studies were carried out for a period of 21 days. The growth parameters such as cell number, biomass [25], the levels of different pigments such as Chlorophyll *a*, Chlorophyll b and carotenoids [26], total carbohydrates [27], proteins [28] and lipids [29] were recorded at every 3 days interval during the study period. It revealed that *T. straiata* Butcher showed good growth and lipid productivity than the rest. Therefore, this alga was chosen for further investigation.

3.3. Molecular characterization

The genomic DNA samples of *T. straiata* were isolated from the lyophilized algal biomass using the PCR kit obtained from GENEI, Bangalore, India. RNA contamination was eliminated by digesting the extract with 10 µg of RNase-A for 30 min at 37°C. The amount of DNA of the sample was quantified by measuring its absorbance at 260 nm in a spectrophotometer. The optical density (OD) of 1.0 corresponds to 50 µg/mL of double stranded DNA [30] and the purity of DNA was determined at 260 and 280 nm. DNA solution that had a value of 1.8 obtained from the data recorded at 260 nm/ 280 nm was considered as pure.

In the present study, the 18S rRNA gene region of the *T. straiata* isolates were subjected for the amplification of the primers (GENEI, Bangalore, India) as described by Richards et al. [31]. The forward primer 5'- GTAACCCGTTGAACCCCATT - 3' and reverse primer 5'-

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CCATCCAATCGGTAGTAGCG - 3' were used as described by Liu et al. [32]. Polymerase chain reaction (PCR) was performed in a ABI thermal cycler (ABI) using a PCR program with initial denaturing at 95℃ for 5 min, followed by 35 cycles of denaturation at 95℃ for 1 min, annealing at 58℃ for 55 seconds and extension at 72℃ for 50 seconds and a final extension at 72℃ for 10 min. The PCR products were separated through agarose gel electrophoresis. The purified PCR products were separated using 1.4 % agarose gels and stained with 0.5 µg/mL ethidium bromide and the gel was viewed and captured using Vilber Loumart Gel Documentation system. Sequences were determined by the chain termination method with the use of Dye Deoxy Terminator Cycle Sequencing Kit (Perkin Elmer Applied Bio - system, UK) using an AV1377 automated DNA sequencer.

3.4. Familiarization of T. straiata Butcher BBRR1 for outdoor condition

The isolate of *T. straiata* BBRR1 grown under laboratory condition produced high biomass yield and lipid content was scaled up to1 and 2 L in flasks for 2 weeks and further scaled up to 10 L in transparent carboys for 2 weeks. The 15 L culture grown under laboratory condition was used as seed material, inoculated in 135 L medium in a 1.0 m^2 of 150 L capacity mini open raceway pond (Length 2.2 m; Width 0.50 m; Depth 0.26 m) floors are coated with FRP (Fibre-Reinforced Polymer) material and incubated for 2 weeks. The raceway ponds are provided with paddle wheel system for the aeration at 10 rpm. The culture was mixed with paddle wheel system during daytime. The alga in the pond was subjected for adaptation to open air condition for 2 cycles (sub-culturing at an intervals of 2 weeks). During the experimental period the evaporation of water was compensated with chlorine treated bore well water. The raceway ponds were protected from dust on the top with transparent polythene sheet.

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3.5. *Mass culture of T. straiata BBRR1 in open raceway pond*

This experiment was conducted in a concrete raceway pond 10.0 m^2 of 1500 L capacity (Length 7 m; Width 1.5 m; Depth 0.26 m) floors are coated with reinforced polymer. The inoculum was raised in the following order: Optimally grown 15 L of *T. straiata* BBRR1 culture obtained from the laboratory condition was inoculated into 1.0 m^2 raceway pond with a biomass concentration of 0.06 g L^{-1} contained 135 L medium and kept for 10 days. Then the culture (150 L) was inoculated to 10.0 m^2 raceway pond contained 1350 L of basal (f/2) medium and the culture height in the pond was maintained at 15 cm level. The algal culture was mixed with paddle wheel system during day time to prevent settling and enhance dissolved CO_2 concentration. This experiment was conducted for a period of 21days in batch mode. At every 3 days interval, pH, biomass, pigments, total carbohydrates, proteins and lipids were analyzed and recorded. Microscopic analysis was carried out daily to check the purity of the culture. The average temperature and light intensity were ranged from 28°C to 35°C and 42000 and 52000 lux, respectively. At the end of the study period, the algal biomass was harvested and analyzed for different parameters.

3.6. *Biomass harvest of T. straiata BBRR1*

In open raceway ponds, the biomass of *T. straiata* BBRR1 was harvested by switch off the paddle wheel for a period of 24 h. The biomass was collected after the medium was drained off. The algal cells settled at the bottom in the open raceway pond was harvested after 12 h through autoflocculation on 21 day. The biomass in the open raceway pond was washed with ground water and the cells were allowed to settle. This process was repeated for 3 times in order to

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remove the excess salt in the algal biomass. The washed algal cells were spread on white plastic sheet and dried over the sun light for 3 h followed by oven drying at 60℃ for 8 h.

3.7. Biomass estimation

Twenty five millilitres of algal culture was taken and washed thrice with 25 mL of isotonic solution containing 0.65 M ammonium formate [25] in order to remove excess salt. Pre weighed Whatman GF/C glass microfiber filters (1.2 µm) was used to obtain the biomass after filtration in the moisture analyser (Mettler Toledo HR83). Then, the filter along with the biomass was placed in the moisture analyzer and its final dry weight was recorded after drying at 100˚ C for ~8 minutes. Dry weight was calculated after subtracting the filter weight and expressed the values as g L^{-1}.

3.8. Extraction of algal oil and fatty acid analysis

Ten grams of dried algal biomass was treated with hexane 1:5 (w/v) and extracted algal oil by using Soxhlet apparatus at the boiling temperature of the solvent for 8 h. The solvents containing the algal oil were filtered through Whatman GF/C filter paper. The solvents were recovered using rotary evaporator at the respective boiling temperature. The algal oil was recorded gravimetrically and expressed the values as ash-free biomass.

3.9. Acid transesterification

Ten gram of total lipid was extracted from the alga by using chloroform and methanol (2:1) and 0.6 mL g^{-1} of sulphuric acid. The reaction mixture was kept at 90˚C in a water bath for 40 min and intermittently mixed. Then, it was allowed to cool at room temperature, and added 2.0 mL g^{-1} of distilled water and mixed for 45 sec. After phase separation the solvent layer

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contained biodiesel of fatty acid methyl ester (FAME) was collected and transferred to a pre weighed glass vial. The solvent was evaporated using liquid N_2, and quantified the amount of biodiesel gravimetrically. The fatty acid composition of the sample was analysed by GC-MS. FAME was analyzes by GC-MS (Agilent 6890 gas chromatograph, 15 m Alltech EC - 5 column (250 µ I.D., 0.25µ film thickness). A JEOL GCmate II bench top double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-2000[1] software was used for all the analyses. Identification of FAME was made by matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instruments software.

3.10. Statistical analysis

Throughout the study period, triplicates were maintained for each experiment. The results were expressed as Mean ± standard deviations. Data were analysed statistically using Origin Pro.v 8.0 for Windows.

4. Conclusion

This study envisaged on researching the feasibility and sustainability of microalgae as biofuel feedstock to meet the energy crisis. In the present attempt, four different species of *Tetraselmis* belong to Chlorophytes were isolated as *T. chuii* Butcher, *T. gracilis* (Kylin) Butcher, *T.straiata* Butcher and *T. tetrathele* (G.S.West) Bucher. Among these isolates, *T. straiata* contained a maximum biomass productivity in indoor and outdoor conditions. This is the first attempt to cultivate *T. straiata* BBRR1 in an open outdoor raceway pond for biomass and biofuel applications. As this isolate can be grown in open ponds without any major

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contaminations, it can be used as a novel biomass feedstock to produce various biofuels. This work revealed the potential of *T. straiata* BBRR1 for biofuel production and could match the demand of energy in future.

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Authors Contributions

A.B.B., M.G.R. and R.R conceived the project and designed the experiments. N.M., C.S. and S.N assisted to wrote the paper. T.J., M.M. and R.R wrote the paper. J.R.S. and C.C.C contributed clarifications and guidance on the manuscript. All authors were involved in editing the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Table captions

Table 1. Biomass (dry wt.) content of *T.tetrahele, T.gracilis, T.chuii* and *T.straiata* grown in laboratory condition.

Table 2. Total lipid content of *T.tetrahele, T.gracilis, T.chuii* and *T.straiata* grown in laboratory condition.

Table 3. Biomass, lipid productivity and lipid percentage of different *Tetraselmis* spp.

Table 4. Comparative biochemical analysis of *T. straiata* BBRR1 grown under laboratory and open raceway pond.

Table 5. Daily productivity, specific growth rate, divisions per day and generation time of *T. straiata* BBRR1 cultivated under open raceway pond.

Table 6. Lipid profile of *T. straiata* BBRR1 grown under open raceway pond

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Figure legends

Figure 1. **(a)** Colonies of *Tetraselmis* spp. on 2% agar f/2 medium on 20th day. (**b)** *Tetraselmis tetrahele* (A), *Tetraselmis straiata* (B), *Tetraselmis chuii* (C), *Tetraselmis gracilis* (D) on 18th day culture.

Figure 2. **(a)** Dry Biomass and **(b)** total lipids of *Tetraselmis* spp. at different intervals.

Figure 3. PCR amplification of 18S rRNA gene of *T. straiata*. Lane 1. DNA ladder (kB); Lane 2. PCR amplified product of *T. straiata.*

Figure 4. **(a)** Chlorophyll *a* **(b)** Chlorophyll b and **(c)** total carotenoids of *T. straiata* BBRR1 in Modified CFTRI ABRR I medium at different intervals in 10.0 m^2 open raceway pond.

Figure 5. Dry biomass of *T. straiata* BBRR1 in Modified CFTRI ABRR I medium at different intervals in 10.0 m^2 open raceway pond

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Table 1

Days	*T.tetrahele* ($g\ L^{-1}$)	*T.gracilis* ($g\ L^{-1}$)	*T.chuii* ($g\ L^{-1}$)	*T.straiata* ($g\ L^{-1}$)
0	0.050 ± 0.02	0.060 ± 0.03	0.030 ± 0.03	0.030 ± 0.02
3	0.140 ± 0.05	0.170 ± 0.06	0.180 ± 0.08	0.200 ± 0.04
6	0.190 ± 0.08	0.200 ± 0.011	0.170 ± 0.013	0.240 ± 0.05
9	0.240 ± 0.08	0.280 ± 0.015	0.230± 0.018	0.300 ± 0.06
12	0.250 ± 0.012	0.310 ± 0.021	0.300± 0.021	0.350 ± 0.011
15	0.250 ± 0.015	0.350 ± 0.025	0.360 ± 0.026	0.380 ± 0.013
18	0.330 ± 0.018	0.330 ± 0.032	0.380 ± 0.031	0.420 ± 0.017
21	0.420 ± 0.022	0.310 ± 0.035	0.330 ± 0.037	0.580 ± 0.021
24	0.320 ± 0.025	0.160 ± 0.037	0.250 ± 0.041	0.310 ± 0.026

Data represent mean ± Standard Error (SE) of three replicates.

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Table 2

Days	*T.tetrahele* (mg L^{-1})	*T.gracilis* (mg L^{-1})	*T.chuii* (mg L^{-1})	*T.straiata* (mg L^{-1})
0	22 ± 3	21 ± 4	30 ± 2	44 ± 2
3	51 ± 4	44 ± 4	34 ± 5	52 ± 4
6	61 ± 3	51 ± 3	55 ± 3	61 ± 5
9	64 ± 4	59 ± 2	63 ± 3	72 ± 4
12	66 ± 4	66 ± 4	67 ± 4	77 ± 3
15	71 ± 2	83 ± 5	82 ± 6	103 ± 2
18	94 ± 3	92 ± 3	86 ± 4	120 ± 3
21	88 ± 3	80 ± 2	112 ± 7	151 ± 3
24	61 ± 2	68 ± 3	72 ± 3	80 ± 2

Table 3

***Tetraselmis* spp.**	**Biomass productivity (g L^{-1} d^{-1})**	**Lipid productivity (mg L^{-1} d^{-1})**	**% Lipid content / Dry biomass**
Tetraselmis chuii	0.023 ± 0.003	5.33 ± 0.005	31.46 ± 1.24
Tetraselmis gracilis	0.024 ± 0.004	5.12 ± 0.004	27.88 ± 1.12
Tetraselmis straiata	0.025 ± 0.004	7.21 ± 0.005	33.60 ± 1.25
Tetraselmis tetrathele	0.021 ± 0.005	5.22 ± 0.004	28.48 ± 1.05

Data represent mean ± Standard Error (SE) of three replicates.

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Table 4

Parameters	Laboratory	Open raceway pond
Lipid (%)	15 ± 0.87	19 ± 0.98
Carbohydrate (%)	17 ± 1.64	21 ± 1.69
Protein (%)	19 ± 0.92	28 ± 0.87

Table 5

Culture condition	Daily productivity ($g\ L^{-1}\ d^{-1}$)	Specific growth rate K (div^{-1})	Div./day	Gen. time (d)
Open raceway	0.063	0.45	0.64	1.55
Laboratory	0.050	0.39	0.56	1.79

Data represent mean ± Standard Error (SE) of three replicates

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Table 6

Fatty acids	Wt. % (%)
7-Octadecene	1.49
Methyltetradecanoate	2.63
Pentadecanoic acid, methyl ester	3.14
Palmitoleic acid, methyl ester	5.94
Palmitic acid, methyl ester	33.14
Hexadecanoic acid, 14-methyl-, methyl ester	2.82
11-Octadecenoic acid, methyl ester	22.64
Heptadecanoic acid, 16-methyl-, methyl ester	21.94
Cyclopropane octanoic acid 2-hexyl-methyl ester	1.64

Data represent mean ± Standard Error (SE) of three replicates

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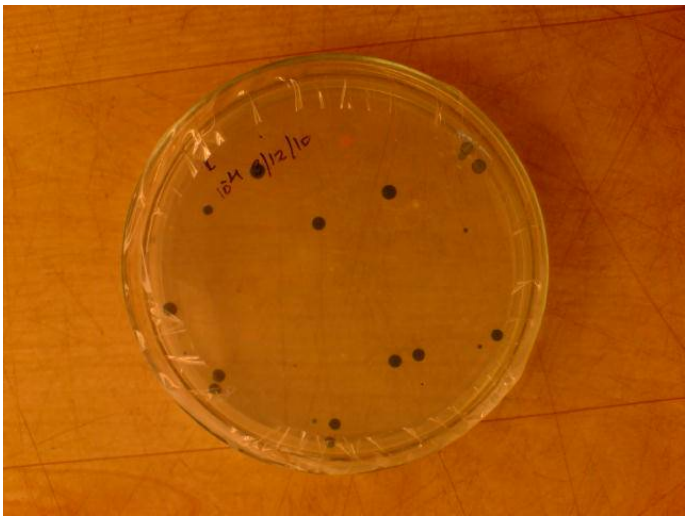

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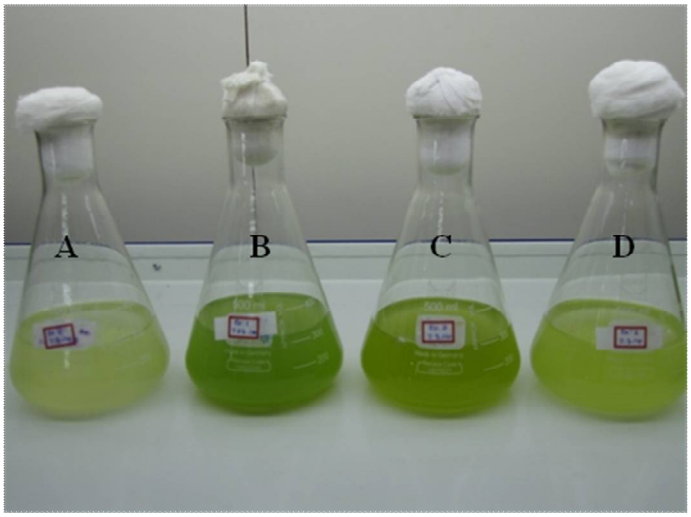

Fig. 1

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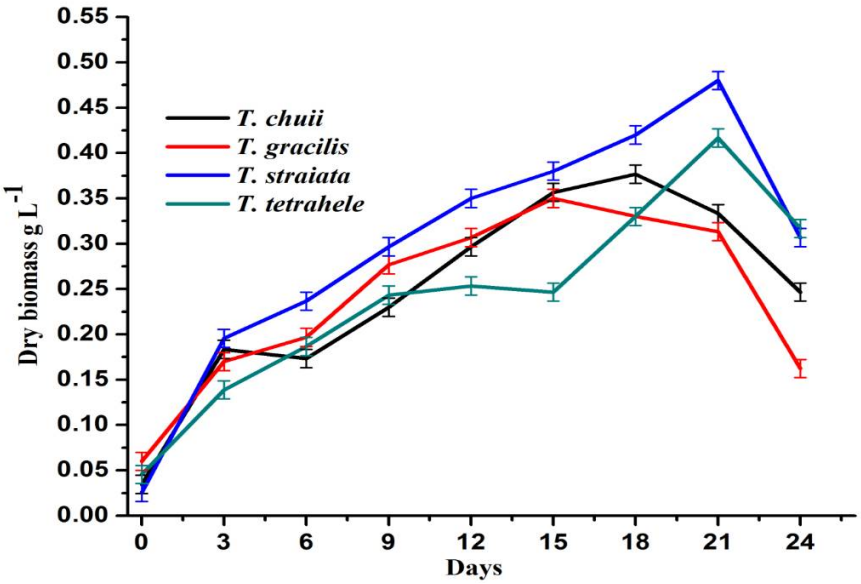

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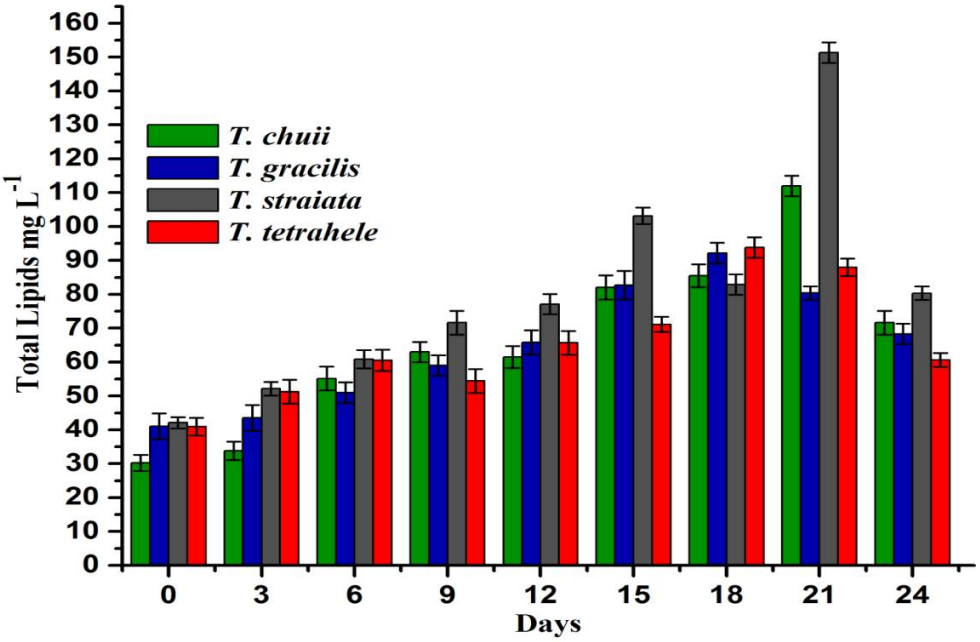

Fig. 2

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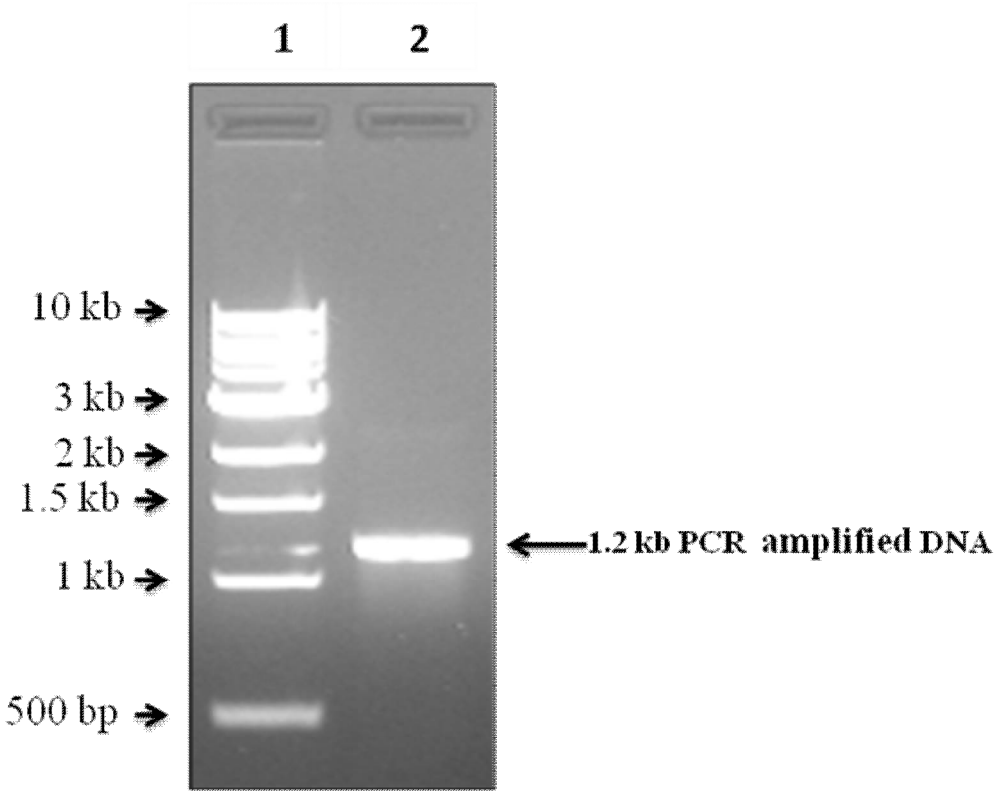

Fig. 3 [28]

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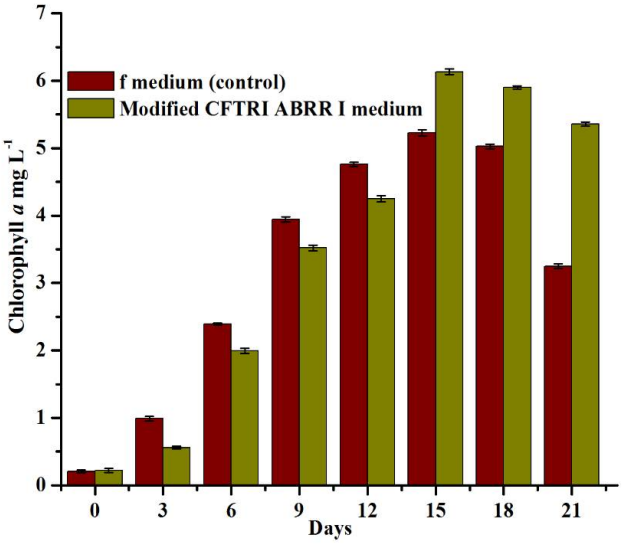

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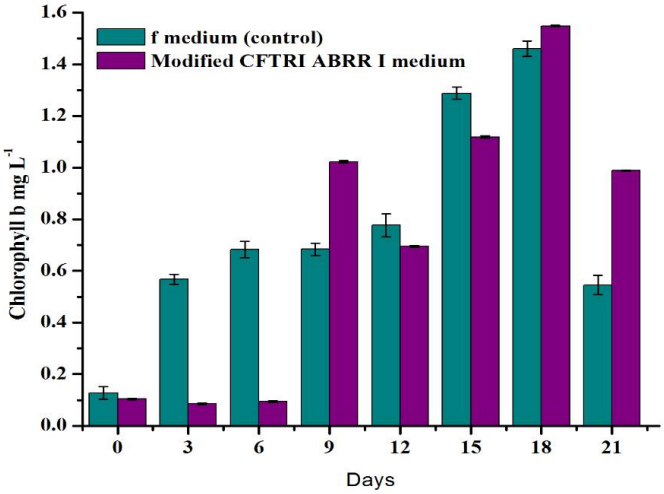

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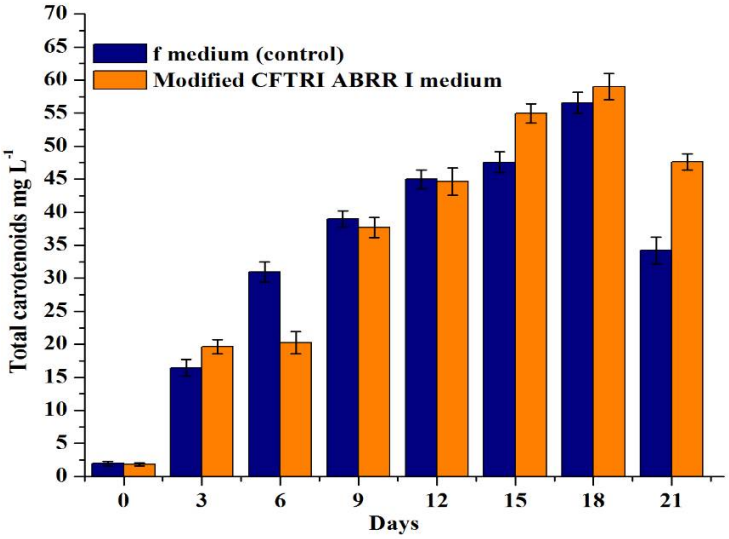

Fig. 4

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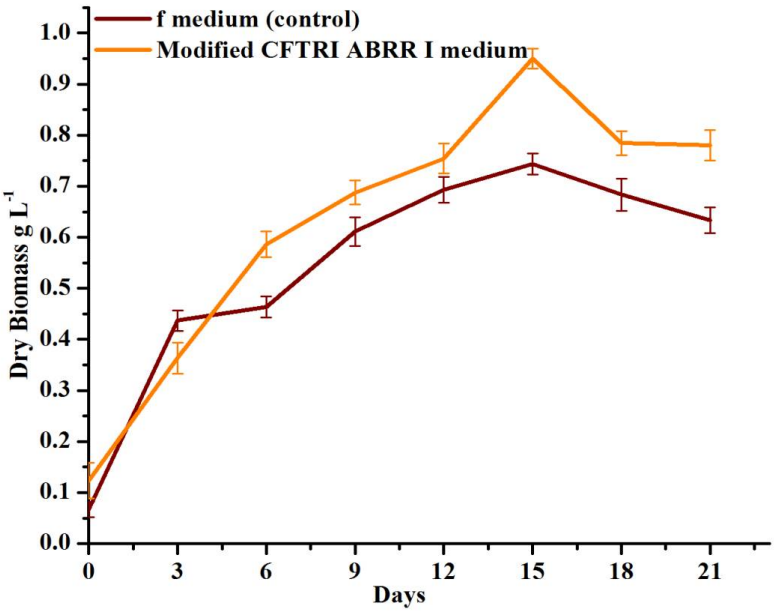

Fig. 5