ISOLATION OF SOME MICROALGAE STRAINS BELONGING TO THE FAMILY SCENEDESMACEAS (CHLOROPHYTA) IN FRESHWATER BODIES IN CENTRAL VIETNAM AND INVESTIGATION INTO THEIR BIOLOGICAL CHARACTERISTICS

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Abstract - Seven microalgae strains from samples collected from freshwater lakes in Central Vietnam were isolated and cultivated in BBM medium for investigating their biological characteristics and their applicability. These strains were identified belonging to 6 species, 3 genera of the family Scenedesmaceae, division Chlorophyta. Their average growth rate was from 0.081 - 0.160 d^{-1}, and the highest cell density of each strain reached from 2.6 x 10^6 - 60 x 10^6 cells/ml. The total content of pigments (including chlorophyll-a, chlorophyll-b, and carotenoid) of all strains was not too high (4.10 - 11.3 µg/mL), but the total lipid content exhibiting noticeably high levels, accounted for 16.6% - 74.1% of dry cell weight. Among isolated strains, *Desmodesmusabundans* and *D.costato-granulatus* were the most prominent with the highest growth rate and density in combination with remarkably high contents of lipids and pigments, and thus were identified as two potentially great candidates for biofuel production and chlorophyll and carotenoid extraction.

Key words - Microalgae; Scenedesmaceae; isolate; biological characteristics; lipid

1. Introduction

Scenedesmaceae is a family of green algae, in the class Chlorophyceae, division Chlorophyta. It is one of the most common microalgae families in freshwater bodies that plays many important roles in aquatic ecosystems. Currently, many species of this family have been reported to be effective as a biological source for producing food, nutritional supplements, and drugs for fish and humans since they contain a lot of nutrients including protein, carbohydrates, lipids, vitamins, as well as antioxidant agents, etc. [1], [2]. Sixteen species of genus Scenedesmus in Himalaya, India were isolated and exploited in fisheries and a single-cell protein source [3]. Furthermore, some microalgae strains such as strains belonging to the genus Scenedesmus have been reported to be effective for the biological treatment of polluted water [4], [5]. Meanwhile, the other Scenedesmus species, especially *Scenedesmus obliquus* were found to have the potential to produce high quality biodiesel that meets the modern industry standards [6], [7], [8]. With such important findings, Scenedesmaceae species continue to be an interesting object of many studies in the world with efforts to isolate and find out more strains for practical applications.

In Vietnam, Scenedesmaceae was also a rather common family in freshwater bodies [9], [10], [11], and some species were even recorded to be dominant in river ecosystems [12]. This is a great valuable potential resource for Vietnam but the information about these species has been very limited. This study aims at isolating some microalgae strains of the Scenedesmaceae family from some freshwater bodies in Central Vietnam and investigating their biological characteristics in order to provide scientific data on this group and evaluate their potential for practical application in Vietnam.

2. Materials and methods

Microalgae samples were collected by a phytoplankton net with a mesh size of 30 µm in three lakes in Da Nang city (Lake Thac Gian - Vinh Trung, Lake Cong vien 29/3, and Lake Bau Trang) and one lake in Thua Thien Hue province (Lake Bau Thiem). Water environmental characteristics (including pH, DO, TDS, NO_2^-, NO_3^-, PO_4^3) at the sampling sites were also identified.

Microalgae samples were grown with sterilized bold basal medium (BBM) [13] under a temperature of 25°C, the fluorescent light intensity of 2500 lux, and day/night lighting time of 16:8. The Scenedesmaceae strains were isolated by the method of inoculation of single colonies on agar with BBM in Petri dishes. The complete purified microalgae species were finally separated and continued growing in 5 mL vials containing BBM medium. Isolated microalgae strains were observed and photographed by a Hund (H6000) microscope and identified by morphological comparison. The major works used for the identification were from Bellinger & Sigee [14] and Tien & Hanh [9].

Spectrophotometric absorption method was used to quantify the density and growth rate of microalgae. Optical density (OD) measurements were carried out at these two wavelengths using a Jasco V-750 UV/VIS Spectrophotometer. Growth rate µ (d-1) of microalgae was calculated according to the following equation [15]:

$$\mu = \frac{\ln(Nt) - \ln(N0)}{(T_t - T_0)}$$

Where, Nt and N0: cell density at T_t and T_0, respectively (cell/mL); T: time (day).

Generation time was calculated as follows [16]:

$$g = \frac{1}{k}\ (\text{hour/generation})$$

with, $k = \frac{\log N2 - \log N1}{0.301\, h}$

where, N2, N1: cell density at T_2 and T_1(cell/mL);

h: period between T_2 and T_1(hour).

The extraction procedure of chlorophyll and carotenoid content was conducted with methanol 90%. The mixture of microalgae cells and methanol was incubated in the dark for 24 hours and was centrifuged for 30 min at 4,000 rpm. The obtained supernatant was then measured by spectrophotometer UV-Vis at 470 nm, 652 nm, and 665 nm. Concentrations of chlorophyll-a, chlorophyll-b, and total carotenoid were calculated as follows [17]:

Chlorophyll-a (Ch-a) =16.72(A_{665}) – 9.16(A_{652})

Chlorophyll-b(Ch-b) =34.09(A_{652}) – 15.28(A_{665})

Total carotenoid content

(C_{x+c}) = [1000(A_{470}) – 1.63Ch-a – 104.96Ch-b] / 221

(Where A: Absorbance)

Lipid extraction and purification were carried out following the method of Bligh and Dyer [18]. Briefly, 15 mL of microalgae cells were mixed with 2 mL of methanol and 1 mL of chloroform before being incubated for 24 hours at 25°C in the dark. The mixture was then mixed with 1 mL of chloroform and 1.8 mL of distilled water and centrifuged at 2,000 rpm for 10 minutes to separate into layers. The lower layer was filtered through a filter paper (Whatman, 0.47 µm pore-size) and the supernatant was weighed (W1). Then, the supernatant was dried at 104°C for 30 minutes to achieve a constant weight (W2). Total lipid content (expressed as a % of dry cell weight (% dcw)) was calculated by subtracting W1 from W2.

Data analysis was performed with R software.

3. Results and discussion

3.1. Morphological characteristics of isolated microalgae strains

The isolation results recorded 7 strains belonging to 6 species, 3 genera of the family Scenedesmaceae, division Chlorophyta (Table 1).

Table 1. *List of isolated microalgae species composition*

Division Chlorophyta
Class Chlorophyceae
Order Sphaeropleales
Family Scenedesmaceae
Genus Scenedesmus
1.*Scenedesmus quadricauda* C2 (Turpin) Brébisson 1835
2.*Scenedesmus quadricauda* C3 (Turpin) Brébisson 1835
Genus Desmodesmus
3.*Desmodesmusabundans* (Kirchner) E.H.Hegewald 2000
4. *Desmodesmusbicaudatus* (Dedusenko) P.M.Tsarenko 2000
5. *Desmodesmuscostato-granulatus*(Skuja) E.Hegewald 2000
Genus Tetradesmus
6. *Tetradesmus obliquus* (Turpin) MJWynne 2016
7. *Tetradesmusincrassatulus* (Bohlin) MJ Wynne 2016

Tetradesmus obliquus **(Turpin) MJWynne 2016** (Figure 1-a)

Description: Coenobium consists of 2 cells arranging linearly and tying together at the middle of the cells. The cell is rhombic with pointed heads. Cell walls are smooth, with 1 starch granule. The cell size is 13.66 ± 1.27 µm in length and 5.15 ± 1.29 µm in width.

Ecology and distribution: Lake Bau Thiem, Thua Thien Hue province. Environmental characteristics at the sampling site are pH = 7.19, PO_4^{3-} = 0.340 mg/L, NO_3^- = 0.62 mg/L, NO_2^- = 0.021 mg/L, TDS = 1.4 mg/L, DO = 6.77 mg/L. This species has been recorded widely distributed in different types of water bodies, especially in moderate polluted water bodies.

Desmodesmusabundans **(Kirchner) E. H. Hegewald 2000** (Figure 1-b)

Description: This species exists in unicellular form or coenobium with 2 - 4 cylindrically oval cells arranged in a row, outer cells with 1 spine at the outer side and 2 spines at both ends which are 5.77 ± 0.42 µm long (reaching a length up to 15 - 18 µm). Cell size is 8.31 ± 0.74 µm in length and 3.55 ± 0.27 µm in width.

Ecology and distribution: Lake Thac Gian - Vinh Trung, Da Nang city. Environmental characteristics at the sampling site: pH = 6.92, PO_4^{3-} = 0.131 mg/L, NO_3^-= 0.148 mg/L, NO_2^- = 0.028 mg/L, TDS = 519 mg/L, DO = 1.85 mg/L. This species has been recorded widely distributed in different water bodies in Europe, North and South America, South Africa and Asia.

Tetradesmusincrassatulus **(Bohlin) MJ Wynne 2016** (Figure 1-c)

Description: Coenobium of 2 - 4 cells arranging linearly. The cell is rhombic, and round at both ends, with 1 pyrenoids, spines were not observed. The cell size is 110.69 ± 0.86 µm in length and 5.66 ± 0.69 µm in width.

Ecology and distribution: Lake Cong Vien 29/3, Da Nang city. Environmental characteristics at the sampling site were aforementioned.

Scenedesmus quadricauda **C2 (Turpin) Brébisson1835** (Figure 1-d)

Description: Coenobium consists of 2 - 4 - 8 cells arranged in a row. The cells are cylindrical and slightly pointed at both ends. Outer cells have 2 slightly curved inward spines at both ends. The cell wall is smooth, with 1 pyrenoids. The cell length is 14.85 ± 0.64 µm and cell width is 5.41 ± 0.22 µm. The spines length is 11.27 ± 0.54 µm.

Ecology and distribution: Lake Thac Gian - Vinh Trung, Da Nang city. Environmental characteristics at the sampling site were already mentioned above. This species has been recorded widely distributed in both floating form and benthic form in all types of water bodies all over the world.

Desmodesmus bicaudatus **(Dedusenko) P. M. Tsarenko 2000** (Figure 1-e)

Description: Coenobium is with 2 linear arranged cells. The cells are nearly ellipsoidal, with 1 straight spine (7.22 ± 0.24 µm length) at one end of each outer cell, cell walls are smooth. The cell size is 10.38 ± 0.07 µm in length and 3.66 ± 0.31 µm in width.

Ecology and distribution: Lake Cong Vien 29/3, Da Nang city. Environmental characteristics at the sampling site are pH = 6.92, PO_4^{3-} = 0.085 mg/L, NO_3^- = 0.128 mg/L, NO_2^- = 0.115 mg/L, TDS = 460 mg/L, DO = 2.59 mg/L. This species has been recorded in several water bodies in Siberia (Russia), Europe (France, Hungary), West Africa, Asia.

Scenedesmus quadricauda**C3 (Turpin) Brébisson1835** (Figure 1-f)

Description: Coenobium consists of 2 - 4 - 8 ellipsoidal cells arranging linearly. Outer cells have 2 spines at both ends. The cell size is 19.88 ± 1.05 µm in length and 10.26 ± 0.58 µm in width. The length of spines is 11.27 ± 0.54 µm.

Ecology and distribution: Lake Thac Gian - Vinh Trung, Da Nang city. Environmental characteristics at the sampling site were aforementioned. This species has been recorded widely distributed in floating form and benthic form of all types of water bodies all over the world.

Desmodesmus costato-granulatus **(Skuja) E. Hegewald 2000** (Figure 1-g)

Description: Coenobium may form or not, consists of 1 - 2 - 4 cells arranging linearly. The cells are egg shaped or elongated egg shape; spines were not observed. Dimensions of cells are 5.54 ± 0.46 µm length and 1.96 ± 0.24 µm width.

Ecology and distribution: Lake Bau Trang, Da Nang city. Environmental characteristics at the sampling site are pH = 6.93, PO_4^{3-} = 0.078 mg/L, NO_3^- = 0.31 mg/L, NO_2^- = 0.028 mg/L, TDS = 7809 mg/L, DO = 6 mg/L. This species has been recorded to be distributed in a wide range of nutritional status of water bodies, from oligotrophic to eutrophic water bodies.

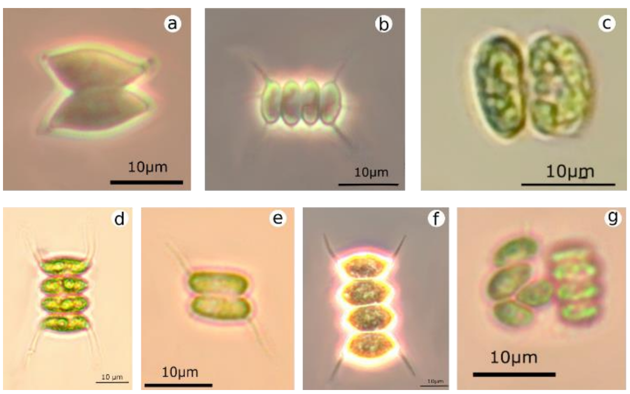

Figure 1. Isolated Scenedesmaceae strains: a. T. obliquus; b. D. abundans; c. T. incrassatulus; d. S. quadricauda C2; e. D. bicaudatus; f. S. quadricauda C3; g. D. costato-granulatus

3.2. Biological characteristics of isolated Scenedesmaceae strains

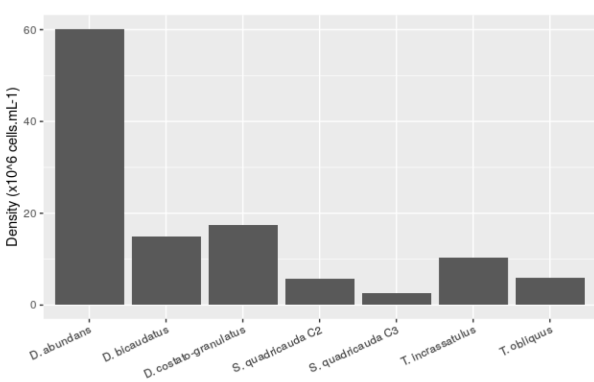

Figure 2. Cell density of isolated microalgae strains

In the seven isolated microalgae strains, *D. abundans* showed an outstanding high density (60 x 10^6 cells/mL) in comparison with the others, followed by *D. costato-granulatus* (17 x 10^6 cells/mL), *D. bicaudatus* (15 x 10^6 cells/mL), and *T. incrassatulus* (10 x 10^6 cells/mL). The three strains *S. quadricauda* C2, *S. quadricauda* C3, and *T. obliquus* had the lowest cell density, which ranged from 2.6 x 10^6 - 5.8 x 10^6 cells/mL (Figure 2).

Furthermore, the difference in growth characteristics in 31 days among isolated microalgae strains was also recognized. Most of the strains developed very slowly in the first 10 days to get familiar with the culture medium condition (Figure 3). Meanwhile, *D. costato-granulatus* and *S. quadricauda* C2 seemly needed a longer time for their adaptation, which was 15 days. The average growth rate in this period was very low and ranged from 0.027 - 0.062 d^{-1}. After that, all of the strains began to grow rapidly that made the cell density at this phase reach very high numbers. *D. abundans* and *S. quadricauda* C2 showed a similar trend in development with the growth rates increased sharply until the 27th day and started to show signs of slowing down its growth from the 28th day. The growth trend of *D. abundans* in this study was rather different from that in an earlier report of El-Sheekh et al. [8], where *D. abundans* changed to the stationary phase on the 16th day. This difference might be due to the differences in strain, culture medium, and isolated condition. The growth curve of the four strains *D. costato-granulatus*, *D. bicaudatus*, *S. quadricauda* C3, and *T. incrassatulus* was different from the other strains with a sharp increase in cell density observing from the 25th day and a continuously increasing trend after the 31st day. These results once again were not consistent with previous studies such as the result of Tuan [19], where *S. quadricauda* reached the stationary phase on the 16th day, or the result of Pena-Castro et al. [20], where *T. incrassatulus* entered the stationary phase on the 14th day. Among the isolated strains, only *T. obliquus* entered the stationary phase from the 29th day after reaching the highest density (5.78x 10^6 cells/mL) on the 28th day, which was a little later than the result of a previous study, where stated that the microalgae started to enter the equilibrium phase on the 21st day and showed signs of entering a recession phase on the 30th day [6].

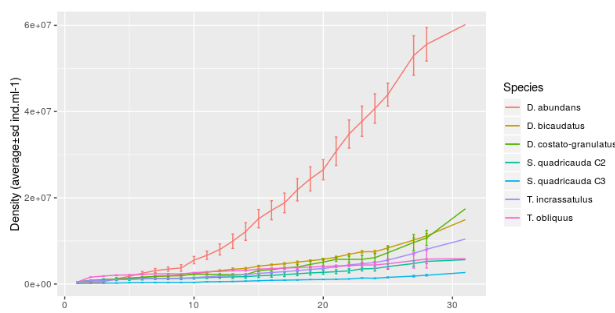

Figure 3. Growth curves of isolated microalgae strains cultivated in BBM medium (25 °C, 2500 lux, 16:8 day/night lighting time)

In general, in the culture medium condition, *D. abundans* was the strain exhibited the highest growth rate and the shortest generation time among the isolated microalgae strains (µ = 0.160 d^{-1}, g = 4.34 days), followed by *D. costato-granulatus* (µ = 0.118 d^{-1}, g = 5.58 days), *D. bicaudatus* (µ = 0.113 d^{-1}, g = 6.12 days), *S. quadricauda* C3 (µ = 0.109 d^{-1}, g = 6.33 days), and *T. incrassatulus* (µ = 0.101 d^{-1}, g = 6.58 days) (Figure 4).

S. quadricauda C2 and *T. obliquus* were the two strains having the lowest average growth rate, which were 0.081 d^{-1} (g = 8.58 days) and 0.082 d^{-1} (g = 8.44 days), respectively (Figure 4).

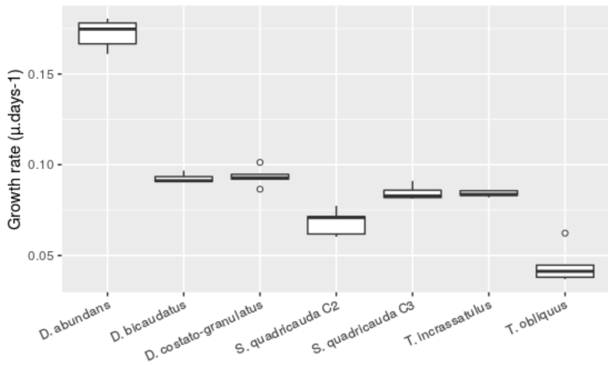

Figure 4. *Typical growth rate of isolated microalgae strains*

3.3. Potential applications of isolated Scenedesmus strains

Chlorophyll-a, chlorophyll-b, and carotenoid concentrations of microalgae strains were determined on the 28th day of the experiment. Among seven isolated strains, *D. abundans* and *D. costato-granulatus* were the two species having the highest level of both chlorophyll (6.28 and 6.54 µg/mL for chlorophyll-a, and 3.57 and 3.00 µg/mL for chlorophyll-b, respectively) and carotenoid (1.45 µg/mL and 1.49 µg/mL) (Figure 5). It is worth noting that they were also strains with the highest growth rate and density. Interestingly, although the growth rate and density of *D. costato-granulatus* (µ = 0.111 d^{-1}; 17.44 x 10^6 cells/mL) was much lower than those of *D. abundans* (µ = 0.16 d^{-1}; 60.17x 10^6 cells/mL), its content of chlorophyll and carotenoid were rather similar and even a little higher. The high growth rate in combination with the high content of chlorophyll and carotenoid of *D. abundans* and *D. costato-granulatus* demonstrated that they are potential strains for the extraction of these two pigment groups.

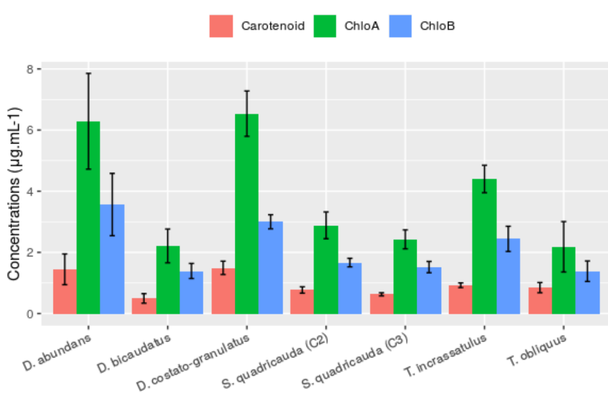

Figure 5. *Concentration of chlorophyll-a, chlorophyll-b, and carotenoid in isolated microalgae strains*

In terms of potential for biofuel production, *D. costato-granulatus* showed a superiority over the other isolated strains with the lipid content accounting for 74.1% of dry cell weight (dcw) (Figure 6). This was a very high lipid content compared to that of other recorded Scenedesmaceae strains such as *S. costatus* (10.19% dcw) [21], *S.deserticola* (18.05% dcw) [22], *Scenedesmus sp.* (19.6% dcw) [23], *Coelastrella sp.* (30.8% dcw) [24], *Desmodesmus sp.* (36.7% dcw) [24], as well as that of reported microalgae species with high lipid productivity such as *Chlorella sorokiniana* (34.56% dcw) [21], *C. vulgaris* (34.18% dcw) [24], *Neochlorisoleoabundans* (35% - 54% dcw) [23]. Furthermore, it should be noted that this lipid level of *D. costato-granulatus* was obtained in a normal condition of culture medium, and it might increase more if this strain is cultivated in an optimal medium for lipid production, making it become a leading candidate for biodiesel production.

The remaining strains had relatively high lipid contents (28.9% - 43.4% dcw), except *S. quadricauda C2* (16.6%), but unfortunately, their growth rates were not high (0.081 - 0.113 d^{-1}). Prominent among these strains was *D. abundans*, which could be considered another potential candidate for lipid production because although its lipid content (32.4 % dcw) was not too high in comparison to that of strains in this study, it was higher than that of many microalgae strains in other works [21], [23], and especially, *D. abundans* exhibited an outstanding growth rate (µ = 0.16 d^{-1}) among the isolated strains.

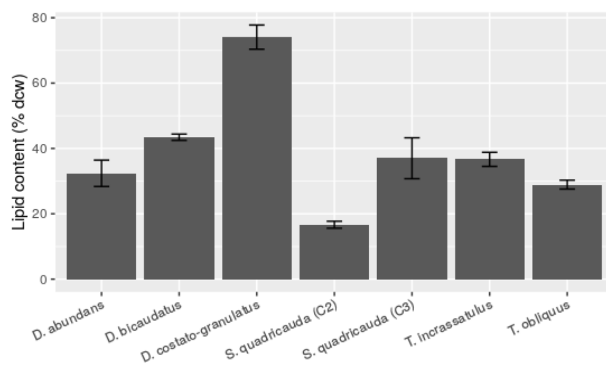

Figure 6. *Lipid content in isolated microalgae strains*

4. Conclusion

In this study, seven microalgae strains belonging to 6 species, 3 genera of the family Scenedesmaceae, division Chlorophyta were isolated and cultivated. In the culture medium condition, these strains showed a growth rate from 0.081 - 0.160 d^{-1}. The cell density was rather high but varied among strains, which ranged from 2.6 x 10^6 - 60 x 10^6 cells/mL.*D. abundans* and *D. costato-granulatus* had the highest growth rate (0.160 and 0.118 d^{-1}) and density (60 x 10^6 and 17 x 10^6 cells/mL) together with the highest pigment contents (chlorophyll-a: 6.28 and 6.54 µg/mL, chlorophyll-b: 3.57 and 3.00 µg/mL, and carotenoid: 1.45 and 1.49 µg/mL, respectively) and an outstanding lipid (32.4% and 74.1% dcw). Therefore, these two strains could be considered as the most potential strains for chlorophyll and carotenoid extraction and lipid production.

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