

GROWTH CHARACTERISATION, PIGMENTS, TOTAL PROTEIN, CARBOHYDRATE AND LIPID CONTENT IN *Cryptomonas* sp. AND *Guinardia* sp. ISOLATED FROM COASTAL REGION OF BANGLADESH

ZANNATUL NAYMA¹, FARDOUS ARA MUKTA², RAZIA SULTANA² AND HELENA KHATOON^{2*}

¹Department of Marine Fisheries and Aquaculture, Faculty of Earth and Ocean Science, Bangabandhu Sheikh Mujibur Rahman Maritime University, Mirpur-12, Pallabi, 1216 Dhaka, Bangladesh. ²Department of Aquaculture, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Khulshi, 4225 Chattogram, Bangladesh.

*Corresponding author: helena@cvasu.ac.bd

Received: 23 July 2023

Accepted: 8 May 2024

<http://doi.org/10.46754/jssm.2024.08.002>

Published: 15 August 2024

Abstract: Microalgae reproducers of different pigments and nutritional components with nutritional and medicinal value. Best-performing local microalgae should be isolated as they adapt well to the local environment. Therefore, to select the best-performing local strains to utilise them effectively, *Cryptomonas* sp. and *Guinardia* sp. were chosen in this study to determine their growth, pigments, and proximate composition. Growth data were collected using cell and optical densities, then mass cultured in commercial Conway medium and harvested at their early stationary phase. Results showed that the onset of the stationary phase (11-12 days) varied between the species. *Cryptomonas* sp. showed significantly higher ($p < 0.05$) chlorophyll a, b, and carotenoid content than *Guinardia* sp. The phycobiliprotein (2.38 ± 0.03 mg/g) and lipid (28.5 ± 0.67 % dry weight) content in *Guinardia* sp. was significantly higher ($p < 0.05$) than *Cryptomonas* sp. On the other hand, *Cryptomonas* sp. showed significantly ($p < 0.05$) higher protein (23.91 ± 0.39 % dry weight), carbohydrate (18.7 ± 0.79 % dry weight), volumetric productivity, areal productivity, and SGR than *Guinardia* sp. Current results could contribute to utilising these two species for different purposes, such as aquaculture, pharmaceuticals, and nutraceuticals industries, as they contain some valuable components.

Keywords: Microalgae, proximate composition, productivity.

Introduction

Microalgae are single-celled microscopic organisms ranging from 1 to 900 μm and mainly composed of eukaryotic organisms and prokaryotic cyanobacteria (blue-green algae), which have multiple applications in several industries, like agriculture, food, pharmaceutical, and biofuel (Kusvuran & Kusvuran, 2019). The rich and balanced nutrients (lipids, proteins, carbohydrates, vitamins) and various bioactive substances in microalgae can meet the nutritional requirements for the development of aquaculture animals at the seedling stage (Sicuro, 2021). Also, microalgae are outstanding for their high pigment content, fast growth rate, ability to grow in stressful conditions, and non-requirement of arable land, making them one of the most promising and competitive sources of natural pigments (Gong & Bassi,

2016). Marine microalgae are more sustainable and economical than freshwater for growing. Additionally, marine microalgae are less prone to contamination by other microorganisms such as protozoa and bacteria because many of them cannot withstand high salt concentrations (Indrayani *et al.*, 2018). *Cryptomonas* sp. are generally brownish or greenish microalgae and feed small zooplankton that can be used as a food source for small fish in fish farms (Parfrey *et al.*, 2011). The biochemical composition of the cryptophytes, especially in fatty acid, phycobiliproteins (PBP), and carbohydrates, has attracted the attention of aquacultures and multiple industrial sectors, such as pharmaceutical, nutraceutical, chemical, and cosmetic industries (Abidizadegan *et al.*, 2021).

Species or strain selection is the preliminary and most important step in the bioprospecting of microalgae for any commercial application (Barclay & Apt, 2013). Isolating and selecting local microalgae species/strains has a significant advantage, especially for those microalgae that are intended to be produced on a large scale outdoors as they are properly adapted to the local climatic environment (Larkum *et al.*, 2012).

Though microalgal industries have several promising advantages, they are not popular in many parts of the world, including Bangladesh, because of insufficient studies on the characterisation and exploitation of microalgae. Although Bangladesh has huge microalgal diversity in seawater, brackish water, and freshwater (Ahmed *et al.*, 2008), its utilisation is negligible due to the lack of adequate studies. Cryptophytes generate different types of secondary metabolites which can be utilised in various industries, but research on them is insufficient and their potential in biotechnological sectors remains unexplored (Abidizadegan *et al.*, 2021). No previous study has been reported on *Guinardia* sp.

Therefore, the objective of this study was to determine the growth rate, pigments and proximate compositions of two different marine microalgae (*Cryptomonas* sp. and *Guinardia* sp.) isolated from the local environment to utilise them in various industrial sectors such as aquaculture, pharmaceuticals and nutraceuticals sectors depend on their nutritional and medicinal value.

Materials and Method

Collection of Marine Isolates

Previously isolated and preserved *Cryptomonas* sp. and *Guinardia* sp. samples were obtained from the Live Feed Research Corner of the Aquaculture Department, Chittagong Veterinary and Animal Sciences University, Bangladesh.

Experimental Design

Two percent of (v/v) *Cryptomonas* sp. and *Guinardia* sp. were separately cultivated in 350 mL of the Conway medium, silicate solution

(1 mL of 20 g/L solution) was additionally added in *Guinardia* sp. culture and adjusted to 25 ppt salinity levels as previously described (Tompkin *et al.*, 1995). Cultures were grown in 350 mL volume in a 500 mL sterile borosilicate Erlenmeyer flask at $24 \pm 1^\circ\text{C}$ temperature with three replicates where 2% pure microalgal stocks were added. Microalgae cultures were grown at 25 ppt salinity, under 24 h photoperiod conditions at $150 \mu\text{Em}^{-2}\text{s}^{-1}$ intensity with continuous gentle aeration at $4.53 \pm 0.53 \text{ mg/L}$. Microalgal growth continued until the death growth phase. The growth curve was determined at this stage based on cell density (cells.mL^{-1}) and optical density (absorbance).

Subsequently, 2 L borosilicate Erlenmeyer flasks containing 1.7 L pure Conway medium were used to obtain sufficient pigment samples and proximate composition analysis. Each microalgae species was cultured in similar conditions, i.e., Temperature: $24 \pm 1^\circ\text{C}$; Light: $150 \mu\text{Em}^{-2}\text{s}^{-1}$ intensities; 25 ppt salinity until the stationary phase. Carotenoids and chlorophyll were analysed from the fresh cultured sample at the end of their exponential phase. Dried cells were used for phycobiliprotein and proximate composition analysis. At the end of the exponential growth phase, microalgae cells were harvested (4,000 rpm for 5 min) by centrifugation (Hitachi* High-speed Refrigerated Centrifuge, himac CR 21g-II) and dried at 40°C temperature using an oven and finally preserved in a refrigerator (4°C) for further use.

Determination of Cell Density

Cell count of selected microalgae was done every day by using a Neubauer hemacytometer (0.0025 mm^2 , 0.1 mm deep chambers, Assistant, Germany) under 40X magnification. Cell density was determined according to the formula of Lavens and Sorgeloos (1996).

Determination of Maximum Absorbance (Optical Density)

For the growth curve analysis, optical density was analysed every day using the maximum

absorbance value for each microalga. The Conway medium without any microalgae cells was used as blank. Maximum absorbance was measured at the wavelength of 480 nm for *Cryptomonas* sp. and 429 nm for *Guinardia* sp. as those wavelengths gave maximum absorbance when the culture samples were scanned between 300 nm to 700 nm, using a NanoDrop spectrophotometer (NanoPlus, WAVE ANALYTICS D-82362 Weilheim, Germany).

Determination of Biomass, Specific Growth Rate, and Productivity

Biomass was determined and calculated according to Ratha *et al.* (2016). For this purpose, 1 mL of microalgae sample was filtered through a pre-weighted (after marking of filter paper, rinsed with 10 mL distilled water and dried at 100°C for 4 h in a hot air oven) glass microfiber filter paper, rinsed with 10 mL distilled water for three times and dried at 100°C for 4 h. Finally, after 15 min desiccation, dry biomass was calculated by subtracting the initial filter paper weight from final weight.

The specific growth rate (mg/day) of the cultured microalga was calculated according to the following formula (Clesceri *et al.*, 1989). For each of the microalgae volumetric (Green *et al.*, 1995), areal (Ugwu *et al.*, 2008), and lipid (Benemann & Tillett, 1987) productivity were calculated by dry biomass and percentage of lipid production at their stationary phase.

Chlorophyll Content

Chlorophyll was extracted according to the procedure described by Dixit (2020). For the extraction of microalgae, 1 mL of MgCO₃ solution (after proper shaking) was filtered through the filter paper (47 mm Ø Whatman® GF/C glass microfiber filter papers), and then 10 mL of each algae sample was filtered through. After that, the filter paper was folded, placed in 10 mL of 90% acetone solution in a 15 mL centrifuge tube, and ground with tissue homogeniser for 2 min. After 1 h of refrigeration in the dark, samples were centrifuged at

3,000 rpm for 10 min, transferred the acetone extract into another centrifuge tube, and again centrifuged at low speed (500 rpm) for 5 min. Lastly, the absorbance of acetone extract was measured against 90% acetone as blank.

Chlorophyll concentration was determined according to Jenkins (1982). The chlorophyll optical density was measured at 664 nm, 647 nm, and 630 nm. Optical density at 750 nm was used as a turbidity correction factor and subtracted from each OD value. The chlorophyll a and b concentrations were calculated based on optical density values using the equation of Jeffrey and Humphrey (1975).

Carotenoid Content

Carotenoid from microalgae species was extracted according to the procedure reported by Khatoon *et al.* (2020). The absorbance of the separated hexane layer was determined using a spectrophotometer (NanoPlus, WAVE ANALYTICS D-82362 Weilheim, Germany) at a wavelength of 450 nm. Subsequently, the carotenoid was determined by multiplying the absorbance $A_{(450)}$ by 25.2 as previously described (Shaish *et al.*, 1992).

Phycobiliproteins Content

Phycobiliproteins were extracted following the procedure reported by Siegelman and Kycia (1978). For this purpose, 40 mg of dried powder was soaked in 10 mL of phosphate buffer (pH 7.0; 0.1 M), mixed properly using a vortex mixture, and stored for 24 h at 4°C. After that, the samples were centrifuged at 6,000 rpm for 10 min. Finally, the supernatant was used for phycobiliprotein quantification based on absorbance and measured at the wavelength of 562 nm, 615 nm, 652 nm, and 720 nm using a spectrophotometer (NanoPlus, WAVE ANALYTICS D-82362 Weilheim, Germany). Absorbance at 720 nm was used as cellular debris, and a PBS buffer was used as a blank. The amount of phycocyanin (PC), and allophycocyanin (APC) and phycoerythrin (PE) was calculated according to Siegelman and Kycia (1978). Total phycocyanin, phycoerythrin,

and allophycocyanin (mg/g) were calculated according to Silveira *et al.* (2007).

Total Protein, Carbohydrate, and Lipid Content

Dubois *et al.* (1956) used the method to quantify carbohydrate content. For this purpose, 5 mg of each freeze-dried cell was homogenised in 25 mL distilled water. For carbohydrate quantification, 1.0 mL of extract was mixed with 1.0 mL of 5% phenol (w/v) and 5 mL (v/v) of sulfuric acid (98% pure). After 30 s, the mixture was chilled in a cold-water bath, and absorbance was taken at 488 nm wavelength using a spectrophotometer (NanoPlus, WAVE ANALYTICS D-82362 Weilheim, Germany) to estimate carbohydrates.

Total protein was quantified using the Lowry *et al.* (1951) method. The homogenised cell, 0.5 mL was mixed with 0.5 mL of 1 N NaOH and heated in a hot water bath (100°C) for 5 min. Subsequently, the mixture was chilled in a cold water bath (10 min) before adding 2.5 mL of mixed reagent (50 mL of Reactive 2 (2 g of Na₂CO₃ in 100 mL of 0.1 NaOH) and 1 mL of Reactive 1 (1% NP tartrate), followed with 0.5 mL of folin reagent. The mixture was kept in a dark place for 30 min and absorptions were read using a spectrophotometer (NanoPlus, WAVE ANALYTICS D-82362 Weilheim, Germany) at 750 nm wavelength. The albumin standard curve was used to determine the protein content.

Bligh and Dyer (1959) methods were used for total lipid content quantification. In a centrifuge tube, 3 mL methanol: chloroform (2:1, v/v) was added to 50 mg of algal sample (diluted 5 times with distilled water), mixed properly and centrifuged at 1,000 rpm for 4 min at 4°C. After centrifugation, the supernatants were transferred into clean centrifuge tubes. In the sample tubes, 3 mL of methanol: chloroform (2:1, v/v) was added and centrifuged in the same condition. The supernatant was transferred to the previous supernatant's tubes, where 1.5 mL of 0.9% NaCl was poured and kept in the refrigerator for 1 hour (4°C). After centrifugation at 1,000 rpm for 10 min (4°C), the lower layer was transferred to the previously

weighted aluminium dish and dried at 60°C in an oven. Finally, lipid content was quantified by subtracting the initial weight from the final weight.

Statistical Analysis

Data were analysed using the IBM SPSS (v. 26.0). The mean differences for cell growth, pigments, and total protein, carbohydrate and lipid content were subjected to a t-test at $p < 0.05$.

Results and Discussion

Growth Phases of Microalgae Species

The observation results of growth in terms of cell density and optical density of the two selected marine microalgae *Cryptomonas* sp. and *Guinardia* sp. differ (Figure 1). Based on the growth curve *Cryptomonas* sp. [Figure 1 (A)] showed the lag phase on days 1 to 3, the exponential phase on days 4 to 12, the stationary phase on days 12, and finally, the phase of death from 13 days. In the present study, *Cryptomonas* sp. showed 4.725×10^6 cells mL⁻¹ of cell density. Similarly, *Guinardia* sp. [Figure 1 (B)] showed the lag phase on 1 to 2 days, the exponential phase on 3 to 11 days, the stationary phase on 11 days and the death phase from 12 days.

In a previous study, Lafarga-De la Cruz *et al.* (2006) reported maximum biomass production in *Rhodomonas* sp., marine microalgae belong to cryptophyte taxon, in day 7 ($1.53 \pm 0.07 \times 10^6$ cells mL⁻¹) with high nutrients in f/2 medium (1323 – 58.5 mM of NaNO₃ and NaH₂PO₄) at $20 \pm 1^\circ\text{C}$, 32 ppt salinity and 92 mmol photon m⁻² s⁻¹ in light intensity. However, in the present study, *Cryptomonas* sp. showed much higher cell density than Lafarga-De la Cruz *et al.* (2006) reported earlier, concluding that *Cryptomonas* sp. mass production can flourish by culturing in Conway medium. No previous study has been reported on *Guinardia* sp. However, Islam *et al.* (2021) found a shorter growth cycle in the case of *Chaetoceros* sp., a brown microalga. Moreover, in the present study, *Cryptomonas* sp. and *Guinardia* sp. showed variation in their growth because microalgae

growth characteristics differed from species to species and were influenced by several factors, such as reactor feature, culture environment and the physiological demand of each microalgal species (Guedes & Malcata, 2012).

Chlorophyll, Carotenoid, and Phycobiliproteins Content

Chlorophyll, carotenoid, and phycobiliproteins content of *Cryptomonas* sp. and *Guinardia* sp. were explored in the study. Chlorophyll a, chlorophyll b, and carotenoid were recorded as µg/mL and represented in Figure 2, where phycobiliprotein content was recorded in mg/g and presented in Table 1. There was a significant difference in chlorophyll content between *Cryptomonas* sp. (5.54 ± 0.17 µg/mL) and *Guinardia* sp. (1.65 ± 0.04 µg/mL); $t(4) = -22.16, p = 0.00$. Also significant difference was observed in the case of chlorophyll b [$t(4) =$

$-23.03, p = 0.00$] and c [$t(4) = 7.379, p = 0.00$] content of both species where 2.60 ± 0.09 µg/mL and 0.39 ± 0.02 µg/mL of chlorophyll b and 0.405 ± 0.07 µg/mL and 0.95 ± 0.02 µg/mL of chlorophyll c were reported from *Cryptomonas* sp. and *Guinardia* sp., respectively. On the other hand, between *Cryptomonas* sp. (2.43 ± 0.02 µg/mL) and *Guinardia* sp. (1.99 ± 0.01 µg/mL) there was a significant [$t(4) = -16.76, p = 0.00$] difference in carotenoid content. Moreover, between the two microalgae *Guinardia* sp. resulted significantly higher [$t(4) = -12.618, p = 0.00$] total phycobiliproteins than *Cryptomonas* sp. whereas Phycocyanine [$t(4) = 25.96, p = 0.00$] and phycoerythrin [$t(4) = 4.42, p = 0.012$] were significantly higher in *Cryptomonas* sp. but allophycocyanin [$t(4) = -19.503, p = 0.00$] was substantially higher in *Guinardia* sp.

In this study, *Guinardia* sp. showed higher chlorophyll c than *Cryptomonas* sp. as

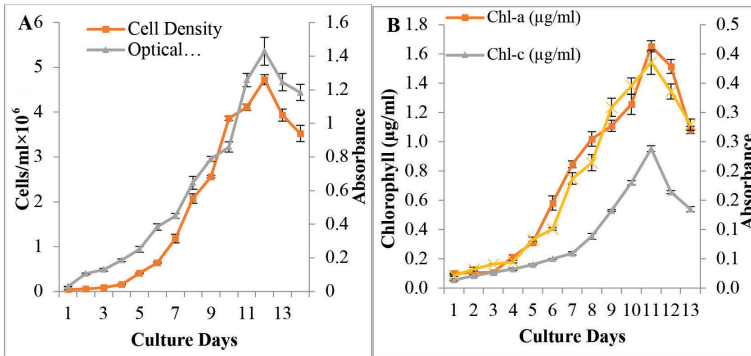


Figure 1: Growth curve in terms of cell density (cells/ml×10⁷) and optical density (Absorbance) for *Cryptomonas* sp. (A), and chlorophyll a, c (µg/ml) and optical density for *Guinardia* sp. (B). Values are means ± standard error

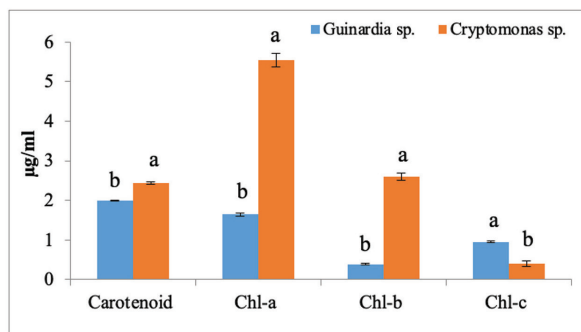


Figure 2: Chlorophyll a, b, and carotenoid content of *Cryptomonas* sp. and *Guinardia* sp. cultured in Conway medium. Values are means ± SE

Table 1: Phycobiliproteins (Phycocyanine, Allophycocyanin, and Phycoerythrin) of *Cryptomonas* sp. and *Guinardia* sp. cultured in Conway medium. Values means \pm SE

Phycobiliproteins	<i>Cryptomonas</i> sp.	<i>Guinardia</i> sp.
Phycocyanine (mg/g)	0.48 \pm 0.01 ^a	0.19 \pm 0.01 ^b
Allophycocyanin (mg/g)	0.62 \pm 0.05 ^b	1.77 \pm 0.02 ^a
Phycoerythrin (mg/g)	0.53 \pm 0.02 ^a	0.41 \pm 0.01 ^b
Total phycobiliprotein (mg/g)	1.63 \pm 0.05 ^b	2.38 \pm 0.03 ^a

photosynthetic organisms contained several types of chlorophylls, but only two forms of chlorophyll like chlorophyll a and c are available in diatoms (Kuczynska *et al.*, 2015). On the other hand, *Cryptomonas* sp. contained much higher chlorophyll a and b than *Guinardia* sp., green microalgae mainly characterised by their chlorophylls a and b content. Moreover, higher chlorophyll concentration in *Cryptomonas* sp. was due to the higher cell density, as according to Lafarga-De la Cruz *et al.* (2006), total chlorophyll concentration is directly correlated with the cellular density and indirectly to the light intensity and nutrient concentration. This study found that *Cryptomonas* sp. can extensively be used as a great source of chlorophyll for the pharmaceutical industry and food colour preparation.

In the case of carotenoid content, the current study reported much higher carotenoid than Islam *et al.* (2021) reported earlier from *Chaetoceros* sp. which was 1.36 ± 0.22 μ g/mL. The present study also concluded that *Cryptomonas* sp. and *Guinardia* sp. both have high carotenoid content and huge potential to contribute to human, animal food industries and aquaculture.

In an earlier study, Mercier *et al.* (2022) reported the highest 7.88 mg/g of total phycobiliprotein from *C. curvata*, and the lowest 0.670 mg/g from *C. ozolinii* at 20°C temperature under white LED lights at 200 μ mol photons $m^{-2}s^{-1}$ light intensity. However, phycobiliprotein in the present study differs from the previous one as PBPs in cryptophyte species differ according to the strain (Cunningham *et al.*, 2019) as well as the abiotic factors like temperature (Chaloub *et al.*, 2015) and light intensity (Heidenreich

& Richardson, 2020). In the case of brown microalgae, Islam *et al.* (2021) found much lower phycobiliprotein in *Chaetoceros* sp. than *Guinardia* sp. in the current study.

Total Protein, Carbohydrate, and Lipid Content

In this study, *Cryptomonas* sp. and *Guinardia* sp. were cultured in Conway medium to determine their protein, carbohydrate and lipid content, represented in Figure 3. Between the two species, *Cryptomonas* sp. ($23.91 \pm 0.39\%$ dry weight) showed significantly higher protein than *Guinardia* sp. ($20.25 \pm 0.5\%$ dry weight); $t(4) = -5.749$, $p = 0.005$. On the other hand, *Guinardia* sp. resulted in significantly higher [$t(4) = 14.049$, $p = 0.00$] lipid than *Cryptomonas* sp. that was accounted to be $28.50 \pm 0.67\%$ and $17.94 \pm 0.35\%$ dry weight, respectively. Moreover, *Cryptomonas* sp. and *Guinardia* sp. showed $18.71 \pm 0.79\%$ and $12.52 \pm 0.5\%$ dry weight of carbohydrate content; $t(4) = -6.645$, $p = 0.003$.

Renaud *et al.* 1999 reported 29% protein content in *Cryptomonas* sp. obtained under 0.88 mM nitrate and 0.036 mM phosphate. Guevara *et al.* (2016) reported maximum 25.4% lipid content for *Rhodomonas salina* (Cryptophyceae) in f/2 medium at 33 ppt salinity. Moreover, Dunstan *et al.* (2005) reported 9% to 24% carbohydrates in different strains of *Rhodomonas salina* (Cryptophyceae). The differences observed herein may be due to differences in the strains and culture conditions applied by several authors that make comparisons difficult to perform (Valenzuela *et al.*, 2005). In a previous study, Nayma *et al.* (2022) reported a similar amount of protein from *Scenedesmus* sp. which was found in *Guinardia* sp. in the current study. According to the findings of the

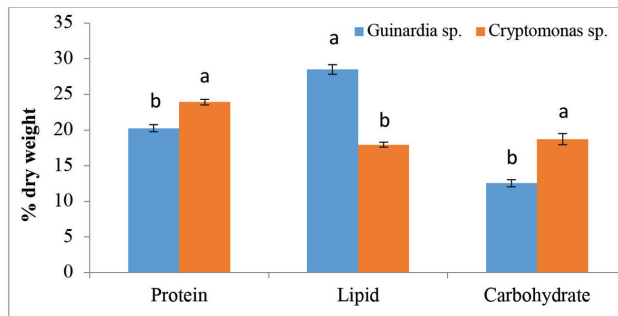


Figure 3: Protein, lipid, and carbohydrate content (% dry weight) of *Cryptomonas* sp. and *Guinardia* sp. cultured in Conway medium. Values means \pm SE

current study, *Cryptomonas* sp. can be utilised as a good protein source for aquaculture or other commercial applications. As well as *Guinardia* sp. can also be used as a potential raw material for crude lipid production.

Productivity of Microalgae Species

Volumetric, areal, and lipid productivity, SGR and cell duplication time varied between the two microalgae (Table 2) where *Cryptomonas* sp. showed significantly higher volumetric productivity [$t(4) = -22.872$, $p = 0.00$], areal productivity [$t(4) = -22.872$, $p = 0.00$], SGR [$t(4) = -7.112$, $p = 0.002$] than *Guinardia* sp. Contrarily, *Guinardia* sp. resulted significantly higher lipid productivity [$t(4) = -6.981$, $p = 0.002$] and cell duplication time [$t(4) = 6.871$, $p = 0.002$] than *Cryptomonas* sp.

Peltomaa *et al.* (2018) reported about 3.4, 2.79, and 1.37 $\text{mgL}^{-1}\text{day}^{-1}$ of volumetric productivity in marine cryptophytes *Chroomonas mesostigmatica*, *Rhodomonas salina* and *Proteomonas sulcata*, respectively. Some previous studies found a 0.68 day^{-1} growth

rate in a marine *Rhodomonas* sp. at 50 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (da Silva *et al.*, 2009). Mercier *et al.* (2022) found the highest 0.322/day SGR in *C. pyrenoidifera* and lowest 0.122/day SGR in *C. sp.* at 20°C under constant illumination by white LED-lights of 200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ intensity in MWC medium at 0 ppt salinity. However, the SGR of the current study varied from earlier studies as growth rates differ among several cryptophyte strains (Peltomaa *et al.*, 2018). Therefore, it is likely that the temperature chosen for the current study was near the optimum. However, some strains might have profited from a lower temperature. Along with this, *Cryptomonas* sp. resulted in the highest cell concentration and lower cell duplication time than *Guinardia* sp. on harvest because smaller size species grow faster than larger species due to their large surface or volume ratio. In this study, the biomass productivity *Cryptomonas* sp. differs from *Guinardia* sp. because it is affected by the microalgae species and the environment where it grows (Mercado *et al.*, 2020). The lipid productivity of microalgae also differs among species because it depends on the biochemical

Table 2: Volumetric, areal and lipid productivity, SGR and cell duplication time of *Cryptomonas* sp. and *Guinardia* sp. cultured in Conway medium. Values are means \pm SE

Productivity parameters	<i>Cryptomonas</i> sp.	<i>Guinardia</i> sp.
Volumetric productivity (mg/L/day)	20.97 \pm 0.38 ^a	9.01 \pm 0.36 ^b
Areal productivity ($\text{mg/cm}^2\text{/day}$)	2.10 \pm 0.04 ^a	0.90 \pm 0.04 ^b
Lipid productivity (mg/L/day)	2.57 \pm 0.15 ^b	3.76 \pm 0.08 ^a
SGR (mg/day)	0.64 \pm 0.00 ^a	0.60 \pm 0.00 ^b
Cell duplication time (day)	1.09 \pm 0.00 ^b	1.15 \pm 0.01 ^a

composition of microalgae (Lucas-Salas *et al.*, 2013). In this study, *Guinardia* sp. contained higher lipid content, which resulted in higher lipid productivity. According to the findings, it can be said that *Cryptomonas* sp. can be utilised for higher microalgal biomass production for different commercial applications as it requires a large amount of biomass for any commercial application of microalgae.

Conclusions

In the current study, both the microalgal strains show slight variation in volumetric, areal, lipid productivity, SGR, and cell duplication time. Based on the result, it can be concluded that, due to the higher volumetric and areal productivity, SGR and lower cell duplication time, *Cryptomonas* sp. will be more suitable for higher microalgal biomass production because biomass is the major factor to utilise microalgae for any commercial application. Additionally, *Cryptomonas* sp. can act as a potential source of pigments to utilise in several applications of the nutraceuticals industry. On the other hand, due to the presence of higher lipid content, *Guinardia* sp. can act as a potential lipid source to utilise in biofuel production.

Acknowledgements

This study was supported by the Krishi Gobeshona Foundation Project ID: TF 100-F/21 and Chattogram Veterinary and Animal Sciences University grants through the University Grant Commission. The authors would like to thank the Microbiology and Diseases Laboratory, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University for providing laboratory facilities for this study.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

- Abidizadegan, M., Peltomaa, F., & Blomster, J. (2021). The potential of cryptophyte algae in biomedical and pharmaceutical applications. *Frontiers in Pharmacology*, *11*, 1-17.
- Ahmed, Z. U., Begum, Z. N. T., Hassan, M. A., Khondker, M., Kabir, S. M. H., Ahmed, M., Ahmed, A. T. A., Rahman, A. K. A., & Haque, E. U. (Eds.). (2008). Vol. 3. Algae. Chlorophyta (Aphanochaetaceae-Zynemataceae). In *Encyclopedia of flora and fauna of Bangladesh* (pp. 812). Asiatic Society of Bangladesh, Dhaka.
- Barclay, W., & Apt, K. (2013). Strategies for bioprospecting microalgae for potential commercial applications. In Richmond, A., & Hu, Q. (Eds.), *Handbook of microalgal culture: Applied phycology and biotechnology* (pp. 69-79). UK: Wiley-Blackwell.
- Benemann, J. R., & Tillett, D. M. (1987). Microalgae lipid production. Energy from biomass and waste XI. Conference Proceeding, Institute of Gas Technology.
- Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*.
- Chaloub, R. M., Motta, N. M. S., de Araujo, S. P., de Aguiar de, P. F., & da Silva, A. F. (2015). Combined effects of irradiance, temperature and nitrate concentration on phycoerythrin content in the microalga *Rhodomonas* sp. (Cryptophyceae). *Algal Research*, *8*, 89-94.
- Clesceri, L. S., Greenberg, A. E., & Trussel, R. R. (1989). *Standard methods for the examination of water and wastewater* (17th ed.). American Public Health Association, American Water Works Association and Water Works Pollution Control Federation, 1015 Washington D.C., USA.
- Cunningham, B. R., Greenwold, M. J., Lachenmyer, E. M., Heidenreich, K. M.,

- Davis, A. C., Dudycha, J. L., & Richardson, T. L. (2019). Light capture and pigment diversity in marine and freshwater cryptophytes. *Journal of Phycology*, *55*, 552-564.
- da Silva, A. F., Lourenço, S. O., & Chaloub, R. M. (2009). Effects of nitrogen starvation on the photosynthetic physiology of a tropical marine microalga *Rhodomonas* sp. (Cryptophyceae). *Aquatic Botany*, *91*, 291-297.
- Dixit, R., Singh, S., & Singh, A. (2020). Effect of nitrogen deficiency on the physiology and biochemical composition of microalga *Scenedesmus rotundus*-MG910488. *Journal of Basic Microbiology*, *60*, 158-172.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350-356.
- Dunstan, G. A., Brown, M. R., & Volkman, J. K. (2005). Cryptophyceae and rhodophyceae; Chemotaxonomy, phylogeny, and application. *Phytochemistry*, *66*, 2557-2570.
- Gong, M., & Bassi, A. (2016). Carotenoids from microalgae: A review of recent developments. *Biotechnology Advances*, *34*, 1396-1412.
- Green, F. B., Lundquist, T., & Oswald, W. (1995). Energetics of advanced integrated wastewater pond systems. *Water Science and Technology*, *3*, 9-20.
- Guedes, A. C., & Malcata, F. X. (2012). Nutritional value and uses of microalgae in aquaculture. In Muchlisin, Z. A. (Ed.), *Aquaculture*. InTech.
- Guevara, M., Arredondo-Vega, B. O., Palacios, Y., Saéz, K., & Gómez, P. I. (2016). Comparison of growth and biochemical parameters of two strains of *Rhodomonas salina* (Cryptophyceae) cultivated under different combinations of irradiance, temperature, and nutrients. *Journal of Applied Phycology*, *28*.
- Heidenreich, K. M., & Richardson, T. L. (2020). Photopigment, absorption, and growth responses of marine cryptophytes to varying spectral irradiance. *Journal of Phycology*, *56*, 507-520.
- Indrayani, Haslianti & Asriyana. (2018). Isolation and screening of marine microalgae from Kendari waters, Southeast Sulawesi, Indonesia suitable for outdoor mass cultivation in hypersaline media. *AAACL Bioflux*, *11*, 1445-1455.
- Islam, Z., Khatoon, H., Minhaz, T. M., Rahman, M. R., Hasan, S., Mahmud, Y., Hossain, M. S., & Sarker, J. (2021). Data on growth, productivity, pigments and proximate composition of indigenous marine microalgae isolated from Cox's Bazar Coast. *Data in Brief*, *35*, 106860.
- Jeffrey, S. W., & Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls a, b, and c, in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen*, *167*, 191-194.
- Jenkins, S. H. (1982). Standard methods for the examination of water and wastewater. *Water Research*, *16*, 1495-1496.
- Khatoon, H., Yuan, G. T. G., Mahmud, A. I., & Rahman, M. R. (2020). Growth and carotenoid production of *Dunaliella salina* (Dunal) Teodoresco, 1905 cultured at different salinities. *Asian Fisheries Science*, *33*, 207-212.
- Kuczynska, P., Jemiola-Rzeminska, M., & Strzalka, K. (2015). Photosynthetic pigments in diatoms. *Marine Drugs*, *13*, 5847-81.
- Kusvuran, A., & Kusvuran, S. (2019). Using of microbial fertilizer as biostimulant alleviates damage from drought stress in Guar (*Cyamopsis Tetragonoloba* (L.) Taub.) seedlings. *International Letters of Natural Sciences*, *76*, 147-157.
- Lafarga-De la Cruz, F., Valenzuela-Espinoza, E., Millán-Núñez, R., Trees, C. C., Santamarí

- Á-del-A´ngel E., & Nu´n ez-Cebrero F. (2006). Nutrient uptake, chlorophyll a and carbon fixation by *Rhodomonas* sp. (Cryptophyceae) cultured at different irradiance and nutrient concentrations. *Aquaculture Engineering*, 35, 51-60.
- Larkum, A. W. D., Ross, I. L., Kruse, O., & Hankamer, B. (2012). Selection, breeding and engineering of microalgae for bioenergy and biofuel production. *Trends in Biotechnology*, 30, 198-205.
- Lavens, P., & Sorgeloos, P. (1996). *Manual on the production and use of live food for aquaculture*. Food and Agriculture Organisation of the United Nations, Rome.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193, 265-275.
- Mercado, I., ´Alvarez, X., Verduga, M., & Cruz, A. (2020). Enhancement of biomass and lipid productivities of *Scenedesmus* sp. cultivated in the wastewater of the dairy industry. *Processes*, 8, 1458.
- Mercier, L., Peltomaa, E., & Ojala, A. (2022). Comparative analysis of phycoerythrin production in cryptophytes. *Journal of Applied Phycology*, 34, 789-797.
- Nayma, Z., Khatoon, H., Rahman, M. R., Mukta, F. A., Sultana, R., & Nuruzzaman, M. (2022). Comparative study of growth, pigments and proximate composition of selected indigenous freshwater microalgae isolated from Bangladesh. *Journal of Innovation in Applied Research*, 5.
- Parfrey, L. W., Lahr, D. J. G., Knoll, A. H., & Katz, L. A. (2011). Estimating the timing of early eukaryotic diversification with multigene molecular clocks. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 13624-9.
- Peltomaa, E., Johnson, M. D., & Taipale, S. J. (2018). Marine cryptophytes are great sources of EPA and DHA. *Marine Drugs*, 16.
- Ratha, S. K., Rao, P. H., Govindaswamy, K., Jaswin, R. S., Lakshmidevi, R., Bhaskar, S., & Chinnasamy, S. (2016). A rapid and reliable method for estimating microalgal biomass using a moisture analyser. *Journal of Applied Phycology*, 28, 1725-1734.
- Renaud, S. M., Thinh, L., & Parry, D. L. (1999). The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. *Aquaculture*, 170, 147-159.
- Shaish, A., Ben-Amotz, A., & Avron, M. (1992). Biosynthesis of β -carotene in *Dunaliella*. Carotenoids Part A: Chemistry, separation, quantitation, and antioxidation. *Methods in Enzymology*, 213, 439-444.
- Sicuro, B. (2021). World aquaculture diversity: Origins and perspectives. *Reviews in Aquaculture*, 133, 1619-1634.
- Siegelman, H., & Kycia, J. (1978). *Algal Bili-Proteins: Handbook of psychological method*. Cambridge: Cambridge University Press. 71-79.
- Silveira, S. T., Burkert, J. F. M., Costa, J. A. V., Burkert, C. A. V., & Kalil, S. J. (2007). Optimisation of phycocyanin extraction from *Spirulina platensis* using factorial design. *Bioresource Technology*, 98, 1629-1634.
- Tompkins, J., De Ville, M. M., Day, J. G., & Turner, M. F. (1995). *Catalog of strains*. Ambleside, UK: Culture Collection of Algae and Protozoa. 204-212.
- Ugwu, C. U., Aoyagi, H., & Uchiyama, H. (2008). Photobioreactors for mass cultivation of algae. *Bioresource Technology*, 99, 4021-4028.
- Valenzuela, E., Lafarga, F., Millán, R., & N´uñez, F. (2005). Crecimiento, consumo de nutrientes y composici3n proximal de *Rhodomonas* sp. cultivada con medio f/2 y fertilizantes agr3colas. *Ciencias Marinas*, 31, 79-89.