Growth Characteristics and Phycobiliprotein Production of Indigenous Cyanobacteria from Malaysia

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Abstract

Some cyanobacteria species have a high capacity for accumulating phycobiliprotein contents in their cells. However, there is a lack of information on the screening of tropical freshwater cyanobacteria, particularly phycobiliproteins. In addition, it is unclear which cellular factor of cyanobacteria (morphological and/or growth) could affect phycobiliproteins production. Hence, the objectives of this study were to isolate and screen the Malaysian indigenous cyanobacteria for the desired growth, biomass and pigment contents of the isolated cyanobacteria and determine the main internal factor that contributed to the variation of phycobiliproteins. The surface/volume (S/V) ratio, specific growth rate, biomass productivity and pigment contents were analysed. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to distinguish the factor responsible for phycobiliprotein variations in cyanobacteria. *Synechocystis* sp. demonstrated significantly higher ($P < 0.05$) specific growth rates and biomass productivity than the other cyanobacteria. For the phycobiliprotein contents, *Arthrospira* sp., *Pseudanabaena* sp., and *Synechococcus elongatus* showed significantly higher ($P < 0.05$) amounts of phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC), respectively, than the other cyanobacteria. The present study suggested that *Arthrospira* sp., *Pseudanabaena* sp. and *Synechococcus elongatus* are suitable candidates for commercial production of PC, PE and APC sources, respectively. Moreover, the current findings showed that the phycobiliprotein contents of cyanobacteria were strain-dependent. Further analyses are required to examine the toxicity of these cyanobacteria to ensure these pigments are safe to be consumed or utilised.

1. Introduction

Cyanobacteria have piqued the interest of researchers in recent years due to their valuable pigment contents, especially phycobiliproteins (Pagels et al., 2019). Phycobiliproteins are the accessory pigments of cyanobacteria that have become the centre of interest for their commercial application. Of note, the phycocyanin (PC) (one of the phycobiliproteins) market alone was worth around USD 100 million by 2020 (Pagels et al., 2021). Phycobiliproteins have been used as bioactive compounds due to their antioxidant, anticancer, and anti-inflammatory properties (Gabr et al., 2020; Wu et al., 2016). They are an alternative to synthetic dye or colourant and are widely utilised in several essential industries encompassing pharmaceuticals, wastewater treatment, cosmetics, food and feed industries (Morais et al., 2018). The increased demand for natural pigments necessitates the development of a more sustainable source. Hence, an economically viable process of pigment production, an effective industrial-scale pigment extraction, and a cost-effective harvesting process are required to accommodate the high demand of various industries (Pagels et al., 2019). A lot of hurdles stand in the way of phycobiliprotein commercialisation and development as a viable cyanobacterial pigment technology (Khan et al., 2018). As a result, a continual search for robust, high-value cyanobacteria strains with high growth rates, biomass productivity, and phycobiliprotein contents for commercial purposes is currently underway (Manirafasha et al., 2016).

Cyanobacteria can adapt to various environments and habitats, from oligotrophic to hypereutrophic, from cold to hot, from mild to extreme conditions including hot springs, deep-sea vents, and polar habitats (Bagul et al., 2018; Tunay Karana, Zekeriya Altunera, 2017). The high biodiversity of cyanobacteria has been reported in tropical regions like Malaysia (Wiśniewska et al., 2019). To our knowledge, commercial strain, *Arthrospira* sp. is only limited to PC production. Moreover, the information on cyanobacteria candidate with a high growth rate, high PE and APC contents are still lacking. Prior to the optimisation of the growth parameters or extraction process and manipulating their genome to achieve higher growth and biomass with phycobiliprotein productions, robust cyanobacteria strains that exhibit desired phycobiliproteins-producing traits should be identified in advance.

Cyanobacteria are diverse in their cell structure, ranging from unicellular to filamentous, branched to unbranched, colonial to complex (Bagul et al., 2018). Basheva et al. (2018) demonstrated that the pigment variation of cyanobacteria might be due to the phenotypic, genetic and taxonomic classification of cyanobacteria. Therefore, the pigment variations in different cyanobacteria species should be analysed to gain insight into the species capacity of accumulating high pigment contents, especially the phycobiliprotein. The signature cyanobacteria that have a high growth rate and high phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE) remain obscure. Hence, the current study aimed to search and identify indigenous cyanobacteria for the desired growth, biomass, and pigment contents, especially phycobiliproteins. Furthermore, multivariate methods including principal component analysis (PCA) and hierarchical cluster analysis (HCA), were employed to identify the major cellular factors affecting the phycobiliprotein content.

2. Materials And Methods

2.1 Sample collection

Samples were separately collected from 20 ponds in the State of Selangor, Malaysia. Water samples were randomly collected using a sampling net with 60 µm pore size from each 10–30 cm deep water sampling point. The samples were transferred to the laboratory in samples bottles with loose cover and kept at room temperature prior to cyanobacterial isolation.

2.2 Strain isolation and culture conditions

Cyanobacteria were isolated through single-celled micro-pipetting under a Axioskop 2 light microscope (Carl Zeiss, Germany) and transferred to two in 6-well plates containing 200 µL sterilized Blue-Green medium, BG11 (Concentration, M in final medium: $NaNO_3$, 1.76×10^{-2}; K_2HPO_4, 2.24×10^{-4}; $MgSO_4 \cdot 7H_2O$, 3.04×10^{-4}; $CaCl_2 \cdot 2H_2O$, 2.45×10^{-4}; Na_2CO_3, 1.89×10^{-4}; K_2HPO_4, 2.24×10^{-4}; $Na_2EDTA \cdot 2H_2O$, 2.79×10^{-6}; Ferric ammonium citrate, 3×10^{-5}; Citric acid, 3.12×10^{-5}; H_3BO_3, 4.63×10^{-5}; $MnCl_2 \cdot 4H_2O$, 9.15×10^{-6}; $ZnSO_4 \cdot 7H_2O$, 7.65×10^{-7}; $CuSO_4 \cdot 5H_2O$, 3.16×10^{-7}; $Na_2MoO_4 \cdot 2H_2O$, 1.61×10^{-6}; $Co(NO_3)_2 \cdot 6H_2O$, 1.70×10^{-7}; pH 7) per well. The streak plate method on BG11 agar was also applied for cyanobacterial isolation. The purity of the monoclonal cyanobacterium was monitored

regularly with a light microscope. Once a single colony of cyanobacterium was obtained after incubating with a normal light-dark cycle for two to three weeks, the colonies were separately enriched and cultured in 500 mL BG-11 liquid media under standard conditions (40 ± 10 µmol photon m^{-2} s^{-1}; 25 ± 1°C; 12: 12 h light : dark) (Tan et al., 2020).

2.3 Morphological identification

Each cyanobacterial isolate was observed under the Axioskop 2 light microscope attached with the charged-coupled device camera (Carl Zeiss, Germany) using 100×, 200×, 400× and 1000× magnification. Morphological features, including shapes of the isolates and their apical cells as well as the size of vegetative cells were used for identification. The width and length of 30 representative vegetative cells were measured and recorded.

2.4 Microalgae species verification through 16s rRNA molecular identification

The identity of cyanobacteria strains isolated was verified using molecular identification techniques. Wizard® Genomic DNA Purification Kit (Promega, United States) was used to conduct genomic DNA extraction following the manufacturer's instructions. Both forward, CF and reverse, CR primers were used to perform PCR (Polymerase Chain Reaction) amplification of the cyanobacterial 16S rRNA gene (Table 1). PCR mixture consisted of 100 ng of DNA template, 1.0 µm of each forward and reverse primer, 12.5 µL MyFi™ Mix, 2x (Bioline) (contain 2.5 U MyFi DNA Polymerase, 0.2 mM dNTPs, 3 mM $MgCl_2$ and enhancers) and 7.5 µL DEPC treated water. The PCR reactions were carried out using a thermal cycler (BioRad T100™, United States) with the following steps: initial denaturation 95°C for 5 min followed by 30 repeating cycles of denaturation (95°C for 30 s), annealing (60°C for 30 s) and extension (72°C for 45 s) before the final extension (72°C for 5 min). The PCR products were then gel-purified with gel/PCR DNA fragment extraction kit (Geneaid, Taiwan) according to the manufacturer's protocol and sent to Apical Scientific Sdn Bhd (Selangor, Malaysia) for Sanger sequencing. Automated sequencing was carried out using an ABI3770 sequencer (Applied Biosystems, USA), where the sequencing was bidirectional with both forward and reverse primers. The sequences generated were analysed using nucleotide BLAST (BLASTN; https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch) and deposited in the NCBI GenBank database with the accession number.

Table 1
PCR primers utilised for amplification of 16S rRNA

Primers set	Sequences (5' – 3')	A (⊠C)	Size (bp)	Target gene
CF	GACGGGTGAGTAACGCGTGAG	60	800	16S rRNA
CR	CGAATTCACYGCAGTATGCTG	57.3		
A: Individual annealing temperature; bp: base pairs				

2.5 Phylogenetic analysis

BioEdit version 7.1.11 software was used to edit and assemble the sequences (Hall, 1999). The sequences generated from the isolates were compared with the sequences in NCBI GenBank using Megablast (Benson et al., 2018). Alignment of all these sequences was performed using Clustal W in the MEGA 6 program package (Tamura et al., 2013). The alignment was cleared of ambiguous features (insertions, deletions, or unidentified sites). A phylogenetic tree for the alignment was constructed with a neighbor-joining approach based on the Jukes-Cantor model at 1000 bootstrap iterations, using the MEGA 6 software package. Bootstrap values calculated from 1000 iterations were performed. Based on the node support for the consensus tree, bootstrap values of 70 or higher indicated significant clustering (Tamura et al., 2013).

2.6 Surface to volume (S/V) ratios of cyanobacteria

The surface area to volume (S/V) ratio of cyanobacteria was calculated based on studies of Patel et al. (2018). The S/V ratio of unicellular cyanobacteria was calculated using Eq. (1) while the S/V ratio of filamentous cyanobacteria was calculated according to Eq. (2). The S/V ratio was calculated using Eq. (3) for the filamentous cyanobacteria with truncated circular cells.

S/V = $\frac{4\pi r^2}{4/3\pi r^3}$ where “r” represents the radius of the circular cells, π is a constant. (1)

S/V = $\frac{2\pi r^2 + 2\pi rL}{\pi r^2 L} = \frac{2}{L} + \frac{2}{r}$, where “r” represents the radius of filament; π is a constant and

“L” represents the length of the filament. (2)

S/V = $\frac{2\pi rD}{\pi(Dr^2 - D^3/12)} = \frac{2r}{r^2 - D^2/12}$, where “r” represents the cell radius; π is a constant and “D” is the

cell length. (3)

2.7 Growth analysis of cyanobacteria

A single colony of cyanobacteria from agar plate stock was transferred to 100 mL BG11 media. Cyanobacterial culture at its exponential phase was used as the inoculum for further subculture. A 100 mL of cyanobacteria culture was then topped up to 1 L with BG 11 media which yielded the final OD_{680nm} of approximately 0.1 and cell count of around 1×10^5 cells. The culture was gently agitated to maintain culture homogeneity before each growth measurement. The growth analysis was carried out by biomass measurement and optical density using UV-1900 UV-VIS Spectrophotometer (Shimadzu, Japan) at OD_{680nm}. A growth assessment was performed daily for 20 days. Specific growth rate (μ) was then calculated according to Eq. (4)

Specific growth rate, $\mu = (\ln N_2 - \ln N_1) / (t_2 - t_1)$, where N_1 and N_2 are the absorbance values of cyanobacteria at the beginning, t_1 and the end, t_2 of the exponential growth

phase, respectively. (4)

Biomass productivity rate, P (mg L^{-1} day^{-1}) was calculated according to Eq. (5) (Apandi et al., 2021).

Biomass productivity rate, P (mg L^{-1} day^{-1}) = $(x_2 - x_1) / (t_x - t_0)$, where x_2 represents the biomass concentrations in g L^{-1} at the end of the cultivation period, t_x and x_1 is the initial biomass concentration at t_0 (day). (5)

2.8 Estimation of pigment contents

2.8.1 Estimation of chlorophyll a content

The content was extracted according to Dharma et al. (2017). The weight of two milliliters of each cyanobacteria culture was collected and centrifuged (Eppendorf, Germany) at 13000 rpm for one minute. The weighed pellet was suspended with 90% methanol (50 mL per gram). Then, the mixtures were incubated in the dark overnight. After incubation, the mixtures were centrifuged at 13000 rpm for 1 minute. The supernatant was measured by UV-1900 UV-VIS Spectrophotometer (Shimadzu, Japan) at the absorbance of 652.4 nm and 665.2 nm with methanol as the blank solution. The chlorophyll *a* content was calculated using the equations (6) described by Lichtenthaler and Buschmann (2005) and the results were expressed in µg mL^{-1}.

Chlorophyll *a*, *Chl a* (µg mL^{-1}) = 16.82 ($A_{665.2\ nm}$) – 9.28 ($A_{652.4\ nm}$) (6)

The absorbances value was corrected for scatter by subtracting the absorbance at 750 nm.

2.8.2 Estimation of total carotenoid contents

The carotenoids were extracted according to Osório et al. (2020) with some modifications. Briefly, the weighed dry biomass of cyanobacteria was suspended with methanol and incubated in the dark. The extracts were centrifuged at 2500 rpm for 10 min. Then, the supernatant was measured by UV-1900 UV-VIS Spectrophotometer (Shidmazu, Japan) at the absorbance of 480 nm with methanol as the blank solution. The total carotenoid contents was calculated using the equations (7) described by Osório et al. (2020) and the result was expressed in mg L^{-1}.

Total carotenoid contents (mg L^{-1}) = 4 x ($A_{480\ nm}$) (7)

The absorbances value was corrected for scatter by subtracting the absorbance at 750 nm.

2.8.3 Estimation of phycobiliprotein contents

Phycobiliprotein contents were extracted using the optimised extraction protocol of Tan et al. (2020). The entire extraction process was carried out in a dark condition by wrapping the cyanobacteria in aluminium foil as phycobiliproteins extracted were sensitive to light exposure. Cyanobacteria cultures were centrifuged at 8000 rpm for 10 min to obtain the pellet. The pellet was then rinsed twice with double

distilled water and dried overnight at 40°C. Phycobiliprotein quantification was carried out using 40 mg of dried cyanobacteria pellet. The pellet was first suspended in 10 mL of double-distilled water (pH 7). The mixture was then frozen at -80 ℃ for two hours before being thawed at 25°C for 24 hours. After the freezing and thawing process, it was centrifuged at 10000 rpm for 5 minutes. The supernatant was filtered using a 25 mm cellulose acetate membrane with 0.2 µm pore size (Bonna-Agela Technologies, United States). The absorbance of the filtrate was measured using a UV-1900 UV-VIS Spectrophotometer at the absorbance of 562 nm, 620 nm, 652 nm and 750 nm. The phycobiliprotein contents were estimated based on the Eqs. (8), (9) and (10) (Bennett, A. & Bogorad, 1973).

$PC\ (mg\ g^{-1}) = [A_{620\ nm} - 0.474\ (A_{652nm})] / 5.34$ (8)

$APC\ (mg\ g^{-1}) = [A_{652\ nm} - 0.208\ (A_{620nm})] / 5.09$ (9)

$PE\ (mg\ g^{-1}) = [A_{562\ nm} - 2.41\ [PC]] - 0.849\ [APC] / 9.62$ (10)

The absorbances value was corrected for scattering by subtracting the absorbance at 750 nm.

2.8.3.1 Stability of extracted phycobiliproteins

The first extracted phycobiliproteins were kept at -20°C. Quantification of phycobiliproteins was repeated after 24 hours based on the Eqs. (8), (9) and (10).

2.8.3.2 Purity of extracted phycobiliproteins

PC purity was determined by using the ratio $A_{620nm/A280nm}$, APC by $A_{652nm/A280nm}$ and PE was determined by $A_{562nm/A280nm}$.

2.9 Statistical analysis

The collected data were analysed using the test of normality ($p > 0.05$), followed by one-way analysis of variance (ANOVA). The Tukey HSD post hoc test at a 95% confidence interval was used to determine the significant differences. The statistical analyses were performed using Minitab 18 Statistical Software (Minitab Inc, State College, PA, United States). Values were expressed in mean ± standard deviation (SD) of three replications ($n = 3$).

2.10 Principle component analysis (PCA) and hierarchical cluster analysis (HCA)

To evaluate the significant factors responsible for variability in pigment content and identify the pattern in the dataset, a PCA was performed by considering the morphological structure, growth, and pigment content of the isolated cyanobacteria strains. The variables were transformed (Log X + 1) and normalised before the PCA was performed using the PRIMER software program (version 6.1.9, PRIMER-E Ltd.). In addition, HCA was used to determine the relationships between cyanobacteria structure and strains and the pigment contents.

3. Results

3.1 Isolation and identification of Malaysian indigenous cyanobacteria

Approximately 100 algae strains were isolated from 20 ponds in the State of Selangor. Only thirteen cyanobacterial strains were successfully cultured and tentatively classified under Chroococcales, Oscillatoriales, Nostocales and Stigonematales based on their morphological characteristics (ESM_1, Fig. 7 and Fig. 8). Four isolates (UPMC-A0091, UPMC-A0093, UPMC-A0097 and UPMC-A0100) in unicellular form were provisionally classified as Chroococcales. UPMC-A0091, UPMC-A0093, UPMC-A0097 and UPMC-A0100 were identified as *Synechococcus elongatus*, *Chroococcus minutus*, *Synechocystis* sp. and *Microcystis aeruginosa*, respectively based on morphological and genetic characteristics (Table 2). On the other hand, seven isolates demonstrated filamentous form and were tentatively grouped under Oscillatoriales. Based on the morphological and genetic characteristics, the filamentous cyanobacteria were identified as *Arthrospira* sp. (UPMC-A0087), *Phormidesmis molle* (UPMC-A0090), *Desertifilum* sp. (UPMC-A0092), *Planktothricoides raciborskii* (UPMC-A0095), *Limnothrix* sp. (UPMC-A0096), *Spirulina subsalsa* (UPMC-A0098) and *Pseudanabaena* sp. (UPMC-A0103). The remaining isolates were tentatively assigned under Nostocales and Stigonematales, respectively. UPMC-A0099 and UPMCA0094 were assigned as *Tolypothrix distorta* and *Fischerella muscicola*, respectively. The phylogenetic tree showed that most isolates were significantly clustered with a particular genus or species (ESM_2).

Table 2
Details of geographical locations of sampling sites in Malaysia and identity of isolated cyanobacteria

Strain	Sampling location	Coordinates	Assigned identity	Genbank Accession number
UPMC-A0087	Bukit Ekspo Lake, UPM	2.987818° N; 101.711013° E	*Arthrospira* sp	MT490212
UPMC-A0090	Ladang 14 Lake, UPM	2.989497° N, 101.730704° E	*Phormidesmis molle*	MW264164
UPMC-A0091	Bukit Ekspo Lake, UPM	2.987818° N; 101.711013° E	*Synechococcus elongatus*	MW264165
UPMC-A0092	Kolej Serumpun Lake, UPM	2.992918° N, 101.717866° E	*Desertifilum* sp.	MW264166
UPMC-A0093	Bukit Ekspo Lake, UPM	2.987818° N; 101.711013° E	*Chroococcus minutus*	MW264167
UPMC-A0094	Bukit Ekspo Lake, UPM	2.987818° N; 101.711013° E	*Fischerella muscicola*	MW264168
UPMC-A0095	Kolej17 Lake, UPM	2.978022° N, 101.714922° E	*Planktothricoides raciborskii*	MW264169
UPMC-A0096	Engineering Lake, UPM	3.007493° N, 101.719816° E	*Limnothrix* sp.	MW264170
UPMC-A0097	Port Dickson	2.466321° N, 101.849614° E	*Synechocystis* sp.	MW264171
UPMC-A0098	Ladang 14 Lake, UPM	2.989497° N, 101.730704° E	*Spirulina subsalsa*	MW264172
UPMC-A0099	Institute of Bioscience Lake, UPM	2.999495° N, 101.723485° E	*Tolypothrix distorta*	MW264173
UPMC-A0100	Putrajaya Lake	2.945026° N, 101.689109°E	*Microcystis aeruginosa*	MW264174
UPMC-A0103	Putrajaya Lake	2.945026° N, 101.689109°E	*Pseudanabaena* sp.	MW267949

3.2 Surface to volume (S/V) ratio

Among the isolated cyanobacteria, *Synechococcus elongates* (2.16 ± 0.11) demonstrated the highest S/V ratio ($p < 0.05$) amongst all the cyanobacterial species (Table 3). This was followed by *Synechocystis* sp. (1.86 ± 0.15) and *Pseudanabaena sp.* (1.81 ± 0.03). In contrast, *Planktothricoides raciborskii* (0.54 ± 0.07) showed the lowest S/V ratio compared to the other cyanobacteria strains, which showed no significant difference ($p > 0.05$) with *Phormidesmis molle* (0.82 ± 0.05), *Arthrospira* sp. (0.63 ± 0.06) and

Planktothricoides raciborskii (0.54 ± 0.07). Overall, unicellular cyanobacteria showed a higher surface-to-volume (S/V) ratio than filamentous cyanobacteria (Table 3).

Table 3
Surface to volume ratio in different species of cyanobacteria. Values annotated with different letters represent a statistically significant difference ($p < 0.05$) in terms of surface to volume (S/V) ratio among the isolated cyanobacteria. The data are means of three replicates

Cyanobacteria	Form	Surface to volume ratio
Chroococcus minutus	Unicellular	$1.15 \pm 0.08^{e,f}$
Microcystis aeruginosa	Unicellular	$1.34 \pm 0.05^{d,e}$
Synechococcus elongatus	Unicellular	2.16 ± 0.11^{a}
Synechocystis sp.	Unicellular	1.86 ± 0.15^{b}
Arthrospira sp.	Filamentous	0.63 ± 0.06^{g}
Desertifilum sp.	Filamentous	$1.35 \pm 0.08^{d,e}$
Fischerella muscicola	Filamentous	$1.40 \pm 0.04^{c,d,e}$
Limnothrix sp.	Filamentous	$1.80 \pm 0.29^{b,c}$
Phormidesmis molle	Filamentous	$0.82 \pm 0.05^{f,g}$
Planktothricoides raciborskii	Filamentous	0.54 ± 0.07^{g}
Pseudanabaena sp.	Filamentous	1.81 ± 0.03^{b}
Spirulina subsalsa	Filamentous	$1.41 \pm 0.26^{c,d,e}$
Tolypothrix distorta	Filamentous	$1.61 \pm 0.22^{b,c,d}$

3.3. Growth analysis

There were no significant differences ($p > 0.05$) in the growth curve in the first two days for all the isolated cyanobacterial strains (Fig. 1(a)). Conspicuous growth differences were observed after the 7th day. Among the 13 isolates, *Synechocystis* sp. demonstrated the fastest growth rate ($p < 0.05$), whereas *Tolypothrix distorta* was the slowest growing species. The growth of the isolated cyanobacteria was also clearly reflected in the specific growth rate (Fig. 1(b)). The specific growth rate of *Synechocystis* sp. (0.13 $\mu\ day^{-1}$) was the highest with significant difference ($p < 0.05$) compared to the other cyanobacteria ($p < 0.05$), while the lowest specific growth rate was observed in *Tolypothrix distorta* (0.05 $\mu\ day^{-1}$). Generally, unicellular cyanobacteria demonstrated higher specific growth rates than the filamentous strains.

3.4 Biomass production

The highest biomass productivity was seen in *Synechocystis* sp. (89.50 ± 0.01 mg L^{-1} day^{-1}) with a significant difference ($p < 0.05$) compared to the other cyanobacteria (Fig. 2). In contrast, *Fischerella muscicola* showed the lowest biomass production rate (3.50 ± 0.01 mg L^{-1} day^{-1}) among the isolated cyanobacteria, which was not significantly different compared to *Desertifilum* sp. (20.70 mg L^{-1} day^{-1}), *Phormidesmis molle* (15.30 mg L^{-1} day^{-1}), *Planktothricoides raciborskii* (11.30 mg L^{-1} day^{-1}), *Chroococcus minutus* (9.30 ± 0.01 mg L^{-1} day^{-1}), *Tolypothrix distorta* (8.30 mg L^{-1} day^{-1}) and *Synechococcus elongatus* (6.20 mg L^{-1} day^{-1}).

3.5 Pigment of the isolated cyanobacteria

3.5.1 Chlorophyll a contents

A variable amount of chlorophyll *a* were identified in the isolated cyanobacteria (Fig. 3). *Microcystis aeruginosa* showed the highest chlorophyll *a* content (13.59 ± 0.78 µg mL^{-1}) with no significant difference ($p > 0.05$) compared to *Synechocystis* sp. (12.81 ± 0.44 µg mL^{-1}). The lowest chlorophyll *a* content (1.520 ± 0.31 µg mL^{-1}) was found in *Chroococcus minutus* with no significant difference ($p > 0.05$) with the content of *Tolypothrix distorta* (1.59 ± 0.48 µg mL^{-1}) and *Planktothricoides raciborskii* (2.37 ± 0.17 µg mL^{-1}). The chlorophyll *a* contents of both unicellular and filamentous cyanobacteria were significantly different for some species (Fig. 3).

3.5.2 Carotenoid contents

The carotenoid contents of the isolated cyanobacteria strains were lower than 2.0 mg L^{-1} except for *Arthrospira* sp. (Fig. 4). Although the current findings showed that there was no significant difference ($p > 0.05$) in the concentrations of carotenoids among most of the cyanobacteria strains, the carotenoid content of *Desertifillum* sp. was the lowest (0.31 ± 0.16 mg L^{-1}). Besides, the carotenoid content of *Arthrospira* sp. (11.85 ± 0.50 mg L^{-1}) was the highest among the other cyanobacteria with a significant difference ($p < 0.05$).

3.5.3 Total phycobiliprotein contents

The total phycobiliproteins within the isolated cyanobacteria fluctuated from 7.11 mg g^{-1} to 219.87 mg g^{-1} (Fig. 5). The total phycobiliprotein contents of *Arthrospira* sp. was the highest (219.87 mg g^{-1}) among the isolated cyanobacteria with significant differences ($p < 0.05$). The lowest total phycobiliprotein contents was observed in *Synechocystis* sp. (7.11 ± 2.79 mg g^{-1}) with no significant difference ($p > 0.05$) to *Planktothricoides raciborskii* (13.95 ± 2.49 mg g^{-1}), *Phormidesmis molle* (12.49 ± 2.64 mg g^{-1}) and *Chroococcus minutus* (8.20 ± 1.02 mg g^{-1}). Generally, the total phycobiliprotein contents of filamentous cyanobacteria were higher than unicellular cyanobacteria

The isolated cyanobacteria showed a range of PC amount from 0.95 mg g^{-1} to 172.84 ± 0.37 mg g^{-1}. The highest amount of PC was observed in *Arthrospira* sp. (172.84 ± 0.37 mg g^{-1}) among the isolated cyanobacteria with significantly higher ($p < 0.05$) (Fig. 5). *Chroococcus minutus* showed the lowest PC content (0.95 ± 0.02 mg g^{-1}) compared to the other cyanobacteria strains. Overall, higher PC content of filamentous cyanobacteria was observed compared to unicellular cyanobacteria.

Apart from that, the isolated cyanobacteria's PE amount ranged from 1.25 mg g^{-1} to 92.57 mg g^{-1} (Fig. 5). *Pseudanabaena* sp. accumulated the highest PE content (92.57 mg g^{-1}) among the other cyanobacteria with a significant difference ($p < 0.05$). *Chroococcus minutus* demonstrated the lowest amount of PE (1.25 ± 0.04 mg g^{-1}) compared to the other cyanobacteria. Generally, filamentous cyanobacteria showed higher PE content compared to unicellular cyanobacteria.

The APC content of the isolated cyanobacteria varied between 0.91 mg g^{-1} and 37.56 mg g^{-1} (Fig. 5). Among all the cyanobacteria strains, the highest APC content was observed in *Synechococcus elongatus* (37.56 ± 3.65 mg g^{-1}) with significant difference ($p < 0.05$), while *Phormidesmis molle* accumulated the lowest amount of APC (0.91 ± 0.20 mg g^{-1}). In general, unicellular cyanobacteria demonstrated higher APC content than filamentous cyanobacteria.

3.5.3.1 Stability of total phycobiliproteins

The stability of total phycobiliproteins after 24 hours of incubation at -20°C varied in the isolated cyanobacteria (Table 4). The loss of phycobiliprotein contents for the isolated cyanobacteria ranged between 4.88%-15.94%. The highest stability of total phycobiliproteins was observed in *Pseudanabaena* sp. (reduced by 4.88%), whereas the extracted phycobiliprotein contents of *Desertifillum* sp. decreased the most by 15.94% over 24 hours.

Table 4
The extracted total phycobiliprotein contents of the isolated cyanobacteria after 24 hours incubated at -20°C

Cyanobacteria	Amount of phycobiliprotein content (mg g^{-1})		
	0 hour	24 hours	Loss of content (%)
Chroococcus minutus	8.20 ± 1.02	6.90 ± 0.68	15.85
Microcystis aeruginosa	103.34 ± 2.87	94.15 ± 4.88	8.89
Synechococcus elongatus	142.19 ± 6.43	130.19 ± 10.70	8.44
Synechocystis sp.	7.11 ± 2.79	6.40 ± 0.33	9.99
Arthrospira sp.	219.87 ± 0.68	205.60 ± 0.94	6.49
Desertifilum sp.	46.10 ± 1.55	38.75 ± 6.25	15.94
Fischerella muscicola	74.76 ± 8.24	63.87 ± 0.13	14.57
Limnothrix sp.	88.08 ± 7.89	81.36 ± 1.88	7.63
Phormidesmis molle	12.49 ± 2.64	11.07 ± 2.35	11.37
Planktothricoides raciborskii	13.95 ± 2.49	11.96 ± 0.35	14.27
Pseudanabaena sp.	160.54 ± 6.15	152.71 ± 3.63	4.88
Spirulina subsalsa	78.4 ± 4.46	66.99 ± 0.32	14.55
Tolypothrix distorta	26.29 ± 1.84	22.41 ± 1.67	14.76

*Values represent mean (± standard deviation) of 3 replicates and the values reported in mg g−1

3.5.3.2 Purity of the extracted PC, PE and APC

The extracted PC, APC, and PE purity were variable for the isolated cyanobacteria (Table 5). The cyanobacteria in the current study demonstrated that the purity of the extracted PC, PE and APC ranged from 0.02–2.07, 0.04–1.11 and 0.01–1.04, respectively. The highest purity of the extracted PC (2.07 ± 0.02) was observed in *Synechococcus elongates*, while *Synechocystis* sp. exhibited the lowest purity of the extracted PC (0.02) among the cyanobacteria. Besides, the highest purity of the extracted PE (1.11 ± 0.19) was identified in *Arthrospira* sp. among the isolated cyanobacteria. *Tolypothrix distorta* was the cyanobacteria that demonstrated the lowest purity of the extracted PE (0.07). On the other hand, *Synechococcus elongatus* exhibited the highest purity of extracted APC (1.04 ± 0.01) while the purity of extracted APC from *Chroococcus minutus* (0.01) and *Synechocystis* sp. (0.01) was the lowest compared to the other cyanobacteria strains.

Table 5
Purity of the extracted phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC) of the isolated cyanobacteria

Cyanoabacteria	PC purity (A620/A280)	PE purity (A562/A280)	APC purity (A652/A280)
Chroococcus minutus	0.08	0.07 ± 0.01	0.01
Microcystis aeruginosa	1.10 ± 0.12	0.45 ± 0.06	0.33 ± 0.03
Synechococcus elongatus	2.07 ± 0.02	0.71	1.04 ± 0.01
Synechocystis sp.	0.02	0.04	0.01
Arthrospira sp.	1.91 ± 0.28	1.11 ± 0.19	0.75 ± 0.14
Desertifilum sp.	0.43 ± 0.10	0.23 ± 0.02	0.22 ± 0.04
Fischerella muscicola	1.13	0.54	0.34
Limnothrix sp.	0.91 ± 0.26	0.39 ± 0.05	0.25 ± 0.04
Phormidesmis molle	0.70 ± 0.02	0.38 ± 0.01	0.21 ± 0.01
Planktothricoides raciborskii	0.20 ± 0.08	0.08 ± 0.03	0.07 ± 0.01
Pseudanabaena sp.	0.36 ± 0.02	1.03 ± 0.14	0.15 ± 0.01
Spirulina subsalsa	0.48	0.34	0.18
Tolypothrix distorta	0.09 ± 0.01	0.07	0.04

* Values represent mean (± standard deviation) of 3 replicates; PC represents phycocyanin content; PE represents phycoerythrin content; APC represents allophycocyanin content.

3.6 Principal component analysis (PCA) and hierarchical cluster analysis (HCA)

The first three components constituted 84.6% of the total variance (PC1: 41.9%; PC2: 27.4%; 15.3%) and eigenvalues greater than 1 (PC1: 3.77; PC2: 2.47; 1.37) (Table 6). PC 1 (Table 6; Fig. 6) demonstrated large negative loadings on the total phycobiliproteins and PC and these were correlated with high amount of total phycobiliproteins and PC of *Arthrospira* sp. On the other hand, *Chroococcus minutus* and *Planktothrix raciborskii* positioned at the opposite side exhibited lower total phycobiliproteins and PC content. The PC2 (Table 6; Fig. 6) showed large positive loadings on biomass productivity and specific growth rate, while large negative loadings on APC and PE were observed in PC2. The positive loadings were well-linked with high biomass productivity and specific growth rate of *Synechocystis* sp. Besides, the negative loadings were correlated with the high APC amount of *Synechococcus elongatus* and the high

PE amount of *Pseudanabaena* sp. PC3 (Table 6; not shown) exhibited large positive loadings on carotenoid contents and these were related to high carotenoid contents of *Arthrospira* sp. In addition, PC3 also demonstrated large negative loadings on the S/V ratio that correlated with high S/V ratio of *Synechococcus elongatus* and *Synechocystis* sp.

The PCA scatter plot (Fig. 6) indicated that all the cyanobacteria strains were not grouped based on cyanobacteria (unicellular and filamentous). The HCA (ESM_3) further supported it. Besides, the cluster analysis showed that *Arthrospira* sp. was highly different from other species and supported comparatively better features to produce pigments, especially phycobiliproteins.

Table 6
Eigenvector derived from principal component analysis of growth, size of cyanobacteria and pigment contents in different cyanobacteria strains

Variables	**Eigenvectors**				
	PC1	**PC2**	**PC3**	**PC4**	**PC5**
SGR	-0.33	0.457	-0.11	0.075	-0.012
BP	-0.245	0.499	-0.136	0.318	0.134
S/V ratio	-0.115	-0.162	-0.718	0.223	-0.516
Chl a	-0.366	0.378	-0.157	-0.314	0.008
Crt	-0.282	0.136	0.604	0.352	-0.274
PC	-0.442	-0.182	0.065	-0.536	-0.04
PE	-0.305	-0.323	-0.178	0.389	0.721
APC	-0.341	-0.371	0.168	0.346	-0.342
TPB	-0.447	-0.281	0.017	-0.25	0.045
Eigenvalues	3.77	2.47	1.37	0.55	0.49
Variation (%)	41.90	27.40	15.30	6.10	5.40
Cumulative Variation (%)	41.90	69.30	84.60	90.70	96.10

*SGR: Specific growth rate; BP: Biomass productivity; S/V ratio: Surface to volume ratio; *Chl a*: Chlorophyll *a*; Crt: Carotenoids; PC: Phycocyanin; PE: Phycoerythrin; APC: Allophycocyanin; TPB: Total phycobiliproteins

Besides, the Pearson Correlation (Table 7) showed the largest positive correlation between phycocyanin and total phycobiliproteins, followed by between specific growth rate and chlorophyll *a*, and between specific growth rate and biomass productivity. All the correlations demonstrated significant difference ($p < 0.01$). On the other hand, the correlation matrices demonstrated that the S/V ratio was the most negatively correlated with carotenoid contents with significant differences ($p < 0.01$).

Table 7
Overall correlation matrices (Pearson Correlation) of growth, size of cyanobacteria and pigment contents in different cyanobacteria strains. Values in bold indicate significant relationship at $p < 0.01$ (n = 39)

	SGR	BP	S/V ratio	*Chl a*	Crt	PC	PE	APC
BP	**0.778**							
S/V ratio	0.079	0.141						
Chl a	**0.893**	**0.767**	0.069					
Crt	0.350	0.310	**-0.411**	0.362				
PC	0.372	0.165	-0.009	**0.442**	**0.734**			
PE	0.156	0.003	-0.377	-0.007	0.022	0.132		
APC	0.069	-0.084	0.193	0.017	**0.542**	**0.701**	0.170	
TPB	0.350	0.112	0.162	0.335	**0.653**	**0.920**	**0.493**	**0.756**

*SGR: Specific growth rate; BP: Biomass productivity; S/V ratio: Surface to volume ratio; *Chl a*: Chlorophyll *a*; Crt: Carotenoids; PC: Phycocyanin; PE: Phycoerythrin; APC: Allophycocyanin; TPB: Total phycobiliproteins

4. Discussion

Nowadays, pigments, especially phycobiliproteins produced from cyanobacteria, have become high-value natural products used in various industries, including pharmaceutical, cosmeceutical, food, and feed industries (Pagels et al., 2019). The high proportion of cyanobacterial bioactive compounds has fueled the research effort for screening more cyanobacteria strains to reveal the promising bioactive source, particularly the pigment contents. To date, most of the screening studies have focused on marine cyanobacteria (Konstantinou et al., 2020; Montalvão et al., 2016). The commercial cyanobacterial species utilised in producing PC is *Arthropira* (Samara C. Silva, Isabel C. F. R. Ferreira & Barreiro, 2020). However, this commercial strain is only limited to PC production. In addition, the signature cyanobacteria candidate with a high growth rate, high PE and APC contents are still underexplored. It is unclear which internal factor of cyanobacteria could be the major factor in producing desired amount and quality of phycobiliproteins. Hence, the relationship between growth parameters, morphology of the cyanobacteria and their pigment contents (particularly, phycobiliproteins) were studied.

An accurate identity of cyanobacteria strains requires a comprehensive identification approach (Manoylov, 2014). Identification solely on morphological features might be difficult since cyanobacteria vary depending on the culture or environmental conditions (Dadheech et al., 2012). Therefore, a polyphasic approach encompassing both morphological and molecular analyses is recommended (Khaw et al., 2020; Martineau et al., 2013). In general, the morphological identification performed in this study corresponded well with the 16S rRNA gene sequence analysis. The small size of Chroococales such as UPMC-A0091 and UPMC-A0097 were challenging to identify based on morphological characteristics due

to their minute size. Besides, the morphological identification of UPMC-A0095 was also challenging as it lacked distinguishing characteristics or features between the *Planktothricoides* genus and *Phormidium* genus. Molecular determination was used in this study for further verification purposes as it can precisely identify the taxa compared to morphological identification (Manoylov, 2014). The identification approach in the present study identified the Malaysian isolates as *Chroococcus minutus, M. aeruginosa, Synechoccus elongatus, Synechocystis* sp, *Arthrospira* sp., *Desertifillum* sp., *Limnothrix* sp., *Phormidesmis molle, Planktothricoides raciborskii, Pseudanabaena* sp., *Spirulina subsalsa, Tolypothrix distorta* and *Fischerella muscicola*.

The specific growth rate and biomass productivity of *Synechocystis* sp. were significantly higher than the other cyanobacteria (Fig. 1). This could be attributed to its higher S/V ratio that this cyanobacterium could uptake more nutrients (Findlay, 1972; Foy, 1980). Besides, the current findings also corroborated with the Foy (1980) study, in which the growth rates of cyanobacteria were positively correlated with the S/V ratio. The difference in specific growth rate and biomass productivity in the isolated cyanobacteria could be ascribed to the structure of cyanobacteria. For instance, a larger surface area of the circular shape of *Synechocystis* sp. led to higher rates of nutrition exchange than the elongated *Synechococcus elongatus* (Patel et al., 2018). In addition, the smaller cell size of *Synechocystis* sp. might be able to exchange nutrients faster from the surroundings and allow it to grow and divide more quickly than the other cyanobacteria (Patel et al., 2018). The lower growth rate and biomass productivity of *Desertifillum* sp., *Phormidesmis molle, Planktothricoides raciborskii* and *Tolypothrix distorta* in the current study could be due to their higher cellular volume. The nutrients might be unable to cross the membrane of filamentous cyanobacteria fast enough to meet the higher cellular volume (Foy, 1980). Moreover, the tightly clumped morphology of filamentous cyanobacteria may cause nutrient competition among the neighbouring thalli. This competition will decrease the overall nutrient uptake rates of cyanobacteria (Den Haan et al., 2016). The growth rates and biomass productivity variation were also found in some cyanobacteria such as *Planktothricoides raciborskii* and *Tolypothrix distorta*. The variation could be attributed to the utilisation of different nutrient transporters or complexes that will alter the nutrient diffusion rate through the plasma membrane (Yang et al., 2020).

Pigments of cyanobacteria are typically divided into three types: chlorophyll, carotenoids, and phycobiliproteins (Samara C. Silva, Isabel C. F. R. Ferreira & Barreiro, 2020). In this study, *Microcystis aeruginosa* and *Synechocystis* sp. demonstrated significantly higher ($p < 0.05$) chlorophyll *a* content compared to the other cyanobacteria. Chlorophyll *a* is the light-harvesting material and the most copious chlorophyll pigment in most cyanobacterial species (Gan & Bryant, 2015). This has corresponded well with the specific growth rate and biomass productivity of these two cyanobacteria (*Microcystis aeruginosa* and *Synechocystis* sp.), as the growth rate and biomass productivity rely on the photosynthesis process. The different structures of cyanobacteria could influence light exposure to the cells. For instance, poor light diffusion and self-shading caused by the larger size of cyanobacteria restrict the amount of light to the cells and reduced specific growth rate and biomass productivity (da Silva et al., 2019).

In this study, a similar amount of carotenoid contents was observed in all the isolated cyanobacteria with no significant differences ($p > 0.05$) except *Arthrospira* sp. Carotenoids are light-harvesting pigments in addition to chlorophylls (Borowitzka, 2018). Moreover, they are also involved in photoprotection (Patel et al., 2018). Higher carotenoid contents of *Arthrospira* sp. could be inferred as a protection mechanism adopted by this cyanobacterium to secure the cells from excessive sunlight by quenching both the singlet and triplet forms of chlorophyll *a* (Saini et al., 2018). Thus, it is postulated that *Arthrospira* sp. might have better adaptation ability in acclimating to diverse environmental conditions compared to the isolated cyanobacteria in the present study (Stahl & Sies, 2002). The current findings showed that the phycobiliprotein contents were strain specific. Phycobiliproteins are embedded in phycobilisomes, found in rows on the thylakoids' outer surface (Giddings et al., 1983). Less amount of thylakoids was found in most of the unicellular cyanobacteria that arranged either parallel to the cytoplasmic membrane (i.e., *Synechococcus* sp.) or in radial form (S*ynechocystis* sp.) (Herrero et al., 2016), resulting in a lower amount of phycobiliproteins in unicellular cyanobacteria. The filaments of many strains, especially the heterocyst-forming cyanobacteria can be hundreds of cells long, which are embedded with more irregular-distributed coiled and entangled phycobilisomes containing a higher number of thylakoid (Herrero et al., 2016; Mareš et al., 2019). Hence, more phycobiliprotein contents were observed in these cyanobacteria. The highest total phycobiliproteins extracted from *Arthrospira* sp. (219.87 ± 0.68 mg g^{-1}) in this study have surpassed the total phycobiliproteins extracted from *Anabaena circinalis* (202.40 mg g^{-1}), *Nostoc* sp. (132.00 mg g^{-1}) and *Spirulina platensis* (199.1 mg g^{-1}) in previous studies (Ajayan et al., 2012; Johnson et al., 2014; Ojit et al., 2015).

In the current study, *Arthrospira* sp., *Pseudanabaena* sp. and *Synechococcus elongatus* demonstrated significantly higher ($p < 0.05$) PC, PE and APC, respectively than the other cyanobacteria. *Arthrospira* sp. was suggested as the desired PC source, albeit the slower growth rate based on the current findings. This was consistent with the previous studies that reported *Arthrospira* sp. as the main source of PC (Borowitzka, 2018; Khandual et al., 2021). PC production has reached 200 tonnes per year; however, the commercial production was solely for PC (https://www.binmei-global.com/about-us/). Nevertheless, the PE and APC sources remained obscure. The current study extracted a total of 92.57 mg g^{-1} (3.70 mg mL^{-1}) PE from the *Pseudanabaena* sp. Recently, the maximum PE recovered from *Porphyridium cruentum* (Ardiles et al., 2020) and *Microcoleus autumnalis* (Basheva et al., 2018) was 33.85 mg g^{-1} and 0.201 mg mL^{-1}, respectively. The PE content in the present study exceeded the amount reported by the previous study, suggesting *Pseudanabaena* sp. could be the potential PE source for commercial application. On the other hand, the current study recovered the APC content of 37.56 ± 3.65 mg g^{-1} (1.50 mg mL^{-1}) from *Synechococcus elongatus*. The APC content in this study surpassed the recommended APC source from *Leptolyngbya boryana* (0.171 mg mL^{-1}) (Basheva et al., 2018), indicating *Synechococcus elongatus* could be employed at the commercial level in producing APC. Generally, the PC, PE and APC contents varied in different cyanobacteria strains and the PC and PE content of most filamentous cyanobacteria was higher than the unicellular cyanobacteria. The study of Konstantinou et al. (2020) stated that most bioactive compounds were extracted from filamentous cyanobacteria strains.

This could be due to the different cell structures of filamentous cyanobacteria, as some filamentous cyanobacteria have three types of cells: climate-resistant akinetes, vegetative cells, and thick-walled heterocysts (Singh et al., 2011).

The stability of extracted total phycobiliprotein contents is one of the crucial factors in determining the acceptability of phycobiliproteins in the industry (Ghosh & Mishra, 2020). The stability assessment on extracted phycobiliproteins was performed at sub-zero temperature, -20 °C because this temperature was reported to be ideal for long-term storage (Ghosh & Mishra, 2020). The current study discovered that the stability of extracted total phycobiliprotein contents was strain-specific. However, the phycobiliprotein contents of *Pseudanabaena* sp. and *Arthrospira* sp. were more stable than the other cyanobacteria. This indicated the possibility of these three cyanobacteria being exploited for commercial utilisation. Purity is another important aspect of commercial applications. The purity of PC, PE and APC were different in the cyanobacteria, suggesting the purity of these phycobiliproteins could be strain-specific. PC purity of 0.7 is considered food-grade. Purity of 3.9 is reactive grade, while purity greater than 4.0 is considered as the analytical grade. The purity of PE and APC is based on the categorisation of PC (Tan et al., 2020). In the present study, food-grade PC, PE and APC were successfully extracted from six, three and two cyanobacteria, respectively, among the 13 isolated cyanobacteria. The purity can be further enhanced by applying a purification approach, which is time-consuming, cost-ineffective and cumbersome (Sharmila Banu et al., 2017). As a result, a robust pigment-enriched cyanobacteria strain plays a crucial role in the low-cost production of high-quality phycobiliproteins.

PCA analysis further discriminated the cyanobacteria strains into particular groups with high growth rates, biomass productivity, S/V ratio, and pigment contents. The results based on the PCA analysis were parallel with the above findings. For instance, both analyses (PCA analysis and characterization analysis) demonstrated high PC and total phycobiliprotein contents in *Arthrospira* sp. whereas *Chroococcus minutus* and *Planktothrix raciborskii* contained a lower amount of these contents. The loadings of *Synechocystis* sp. on the positive axis PC2 showed this cyanobacterium contained the highest specific growth rate and biomass productivity. On the other hand, the loadings of *Synechococcus elongatus* and *Pseudanabaena* sp. on the negative axis PC2 were found to contain high amount of APC and PE, respectively. PCA and HCA are the multivariate explanatory methods for reducing the number of parameters and retaining only the most important ones in explaining a phenomenon (Milovanovi et al., 2015). The PCA scatter plot and the dendrogram of the HCA in this study indicated that the form of cyanobacteria strains was not the factor in determining the pigment variation, yet the variation was mainly strain specific. All the cyanobacteria strains were scattered randomly according to their pigment amount. This is correlated with Basheva et al. (2018) studies, which stated that the phycobiliprotein production was species-dependent and appropriate to serve as a taxonomic criterion.

The PCA analysis revealed that the filamentous genera *Arthrospira* and *Pseudanabaena* produced higher total phycobiliproteins than the other filamentous strains of genera *Planktothrix* and *Phormidesmis*, which contained low concentrations of phycobiliproteins in general. This evidenced that total phycobiliprotein production was strictly individual and was most likely related to the characteristics of

each cyanobacterial strain rather than the strain form. The type and structure of cells, such as vegetative cells, akinetes, and heterocysts, which alter the rate of nutrient diffusion across the plasma membrane, may affect phycobiliprotein production, as previously discussed (Herrero et al., 2016; Mareš et al., 2019). Besides, Azaman et al. (2017) study found that different microalgae species respond to varying environmental conditions by producing different types of metabolites including pigments. In addition, Basheva et al. (2018) study further demonstrated that the differences in the quantitative distribution of the phycobiliproteins were observed not only among representatives of different genera but also within one species. This further proved that the pigment variations were strictly strain-specific. Considering the same growth conditions of the strains, pigment variation in this study was mainly due to the characteristics of each cyanobacteria strain.

According to Patel et al. (2018), different cell types in the cyanobacteria could be used to establish the relationships between cell diameter, cell surface area and its volume to biomass productivity and other physiological characteristics. The Pearson correlation in this study revealed that the S/V ratio was negatively correlated with carotenoid contents. This might be due to the structure difference of cyanobacteria strains that exhibited lower S/V ratio and better acclimated towards diverse environmental conditions through the high carotenoid contents (Stahl & Sies, 2002). For example, Kula-maximenko et al. (2021) showed that the more flattened and ellipsoidal *Gloeobacter violaceus* cell could maximise light penetration through the cell and harvesting by photosynthesis apparatus, resulting in increased metabolite production. On the other hand, PC and total phycobiliproteins have a significant positive correlation. This is because PC is the major phycobiliproteins in cyanobacteria; hence, the increase in PC could majorly affect the total phycobiliproteins (Kuddus et al., 2013). In addition, the specific growth rate of this study was found correlated with the chlorophyll *a* and biomass productivity. The high chlorophyll *a* content could increase the photosynthesis rate resulting in rapid growth and biomass production (Patel et al., 2018). Therefore, higher biomass-producing strains were discriminated from the others based on their specific growth rate and chlorophyll *a* content. Several previous studies have used chlorophyll content and biomass to measure the growth rate of cyanobacteria (Barbera et al., 2019; Coles & Jones, 2000).

5. Conclusion

Some tropical cyanobacteria have the ability in accumulating the desired amount of phycobiliproteins. The present study isolated, identified the Malaysian indigenous cyanobacteria and assessed the potential of tropical freshwater cyanobacteria isolates, representing different taxa of Chroococcales, Oscillatoriales, Nostocales and Stigonematales orders, to produce the pigments, especially phycobiliproteins. A total of 13 Malaysian cyanobacteria were isolated and identified. Among all the cyanobacteria strains, *Synechococcus elongatus* exhibited a significantly higher S/V ratio, while *Synechocystis* sp. showed a significantly higher specific growth rate and biomass productivity. Carotenoid contents were almost similar in all cyanobacteria strains except for *Arthrospira* sp. The current study also reported that the highest amount of phycobiliproteins and PC was observed in *Arthrospira* sp. In addition, the present study highlighted *Pseudanabaena* sp. and *Synechococcus*

elongatus as the potential source of PE and APC, respectively. The PCA analysis and hierarchical cluster analysis showed that phycobiliprotein variations were mainly strain specific. Besides, this study showed a positive correlation between specific growth rate with biomass productivity and *chl a* content and between phycocyanin with total phycobiliprotein. A negative correlation has been shown between the S/V ratio and carotenoid contents. The stability and purity of phycobiliproteins were also strain-specific. Toxicity assessments are required to ensure the safety of these potential sources for commercial purposes. Although these findings should be regarded as indicative, they highlighted the potential of some freshwater cyanobacteria isolates to produce phycobiliproteins and allow the selection of the most promising strains for further exploration. Besides, the current findings could offer insight into cyanobacteria characteristics and serve as a fundamental in selecting desired strains for commercial production of interest pigment. With the robust strain, a time-saving and cost-effective pigment production method could be developed.

6. Declarations

6.1. Ethical Approval and Consent to participate: Not applicable

6.2. Human and Animal Ethics: No approval of research ethics committees was required to accomplish the goals of this study.

6.3 Consent for publication: Not applicable

6.4. Availability of supporting data: The datasets generated or analyzed during this study are available from the corresponding author on reasonable request.

6.5. Competing interests: The authors have no relevant financial or non-financial interests to disclose. The authors declare no conflict of interest.

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Figures

Figure 1

(**a**) Growth curves of the isolated cyanobacteria. The error bars represent standard deviation (SD) and the data are means of three replicates; (**b**) Specific growth rate of the isolated cyanobacteria. The error bars represent standard deviation (SD) and values annotated with different letters represent a statistically significant difference ($p < 0.05$) in terms of specific growth rates among the isolated cyanobacteria

Figure 2

Biomass production rate of the isolated cyanobacteria. Values annotated with different letters represent a statistically significant difference ($p < 0.05$) in terms of biomass production rate among the isolated cyanobacteria

Figure 3

Chlorophyll *a* content of the isolated cyanobacteria. Values annotated with different letters represent a statistically significant difference ($p < 0.05$) in terms of chlorophyll *a* content among the isolated cyanobacteria

Figure 4

Carotenoid contents of the isolated cyanobacteria. Values annotated with different letters represent a statistically significant difference ($p < 0.05$) in terms of carotenoid contents among the isolated cyanobacteria

Figure 5

Phycobiliprotein contents of the isolated cyanobacteria. Values annotated with different letters represent a statistically significant difference ($p < 0.05$) in terms of phycobiliprotein contents among the isolated cyanobacteria

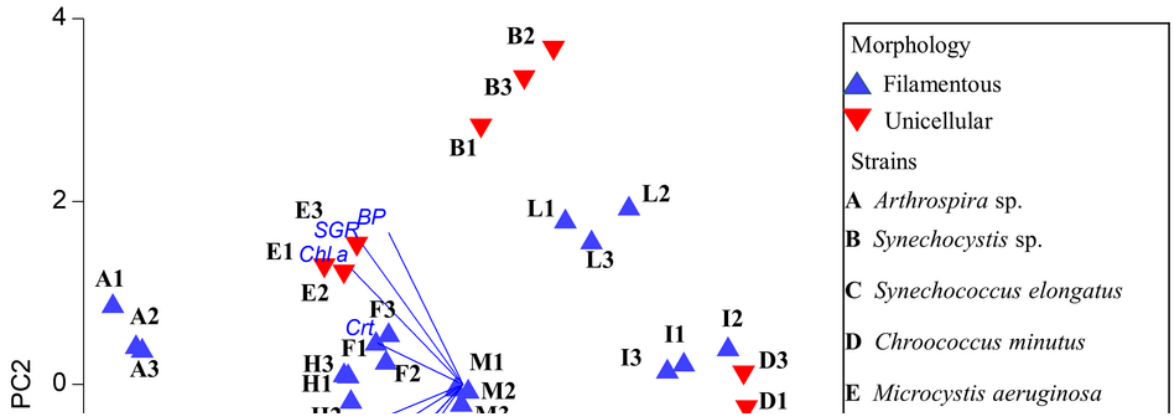

Figure 6

Principal component analysis of growth, size of cyanobacteria and pigment contents in different cyanobacteria strains. *SGR: Specific growth rate; BP: Biomass productivity; S/V ratio: Surface to volume ratio; *Chl a*: Chlorophyll *a*; Crt: Carotenoids; PC: Phycocyanin; PE: Phycoerythrin; APC: Allophycocyanin; TPB: Total phycobiliproteins

Figure 7

Photomicrographs of unicellular cyanobacteria strains isolated in this study **(a)** UPMC-A0091 *Synechococcus elongatus*.; **(b)** UPMC-A0093 *Chroococcus minutus*; **(c)** UPMC-A0097 *Synechocystis* sp.; **(d)** UPMC-A0100 *Microcystis aeruginosa*

Figure 8

Photomicrographs of filamentous cyanobacteria strains isolated in this study **(a)** UPMC-A0087 *Arthrospira* sp.; **(b)** UPMC-A0090 *Phormidesmis molle*; **(c)** UPMC-A0092 *Desertifillum* sp.; **(d)** UPMC-A0096 *Limnothrix* sp. **(e)** UPMC-A0098 *Spirulina subsalsa* (f) UPMC-A0103 *Pseudanabaena* sp. **(g)** UPMC-A0095 *Planktothricoides raciborskii* (h) UPMC-A0099 *Tolypothrix distorta* (i) UPMC-A0094 *Fischerella muscicola*

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