GROWTH AND MORPHOLOGICAL FEATURES OF LOCALLY ISOLATED MICROALGAE *CYCLOTELLA* AND *PAVLOVA*

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Abstract. Microalgae are a vast class of simple unicellular or multicellular autotrophic life forms with non-complex growth demands. Two factors that are important in preliminary assessments of microalgae are their growth profile and morphological characteristics. This study aimed to analyze the growth profile and the morphological characteristics of two local microalgae; *Cyclotella meneghiniana* and *Pavlova noctivaga.* Both microalgae were grown in Bold's Basal Medium (BBM) with 10% inoculation and incubated at constant room temperature (25±1 °C) under the light (100 µmol photons m^{-1} s^{-1}) in a 12h dark/12h light cycle for 21 days. Growth parameters such as specific growth rate and generation time were determined and their morphological feature was investigated using a light microscope and scanning electron microscope (SEM). From this study, *C. meneghiniana* showed 0.811 day^{-1} specific growth rate and 0.854 day^{-1} generation time, while *P. noctivaga* showed specific growth rate and generation time at 0.506 day^{-1} and 1.37 day^{-1}, respectively. Preliminary morphological characterization using a scanning electron microscope for *P. noctivaga* showed a spherical shape with two unequal flagella. Meanwhile, *C. meneghiniana* showed a cylindrical shape under the light microscope. Besides, there are 12 radiating striae with indistinctive fultoportula in the center region, at both ends of the *Cyclotella* cell which was clearly seen under the scanning electron microscope. The information gained from the growth profiling study such as the growth rate, doubling time, specific growth rate and the morphology of the microalgae can be used and manipulated for future research.

Keywords: Growth profile, morphology, *Cyclotella*, *Pavlova*

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Introduction

Microalgae are tiny phytoplanktonic organisms that range in size from 1 to 2 mm and include cyanobacteria, diatoms, dinoflagellates, and green algae. Generally, microalgae consist of two big groups that vary in terms of their cellular structure: the prokaryotic group (Cyanophyta and Prochlorophyta) and the eukaryotic group (Glaucophyta, Euglenophyta, Ochrophyta, Rhodophyta, Chlorarachniophyte, Cryptophyta, Haptophyta, Dinophyta, and Chlorophyta). The groups that are exceptional for biotechnological exploitation include Cyanophyta, Chlorophyta and Ochrophyta [1]. Meanwhile, in regards to their abundance, these four groups stand out the most: Chlorophyceae (green algae), Chrysophyceae (golden algae), Bacillariophyceae (diatoms) and Cyanophyceae (blue-green algae).

In terms of their morphology, physiology and structural characterization, microalgae have astounding adaptive capacity to survive in diverse types of environments. They have developed various adaptive and defense mechanisms that range from changing the ratio and composition of the main structural and functional elements of the cell (proteins, carbohydrates, lipids, pigments), activating non-enzymatic and enzymatic defense strategies to the production of substances that have cytotoxic, antibacterial and allelochemical properties, including various secondary metabolites [2]. The growth rate, doubling time, specific growth rate and duration of their life cycle are very important as preliminary assessments of the microalgae before they can be manipulated for various applications. Microalgal growth is typically demonstrated as biomass, by the number of cells or by evaluating pigments and protein contents throughout a specific period. The growth rate is observed through the rate of cell division. Besides growth parameters, morphological features are usually linked to the physiological and chemical behavior of the microalgae. In the previous study, the changes in the biochemical contents of microalgae have been proved related to their morphological features, where different environmental conditions affect the morphology as well as biochemical contents of the microalgae [3].

In this study, two species of locally isolated microalgae; *Cyclotella meneghiniana* and *Pavlova noctivaga* were examined for their morphological characters and growth profiles. *Pavlova noctivaga* were originally collected from the Institute of Bioscience Lake, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Meanwhile, *Cyclotella meneghiniana* was collected from Lukut Lake, Seremban, Negeri Sembilan, Malaysia. *C. meneghiniana* is a member of the class Bacillariophyceae (or diatom). Generally, diatom is present as either unicellular or colonial of various forms such as filaments. The genus *Cyclotella* is widely distributed in various environmental conditions. However, only eight of the species (such as *C. caspia, C. choctawhatcheeana, C. cryptica, C. quillensis, C. litoralis, C. meneghiniana, C. striata and C. stylorum*) have been found to thrive in water with high concentrations of salt [4]. Their cells are regular in shape, and they typically appear straight, however, when cross sectioned, they can appear as circular, elliptical, specular or lobed shapes. They possess cell walls that form shells called frustule that are comprised of overlapping halves (hypotheca and epitheca) perforated by holes that give access to water, dissolved materials and solids to pass in and out of the cells [5]. Meanwhile, *P. noctivaga* is classified under haptophytes group. This group of microalgae contains chlorophyll a and c, and the accessory pigments diadinoxanthin and fucoxanthin that contribute to their golden-brown color. The genus *Pavlova* may possess an eyespot, knob-scales on the plasma

membrane and flagella with unequal lengths. The longer flagellum possesses hairs as well as scales and a very small haptonema [6].

Generally, wild type strains have characteristics based on the acclimatization to their origin environment. When these strains are sub-cultured continuously to ensure their viability, they become adapted to the environment of cultivation and may lose some competitive advantageous characteristics. This is because some microalgae strains exhibit metabolic shifts when responding to changes in environmental conditions [7]. Thus, the changes in biochemical contents from the wild type strain to the cultured strain are also affected. This is called the evolutionary adaptation of microalgae towards the changing environment. Investigating the growth parameters of microalgae in a new environment is fundamental steps that support their successful manipulation and application in various fields. It ensures that the cultivation and use of microalgae are based on a solid understanding of their biology and ecology. Furthermore, knowledge gain from this study might help in designing larger-scale production systems, understanding the growth requirements of microalgae for optimization strategies to enhance biomass yield and help in strain selection that are well-suited for specific applications. For instance, *Pavlova* sp. are popular in producing valuable pigments; fucoxanthin, which possess numerous biological and health-stimulating properties such as antidiabetic, anti-obesity, anticancer and antioxidant [8]. Meanwhile, diatom like *Cyclotella* sp. gain many attentions due to their ability to incorporate desired substance into their frustule, making them a great use in producing hybrid biosensors, bioreactors, nanomedicine, photonic devices and microfluidics [9]. Thus, this preliminary study aimed to gain insights into both locally isolated microalgae when cultured under an artificial cultivation medium, which can be later manipulated for further exploration.

Materials and Methods

Microalgae Species

The microalgae used in this study; *C. meneghiniana* and *P. noctivaga*, were obtained from the Institute of Bioscience, Universiti Putra Malaysia. Originally, *P. noctivaga* was collected from a lake located at Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Meanwhile, *C. meneghiniana* was collected from a lake located at Lukut, Seremban, Negeri Sembilan, Malaysia.

Cultivation of Microalgae

The microalgae were grown in Bold's Basal Medium (BBM) (PhytoTechnology Laboratory®, USA) with 10% inoculation. The microalgae cultures were incubated at constant room temperature (25±1 °C) under the light (100 µmol photons m^{-1} s^{-1}) in a 12h dark/12h light cycle for 21 days. The growth profile of the microalgae was recorded using Marienfeld haemocytometer (Paul Marienfeld GmbH & Co. KG, Germany) and UV-Vis spectrophotometer (Shimadzu Corporation, Japan) at 680 nm wavelength. The specific growth rate and generation time for each microalgae species were determined as follows:

$$\text{Specific growth rate, } \mu = \frac{\ln N_2 - N_1}{t_2 - t_1} \quad (1)$$

where, N_2 and N_1 were the cell numbers for each mL at day t_2 and t_1, respectively. This specific growth rate was taken from the exponential growth phase of each species.

$$\text{Generation time, } t_d = \frac{\ln 2}{\mu} \quad (2)$$

where, μ is the specific growth rate.

Morphological Observation using Light and Electron Microscopy

The morphology of the microalgae under the light microscope was determined using a Zeiss Axioskop 2 microscope (Carl Zeiss, Germany) using a 100x bright field objective lens. Pictures were obtained using a camera and the sizes of the cells were measured using ZEN software. Meanwhile, the morphology of the microalgae under a scanning electron microscope was determined by scanning electron microscope (SEM) JSM-7500 (JEOL, Japan) at 15 kV. The sample preparation of microalgae for the scanning electron microscope analysis was based on the methods performed previously by Azaman et al. [3].

Results and Discussion

Analysis of Growth Profile

The cell growth was recorded from 0 to 21 days and the growth curve for each species was constructed. The growth curve was developed by plotting the number of cells against growing days. The growth profiles of *C. meneghiniana* and *P. noctivaga* are shown in Figures 1 and 2.

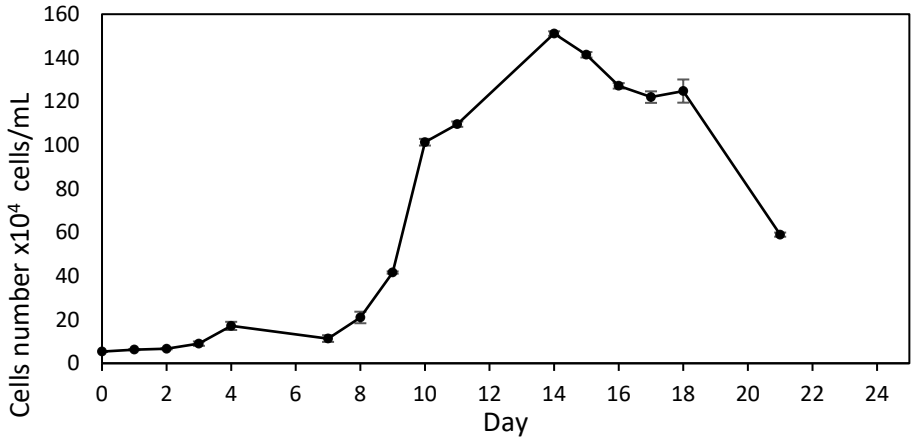

Figure 1: Growth profile of *C. meneghiniana*

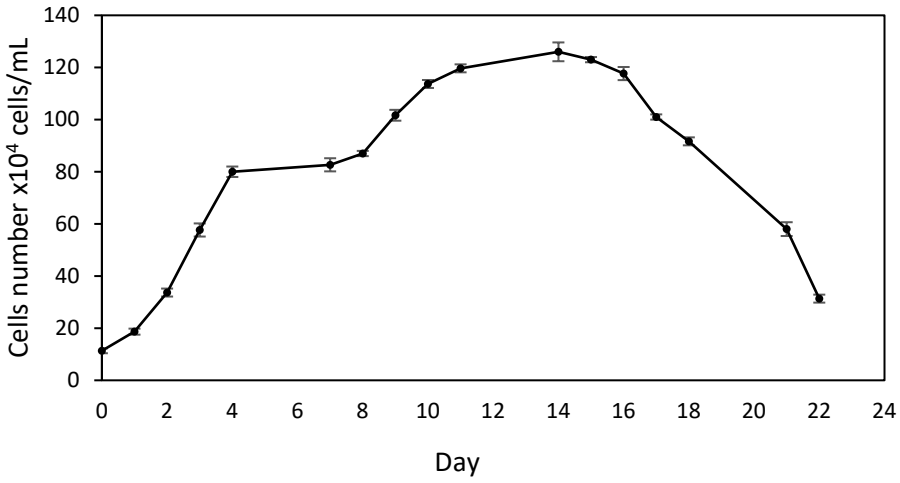

Figure 2: Growth profile of *P. noctivaga*

The growth profile of *C. meneghiniana* in Figure 1 showed a long lag phase which was recorded from day 0 until day 7. Starting from day 8, the cell's number of *C. meneghiniana* started to increase, indicating the entering of the exponential phase. The exponential phase lasted from day 8 until day 14. During this phase, the specific growth rate and generation time were 0.811 day^{-1} and 0.854 day^{-1}, respectively. The maximum cell concentration was 151 x 10^4 cells/mL, which was on the last day of the exponential phase. Starting from day 15, the growth profile demonstrated a drop which indicated that the cells were in the death phase. The shorter period of growth for *C. meneghiniana* might due to several reasons. Besides unoptimised culture conditions, the nutrient content might affect the growth of this microalgae. The requirements of diatom like *C. meneghiniana* with silicon for cell division process is very important [10]. In this case, diatoms polymerize orthosilicic acid into biogenic silica that is utilized to build their cell walls, which is known as the frustules [10]. To ensure the deposition of silica in the diatoms, nutrient availability plays a huge factor as well as the dynamics of cell population.

Meanwhile, for *P. noctivaga* (Figure 2), there was no indication of an initial lag phase, but rather, the cells were seen to have rapidly grown and increased exponentially from day 0 until day 4. The cells continued to grow exponentially until day 11 with the highest number of cells at 120 x 10^4 cells/mL. The specific growth rate and generation time were 0.506 day^{-1} and 1.37 day^{-1}, respectively. Starting from day 15, the microalgae experienced the death phase as the number of cells gradually dropped until the end of the culture time. Based on a study done by Fernandes et al. [11], who also investigated the growth profile of marine *Pavlova; Pavlova pinguis*, the lag phase of the microalgae was also undetected. This was similar with the present study in which the microalgae showed an exponential stage from day 0 until day 5. After that, the growth rate was reduced prior to the stationary phase. The growth rate and maximum number of cells of *P. pinguis* were 0.8 day^{-1} and 8.46 × 10^6 cells/mL respectively. The maximum number of cells of *P. pinguis* was higher than *P. noctivaga* recorded in the present study. Steinrücken et al. [12] reported that, if *Pavlova* sp. has a growth rate higher than 0.7 day^{-1}, that species was considered as a higher growth species. In addition, the use of suitable medium has a great influence on the growth of microalgae being cultured.

Table 1: Comparison of *C. meneghiana* and *P. noctivaga* growth parameters

Growth parameters	*C. meneghiniana*	*P. noctivaga*
Maximum cell number	151 x 10^4 cells/mL	120 x 10^4 cells /mL
Specific growth rate, μ	0.811 day^{-1}	0.506 day^{-1}
Generation time, td	0.854 day^{-1}	1.37 day^{-1}

Based on Table 1, the growth parameters (such as maximum number of cells, specific growth rate and generation time) of *C. meneghiniana* was higher than *P. noctivaga*. Although adaptation period (or lag phase) for *C. meneghiniana* was longer than *P. noctovaga*, *C. meneghiana* showed doubling time almost double from *P. noctivaga*. Generally, the duration of the lag phase is affected by several factors which include the size of the inoculum, the physiological background of the cells and the accurate physiochemical environment of both the initial and recent growth mediums [13]. Temperature can also influence the period of the lag phase as the cell's metabolic rate controls the duration necessary for the cells to adjust to new environmental conditions. Thus, as the temperature decreases from the temperature of original habitat (outdoors) to the temperature in the lab, the duration of the lag phase increases. In this study, both microalgae species showed different patterns of the lag phase which warrants the need for further investigation.

On the other hand, both species were able to attain the highest number of cells between 10 to 18 days of culture. Optimized culture conditions and media composition might improve their growth and productivity. The specific growth rate for *C. meneghiniana* and *P. noctivaga* found in this study were comparable with literature [14]. Notably, the growth rate of *C. meneghiniana* was higher than what was reported by Li et al. [14]. This finding indicated that without the optimization of medium composition and culture condition *C. meneghiniana* and *P. noctivaga* showed similar growth activities with other reported findings.

Microalgae Morphology

The morphological study of *C. meneghiniana* and *P. noctivaga* was performed using a light microscope and SEM with different magnifications. The morphological characteristics of *C. meneghiniana* observed under the light microscope are shown in Figure 3. The shape of this microalgae was found to be cylindrical. Meanwhile, from the valve view (Figure 3(d)), the cells appeared round. They can be seen as solitary (Figure 3(a)) or in a chain of cells (Figure 3(b)).

The images of *C. meneghiniana* obtained from SEM are shown in Figure 4. From the valve view (Figure 4(c)), the valve diameter was approximately 10 μm in length. The central part of *C. meneghiniana* (3 μm) was smooth, defined and tangentially undulated. The areolae were also grouped into fascicles that oriented radially. In total, there were 12 striae present with each of them being roughly 2 μm in length. The striae were radiating, elevated and arranged in an alternating pattern with the depressed interstriae.

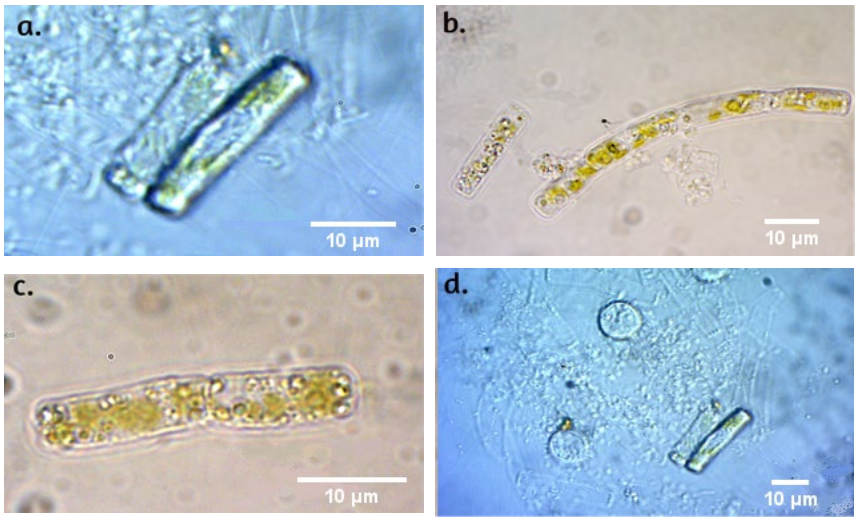

Figure 3: Morphology of *C. meneghiniana* under the light microscope. (a) solitary cell, (b) chain of cells, (c) girdle view, and (d) both girdle and valve view

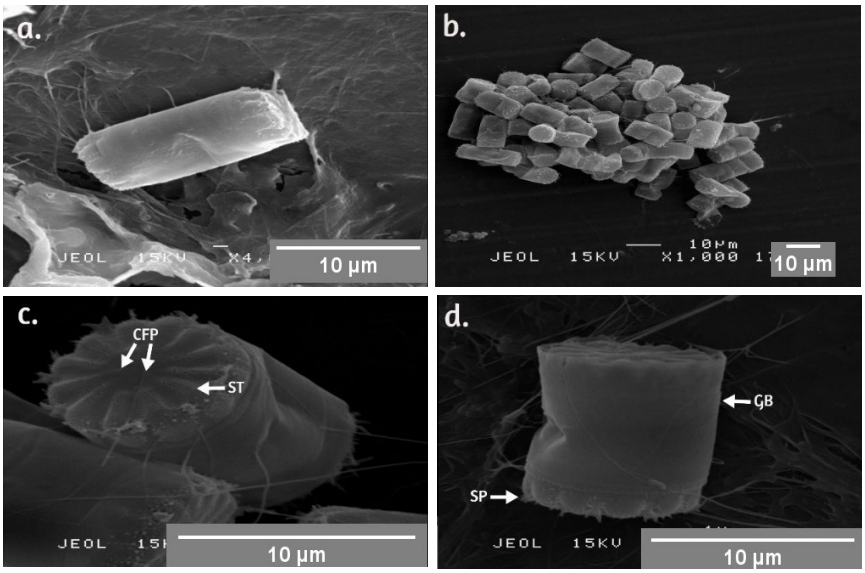

Figure 4: SEM images of *C. meneghiniana* under (a) 4000x, (b) 1000x, (c) 7000x and (d) 6000x magnifications. Abbreviation: CFP: central fultoportula, GB: girdle band, SP: spine, ST: striae

The fultoportula was quite indistinct, but two of them were present in the central region. In most specimens of *C. meneghiniana*, one or more fultoportulae can be found on the raised sections of the valve face near the marginal zone [10]. In regards to rimoportula and marginal fultoportulae, they were not observed in the images. Small spines, though quite unnoticeable, can be observed protruding out of the valve face. From the girdle view (Figure 4(d)), the frustule was cylindrical in shape. However, it had a small dent on the left side. The frustule of this species was also observed to resemble a drum shape with several girdle bands. From the electron micrograph structure obtained for *C. meneghiniana*, the diameter was within the range of reported *C. meneghiniana* by Solak et al. [15]. Most of the diameter of *C. meneghiniana* ranges between 7.7 to 22.8 µm. Occasionally, the cells are covered with mucous [16]. Meanwhile, the length of

marginal striae of *Cyclotella* is generally about 3.5 µm. However, some of them also possess striae with a length between 2.5 µm to 6 µm [17].

The cell wall of diatoms consists of silica, producing a structure known as frustule, in which hydrated amorphous silica is present with two interlocking halves [10]. The silicification of cell walls may vary depending on several environmental factors such as grazing pressure or due to limited growth by insufficient light, nitrogen, iron, or phosphorus [18-19]. A report by Hoops & Floyd [20] states that *C. meneghiniana* is a tiny discoid species of diatom that becomes longer and cylindrical as the diameter of the valve diminishes due to vegetative division. The changing shape of the diatom is due to the changing of certain parts of the cellular structure.

The morphological characteristics of *P. noctivaga* observed under the light microscope are shown in Figure 5. Their shapes were round to oblong with a diameter of approximately 9 µm. They appeared either as single cells (Figure 5(a)) or formed aggregations that were not motile (Figure 5(d)). Based on Figure 5(c), some cells clearly show their flagella.

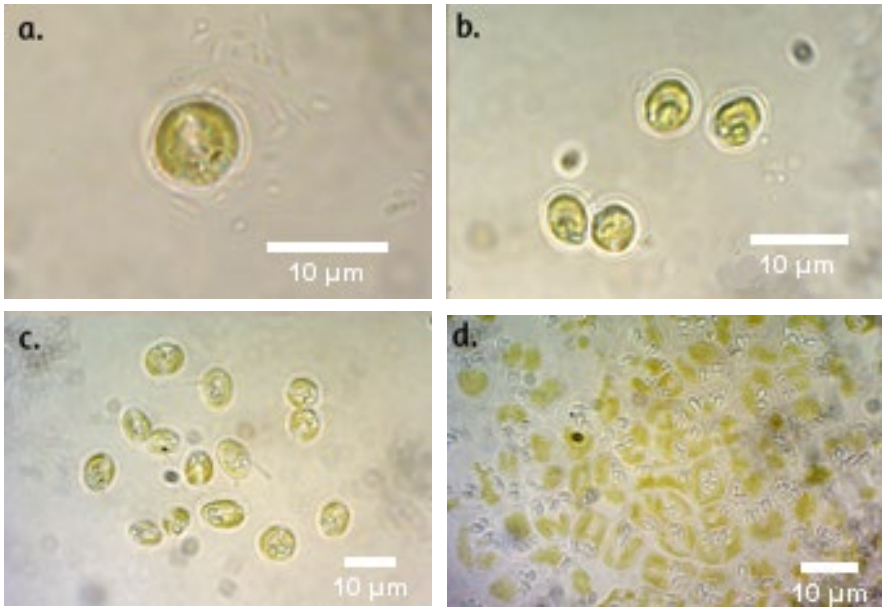

Figure 5: Morphology of *P. noctivaga* under the light microscope. (a) single cell, (b) four cells, (c) multiple cells and (d) a group of cells clustered together

The images of *P. noctivaga* obtained from SEM are shown in Figure 6. From the figure, *P. notivaga* was spherical in shape. Among haptophytes, motile unicell may occur as spherical, saddle, conical or bell-like shapes. The surface of *P. noctivaga* was irregular (or not smooth), with the presence of some folding and vein-like furrows that extended throughout the outer surface. In Figure 6(b), the flagellum of *Pavlova* had two distinct structures; one longer anterior flagellum and another shorter posterior flagellum. Interestingly, the electron micrograph of *P. noctivaga* in this study did not show the presence of hair on the surface of one flagellum (Figure 6(b)).

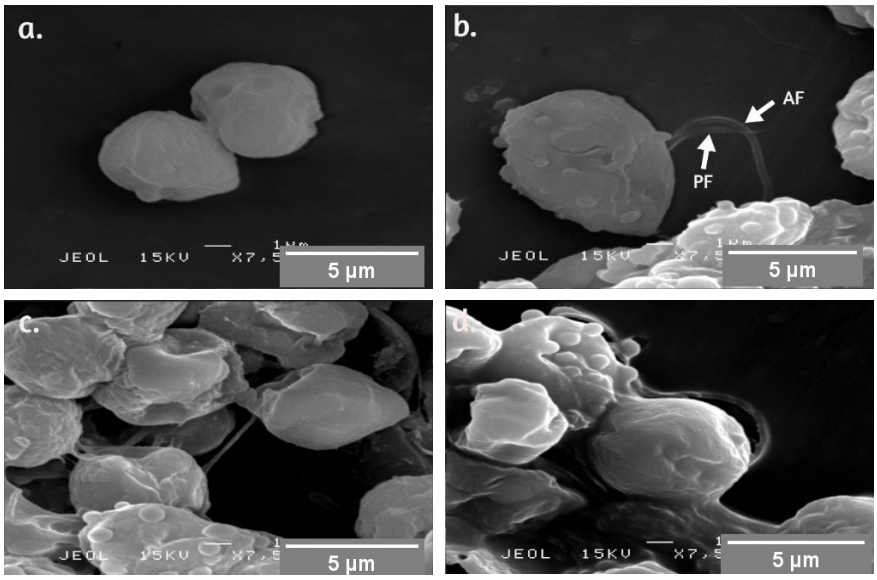

Figure 6: SEM images of *P. noctivaga* under 7500x magnification. (a) cells without flagella observed, (b) presence of anterior and posterior flagellum, (c) cluster of cells and (d) anterior flagellum. Abbreviation: AF: anterior flagellum, PF: posterior flagellum

Meanwhile, the ultrastructure of *P. noctivaga* showed tail-like structures were present in some cells. However, it was not confirmed whether the structures were flagella or haptonema. This is because haptonema is quite similar to flagellum in terms of its appearance, where the differences are only in terms of their microtubular arrangement and function. The haptonema can be very short or rudimentary in some groups of haptophytes, including the Pavlovales. If heterokont flagella are present, they can be found in the subapical or ventral region of the cell, surrounding the haptonema [6]. Further analysis and observation of the microalgal morphology and growth characteristic need to be carried out in future studies to gain more insight.

Conclusions

In this study, several growth parameters such as profile, specific growth rate and morphological characteristics were obtained from two locally isolated microalgae; *C. meneghiniana;* and *P. noctivaga.* The growth of both microalgae were influenced by the medium and the environmental factors. Morphologically, *P. noctivaga*, were seen to be spherical in shape while *C. meneghiniana* appeared round from the valve view and cylindrical from the girdle view. Several basic structures of each microalga were also observed such as haptonema in *P. noctivaga* which aids in feeding, attachment and protection, and two unequal lengths of flagella in *Pavlova* sp. which is involved in locomotion. This preliminary data can be used in future exploration of these microalgae for various applications.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards

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