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Microalgae From Antarctic Soil

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Abstract

Antarctic microalgae are rich in valuable fatty acids such as omega-3 PUFAs which are significant in the fields of medicine, pharmaceuticals, and aquaculture. Five soil samples from the Antarctic, S6, S10, S11, S21, and S26 were collected and cultured in four different liquid media namely Bold Basal media (BBM), BBM with 3-fold of nitrate and additional vitamins (3N-BBM+V), Jaworski (JM), and Chu 13. After two months, only the S26 soil sample with fine-grained sandy loam texture has positively influenced the revival of microalgae in every nutrient medium except for Jaworski medium (JM). The morphological traits such as spherical shape, smooth cell wall, and parietal chloroplast of the microalgae were observed under light microscopy. The microalgae showed similarities with microalgae belonging to the Chlorophyta group. These findings create further possibilities for further analyses such as molecular identification and chemical compound manipulation.

Keywords: Microalgae, Antarctic soil, isolation, morphological characterisation.

Abstrak

Mikroalga Antartika kaya dengan asid lemak berharga seperti Omega-3 PUFA yang berharga dalam bidang perubatan, farmaseutikal dan akuakultur. Lima sampel tanah dari Antartika, S6, S10, S11, S21, dan S26, dikumpulkan dan dikultur di dalam empat cecair media yang berbeza iaitu **media Bold Basal (BBM), BBM dengan 3 kali ganda nitrat dan vitamin tambahan (3N-BBM+V), Jaworski (JM) dan Chu 13.** Selepas dua bulan, hanya sampel tanah S26 yang mempunyai tekstur tanah pasir lempung yang halus telah memberi kesan positif kepada pemulihan mikroalga dalam setiap media nutrien kecuali medium Jaworski (JM). Ciri-ciri morfologi mikroalga seperti bentuk sfera, dinding sel yang licin, dan kloroplas parietal telah diperhatikan di bawah mikroskop cahaya. Mikroalga menunjukkan persamaan dengan mikroalga yang tergolong dalam kumpulan Chlorophyta. Penemuan ini membolehkan kemungkinan untuk analisis lanjut seperti pengenalpastian secara molekul dan manipulasi sebatian kimia.

Kata kunci: Mikroalga, tanah Antartika, pengasingan, pencirian morfologi

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Introduction

Microalgae are primitive organisms that include eukaryotes and prokaryotes. The simple-structured unicellular organisms are incredibly tiny, ranging in size from 2 to 200 µm. They have a variety of forms that maximise the ratio of cell surface area to volume, thus enhancing the flow

uptake of nutrients from their environment (Nybakken & Bertness, 2005; Morrissey et al., 2018). The groups of microalgae, such as Chlorophyta, Cyanophyta, Glaucophyta, Cryptophyta, Rhodophyta, and Dinoflagellates, are typically found in photic zones. These phytoplanktons are photoautotrophic since they contain chlorophyll *a* and are capable of photosynthesis similar to that of higher plants. The wide distribution of microalgae colonies in various habitats, including sea ice, soil crust, wastewater, tree barks, and freshwater, demonstrates their diversity (Christi, 2020).

Recently, microalgae have gained interest as a future bioenergy source in various major industries such as nutraceuticals, cosmetics, sustainable aquafeeds, and biofuels. Microalgae can produce essential bioactive metabolites such as fatty acids, polymers, chlorophyll, astaxanthin, peptides, and sterols to help improve human and animal health (Venkatesan et al., 2015). These microscopic organisms are well-recognised as promising alternatives for the fish oil that was formerly known as the primary source of essential polyunsaturated fatty acids (PUFAs) as it makes up 10–70% of their total fatty acids (Kumari et al., 2013). Since fish is often consumed directly by humans as part of their diet, there has been a shift away from using fish and towards the rapid usage of microalgae in producing goods of high nutritional value for humans and animals. Furthermore, the microalgae are a plant-based source of omega-3 fatty acids because these microalgae are autotrophic microorganisms like higher plants. Thus, the microalgae are free from heavy metal contaminants and cholesterols (Dolganyuk et al., 2020; Ryckebosch et al., 2012). Besides PUFAs, microalgae such as *Chlorella vulgaris, Haematococcus pluvialis, Dunaliella salina,* and *Spirulina platensis* are cultivated commercially as producers of functional metabolites such as proteins, polysaccharides, carotenoids, vitamins, and minerals as they exhibit antioxidant, antiviral, and antibacterial effects (Patra et al., 2019).

The Arctic and the Antarctic regions are populated by microbes like microalgae that make up a well-developed food web in the sea ice communities (John et al., 2011; Nybakken & Bertness, 2005). Psychrophilic or polar microalgae can survive in permanent and frigid environments by accumulating fatty acids in their membrane as an adaptive mechanism (Sun et al., 2018; Morrisey et al., 2018). The high accumulation PUFAs such as oleic acid (C18:1 cis-9) and palmitic acid (C16:1) in polar microalgae can maintain the structural integrity and fluidity of the cell membrane under freezing conditions (Kumari et al., 2013). The species and the nutritional content of culture media substantially affect the fatty acid profiles of the microalgae. For instance, C18:1 n-9 is common in Chlorophytes, while C16:1 n-7 is abundant in diatoms (Mimouni et al., 2018). Identifying the isolated microalgae is crucial for addressing the diversity of microalgae and their immense potentials as a sustainable biosource of multiessential businesses based on their fatty acid profiles. Thus, this study was conducted by using various culture media to isolate the distinct microalgal genera from Antarctic soil.

Materials And Methods

Sample Collection

Five soil samples from the different sample stations in Antarctic regions were collected by Dr Noor Faizul Hadry of the International Institute for Halal Research and Training (INHART), International Islamic University Malaysia (IIUM) and stored in a −20 °C freezer. The soil samples were labelled as S6, S10, S11, S21, and S26.

Isolation of Microalgae and Culture Conditions

Approximately 0.5g of Antarctic soil samples (Figure 1) were added into 100 ml culture media broth in a 250 ml sterile conical flask

followed by 2 months of incubation (Kirrolia et al., 2012). The culture media that were applied includes bold basal media (BBM), BBM with 3-fold of nitrate and additional vitamins (3N-BBM+V), Jaworski (JM), and Chu 13. The cultured samples were incubated at 120 rpm and conditioned into periodic cool white illumination of 12:12 hours light: dark at 25 ± 1 °C. The well-grown microalgae in liquid media with soil samples were transferred to the new and soilless liquid media every 3 weeks to reduce the possible contamination of bacteria.

Figure 1 The Antarctic soil samples **a:** S6 **b:** S10 **c:** S11 **d:** S21 and **e:** S26

Isolation of Microalgae from S26 Soil Sample

The suspension containing microalgae from broth media BBM, 3N-BBM+V, and Chu 13 were transferred in the amount of 50 µl–100 µl onto the respective solid media. The agar plates were incubated for 2 to 3 weeks, and the culture condition was set to 25 ± 1 °C with a photoperiod of 12 hours light: dark with 2,000 l× white luminescence intensity (Senousy et al., 2020). After the colonies of microalgae were observed, single microalgae colonies from each agar plate were transferred into 25 ml of respective liquid media and incubated for a month at 25 ± 1 °C with a photoperiod of 12 hours light/dark.

Morphological Identification

Microalgae isolated were morphologically identified using a light microscope. Briefly, a pipette was used to transfer a drop of liquid culture to a microscope slide. The sample suspension cells were smeared and allowed to dry before being heat-fixed by passing the slide through a Bunsen burner flame for a few times. After adding a drop of methylene blue to the sample, the sample was covered with a glass slipcover. The sample was then examined using a light microscope at 40× and 100× magnifications. The morphological characteristics such as colour, size, and shape were observed.

Results and Discussion

The Antarctic continent is unique due to its diverse communities of benthic and microbial organisms including microalgae. Different groups of polar microalgae can be located predominately on bedrock, soft-bottom sediments, soil, melting snow surfaces, or epiphytes (Wulff et al., 2009; Broady, 1996). Nybakken and Bertness (2005) stated that polar microalgae, together with other primary microscopic organisms such as protozoa, bacteria, and viruses, are crucial for the established marine food web as they maintain the atmospheric nitrogen fixation and promote soil aggregation by synthesising organic matters such as polysaccharides (Pushkareva et al., 2016).

The presence of polar microalgae is likely to be affected by the soil texture, stability, temperature, and micronutrient availability. The type and texture of the Antarctic have a major influence on the presence of microalgae communities that heavily depend on soil fertility and stability (Colacevich et al., 2009). During this experiment, only S26 soil samples showed changes in all liquid media except for S26 in JM after two months of incubation. The changes were from colourless to light green, indicating the presence of microalgae. The S6,

S10, S11, and S21 soil samples were sandy with a coarsely-grained texture, while the S26 soil samples were sandy loam with a fine-grained texture (Figure 1). The aggregates of loamy soil were composed of silt and tiny particles of clay that provided a soft and stable soil condition.

In addition, the clay particles contain a negative charge that can bind to the positive charge of the micronutrients, thus increasing the nutrient availability in the soil inhabited by the polar microalgae and enhancing soil fertility. Additionally, the loamy soil particles can bind to minerals and organic matters such as the mucilaginous sheath of polar microalgae (Pushkareva et al., 2016; Belnap et al., 2001). Hence, the texture of the sandy soil is favourable for the development and reproduction of polar microalgae. In contrast to fine-grained soil, coarse-grained soil is highly permeable to water and has better aeration. However, due to its inability to retain micronutrients and water, this soil condition is not ideal for developing polar microalgae. Thus, coarse-grained soil has negatively affected the development of microalgae in the Antarctic soil.

The availability of micronutrients significantly influences marine microalgae's survival in their native ecosystem, particularly nitrogen and phosphorus (Quigg, 2016). Therefore, the components of the artificial growth medium should either mimic or improve upon the nutrients of the prior habitat to cultivate the microalgae successfully for research or commercial purposes. The salts chlorides, nitrates, and sulphates make up the majority of the growth media, which can either be a liquid or agar-based media. The total salt chloride in the Antarctic soils is mainly composed of 55.01% sodium (Na^+), 30.40% sulphate (SO^{-2}_4), 7.67% magnesium (Mg^{2+}), 3.91% calcium (Ca^{2+}), 1.16% potassium (K^+), and 1.10% boric acid (H_3Bo_3) (Milne, 1995). Microalgae isolated from the S26 soil samples were able to thrive in BBM, 3N-BBM+V, and Chu 13 due to the availability of optimal concentrations of particular salt chloride and vitamins, which likely provided growth conditions similar to those in their native environments. Different microalgae genera are selective to the particular combination of nutrients and growth conditions (Broady, 1996). Moreover, the microalgae cultured in the salt broth including BBM, 3N-BBM+V, Chu 13, and JM need to be widely dispersed by shaking it in the liquid media to increase their ability to absorb dissolved nutrients in the liquid media (Morrissey et al., 2018). Hence, several microalgae genera including *Botryococcus* sp. and *Chlorella* sp. were targeted to be grown in the liquid media used in this research, as proven by a previous study (Figueroa-Torres et al., 2016).

Generally, microalgae can be identified by their forms, sizes, photosynthetic pigments, and cell wall encapsulation. There are several distinct morphological observations of the cultured polar microalgae under light microscopy. Based on the shapes, sizes, and colours, the polar microalgae from S26 soil samples (Figure 1) were likely to be classified under the group of Chlorophyceae or commonly known as green algae. This result is mainly an inference to the visible green pigmentation of the microalgae due to the presence of chlorophyll *a*, chlorophyll *b*, and beta carotene (Dolganyuk et al., 2020). The cell unit arrangements are unicellular and colonial by attachment to the adjacent cell walls either in longitudinal or irregular clusters.

Based on the observation, the microalgae cultured in BBM (Figure 1) grew in a discoid shape, had a smooth cell wall, and the nuclei were visible. The microalgae were both observed to be unicellular and formed colonies. Several genera under the group of chlorophytes have a disc-like shape, such as *Actinochloris*, *Scenedesmus*, *Bracteacoccus*, *Neochloris*, and *Coelastrum* (John et al., 2011). The morphology of the observed

microalgae was comparable to the identified genera of microalgae by John et al. (2011), such as *Bracteacoccus*, *Actinochloris*, and *Chlorella*, based on the visible chloroplast and coccoidal shapes that can be both unicellular and irregular formed by the masses of colonies.

The morphological characteristics of the microalgae isolated in media 3N showed a distinguished shape of spherical and broadly ellipsoidal for the matured microalgae that had grown in size. Additionally, the solitary and matured vegetative cells of microalgae (Figure 3**b** & Figure 3**d**) were observed to evolve a pair of flagella and to have a visible single pyrenoid in the middle part, nucleus at the anterior part of the cell, and smooth cell wall (Proeschold et al., 2018). The isolated microalgae were more likely to have morphological traits similar to that of microalgae belonging to *Chlamydomonas* sp. as described by Dang et al. (2021). Numerous research has also utilised 3N media and Culture Collection of Algae and Protozoa (CCAP) to cultivate *Chlamydomonas* species (Rahman et al., 2019; Liu & Nakamura, 2019).

Furthermore, the isolated microalgae from Chu 13 media may closely resemble the microalgae grown in BBM due to the typical shape of spherical to ovoid, smooth cell wall, and lack of flagella, which categorised the microalgae as non-motile. The microalgae grown in BBM showed a visible nucleus in the centre of the cell, a cup-shaped chloroplast, and a varying number of cell units that made up a floral-like cluster and a singular cell. These forms closely resembled the morphological traits of *Coelastrum* sp. and *Botryococcus* sp. Meanwhile, the microalgae grown in Chu 13 media also showed an apparent nucleus or pyrenoids in the centre of the cell and parietal chloroplast, which were comparable to the microalgae under the group of Chlorophyta such as *Chlorococcum* sp. and *Chlorella* sp. The daughter cells were developed from the parent cells through cell mitosis (Figure 4**c**), demonstrating that the microalgae reproduced asexually (Safi et al., 2014). However, molecular identification and gene sequencing are required to validate the findings further, considering that all morphological characteristics were likely to match.

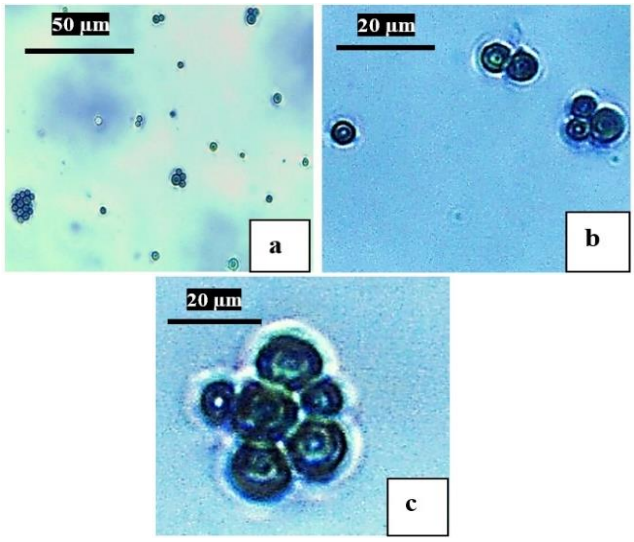

Figure 2: The morphological observations of microalgae cultured in BBM (**a-c**). Light microscope (40× and 100× magnification)

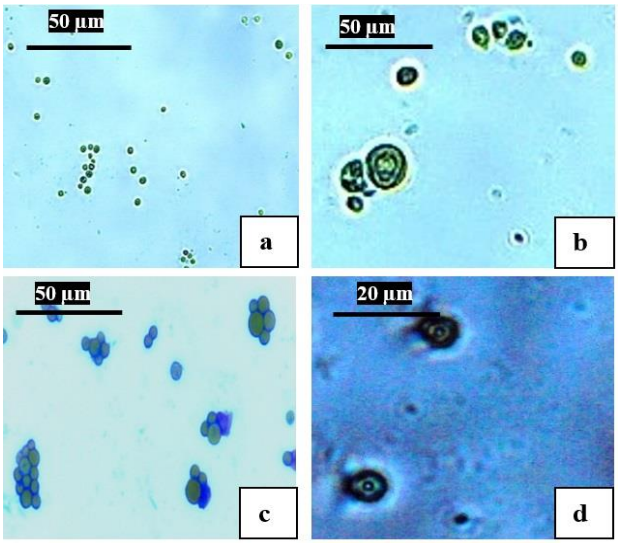

Figure 3: The morphological observations of microalgae cultured in 3N-BBM+V (**a-d**). Light microscope (40× and 100× magnification)

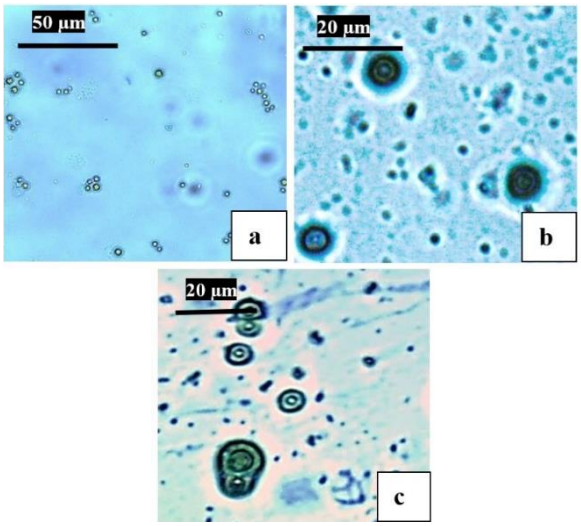

Figure 4: The morphological observations of microalgae cultured in Chu 13 medium (**a-c**). Light microscope (40x and 100x magnification)

Conclusion

In conclusion, collecting soil samples with a high probability of microalgae presence from sandy loam types of soil with fine-grained textures is recommended. The softer texture of the tiny clay particles in loamy soil makes the environment more stable and improves the availability of nutrients for the growth of the polar microalgae. Furthermore, BBM, 3N-BBM+V, and Chu 13 are favourable in culturing the Antarctic microalgae for maintenance, and they have also been used in many other studies extensively. Based on the observed morphological characteristics such as the presence of visible green-pigmented chloroplast, spherical to the ovoid, smooth cell wall, double-enveloped membrane, and flagella, the isolated microalgae are most likely to be considered Chlorophyta. Green algae, or chlorophytes, are the most prevalent species in many ecosystems, especially in the polar zone. However, further confirmation of the microalgae's species through molecular identification is required. The presence of lipids and other secondary metabolites in the discovered polar microalgae species can be investigated further.

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