Revelation and Science
Vol. 13, No. 02 (1445H/2023) 76-83

Fatty Acids Profiling from An Antarctic Microalga, *Pseudococcomyxa* sp.

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ABSTRACT
The main source of valuable fatty acids such as eicosapentaenic acid (EPA) and docosahexaenoic acid (DHA) that are important for human health are from marine fish. However, fatty acids derived from fish have many downsides such as contaminated with environmental pollution. Plus, the marine fish sources are limited due to several reasons. Hence, microalgae are seen to be potential alternative source of these valuable fatty acids. Microalgae are equipped with distinctive adaptive mechanisms that allow them to survive in all sorts of habitat even in subzero temperature such as in Antarctic. One of them is increased in their fatty acid levels. This research aims to characterize one species of Antarctic microalga isolated from polar ice of genus thought to be *Pseudococcomyxa* sp. and to profile its fatty acid compositions. The fatty acid methyl ester (FAME) was profiled using gas chromatography mass spectrometer (GC-MS) using methanol as solvent. Generally, the morphological characteristics of possible genus of *Pseudococcomyxa* sp. matched the actual *Pseudococcomyxa* sp. The total fatty acids percentage in *Pseudococcomyxa* sp. cultured in Bold's Basal Medium (BBM) had been successfully identified using methanol as solvent which is 53.13% with linolelaidic acid as the highest percentage (27.15%). The fatty acid compositions of Antarctic microalga, *Pseudococcomyxa* sp. which were successfully profiled have many potentials to be exploited in the future especially in pharmaceutical and food industry.
Keywords: Antarctic, fatty acids, food, microalgae, pharmaceutical

ABSTRAK
Sumber utama asid lemak yang bernilai seperti asid eikosapentaenik dan asid dokosahexaenoik yang penting bagi manusia adalah dari ikan laut. Walau bagaimanapun, asid lemak daripada ikan mempunyai banyak kelemahan seperti sudah tercemar dengan pencemaran alam sekitar. Tambahan pula, asid lemak ikan adalah terhad kerana beberapa sebab. Oleh itu, mikroalga dilihat sebagai sumber alternatif untuk memperoleh asid lemak bernilai ini. Mikroalga dilengkapi dengan mekanisma penyesuaian tersendiri yang membolehkannya untuk hidup dalam pelbagai jenis habitat walaupun dalam keadaan suhu bawah kosong seperti di Antartika. Salah satu mekanisma adaptasi adalah dengan meningkatkan tahap asid lemak. Kajian ini bertujuan untuk mengenalpasti satu spesies mikroalga dari Antartika yang diasingkan dari litupan ais kutub yang pada mulanya dikenalpasti sebagai genus *Pseudococcomyxa* sp. dan juga untuk mencirikan kandungan asid lemak. Metil ester asid lemak (FAME) telah diprofilkan menggunakan spektrometer jisim gas kromatografi (GC-MS) dengan metanol sebagai pelarut. Secara umumnya, padanan morfologi sampel yang pada mulanya dikenalpasti sebagai *Pseudococcomyxa* sp. adalah selari dengan morfologi sebenar *Pseudococcomyxa* sp. Jumlah peratusan asid lemak di dalam *Pseudococcomyxa* sp. yang membiak di dalam media BBM telah berjaya diperoleh menggunakan metanol sebagai pelarut iaitu 53.13% dengan metil etser asid linolelaidik sebagai peratus tertinggi (27.15%). Komposisi asid lemak mikroalga *Pseudococcomyxa* sp. dari Antartika telah berjaya diprofilkan dan mempunyai banyak potensi untuk dimanfaatkan pada masa hadapan terutamanya dalam industri farmaseutikal dan makanan.
Kata Kunci: Antartika, asid lemak, makanan, mikroalga, farmaseutikal

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1. INTRODUCTION

Microalgae encompass of microorganisms that could harvest energy from the sun through the process of photosynthesis and commonly represent as eukaryotic models for the high production of lipids. According to Siqueira *et al.* (2018), the large-scale commercial exploration of microalgal intracellular product has started since 1950 due to the high protein biomass that were utilized as an alternative food source. Since then, it has opened a wide range of passable products to be explored. Microalgae can produce valuable metabolites such as antioxidants, antimicrobials, anticarcinogenic, fatty acids, vitamins, pigments, and enzymes (Kaur *et al*., 2023) and have been studied as potential source for commercial production of valuable fatty acids and oil as alternative to animal and higher plant resources (Fawcett *et al*., 2022).

The ephemeral nature of polar microalgae where new ones will regularly drive out the old, and also their ability to continually acclimating and adapting to the extreme low temperature environment of the Antarctic promotes fast evolution through horizontal exchange and recombination of genetic material. Thus, these organisms usually represent as a resource for identification of new species, new physiological mechanisms of adaptation and new genes (Lyon & Mock, 2014). The increase in the degree of unsaturation of membranal fatty acids upon reduction in temperature is a universal phenomenon. It has been reported that enhancement of the polyunsaturated fatty acid (PUFA) content of microalgae membrane is one of the mechanisms for the adaptation to low temperature (Gao *et al*., 2023). The species of microalgae from the polar region therefore constitute high level of PUFAs in their fatty acid compositions (Suzuki *et al*., 2019; Morales-Sánchez *et al*., 2020). Eicosapentaneic acid (EPA, 20:5 ω3) and docosahexaenoic acid (DHA, 22:6 ω3) and arachidonic acid (AA, 20:4 ω6) are the important omega 3 and omega 6 PUFAs, respectively. They have numbers of nutraceutical and pharmaceutical applications (Oliver *et al*., 2020). However, PUFAs cannot be synthesized by human and can only be obtained through the diet (Mariamenatu & Abdu, 2021). EPA has been proven to reduce blood cholesterol and prevent coronary heart disease while DHA plays an important role in the development of the central nervous system of infants. Meanwhile AA is an important precursor that plays an important role in circulatory and central nervous systems (Handayani & Ariyantib., 2011). EPA and DHA are also important in treatment of cancer, inflammation, Alzheimer, neurodegenerative and cardiovascular diseases (Jesionowska *et al*., 2023). The reasons that qualify PUFAs as aids in primary prevention of many health conditions are their anti-inflammatory, antioxidant (Oppedisano *et al*., 2020), anti-atherogenic, anti-amyloid and neuroprotective properties (Wen *et al*., 2024). Thus, much research had been done regarding fatty acid productions in microalgae. However, the ability of Antarctic microalgae to produce high number of fatty acids is yet to be fully investigated. Hence the objective of this study is identifying a microalga isolated from Antarctic and to investigate its ability for high fatty acid production.

2. MATERIAL AND METHODS

2.1 Microalgal sample

Possible genus *Pseudococcomyxa* sp. isolated from polar ice in Antarctic, was obtained from our collaborators at Universiti Sains Malaysia (USM). The sample was labelled as *Pseudococcomyxa* sp. and was maintained on agar plate of Bold's Basal Medium (BBM) at 23°C.

2.2 Enrichment of *Pseudococcomyxa* sp.

The microalga *Pseudococcomyxa* sp. on agar media was subcultured into conical flask containing BBM liquid media. The flask was incubated in a shaker incubator at 23°C with rotation of 120 rpm. The microalga was then subcultured to a fresh BBM liquid medium every 2 weeks to reduce bacterial contamination. The microalga that was used in every experiment was grown for 1 month.

2.3 Morphological characterization of microalga

After one month of culture, the growth of *Pseudococcomyxa* sp. was observed using the naked eyes. The morphological characteristics of possible genus *Pseudococcomyxa* sp. was observed under a light microscope. About one drop of liquid culture was pipetted to a microscope slide. Next, the cells were smeared and let dried followed by heat fixing the cells by passing the slide through a Bunsen burner flame for few times. Then, a drop of methylene blue was added to the sample and then covered with a glass slipcover. The excess methylene blue was wiped using a clean paper towel gently. The sample was then viewed under light microscope at magnifications of 40x-100x. The morphological characteristics such as size, shape of colonies and mucilage were noted as proposed by Novis *et al*. (2008).

2.3 Fatty acid profiling

Fatty acid was extracted from *Pseudococcomyxa* sp. grown in BBM following Sasser (2001). Firstly, 20 ml of *Pseudococcomyxa* sp. cultures were placed into 50 ml of centrifuge tubes. The tubes were centrifuged at 4000x *g* for 5 minutes. The upper aqueous phase was discarded, and the lower part was left aside. Approximately, 40 mg microalgal cells were placed in a clean test tube. 1 ml of Reagent 1 (Saponification: 45 g NaOH, 150 ml CH_3OH and 150 ml dH_2O) was added to the test tube that contained the microalga and the tube was vortexed briefly and heated in a boiling water bath for 5 minutes, at which time the tube was vigorously vortexed for 5-10 seconds and returned to the water bath to complete the 30 minutes heating. After that, 2 ml of Reagent 2 (Methylation: 325 ml 6M HCl and 275 ml CH_3OH) was added. The tube was heated for 10 ± 1 minutes at 80°C. Next, 1.25 ml of Reagent 3 (Extraction: 200 ml hexane and 200 ml methyl tert-butyl ether) was added to the cooled tube, followed by recapping and gentle tumbling on a clinical rotator for about 10 minutes. The aqueous at the bottom layer was pipetted out and discarded. Reagent 4 (Sample cleanup: 10.8 g NaOH and 900 ml dH_2O) was added about 3 ml to the organic phase remaining in the tube. The tube was recapped, and the tumbled for 5 minutes. About 1/3 of the organic phase was pipetted into a vial and send for analysis. Determination of the fatty acid profiles of the *Pseudococcomyxa* sp. was carried out using gas chromatography mass spectrometer (GC-MS). Methanol was the solvent being used GC-MS for profiling the fatty acid. 20 µl of sample was placed into a vial, followed by solvent, added up until 1 ml. The blank consisted of only the solvent. The GC-MS analysis was run for 40 minutes, temperature of 30 ºC per minute and increase at 5°C per minute until it reaches 200°C with holding time of 5 minutes.

3. RESULTS AND DISCUSSION

The morphological observation of the cultured microalga possible to be *Pseudococcomyxa* sp. was obtained by using light microscope under 40x and 100x magnifications. The observed microalgal cells were green in colour, unicellular, elongated, have smooth cell walls and appeared as irregularly elliptical vegetative cells (Figure 1). Based on the observed features, all the characteristics matched with the genus *Pseudococcomyxa* sp. as

recorded in AlgaeBase (2019). The dividing cells observed under the light microscope of the cultured microalga also matched with the same genus, *Pseudococcomyxa* sp. as noted by Novis *et al*. (2008) (Figure 2).

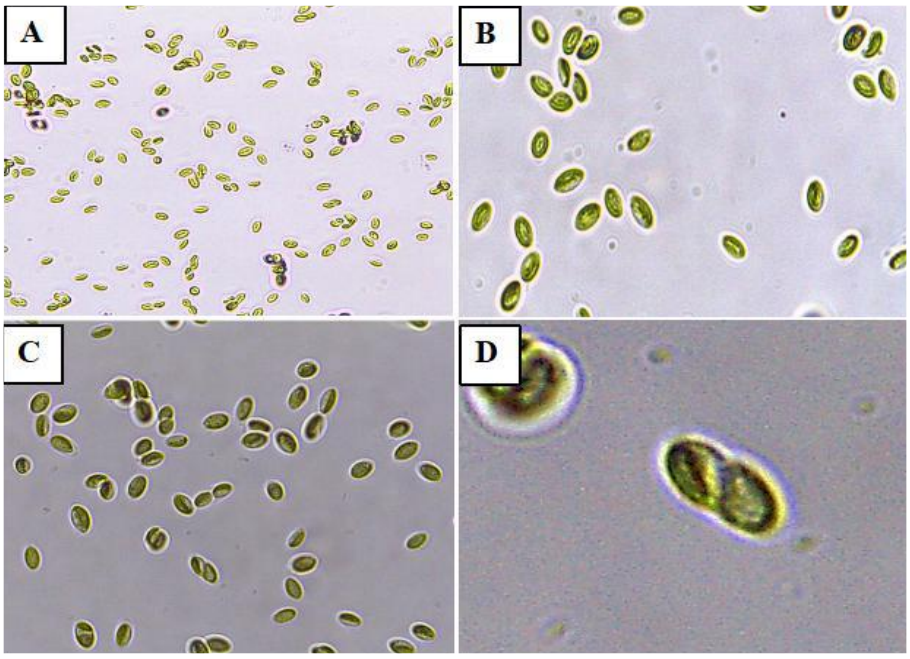

Figure 1: Morphological characteristics of possible *Pseudococcomyxa* sp. isolated from polar ice in Antarctic. A, 40x magnification; B-C, 100x magnification; D, dividing cells

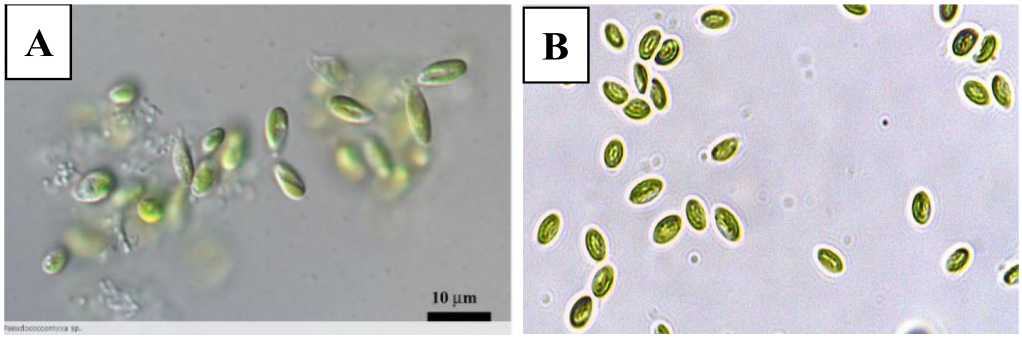

Figure 2: Comparative photos of A: *Pseudococcomyxa* sp. obtained from CCALA: Culture Collection of Autotrophic Organisms, http://ccala.butbn.cas.cz/en/pseudococcomyxa-sp-1and B: *Pseudococcomyxa* sp. isolated from Antarctic (100x magnification)

For fatty acid profiling, the GCMS analysis was performed for fatty acids extracted from *Pseudococcomyxa* sp. in BBM with 50 µl sample. Figure 3 showed the GC-MS peaks for fatty acid profile and Table 1 showed the fatty acid profile identified in the sample and the total percentage of fatty acids content detected. Using methanol as a solvent, 5 types of fatty acid methyl ester (FAME) which is an ester of the fatty acid itself were detected. Based on the absorbance value of the resulting peaks, there are 2 saturated FAMEs which are palmitic acid and stearic acid methyl ester, 1 monounsaturated FAME, methyl oleate and 2 polyunsaturated FAME which are linolelaidic acid and linolenic acid methyl ester. The total amount of FAME in the *Pseudococcomyxa* sp. sample is 53.13% which comprises of 3.75% palmitic acid methyl ester, 1.98% stearic acid methyl ester, 4.46% methyl oleate, 27.15%

linolelaidic acid methyl ester and 15.79% linolenic acid methyl ester. The amount of unsaturated FAME, 47.4% is much higher than the saturated FAME which only total up to only 5.73%. This is probably due to the origin of the *Pseudococcomyxa* sp. sample which is an Antarctic microalga. The extreme cold temperature of the Antarctic may induce the desaturation of fatty acid in its membrane to increase the membrane fluidity.

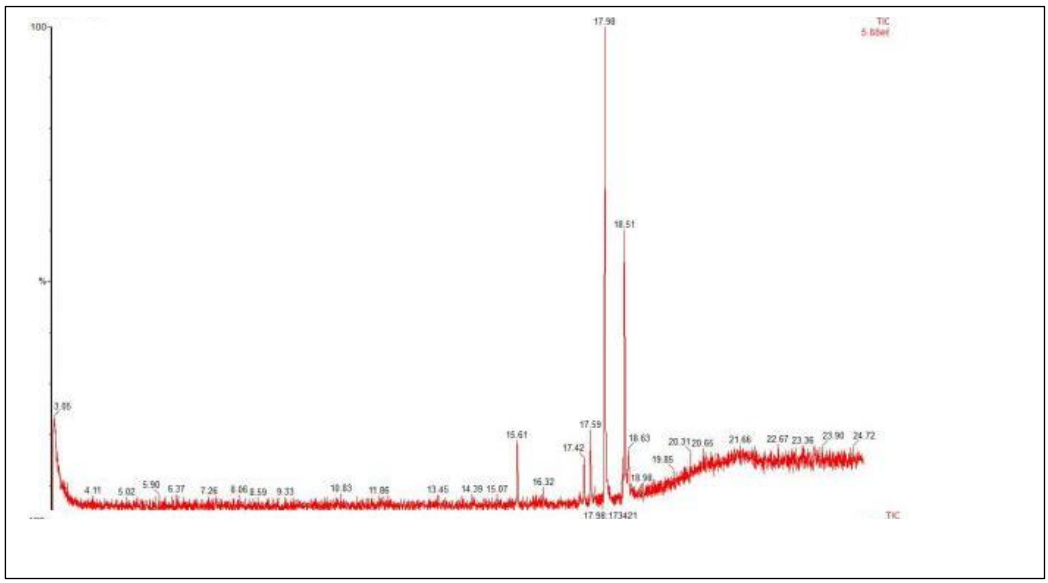

Figure 3: GC-MS chromatogram result showing peaks of fatty acids for *Pseudococcomyxa* sp. grown in BBM after 25 minutes retention time with pure methanol as solvent.

Table 1: Fatty acid profiles and their percentage of *Pseudococcomyxa* sp. cultivated in BBM medium

Solvent	Fatty acid methyl esters (FAME)			Percentage of fatty acid methyl esters (FAME) (%)
Methanol	Common name	Molecular formula	Systematic name	*Pseudococcomyxa* sp.
	Saturated FAME			
	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	n-Hexadecanoic acid methyl ester (16:0 ME)	3.75
	Stearic acid. Methyl ester	$C_{19}H_{38}O_2$	Octadecanoic acid methyl ester (18:0 ME)	1.98
	Unsaturated			
	Monounsaturated FAME			
	Methyl Oleate	$C_{19}H_{36}O_2$	9(Z)-Octadecenoic acid methyl ester (18:1 ME)	4.46
	Polyunsaturated FAME			

	Linolelaidic acid, methyl ester	$C_{19}H_{34}O_2$	9(E), 12(E)-Octadecadienoic acid methyl ester (18:2 ME)	27.15
	Linolenic acid methyl ester	$C_{19}H_{32}O_2$	9(Z),12(Z),15(Z)-Octadecatrienoic acid methyl ester (18:3 ME)	15.79
	Total FAME (%)			**53.13**

Based on the fatty acid profiling, besides the fact that the Antarctic microalga in this study contains high amount of unsaturated fatty acid compared to saturated fatty acid, the fatty acid profiling may be affected by the type of solvent used during GC-MS analysis. Generally, fatty acids are attracted to non-polar solvent. However, according to Japir *et al*. (2018), unsaturated fatty acids are more polar than saturated fatty acid, hence unsaturated fatty acids are highly soluble in methanol and ethanol compared to saturated fatty acids. Different type of solvents and solvent combinations should be used for diverse range of fatty acids identification.

4. CONCLUSION

In this study, possible genus *Pseudococcomyxa* sp. was characterized by its morphological shape. The profiling of fatty acids of the *Pseudococcomyxa* sp. grown in BBM was determined using methanol as solvent. Polyunsaturated fatty acids (PUFAs) are the most dominant fatty acid that can be found in the microalgal samples. The fatty acids present in the microalgae have potentials to be applied in various fields such as healthcare, pharmaceutical, aquaculture and food industries which will lead to microalgae as the new renewable sources of biomaterials.

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Article History
Received: 30/10/2023
Accepted: 12/12/2023