ORIGINAL ARTICLE

Screening and optimization of oleaginous marine fungi for lipid production as a possible source for biodiesel

Kanokkorn Longmatcha,[a] Worawut Chaiyasaeng,[a,b] Thamasak Yeemin,[c] Makamas Sutthacheep,[c] Jamrearn Buaruang[a,*]

[a]Marine Microbe Environment Research Unit, Division of Environmental Science, Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok, Thailand, 10240
[b]Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok, Thailand, 10240
[c]Marine Biodiversity Research Group, Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok, Thailand, 10240

**Corresponding author: jamrearn.b@rumail.ru.ac.th*

Received:17 August 2022 / Revised: 29 August 2022 / Accepted: 29 August 2022

Abstract. This research aims to screen and find appropriate culture media conditions for oleaginous marine fungi. In the experiment, the marine fungi were isolated from marine organisms that were collected from Mu Koh Yao and Mu Koh Samaesarn using the tissue transplanting method and screened by Sudan Black B staining and extracting lipids from fungi using chloroform with methanol. Studies for five parameters namely, glucose concentration, peptone concentration, incubation period, pH, and water type for optimum conditions. The result of fungi isolated from both sites found fifty-five isolates. The lipid content in the marine fungal, *Aspergillus* sp. MMERU 24, isolated from sponge (*Amphimedon* sp.) at Mu Koh Yao showed lipid content at 20.90±1.71% per dried biomass. Glucose concentration, peptone concentration, incubation period, pH, and water type of 30 g/L, 1 g/L, 8 days, 5, and distilled water 100% produced the highest lipid content at 25.47%, 23.02%, 26.67%, 19.23%, and 20.1%, respectively, were the optimum culture media conditions of *Aspergillus* sp. MMERU 24. From these experiments, we will be able to develop fungal biodiesel production in the future, which is environmentally friendly and has fewer emissions compared to other fuels, and follows the policy toward net-zero emissions.

Keywords: *Aspergillus* sp. MMERU 24, Biodiesel, Marine fungi, Oleaginous, Single-cell oils.

1. Introduction

Most countries' energy systems, either developed or in development, are based on fossil fuels, and demand for alternative fuels has grown significantly because control of greenhouse gas emissions is becoming more important than ever before. Biodiesel is derived from oils or fats, which are contained in plant seeds and animal fats (Martins et al., 2019; Zhang et al., 2014). Both feedstocks can be used in cooking as a result increasing the cost of food products. These drawbacks constrained users to shift to further alternate sources for feedstock of biodiesel production (Singh et al., 2020). Single-cell oils (SCOs) are produced by oleaginous microorganisms such as microalgae, bacteria, yeast, and fungi. They can easily grow in a controlled environment without being dependent on the climate (Ochsenreither et al., 2016; Patel et al.,2020).

Fungi can be classified into different types, such as endophyte fungi, soil fungi, marine fungi, etc., depending on the host of fungi living. Marine fungi are remarkable due to their little studied usefulness. They are growing under saline conditions requires adaptation to the habitat by creating polyols such as mannitol, arabitol, glycerol, etc., to control the salt balance inside and outside the cell (Wethered et al., 1985). In addition, lipids also play a role in cell equilibrium, which is commonly found in cell membranes and cell walls, but when excess lipid is stored in the form of triglycerides, etc. within the filamentous (Akpinar-Bayizit, 2014). Oleaginous microorganisms are species that are known for their ability to accumulate inside their cells or mycelia amounts of lipid

higher than 20% w/w in their dry cell weight (Athenaki et al., 2018). However, some species of fungi can be accumulated by more than 20% with modifying conditions such as pH, carbon (glucose) concentration, nitrogen (peptone) concentration, incubation period, water type, etc. (Kumar et al., 2020; Mhlongo et al., 2021).

Leesing and Baojungharn (2011) studied different glucose concentrations for *Torulaspora globosa* YU5/2, and the result glucose concentrations at 80 g/L produced lipids that reached a maximum of 4.31 g/L or 45.7%. Indeed, glucose concentration is the major impact factor for oil accumulation by the oleaginous microorganisms. Abou-Zeid et al. (2019) in this experiment nitrogen concentrations of glutamic acid at 0.5 g/L for *Aspergillus versicolor* showed the highest lipid content at 40.99 ± 1.41% and sodium nitrate at 1.0 g/L ($NaNO_3$) for *Aspergillus niveus* showed the highest lipid content at 43.06 ± 0.84%. Nitrogen concentration limitation is considered the most effective condition for most oleaginous fungal species in lipogenesis.

And the research of Hashem et al. (2020) studied the pH and incubation period optimum of *Syncephalastrum racemosum*, with the result that pH of 5 showed lipid content at 27.64%. The incubation period is determined by the fungi species and other factors, such as *S. racemosum*, which can accumulate the most lipids (28.08 ± 0.28%) after 11 days. While Subhash and Mohan (2014) studied appropriate conditions of *Aspergillus awamori* at 72 hours, the most accumulated lipids was 34.5% while other factors such as glucose, proteins, pH, nitrates, NaCl, phosphorous, and incubating temperature were 40 g/l, 5 g/l, 5.5, 4 g/l, 1.5 g/l, 0.5 g/l, and 30°C, respectively. The relative influence of the factors on lipid productivity can be summarized in the following: (1) pH (2) glucose (3) temperature (4) incubation time (5) peptone (6) nitrates and (7) NaCl, especially pH showed a stronger influence on lipid productivity.

However, each species of fungi collects a different amount and type of lipid depending on the factors mentioned above. Therefore, it is necessary to adjust appropriate conditions for each species of fungi to the potential for biodiesel production. This research aims to screen and find appropriate conditions of oleaginous marine fungi for development in biodiesel production, which is environmentally friendly and has fewer emissions compared to other fuels, and follows the policy toward net-zero emissions.

2. Materials and Methods

2.1 Sample collection

Marine organisms (sponges and algae) were collected from the coral reef in Mu Koh Yao, Phang Nga Province, and Mu Koh Samaesarn, Chonburi Province, by SCUBA diving (collected samples by random in study sites with a focus on sponge and algae). The samples were photographed with a waterproof digital camera kit and labeled with the numbers and location of the samples on waterproof paper and placed in a polyethylene bag with seawater. Finally, the sample is put in the icebox for one night before isolation and then immediately transported to the laboratory (Buaruang et al., 2015).

2.2 Culture media

Three different culture media were used for isolating and screening fungi namely, (1) Malt Extract Agar (MEA) with 70% seawater containing: malt extract powder 20.0 g, peptone 1.0 g, glucose 20.0 g, agar 15.0 g, seawater 700 ml, and distilled water 300 ml, (2) Yeast Peptone Agar (YPA) containing: yeast extract 1.0 g, peptone 1.0 g, agar 15.0 g, and distilled water 1 L (Add streptomycin sulfate 10 mg/L into both media after autoclaved and decrease temperature for 45-50°C), and (3) Malt Extract Broth (MEB) with 70% seawater containing: malt extract powder 20.0 g, peptone 1.0 g, glucose 20.0 g, seawater 700 ml, and distilled water 300 ml (Buaruang et al., 2015).

2.3 Isolation of marine fungi

Isolation of marine fungi from samples using the tissue transplanting method. The samples were washed in 0.06% sodium hypochlorite (NaOCl) for 1 min and three times in sterilized seawater were cut into 0.5×0.5 cm and placed on MEA and YPA incubated at 28°C for 5-7 days. The hypha tips were transferred from the colony to deep MEA and kept as a pure culture for further investigation (Buaruang et al., 2015).

2.4 Morphological and DNA identification of oleaginous marine fungi

Morphological characteristics of colonies were determined, such as growth pattern, color, and texture on media. Incubated marine fungi on MEA for 7 days. Colony characteristics were examined under a light microscope with a Dino-eye microscope and taken a photo. The ITS regions were used to analyze the DNA of oleaginous marine fungi by picking up some of the fungi in an Eppendorf tube and were carried out by Macrogen Inc., Korea (Buaruang et al., 2015; Paul et al., 2020).

2.5 Lipid analysis

The selection of lipid accumulation was performed both qualitatively and quantitatively. Lipid analysis was to assess fungal lipids that suitability to be precursors for biodiesel.

2.5.1 Qualitative selection of lipids in the marine fungus isolate

Incubated marine fungi on MEA for 7 days. Sudan Black B (prepare with 0.3 g of the powdered stain in 100 ml of 70 percent ethyl alcohol (Burdon, 1946)) is a lysochrome (fat-soluble dye) diazo dye used for staining lipid on the glass slide and covered by a coverslip. The stained marine fungi were observed with a light microscope using oil immersion to detect the presence of blue or grayish-colored lipid globules within the cells (Cheirsilp and Kitcha, 2015).

2.5.2 Quantitative selection of lipids in the marine fungus isolate

After the fermentation period in MEB for 7 days, cultures were harvested by filtration (Whatman No.1) and rinsed thoroughly with distilled water to remove media from the cell. Dries biomass in a hot air oven at 60°C for 1 day and take the cell dry weight of 200 mg for extraction by 2:1 (v/v) chloroform:methanol and calculates the percentage of lipid content per cell dry weight together with standard deviation (Vicente et al., 2009; Ali and El-Ghonemy, 2014). Lipid content was determined as (Reis et al., 2019):

$$Lipid\ content\ (\%) = \frac{Total\ extracted\ lipids\ (mg/200mL)}{Dry\ biomass\ weight\ (mg/200mL)} \times 100\%$$

2.6 Increased lipid production due to optimal culture media conditions

Optimization of culture medium for lipid accumulation was carried out for the potent oleaginous marine fungal, *Aspergillus* sp. MMERU 24 was characterized through lipid analysis, which showed higher lipid content as a percentage of lipid in the dried biomass. The oleaginous marine fungal was transferred to MEB for 7 days (static condition) (Abou-Zeid et al., 2019; Paul et al., 2020).

2.6.1 The influence of glucose concentration

This experiment used different glucose concentrations starting from 10, 15, 20, 25, 30, 35, 40, and 45 g/L. Incubate in MEB (use as previously mentioned except amount of glucose) using Erlenmeyer flask 500 mL contains culture media at 200 mL for 7 days. Finally, calculate lipid content was fungal at different glucose concentrations.

2.6.2 The influence of peptone concentration

This experiment used different peptone concentrations starting from 0.5, 1, 1.5, 2, 2.5, 3, and 3.5 g/L. Incubate in MEB (use as previously mentioned except amount

of peptone) using Erlenmeyer flask 500 mL contains culture media at 200 mL for 7 days. Finally, calculate lipid content was fungal at different peptone concentrations.

2.6.3 The influence of incubation period

The fungal was grown in an MEB using an Erlenmeyer flask 500 mL containing culture media at 200 mL. Incubate time from 4 days to 15 days. Finally, the calculate lipid content was fungal at different days.

2.6.4 The influence of pH conditions

The pH of the MEB in an Erlenmeyer flask 500 mL containing culture media at 200 mL was adjusted to different values starting from 3, 4, 5, 6, 7, and 8 using 1N of NaOH and HCl then inoculated the fungal and incubated for 7 days. Finally, the calculate lipid content was fungal at different pH conditions.

2.6.5 The influence of water types in culture media

The selected fungal strains were grown on MEB in an Erlenmeyer flask 500 mL containing culture media at 200 mL using different waters such as seawater 70% (SW 70%), seawater 100%, distilled water 70%, and distilled water 100% incubated for 7 days. Finally, the calculated lipid content was fungal under different water types in culture media conditions.

3. Results

3.1 Isolation of marine fungi

Ten sponge samples (*Amphimedon* sp., *Clathria* sp., *Coelocarteria singaporensis*, *Dysidea* sp., *Haliclona cymaefomis*, *Hytios erecta*, *Neopetrosia* sp., *Oceanapia sagittaria*, *Spheciospongia* sp., and *Xestospongia* sp.) and four algae samples (*Acanthophora* sp., *Padina* sp., *Sargassum prismaticum*, and *Tricleocarpa* sp.) were collected from Mu Koh Yao, Phang Nga Province. Forty-eight isolates of marine fungi were found from marine organisms comprising *Alternaria* sp., *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus* spp., *Penicillium* spp., *Syncephalastrum racemosum*, *Trichoderma* spp., and sterile mycelium. Overall, *Amphimedon* sp. found the most marine fungi were nine isolates and *Aspergillus* spp. was found thirty isolates, which have the largest amount and can be considered the dominant species. However, *Aspergillus* spp. isolated from *Amphimedon* sp. more than other samples to seven isolates (Figure 1 and Table 1). *Amphimedon* sp. was the sponge that obtained food by filter feeder effect some of the sediment and microorganisms that in the water to stick in the body of the sponge and the study site found this sponge living close to coastal as the highest sediment accumulation areas as influenced by fresh water from the island which resulted in the highest marine fungi from this sponge.

Moreover, two sponge samples (*Clathria* sp. and *Xestospongia* sp.) and one algae, *Laurencia* sp., were collected from Mu Koh Samaesarn, Chonburi Province. Seven isolates of marine fungi were found, comprising *Aspergillus japonicus*, *Aspergillus* spp., and *Trichoderma* spp. (Figure 2 and Table 1).

3.2 Morphological and DNA identification of oleaginous marine fungi

The morphology of *Aspergillus* sp.1-5 isolated from marine sponge (*Amphimedon* sp.) (Figure 3a) which is oleaginous marine fungi the colony growth on MEA with a diameter of 4.5 cm after 7 days the colony color is cinnamon brown (Figure 3b). Conidiophores are typically long, columnar, colorless (hyaline) and smooth giving rise to sub-spherical vesicles that are biseriate (Figure 3c) and conidia are smooth-walled, globose to slightly elliptical, and striate (Figure 3d). However, the morphology data

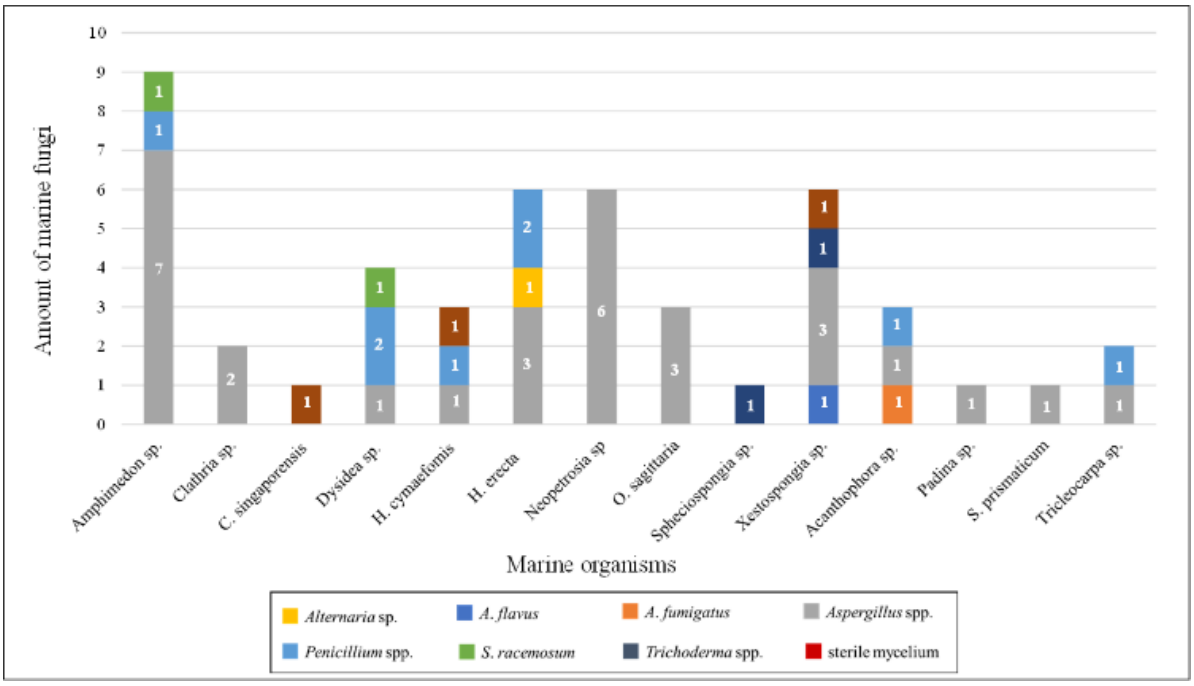

Figure 1. Bar diagram showing marine-derived fungi isolated from marine organisms at Mu Koh Yao, Phang Nga Province

of *Aspergillus* sp.1-5 analogous with *Aspergillus terreus*, which corresponds to DNA analysis, BLAST search of ITS gene sequences and phylogenetic analysis affiliated the oleaginous marine fungi to the closest similar *Aspergillus terreus* 99%. Hence, the results of morphological and DNA analysis, *Aspergillus* sp. 1-5 isolated from sponge (*Amphimedon* sp.) was *Aspergillus* sp. MMERU 24.

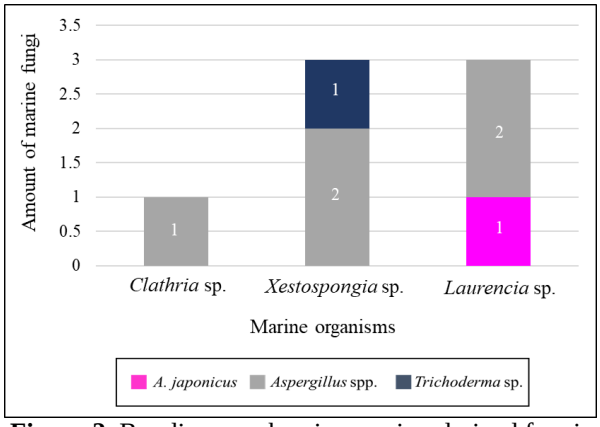

Figure 2. Bar diagram showing marine-derived fungi isolated from marine organisms at Mu Koh Samaesarn, Chonburi Province

3.3 Lipid analysis

In the qualitative selection method using Sudan Black B staining, 55 isolates of marine fungi were tested. The results showed that 38 isolates were detected with lipid in the mycelium. Quantitative selection by extraction for lipid content found *Aspergillus* sp. MMERU 24 had a lipid content of more than 20% per dried biomass weight, an indicator of being oleaginous, followed by Table 1. The result of lipid analysis indicates that *Aspergillus* sp. MMERU 24 was an oleaginous marine fungus by Figure 4, showing lipid globules uptake the color dye in the mycelium.

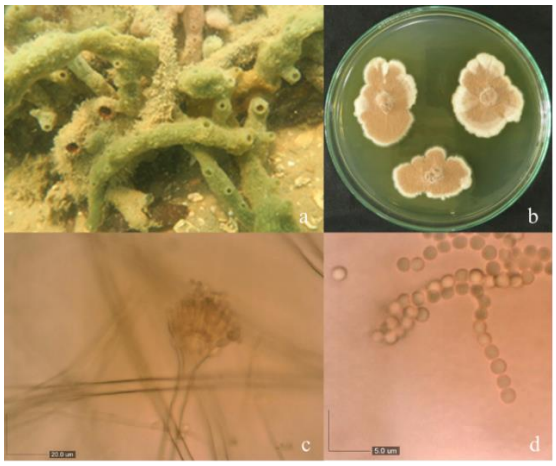

Figure 3. (a) *Amphimedon* sp. is the host of *Aspergillus* sp.1-5, (b) *Aspergillus* sp.1-5 on MEA for 7 days, (c) vesicle, metulae, and phialide (scale bar 20 µm), and (d) conidia (scale bar 5 µm)

Table 1. The result of marine fungi isolated from marine organisms, qualitative selection, and quantitative selection

Location	No.	Marine organisms	Isolate	Qualitative		Quantitative
				Stained	Not Stained	Lipid content (%)
Mu Koh Yao	1	*Amphimedon* sp.	*Aspergillus* sp.1-1		✓	
			Aspergillus sp.1-2		✓	
			Aspergillus sp.1-3	✓		4.35±1.20*
			Aspergillus sp.1-4		✓	
			Aspergillus sp.1-5	✓		20.90±1.71
			Aspergillus sp.1-6		✓	
			Aspergillus sp.1-7	✓		2.97±0.59
			Penicillium sp.1-8		✓	
			*S. racemosum*1-9	✓		11.58±2.71
	2	*Clathria* sp.	*Aspergillus* sp.2-1		✓	
			Aspergillus sp.2-2		✓	
	3	*C. singaporensis*	Sterile mycelium3-1	✓		3.95±1.40
	4	*Dysidea* sp.	*Aspergillus* sp.4-1	✓		3.17±0.59
			Penicillium sp.4-2	✓		11.27±2.90
			Penicillium sp.4-3	✓		11.82±2.55
			*S. racemosum*4-4	✓		12.78±2.97
	5	*H. cymaefomis*	*Aspergillus* sp.5-1	✓		4.35±0.62
			Penicillium sp.5-2	✓		13.77±3.28
			Sterile mycelium5-3	✓		12.60±4.70
	6	*H. erecta*	*Alternaria* sp.6-1	✓		10.17±3.36
			Aspergillus sp.6-2		✓	
			Aspergillus sp.6-3		✓	
			Aspergillus sp.6-4		✓	
			Penicillium sp.6-5	✓		8.45±1.01
			Penicillium sp.6-6	✓		17.08±2.34
	7	*Neopetrosia* sp.	*Aspergillus* sp.7-1	✓		5.83±0.61
			Aspergillus sp.7-2	✓		4.10±0.53
			Aspergillus sp.7-3	✓		5.83±0.61
			Aspergillus sp.7-4		✓	
			Aspergillus sp.7-5		✓	
			Aspergillus sp.7-6	✓		3.12±0.25
	8	*O. sagittaria*	*Aspergillus* sp.8-1	✓		4.85±1.06
			Aspergillus sp.8-2	✓		8.07±1.44
			Aspergillus sp.8-3		✓	
	9	*Spheciospongia* sp.	*Trichoderma* sp.9-1	✓		3.67±3.16
	10	*Xestospongia* sp.	*A. flavus*10-1	✓		6.83±2.93
			Aspergillus sp.10-2	✓		4.17±0.06
			Aspergillus sp.10-3	✓		3.85±0.85
			Aspergillus sp.10-4	✓		5.05±2.67
			Trichoderma sp.10-5	✓		3.97±2.47
			Sterile mycelium10-6	✓		12.27±3.74
	11	*Acanthophora* sp.	*A. fumigatus*11-1	✓		4.35±0.10
			Aspergillus sp.11-2	✓		15.25±3.22
			Penicillium sp.11-3	✓		13.67±3.25
	12	*Padina* sp.	*Aspergillus* sp.12-1	✓		8.25±1.48
	13	*S. prismaticum*	*Aspergillus* sp.13-1	✓		3.02±1.65
	14	*Tricleocarpa* sp.	*Aspergillus* sp.14-1		✓	
			Penicillium sp.14-2	✓		7.28±1.65
Mu Koh Samaesarn	15	*Clathria* sp.	*Aspergillus* sp.15-1		✓	
	16	*Xestospongia* sp.	*Aspergillus* sp.16-1	✓		8.12±1.36
			Aspergillus sp.16-2		✓	
			Trichoderma sp.16-3	✓		5.82±1.40
	17	*Laurencia* sp.	*A. japonicus*17-1	✓		4.43±0.75
			Aspergillus sp.17-2	✓		16.60±1.59
			Aspergillus sp.17-3		✓	

*±standard deviation

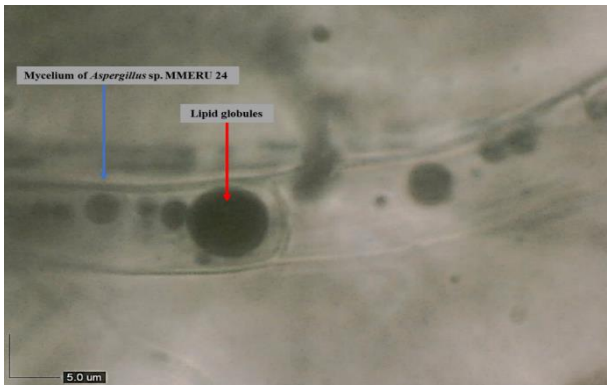

Figure 4. The Sudan Black B staining lipid globules (red arrow) in the mycelium of *Aspergillus* sp. MMERU 24 (blue arrow)

3.4 Increased lipid production due to optimal culture media conditions

The effective parameters for lipid accumulation in oleaginous fungi are glucose concentration, peptone concentration, pH, incubation period, and water type. These are important parameters that determine the worthiness of fungi lipids.

3.4.1 The influence of glucose concentration

The synthesis of lipids in fungi was influenced by the supply of glucose in the growth medium. If enough glucose, the lipid production rate and accumulation will increase up to a maximum level. However, the use of higher glucose concentrations may be an inhibitory factor for microbial growth. For *Aspergillus* sp. MMERU 24, a glucose concentration of 30 g/L produced the highest lipid content at 25.47±0.87% (Table 2).

Table 2. The lipid content of *Aspergillus* sp. MMERU 24 under different glucose concentrations

Glucose concentrations (g/L)	Lipid content (%w/w)
10	14±0.26*
15	18±1.45
20	21.1±1.48
25	22.52±1.46
30	25.47±0.87
35	24±1.15
40	21.05±0.17
45	19.22±0.55

*±standard deviation

3.4.2 The influence of peptone concentration

The effect of peptone plays an important role in motivating lipid accumulation. The effect of peptone is contrary to the accumulation of lipids by oleaginous fungi. For *Aspergillus* sp. MMERU 24, peptone concentration of 1 g/L produced the highest lipid content at 23.02±0.47% (Table 3).

Table 3. The lipid content of *Aspergillus* sp. MMERU 24 under different peptone concentrations

Peptone concentrations (g/L)	Lipid content (%w/w)
0.5	18.43±0.72*
1.0	23.02±0.47
1.5	20.17±0.55
2.0	19.03±0.42
2.5	17.48±1.14
3.0	15.42±0.61
3.5	12.50±0.10

*±standard deviation

3.4.3. The influence of incubation period

The effect of the incubation period of fungal lipid products may depend on other factors such as carbon source, nitrogen source, incubation time, etc. In this experiment, *Aspergillus* sp. MMERU 24, the incubation period of 8 days produced the highest lipid content at 26.67±1.46% (Table 4).

Table 4. The lipid content of *Aspergillus* sp. MMERU 24 under different incubation periods

Incubation periods (days)	Lipid content (%w/w)
4	18.17±0.35*
5	20.8±0.67
6	22.52±0.33
7	23.17±0.60
8	26.67±1.46
9	22.38±0.31
10	20.82±0.85
11	19.9±0.60
12	20.9±0.87
13	18.9±0.40
14	18.6±0.44
15	19.2±0.87

*±standard deviation

3.4.4 The influence of pH conditions

Normally, fungi produce lipids over a wide range of pH, but most of the ability of lipase production to increase at a highly acidic

pH, that the pH optimum differs for each oleaginous fungi. For *Aspergillus* sp. MMERU 24, the pH at 5, produced the highest lipid content 19.23±0.51% (Table 5).

Table 5. The lipid content of *Aspergillus* sp. MMERU 24 under different pH conditions

pH	Lipid content (%w/w)
3	8.1±0.10*
4	18.32±0.12
5	19.23±0.51
6	15.28±0.21
7	16.22±1.64
8	12.17±0.06

*±standard deviation

3.4.5 The influence of water type in culture media

Interestingly, the culture media with distilled water 100% showed that *Aspergillus* sp. MMERU 24 produced the highest lipid content at 20.1±0.44%, while seawater of 100% can produce lipid content at 13.77±0.92% (Table 6).

Table 6. The lipid content of *Aspergillus* sp. MMERU 24 under different water types in culture media

Water type in culture media (%)	Lipid content (%w/w)
Sea water 70%	18.37±0.21*
Sea water 100%	13.77±0.92
Distilled water 70%	18.65±0.17
Distilled water 100%	20.1±0.44

*±standard deviation

4. Discussion

The marine fungi isolated from Mu Koh Yao found forty-eight isolates and seven isolates from Mu Koh Samaesarn. In the experiment from both study sites, marine fungi from Mu Koh Yao more than Mu Koh Samaesarn (when comparing the amount of the marine fungi isolated from the same samples were *Clathria* sp. and *Xestospongia* sp.) due to Mu Koh Yao has abundant ecosystem than Mu Koh Samaesarn, thus resultant micro-organisms like fungi, bacteria, etc. including macro-organisms such as fish, etc., more than diverse Mu Koh Samaesarn. The result of selection was *Aspergillus* sp. MMERU 24 isolated from *Amphimedon* sp. at Mu Koh Yao had the highest lipid content of 20.90±1.71% an indicator of being oleaginous. When developed for optimized culture conditions, the glucose concentration of 30 g/L produced the highest lipid content at 25.47±0.87%, while using lower and higher glucose concentrations, as a result, inhibited lipid production according to Abou-Zeid et al. (2019) described the use of higher glucose concentrations could act as an inhibitory factor for microbial growth and lipid synthesizing. For peptone concentration of 1 g/L, the fungal produced the highest lipid content at 23.02±0.47% from tenet the nitrogen concentration plays a crucial role in stimulating lipid accumulation, the effect of nitrogen is inverse as the accumulation of lipids by oleaginous fungi is triggered by the exhaustion of the exogenous nitrogen in the culture medium (Mhlongo et al., 2021). The incubation period of 8 days produced the highest lipid content at 26.67±1.46%. The pH factor was the most significant for lipid production. From the experimental data, pH 5 was found to have a lipid content of 19.23±0.51% to be suitable for lipid accumulation. The observance was also supported by the study of Subhash and Mohan (2014). Finally, the water type in the experiment showed the highest lipid content at 20.1±0.44%, when using distilled water at 100% as agreeable with the experiment of Subhash and Mohan (2014) explain that sodium chloride (NaCl) is the seventh most important out of eight parameters, and that lipid production, which was the least impacting factor at individual levels maximizes lipid productivity in combination.

Summary, *Aspergillus* sp. MMERU 24 was an oleaginous marine fungus with optimum culture conditions using glucose concentration of 30 g/L, peptone concentration of 1 g/L, an incubation period of 8 days, pH at 5, and the water type in the culture media was distilled water of 100%. The conditions of *Aspergillus* sp. MMERU 24 produce the highest lipid content, so it is appropriate for the oleaginous marine fungi. This research was on the screen, and optimum culture media conditions of oleaginous marine fungi for developing

biodiesel production using the conditions incubated of *Aspergillus* sp. MMERU 24 to study the suitable biodiesel production from the fungal for the further as a possible source for biodiesel.

Acknowledgements

This project is funded by National Research Council of Thailand and the Plant Genetic Conservation Project under the Royal initiative of HRH Princess Maha Chakri Sirindhorn and to the Naval Special Warfare Command, the Royal Thai Fleet, the Royal Thai Navy for their assistance in collecting the marine organism samples. We thank Dr. Sumaitt Putchakarn of the Institute of Marine Science, Burapha University for the identification of the sponge. We are most grateful to the staffs of the Marine Microbe Environment Research Unit (MMERU), Division of Environmental Science, Faculty of Science, Ramkhamhaeng University for their field work assistance.

References

Abou-Zeid AM, Abd El-Zaher EH, Saad-Allah KM, Ahmed, RU (2019) Screening and optimization of some Egyptian soil-born fungi for lipids production as possible source for biofuel. The Egyptian Society of Experimental Biology 15:243-250

Akpinar-Bayizit A (2014). Fungal lipids: the biochemistry of lipid accumulation. International Journal of Chemical Engineering and Applications 5(5):409

Ali TH, El-Ghonemy DH (2014) Optimization of culture conditions for the highest lipid production from some oleaginous fungi for biodiesel preparation. Asian Journal of Applied Sciences 2(5):600-609

Athenaki M, Gardeli C, Diamantopoulou P, Tchakouteu SS, Sarris D, Philippoussis A, Papanikolaou S (2018) Lipids from yeasts and fungi: physiology, production and analytical considerations. Journal of Applied Microbiology 124(2):336-367

Buaruang J, Manoch L, Chamswarng C, Piasai O, Yaguchi T, Kijjoa A (2015) Species of *Aspergillus* Section *Fumigati* from the Coral Reefs in the Gulf of Thailand and Andaman Sea and their Antagonistic Effects Against Plant Pathogenic Fungi. Thai Journal of Agricultural Science 48(2):87-107

Burdon KL (1946) Fatty material in bacteria and fungi revealed by staining dried, fixed slide preparations. Journal of bacteriology 52(6):665-678

Cheirsilp B, Kitcha S (2015) Solid state fermentation by cellulolytic oleaginous fungi for direct conversion of lignocellulosic biomass into lipids: fed-batch and repeated-batch fermentations. Industrial Crops and Products 66:73-80

Hashem AH, Suleiman WB, Abu-elreesh G, Shehabeldine AM, Khalil AMA (2020) Sustainable lipid production from oleaginous fungus *Syncephalastrum racemosum* using synthetic and watermelon peel waste media. Bioresource Technology Reports 12:100569

Kumar SPJ, Avanthi A, Chintagunta AD, Gupta A, Banerjee R (2020) Oleaginous lipid: a drive to synthesize and utilize as biodiesel. Springer, New Delhi, pp 105-129

Leesing R, Baojungharn R (2011) Microbial oil production by isolated oleaginous yeast *Torulaspora globosa* YU5/2. World Academy of Science, Engineering and Technology 76(52):799-803

Martins F, Felgueiras C, Smitkova M, Caetano N (2019) Analysis of fossil fuel energy consumption and environmental impacts in European countries. Energies 12(6):964

Mhlongo SI, Ezeokoli OT, Roopnarain A, Ndaba B, Sekoai PT, Habimana O, Pohl CH (2021) The potential of single-cell oils derived from

filamentous fungi as alternative feedstock sources for biodiesel production. Frontiers in Microbiology 12:57

Ochsenreither K, Glück C, Stressler T, Fischer L, Syldatk C (2016) Production strategies and applications of microbial single cell oils. Frontiers in microbiology 7:1539

Patel A, Karageorgou D, Rova E, Katapodis P, Rova U, Christakopoulos P, Matsakas L (2020) An overview of potential oleaginous microorganisms and their role in biodiesel and omega-3 fatty acid-based industries. Microorganisms 8(3):434

Paul S, Bhagobaty RK, Nihalani MC, Joshi SR (2020) Characterization of oleaginous endophytic fungi of biodiesel plants as potential biofuel minifactories. Biomass and Bioenergy 142:105750

Reis CER, Carvalho AKF, Bento HB, de Castro HF (2019) Integration of microbial biodiesel and bioethanol industries through utilization of vinasse as substrate for oleaginous fungi. Bioresource Technology Reports 6:46-53

Singh D, Sharma D, Soni SL, Sharma S, Sharma PK, Jhalani A (2020) A review on feedstocks, production processes, and yield for different generations of biodiesel. Fuel 262:116553

Subhash GV, Mohan SV (2014) Lipid accumulation for biodiesel production by oleaginous fungus *Aspergillus awamori*: influence of critical factors. Fuel 116:509-515

Vicente G, Bautista LF, Rodríguez R, Gutiérrez FJ, Sádaba I, Ruiz-Vázquez RM, Torres Martínez S, Garre V (2009) Biodiesel production from biomass of an oleaginous fungus. Biochemical Engineering Journal 48(1):22-27

Wethered JM, Metcalf EC, Jennings DH (1985) Carbohydrate metabolism in the fungus Dendryphiella salina: VIII. The contribution of polyols and ions to the mycelial solute potential in relation to the external osmoticum. New phytologist 101(4):631-649

Zhang X, Yan S, Tyagi RD, Drogui P, Surampalli RY (2014) Ultrasonication assisted lipid extraction from oleaginous microorganisms. Bioresource technology 158:253-261