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Research article

Growth, total lipid content and fatty acid composition of *Amphora* sp. isolated from Gulf of Thailand as alternative lipid source in larviculture

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Abstract

Importance of the work: *Amphora* sp. strains contain a high lipid content that could be an excellent substitute ingredient in fish meal and fish oil.

Objectives: To evaluate the growth performance and lipid content of four *Amphora* sp. strains isolated from four different locations in the Gulf of Thailand.

Materials & Methods: Four *Amphora* sp. strains—Sichang Island (ASC), Samaesarn Island (ASS), Pran Buri (APB) and Laemyai (ALY)—isolated from four different locations in the Gulf of Thailand were cultured in F/2 medium for 13 d. Biomass and the specific growth rate (SGR) of the experimented *Amphora* sp. were determined. Two *Amphora* sp. strains with high biomass performance were further investigated for their lipid content and fatty acid composition.

Results: Among the *Amphora* sp. strains, ASC had the highest biomass production (1.01×10^6 cells/cm^2), followed by ALY (3.75×10^5 cells/cm^2), with the biomass performance of these two strains being significantly higher than for the other two strains. ALY had the fastest growth rate, while ASC grew more slowly, but was more stable. The mean (±SD) maximum lipid content of ALY was 74.35±2.46%, while that of ASC was 45.38±4.18%. The major UFAs detected in both strains included eicosapentaenoic acid, docosapentaenoic acid, docoxahexaenoic acid and stearidonic acid.

Main finding: ALY, with the fastest growth rate and the highest lipid content was the most efficient candidate for mass production, while ASC was more tolerant as an aged culture, which should be useful for long-term mass production.

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Introduction

Fishmeal and fish oil contain an optimal mix of essential nutrients and have been used as major feed ingredients in aquaculture for many years (Miles and Chapman, 2021). However, over the past few decades, supplies of fish-based materials in aquaculture feeds have decreased substantially. Therefore, it has become necessary to search for alternative feed ingredients (Araújo et al., 2019).

Microalgae are one of the promising alternatives to fish meal ingredients due to their combination of essential amino acids, healthy triglycerides and abundant levels of omega-3 fatty acids, carbohydrates, vitamins and pigments. These attributes make microalgae a potential source for promoting fish health (Sarker et al., 2020). Several microalgal species, such as *Schizochytrium* sp., *Isochrysis* sp., *Spirulina* sp., *Pavlova* sp., *Chlorella* sp., *Chlamydomonas* sp. and *Nannaochloropsis* sp., have been extensively studied as alternative lipid sources due to their potential enhancement of growth and their ability to synthesize essential fatty acid (Macias-Sancho et al., 2014; Nagappan et al., 2021).

Diatom is a member of the microalgae that can produce and accumulate lipid up to 40–60% of its dry biomass (Chen, 2012; Sayanova et al., 2017; Xue et al., 2017; Zulu et al., 2018). The main proportion of these lipids is unsaturated fatty acids (UFAs), especially long-chain fatty acids, which are necessary for the growth and health of aquatic animals. Among various diatom species, *Amphora* sp. is one of the most interesting, especially for larvae, such as abalone, sea urchin and crustaceans, during their grazing life stage, due to its rapid growth, high lipid content potential and its suitable size for grazing (Peterson, 2002; de la Peña, 2007; Khatoon et al., 2009; Zhang et al., 2010; Khwancharoen et al., 2020; Gomes et al., 2021). However, the lipid content, productivity and biosynthesis varies among species, growth stages and environmental parameters (Upadhyay et al., 2017; Cointet et al., 2019a). Ideally, strains of microalgae suitable for candidates as a lipid source in aquaculture should have the ability to grow rapidly under a wide variety of growth conditions, with a good balance between cost and productivity and industrial scale application. As benthic diatoms, most identified *Amphora* are used as live feed for grazers, such as bivalves and molluscs (Brown, 2002; Chiu et al., 2007), with very few used as an alternative ingredient in the artificial feed of larvae and the juvenile stage of commercial crustacean species, such as shrimp and crabs. Additionally, *Amphora* sp. strains have recently been found to possess several useful nutritional properties, in particular, polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA), docoxahexaenoic acid (DHA) and arachidonic acid (AA), which are known to be essential for various larvae and are found in *Amphora* sp. in adequate proportions (Courtois de Viçose et al., 2012). However, not all *Amphora* sp. have a high lipid content, which can vary greatly in the range 18–40%, depending on species or strains, or both (Govindan et al., 2021). These factors have driven the present study to evaluate the feasibility of using novel *Amphora* isolates as valuable lipid source. Therefore, this study aimed to determined growth, total lipid content and fatty acid composition of four *Amphora* strains isolated from different locations in the Gulf of Thailand as criteria to verify the potential use of these diatoms as an alternative lipid source in shrimp larviculture.

Materials and Methods

Isolation of diatoms

Seawater samples were collected from Sichang Island (ASC), Samaesarn Island (ASS), Pran Buri (APB) and Laemyai (ALY), as shown in Fig. 1. The isolation of diatom cells was carried out using the micropipette washing technique (Parvin et al., 2007); subsequently, the isolated diatoms were separately cultured in F/2 medium. Each isolation was identified by the size and morphological characteristics of their semi-elliptical and dorsiventral valves and dorsal margin curve using a light microscope (Wang et al., 2014; Khumaidi et al., 2020). Growth, total lipid content and fatty acid content were monitored during the cultivation (Arroussi et al., 2017).

Diatom culture conditions

Each of the isolated diatoms was grown in separate 1 L glass bottles containing 30 parts per trillion sterilized seawater enriched with F/2 medium at 25°C under 12 hr:12 hr of dark: light period (Supramaetakorn et al., 2019) using a light intensity of 19–35 µmol photons/m^2/s provided by a cool daylight LED. The ASC, ASS, APB and ALY samples were inoculated with mean (±SD) values of 79±36 cells/glass bottle, 79±27 cells/glass bottle, 95±23 cells/glass bottle and 111±36 cells/glass bottle, respectively. Ten acrylic plates (20 mm × 200 mm × 1 mm) were placed inside each glass bottle for diatom attachment. Two plates were collected on days 2, 6, 9 and 13 of culture.

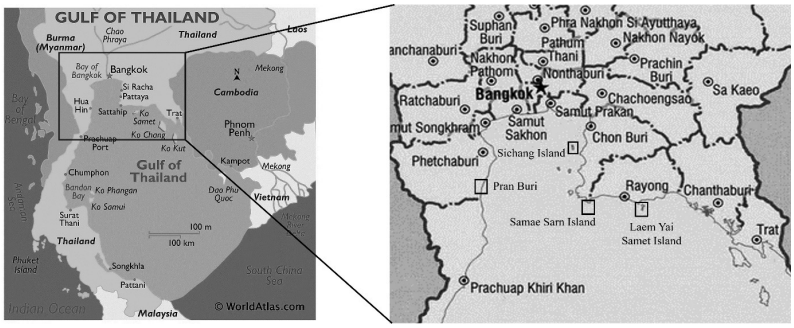

Fig. 1 Sampling locations of four *Amphora* sp. strains in Gulf of Thailand

Biomass determination

Diatom growth was determined based on *in vivo* chlorophyll analysis and a cell counting method (LeGresley and McDermott, 2010). *In vivo* chlorophyll analysis was conducted using a handheld fluorometer (AquaFluor® Handheld Fluorometer and Turbidimeter, 2023). Algal medium was used as a blank. Chlorophyll extracted from spinach was used as a standard for calibration. The chlorophyll content was calculated according to Equation 1 and was presented as relative fluorescence chlorophyll (AquaFluor® Handheld Fluorometer and Turbidimeter, 2023):

$$\textit{Relative fluorescence chlorophyll} = A - B \quad (1)$$

where A is the relative fluorescence chlorophyll measured from the sample and B is the relative fluorescence chlorophyll measured from the blank culture medium.

Cell counting was performed using a hemocytometer and an optical light microscope to investigate cell density (Cointet et al., 2019a; Supramaetakorn et al., 2019) on days 0, 2, 6, 9 and 13 of culture. Algal cells (measured as the number per square centimeter) were calculated using Equation 2:

$$\text{Algal cells / cm}^2 = \frac{(A)(B)}{C} \quad (2)$$

where A is the number of cells counted in 1 mL of medium (measured in cells per milliliter), B is the sampling volume (measure in milliliters) and C is the sampling area (measured in square centimeters).

The cell density was counted from days 2–13 and was used for calculation of the specific growth rate (SGR) according to Santos-Ballardo et al. (2015), Cointet et al. (2019a) and Supramaetakorn et al., 2019 as shown in Equation 3:

$$\mu = \frac{\ln X_t - \ln X_0}{t} \quad (3)$$

where μ is the SGR, X_0 is the initial biomass, X_t is the final biomass and t is the time (measured in days).

Determination of total lipid content

The two diatom strains with the highest biomass were selected for analysis to determine their total lipid contents and fatty acid compositions. These analyses were carried out on days 6, 9 and 13 during the stationary and death phases of growth.

Total lipid extraction was conducted based on two-step chloroform/methanol extraction, according to Li et al. (2015) and Nomaguchi et al. (2018), with some modifications. In the first step, 25 mg of each dried diatom (W) was mixed with 1.5 ml chloroform-to-methanol solution (2:1 volume per volume) and vortexed for 2 min. Next, the samples were stored at room temperature for 24 hr. Then, the mixtures were centrifuged at 12,000 revolutions per minute for 3 min. Finally, the supernatants were transferred into pre-weighed microcentrifuge tubes (W_1). For the second step, the residual dried algae were re-extracted with 1.5 mL of chloroform-to-methanol (2:1 volume per volume) following the method as described above. After the second step, the supernatant was combined with the preceding one and then dried in an oven at 70°C until a constant weight (W_2) was achieved. The total lipid content (measured in milligrams per 100 mg of algal dried weight of each sample was calculated gravimetrically using Equation 4:

$$\text{Total lipid content} = \frac{(W_2 - W_1) \times 100}{W} \quad (4)$$

where W is the dried algal weight, W_1 is the initial weight of the microcentrifuge tube and W_2 is the constant weight of the microcentrifuge tube after drying.

Determination of fatty acids composition (fatty acid methyl ester analysis)

The total lipid extracted from the diatom cells was dissolved in 0.5 mL of hexane (Chen, 2012) and *trans*-esterified by adding 1 mL of 0.4 M KOH-MeOH and incubated for 30 min at 25°C, according to Li et al. (2018) and Orefice et al. (2016). The fatty acid methyl ester (FAME) solution (5 µL) was injected into a gas chromatograph (GC; Shimadzu GC-2010 Plus; Shimadzu, Kyoto, Japan) for analysis.

The GC conditions consisted of a Framewax (Crossband® Polyethylene glycol; RESTEK; New Jersey, United States) column (30 m length, 0.32 mm internal diamter and 0.25 µm film thickness) coupled with a flame ion detector. The injector and detector temperature were set at 250°C. The column temperature was programmed with the temperature held at 150°C for 1 min, then increased to 190°C at 5°C/min and held for 5°C, followed by an increase to 240°C at 5°C/min and holding for 6 min. Helium was used as the carrier gas at a flow rate of 22.0 mL/min. Chromatogram peak areas were analyzed and quantified using the LabSolution software. (Arroussi et al., 2017; Cointet et al., 2019; de Jesús-Campos et al., 2020).

The FAME standards were: C14:0, C16:0, C16:1ω7, C18:1ω7, C18:1ω9, C18:26, C18:4ω3, C20:1ω9, C22:1ω11, C22:19, C20:5ω3, C22:5ω3, C22:6ω3 (Matreya LLC; Michigan, United States). The standard fatty acid mixture used was a natural, fish oil source. All experiments were carried out with three replications (Cointet et al., 2019b).

Statistical analysis

Biomass as represented by relative fluorescence, cell density, SGR, total lipid content and fatty acids were presented as mean ± SD values of three replicates.

The data were analyzed using one-way analysis of variance. Post hoc pairwise comparisons among diatom strains were conducted using Duncan's multiple range test. All tests were considered significant at $p < 0.05$. The SPSS Statistic22 software (IBM Corp, Armonk, NY, USA) was used for all analyses.

Results

Biomass

Chlorophyll

The three growth parameters differed among the four diatom strains investigated. The chlorophyll contents of ALY at day 6 (1,464±238) and ASC at day 6 and day 9 (539±120 and 898±400, respectively) were significantly higher than for APB and ASS throughout the experimental period with a range of 0.44–197. The chlorophyll peak of ALY was detected on day 6, while that of ASC was detected on day 9. Thereafter, their chlorophyll contents decreased and remained similar at the end of the experiment (day 13). The chlorophyll contents of APB and ASS slightly and constantly increased throughout the whole experiment but were not significantly different during this period, indicating that the highest point of their chlorophyll contents was probably beyond 13 days. These results indicated that ALY was the fastest strain to reach the highest maximum chlorophyll content followed by ASC, with both APB and ASS being lower (Fig. 2A).

Cell density

The differences in the cell densities of all four strains were first detectable at day 2 of the experiment. At day 6, the cell density of ALY was significantly higher than for the other strains ($3.75\times10^5 \pm 9.38\times10^4$ cells/cm^2). After that, it slowly declined until the end of the experiment. The cell density of ASC gradually increased during day 2 ($4.19\times10^3 \pm 7.28\times10^2$ cells/cm^2) – day 9 ($1.95\times10^5 \pm 2.41\times10^4$ cells/cm^2) of the experiment and significantly surged at the end of the experiment (day 13: $1.01\times10^6 \pm 1.03\times10^5$ cells/cm^2). The cell density of APB (day 2: $5.95\times10^3 \pm 2.18\times10^3$ – day 13: $1.51\times10^5 \pm 8.86\times10^4$ cells/cm^2) and ASS (day 2: $4.07\times10^3 \pm 1.32\times10^3$ – day13: $9.77\times10^4 \pm 4.01\times10^4$ cells/cm^2) slowly increased throughout the experiment period, as shown in Fig. 2B.

The cell density results revealed distinct growth patterns for each strain. ALY had an exponential phase from days 2 to 6, followed by an abrupt entry into the death phase. ASC and APB both initiated a slight exponential phase between days 2 to 6, persisting in exponential growth until the experiment's conclusion. Notably, ASC experienced a significant surge in exponential growth from days 6 to 13, while APB maintained a slight increase in the exponential phase. ASS entered the exponential phase between days 6 to 9, transitioning to a stationary phase that persisted until the end of experiment at day 13.

Specific growth rate

The SGR was calculated during days 2–6, 6–9 and 9–13 of the culture period using the cell density. During days 2–6, ALY and ASC had the maximum SGRs with 0.85±0.01 µ/d and 0.56±0.01 µ/d, respectively, while ASS and APB had their maximum SGRs during days 6–9 with 1.03±0.34 µ/d and 0.9±0.36 µ/d, respectively. ASS and APB had higher SGRs compared to ASC and ALY; however, ASS and APB spent a longer period to reach their maximum SGRs. (Fig. 2C).

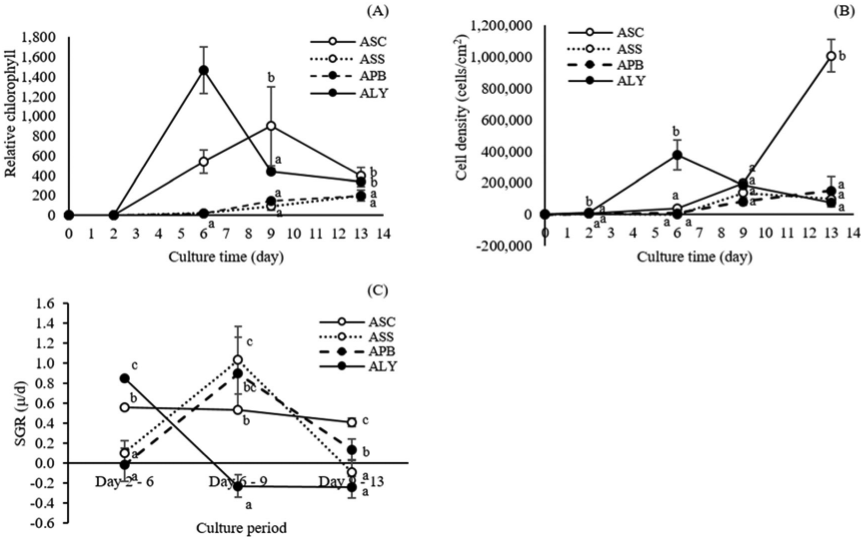

Fig. 2 Growth data for four diatom strains: (A) biomass; (B) relative fluorescence, with cell density determined on days 0, 2, 6, 9 and 13 of culture; (C) specific growth rate (SGR), determined during the intervals of days 2–6, 6–9 and 9–13 of culture. Data points (mean ± SD) with different lowercase letters are significantly ($p < 0.05$) different between *Amphora* sp. strains on each culture day.

Based on the cell density and SGR results, the ASC and ALY strains were chosen for further analysis, including total lipid contents and fatty acid composition.

Total lipid content

Diatom samples were collected during the stationary and death phases (days 6, 9 and 13). From day 6 to day 9, the total lipid content of both strains increased slightly; however, the total lipid content of ALY at day 6 (60.71±4.82 mg/100 mg algal dried weight) was significantly higher than that of ASC (43.67±5.03 mg/100 mg algal dried weight). On day 9, when ALY entered the death phase, while the ASC strain remained in the exponential phase, the total lipid content of ALY (74.34±4.26 mg/100 mg algal dried weight) was still significantly higher than for ASC (45.38±7.25 mg/100 mg algal dried weight). On the last day of the culture period (day13), the total lipid contents of both strains were similar (ASC: 36.24±2.20 mg/100 mg algal dried weight and ALY: 31.71±8.15 mg/100 mg algal dried weight) and lower than for the previous period (Fig. 3A).

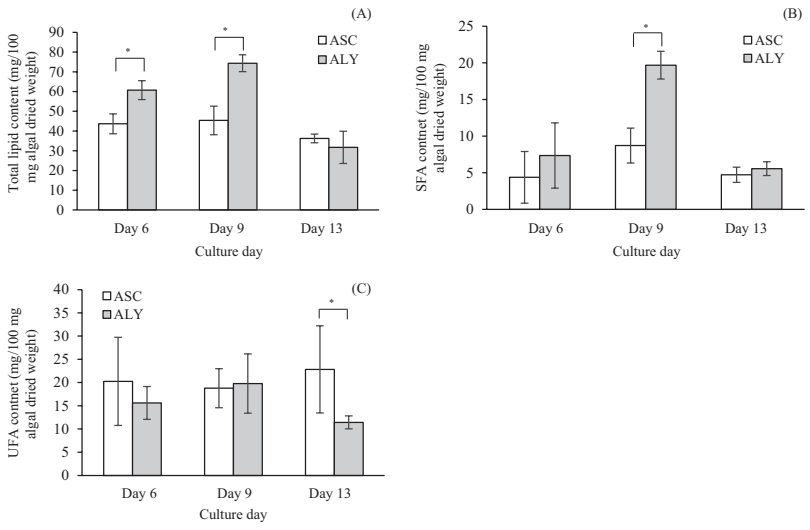

Fig. 3 Lipid and fatty acid contents of two diatom strains determined on days 6, 9 and 13 of culture: (A) total lipid content; (B) saturated fatty acid (SFA) content; (C) unsaturated fatty acid (UFA), where values (mean ± SD) with asterisk are significantly ($p < 0.05$) different between *Amphora* sp. strains on each culture day

Fatty acid composition

The total amounts of SFAs in both the ASC and ALY strains showed similar patterns, with the SFAs increasing from day 6 to day 9 and declining thereafter (Fig. 3B) while the total amounts of UFAs showed different patterns of alteration. The UFAs of the ASC strain remained at similar levels from day 6 to day 9 to day, 13 while the UFAs of the ALY strain increased from day 6 to day 9 and then declined from day 9 to day 13 (Fig. 3C).

The results showed that main SFAs detected from the collected samples were myristic acid (C14:0) and palmitic acid (C16:0). The main UFAs detected from the collected samples were palmitoleic acid (C16:1ω7), arachidic acid (C20:1ω9), cis-11-docosenoic acid (C22:1ω11), eicosapentaenoic acid EPA (C20:5ω3), docosapentaenoic acid DPA (C22:5ω3) and docoxahexaenoic acid DHA (C22:6ω3), as shown in Table 1.

Discussion

Biomass and growth of Amphora sp. strains

The average cell density and generation time obtained from this study were in close proximity to the results of other reports (Romero-Romero and Sánchez-Saavedra, 2017; Khwancharoen et al., 2020; Rani et al., 2022). Therefore, the culture conditions in the current study were acceptable.

The results for the cell density and the chlorophyll content clearly showed that ALY (*Amphora* sp. isolated from Laem Yai), achieved the best biomass production performance, followed by ASC (*Amphora* sp. isolated from Sichang Island), ASS (*Amphora* sp. isolated from Samaesarn Island) and APB (*Amphora* sp. isolated from Pranburi), respectively.

ALY reached the maximum cell density within 6 d when its cell density was 10 times higher than for ASC and about 51 and 57 times higher than for ASS and APB, respectively. However, the cell density of ALY dropped quickly to the death phase within 9 days, while that of ASC increased with culture time. Therefore, the cell density of ASC at the end of the experiment was significantly higher than that of ALY. On the other hand, it can be inferred that ASC required a longer period (13 d) of culture to achieve the same amount of biomass as the ALY strain (6 d). For this reason, ALY could be considered as the best productivity strain due to it requiring only 6 days of culture to reach the maximum growth rate, which was the fastest growth strain compared to the other three strains.

In addition, the chlorophyll content of ALY peaked at day 6 of culture and was significantly higher than for the other three strains, with ALY being twice as high as ASC and 10 folds and 7 folds higher than APB and ASS, respectively. Considering the maximum amounts of chlorophyll content and cell density, the peaks of the chlorophyll levels and cell density occurred in the same period for ALY, ASS and APB. These results indicated that the biomass of these three *Amphora* sp. strains correlated with their chlorophyll contents. On the other hand, the chlorophyll content of ASC reached the maximum level within 9 days but decreased notably, while its cell density remained unchanged (day 13). This was probably due to the longer period of culture causing the depletion of some essential nutrients which can be commonly found in various species of

Table 1 Fatty acid composition determined for two strains of *Amphora* sp. at different periods of culture

Fatty acid	ASC			ALY		
	Day6	Day9	Day13	Day6	Day9	Day13
Saturated fatty acids						
Myristic acid (C14:0)	1.10±0.84^{a}	4.07±1.10^{a}	2.77±1.07^{b}	1.36±1.21^{a}	10.58±0.94^{c}	3.15±0.27^{b}
Palmitic acid (C16:0)	3.28±4.18^{a}	4.64±1.30^{ab}	1.94±0.95^{a}	5.99±4.42^{ab}	9.11±2.44^{b}	2.40±1.11^{a}
Unsaturated fatty acids						
Palmitoleic acid (C16:1ω7)	0.30±0.36^{a}	7.63±1.19^{b}	7.74±3.26^{b}	1.83±2.26^{ab}	6.03±6.29^{ab}	5.38±0.70^{ab}
Oleic acid (C18:1ω9)	1.25±0.54^{ab}	1.24±0.59^{ab}	1.52±0.80^{ab}	2.49±1.48^{b}	1.90±0.60^{ab}	0.52±0.36^{a}
Oleic acid (C18:1ω7)	0.05±0.09^{a}	0.90±0.16^{a}	0.08±0.13^{a}	0.07±0.12^{a}	0.15±0.25^{a}	0.08±0.14^{a}
Arachidic acid (C20:1ω9)	1.23±0.60^{ab}	2.21±0.67^{bc}	2.86±0.99^{bc}	2.05±1.22^{abc}	3.86±1.96^{bc}	0.03±0.05^{a}
Erucic acid (C22:1ω9)	0.22±0.38^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
cis-11-Docosenoic acid (C22:1ω11)	4.65±6.30^{a}	0.56±0.18^{a}	0.35±0.12^{a}	3.75±6.22^{a}	1.19±0.51^{a}	1.23±0.67^{a}
Linoleic acid (C18:2ω6)	0.89±0.38^{ab}	0.46±0.05^{ab}	0.74±0.15^{ab}	0.32±0.20^{a}	0.58±0.26^{ab}	1.02±0.67^{b}
Stearidonic acid (C18:4ω3)	0.18±0.18^{a}	0.96±1.67^{a}	2.98±1.15^{a}	0.19±0.17^{a}	0.00±0.00^{a}	0.01±0.02^{a}
Eicosapentaenoic acid EPA (C20:5ω3)	0.88±0.78^{a}	1.24±1.08^{a}	1.12±0.97^{a}	0.95±0.82^{a}	2.14±1.85^{a}	1.00±0.93^{a}
Docosapentaenoic acid DPA (C22:5ω3)	2.11±1.73^{a}	3.08±2.65^{a}	2.37±1.91^{a}	1.89±1.51^{a}	4.99±4.12^{a}	1.81±1.43^{a}
Docoxahexaenoic acid DHA (C22:6ω3)	11.85±9.77^{a}	18.42±17.60^{a}	13.43±10.32^{a}	10.74±7.86^{a}	29.08±25.24^{a}	10.86±8.27^{a}

Mean ± SD in each row superscripted with different lowercase letters are significantly ($p < 0.05$) different between culture days for each fatty acid.

microalgae cultured in controlled condition. This would also explain why there were differences in the cell density and chlorophyll contents between ALY and ASC, with the former showing a decline in both the cell density and chlorophyll contents, while the latter had a decline only in the chlorophyll contents.

From the literature, chlorophyll concentrations often dropped from the exponential to the stationary growth phase in microalgal culture after long culture, causing nutrient starvation (Ruivo et al., 2011; Lim et al., 2017; Young et al., 2022). Abiotic stress factors, such as temperature, CO_2, light and salinity can significantly affect the biochemical composition of algal cells, including the protein concentration, lipid production and photosynthetic components (Chen et al., 2011; He et al., 2015; da Silva Ferreira, 2017).

Under these circumstances, microalgae stop dividing but are still able to perform photosynthesis and the accumulation of triacylglycerides, which is considered a survival strategy to endure adverse conditions (Schenk et al., 2008; Breuer et al., 2012; Liu and Benning, 2013).

The results herein indicated that ASC had the longest stationary phase (>11 d), which meant it could be cultured for a longer period and its biochemical contents (in this study, chlorophyll) could be processed with no significant impact on its growth rate for such a period. The ability of the long-term culture to tolerate nutrient exhaustion and the ability to adjust its biochemical composition with no obvious effect on growth are considered desirable characteristics for potential commercial microalgal biomass production (Bumbak et al., 2011; Mohammad Mirzaie et al., 2016). However, the downside to ASC from this viewpoint is that it is a slow-growing algae compared to ALY.

Total lipid content

It was clear that the maximum biomass levels reached by ALY and ASC were significantly higher than for the other *Amphora* sp. strains in this study. Therefore, the determination of lipid composition focused only on these two strains.

The total lipid contents in ALY at day 6 and day 9 of culture were significantly higher (1.6 folds) than those in ASC; however, the levels of lipids in ASC remained the same throughout the experiment. The specific increase in the lipid content observed in ALY might have resulted from the response to nutrient depletion. There are increasing numbers of studies confirming that a lipid increase in microalgae during culture depended on the depletion or removal of nutrients (Yu et al., 2009; d'Ippolito et al., 2015; Cointet et al., 2019b). Several of studies have indicated that various diatoms were able to produce lipid amounting to up to 40–60% of their dry biomass (Chen, 2012; Sayanova et al., 2017; Xue et al., 2017; Zulu et al., 2018).

In the present study, the total lipid content in ALY peaked at greater than 70% of the dried weight which was the highest amount found in diatom documentation, while that of ASC was about 45% of the dried weight, which was close to the results from other studies (44.33 % in *Chaetoceros sp.* (Saxena et al., 2021), 29.66 % in *Thalassiosira sp*. (Saxena et al., 2021), 57.6 % in *C. gracilis* (Saxena et al., 2022) and 50.4 % in *T. weissflogii* (Saxena et al., 2022)).

ALY and ASC possessed different characteristics of lipid production, with ALY requiring 6 d to produce its maximum total lipid content, while ASC needed 9 d of culture. Thus, ALY would be the best candidate for lipid production based on its higher yield and harvesting batches. Additionally, ALY showed greater potential for lipid production compared to other diatoms in other reports, such as *Chaetoceros* sp., *Chaetoceros gracilis*, *Thalassiosira* sp. and *Thalassiosira weissflogii* (Saxena et al., 2021). In contrast, ASC appeared to be inferior in lipid production but could produce lipid consistently during long and stressful conditions, such as nutrient deprivation, suggesting ASC as an optional candidate for culturing over an extended culture period or for specific purpose biomass production.

Fatty acid composition

The major UFAs found in this study were n-3 PUFAs—DHA, DPA, EPA and stearidonic acid (C18:4ω3)—while the minor UFA was linoleic acid (LA). The level of UFAs was higher than for SFAs in both strains and the same proportion of these two was evident throughout the experiment. Both the UFAs and SFAs in ALY increased at the beginning and dropped at the end of the experiment, while those of ASC remained constant throughout the experiment. The fatty acid profiles in ALY and ASC were similar to the results from other studies on diatoms. Compared to other diatoms, the DHA contents of ASC and ALY were superior to those reported for *Amphora* sp. and most other diatoms, while the EPA contents of both ALY and ASC were inferior to those of other diatoms (Table S1).

The high DHA content in ASC could be useful for DHA enrichment in live feed, such as copepods (Reitan et al., 1997; McKinnon et al., 2003) or as an alternative source of DHA to some fish oils that contain high DHA content, such as tuna oil (Sprague et al., 2015; Fard et al., 2020). Omega 3 (ω-3) and

omega 6 (ω-6) fatty acids are PUFAs that are essential for the human body and animal to function properly, establishing normal growth and development through all stages of life (González-Félix et al., 2002; Turchini et al., 2010; Araújo et al., 2019; DurmuŞ, 2019; An et al., 2020; Zhang et al., 2022; Zhu et al., 2023).

Compared to other microalgae, the ω-3 levels in both ALY and ASC were higher than the reported in other *Amphora* sp. strains but still lower than in some other diatoms (*Chaetoceros* sp. and *Thalassiosira* sp.). In contrast, the ω-6 content of both strains was much lower than for other *Amphora* sp. strains. but clearly surpassed the other diatoms (Table S2).

The activities of ω-3 and ω-6 carry out opposite physiological functions; therefore, an imbalance in the amounts and ratio of these fatty acids can cause detrimental health effects. For this reason, adequate amounts and the optimal ratio of these fatty acids in the diet are crucial.

In the current study, the ω-6-to-ω-3 ratios of ALY and ASC were 0.18 and 0.15, respectively, which were in a similar range for this ratio commonly found in most microalgal and marine organisms. These ratios are considered as ideal indicators of quality for lipid ingredients in the aquaculture feed industry, as such low ω-6-to-ω-3 ratios can be easily optimized into feed formulations, which is desirable for fish meal or fish oil replacement.

In marine animals, the ω-6-to-ω-3 ratio varies vastly, from 0.02 in European squid to 0.48 in striped piggy (Özogul et al., 2009). In microalgae, the ratio varies from 0.04 in Dinophyta to 0.40 in Cyanobacteria, while the average ω-6-to-ω-3 ratio of diatoms was reported as 0.06 (Jónasdóttir, 2019). In the current study, the ω-6-to-ω-3 ratios for ALY and ASC were 0.18 and 0.15, respectively, which were within the range for this ratio commonly found in most diatoms (or microalgae) and marine organisms.

According to their fatty acid profiles, ALY and ASC would be considered as n-3 PUFA-producing diatoms. Based on their growth performance levels, ALY and ASC had the highest biomass production compared to the other two strains. ALY should be considered the best candidate for biomass production over a shorter period, while ASC requires a longer time but is more tolerant to nutrient depletion than the other strains. In summary, this recent study provides basic knowledge to help guide biomass production of *Amphora* sp. Strains isolated from the Gulf of Thailand and cultured indoors. Two potential strains (ALY and ASC) were analyzed for their lipid contents and fatty acid profiles, which revealed that these diatoms produced high levels of n-3 PUFAs, suggesting that the ALY and ASC *Amphora* sp. strains could be considered for further application.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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