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Squalene-producing thraustochytrids isolated from mangrove habitats in the Sidoarjo District, Indonesia, and the antioxidant activity of the isolated strain

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Abstract. *Arifin NB, Fakhri M, Yuniarti A, Musa M, Hariati AM. 2025. Squalene-producing thraustochytrids isolated from mangrove habitats in the Sidoarjo District, Indonesia, and the antioxidant activity of the isolated strain. Biodiversitas 26: 1975-1982.* Squalene, a polyunsaturated triterpenic hydrocarbon, is commonly used in the pharmaceutical, cosmetic, and nutraceutical industries because of its antioxidant and anti-inflammatory properties; however, traditional sources such as deep-sea shark liver oil and plant-based alternatives such as the olive *Olea europaea* present sustainability challenges. This study aimed to isolate and characterize squalene-producing thraustochytrids from mangrove habitats in Sidoarjo, Indonesia, and evaluate their potential antioxidant activity. Isolation was performed from fallen mangrove leaves and was identified based on morphological and molecular analysis using 18S rRNA sequencing. The lipid and squalene contents were quantified through solvent extraction and high-performance liquid chromatography, whereas the antioxidant activity was assessed using the DPPH radical scavenging assay. A total of 14 strains were successfully isolated, which showed cell morphologies including circular and/or ameboid-like shapes and the presence of tetrad cell division. Molecular analysis showed that all isolates were close to the genus *Aurantiochytrium.* Seven of the isolated strains exhibited high lipid contents (>35% of dry cell weight). Among these strains, AB12 produced the highest lipid content (54.77%), whereas AB14 exhibited the highest squalene yield (2.18 mg g^{-1} dry cell weight). Antioxidant analysis revealed that lipid extracts from AB12 displayed significant radical scavenging activity, outperforming pure squalene, thereby indicating potential synergistic effects with other bioactive compounds. These Indonesian mangrove-derived isolates represent a promising alternative for sustainable squalene production, addressing the limitations of conventional sources. This study provides novel insights into squalene-producing *Aurantiochytrium* strains from Indonesian mangrove forests.

Keywords: Antioxidant activity, *Aurantiochytrium*, mangrove, squalene, thraustochytrids

INTRODUCTION

Thraustochytrids, marine unicellular protists, have recently gained attention owing to their ability to produce essential nutrients for higher marine and terrestrial organisms (Tran et al. 2020; Pawar et al. 2021). Several of these are recognized as polyunsaturated fatty acids, eicosapentaenoic acid, docosahexaenoic acid, docosapentaenoic acid, and squalene producers (Otagiri et al. 2017). The genera *Ulkenia, Thraustochytrium*, *Schizochytrium*, and *Aurantiochytrium* are the most well-studied among the nine genera belonging to Thraustochytrids (Tran et al. 2020). The genus *Aurantiochytrium* has been known as a squalene-producing thraustochytrid because it yields squalene during lipid metabolism (Kaya et al. 2011).

Squalene is a triterpenic hydrocarbon with six double bonds that has various biological functions (Du et al. 2024). Several biological properties of squalene include immune system enhancement (Sánchez-Quesada et al. 2018), increasing reproductive performance (Xu et al. 2022), antibacterial activity (Bhat et al. 2023), and anti-inflammatory and antioxidant (Zhang et al. 2023a). Squalene's antioxidant ability is related to its ability to protect cells from free radicals and reactive oxygen species. (Kaya et al. 2011). Owing to its multiple uses in the pharmaceutical industry, the squalene market was worth USD 149.4 million in 2023 and is projected to increase approximately 10% annually from 2024 to 2030 (www.grandviewresearch.com).

Generally, squalene is produced by both plants (Zhang et al. 2019) and animals (Flieger et al. 2021). The liver of deep-sea sharks is a major source of squalene, resulting in approximately 600 g kg^{-1} on average. However, squalene production resulting from shark liver oil extraction is unsustainable owing to the declining deep-water shark population (Zhang et al. 2021; Finucci et al. 2024). In contrast, the production of plant-based squalene, such as olive *Olea europaea* and amaranth *Amaranthus cruentus* oils, is limited because of environmental factors and land use (Patel et al. 2020; Sayed-Ahmad et al. 2022). Therefore, squalene production from thraustochytrids, particularly from the genus *Aurantiochytrium,* is promising.

The investigation of squalene-producing thraustochytrids has been reported in several studies with considerable results (Nakazawa et al. 2014; Aasen et al. 2016). For example, a strain of *Aurantiochytrium* sp. 18W-13a obtained from a mangrove area in Okinawa Prefecture, Japan, showed a high squalene content (198 mg g^{-1} of Dry Cell Weight (DCW)) (Kaya et al. 2011). Meanwhile, Otagiri et al. (2017) reported that 132 *Aurantiochytrium* isolates producing more than 0.1 mg g^{-1} were obtained from the same mangrove area, with the highest-producing one reaching 13.9 mg g^{-1} DCW. Furthermore, similar squalene content (up to 13 mg g^{-1} DCW) has resulted from the newly isolated *Aurantiochytrium* in Victoria, Australia (Tran et al. 2020). Mostly, squalene-producing thraustochytrids are isolated from mangrove habitats (Krzynowek 2021) because they have high organic matter from the litter of mangrove trees (Kaliyamoorthy et al. 2025). Moreover, under fluctuating environmental conditions such as salinity and temperature, thraustochytrids found in mangrove habitats produce antioxidants such as squalene as an adaptive response to environmental stress (Zhang et al. 2022; Zhang et al. 2023b; Schütte et al. 2024). However, few studies have investigated squalene-producing thraustochytrids in Indonesia (Suhendra et al. 2023).

Indonesia, with its extensive mangrove ecosystems spanning approximately 3.31 million hectares, represents one of the largest and most diverse mangrove habitats globally (Richards and Friess 2016; Arifanti et al. 2022). Despite this remarkable biodiversity potential, Indonesian mangrove forests remain relatively unexplored as sources of thraustochytrids and their bioactive compounds. The Sidoarjo District in East Java, Indonesia, features distinctive mangrove ecosystems influenced by natural environmental factors and anthropogenic impacts, including the mud volcano eruption (known as the Lapindo mudflow), which began in 2006 (Maryantika and Lin 2017). The mangrove ecosystems in this area may have high environmental pressures because of the direct discharge of mud into the river, which flows to the mangrove (Maryantika and Lin 2017). As a result of high environmental pressure, there may be thraustochytrid strains with novel metabolic capabilities from mangroves in the Sidoarjo District. The increasing environmental stress could enhance squalene production and other antioxidant compounds (Zhang et al. 2022; Zhang et al. 2023b; Schütte et al. 2024). The present study aimed to isolate and characterize squalene-producing thraustochytrids from mangrove habitats in the Sidoarjo District, Indonesia, and to evaluate the antioxidant activity of selected strains.

MATERIALS AND METHODS

Sample collection and isolation of thraustochytrids

Fallen mangrove leaves were collected from the Sidoarjo District (latitude: −7.527020; longitude: 112.859131), East Java, Indonesia. The leaf samples were then placed into a sterile plastic bag and kept in a cool box until they were processed at the laboratory. Sterile Natural Seawater (NSW) containing antibiotics (penicillin G and streptomycin sulfate: 500 mg L^{-1} of each, Himedia) was used to wash the leaf samples (Unagul et al. 2017). The leaf samples were then cut into 1 × 1 cm^2 and either plated on agar or liquid medium containing 5 g L^{-1} glucose (Himedia), 1 g L^{-1} mycological peptone (Himedia), 1 g L^{-1} yeast extract (Himedia), antibiotics (penicillin G and streptomycin sulfate: 300 mg L^{-1} of each, nystatin: 10 mg L^{-1}, Himedia), diluted in half-strength NSW (1:1 NSW/distilled water). For the liquid medium containing the leaf samples, the liquid was plated on new agar after 48 h of incubation at room temperature, followed by plating on agar.

Morphological identification

The colonies that grew on the plate were observed morphologically as described in (Yokoyama and Honda 2007). The colonies identified as thraustochytrids streaked repeatedly until the axenic one was obtained. The isolates were then observed under an inverted microscope (Olympus IX53) to determine cell characteristics (Yokoyama and Honda 2007). Strains showing thraustochytrid-like features microscopically were further identified molecularly.

Molecular identification

DNA was extracted from 48-h incubated cultures using a DNA extraction kit (Promega, USA). The universal primers 18S Univ F-5′ -TGGTTGATCCTGCCAGT-3′ and 18S Univ R-5′-TAATGATCCTTCCGCAGGTTCACCT-3′ were used to amplify the 18S rRNA gene fragment (Jaseera et al. 2019). Amplification was performed using Polymerase Chain Reaction (PCR) (Biorad, USA). PCR reaction of 30 µL consisting of 15 µL master mix (Gotaq green, Promega, USA), 11 µL ddH_2O, 1 µL of each forward and reverse primer was set to amplify 2 µL DNA template. Amplification was performed using the following setup: initial denaturation at 95°C for 3 min, denaturation at 94°C for 45 s, annealing at 53°C for 30 s for 35 cycles, extension at 72°C for 2 s, and final extension at 72°C for 10 min. The PCR product was visualized on the agarose gel via electrophoresis at approximately 1800 bp; moreover, it was then sequenced (18S Univ F and 18S Univ R). BLAST analysis was performed to identify the 18S rRNA gene sequence. For phylogenetic analysis, alignment using MEGA11 software was conducted to compare the results with those of related species from the NCBI GenBank database (Jaseera et al. 2019).

Growth, biomass, lipid, and squalene determination

Strains identified molecularly were then screened based on lipid content. The strain with high-lipid content (>35% Dry Cell Weight (DCW)) was further examined for squalene production. Strains were grown in a medium modified from Nakazawa et al. (2014) containing half-strength NSW, 20 g L^{-1} glucose, 1 g L^{-1} mycological peptone, 1 g L^{-1} yeast extract, and antibiotics (penicillin G and streptomycin sulfate: 300 mg L^{-1} of each, nystatin: 10 mg L^{-1}). The half-strength NSW and 20 g L^{-1} glucose were optimum for squalene production of the thraustochytrid *Aurantiochytrium* sp. strain 18W-13a (Nakazawa et al. 2014). The medium was then inoculated with 0.1% of the fast-growing inoculum and incubated for 72 h in an incubator shaker (215 rpm) at room temperature. The optical density of the culture was measured using a spectrophotometer (Thermo Scientific GENESYS 20, USA) at 660 nm to monitor the growth of the referral strains (AB12 and AB14) during the culture period (Jaseera et al. 2019). After 72 h of incubation, the cultures were centrifuged and freeze-dried. The biomass production, lipid content, and squalene content were evaluated as described by Yang et al. (2022). The biomass was examined gravimetrically, and the lipid and squalene contents were extracted using solvent-based methods. The freeze-dry cell (100 mg) was immersed in 16 mL of chloroform/methanol (2:1) in a clean glass test tube. The samples were then homogenized in an ultrasonic cleaner (Branson, USA) for 5 min at room temperature and incubated overnight. Subsequently, filtration using No. 2 filter paper (Advantec Toyo, Tokyo, Japan) was used, and the filtrate was added to 4 mL of a 0.9% (w/v) NaCl solution. The organic phase in the lower layer was transferred into a vial and evaporated using nitrogen gas. The vial containing the lipids was weighed, and the lipid content was calculated. For squalene determination, a vial containing lipid was mixed with 1.5 mL of hexane. The solution was further diluted with ethanol a hundred times. After that, the next ten times dilution was also performed using acetonitrile before it was injected into a High-Performance Liquid Chromatography (HPLC) (UFLC Shimadzu). The system was equipped with a C_{18} column (SHIMPACK VP-ODS C-18 250 × 4.6 mm, Shimadzu) and a UV/Vis detector. A 100% acetonitrile was used as the mobile phase at a flow rate of 1.0 mL/minute for 8 minutes, running under isocratic conditions. A 20 microliter of sample was injected, and the temperature column was set at 40°C. Authentic and extracted squalene were detected at 195nm. The authentic squalene (Wako Chemical, Japan) was used for constructing a squalene standard curve. To confirm the squalene content, Gas Chromatography-Mass Spectrophotometry (GC-MS) (GCMS-QP2020NX, Shimadzu) was also performed on a selected strain extract.

Determination of antioxidant activity

The antioxidant activity was examined in terms of Radical Scavenging Activity (RSA) (Zhang et al. 2023a). The antioxidant activity of the selected strain was evaluated. The lipid extract from the selected strain was tested for its scavenging ability against 2,2-diphenyl-picrylhydrazyl (DPPH). Different concentrations of the lipid extract (100, 200, 300, 400, 500, 1000, 1500, and 2000 ppm) diluted in 4 mL of methanol were added to 1 mL of a methanol solution of DPPH (0.4 mM). The solution was then homogenized and incubated for 30 min in the dark at room temperature. The result was measured at an absorbance of 517 nm. The RSA calculation was expressed as percentages of DPPH discoloration using the following equation (Zhang et al. 2023a):

$$\text{DPPH radical scavenging (\%)} = \frac{(\text{A}_{\text{control}} - \text{A}_{\text{sample}})}{\text{A}_{\text{control}}} \times 100$$

Where :

$\text{A}_{\text{control}}$: Absorbance of the DPPH solution alone

A_{sample} : Absorbance of the samples added to the DPPH solution.

All analyses were conducted in duplicate.

Data analysis

IBM SPSS Statistics version 26 was used to perform the statistical analyses. The biomass, lipid, and squalene contents were analyzed using One-Way ANOVA ($\alpha<0.05$ of confidence level). The means±standard deviation were used to express all data.

RESULTS AND DISCUSSION

Isolation and identification

In the present study, we isolated thraustochytrids from a mangrove habitat near the estuary of the Porong River, East Java, Indonesia, approximately 19 km east of the Sidoarjo mud flow, East Java, Indonesia. We found that bacteria and fungi still grew on the plate, although a broad spectrum of antibiotics was supplemented in the media (Figure 1.A), which is similar to the findings by Jaseera et al. (2019) and Pawar et al. (2021). Bacterial and fungal contamination indicated that they are resistant to antibiotics (Jaseera et al. 2019). Contamination is the major bottleneck in thraustochytrids isolation (Pawar et al. 2021). A total of 14 colonies were obtained in the present study. The colony's morphology, including its circular form, white, cream, and orange color, and raised elevation, was similar to that reported by Unagul et al. (2017) and Malawet et al. (2020) (Figures 1.B and 1.C). Moreover, microscopic observation revealed several characteristics of the cell, including 5-34 μm in diameter, circular and/or ameboid-like shape (Figures 1.D-1.F), and the presence of tetrad cell division (Figure 1.F). The colonies and cell characteristics indicated that all the isolated strains were close to the genus *Aurantiochytrium*, as described in (Yokoyama and Honda 2007); therefore, molecular analysis using 18S rRNA was performed to confirm all the isolated strains.

Molecular analysis showed that all isolates were close to the genus *Aurantiochytrium* (Figure 2). BLAST analysis revealed that all isolates were similar to this genus, as reported by Jaseera et al. (2019). The phylogenetic tree constructed here has a typology similar to that of Jaseera et al. (2019). The isolated strains formed clusters with other *Aurantiochytrium* species retrieved from GenBank; this cluster was separated from other genera in the Thraustochytriaceae family, for example, *Thraustochytrium*, *Schizochytrium*, and *Ulkena*.

The close relationship between the isolated strains and *Aurantiochytrium* sp. CMFRIMBTDJMVL1 (Jaseera et al. 2019) is presented in the constructed phylogenetic tree; the similarity between *Aurantiochytrium* sp. CMFRIMBTDJMVL1 and the isolated strain were 90%. It indicates that there may be a novel strain. We named our isolates *Aurantiochytrium* sp. AB01 to AB14. *Aurantiochytrium* is the most predominant genus in mangrove habitats (Otagiri et al. 2017; Jaseera et al. 2019). Moreover, the genetic relationships of the isolated strains with squalene-producing *Aurantiochytrium* species, such as *A. limacinum* (Patel et al. 2020) indicated that they are squalene producers. Thus, the screening of isolated strains may provide novel candidates for squalene production.

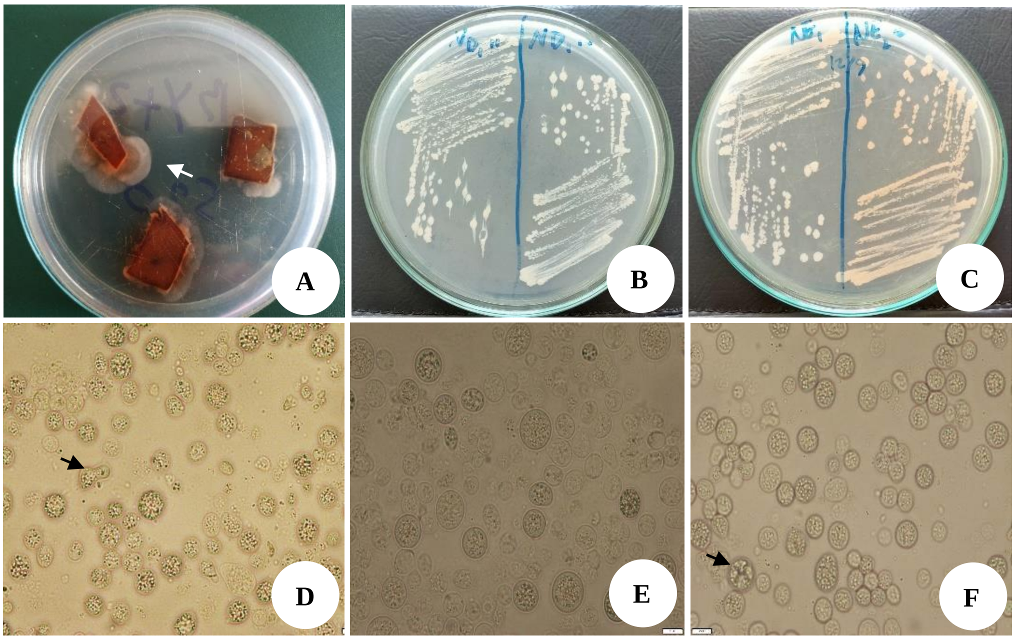

Figure 1. Colonies and cells of isolated strains. A. Other microbial contamination (arrow); B. White colonies; C. Cream and orange colonies; D. Cells of white colonies (arrow indicates ameboid-like shape); E. Cells of cream colonies; F. Cells of orange colonies (arrow indicates tetrad cell division) (scale bar: 20µm)

Growth, biomass, and lipid and squalene content

The growth of the referral strains AB12 (white colonies) and AB14 (orange colonies) showed similar patterns (Figure 3). The lag phase of both strains was observed before the 12-h cultivation period. Similarly, Jaseera et al. (2019) reported that the lag phase of the *Aurantiochytrium* strains JMVL1, JMVL3, and JMVL4 was observed at 1-3 hours of incubation. However, the lag phase may be undetected under optimal culture conditions (Perveen et al. 2006). Following the lag phase, strains AB12 and AB14 underwent exponential growth and reached maximum growth at approximately 48 h of culture. The period to reach maximum growth may vary from approximately 33 h (Jaseera et al. 2019) to 2 days of culture (Perveen et al. 2006). In the present study, the maximum growth of strain AB12 was higher than that of strain AB14; therefore, AB12 produced a higher biomass than AB14 (Table 1). The higher biomass production of strain AB12 demonstrates its resilience under present culture conditions. Because the strain has not yet been optimized, environmental conditions such as temperature and salinity may affect its growth. The temperature and salinity in the present study were 27.5 ± 2°C and 16.2 ± 1 ppt, respectively. Low temperature can increase Polyunsaturated Fatty Acid (PUFA) production, although it may also reduce growth by downregulating energy metabolism pathways (Song et al. 2022). Moreover, certain strains demonstrate resilience to varying salinity levels; however, excessive salinity can reduce biomass during the exponential growth phase (Moon et al. 2019). On the contrary, the highest biomass and lipid content of *A. limanicum* were produced in 100% saline water (Pawar et al. 2021); meanwhile, the addition of sea salt 2% (w/v) increased the lipid content of *Thraustochytrium* sp. BM2 (Chen et al. 2020). The results of the present study, along with those of the previous one, indicated that each strain had a different optimum culture condition.

Thraustochytrids are oleaginous and fast-growing microorganisms (Jaseera et al. 2019; Morabito et al. 2019). The lipid content of thraustochytrids can reach up to 55% DCW (Sun et al. 2021). The present study found that 7 of 14 strains possessed a significant lipid content (>35% DCW) (Table 1). These seven high-lipid-content strains produced biomass between 3.3 and 7.23 g L^{-1}, with the strain of AB10 being the highest biomass producer. The AB12 strain had the highest lipid content (54.77% DCW) among the isolated strains. This result was comparable to that reported by Gao et al. (2013), in which the lipid content of *Aurantiochytrium* isolates was up to 49.3% DCW. However, a higher lipid content (58% DCW) of *A. limacinum* was obtained under the optimized culture conditions (Pawar et al. 2021).

The biomass obtained in the present study was similar to that reported by Unagul et al. (2017) and Otagiri et al. (2017), who obtained biomasses varying from 0.3 to 8.9 g L^{-1} and 2.36 to 7.40 g L^{-1}, respectively. However, a higher biomass was produced by several thraustochytrid strains isolated from some countries, for example, Thailand (up to 17.4 g L^{-1} of biomass) (Malawet et al. 2020), India (up to 14 g L^{-1} of biomass) (Jaseera et al. 2019; Pawar et al. 2021), China (9.47 g/L of biomass) (Zhang et al. 2019), and Australia (up to 18.1 g/L of biomass) (Tran et al. 2020). Strain-specific might be the possible explanation for the varying biomass and lipid content obtained in the present study. In addition to being strain-specific, the biomass and lipid content of thraustochytrids could be due to nutrient availability (Anwar et al. 2024).

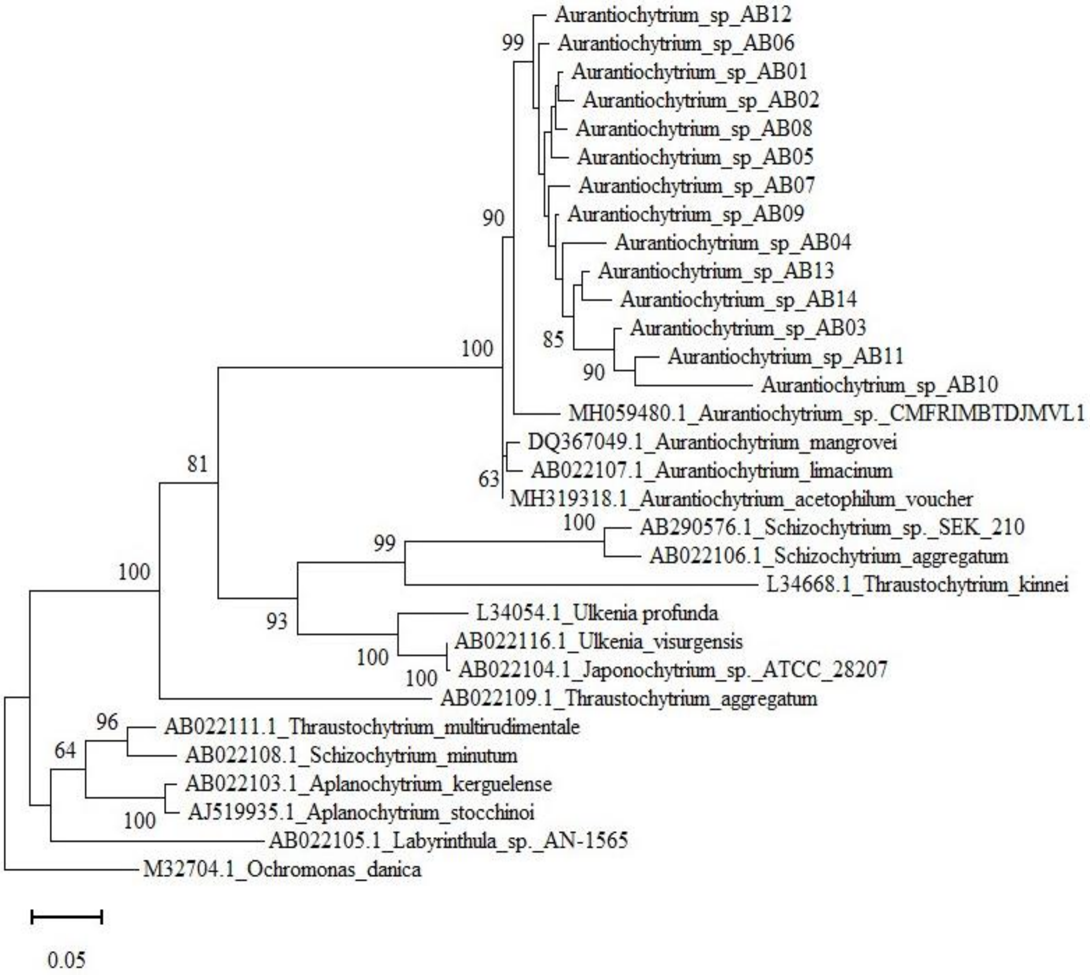

Figure 2. Molecular analysis of isolated strains using the 18S rRNA gene. Several genera belonging to the Thraustochytriaceae family were used as phylogenetic trees. *Ochromonas danica*, a diatom species, was used as an outgroup

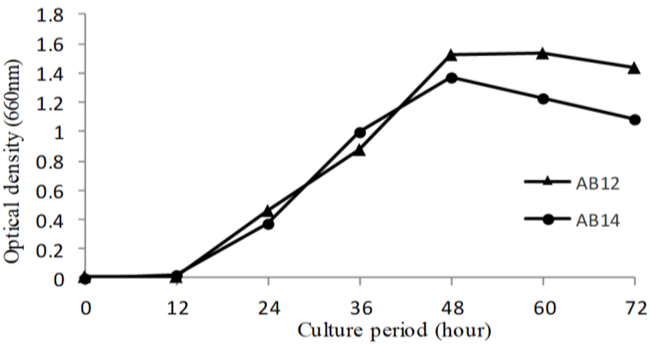

Figure 3. Growth patterns of AB12 and AB14 strains during the 72-h culture period

Tran et al. (2020) demonstrated that increasing the carbon sources (glucose, fructose, sucrose, and glycerol) from 0.5% to 3% enhanced the biomass and lipid content up to 4.5-fold and 40% DCW, respectively. In the present study, glucose was used as a carbon source at 2%. The C/N ratio of the culture medium influenced the lipid content of the microorganisms; enhancing the C/N ratio increased the lipid content of the cells (Gao et al. 2013). Lipid accumulation began during nitrogen limitation in the culture medium. As a result, cells assimilate more carbon for lipid biosynthesis (Wynn and Ratledge 2005). The enzymes that catalyze lipid biosynthesis include citrate lyase and malate dehydrogenase, exhibit high activity in oleaginous microorganisms (Wynn and Ratledge 2005).

In the present study, squalene was detected in the HPLC and GC-MS analyses (Figure 4). The HPLC analysis detected squalene in approximately 3 min of retention time. The squalene determination was also detected in approximately 12 min of retention time using GC-MS analysis. All seven high-lipid-content strains produced squalene (Table 1). The quantitative analysis using HPLC indicated that the squalene content varied from 0.57 to 2.18 mg g^{-1}, with the strain of AB14 exhibiting the highest squalene content. A high variation in squalene content (from 2.9 to 317.7 mg g^{-1} DCW) was also observed in 176 strains of thraustochytrids isolated from several locations in Asia (Nakazawa et al. 2014). In contrast, several thraustochytrid strains from Japan and Thailand had low squalene content (less than 0.1 mg g−1 DCW). (Otagiri et al. 2017; Malawet et al. 2020). It appears that thraustochytrids have a large range of squalene contents, depending on the strain origin. The culture period may also affect squalene content because it is an intermediate in sterol biosynthesis, such as cholesterol (Aasen et al. 2016). Tran et al. (2020) reported that the squalene content of the thraustochytrid strain isolated from Australia decreased by day 7 of the culture period. In the present study, squalene was determined on day 3 of each culture period. The present study is the first report of a squalene-producing thraustochytrid isolated from a mangrove habitat in Sidoarjo, Indonesia.

Table 1. Lipid and squalene contents of the *Auantiochytrium* strains. Data were mean±standard deviation

***Aurantiochytrium* strains**	**Biomass (g/L)**	**Lipid content (%)**	**Squalene content (mg/g)**
AB02	4.73 ± 0.13^{d}	41.54 ± 0.66^{c}	0.57 ± 0.01^{a}
AB04	4.09 ± 0.05^{b}	46.56 ± 0.75^{d}	2.02 ± 0.10^{de}
AB05	3.30 ± 0.08^{a}	53.34 ± 0.56^{f}	2.07 ± 0.08^{de}
AB10	7.23 ± 0.13^{f}	47.91 ± 0.14^{e}	1.28 ± 0.03^{b}
AB11	4.39 ± 0.20^{c}	38.17 ± 0.34^{b}	1.91 ± 0.03^{cd}
AB12	5.24 ± 0.11^{e}	54.77 ± 0.37^{g}	1.76 ± 0.08^{c}
AB14	4.21 ± 0.06^{bc}	36.00 ± 0.03^{a}	2.18 ± 0.12^{e}

Note: A different superscript in the same column indicated significantly different ($p<0.05$)

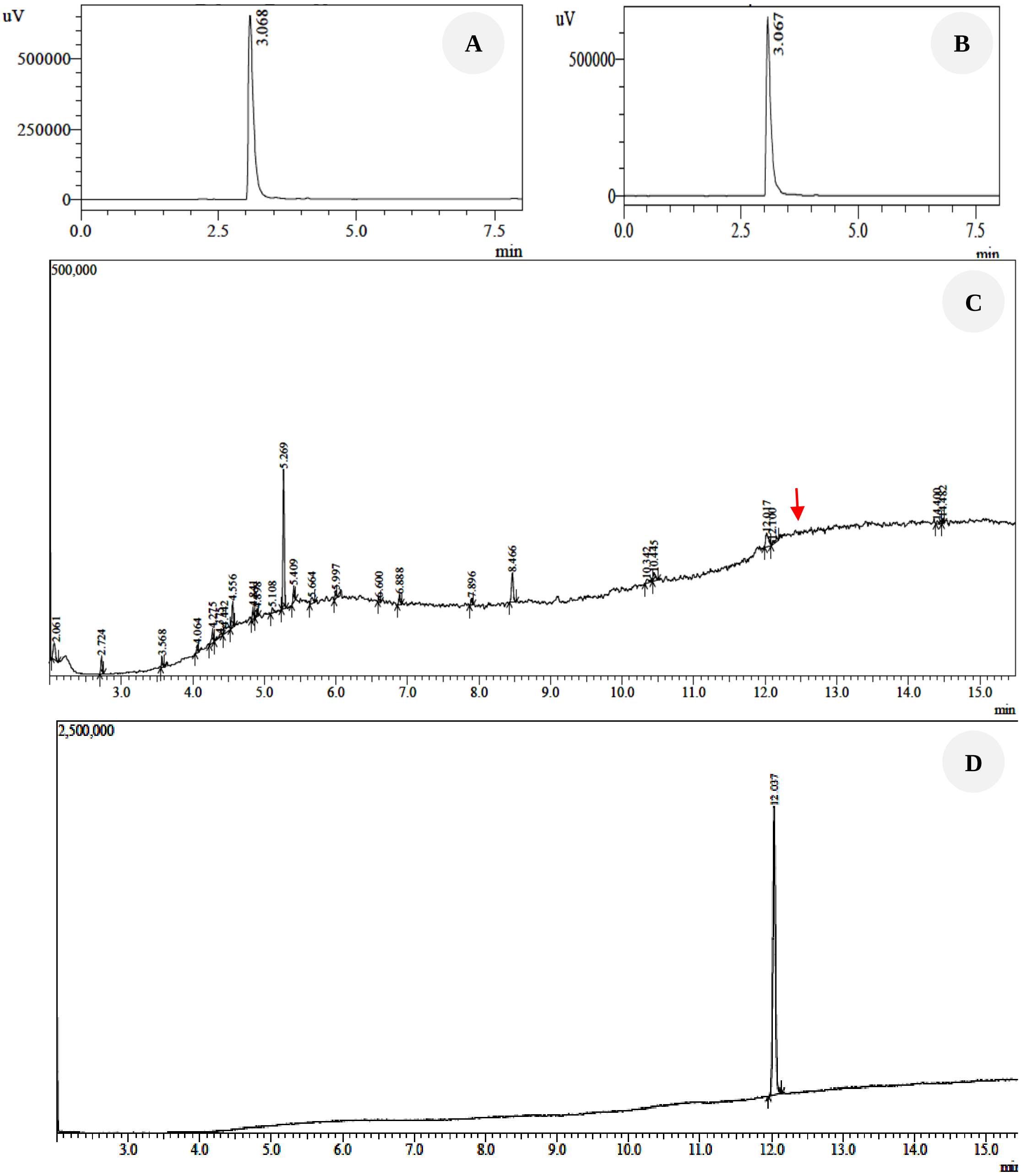

Figure 4. HPLC and GC-MS analysis of *Aurantiochytrium* sp. extract and authentic squalene: A. HPLC chromatogram of *Aurantiochytrium* sp. extract; B. HPLC chromatogram of authentic squalene; C. GC-MS chromatogram of *Aurantiochytrium* sp. extract (red arrow indicates squalene); and D. GC-MS chromatogram of authentic squalene

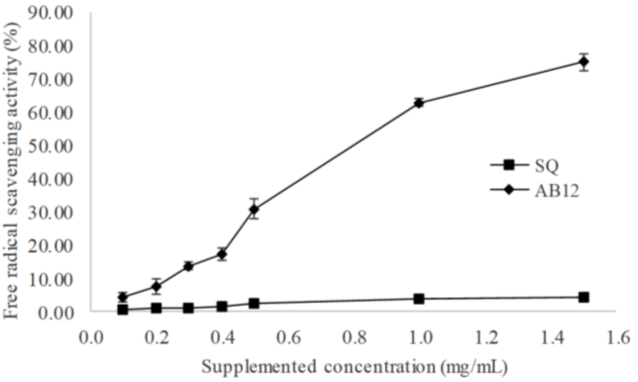

Figure 5. Determination of free RSA using DPPH of *Aurantiochytrium* sp. AB12 extract (AB12) and authentic squalene (SQ). Data were mean±standard deviation

Antioxidant activity of the selected strain

The free RSA using DPPH of *Aurantiochytrium* sp. AB12 extract (AB12) and authentic squalene (SQ) is presented in Figure 5. AB12 was selected for the antioxidant activity analysis because of its superior lipid content. The lipid extract of the diatoms had antioxidative properties against DPPH and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) (Pekkoh et al. 2022). The free RSA increased with increasing concentrations of the AB12 extract and SQ. The AB12 extract had higher free RSA than the SQ. The dose-dependent scavenging activity was also reported by Zhang et al. (2023a). They found that the highest (32%) scavenging activity of the DPPH radical was observed for 1% squalene. Moreover, SQ could not scavenge DPPH even when supplemented at 500 µg/mL (Ko et al. 2002). This indicates that the SQ might have a limited capacity to scavenge DPPH and scavenge more singlet oxygen (Kohno et al. 1995; Ko et al. 2002). The higher free RSA of AB12 might be because of the presence of other extracted components, as shown in Figure 4.C. The two predominant compounds, including hexadecanoic acid (at 5.269 retention time; 28.56% of compound compositions) and doconexent (at 8.466 retention time; 8.58% of compound compositions), a synonym of Docosahexaenoic Acid (DHA), have been known to have antioxidant properties (Li et al. 2021; Ganesan et al. 2024; Igwe et al. 2024).

The DPPH scavenging activity of the n-hexadecanoic acid extracted from the leaves of *Ipomoea eriocarpa* was 30.19-89.13% at 100-500 µg/mL (Ganesan et al. 2024). Moreover, the antioxidant capacity of HepG2 cells increased by approximately 50% after it was treated with 100 µM DHA for 48 hours (Li et al. 2021). This study identified a new thraustochytrid isolate with potential antioxidant activity.

This study concludes that mangrove-derived *Aurantiochytrium* isolates from the Sidoarjo district, East Java, Indonesia, can serve as promising sustainable squalene sources. Specifically, strain AB12 exhibited notable lipid content and antioxidant activity, whereas strain AB14 exhibited the highest squalene yield. The different squalene production obtained in the present study from other previous reports may be because of different isolated strains and culture conditions. These findings have the potential to impact squalene production significantly. Further study is still needed to optimize environmental culture conditions for enhancing the growth and squalene production of isolated strains.

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