Agriculture and Natural Resources 52 (2018) 24–32

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original Article

# Species diversity and polyunsaturated fatty acid content of thraustochytrids from fallen mangrove leaves in Chon Buri province, Thailand

Somtawin Jaritkhuan,[a, *] Sudarat Suanjit[b]

[a] Department of Aquatic Science, Faculty of Science, Burapha University, Bangsaen, Chon Buri, 20131, Thailand
[b] Department of Microbiology, Faculty of Science, Burapha University, Bangsaen, Chon Buri, 20131, Thailand

ARTICLE INFO

*Article history:*
Received 15 January 2017
Accepted 23 October 2017
Available online 17 May 2018

*Keywords:*
Docosahexaenoic acid (DHA)
Eicosapentaenoic acid (EPA)
Mangrove forest
Polyunsaturated fatty acids
Thraustochytrids

ABSTRACT

Screening and isolation were carried out of thraustochytrids from fallen, senescent mangrove leaves from three mangrove forests in Chon Buri province, Thailand. In total, 715 thraustochytrid isolates were obtained and classified into 10 species: *Aurantiochytrium mangrovei*, *A. limacinum*, *Aurantiochytrium* sp.1, *Aurantiochytrium* sp.2, *Aurantiochytrium* sp.3, *Aurantiochytrium* sp.4, *Aurantiochytrium* sp.5, *Aurantiochytrium* sp.7, Unknown 1 and Unknown 2. The frequency of occurrence of thraustochytrids ranged from 2.50% to 57.50% and was higher in the dry season than the rainy season. The dominant species found in these areas were *A. mangrovei* and *A. limacinum*, and the leaves of *Avicennia alba* had the greatest abundance of *A. mangrovei* (57.50%) and *A. limacinum* (28.75%). The biomass of *A. mangrovei* and *A. limacinum* was in the range 6.88–22.49 g/L, and 9.39–20.71 g/L, respectively. The highest content of polyunsaturated fatty acids (PUFA) was docosahexaenoic acid (DHA, C22:6n-3) in *A. limacinum* and *A. mangrovei* at 1.43–29.67% and 0.84–31.09% of total fatty acid, respectively. The arachidonic acid (ARA, C20:4n-6) and eicosapentaenoic acid (EPA, C20:5n-3) contents were highest in *A. limacinum* (0.03–0.10% of total fatty acid), and *A. mangrovei* (0.13–0.60% of total fatty acid), whereas the amount of docosapentaenoic acid (DPA, C22:5n-3) was similar in *A. limacinum* (0.41–6.08% of total fatty acid) and *A. mangrovei* (0.23–7.51% of total fatty acid). The results from this study add to the database of biodiversity of thraustochytrids in Thailand and showed that high amounts of C22:6n-3 in some selected strains have potential for use in aquaculture or commercial use.



## Introduction

Thraustochytrids are non-photosynthetic marine protists of the eucaryota with monocentric thalli, an ectoplasmic net and biflagellate zoospores. The thallus possesses a multi-layered wall of scales composed predominantly of L-galactose (Bongiorni, 2012). They have been classified into the Class Labyrinthula of the Kingdom Chromista or Straminipila (Burja et al., 2006; Raghukumar, 2002), and not as fungi, based on the taxonomy of the phylogenetic analysis through 18S rRNA sequencings (Cavalier-Smith et al., 1994), but they are closely related to the red and brown algae (Mannella et al., 1987). They can be found in seawater, algae, seagrass, coral reef, estuarine, mangrove forest, sediment and oceanic habitats worldwide (Leaño, 2001; Fan et al., 2002; Shene et al., 2010). They play an important role as a decomposer in marine environments, especially in the decomposition of leaf materials in mangrove forests, as a food source for filter feeders and detritus feeders in mangrove food webs and also as secondary producers in coastal zones (Raghukumar, 2002; Leaño, 2001; Wong et al., 2005; Perveen et al., 2006; Chang et al., 2012) and additionally as detritivores, bacterivores or pathogens of edible marine invertebrates (Mo and Rinkevich, 2001; Scharer et al., 2007). Another important feature of thraustochytrids is the ability to degrade hydrocarbons following oil spill events (Raikar et al., 2001).

Thraustochytrids produce high amounts of n-3 polyunsaturated fatty acids (n-3 PUFA), especially C22:6n-3 (DHA) and C20:5n-3 (EPA) which are essential for human health. C22:6n-3 is important for brain, eye and heart health, while C20:5n-3 is a precursor for prostaglandin-3 (inhibits platelet aggregation), thromboxane-3, and leukotriene-5 groups (Jain et al., 2007). *Thraustochytrium aureum* Goldstein ATCC 34304 and ATCC 28211 were reported to contain 47.40% C22:6n-3 and 52.30% total fatty acid (Bajpai et al., 1991). Numerous studies on thraustochytrids mentioned that

* Corresponding author.
*E-mail address:* jsomtawin@yahoo.com (S. Jaritkhuan).

https://doi.org/10.1016/j.anres.2018.05.002


**Fig. 1.** Study sampling sites.

they were abundant on fallen, decaying leaves of *Kandelia candel* (L.) Druce and in the sediments in Futian National Nature Reserve, China (Wong et al., 2005), and also on leaves of *Kandelia candel* as well as in Hong Kong (Fan et al., 2002). A new species (*Thraustochytrium gaertnerium*) was found from mangrove forest in Goa, India (Bongiorni et al., 2005). However, there have been few studies of thraustochytrid diversity in Thailand; Juntaban et al. (2007) found *Schizochytrium mangrovei* Raghuk., *Schizochytrium limacinum* Honda et Yokochi, *Schizochytrium* sp.2 and *Ulkenia visurgensis* (Ulken) A. Gaertn. from fallen leaves of mangrove forest at Bang Khun Thean, Bangkok. The current study aimed to determine the species diversity of thraustochytrids from the fallen leaves of mangrove forests in Chon Buri province, Thailand and also the amount of n-3 PUFA in this group, which might be beneficial for database expansion and commercial uses.

## Materials and methods

### *Sampling sites*

Three mangrove forests (Mangrove Forest Conserve and Natural Study Center (Station 1, as a conservation and planting area, 13°20′33.78″ N and 100°56′32.65″ E), Klong Prong Mangrove forest, Ang Sila (Station 2, as a community area, 13°18′54.59″ N and 100°55′2.85″ E), and Sattahip Naval Base, Sattahip (Station 3, as a conservative area, 12°38′46″ N and 100°55′49″ E)) were the sites for collecting fallen, senescent mangrove leaves (Fig. 1). The sampling occurred in March 2014 (dry season) and September 2014 (rainy season). There were 6, 7 and 10 mangrove species collected from Stations 1, 2 and 3, respectively (Table 1). Dissolved oxygen (DO), pH, water temperature and salinity in the study areas were in the ranges 1.99–2.46 mg/L, 7.35–8.82, 24.9–30.0 °C and 12–15 psu, respectively.

### *Sample collection and isolation*

Twenty samples of fallen leaves in each mangrove species were collected and placed in plastic zip lock bags and brought to the laboratory for screening and isolation of thraustochytrids. Each leaf was cut into nine small pieces (0.25 $cm^2$) and washed twice in sterile seawater. Then leaf pieces were placed onto glucose-yeast extract-peptone HIMEDIA agar (GYP agar medium containing 1 g/L each of glucose, yeast extract and peptone with antibiotics) and overlaid with a thin layer of sterile seawater. Three GYP plates were prepared per leaf sample (60 plates/mangrove species). These plates were incubated at room temperature (29–32 °C) and

**Table 1**
Mangrove species at three sampling sites in Chon Buri province, Thailand.

| Mangrove species | Station 1 | Station 2 | Station 3 |
|---|---|---|---|
| *Rhizophora apiculata* Blume (RA) | + | + | + |
| *Rhizophora mucronata* Lam. (RM) | + | + | + |
| *Hibiscus tiliaceus* L. (HT) | | | + |
| *Ceriops decandra* (Griff.) Ding Hou (CD) | | | + |
| *Lumnitzera racemosa* Willd. (LR) | | | + |
| *Thespesie populnea* (L.) Sol. ex Corrêa (TP) | + | + | + |
| *Sonneratia caseolaris* (L.) Engl. (SC) | | + | |
| *Avicennia alba* Blume (AA) | + | + | + |
| *Avicennia officinalis* L. (AO) | + | + | |
| *Avicennia marina* (Forsk.) Vierh (AM) | + | + | + |
| Total | 6 | 7 | 8 |

Remark: Station 1 Natural and Mangrove Reservation Study Center (S1).
Station 2 Klong Prong Mangrove Forest, Ang Sila (S2).
Station 3 Sattahip Naval Base, Sattahip (S3).
+ Species was found at that station.

**Table 2**
Morphological features of thraustochytrids found in mangrove forest, Chon Buri province, Thailand.

| Tharustochytrids | Colony morphology | Cell feature | | | | |
|---|---|---|---|---|---|---|
| | | Cell wall | Amoeboid cells | Shape and size of vegetative cell (diameter) | Shape and size of a zoosporangium (diameter) | Binary cell division |
| *Aurantiochytrium mangrovei* | Colonies white opaque, circular in form, undulate margin and flat in elevation | Thin-walled | Present | Spherical, 5–15 µm | Spherical, 10–35 µm | Present |
| *Aurantiochytrium limacinum* | Colonies white opaque, irregular spread in form, undulate margin and flat in elevation | Thin-walled | Present | Spherical, 5–10 µm | Spherical, 7–25 µm | Present |
| *Aurantiochytrium* sp.1 | Colonies pale orange, circular in form, undulate margin and raised in elevation | Thin-walled | Present | Spherical, 5–10 µm | Spherical, 10–40 µm, cell aggregated | Present |
| *Aurantiochytrium* sp.2 | Colonies pale yellow, circular in form, undulate margin and raised in elevation | Thin-walled | Present | Spherical, 5–20 µm | Spherical, 10–50 µm, cell aggregated | Present |
| *Aurantiochytrium* sp.3 | Colonies white opaque, circular in form, entire margin and raised in elevation | Thin-walled | Present | Spherical, 5–15 µm | Spherical, 10–50 µm, cell aggregated | Absent |
| *Aurantiochytrium* sp.4 | Colonies white, irregular in form, undulate margin and flat in elevation | Thin-walled | Present | Spherical, 5–10 µm | Spherical, 10–25 µm | Present |
| *Aurantiochytrium* sp.5 | Colonies white, circular in form, undulate margin and raised in elevation | Thin-walled | Present | Spherical, 5–20 µm | Spherical, 10–60 µm, cell aggregated | Present |
| *Aurantiochytrium* sp.7 | Colonies white, circular in form, undulate margin and flat in elevation | Thin-walled | Present | Spherical, 5–15 µm | Spherical, 10–30 µm | Present |
| Unknown 1 | Colonies pale orange, circular in form, entire margin and convex in elevation | Thick-walled | Present | Spherical, 3–8 µm | Spherical, 10–20 µm | Absent |
| Unknown 2 | Colonies white, circular in form, undulate margin and raised in elevation | Thin-walled | Absent | Spherical, 4–10 µm | Spherical, 10–75 µm | Absent |

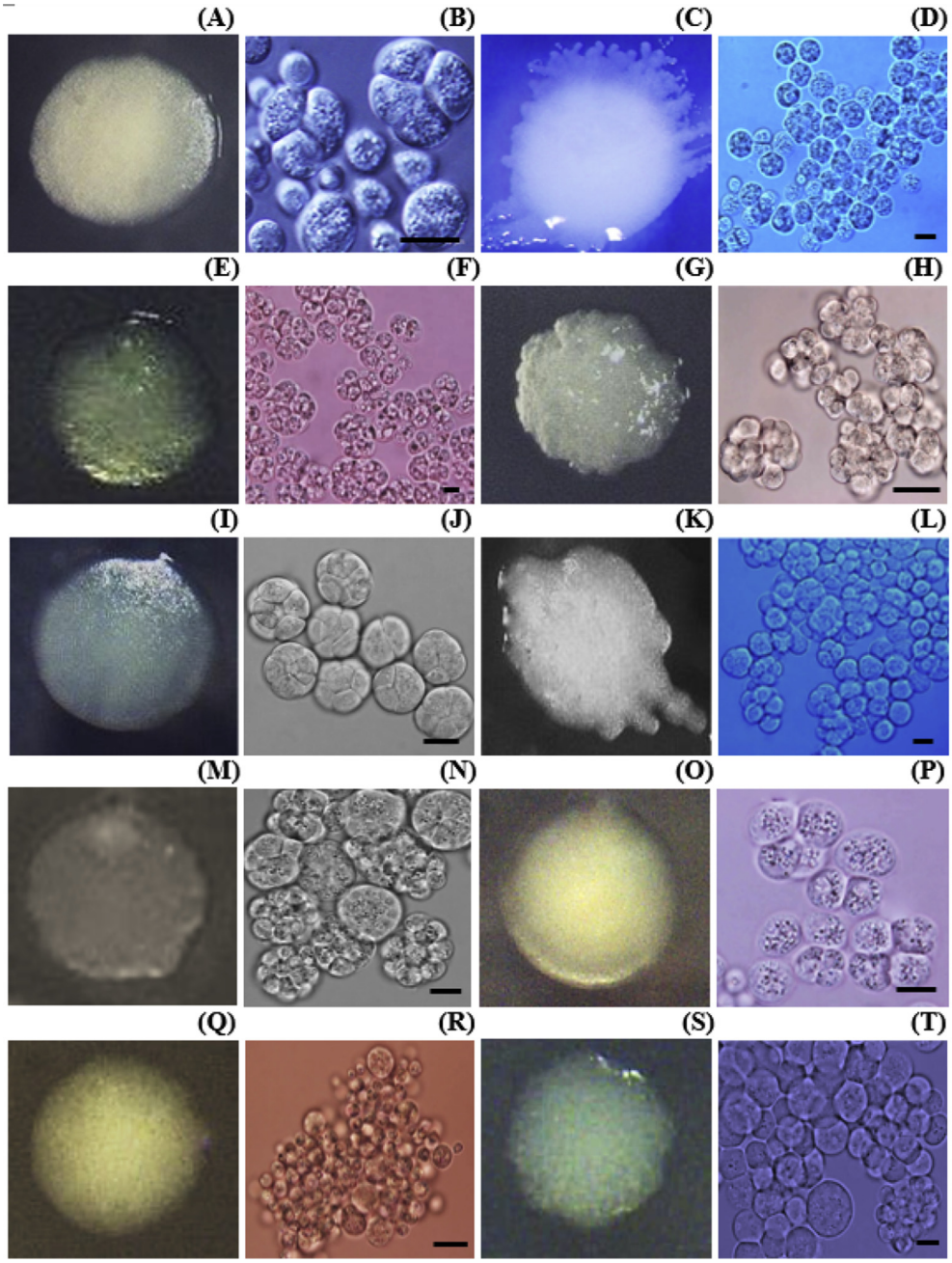


**Fig. 2.** Thraustochytrid colony and cells, *Aurantiochytrium mangrovei* (A–B), *Aurantiochytrium limacinum* (C–D), *Aurantiochytrium* sp.1 (E–F), *Aurantiochytrium* sp.2 (G–H), *Aurantiochytrium* sp.3 (I–J), *Aurantiochytrium* sp.4 (K–L), *Aurantiochytrium* sp.5 (M–N), *Aurantiochytrium* sp.7 (O–P), Unknown 1 (Q–R) and Unknown 2 (S–T). (scale bar = 20 μm).

monitored daily for the appearance of thraustochytrids. Colonies of thraustochytrids were picked up and the isolates were purified on GYP agar. Thraustochytrid isolates were identified based on: morphological features (formation of zoospores, cell division); zoosporangial characteristics (release of zoospores); and colony formation, using the identification keys of Honda (2001), Hunt (2000), Leander and Porter (2000), Yokoyama and Honda (2007) and Yokoyama et al. (2007).

The thraustochytrid species were characterized using their frequency of occurrence (%) in each mangrove species (Equation (1)):

$$\text{Frequency of occurrence (\%)} = \frac{\text{Number of specific thraustochytrid observed}}{\text{Total number of leaf samples collected (per mangrove species)}} \times 100 \quad (1)$$

*Biomass and fatty acid composition analysis*

Thraustochytrid isolates were grown with glucose and yeast extracts in the ratio 6:1 (6 g/L of glucose and 1 g/L of yeast extract with antibiotics) in sterile seawater at 15 psμ, temperature 25 °C and stirring at 200 rpm for 4 d to determine the biomass (harvested using centrifugation and lyophilization) and fatty acid composition. Fatty acids of thraustochytrid were analyzed using direct transmethylation with 2% sulfuric acid in methanol and then heated at 80 °C water bath for 2 h (modified from Shimizu et al., 1988) and fatty acid methyl esters were separated using gas chromatography (HP 6890 Series GC System; Wilmington, DE, USA) equipped with a flame ionization detector. A capillary column was used HP-INNO-Wax polyethylene glycol; 25 m × 200 μm × 0.40 μm; Agilent Technologies; Santa Clara, CA, USA. Helium was used as the carrier gas and the injector and detector temperatures were both 250 °C. The column temperature was initially programmed to be 150 °C for 1 min, and increased at a rate of 15 °C/min to180 °C, holding for 1 min, then 1 °C/min to 210 °C, holding for 3 min, and 2 °C/min to a final temperature of 250 °C, holding for 10 min. Peak identification was performed by means of the retention time of known standard fatty acids (Sigma Chemical Co.; St. Louis, MO, USA). The fatty acid compositions were determined by comparing their peak areas with that of the internal standard (19:0) which had been added in the sample before extraction (modified from Shimizu et al., 1988).

## Results and discussion

In total, 715 thraustochytrid isolates were obtained, classified into 10 species: *Aurantiochytrium mangrovei*, *Aurantiochytrium limacinum*, *Aurantiochytrium* sp.1, *Aurantiochytrium* sp.2, *Aurantiochytrium* sp.3, *Aurantiochytrium* sp.4, *Aurantiochytrium* sp.5, *Aurantiochytrium* sp.7, Unknown 1 and Unknown 2 (Table 2 and Fig. 2). The dominant species were *A. mangrovei* and *A. limacinum*. This agreed with Juntaban et al. (2007) who found *Schizochytrium mangrovei* (syn. *Aurantiochytrium mangrovei*), *Schizochytrium limacinum* (syn. *Aurantiochytrium limacinum*), *Schizochytrium* sp.2 (syn. *Aurantiochytrium* sp.2), and *Ulkenia visurgensis* on fallen leaves from a mangrove forest in Bang Khun Thean, Bangkok, Thailand. Six thraustochytrids (*Schizochytrium* sp. KF-1, *Schizochytrium mangrovei* KF-2, KF-7, KF-12, *Thraustochytrium striatum* KF-9 and *Ulkenia* KF-13) were found on fallen, senescent leaves of *Kandelia candel* in Hong Kong (Fan et al., 2002).

The average frequency of occurrence of thraustochytrids from these three mangrove forests was in the range 2.50–57.50%, with *A. mangrovei* being the main species found (5.00–57.50%), followed

**Table 3**
Frequency of occurrence (%) of thraustochytrids during dry and rainy seasons from different mangrove species in Chon Buri province, Thailand.

| Mangrove species | Thraustochytrids | Frequency of occurrence (%) | | |
|---|---|---|---|---|
| | | Dry season | Rainy season | Average |
| *Rhizophora apiculata* (RA) | *A. mangrovei* | 70.00 | 20.00 | 45.00 |
| | *A. limacinum* | 18.33 | 10.00 | 14.16 |
| | *Aurantiochytrium* sp.1 | 5.00 | – | 2.50 |
| | *Aurantiochytrium* sp.4 | – | 7.50 | 3.75 |
| *Rhizophora mucronata* (RM) | *A. mangrovei* | 66.67 | 37.50 | 52.08 |
| | *A. limacinum* | 18.33 | 18.33 | 18.33 |
| | *Aurantiochytrium* sp.3 | – | 10.00 | 5.00 |
| | *Aurantiochytrium* sp.4 | – | 25.00 | 12.50 |
| *Hibiscus tiliaceus* (HT) | *A. mangrovei* | 15.00 | – | 7.50 |
| *Ceriops decandra* (CD) | *A. mangrovei* | 20.00 | 10.00 | 15.00 |
| | *A. limacinum* | – | 10.00 | 5.00 |
| | *Aurantiochytrium* sp.4 | – | 5.00 | 2.50 |
| | Unknown 2 | 10.00 | – | 5.00 |
| *Lumnitzera racemosa* (LR) | *A. mangrovei* | 10.00 | – | 5.00 |
| | *Aurantiochytrium* sp.5 | 5.00 | – | 2.50 |
| *Thespesia populnea* (TP) | *A. mangrovei* | 72.50 | 35.00 | 53.75 |
| | *A. limacinum* | – | 15.00 | 7.50 |
| | *Aurantiochytrium* sp.1 | – | 17.50 | 8.75 |
| | *Aurantiochytrium* sp.7 | – | 5.00 | 2.50 |
| *Sonneratia caseolaris* (SC) | *A. mangrovei* | 90.00 | – | 45.00 |
| | *A. limacinum* | 20.00 | 10.00 | 15.00 |
| | *Aurantiochytrium* sp.4 | – | 5.00 | 2.50 |
| *Avicennia alba* (AA) | *A. mangrovei* | 85.00 | 30.00 | 57.50 |
| | *A. limacinum* | 45.00 | 12.50 | 28.75 |
| | *Aurantiochytrium* sp.1 | 10.00 | – | 5.00 |
| | *Aurantiochytrium* sp.3 | – | 5.00 | 2.50 |
| | *Aurantiochytrium* sp.4 | – | 7.50 | 3.75 |
| | *Aurantiochytrium* sp.5 | 5.00 | – | 2.50 |
| | Unknown 1 | 5.00 | – | 2.50 |
| *Avicennia officinalis* (AO) | *A. mangrovei* | 52.50 | 35.00 | 43.75 |
| | *A. limacinum* | 40.00 | 15.00 | 27.50 |
| | *Aurantiochytrium* sp.1 | 10.00 | – | 5.00 |
| | *Aurantiochytrium* sp.2 | 10.00 | – | 5.00 |
| | *Aurantiochytrium* sp.3 | – | 5.00 | 2.50 |
| | *Aurantiochytrium* sp.4 | – | 10.00 | 5.00 |
| *Avicennia marina* (AM) | *A. mangrovei* | 67.50 | 36.67 | 52.08 |
| | *A. limacinum* | 30.00 | 18.33 | 24.16 |
| | *Aurantiochytrium* sp.3 | – | 10.00 | 5.00 |
| | *Aurantiochytrium* sp.4 | – | 7.50 | 3.75 |
| | *Aurantiochytrium* sp.5 | 15.00 | – | 7.50 |
| | *Aurantiochytrium* sp.7 | – | 5.00 | 2.50 |
| | Unknown 1 | 5.00 | – | 2.50 |
| | Unknown 2 | 22.50 | – | 11.25 |

by *A. limacinum* (5.00–28.75%; Table 3 and Fig. 3). This was in agreement with Raghukumar et al. (1994) who stated that thraustochytrids were found in large numbers in mangrove detritus. All mangrove leaf samples from Panay Island, the Philippines had few-to-abundant thraustochytrid colonies and *Schizochytrium mangrovei* (*A. mangrovei*) was the most abundant (40–100%; Leaño, 2001).

The highest frequency of occurrence of thraustochytrids was mostly in the dry season rather than the rainy season (Table 3). *Aurantiochytrium* sp.4, *Aurantiochytrium* sp.3 (5.00–10.00%) and *Aurantiochytrium* sp.7 (5.00%) were found only in the rainy season (5.00–25.00%), while *Aurantiochytrium* sp.5 was found only in the dry season (5.00–15.00%). This might have been due to differences in adaptation and tolerance of thraustochytrid species to fluctuating salinities and others physical factors in the mangrove environments. This result was consistent with the findings of Nakazawa et al. (2012) who mentioned that *Aurantiochytrium* sp. usually inhabits mangrove areas where daily and seasonal temperature and salinity fluctuation occurred. Some strain of thraustochytrids may have higher potential to adapt to these conditions than others.

The thraustochytrids in this study were found on a wide variety of mangrove leaf species. Leaves of *Avicennia alba* had the greatest abundance of *A. mangrovei* (57.50%), followed by *Thespesia populnea* (53.75%), and *Avicennia marina* and *Rhizophora mucronata* (52.08%), while *Lumnitzera racemosa* had the lowest (5.00%; Table 3 and Fig. 3). It was found that the diversity and frequency of occurrence of thraustochytrids for each mangrove leaf species depended on leaf characteristics such as succulence (thin and thick), leaf senescence or decay (yellow or yellowish-brown or black-colored leaves), leaf humidity (internal water storage tissue), salinity, inundation, waterlogging and the season. The brown or yellowish-brown color of the wet leaf samples collected in this study had higher percentages of occurrence of thraustochytrids which could be found inhabiting all mangrove leaf species, as the thraustochytrids are decomposers and can secrete high amounts of extracellular enzymes (degradative enzymes), such as cellulase, amylase, polygalacturonase, protease, lipase and xylanase that can degrade mangrove leaves (Raghukumar et al., 1994; Bremer and Talbot, 1995; Bongiorni et al., 2005) and also act as a food source for detritus and picoplankton feeders in mangrove ecosystems (Wong et al., 2005).

The biomass production of some thraustochytrid isolates from the three mangrove forests was in the range 6.88–22.49 g/L at day 4 (Fig. 4). The selected isolates with high yields of biomass for further commercial use were: *A. mangrovei* BUCHSC 021, *A. mangrovei* BUCHSC 103, *A. mangrovei* BUCHSC 123, *A. mangrovei* BUCHSC 142 and *A. mangrovei* BUCHHT 172 (20.11–22.49 g/L); and *A. limacinum* BUCHHT 093 (20.71 g/L). The remaining thraustochytrid species had biomass of less than 20 g/L. The biomass amounts from this study were higher than those reported by Kamlangdee and Fan (2003) who stated that five isolates of *Schizochytrium* sp. from mangrove forest (*Kandelia candel*) in Hong Kong had biomass yields in the range 10.8–13.2 g/L. However, different growth conditions affect the biomass yield such as salinity, temperature, pH and the media used; Fan et al. (2002) stated the optimal growth temperature and salinity for six thraustochytrids isolated from senescent leaves of *Kandelia candel* in Hong Kong were 20–25 °C and 7.5–30.0 psu, respectively, and also the optimal salinity for thraustochytrids was 50–100% seawater (Raghukumar, 2008).

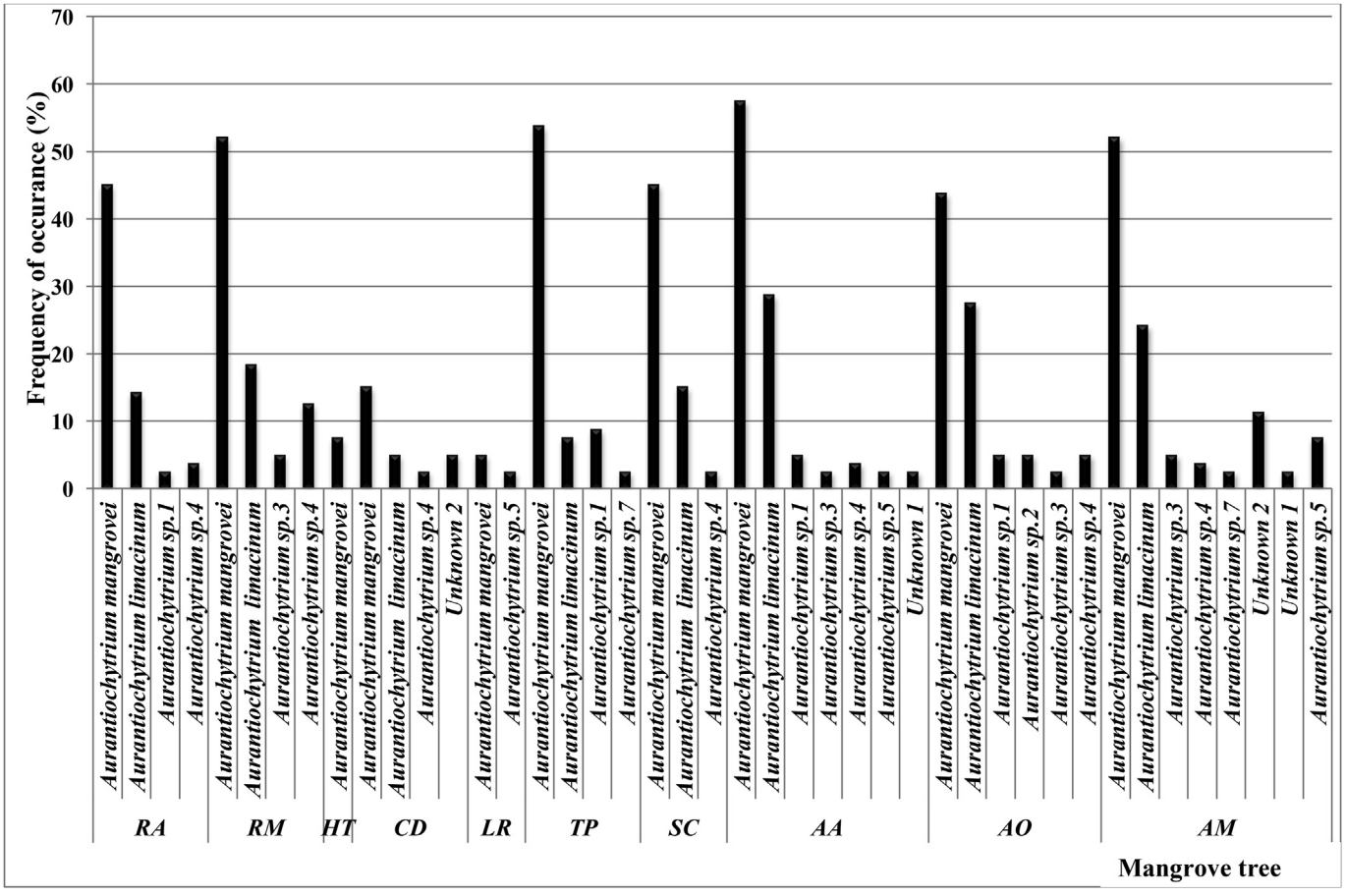


**Fig. 3.** Average frequency of occurrence of thraustochytrids from different mangrove species, Chon Buri province, Thailand.

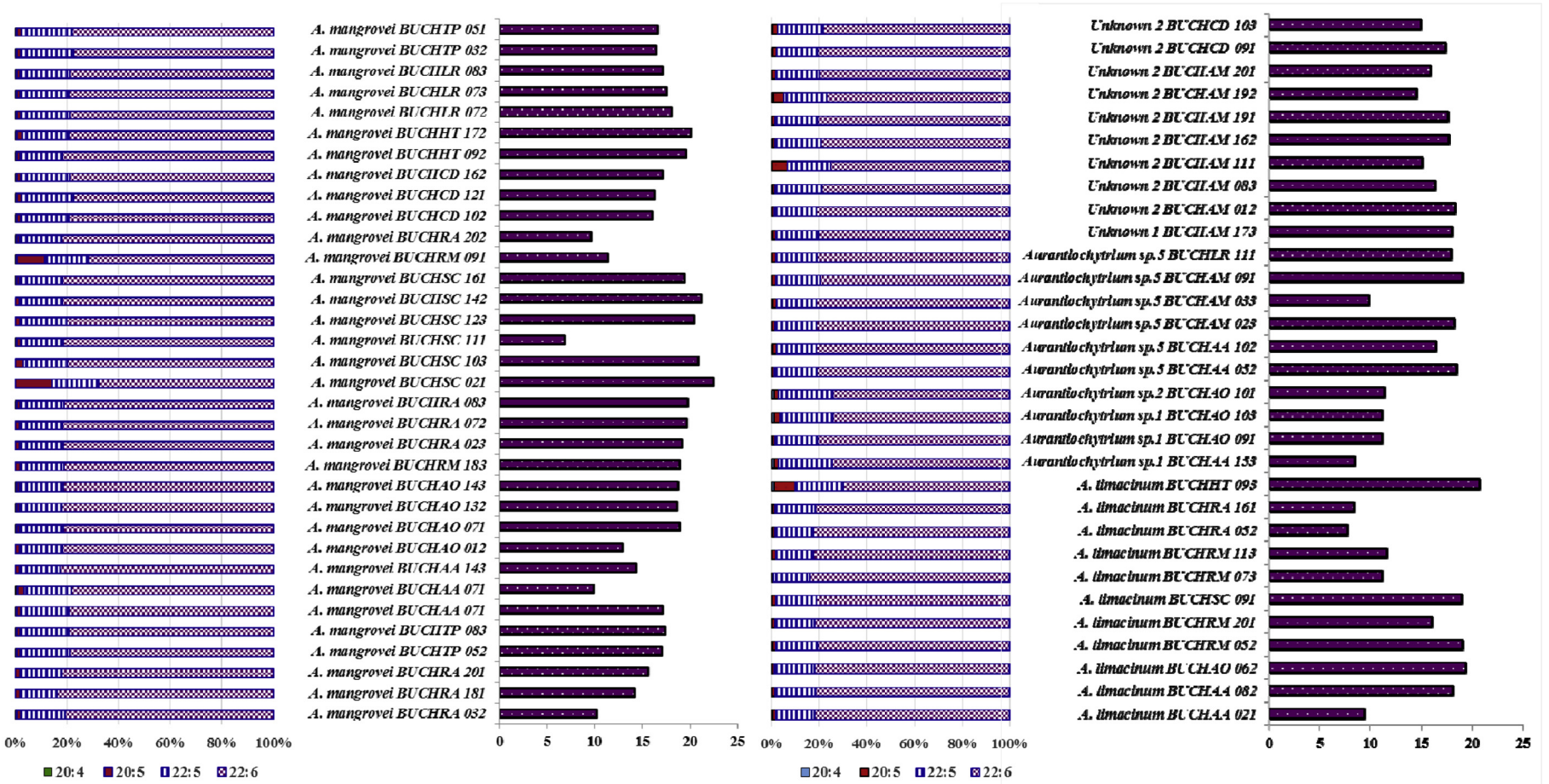


**Fig. 4.** Proportion of arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3) docosapentaenoic acid (C22:5n-3) and docosahexaenoic acid (C22:6n-3) of thraustochytrids (a, c) and biomass (b, d) from mangrove forest in Chon Buri province, Thailand.

*Fatty acids content in thraustochytrids*

The C13:0, C14:0, C16:0, C17:0 and C18:0 fatty acids were the dominant saturated fatty acids (SFAs), while the polyunsaturated fatty acids (PUFAs) were dominated by C22:6n-3, followed by C22:5n-3, C20:5n-3 and C20:4n-6, respectively. The PUFA composition was dominated by n-3 rather than n-6 fatty acids. This was in agreement with previous articles which reported that the principal fatty acids were palmitic acid (C16:0) and C22:6n-3 in thraustochytrids (Liu et al., 2014; Ludevese-Pascual et al., 2016) but the majority of PUFAs in *Thraustochytrium* sp. ATCC 26185 were C22:5n-3 (21–24%) and C22:6n-3 (68–71%) (Furlan et al., 2012).

C20:4n-6 and C20:5n-3 were found in lower amounts with C20:4n-6 being highest in *A. limacinum* (0.09–0.55 mg/g dry weight (DW); 0.03–0.10% of total fatty acids), and the amount of C20:5n-3 was highest in *A. mangrovei* (0.52–5.67 mg/g DW, 0.13–0.60% of total fatty acids). These results were quite similar to Ludevese-Pascual et al. (2016) who reported that *Schizochytrium* sp. LEY7 had low amounts of C20:4n-6 (0.90%) and C20:5n-3 (1.01%), and also 0.30% and 0.90% of C20:4n-6 and C20:5n-3, respectively (Kamlangdee and Fan, 2003). High amounts of C22:5n-3 were similar in *A. limacinum* (1.37–37.71 mg/g DW, 0.41–6.08% of total fatty acids) and *A. mangrovei* (4.74–41.87 mg/g DW, 0.23–7.51% of total fatty acids; Fig. 4). C22:5n-3 plays an important role in the cell physiology of thraustochytrids, aplanochytrids and labyrinthulids and it is quite rare in other organisms but commonly found in the Labyrinthulomycetes and can be considered as a signature fatty acid (Huang et al., 2003).

The dominant fatty acid in thraustochytrids was C22:6n-3 which had the highest content in *A. limacinum* (4.71–191.07 mg/g DW, 1.43–29.67% of total fatty acids) and *A. mangrovei* (20.75–175.34 mg/g DW, 0.84–31.09% of total fatty acids). This was in agreement with previous work reporting *Aurantiochytrium* sp. had a C22:6n-3 content of 27.90% (Nakazawa et al., 2012), and 145 mg/g DW (30.30% of total fatty acids; Choi et al., 2014), *Schizochytrium* sp. isolate had N-2 (157.90–203.60 mg/g DW, 36.10% of total fatty acid; Kamlangdee and Fan, 2003) and *Schizochytrium* sp. LEY7 (39.92% of total fatty acid; Ludevese-Pascual et al., 2016). *Thraustochytrium* spp. and *Schizochytrium* spp. had a C22:6n-3 content in the range 1.50–35.00% of total fatty acids (Singh and Ward, 1997), while *Aurantiochytrium* sp. SD116 had 50.90% of total fatty acids (Gao et al., 2013).

The biomass and C22:6n-3 production of thraustochytrids in this study ranged from 6.88 to 22.49 g/L and 0.84–31.09% of total fatty acids, respectively, which were incorporated with the composition of the growth medium as mentioned in *A. limacinum* SR21 (Yokochi et al., 1998), while the *Aurantiochytrium* strain, 4W-1b, had fatty acid production and yields of C22:6n-3 and C16:0 of 9 g/L, 1.5 g/L and 4.8 g/L, respectively (Nakazawa et al., 2012). In addition, *Schizochytrium* sp. had the highest C22:6n-3 production at 44% of total fatty acids with biomass and a C22:6n-3 yield of 7.1 and 1.6 g/L, respectively (Liu et al., 2014). These previous articles mentioned that the strains obtained from various areas had different amounts of fatty acids; for example, the Australian strains had a higher C22:6n-3 content (17–31% of total fatty acids) than Indian strains (Gupta et al., 2016). The production depended on strain specificity and growth conditions such as media, salinity, temperature, pH and growing period. These results indicated that thraustochytrids are considered as marine microorganisms that can commercially produce C22:6n-3 and can be used as alternative source of polyunsaturated fatty acids, especially C22:6n-3 for aquaculture or further commercial uses.

This study isolated thraustochytrids from fallen, senescent mangrove leaves in Chon Buri province belonging to 10 species with 2.50–57.50% occurrence. *A. mangrovei* BUCHSC 021 and *A. limacinum* BUCHHT 093 showed high potential as sources of C20:5n-3 and C22:6n-3, while *A. mangrovei* BUCHSC 103, *A. mangrovei* BUCHSC 123, *A. mangrovei* BUCHSC 142, and *A. mangrovei* BUCHHT 172 were sources of C22:6n-3.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Acknowledgements

The authors would like to record their gratitude to Burapha University for grant support and thank Miss Naruemon Thoobbucha, Miss Tiwapon Thongprasom and the aquatic science students for assisting in the laboratory and field study.

## References

Bajpai, P., Bajpai, K., Ward, P.O., 1991. Production of docosahexaenoic acid by *Thraustochytrium aureum*. Appl. Microbiol. Biotechnol. 35, 706–710.
Bongiorni, L., 2012. Thraustochytrids, a neglected component of organic matter decomposition and food webs in marine sediments. Prog. Mol. Subcell. Biol. 53, 1–13.
Bongiorni, L., Jain, R., Raghukumar, S., Aggarwal, R.K., 2005. *Thraustochytrium gaertnerium* sp. nov.: a new thraustochytrid stramenopilan Protist from mangroves of Goa, India. Protist 156, 303–315.
Bremer, G.B., Talbot, G., 1995. Cellulolytic enzyme activity in the marine protest *Schizochytrium aggregatum*. Bot. Mar. 38, 37–41.
Burja, A.M., Radianingtyas, H., Windust, A., Barrow, C.J., 2006. Isolation and characterization of polyunsaturated fatty acid producing *Thraustochytrium* species: screening of strains and optimization of omega-3 production. Appl. Microbiol. Biotechnol. 72, 1161–1169.
Cavalier-Smith, T., Allsopp, M.T.E.P., Chao, E.E., 1994. Thraustochytrids are chromists, not fungi: 18S rRNA signatures of Heterokonta. Philos. T. Roy. Soc. B 346, 387–397.
Chang, K.J.L., Dunstan, G.A., Abell, G., Clementson, L., Blackburn, S., Nichols, P.D., Koutoulis, A., 2012. Biodiscovery of new Australian thraustochytrids for production of biodiesel and long-chain omega-3 oils. Appl. Microbiol. Biotechnol. 93, 2215–2231.
Choi, S.A., Jung, J.Y., Kim, K., Kwon, J.H., Lee, J.S., Kim, S.W., Park, J.Y., Yang, J.W., 2014. Effects of molten-salt/ionic-liquid mixture on extraction of docosahexaenoic acid (DHA)-rich lipids from *Aurantiochytrium* sp. KRS101. Bioprocess Biosyst. Eng 37, 2199–2204.
Fan, K.W., Vrijmoed, L.P.P., Jones, E.B.G., 2002. Physiology studies of subtropical mangrove thraustochytrids. Bot. Mar. 45, 50–57.
Furlan, V.J.M., Paulo, M.C., Batista, I., Bandarra, N.M., Santo, M.L.E., Prentice, C., 2012. Effect of the concentration of glucose in the docosahexaenoic acid (DHA) production by *Thraustochytrium* sp., ATCC 26185. Adv. J. Food Sci. Technol. 4, 257–264.
Gao, M., Song, X., Feng, Y., Li, W., Cui, Q., 2013. Isolation and characterization of *Aurantiochytrium* species: high docosahexaenoic acid (DHA) production by the newly isolated microalga, *Aurantiochytrium* sp. SD116. J. Oleo Sci. 62, 143–151.
Gupta, A., Singh, D., Byreddy, A.R., Thyagarajan, T., Sonkar, S.P., Mathur, A.S., Tuli, D.K., Barrow, C.J., Puri, M., 2016. Exploring omega-3 fatty acids, enzymes and biodiesel producing thraustochytrids from Australian and Indian marine biodiversity. Biotechnol. J. 11, 345–355.
Honda, D., 2001. Phylogeny and taxonomy of labyrinthulids. Aquabiology 132, 7–18.
Huang, J., Aki, Y., Yokochi, T., Nakahara, T., Honda, D., Kawamoto, S., Shigeta, S., Ono, K., Suzuki, O., 2003. Grouping newly isolated docosahexaenoic acid-producing thraustochytrids based on their polyunsaturated fatty acid profile and comparative analysis of 18S rRNA gene. Mar. Biotechnol. 5, 450–457.
Hunt, E.A., 2000. The Production of N-3 Polyunsaturated Docosahexaenoic Acid by Member of the Marine Protistan Group the Thraustochytrids. Doctoral Dissertation, Biological Sciences. University of Portsmouth, Portsmouth, UK.
Jain, R., Raghukumar, S., Sambaiah, K., Kumon, Y., Nakahara, T., 2007. Docosahexaenoic acid accumulation in thraustochytrids: search for the rationale. Mar. Biol. 151, 1657–1664.
Juntaban, J., Jaritkhuan, S., Suanjit, S., 2007. Diversity of thraustochytrids isolated from mangrove leaves at Bang Khun Thean, Bangkok. In: Proceedings of 8th National Grad Research Conference. Mahidol University, Salaya, Thailand, pp. 1–10.
Kamlangdee, N., Fan, K.W., 2003. Polyunsaturated fatty acids production by *Schizochytrium* sp. isolated from mangrove. Songklanakarin J. Sci. Technol. 25, 643–650.
Leaño, M., 2001. Straminipilous organism from fallen mangrove leaves from Panay Island, Philippines. Fungal Divers. 6, 75–80.
Leander, C.A., Porter, D., 2000. Redefining the genus Aplanochytrium (Phylum Labyrinthulomycota). Mycotaxon 25, 439–444.
Liu, Y., Singh, P., Sun, Y., Luan, S., Wang, G., 2014. Culturable diversity and biochemical features of thraustochytrids from coastal waters of Southern China. Appl. Microbiol. Biotechnol. 98, 3241–3255.

Ludevese-Pascual, G., Pena, M.D., Tornalejo, J., 2016. Biomass production, proximate composition and fatty acid profile of the local marine thraustochytrid isolate, *Schizochytrium* sp. LEY7 using low-cost substrates at optimum culture conditions. Aquacult. Res. 47, 318–328.

Mannella, C.A., Frank, J., Delihas, N., 1987. Interrelatedness of 5S RNA sequences investigated by correspondence analysis. J. Mol. Evol. 24, 228–235.

Mo, C., Rinkevich, B., 2001. A simple, reliable, and fast protocol for thraustochytrid DNA extraction. Mar. Biotechnol. 3, 100–102.

Nakazawa, A., Matsuura, H., Kose, R., Ito, K., Ueda, M., Honda, D., Inouye, I., Kara, K., Wayanabe, M.M., 2012. Optimization of biomass and fatty acid production by *Aurantiochytrium* sp. strain 4W-1b. Procedia Environ. Sci. 15, 27–33.

Perveen, Z., Ando, H., Ueno, A., Ito, Y., Yamamoto, Y., Yamada, Y., Takagi, T., Kaneko, T., Kogame, K., Okuyama, H., 2006. Isolation and characterization of a novel thraustochytrid-like microorganism that efficiently produces docosahexaenoic acid. Biotechnol. Lett. 28, 197–202.

Raikar, M., Raghukumar, S., Vani, V., David, J.J., Chandramohan, D., 2001. Thraustochytrid protists degrade hydrocarbons. Indian J. Mar. Sci. 30, 139–145.

Raghukumar, S., 2002. Ecology of the marine portists, the Labyrinthulomycetes (thraustochytrids and labyrinthulids). Eur. J. Protistol. 38, 127–145.

Raghukumar, S., 2008. Thraustochytrid marine Protists: production of PUFAs and other emerging technologies. Mar. Biotechnol. 10, 631–640.

Raghukumar, S., Sharma, S., Raghukumar, C., Sathe-Pathak, V., 1994. Thraustochytrid and fungal component of marine detritus. IV. Laboratory studies on decomposition of leaves of the mangrove *Rhizophora apiculata* Blume. J. Exp. Mar. Biol. Ecol. 183, 113–131.

Scharer, L., Knoflach, D., Vizoso, D.B., Rieger, G., Peintner, U., 2007. Thraustochytrids as novel parasitic protists of marine free-living flatworms: *Thraustochytrium caudivorum* sp. nov. parasitizes *Macrostomum lignano*. Mar. Biol. 152, 1095–1104.

Shene, C., Leyton, A., Esparza, Y., Flores, L., Quilodrán, B., Hinzpeter, I., Rubilar, M., 2010. Microbial oils and fatty acids: effect of carbon source on docosahexaenoic acid (C22:6 n-3, DHA) production by Thraustochytrid strains. J. Soil Sci. Plant Nutr. 10, 207–216.

Shimizu, S., Kawashima, H., Shinmen, Y., Akitomo, K., Yamada, H., 1988. Production of eicosapentaenoic acid by *Mortierella* fungi. J. Am. Oil Chem. Soc. 65, 1455–1459.

Singh, A., Ward, O.P., 1997. Microbial production of docosahexaenoic acid (DHA, C22:6). In: Neidleman, S.L., Laskin, A.I. (Eds.), Advances in Applied Microbiology. Academic Press, New York, NY, USA.

Wong, M.K.M., Vrijmoed, L.P.P., Au, D.W.T., 2005. Abundance of thraustochytrids on fallen decaying leaves of *Kandelia candel* and mangrove sediment in Futian National Nature Reserve, China. Bot. Mar. 48, 374–378.

Yokochi, T., Honda, D., Higashihara, T., Nakahara, T., 1998. Optimization of docosahaexaenoic acid production by *Schizochytrium limacinum* SR21. Appl. Microbiol. Biotechnol. 49, 72–76.

Yokoyama, R., Honda, Y., 2007. Taxonomic rearrangement of the genus *Schizochytrium sensu lato* based on morphology, chemotaxonomic characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomycetes): emendation for *Schizochytrium* and erection of *Aurantiochytrium* and *Oblongichytrium* gen. nov. Mycoscience 48, 199–211.

Yokoyama, R., Salleh, B., Honda, Y., 2007. Taxonomic rearrangement of the genus *Ulkenia sensu lato* based on morphology, chemotaxonomical characteristics, and 18S rRNA gene phylogeny (Thraustochytriaceae, Labyrinthulomycetes): emendation for *Ulkenia* and erection of *Botryochytrium*, *Parietichytrium*, and *Sicyoidochytrium* gen. nov. Mycoscience 48, 329–341.