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^[19] Single cell oil of oleaginous marine microbes from Saudi Arabian mangroves as a potential
feedstock for biodiesel production

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Abstract

This study aims to explore microbes from mangrove in Saudi Arabia for their abilities to produce high level of lipids. Mangroves are seldom investigated for oleaginous microbes.^[23] A total of 961 isolates of yeasts and filamentous fungi were isolated from 144 submerged marine samples include: 68 decaying leaves of *Avicennia marina*, 33 decaying thalli of *Zostera marina*, 14 decaying pneumatophores of *Avicennia marina*, 9 crab shells, 8 sediment, 7 decaying thalli of *Turbinaria ornata* and 5 decaying thalli of *Cystoseira myrica*.^[9] Samples were collected from four mangrove sites:^[9] Al-Leith, Jeddah and Yanbu at the Red Sea coast and the Syhat mangroves at the Arabian Gulf coast. Isolated fungi were grouped into 62 morphological types that include: 21 yeasts and 41 filamentous fungi. Fifty-four isolates of thraustochytrids were cultured from the four mangrove sites and were grouped into 22 strains. Two oleaginous yeasts: *Hortaea werneckii* (AL-19) and *Rhodotorula mucilaginosa* (AL-14) and four *Aurantiochytrium* strains: AL-22, AL-23, SY-78 and SY-85 produced high dry weight ranged between 32 and 49.3 g/L of which 35.2–62 % lipid and their fatty acid profile were determined using GC/MS.^[11] Palmitic acid was the major fatty acid in the lipid of the four thraustochytrid isolates and ranged between 5.71 to 82% of the total fatty acids, while it was not recorded from the lipid of the two yeast isolates, 9-Octadecenamide, (Z)- was the major fatty acid amide in the lipid of the two yeast isolates and two thraustochytrid isolates and ranged between 26.94 and 56.63%, followed by 13-Docosenamide, (Z)- (20.44–34.99%) from the same four isolates. Other major lipid compounds were: Hexadecanamide (4.35–7.19 %), Cholesterol (7.24–15.07 %), Butylated Hydroxytoluene (3.46–15.76 %), Octadecanamide (3.95–7.9%), Phenol, 2-(1,1-dimethylethyl)-5-methyl- (1.78–10.33%) and Pentadecanoic acid (7.47%).

Keywords: Heterokonta; filamentous fungi; Labyrinthulomycetes, molecular phylogenetics; subtropics; red yeasts.

1. Introduction

Fungi as a source of oil has advantages over conventional plant and algal resources as they can be easily grown in bioreactors, have short life cycles, display rapid growth rates, are unaffected by space, light or climatic variations. Fungi are easier to scale up and can be grown on a wide range of inexpensive renewable carbon sources, e.g. lignocellulosic biomass and agro industrial residue (Khot et al., 2012). Major efforts have been made to replace fossil fuels that has had serious environmental issues especially the increased greenhouse effect through the emission of CO₂ and NO_x gasses that consequently rise global temperature. Combustion of fossil fuels has contributed to the acidification of the oceans that significantly changes marine life.

^[46] Biofuels are an attractive alternative as they can be used as transportation fuels with little change to the current technologies (Carere et al., 2008). Liquid transportation biofuels include: bioethanol and biodiesel (Demirbas, 2011). Most vehicles can use from 10% to 85 % ethanol blends for fuels. Bioethanol is produced by fermentation of corn glucose in the United States or sucrose in Brazil (Rosillo-Calle and Cortez, 1998). The International Energy Agency expects that biofuels will contribute 6% of total fuel use by 2030.

Recently lipids synthesis by oleaginous fungi from raw cheap materials has received great attention (Sergeeva et al., 2008; Zheng et al., 2012; Kyung et al., 2013; Venkata et al., 2014; Venkata Subhash and Venkata Mohan, 2014; Ranjan, 2015; Yang et al., 2014).^[7] Those produced lipids can be used for biodiesel production (Economou et al., 2011; Papanikolaou et al., 2011). Fungal lipids have advantages over algal lipids because fungi are fast growing with short span, light independent and can degrade a wide range of carbon sources (Pant and Adholeya, 2010; Yousuf et al., 2010; Chen et al., 2012). Lipid production by oleaginous fungi using renewable carbon sources such as: glycerol, sewage water, whey and molasses were reported (Easterling et al., 2009; Chtzifragkou et al., 2010; Subramaniam et al., 2010; Bellou et al., 2012; Peng et al., 2013). Oleaginous microorganisms can convert a wide variety of carbon sources into stored lipids (Ratledge, 2004). Vegetable oils, animal fats and waste cooking oils

were traditionally used for biodiesel production (Venkata Subhash and Venkata Mohan, 2014). However, those traditional sources are unsustainable and expensive.

Thraustochytrids are oleaginous microorganisms that can utilize a wide range of substrates including: glucose, galactose, fructose, mannose, sucrose (Yokochi et al., 1998; Shene et al., 2010), complex organic matter (Bongiorni et al., 2005) and cellulosic biomass (Hong et al.^[3], 2012), for the production of polyunsaturated fatty acids (PUFAs)^[6]. Thraustochytrids are lipid rich biomass that can be used for biodiesel and PUFA production (Johnson and Wen, 2009; Gupta, 2012; Abdel-Wahab et al., 2021a,b, 2022).

^[10] 2. Materials and methods

^[9] 2.1. Sampling sites and sample collection

One hundred and forty-four samples were collected from four mangrove sites along the Red Sea and the Arabian Gulf in Saudi Arabia namely: Al-Leith (20° 49' 10" N 39° 27' 26" E), Jeddah (22° 46' 32" N 39° 48' 25" E), Syhat (26° 29' 32" N 50° 02' 46" E) and Yanbu (24°02.55' N 38°68.89' E). Collected samples included: 68 decaying leaves of *Avicennia marina*, 33 decaying thalli of *Zostera marina*, 14 decaying pneumatophores of *Avicennia marina*, 9 crab shells, 8 sediment, 7 decaying thalli of *Turbinaria ornata* and 5 decaying thalli of *Cystoseira myrica*.

2.2. Isolation of filamentous fungi, yeasts and thraustochytrids

Decaying leaves and pneumatophores of *Avicennia marina* and decaying thalli of seaweeds were placed in clean plastic bags and brought to the laboratory on the same day. In the laboratory, samples were washed using sterile sea water, cut into small segments (ca 1 cm in length), surface sterilized using 5% sodium hypochlorite for 1 min, transferred into sterile sea water (1 min), surface dried using sterile filter paper and placed on the surface of GYPTA medium (1 g glucose, 1 g yeast extract, 1 g peptone, 10 g tomato juice, 2 g monosodium glutamate, 1 g tween 80, 0.2 g KH₂PO₄, 0.5 g chloramphenicol, 15 g agar in 1 l of half-strength natural seawater). Plates were incubated at 25 °C and examined every 24 h. Microbial growth were transferred to new plates and further purified until axenic cultures were obtained.

Pure cultures were maintained on GYP slants (30 g glucose, 5 g yeast extract, 10 g peptone in 1 L of 50% seawater) and re-subculture every two months. We also preserved them by cryopreservation at -80°C and re-subculture every 4 months.

2.3. Sudan Black B test of the isolated microbes

The 84 morphological strains isolated during the present study were tested for their abilities to produce high level of oil using Sudan Black B. Positive strains for oil production were further grown on a larger scale and the percentage of oil were determined using Sulfo-Phospho-Vanlin (SPV) method.

2.4. Quantification of microbial lipids using sulfo-phospho-vanillin (SPV) method

Phosphovanillin (PV) reagent was prepared by dissolving six milligrams of vanillin in 100 mL of hot water and further diluted to 500 mL with phosphoric acid. Oleic and palmitic acids were used as standard and diluted properly with concentrated sulfuric acid to reach 1 mg/mL. Reagents (oleic, palmitic, phosphoric and sulfuric acids and vanillin) were purchased from local company. Twenty-five mg of microbial cells were dissolved in one mL concentrated sulfuric acid, of which 20 µL of the samples were diluted in 180 µL of concentrated sulfuric acid, incubated at 100 °C for 10 min. Samples were cooled to room temperature and 0.5 mL of phosphovanillin reagent was added, incubated at 37 °C for 15 min, cooled to room temperature and stored for 45 min in a dark box for color development. The absorbance was measured at 530 nm in a spectrophotometer (Anschau et al., 2017, Abdel-Wahab et al. 2022).

2.5. Lipid extraction and analysis of fatty acids

Biomass and their dry weights were calculated and the lipid was extracted as previously described by Abdel-Wahab et al. (2022). Fatty acids profile of the extracted lipid was determined using methyl esters boron tri-fluoride method (Folch et al., 1957; AOAC, 2012; Abdel-Wahab et al., 2021a, 2022).

2.6. ^[32] DNA sequencing and phylogenetic analyses

Pure microbial strains were grown in liquid medium and DNA was extracted using MOBIO microbial DNA extraction kit according to the manufacturer's instructions. ITS region was amplified and sequenced using ITS1 and ITS4 primers. Partial SSU and LSU rDNA were amplified and sequenced using primers pairs NS1/NS4 and LROR/LR7 respectively (White et al., 1990; Bunyard et al., 1994).

PCR amplification and DNA sequencing were carried out by SolGent Inc., South Korea. The obtained sequences were deposited in GenBank (Figs. 2–3, 5). Sequences were aligned using ClustalX (Thompson et al., 1997). Phylogenetic analyses were carried out using MEGA X (Kumar et al., 2018). Bayesian phylogenetic analysis was performed using MrBayes 3.1.2^[3] (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). MrModeltest 2.2 was used to determine the best-fit model for the sequences dataset (Nylander, 2004). The phylogenetic tree in Figure 2 was visualized using Njplot (Perrière and Gouy, 1996) and edited using Adobe Illustrator CS6.

3. Results and discussion

3.1. Diversity of oleaginous filamentous fungi and yeasts from Saudi mangroves

A total of 961 isolates (include 759 filamentous fungi and 202 yeasts) were isolated from 144 submerged marine samples that include: 68 decaying leaves of *Avicennia marina*, 33 decaying thalli of *Zostera marina*, 14 decaying pneumatophores of *Avicennia marina*, 9 crab shells, 8 sediment, 7 decaying thalli of *Turbinaria ornata* and 5 decaying thalli of *Cystoseira myrica*. Samples were collected from four mangrove sites:^[9] Al-Leith, Jeddah and Yanbu (Red Sea) and Syhat (Arabian Gulf). Isolated fungi were grouped into 62 morphological types that include: 21 yeasts and 41 filamentous fungi. *Aspergillus* and *Penicillium* species were the most common fungi and represented by 337 and 330 isolates respectively. Other recorded genera were: *Cladosporium*, *Lasioidiplodia*, *Mortierella* and *Rhizopus*. Forty-three isolates did not fruit in culture and were grouped into ten morphological types (Table 1).

The 202 isolates of yeasts were cultured from 48 samples of decaying leaves of *Avicennia marina* and seaweeds. The yeast isolates were grouped into 21 morphological types, of which four morphological types were identified using molecular phylogenetics of ribosomal genes.

3.2. Diversity of oleaginous thraustochytrids from the Saudi mangroves

Fifty-four isolates of thraustochytrids were cultured from the four mangrove sites namely: Al-Leith, Jeddah and Yanbu (Red Sea) and Syhat (Arabian Gulf). Isolated thraustochytrids were grouped into 22 strains.^[0] Isolated thraustochytrids belonged to two genera: *Aurantiochytrium* and *Thraustochytrium*

(Table 2). Thraustochytrids that produced high biomass and high levels of lipid were chosen for further study. Selected strains were grown on a larger volume of culture media and their lipid were extracted and the fatty acids and their derivatives were determined using GC/MS.

3.3. Molecular phylogenetics of yeasts and thraustochytrids

Four oleaginous yeast strains (AL-14, AL-15, AL-19 and AL-20) were selected and their taxonomical placements were determined based on LSU rDNA and ITS and were placed in *Rhodotorula mucilaginosa* and *Hortaea werneckii* (Table 1, Figures 1-3). DNA was extracted from the most promising thraustochytrid isolates (5 strains: AL-22, AL-23, SY-78, SY-85 and YB-39) and their taxonomical placements were determined based on ITS sequences. Thraustochytrids from the Arabian Gulf and the Red Sea formed a separate node basal to other known *Aurantiocytrium* species and might represent undescribed taxa (Figures 4-5).

3.4. Lipid profile of yeasts and thraustochytrid strains

Two oleaginous yeasts: *Hortaea werneckii* (Horta) Nishim. & Miyaji (AL-19) and *Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison (AL-14) and four *Aurantiocytrium* strains: AL-22, AL-23, SY-78 and SY-85 produced high dry weight ranged between 32 and 49.3 g/L of which 35.2-62 % lipid. These promising results can be improved by changing the growth conditions using different combinations of different C/N ratio. Fatty acid profiles of the six strains on GC/MS were determined. Palmitic acid was the major fatty acid in the lipid of the four thraustochytrid isolates and ranged between 5.71 to 82% of the total fatty acids, while it was not recorded from the lipid of the two yeast isolates, 9-Octadecenamide, (Z)- was the major fatty acid amide in the lipid of the two isolates and two thraustochytrid isolates and ranged between 26.94 and 56.63%, followed by 13-Docosenamide, (Z)- (20.44-34.99%) from the same four isolates. Other major lipid compounds were: Hexadecanamide (4.35-7.19 %), Cholesterol (7.24-15.07 %), Butylated Hydroxytoluene (3.46-15.76 %), Octadecanamide (3.95-7.9%), Phenol, 2-(1,1-dimethylethyl)-5-methyl- (1.78-10.33%) and Pentadecanoic acid (7.47%) (Tables 3).

4. Discussion

Palmitic acid was the major fatty acid in the four studied strains of thraustochytrids and ranged between 5.71 to 82.02% of the total fatty acids.^[17] The acid was previously reported in high levels from *Aurantiochytrium* species (Nagano et al., 2009; Ramos et al., 2009). Palmitic acid produced by thraustochytrids can be used to produce high quality biodiesel due to its high octane number, low iodine content and high oxidation stability. Genera of thraustochytrids were reported to accumulate commercially and biologically important fatty acids (Huang et al., 2003;^[3] Ward and Singh, 2005; Chang et al., 2014).^[27] The major fatty acids of thraustochytrids are palmitic acid C16, arachidonic acid C20:^[3]4, eicosapentaenoic acid C20:^[3]5, docosapentaenoic acid C22:^[3]5, and docosahexaenoic acid C22:6 (DHA) (Kobayashi et al., 2011). Among these fatty acids, palmitic acid has been reported to be potential alternative materials for microbial-derived biodiesel (Kobayashi et al., 2011). Velmurugan et al. (2021) studied the fatty acids composition of two strains of *Aurantiochytrium* cultured from Taiwan mangroves. Major fatty acids reported were the saturated acids: pentadecanoic (15:0), palmitic (C16:0), and unsaturated DHA (C22:6) which is consistent with previous results (Liu et al., 2014; Ludevese-Pascual et al., 2016; Jaritkhuan and Suanjit, 2018). Bai et al.^[5] (2022) isolated 58 thraustochytrid strains isolated from the coastal waters of Qingdao, China and studied their fatty acid profile. Isolated strains were phylogenetically affiliated with the genera:^[5] *Botryochytrium*, *Oblongichytrium*, *Schizochytrium*, *Thraustochytrium*, and *Sicyoidochytrium*.^[5] Palmitic acid was the most abundant fatty acid in the studied 58 strains and represented 7.90–37.12% of the total fatty acids.

Oleaginous yeast and thraustochytrid strains produced 9-Octadecenamide, (Z)- as the major fatty acid derivative (ranged between 26.94 and 56.63 % of the total fatty acids). 9-Octadecenamide, (Z)- (Oleamide) is the amid of Oleic acid and was first detected in human plasma and was shown to induce sleep in animals (Cravatt et al., 1995; Mckinney and Cravatt, 2005).^[30] Oleamide can be used for the treatment of mood and sleep disorders (Mechoulam et al., 1997). The third major fatty acid derivative was 13-Docosenamide, (Z)- (ranged between 20.44 and 34.99 % of the total fatty acids) and recorded from both yeasts and thraustochytrids.^[31] 13-Docosenamide is the amide of docosenoic acid (Erucic acid) and it was first identified from cerebrospinal fluid of sleep-deprived cats and from cerebrospinal fluid of rats

and humans.^[31] 13-Docosenamide causes reduced mobility and slightly lessened awareness in rats (Cravatt et al., 1995; Li et al., 2017). Both 9-Octadecenamide, (Z)- and 13-Docosenamide, (Z)- have antimicrobial and anticancer activities (Tayung et al., 2011; Sharma et al., 2019).

5.^[1] Conclusion

Microbes (filamentous fungi, yeasts and thraustochytrids) isolated from four mangrove sites along the coasts of the Arabian Gulf and the Red Sea in Saudi Arabia were screened for their abilities to produce high level of lipids. Isolated fungi were grouped into 62 morphological types that include: 21 yeasts and 41 filamentous fungi. Also, fifty-four isolates of thraustochytrids were cultured from the four mangrove sites and were grouped into 22 strains. The 84 morphological strains isolated during the present study were tested for their abilities to produce high level of oil using Sudan Black B. Positive strains for oil production were further grown on a larger scale and the percentage of oil were determined using Sulfo-Phospho-Vanlin (SPV) method. Two oleaginous yeasts: *Hortaea werneckii* and *Rhodotorula mucilaginosa* and four *Aurantiochytrium* strains produced high dry weight ranged between 32 and 49.3 g/L of which 35.2–62 % lipid and their fatty acid profile were determined using GC/MS. Palmitic acid was the major fatty acid in the lipid of the four thraustochytrid isolates and ranged between 5.71 to 82% of the total fatty acids, while it was not recorded from the lipid of the two yeast isolates, 9-Octadecenamide, (Z)- was the major fatty acid amide in the lipid of the two yeast isolates and two thraustochytrid isolates and ranged between 26.94 and 56.63%, followed by 13-Docosenamide, (Z)- (20.44–34.99%) from the same four isolates. Other major lipid compounds were: Hexadecanamide, Cholesterol, Butylated Hydroxytoluene, Octadecanamide, Phenol, 2-(1,1-dimethylethyl)-5-methyl and Pentadecanoic acid.

^[62] Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Fig. 1. a–f *Hortaea werneckii* (AL–19) isolated from decaying leaves of *Avicennia marina*, Al-Leith mangroves, Red Sea. Various shaped vegetative cells with or without buds and pseudo-hyphae. g–i *Rhodotorula mucilaginosa* (AL–14) isolated from decaying leaves of *Avicennia marina*, Al-Leith mangroves, Red Sea. g–i Vegetative cells with or without buds. Bars: a–i = 5 μm .

Fig. 2. Phylogenetic relationship of *Hortaea werneckii* strains (AL–19 and AL–20) isolated during the current study. Phylogenetic analyses based on the nucleotide sequences of the LSU rDNA placed the two strains among the other strains of the species. The maximum likelihood (ML) tree (-ln likelihood = 3611.05) was constructed in MEGA X (Kumar et al., 2018). Phylogenetic trees obtained from ML, maximum parsimony (MP) and Bayesian inference posterior probabilities (BYPP) were similar in topology. Bootstrap support on the nodes represents ML and MP $\geq 50\%$. Branches with a BYPP of $\geq 95\%$ are in bold. The two sequences of *Hortaea werneckii* generated in this study are in green, previous strains of the species from Red Sea are in red.

Fig. 3. Phylogenetic relationship of *Rhodotorula mucilaginosa* strains (AL–14 and AL–15) isolated during the current study. Phylogenetic analyses based on the nucleotide sequences of the LSU rDNA placed the two strains among the other strains of the species. The maximum likelihood (ML) tree (-ln likelihood = 2318.03) was constructed in MEGA X (Kumar et al., 2018). The maximum parsimonious data set consisted of 29 taxa include: 18 sequences of *Rhodotorula mucilaginosa* strains, 4 sequences of other *Rhodotorula*, 5 sequences of other taxa of Sporidiobolaceae and two taxa belong to Chrysozymaceae (outgroup). Phylogenetic trees obtained from ML, maximum parsimony (MP) and Bayesian inference posterior probabilities (BYPP) were similar in topology. Bootstrap support on the nodes represents ML and MP $\geq 50\%$. Branches with a BYPP of $\geq 95\%$ are in bold. The two sequences of *Rhodotorula mucilaginosa* generated in this study are in red.

Fig. 4. *Aurantiochytrium* spp. isolated from decaying leaves of *Avicennia marina* from Syhat mangroves. a–c *Aurantiochytrium* sp. (SY–78) Various shaped sporangia. d–g *Aurantiochytrium* sp. (SY–85). d–e Various shaped sporangia. f–g Zoospores. Bars: a–g = 5 μm .

Fig. 5. Phylogenetic relationship of oleaginous thraustochytrid strains (AL–22, AL–23, SY–78, SY–85 and YB–39) isolated during the current study from Al-Leith, Syhat and Yanbu mangroves. Phylogenetic analyses based on the nucleotide sequences of the ITS region placed the five strains in a well-supported node basal to other strains of the genus *Aurantiochytrium*. The maximum likelihood (ML) tree (-ln likelihood = 1185.76) was constructed in MEGA X (Kumar et al., 2018). Phylogenetic trees obtained from ML, maximum parsimony (MP) and Bayesian inference posterior probabilities (BYPP) were similar in topology. Bootstrap support on the nodes represents ML and MP $\geq 50\%$. Branches with a BYPP of $\geq 95\%$ are in bold. The newly generated sequences in this study are in red.

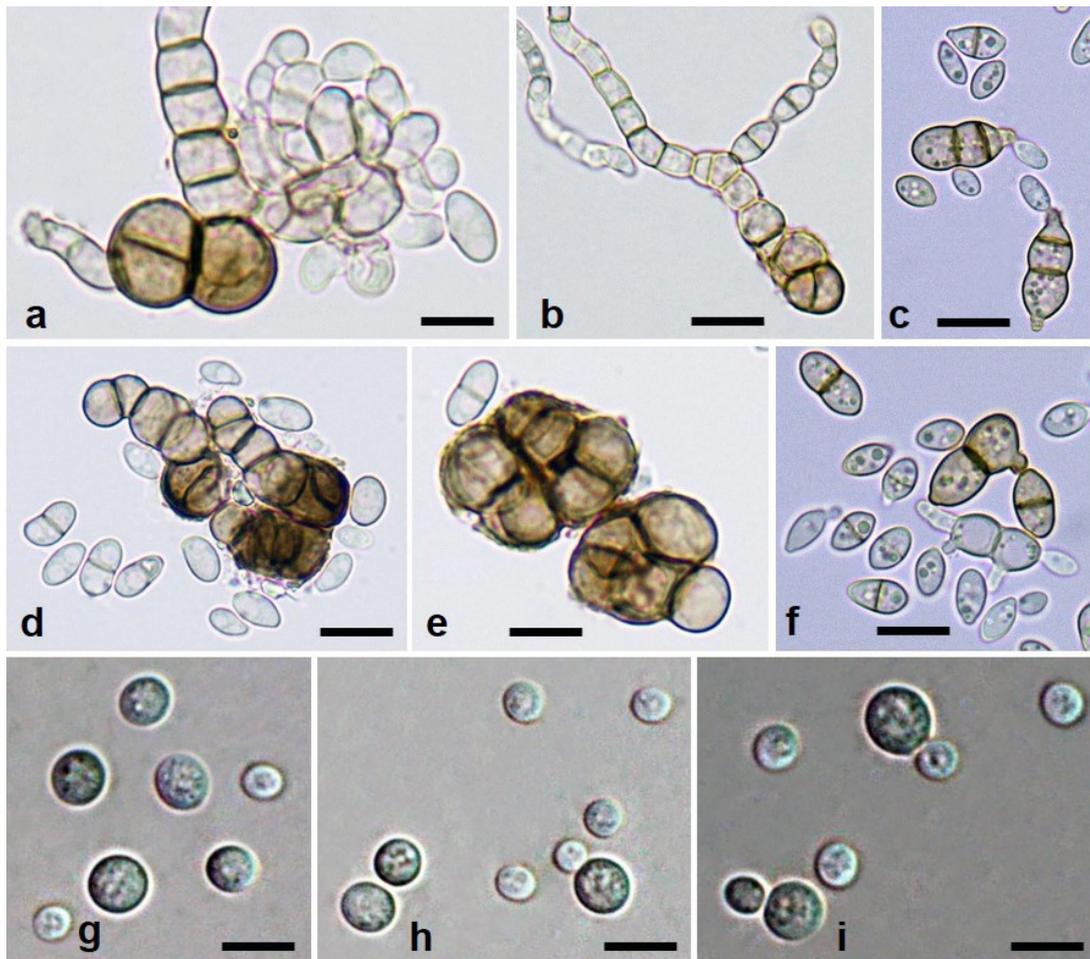


Fig. 1. a–f *Hortaeta werneckii* (AL-19) isolated from decaying leaves of *Avicennia marina*, Al-Leith mangroves, Red Sea. Various shaped vegetative cells with or without buds and pseudo-hyphae. g–i *Rhodotorula mucilaginosa* (AL-14) isolated from decaying leaves of *Avicennia marina*, Al-Leith mangroves, Red Sea. g–i Vegetative cells with or without buds. Bars: a–i = 5 µm.

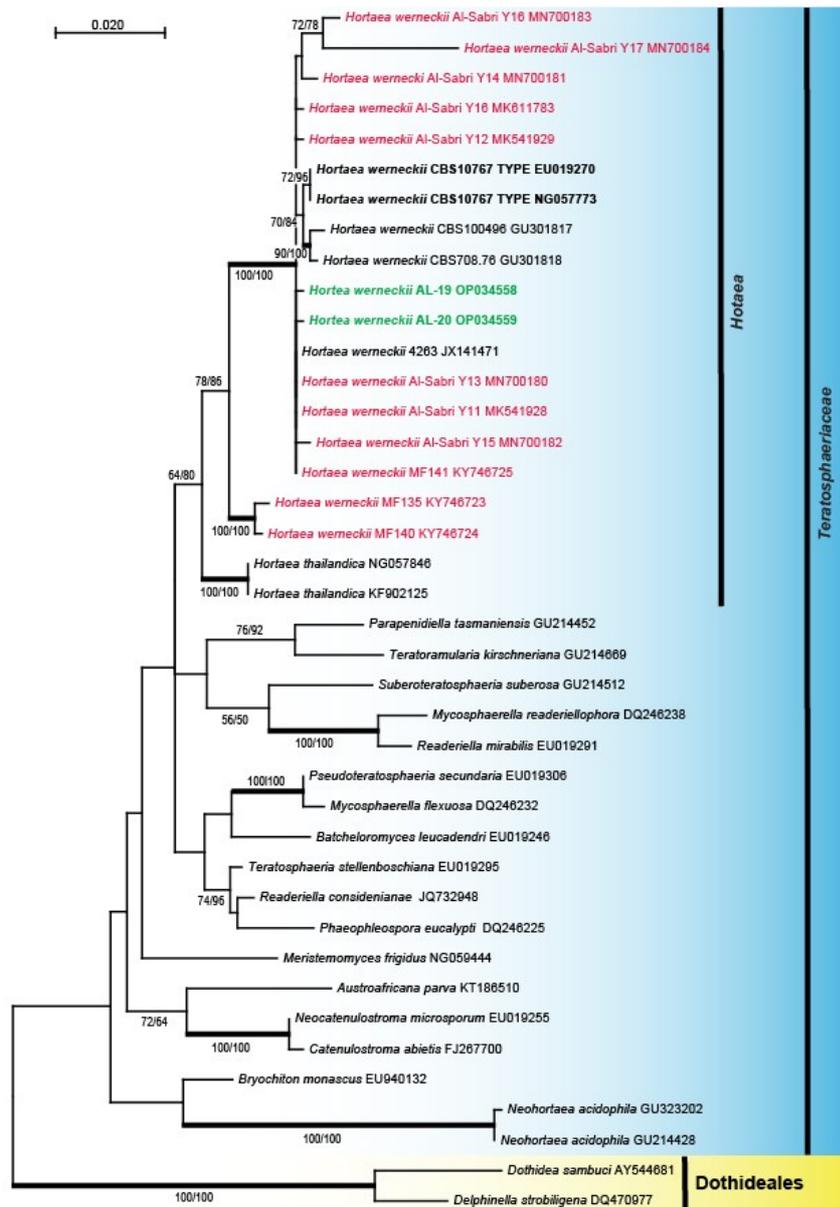


Fig. 2. Phylogenetic relationship of *Hortaea werneckii* strains (AL-19 and AL-20) isolated during the current study. Phylogenetic analyses based on the nucleotide sequences of the LSU rDNA placed the two strains among the other strains of the species. The maximum likelihood (ML) tree (-ln likelihood = 3611.05) was constructed in MEGA X (Kumar et al., 2018). Phylogenetic trees obtained from ML, maximum parsimony (MP) and Bayesian inference posterior probabilities (BYPP) were similar in topology. Bootstrap support on the nodes represents ML and MP $\geq 50\%$. Branches with a BYPP of $\geq 95\%$ are in bold. The two sequences of *Hortaea werneckii* generated in this study are in green, previous strains of the species from Red Sea are in red.

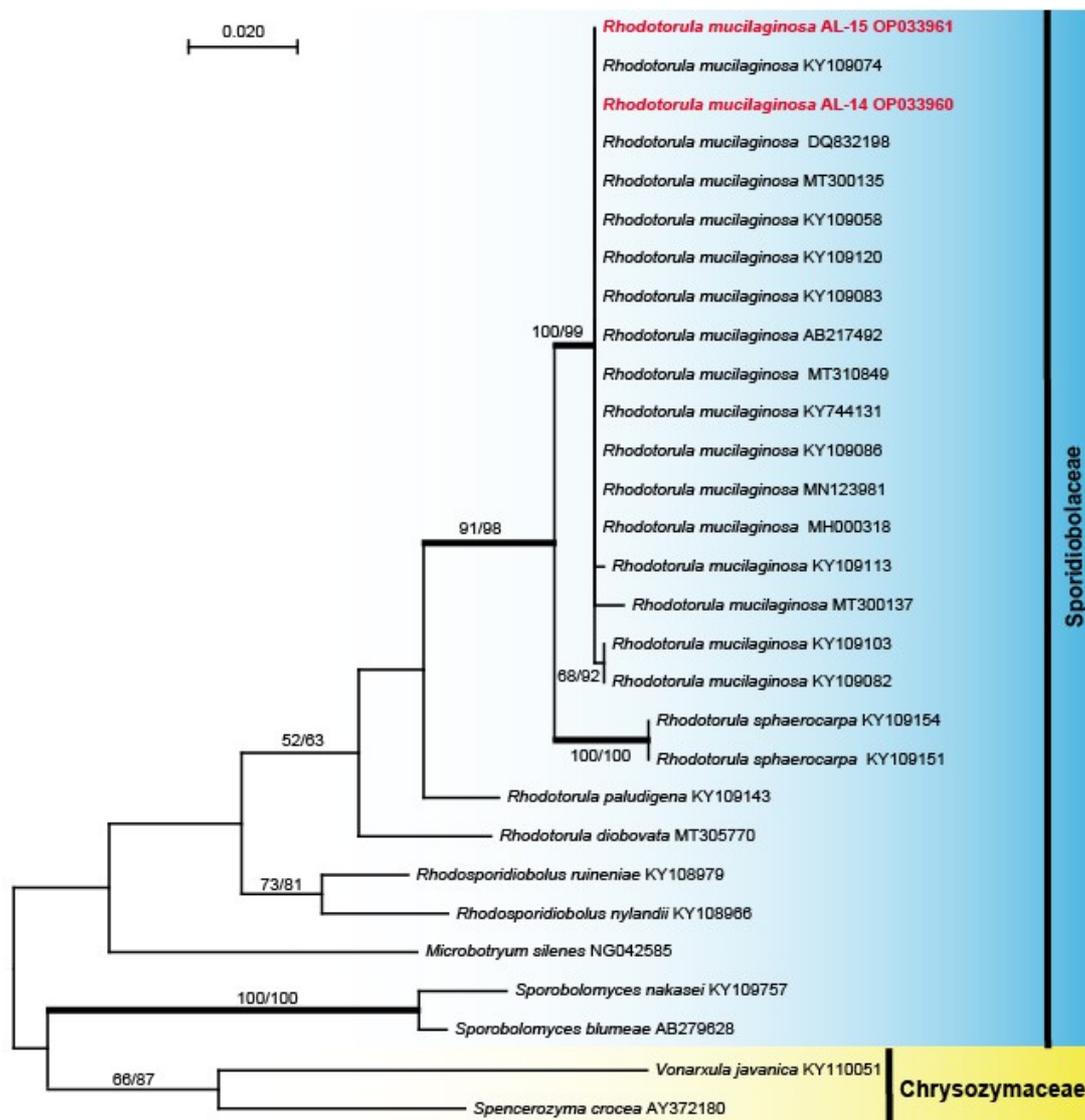


Fig. 3. Phylogenetic relationship of *Rhodotorula mucilaginosa* strains (AL-14 and AL-15) isolated during the current study. Phylogenetic analyses based on the nucleotide sequences of the LSU rDNA placed the two strains among the other strains of the species. The maximum likelihood (ML) tree (-ln likelihood = 2318.03) was constructed in MEGA X (Kumar et al., 2018). The maximum parsimonious data set consisted of 29 taxa include: 18 sequences of *Rhodotorula mucilaginosa* strains, 4 sequences of other *Rhodotorula*, 5 sequences of other taxa of Sporidiobolaceae and two taxa belong to Chrysozymaceae (outgroup). Phylogenetic trees obtained from ML, maximum parsimony (MP) and Bayesian inference posterior probabilities (BYPP) were similar in topology. Bootstrap support on the nodes represents ML and MP $\geq 50\%$. Branches with a BYPP of $\geq 95\%$ are in bold. The two sequences of *Rhodotorula mucilaginosa* generated in this study are in red.

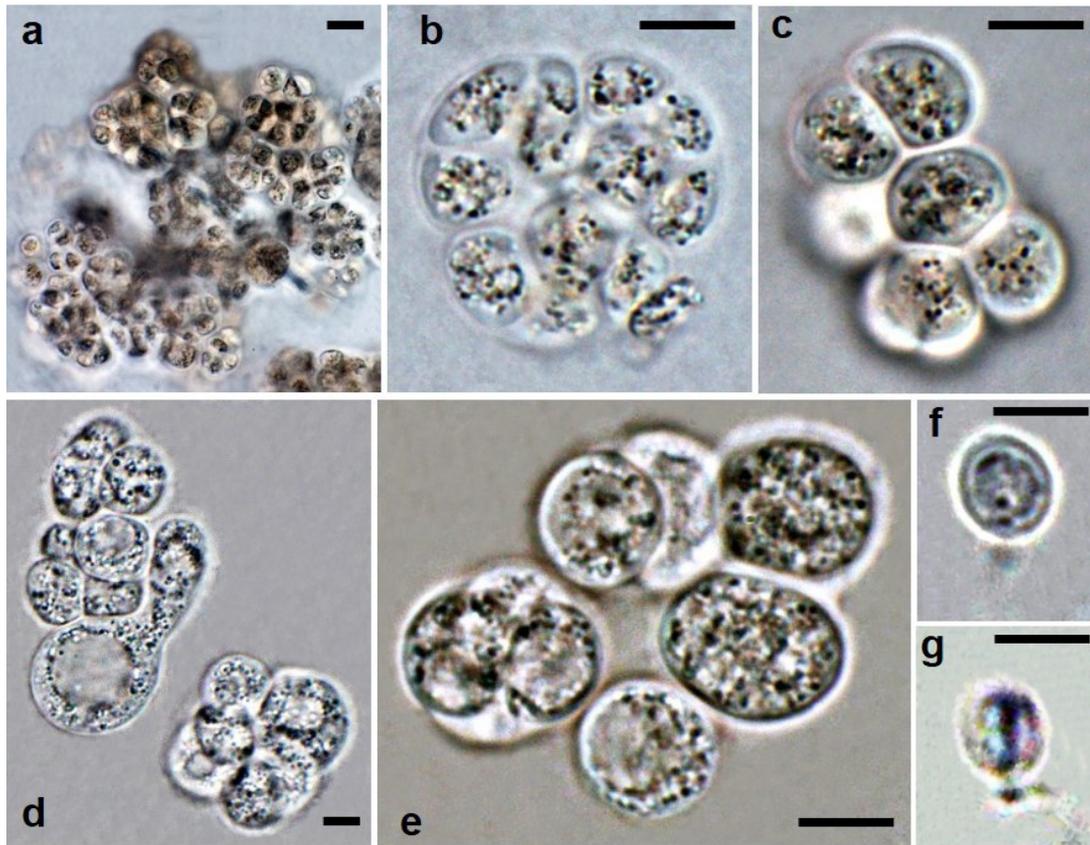


Fig. 4. *Aurantiochytrium* spp. isolated from decaying leaves of *Avicennia marina* from Syhat mangroves. a–c *Aurantiochytrium* sp. (SY–78) Variously shaped sporangia. d–g *Aurantiochytrium* sp. (SY–85). d–e Variously shaped sporangia. f–g Zoospores. Bars: a–g = 5 μ m.

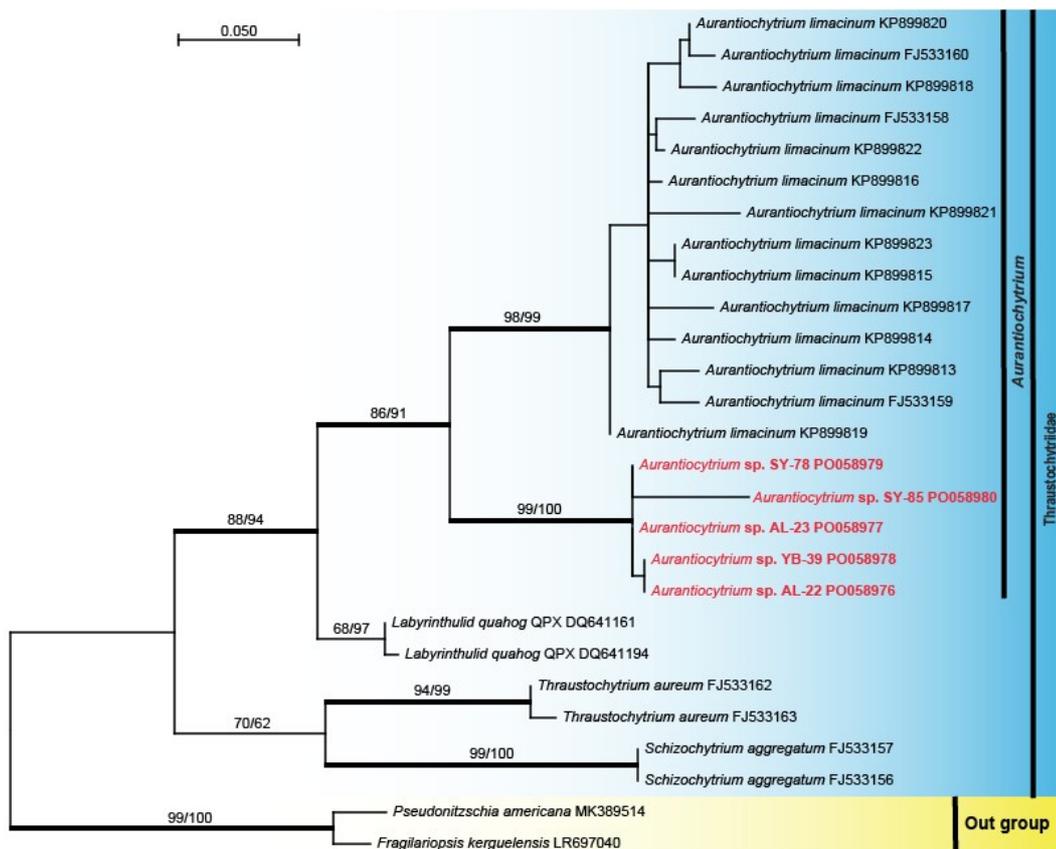


Fig. 5. Phylogenetic relationship of oleaginous thraustochytrid strains (AL-22, AL-23, SY-78, SY-85 and YB-39) isolated during the current study from Al-Leith, Syhat and Yanbu mangroves. Phylogenetic analyses based on the nucleotide sequences of the ITS region placed the five strains in a well-supported node basal to other strains of the genus *Aurantiochytrium*. The maximum likelihood (ML) tree (-ln likelihood =1185.76) was constructed in MEGA X (Kumar et al., 2018). Phylogenetic trees obtained from ML, maximum parsimony (MP) and Bayesian inference posterior probabilities (BYPP) were similar in topology. Bootstrap support on the nodes represents ML and MP $\geq 50\%$. Branches with a BYPP of $\geq 95\%$ are in bold. The newly generated sequences in this study are in red.

Table 1 Filamentous fungi and yeasts isolated from Saudi mangroves:

Strain No.	Microorganism name	No. of isolates	Substrate	Dry weight (g/L)	Lipid (% w/w)
#AL-14	<i>Rhodotorula mucilaginosa</i> (A. Jörg.) F.C. Harrison	4	Decaying thallus of <i>Padina pavonica</i>	36.2	35.2
#AL-15	<i>R. mucilaginosa</i>	4	Decaying thallus of <i>Zostera marina</i>	34.9	30.1
AL-16	Yeast	2	Decaying thallus of <i>Sargassum</i>		
AL-17	Yeast	2	Decaying thallus of <i>Sargassum platycarpum</i>		
AL-18	Yeast	1	Decaying thallus of <i>Sargassum platycarpum</i>		
#AL-19	<i>Hortaea werneckii</i> (Horta) Nishim. & Miyaji	10	Decaying leaves of <i>Avicennia marina</i>	42	46.2
#AL-20	<i>H. werneckii</i>	2	Decaying leaves of <i>Avicennia marina</i>	44.9	45
JD-11	Yeast	5	Decaying leaves of <i>Avicennia marina</i>		
JD-12	Yeast	5	Decaying leaves of <i>Avicennia marina</i>		
JD-13	Yeast	9	Decaying leaves of <i>Avicennia marina</i>		
JD-14	Sterile mycelium	7	Decaying leaves of <i>Avicennia marina</i>		
JD-15	Yeast	37	Decaying leaves of <i>Avicennia marina</i>		
JD-16	<i>Aspergillus</i> sp.	67	Decaying leaves of <i>Avicennia marina</i>		
JD-17	Yeast	5	Decaying thallus of <i>Zostera marina</i>		
JD-18	<i>Aspergillus flavus</i> Link	8	Decaying thallus of <i>Zostera marina</i>		
JD-19	Yeast	10	Decaying thallus of <i>Zostera marina</i>		
JD-20	Yeast	23	Decaying leaves of <i>Avicennia marina</i>		
JD-21	<i>Penicillium citrinum</i> Thom	15	Decaying leaves of <i>Avicennia marina</i>		
JD-22	Yeast	10	Decaying leaves of <i>Avicennia marina</i>		
JD-23	Yeast	12	Decaying leaves of <i>Avicennia marina</i>		
JD-24	Yeast	22	Decaying leaves of <i>Avicennia marina</i>		
JD-25	Sterile mycelium	9	Decaying leaves of <i>Avicennia marina</i>	42.7	19.25
JD-26	Yeast	11	Decaying leaves of <i>Avicennia marina</i>		
JD-27	Yeast	10	Decaying leaves of <i>Avicennia marina</i>		
JD-28	Sterile mycelium	8	Decaying leaves of <i>Avicennia marina</i>		
JD-29	<i>Cladosporium</i> sp.	12	Decaying thallus of <i>Zostera marina</i>		
JD-30	Yeast	11	Decaying leaves of <i>Avicennia marina</i>	;	;

Table 1 Cont.

JD-31	Penicillium sp.	6	Decaying leaves of Avicennia marina		
JD-32	Aspergillus terreus Thom	147	Sediment		
JD-33	Rhizopus sp.	2	Sediment	18.9	
JD-34	Mortierella sp.	6	Decaying leaves of Avicennia marina		
JD-35	Sterile mycelium	5	Decaying thallus of Sargassum platycarpum		
JD-36	Mortierella sp.	8	Sediment	23.1	
JD-37	Aspergillus sp.	21	Sediment		
JD-38	Aspergillus sp.	10	Decaying thallus of Sargassum platycarpum		
JD-39	Penicillium sp.	12	Decaying leaves of Avicennia marina		
#SY-69	Lasiodiplodia theobromae (Pat.) Griffon & Maubl.	3	Decaying leaves of Avicennia marina	48.2	34.1
#SY-70	Aspergillus sp.	7	Decaying leaves of Avicennia marina	43.1	48.23
SY-71	Rhizopus sp.	4	Decaying leaves of Avicennia marina	62.3	50.85
SY-72	Sterile mycelium	1	Decaying leaves of Avicennia marina		
SY-73	Sterile mycelium	1	Decaying wood of Avicennia marina		
SY-74	Penicillium sp.	3	Decaying leaves of Avicennia marina		
SY-75	Sterile mycelium	4	Decaying leaves of Zostera marina		
SY-76	Sterile mycelium	1	Decaying leaves of Avicennia marina		
YB-21	Yeast	7	Decaying leaves of Avicennia marina		
YB-22	Penicillium sp.	15	Decaying leaves of Avicennia marina		
YB-23	Sterile mycelium	2	Sediment		
YB-24	Penicillium sp.	15	Decaying thallus of Zostera marina		
YB-25	Penicillium sp.	21	Decaying thallus of Turbinaria ornata		
YB-26	Aspergillus sp.	6	Decaying thallus of Cystoseira myrica	21.6	
YB-27	Penicillium sp.	10	Decaying leaves of Avicennia marina		
YB-28	Penicillium sp.	9	Decaying leaves of Avicennia marina		
YB-29	Aspergillus sp.	13	Decaying thallus of Zostera marina		
YB-30	Aspergillus sp.	30	Sediment		
YB-31	Aspergillus sp.	25	Decaying pneumatophores of Avicennia marina	16.4	26.86
YB-32	Penicillium sp.	35	Decaying leaves of Avicennia marina	;	;

Table 1 Cont.

YB-33	Penicillium sp.	55	Crab shell	16.3	30.54
YB-34	Penicillium sp.	60	Sediment		
YB-35	Penicillium sp.	55	Sediment		
YB-36	Aspergillus niger Tiegh.	16	Decaying leaves of Avicennia marina		
YB-37	Sterile mycelium	5	Decaying leaves of Avicennia marina	7.1	
YB-38	Penicillium sp. 20		Decaying leaves of Avicennia marina		21.
				3	;

AL: Al-Leith mangroves, Al-Leith City, Re Sea, Saudi Arabia.

JD: Jeddah mangroves, Jeddah City, Red Sea, Saudi Arabia.

SY: ¹⁰Syhat mangroves, Dammam city, Arabian Gulf, Saudi Arabia.

YB: Yanbu mangroves, Yanbu City, Red Sea, Saudi Arabia.

#Supported by molecular data.

Studied strains in bold.

Table 2 Thraustochytrids isolated from Saudi mangroves:

Strain No.	Microorganism name	No. of isolates	Substrate	Dry weight (g/L)	Lipid (% w/w)
AL-21	Aurantiochytrium sp.	3	Decaying leaves of <i>A. marina</i>		
#AL-22	Aurantiochytrium sp.	2	Decaying leaves of <i>A. marina</i>	46	62
#AL-23	Aurantiochytrium sp.	4	Decaying leaves of <i>A. marina</i>	32	44.8
AL-24	Thraustochytrium sp.	1	Decaying leaves of <i>A. marina</i>		
AL-25	Thraustochytrium sp.	2	Decaying leaves of <i>A. marina</i>		
AL-26	Aurantiochytrium sp.	2	Decaying leaves of <i>A. marina</i>	50.1	66.5
AL-27	Aurantiochytrium sp.	7	Decaying leaves of <i>A. marina</i>	43	52
AL-28	Aurantiochytrium sp.	1	Decaying leaves of <i>A. marina</i>	38	54.1
AL-29	Aurantiochytrium sp.	1	Decaying leaves of <i>A. marina</i>	43	57
AL-30	Aurantiochytrium sp.	4	Decaying leaves of <i>A. marina</i>		
JD-40	Aurantiochytrium sp.	6	Decaying leaves of <i>A. marina</i>	48.2	71.42
SY-77	Aurantiochytrium sp.	1	Decaying leaves of <i>A. marina</i>	16.1	38.3
#SY-78	Aurantiochytrium sp.	3	Decaying leaves of <i>A. marina</i>	32	44
SY-79	Aurantiochytrium sp.	1	Decaying leaves of <i>A. marina</i>	26.6	47.2
SY-80	Aurantiochytrium sp.	1	Decaying leaves of <i>A. marina</i>	21.8	39
SY-81	Aurantiochytrium sp.	3	Decaying leaves of <i>A. marina</i>	28	49
SY-82	Aurantiochytrium sp.	1	Decaying leaves of <i>A. marina</i>	13.4	36
SY-83	Aurantiochytrium sp.	4	Decaying leaves of <i>A. marina</i>		
SY-84	Aurantiochytrium sp.	2	Decaying leaves of <i>A. marina</i>	28.9	46.1
#SY-85	Aurantiochytrium sp.	1	Decaying thalli of <i>Zostera marina</i>	49.3	59.9
#YB-39	Aurantiochytrium sp.	3	Decaying leaves of <i>A. marina</i>	49.9	64.96
YB-40	Aurantiochytrium sp.	1	Decaying leaves of <i>A. marina</i>	52.8	60.39

AL: Al-Leith mangroves, Al-Leith City, Re Sea, Saudi Arabia.

JD: Jeddah mangroves, Jeddah City, Red Sea, Saudi Arabia.

SY: Syhat mangroves, Dammam city, Arabian Gulf, Saudi Arabia.

YB: Yanbu mangroves, Yanbu City, Red Sea, Saudi Arabia.

#Supported by molecular data.

Studied strains in bold.

Table 3 GC/MS analyses of fatty acids and their derivatives of promising strains:

Microbes/fatty acids (%)	Hortaea werneckii (AL-19)	Rhodotorula mucilaginosa (AL-14)	Aurantiochytrium sp. (AL-22)	Aurantiochytrium sp. (AL-23)	Aurantiochytrium sp. (SY-78)	Aurantiochytrium sp. (SY-85)
Palmitic acid (C ₁₆ H ₃₂ O ₂)	-	-	5.71	7.52	71.92	82.02
9-Octadecenamide, (Z)- (C ₁₈ H ₃₅ NO)	49.68	56.63	37.73	26.94	-	-
13-Docosenamide, (Z)- (C ₂₂ H ₄₃ NO)	27.87	34.99	24.32	20.44	-	-
Hexadecanamide (C ₁₆ H ₃₃ NO)	7.06	7.19	6.51	4.35	-	-
Cholesterol (C ₂₇ H ₄₆ O)	-	-	7.24	15.07	-	-
Butylated Hydroxytoluene (C ₁₅ H ₂₆ O)	-	-	-	-	15.76	3.46
Octadecanamide (C ₁₈ H ₃₇ NO)	7.9	-	4.08	3.95	-	-
Phenol, 2-(1,1-dimethylethyl)-5-methyl- (C ₁₁ H ₁₆ O)	-	-	-	-	1.78	10.33
Pentadecanoic acid (C ₁₅ H ₃₀ O ₂)	-	-	7.47	-	-	-
Stigmasterol (C ₂₈ H ₄₈ O)	-	-	2.23	4.36	-	-
1-Heptatriacotanol (C ₃₇ H ₇₆ O)	1.33	1.19	-	3.09	-	-
Methyl tetradecanoate (C ₁₅ H ₃₀ O ₂)	-	-	-	-	4.41	-

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