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Original article

Utilization of low-cost substrates for the production of high biomass, lipid and docosahexaenoic acid (DHA) using local native strain *Aurantiochytrium* sp. YB-05

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ABSTRACT

This study explores low-cost substrates for the production of high biomass, lipid and DHA by local native strain of thraustochytrid that was isolated from decaying leaves of *Avicennia marina* collected from Yanbu mangroves along the Red Sea coast of Saudi Arabia. Molecular phylogenetic analyses based on 18S rDNA sequences placed the thraustochytrid strain (YB-05) in the *Aurantiochytrium*. The studied strain was grown on three different types of raw material available in the local market and compared to pure glucose for high biomass, lipid and DHA production. *Aurantiochytrium* sp. YB-05 was grown on: date fruit, glycerol, cane molasses and pure glucose at four concentrations: 10, 20, 40 and 80 g/L. Produced dry cell weight increased as the carbon sources: date fruit, molasses and glucose increased from 10 to 80 g/L. Both date fruit and molasses gave a higher biomass than pure glucose and glycerol at 40 and 80 g/L concentration, while glucose gave higher biomass at 10 and 20 g/L than the other three carbon sources. Date fruit gave the highest biomass (42.6 g/L) at the 80 g/L concentration and also increased the level of DPA (decosapentaneoic acid) and DHA from 0.64 and 4.89% TFA (total fatty acids) with glucose to 7.13 and 24.85% with date fruit respectively. Ten g/L glycerol gave the highest percentage of lipids (85.6% of the biomass), followed by 40 g/L glucose (80.1%), 20 g/L glycerol (70%), 80 g/L glucose (59.3%) and 80 g/L date fruit (56.4%). The percentage of lipid in the biomass increased with increasing the weight of date fruit and molasses from 10 g/L (24.7% and 15.6% respectively) to 80 g/L (56.4% and 45.7% respectively). This study shed a light on the potential use of date fruits for the production of value-added products using microbes. Saudi Arabia is the second largest date producer in the world after Egypt. The present research also studied the effect of four types of oily seeds in a combination with date fruit on the production oil, DHA and the major fatty acids produced by *Aurantiochytrium* sp. YB-05. The fatty acid profile of *Aurantiochytrium* sp. YB-05 grown on date palm, pure glucose and seeds were determined.

1. Introduction

The genus *Aurantiochytrium* (Thraustochytriaceae, Stramenopiles) is widely distributed in the marine habitats especially mangroves with even higher biomass than bacteria (Marchan et al., 2018; Abdel-Wahab et al., 2021a,b). Species of the marine oleaginous protist *Aurantiochytrium* which have been used as important DHA producers for many years are the ideal substitute for the traditional fish oil resource (Yokoyama and Honda, 2007). Marine fish oil industry, the traditional source

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of DHA, face a series of problems, e.g. resource shortage, environmental pollution, and unpleasant odors. Moreover, marine fishes are not the primary producer of DHA and do not have the ability to synthesize DHA de novo. Thus, exploring thraustochytrids (the primary DHA producers) for commercial DHA production using green and renewable sources is critical for improving the human health and DHA industry (Nagano et al., 2009; Li et al., 2015).

However, the cost of the DHA products derived from *Aurantiochytrium* is still high, limiting their wide applications. The cost of DHA production by *Aurantiochytrium* spp. is mainly derived from the medium compositions, including the carbon and nitrogen resources. Thus, exploring the low-cost and renewable feedbacks for DHA production or further improving biomass and DHA production from thraustochytrids is a crucial strategy to reduce the cost of DHA products and accelerate the development of DHA industry (Xu et al., 2020). Species of *Aurantiochytrium* can produce various extracellular enzymes (e.g. proteases, cellulases, amylases, lipases, gelatinases, chitinase phosphatases, β-glucosidases) and utilize various substrates to grow fast and accumulate high level of DHA in their cells. DHA derived from microbes have largely alleviated the severe problems caused by the traditional fish oils (Raghukumar, 2002; Nagano et al., 2009; Marchan et al., 2018).

Date palm (*Phoenix dactylifera* L.) is the most widely grown fruit tree in Saudi Arabia and the country is ranked as the second largest date producer in the world after Egypt (FAOSTAT, 2012; Assirey, 2015). The Saudi production of date fruits represent 17% of the total world, with the aim to become the largest exporter of dates in the world in its 2030 Vision (Saudi Gazette, 2021). Saudi Arabia produce 1,539,755 tons of dates annually, of which only exports 184,000 tons (12% of the total production) at a value of SR860 million. Date fruits consumption in Saudi Arabia represents 13% of the total production (Saudi Gazette, 2021). The sum of exports and consumption is 25% of total production of dates in Saudi Arabia which means that 75% of the production is surplus that can be used for value-added production.

Date fruits are rich in mineral salts and vitamins, excellent source of refined sugar, juice, pastes and fermented products (Samarawira, 1983; Booij et al., 1992). Dates contain small amounts of vitamins C, B1 thiamine, B2 riboflavin and nicotinic acid (Al-Shahib and Marshall, 2002). Dates have strong antioxidant, anticancer and antiviral activities (Vayalil, 2002; Ishurd et al., 2004; Al-Farsi et al., 2005). Assirey (2015) determined the chemical composition of ten types of date fruits cultivated in Saudi Arabia. She recorded high levels of sugars (71.2–81.4% dry weight) that is mainly glucose and fructose, low percentage of protein (1.72–4.73%) and lipid (0.12–0.72%). Ash represented 1.68–3.94% dry weight with potassium was the predominant mineral. She recorded 15 amino acids at different concentrations.

Sugar-cane molasses generally has 38–48% (w/v) content of fermentable sugars (Inamdar, 1994; Mostafa and Zohri, 1997). Kelly (1954) determined the glucose and fructose percentage in five samples of cane molasses collected from Queensland, Australia. Glucose ranged between 3.2 and 12.5%, while fructose ranged between 10.5 and 15.8%.

This study aimed to assess the potential of different raw materials as a carbon source for cost-efficient biomass, lipid and DHA production by local native strain *Aurantiochytrium* sp. YB-05. Explored raw material include: date fruit, glycerol and cane molasses. Also four types of oily seeds (as a precursor of fatty acids) were used in a combination with date fruits to test their abilities to increase the level of produced oil and their major fatty acids in the *Aurantiochytrium* sp. YB-05 biomass.

2. Materials and methods

2.1. The Aurantiochytrium strains

Aurantiochytrium sp. YB-05 was isolated from decaying leaves of *Avicennia marina* (Forssk.) Vierh. from Yanbu mangroves (24°02.55′ N 38°68.89′ E) along the coast of the Red Sea, Saudi Arabia (Abdel-Wahab et al., 2021b). The pure culture was preserved on GYPA slants (30 g glucose, 10 g peptone, 5 g yeast extract, 2 g monosodium glutamate, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.5 g KH_2PO_4, 0.5 g chloramphenicol, 15 g agar in 1 L of half-strength natural seawater) and sub-cultured every two months. Pure culture of the microbe was also preserved in 20% glycerol at −80 °C. Cells were cultivated in GYP broth (20 g glucose, 5 g yeast extract, 5 g peptone in 1 L of 50% seawater) in 250 mL flasks with a 50 mL working volume at 26 °C, while shaking at 120 rpm.

2.2. DNA sequencing and phylogenetic analysis

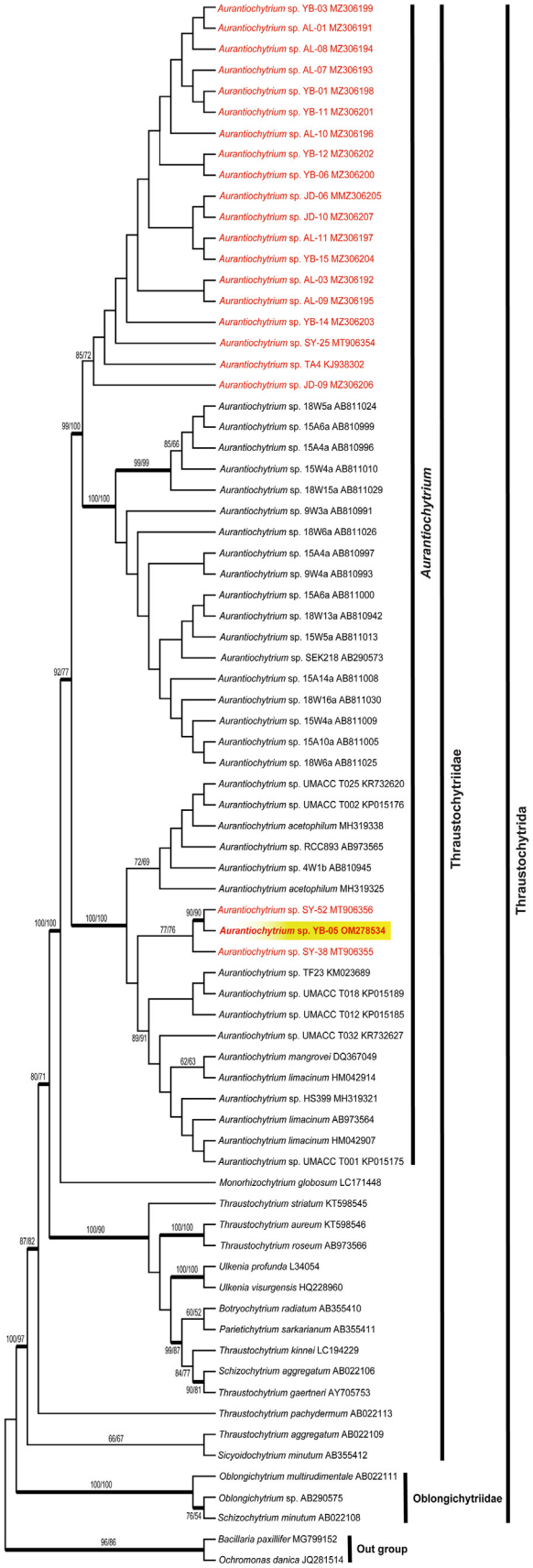
DNA extraction, 18S rDNA sequencing and phylogenetic analyses were previously described in detail (Abdel-Wahab et al., 2021a). PCR amplification and DNA sequencing were carried out by SolGent Inc., South Korea. The obtained sequences were deposited in GenBank (Fig. 1), and aligned with sequences of *Aurantiochytrium*, other genera of Thraustochytriidae and the outgroup taxa *Bacillaria paxillifer* and *Ochromonas danica* using ClustalX (Thompson et al., 1997). Maximum-parsimony (MP) and maximum-likelihood (ML) phylogenetic analyses were carried out using MEGA X (Kumar et al., 2018). The MP tree was obtained using the Subtree-Pruning-Redrafting (SPR) algorithm (Nei and Kumar, 2000) with search level 1 in which the initial trees were obtained by random addition of sequences and bootstrap analysis was done with 1000 replicates. The evolutionary history was inferred by using the Maximum Likelihood method was carried out in MEGA X using Tamura-Nei model (Tamura and Nei, 1993). Bayesian phylogenetic analysis was performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) with the GTR model that was determined using MrModeltest 2.2 (Nylander, 2004).

2.3. Preparation of raw materials and growth condition

Date fruits, glycerol, cane molasses were purchased from the local market. Glucose was purchased from Sigma-Aldrich (St. Louis, USA). The four carbon sources were used at four concentrations: 10, 20, 40 and 80 g/L. Date fruits were boiled in water for 10 min and the filtrate was completed to 1 L with 50% sea water (500 mL distilled water and 500 mL natural sea water), 5 g yeast extract, 5 g peptone and 0.5 g chloramphenicol was added. The culture media was distributed in 250 mL flasks, 50 mL each, autoclaved, inoculate with *Aurantiochytrium* sp. YB-05 and incubated at 26 °C in a shaking incubator at 120 rpm for 4 days. Cell growth was determined by measuring the dry cell weight (DCW).

2.4. Date fruit water extract in combination with water extract of oily seeds

In another experiment, water extract of date fruits at 80 g/L concentration was used along with the water extract of seed oils at four concentrations: 50, 100, 150 and 200 g/L. The used seeds were: cotton, flax, peanut and sesame. Seeds were boiled for 10 min and filtered. The media composition was: date fruit and



seed filtrates and complete it to one liter 50% sea water, 5 g yeast extract, 5 g peptone and 0.5 g chloramphenicol.

2.5. Quantification of thraustochytrid lipids using sulfo-phospho-vanillin (SPV) method

Phosphovanillin (PV) reagent was prepared by using six milligrams of vanillin dissolved in 100 mL of hot water and further diluted to 500 mL with o-phosphoric acid. Oleic and palmitic acids were used as standard and diluted properly with concentrated sulfuric acid to reach 1 mg/mL. Concentrated sulfuric acid and o-phosphoric acid were purchased from Alpha chemical Egypt. Vanillin, palmitic and oleic acids were purchased from Sigma-Aldrich. 25 mg of thraustochytrid cells were dissolved in 1 mL concentrated sulfuric acid, of which 20 µL of the samples were diluted in 180 µL of concentrated sulfuric acid in test tubes and incubated at 100 °C for 10 min. Then, the tubes were cooled to room temperature (26–28 °C) and 0.5 mL of PV reagent was added for color development. The mixture was incubated at 37 °C for 15 min and stored for 45 min in a dark box. Then, the absorbance was measured at 530 nm in a spectrophotometer (UV-1600 spectrophotometer, Abbota, China) and reads were converted into g/L (Anschau et al., 2017).

2.6. Lipid extraction and analysis of fatty acid

The entire mass of thraustochytrids in the flask was transferred to a pre-weighed centrifuge tube and harvested by centrifugation at 6000 g for 20 min and the supernatant discarded. Harvested cells were subsequently washed with sterile distilled water and centrifuged at 6000 g for 20 min and the supernatant discarded. This rinsing centrifugation process was repeated three times, then the washed cells were freeze-dried for 24 h for biomass determination. Lipids were extracted from freeze-dried cells with a mixture of chloroform/methanol (2:1, vol/vol) (Folch et al., 1957). Saturated, unsaturated and total fatty acids were determined in the lipid by using methyl esters boron tri-fluoride method (AOAC, 2012). The lipid was saponified with sodium hydroxide in methanol. The fatty acids were methylated with boron tri-fluoride in methanol, extracted with heptane and were determined on a gas chromatograph with FID detector (PE auto system XL) with auto sampler and Ezchrom integration system. Helium was used as the carrier gas with the following settings: 25 Psi – air 450 mL/min – Hydrogen 45 mL – split 100 mL/min (Abdel-Wahab et al., 2021a).

3. Results

3.1. Phylogenetic placement of Aurantiochytrium sp. YB-05

The 18S rDNA dataset included 75 sequences: 56 *Aurantiochytrium*, 14 from other genera in Thraustochytriidae, three from Oblongichytriidae and *Ochromonas danica* and *Bacillaria paxillifer* were used as outgroup taxa (Fig. 1). The maximum parsimony

Fig. 1. Maximum likelihood (ML) phylogenetic tree based on 18S rDNA of *Aurantiochytrium* sp. YB-05 and other species and strains of the genus, other genera of the family Thraustochytriidae and representatives of Oblongichytriidae. The tree is rooted with *Bacillaria paxillifer* and *Ochromonas danica*. Bootstrap support on the nodes represents ML and MP $\geq$ 50%. Branches with a BYPP (Bayesian posterior probabilities) of $\geq$ 95% are in bold. *Aurantiochytrium* strains isolated from mangroves in the Arabian Gulf and the Red Sea are in red. The sequence of *Aurantiochytrium* sp. YB-05 in yellow box.

dataset consisted of a total of 721 characters, of which 265 were constant, 101 variables and parsimony-uninformative, and 355 were counted as parsimony-informative. The most parsimonious tree had a length of 1673 steps, a consistency index of 0.470,550, a retention index of 0.800,342, and a composite index of 0.409,021. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree with the highest log likelihood (-8694.75) is shown in Fig. 1. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Bayesian analysis yielded two trees similar in topology to the ML phylogenetic tree shown in Fig. 1.

Aurantiochytrium sp. YB-05 grouped consistently with *Aurantiochytrium* sp. SY-52 with high statistical support (98/99/100 for ML/MP/BYPP, respectively), while *Aurantiochytrium* sp. SY-38 formed a basal branch with a moderate statistical support (77/78/83 for ML/MP/BYPP, respectively). The last two *Aurantiochytrium* strains were previously isolated from decaying leaves of *Avicennia marina* and sea water samples collected from Syhat mangroves, Arabian Gulf, Saudi Arabia (Abdel-Wahab et al., 2021a). The three strains formed a distinct clade within the genus *Aurantiochytrium*. Another 19 strains isolated previously from mangroves in the Arabian Gulf and the Red Sea formed another distinct clade within the genus *Aurantiochytrium* (Fekrat and Shakeri, 2015; Abdel-Wahab et al., 2021a).

3.2. Morphology of Aurantiochytrium sp. YB-05

Colony color yellow-orange to reddish-orange, vegetative cells 12–24 μm, hyaline to reddish-orange, globose or subglobose, with thick-walled gelatinous sheath, producing 8–64 zoospores. Zoospores 2–4 μm in diameter, globose or subglobose. Cyst 12–14 μm long, 6–8 μm wide, thick-walled, pale-brown to brown in color. Amoebae 22–29 μm long, 8–10 μm diam., hyaline, irregular in shape, thick-walled (Fig. 2).

3.3. Effect of carbon source concentration on biomass production

Produced dry cell weight increased as the carbon sources: date fruit, glucose and molasses increased from 10 to 80 g/L. Date fruit and molasses gave slightly higher biomass at 40 and 80 g/L than glucose, while higher biomass were obtained at 10 and 20 g/L glucose than date fruit and molasses at the same concentrations. Glycerol and molasses gave the highest biomass at 40 g/L, while at 80 g/L there was a decline in the biomass produced (Table 1, Fig. 3).

3.4. Effect of carbon source concentration on lipid production

Ten g/L glycerol gave the highest percentage of lipids (85.6% of the biomass), followed by 40 g/L glucose (80.1%), 20 g/L glycerol (70%), 80 g/L glucose (59.3%) and 80 g/L date fruit (56.4%). The percentage of lipid in the biomass decreased as the weight of glycerol increased in the medium. The percentage of lipid in the biomass increased with increasing the weight of date fruit and molasses from 10 g/L (24.7% and 15.6% respectively) to 80 g/L (56.4% and 45.7% respectively) (Table 1, Fig. 3).

3.5. Morphology of Aurantiochytrium sp. YB-05

Colony color yellow-orange to reddish-orange, vegetative cells 12–24 μm, hyaline to reddish-orange, globose or subglobose, with thick-walled gelatinous sheath, producing 8–64 zoospores. Zoospores 2–4 μm in diameter, globose or subglobose. Cyst 12–14 μm long, 6–8 μm wide, thick-walled, pale-brown to brown in color. Amoebae 22–29 μm long, 8–10 μm diam., hyaline, irregular in shape, thick-walled (Fig. 2).

3.6. Effect of carbon source concentration on biomass production

Produced dry cell weight increased as the carbon sources: date fruit, glucose and molasses increased from 10 to 80 g/L. Date fruit and molasses gave slightly higher biomass at 40 and 80 g/L than glucose, while higher biomass were obtained at 10 and 20 g/L glucose than date fruit and molasses at the same concentrations. Glycerol and molasses gave the highest biomass at 40 g/L, while at 80 g/L there was a decline in the biomass produced (Table 1, Fig. 3).

3.7. Effect of carbon source concentration on lipid production

Ten g/L glycerol gave the highest percentage of lipids (85.6% of the biomass), followed by 40 g/L glucose (80.1%), 20 g/L glycerol (70%), 80 g/L glucose (59.3%) and 80 g/L date fruit (56.4%). The percentage of lipid in the biomass decreased as the weight of glycerol increased in the medium. The percentage of lipid in the biomass increased with increasing the weight of date fruit and molasses from 10 g/L (24.7% and 15.6% respectively) to 80 g/L (56.4% and 45.7% respectively) (Table 1, Fig. 3).

3.8. Effect of carbon source and seed water extract on docosahexaenoic acid (DHA) production

The production of DHA by *Aurantiochytrium* sp. YB-05 was compared between glucose and date fruit at 80 g/L concentration and the effect of the addition of seed water extracts in combination with 80 g/L date fruit on DHA production was also tested. A higher level of DHA (24.85% total fatty acids "TFA") was produced by *Aurantiochytrium* sp. YB-05 when grown on date fruit than on glucose (4.89% TFA) (Tables 1 and 2). Also docosapentaenoic acid (DPA) increased from 0.64%.

TFA on glucose to 7.13% TFA on date fruit. DPA and DHA were the only polyunsaturated fatty acids (PUFA) in the fatty acid profile of the *Aurantiochytrium* sp. YB-05 grown on date fruit. The addition of seed water extracts did not increase the level of DHA level compared to date fruits alone (Tables 1 and 2). However, it affect the fatty acids composition. The addition seed extracts increased the level of total monosaturated fatty acids (MUFA) and ranged between 18.08% TFA (Flax) to 53.56% TFA (Cotton). MUFA was only 0.64% TFA on date fruit alone. Remarkably, oleic acid was reported at high percentage between 15.05% TFA (Flax) and 28.91% TFA (Cotton) (Table 2).

4. Discussion

This study shed a light on the potential use of date fruits industry by-products for the production of value-added products using microbes. Results of the current study showed that date fruits, glycerol and molasses can be a good candidates for replacing glucose in the commercial production of biomass, lipids and DHA from thraustochytrids. Further studies are needed in order to evaluate date fruits and their by-products from different factories in the Saudi Arabia in order to be used for the commercial production of value-added products e.g. biodiesel and DHA from thraustochytrids. Wastes of the palm oil industry are rich source of carbohydrates, organic matter and amino acids that can be used for growing microorganisms (Ahmed et al., 2015).

High diversity of *Aurantiochytrium* species was recorded from mangroves in the Arabian Gulf and the Red Sea in Saudi Arabia. Phylogenetic analyses based on 18S rDNA of 22 strains isolated

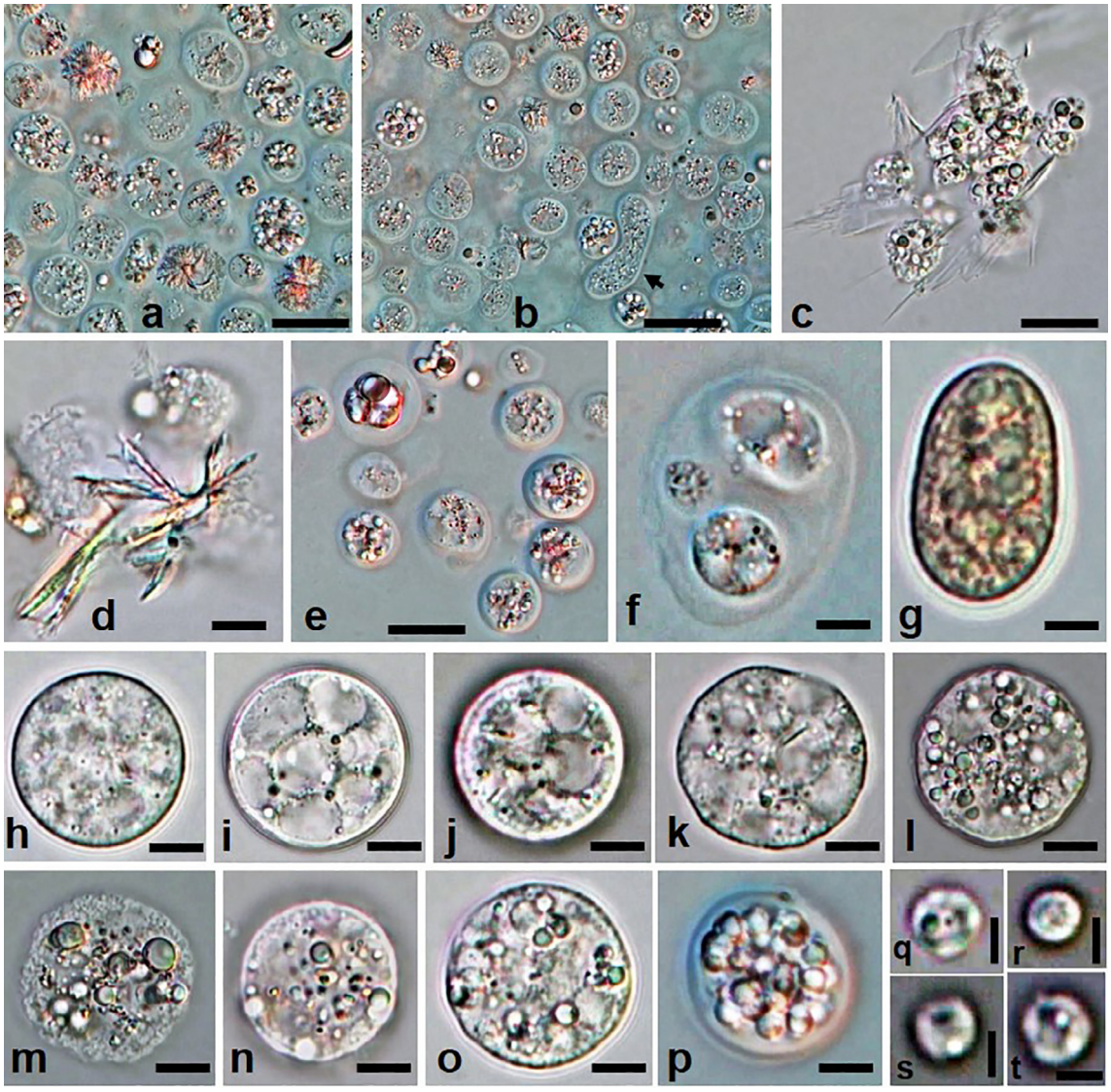


Fig. 2. *Aurantiochytrium* sp. YB-05 isolated from decaying leaves of *Avicennia marina*. a–f, h–p Zoosporangia at different stages of maturity. b Amoeboid cell, arrowed. c–d Zoosporangia with ectoplasmic net. g Cyst. q–t Zoospores. Bars: a–c, e = 20 μm, d, f–p = 10 μm, q–t = 2 μm.

Table 1
Comparison of cell dry weight, lipid and DHA production by *Aurantiochytrium* sp. YB-05 using different raw materials.

Carbon source conc. (g/L)	Dry cell weight (g/L)	Lipid (%)	DHA (% TFA)	Seed source conc. (g/L) + 80 g/L date fruit	Dry cell weight (g/L)	DHA (% TFA)
Date fruit				Cotton		
10	17.2	24.7		50	52.4	
20	17.4	31.6		100	59.6	
40	34.8	39.2		150	50.4	2.88
80	42.6	56.4	24.85	200	41	
Glucose				Flax		
10	26.4	36.4		50	37.6	
20	27.2	50.1		100	38.8	
40	28	80.1		150	39.8	12.06
80	38.4	59.3	4.89	200	43	
Glycerol				Peanut		
10	9.4	85.6		50	36.2	
20	25.8	71		100	43.2	
40	26.8	55.8		150	44.8	5.45
80	9	15.38		200	56.6	
Molasses				Sesame		
10	11.4	15.6		50	31.6	
20	15.2	22		100	37.6	
40	42	31.2		150	37.7	16.24
80	38.4	45.7		200	38	

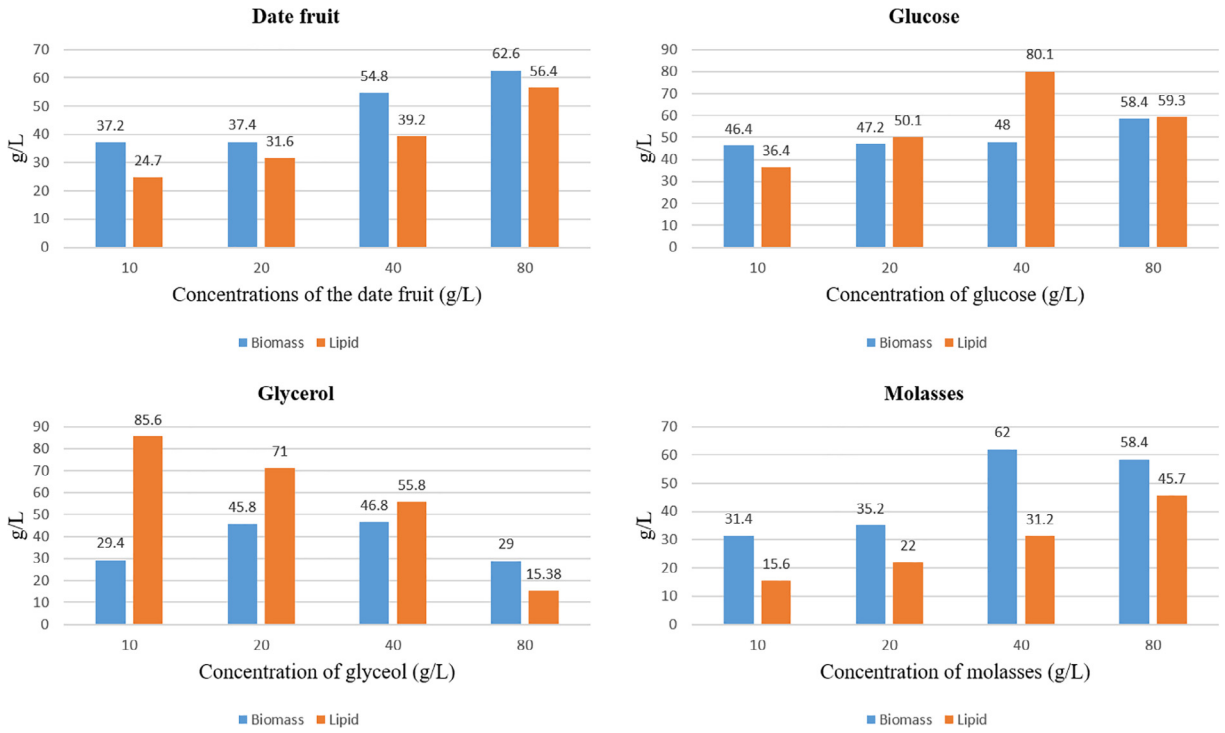


Fig. 3. Dry cell weight and lipid production by *Aurantiochytrium* sp. YB-05 on three low-cost substrates and glucose at four different concentrations.

Table 2
Fatty acid composition (% of total FA) of the *Aurantiochytrium* YB-05 grown on different.

Carbon and seed sources:							
Fatty acids	Name	Glucose	Date fruit syrup	Cotton	Flax	Peanut	Sesame
C10:0	Capric acid	–	0.67	0.23	0.6	–	0.42
C11:0	Undecanoic acid	1.3	4.81	0.21	2.9	0.83	2.18
C12:0	Lauric acid	6.7	20.8	2.46	12.62	3.92	7.78
C14:0	Myristic acid	0.89	1.7	4.69	4.83	4.51	3.86
C15:0	Pentadecanoic acid	3.1	1.11	0.91	1.3	0.79	1.48
C16:0	Palmitic acid	24.02	36.11	24.12	28.87	25.23	32.09
C16:1ω9	Palmitoleic acid	–	–	0.21	–	–	–
C16:1ω7	Palmitoleic acid	0.69	–	5.63	0.68	3.97	0.65
C16:1ω5	Palmitoleic acid	–	–	0.17	–	–	–
C16:2ω4		–	–	0.62	–	0.47	–
C16:3ω4	Hexagonic acid	–	–	0.3	–	–	0.23
C17:0	Heptadecanoic acid	1.41	0.38	1.92	0.67	8.28	1.95
C18:0	Stearic acid	6.96	1.8	4.55	6.28	4	5.64
C18:1ω9	Oleic acid	25.01	0.64	28.91	15.05	24.33	20.72
C18:1ω7	Vaccenic acid	1.17	–	3.95	1.11	3.16	1.05
C18:1ω5	Octadecosaenoic acid	–	–	0.25	–	–	–
C18:2ω6	Linoleic acid	20.72	–	2.79	8.22	3.01	2.92
C18:3ω3	Linolenic acid	1.4	–	–	0.49	–	–
C20:0	Arachidic acid	0.5	–	0.23	–	–	–
C20:1ω11	Gadolic acid	–	–	1.26	–	0.98	–
C20:1ω9	Gondoic acid	0.6	–	7.12	–	5.77	–
C20:1ω7	Eicosaenoic acid	–	–	0.23	1.24	–	–
C22:1ω11	Cetoleic acid	–	–	5.36	–	4.17	–
C22:1ω9	Erucic acid	–	–	0.47	–	–	–
C22:2ω6	Docasadienoic acid	–	–	–	1.47	–	–
C22:5ω6	Docosapentaenoic acid (DPA)	0.64	7.13	0.53	1.61	1.13	2.79
C22:6ω3	Docosahexaenoic acid (DHA)	4.89	24.85	2.88	12.06	5.45	16.24
Total saturated fatty acids (SFA)		44.88	67.38	39.32	58.07	47.56	55.4
Total monounsaturated fatty acids (MUFA)		27.47	0.64	53.56	18.08	42.38	22.42
Total polyunsaturated fatty acids (PUFA)		27.65	31.98	7.12	23.85	10.06	22.18

from mangroves in the Arabian Gulf and the Red Sea placed them as two distinct clades within the genus *Aurantiochytrium* (Fekrat and Shakeri, 2015; Abdel-Wahab et al., 2021a). Fatty acid profile analyses of six *Aurantiochytrium* strains within those two distinct clades from the Middle East (Abdel-Wahab et al., 2021a,b; this study) showed that they produced high percentages of oleic and linoleic acids in total fatty acid and this might be the characteristic feature of those two *Aurantiochytrium* clades. Among thraustochytrid species, *Aurantiochytrium* is considered a potential candidate for DHA production because of its ability to produce high

levels of lipid and DHA (Hong et al., 2011; Chen et al., 2020). Several studies have been carried out to maximize the DHA production through optimization of nutritional sources (Ryu et al., 2013; Chen et al., 2020).

Species of *Aurantiochytrium* are well known for their utilization of various carbon and nitrogen sources (Hong et al., 2011). Although several attempts have been made to replace glucose with a lower cost carbon source, high DHA yields are typically achieved using other sugars which are still relatively expensive (Nagano et al., 2009; Hong et al., 2013; Li et al., 2015; Cho et al., 2018; Ju et al., 2018; Xu et al., 2020). Thus, the identification of inexpensive and effective carbon sources is necessary to further reduce the costs of commercial thraustochytrid DHA production.

In the present study, date fruits and cane molasses gave comparable biomass at 10 and 40 g/L, while they gave a higher biomass at 40 and 80 g/L than those from pure glucose. Both date fruits and cane molasses have high percentages of fermentable sugars beside other nutrients (Kelly, 1954; Samarawira, 1983; Booij et al., 1992; Inamdar, 1994; Mostafa and Zohri, 1997; Assirey, 2015).

Several raw materials were used in previous studies for the production of DHA by *Aurantiochytrium* isolates. Liang et al. (2010) used sweet sorghum juice at four concentrations: 100%, 75%, 50%, and 25% for biomass, lipid, and DHA production by *Aurantiochytrium limacinum* SR21. At 50% juice concentration, produced biomass was similar to that from pure glucose, but with 75% juice concentration, lipid content and DHA level was higher than those from pure glucose. Orange peel extract optimized with supplemental $NaNO_3$ (1.2 g/L) resulted in a DHA yield of 0.63 g/L, which was 2.5 fold greater than the yield obtained using the conventional basal medium (Park et al., 2018). The use of Jerusalem artichoke hydrolysate (JAH) as a carbon source for growing *Aurantiochytrium* sp. KRS101 resulted in 27.8% improvement in biomass productivity compared with the basal medium control (Heo et al., 2020). *Aurantiochytrium mangrovei* Sk-02 produced 28 g/L biomass using 33% (v/v) coconut water (Unagul et al., 2007). Produced biomass in the present study are higher than those from previous studies using raw materials.

5. Conclusion

In the present study, we used three low-cost substrates to replace pure glucose for economical production of biomass, lipid and DHA using *Aurantiochytrium* sp. YB-05. Date fruit and cane molasses produced comparable levels of biomass and lipid to those produced by the *Aurantiochytrium* using pure glucose. However, a higher level of DHA and DPA were obtained using date fruits. Further studies are needed to evaluate the use of date fruits and their by-products industry to use the surplus in date production in the Saudi Arabia for the production of value-added products especially DHA using microorganisms.

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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