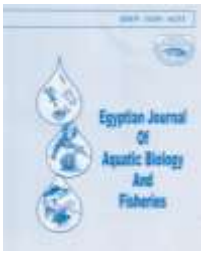
Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 - 6131
Vol. 22(4): 195- 207 (2018)
ejabf.journals.ekb.eg



Enhancement of lipid accumulation in oleaginous marine yeast; *Candida* sp. for biodiesel production and antimicrobial properties

Sahar W.M. Hassan, Khouloud M. Barakat and Hassan A.H. Ibrahim*
Marine Microbiological Lab., Environmental Division, National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt
*Corresponding author: drhassan1973@yahoo.com

ARTICLE INFO
Article History:
Received:Aug. 2, 2018
Accepted:Sept. 13, 2018
Available online: Sept.20, 2018

Keywords:
Lipid accumulation
oleaginousmarine yeast
Candida sp.
Biodiesel production
Antimicrobial activity

ABSTRACT

Fatty acids, both free or as part of complex lipids, are essential components of all vital membranes. Thus, the aim of the present study is the production of fatty acids by marine yeast using some natural of low price sources. Marine *Candida* sp. isolated from SidiBishr, Mediterranean Sea, Egypt, showed the highest capabilities of lipid accumulation by 42.8% in 1.9 gl^{-1} dry biomass. Optimization of the fermented conditions for lipid production was carried out by using one factor at time. Molasses and urea as carbon and nitrogen sources showed the highest lipid content 71% in 6 gl^{-1} biomass at pH 5, 25^{o}C and 20% inoculum size, where, lipid accumulation and dry biomass of marine *Candida* sp. increased by 1.7 and 3.2 fold, respectively. Esterification of the accumulative lipid was performed, resulting in 34 fatty acid methyl esters (FAMEs) detected by Gas chromatographic analysis. Biodiesel properties were estimated showing the Degree of Unsaturation (DU), 98.87; Long Chain Saturation Factor (LCSF), 13.96; Iodine Value (IV), 105.07; Saponification Value (SV), 190.34; the Cetane Number (CN), 57.43; Kinematic Viscosity (υ), 4.52; Density (ρ), 0.87 and the Higher Heating Value (HHV), 40.05. Antibacterial activity of the extracted lipid showed inhibition zone diameters ranged from 10 to 17 mm. Moreover antifungal activity against some pathogens ranged from 50 -100%.

INTRODUCTION

Microbial lipid as superseding source of lipid has attracted attention from the first years of twentieth century (Ratledge, 2005, Liang and Jiang, 2013). Oleaginous yeasts are preferable than the other microbial sources for the production of triglycerides, surfactants and poly unsaturated fatty acids (Yamada *et al.*, 2017) due to their easy cultivation and the time that needed to become two fold biomass is less than one hour. Yeast applicable for lipid production are *Candida, Yarrowia, Rhodotorulla, Rhodosporidium, Cryptococcus, Trichosporon* and *Lipomyces*, which can accumulate different types of lipids or fatty acids according to the medium composition (Ageitos *et al*., 2011).

Nowadays, an increased demand for clean and non-toxic energy was targeting due to the rapid growth of world's inhabitance and crisis of fuels (Vairavan *et al*., 2010, Helwani *et al.*, 2009).

Currently,marine yeasts have been reported as promising alternative for generation of biodiesel (Obara *et al*., 2012; Khambhaty *et al.*, 2013, Zaky *et al*., 2014; Park *et al*., 2018).

Another economic problem is the limited availability of omega-3 fatty acids, because the production of them is by marine fisheries, which cannot face demands of the growing market (Xue *et al.*, 2013). Thus there is a need for another non-animal source of omega-3 fatty acids. Scholza *et al.* (1999) reported the better survival and effective clearance of the pathogenic bacteria from the hemolymph of *Penaeus vannamei* (shrimp) reared on two depended fatty acid yeast strains; *Saccharomyces cerevisiae* and *Phaffia rhodozyma.*

Thus, the goal of the present study was isolation of marine yeast strainwhich capable for lipid production using low cost substrates through the optimized conditions. Trans-esterification of fatty acids and composition of final products were investigated using GC-MS analysis. Biodiesel generation and antimicrobial properties were investigated as potential applications.

MATERIAL AND METHODS

Collectionof samples and marine yeast isolation

Water samples were gathered from different sites of Mediterranean coasts, Egypt, in a sterile screw capped bottles, transferred to laboratory, and stored at 4^{o}C. Different dilutions were prepared, one hundred microliter of each dilution was spread and plated on to sterile plates containing potato dextrose agar (PDA) (Cat.1261.00, CONDA), final pH 5±0.2 at 25^{o}C. Colonies distinct in size and morphology were selected, picked up and purified on potato dextrose slants and kept at 4^{o}C for further studies.

Indicator pathogenic microorganisms

The pathogenic microbial strains used to estimate the antimicrobial activity included**:** *Aeromonas hydrophila, Pseudomonas aeruginosa, Pseudomonas* sp., *Vibrio cholerae, V. fluvialis, V. parahaemolyticus* and *Escherichia coli, Helminthosporium sp., Rhizoctonia solani, Rhizopus oryzae* and *Fusarium solani.* They were kindly provided by Marine Microbiology Department, Environment division, National Institute of Oceanography and Fisheries, Alexandria, Egypt

Growth conditions

Colonies of yeast were incubated on PDA slants for 48 h, and thenused to inoculate 100 ml of medium containing (gl^{-1}): Glucose, 20; peptone, 5; yeast extract,15 and incubatedat 25ºC for 4 days under shaking condition (Dai *et al*., 2007).

Determination of yeast dry mass

The fermented cultures were centrifuged at 6000 g for twenty minutes. Then washing of the obtainedmass was carried outwith distilled water and then dried at 60ºC. Gravimetric method was used to determine the biomass (El-Fadaly *et al.*, 2009).

Total lipid extraction

Lipid extraction was done according to Kraisintu *et al.* (2010); first,washing the centrifuged yeast cellswith 40 ml of distilled water for two times, acid hydrolysis using 8 ml of 4M hydrochloric acidat 60ºC for two hours, followed by stirring with mixture of methanol /chloroform (1:1) for three hours. Then centrifugation at 5000 g for five minutes was carried out for separation of the lower phase and aqueous upper phase. Then the lower phase was evaporated for recovery of lipid. After that the dry

lipid was determined gravimetrically. The total extracted lipid yield (%w/w) was determined as in the following equation (Gutierrez *et al.*, 2008).

$$\text{Total lipid extraction yield (\%)} = \frac{\text{Weight of lipid extracted (g)}}{\text{Weight of yeast biomass (g)}} \text{X } 100$$

Identification of promising oleaginous yeast

The most potent yeast isolate with the highest lipid accumulationwas identified according to morphological, biochemical and physiological characteristics using Analytical Profile Index (API-20 AUX V4.0) covering 20 different biochemical reactions (Barnett *et al.*, 1990).

Optimization of fermentation conditions

Effect of different carbon andnitrogensources

Yeast isolate was screened for production of lipid using different carbon sources:(20 gl^{-1}) molasses, bran, corn, oat and rice straw. Rice, bran, oat and corn were hydrolyzed by HCl (5%) and incubated at 110ºC for 20 min before use. Ten ml of hydrolyzed mass in 40 ml of distilled water were added to the production media at equivalent weight. Using the potent carbon source, different nitrogen compounds: sodium nitrate, ammonium nitrate, ammonium sulphate and urea were added at equivalent weight to the production medium.Both experimentswere separatelyinoculated with 1% yeast culture (A_{600}= 1) in the medium and incubated at 30^{o}C for 5 days under shake conditions. Dry biomass and content of lipid were determined (Enshaeieh *et al.*, 2012).

Effect of different pH, temperature and inoculum size

Yeast isolatewas cultivated in the production media containing the best carbon and nitrogen sources at different pH values (3, 4, 5, 7, and 8) at 25^{o}C,temperatures (5, 25, 30, and 45^{o}C)and volumes of yeast culture (5, 10, 20, and 30 ml) under shake conditions.Each experiment was separately performed.Drybiomass and content of lipid were determined in each case (Devi and Velayutham, 2011).

Fatty acids profile

The obtained yeast biomass (45 g) under the optimized culture conditions was used for extraction of fatty acidand esterification according to Lewis *etal*. (2000). Profile of the fatty acidsin the extracted lipid was detected using gas chromatography analysis system (GC-QqQ/MS triple Quade, Agilent 7890A series GC system coupled with an Agilent 7000B QqQMS; Agilent Technologies Inc., USA).

Biodiesel properties

The physical properties of biodiesel products were calculated according to Islam *et al.* (2013) and Saravanan *et al*. (2013) depending on profile of the fatty acids.

Antibacterial activity

Antibacterial activityof the extracted fatty acids against different pathogens was performed usingthe well-cut diffusion technique. Briefly, wells were punched inagar plates (using a sterile cork borer) seeded with pathogens, 100 µlof the extracted fatty acids was added in each well. After incubation at 30^{o}C for 24h, the positive result was detected as clear zone around wells (El-Masry*et al*., 2002).

Antifungal activity

Antifungal activity of the extracted fatty acids was tested against *F. solani*, *R. solani*, *R. oryzae*, and *Helminthosporium* sp. using the method recommended by Kumar *et al*. (2011).

Statistical analysis

Data analysis was performed with the software package Microsoft Excel, Version 2003. Statistically significant difference was determined using paired Student's-test and $P \leq 0.05$ was used as a limit to indicate statistical significance.

RESULTS AND DISCUSSION

Lipid production by marine yeast isolates

Different marine yeast strains were isolated from different stations of Mediterranean Sea, Egypt, and screened for their capabilities to accumulate lipid. Out of 10 isolates, S2 strain from SidiBishr coast showed promising capability for lipid accumulation. The isolate S2 exhibited the highest dry weight (1.9 gl^{-1}) and lipid content (42.8%) upon growing on medium containing glucose.

Identification of S2 yeast strain

Biochemical and physiological analyses in Table 1 indicated that S2 strain is identified as *Candida* sp. It appears off white on Chrom Agar Candida medium. Figure 1 represents scanning electron micrograph of *Candida* sp. that was previously proven for lipid accumulation (Kolouchová *et al.*, 2016).

Table 1: Physiological characteristics of the potent yeast isolate (S2)

Characteristic	**Observation**
Utilization of	
Calcium 2-ceto-	+
L-arabinose	+
D-xylose	-
Adontol	+
Xylitol	+
D-galactose	-
Inosol	+
D-sorbitol	-
Methyl-D-	+
N-Acetyl-glucosmine	+
D-celobiose	+
D-lactose	+
D-maltose	+
D-saccharose	+
D-trehalose	+
D-meleztose	+
D-rafinose	+

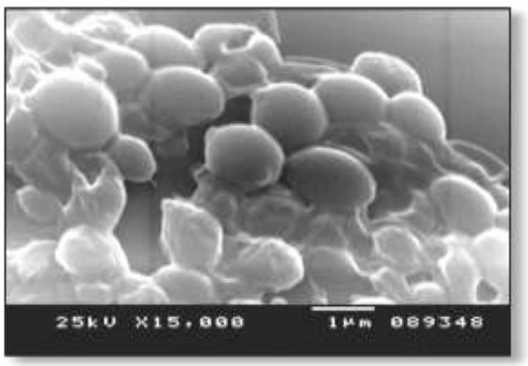


Fig. 1: Electron micrograph showing cells of *Candida* sp.

Optimization of fermentation conditions for lipid production by *Candida* sp.

Synthesis of lipids is affected by culture conditions such as medium pH, incubation temperature, nutrient limitation (Sitepu *et al*., 2013; Carsanba *et al*., 2018). So, it is possible to enhance lipid production by manipulating the culture conditions.

Effect of different carbon sources

Glucose as carbon source was used for lipid production in different studies (Fei *et al*., 2011). Different low cost wastes were promising for production of lipid (Kosa and Ragauskas, 2011). In the present study, the marine *Candida* sp. was screened for production of lipid using different natural and cheap carbon sources such as molasses, hydrolysates of bran, rice straw, oat and corn. As shown in Figure 2a, *Candida* sp. exhibited the highest lipid content (55.4%) in 4 gl^{-1} dry biomass upon using molasses as carbon source, while the lowest biomass (1.8 gl^{-1}) and lipid content (10%) was in case of growing in medium containing rice as carbon source. The same finding was observed by Dai *et al.* (2007) who reported that straw hydrolyte was unfavorable for growth of the oleaginous yeast; *Rhodotorula glutinis*, while molasses was favorable for production of lipid by *Cryptococcus curvatus* NRRLY-1511 (El-Fadaly *et al.*, 2009).

Effect of different nitrogen sources

Effect of nitrogen sources on dry biomass and content of lipid was investigated. Results in Figure 2b showed that urea realized the highest lipid content (60%) and biomass 4.5 gl^{-1}. In accordance, Zhu *et al.* (2008) recommended the organic nitrogen "urea"as good source for lipid production, whilemineral nitrogenhad good effect on biomass yield of *Trichosporon fermentans* CICC 1368. The same was shown by Azad *et al.* (2014). Another investigation revealed that ammonium sulfate and yeast extract were preferable for lipid production by *Rhodotorula* yeast (Enshaeieh *et al.*, 2012).

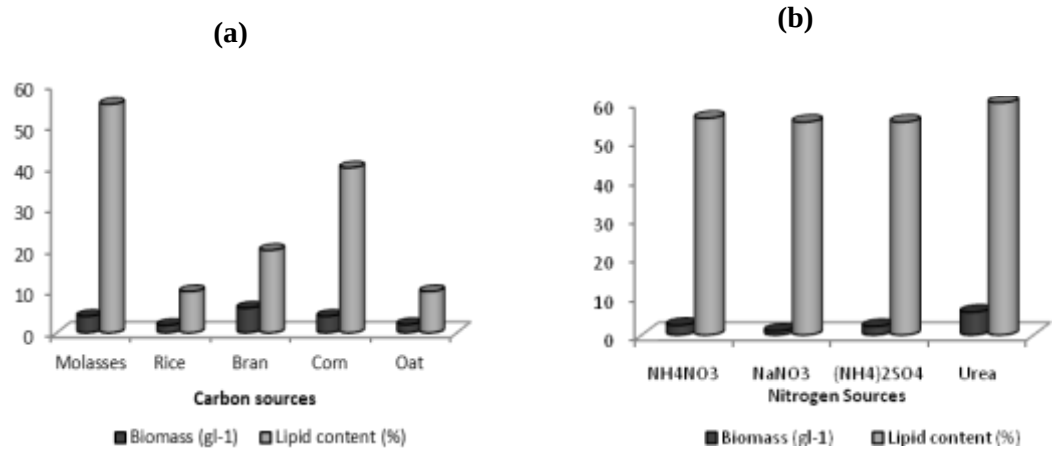


Fig. 2: Effect of different carbon (a) and nitrogen (b) sources on lipid accumulation and dry biomass by *Candida* sp.

Effect of different pH

The pHof the medium affects growth and product formation (Barnett *et al*., 1990). Evaluation of lipid production and biomass was investigated at different pH (3, 4, 5, 7, and 8). Results in Figure3a indicated that the highest lipid content (61.5%) and dry biomass (5.2 gl^{-1}) were observed at pH 5, while lipid content was gradually decreasedat pH lower or higher than 5. Dias *et al.* (2016) reported that the

Rhodosporidium toruloides NCYC 921 highest biomass and lipid content were 5.90 gl^{-1} and 21.85%, w/w, respectively, at pH 4.0. El-Fadaly *et al.* (2009) considered the highest production of lipidby *Cryptococcus curvatus* NRRLY-1511 strain at pH 5.5.

Effect of temperature

Change in temperature is an important factor affecting lipid accumulation (Taskin*et al.*, 2015). This experiment was carried out to evaluate the effect of different temperature (5, 25, 30, and 45^{o}C) on accumulation of lipid by *Candida* sp. As shown in Figure 3b, there was a general trend towards an increased relative amount of lipid content and biomass with increasing growth temperature until reaching 25^{o}C with 65% lipid content and 5 gl^{-1} for biomass. Similar feature was documented by Huang *et al.* (2012) for oleaginous yeast; *Trichosporon fermentans*. Contrary, 15°C was favorable for oleaginous yeast *Yarrowia lipolytica* B9 (Taskin *et al.*, 2015).

Effect of inoculum size

The effect of inoculum size (5, 10, 20, and 30%) on lipid production by *Candida* sp. was investigated. It is clear from Figure 3c that the maximum lipid content (71%) and biomass (6 gl^{-1}) was obtained with an inoculum size of 20% and nearly equal lipid content was obtained upon using 5% and 10% inoculum size. More inoculum size (30%) decreased the lipid accumulation, where molasses was preferable as energy source instead of production of lipid (Christophe *et al.*, 2012). Dai *et al.* (2007) stated that 5% was the most suitable inoculum size for better biomass and lipid production. The same finding was observed by Huang *et al.* (2012). Another study by Taskin *et al.* (2015) showed that the highest content of lipid and biomass of oleaginous yeast *Yarrowia lipolytica* B9 were (28%) and (4.38 gl^{-1}) with an inoculum size of 3 %. Jiru *et al.* (2017) reported that 10% v/v inoculum size was the optimum inoculum size for lipid accumulation by *Rhodotorula kratochvilovae*.

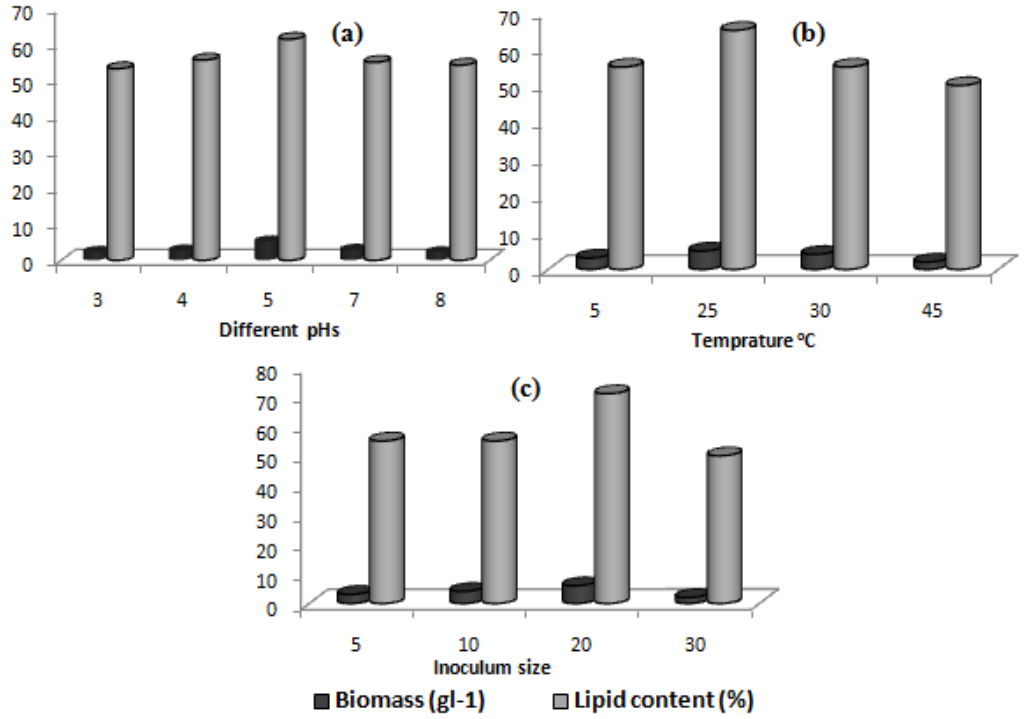


Fig. 3: Effect of temperature (a), pH (b) and inoculum size (c)on lipid accumulation and dry biomass by *Candida* sp.

Generally, the results of the current study concluded that 1.7 and 3.2 fold increase in lipid content (71%) and dry biomass (6 gl^{-1}) of the marine yeast *Candida* sp. was achieved up on growing under the optimized culture conditions compared tothebasal conditions (42.8 % and 1.9 gl^{-1}), respectively.ANOVA test ($P \leq 0.05$) was used to predict the most significant factors affecting lipid accumulation by *Candida* sp. Accordingly, it was revealed that among the tested parameters; carbon sources (P= 0.035), pH (P = 0.046) and inoculum size (P = 0.002)were the most significant factors affecting the bioaccumulation of lipid by *Candida* sp.

Analysis of extracted lipids using GC-MS

The fatty acids in the lipid content of *Candida* sp. were determined using GC-MS according to standard methods. Data presented in Table 2 showed 34 fatty acids; sixteen saturated;seven monosaturatedand eleven polyunsaturated.

Table 2: Analysis of extracted lipid profile from marine *Candida* sp. using GC-MS

Type	Compound	Formula	Molecular weight (g/mol)	% fatty acids
Saturated	Caproic acid methyl ester (C6)	$C_6H1_2O_2$	116.16	0.34
	Caprylic acid methyl ester (C8)	$C_8H_{16}O_2$	144.21	0.20
	Capric acid methyl ester (10)	$C_{10}H_{20}O_2$	172.26	0.24
	Undecanoic acid methyl ester (C11)	$CH_3(CH_2)_9COOH$	186.29	0.18
	Lauric acid methyl ester (C12)	$CH_3(CH_2)_{10}COOH$	200.32	0.20
	Tridecanoic acid methyl ester (C13)	$CH_3(CH_2)_{11}CO_2H$	214.34	0.06
	Myristic acid methyl ester (C14)	$CH_3(CH_2)_{12}COOH$	228.37	0.59
	Pentadecanoicame (C15)	$C_{16}H_{32}O_2$	242.40	0.64
	Palmiticame (C16)	$C_{16}H_{32}O_2$	256.43	7.43
	Heptadecanoicame (C17)	$CH_3(CH_2)_{15}COOH$	270.45	0.88
	Stearic acid methyl ester (C18)	$C_{18}H_{36}O_2$	284.48	6.30
	Arachidic acid methyl ester (C20)	$C_{20}H_{40}O_2$	312.54	0.25
	Heneicosanoicame (C21)	$CH_3(CH_2)_{19}COOH_3$	326.56	0.41
	Tricosanoicame (C23)	$C_{23}H_{46}O_2$	354.35	0.44
	Behenoic acid methyl ester (C22)	$C_{23}H_{46}O_2$	340.59	0.60
	Lignoceric acid methyl ester (C24)	$C_{24}H_{48}O_2$	368.63	4.40
Monounsaturated	Myristoleic acid methyl ester (C14)	$CH_3(CH_2)_3CH{=}CH(CH)_7CO_2H$	226.36	0.08
	cis-10-Pentadecenoic ame (C15)	$C_{15}H_{28}O_2$	240.38	1.88
	Palmitoleicame (C16)	$C_{16}H_{30}O_2$	254.41	4.69
	cis-10-Heptadecenoic ame (C17)	$CH_3(CH_2)_{15}COOH$	268.43	1.43
	Elaidic acid methyl ester (C18)	$C_{18}H_{34}O_2$	282.46	1.12
	Erucic acid methyl ester (C22)	$C_{22}H_{42}O_2$	338.58	0.47
	Docosadienoicame (C22)	$C_{22}H_{40}O_2$	336.56	0.33
Polyunsaturated (ω−3)	Linolenicame (C18)	$C_{18}H_{30}O_2$	278.43	18.28
	Eicosapentaenoicame (C20)	$C_{20}H_{30}O_2$	302.45	0.16
	Eicosatrienoicame (C20)	$C_{20}H_{34}O_2$	306.49	0.39
	Eicosenoicame (C20)	$C_{20}H_{38}O_2$	310.51	0.51
	Ecosatrienoicame (C20)	$C_{20}H_{34}O_2$	306.49	0.34
Poly-unsaturated (ω−6)	gama-Linolenicame (C18)	$C_{18}H_{30}O_2$	278.44	0.36
	Arachidonicame (C20)	$C_{20}H_{32}O_2$	304.47	0.83
	Eicosadienoicame (C20)	$C_{20}H_{36}O_2$	506.00	0.35
	Docosahexaenoicame (C22)	$C_{22}H_{32}O_2$	328.49	0.44
Poly-unsaturated (ω−9)	Oleic acid methyl ester (C18)	$C_{18}H_{34}O_2$	282.46	46.82
	Nervonic acid methyl ester (C24)	$C_{24}H_{46}O_2$	366.62	0.22

The highest % of fatty acid was presented by oleic acid methyl ester (C18, 46.82%) followed by linolenic acid methyl ester (C18, 18.28%), however, the lower values were recorded for palmitic acid methyl ester (C16, 7.43%) and stearic acid methyl ester (C18, 6.30%). The lowest records were from 0.1-4.4 %. Different cultivation conditions are known to affect the composition of fatty acids in cultivated yeast (Makri *et al*., 2010). Recently, Tsakraklides *et al.* (2018) reportedthe production of high-oleatetriacylglyceride oil by oleaginous yeast *Yarrowia lipolytica* for lubricant applications.

Biodiesel production from microbial lipids and the resulted biodiesel properties

Trans-esterification procedures of the extracted lipids from *Candida* sp. were used to detect biodiesel properties. The best physicochemical properties of the produced biodiesel are represented in Table 3.Cetane Number (CN) of the fuel is an important parameter and responsible for the delay period. Higher cetane number cause lower delay period and smoother engine operation. In the current investigation, the obtained biodiesel has a CN (57.43), which in the range of the British biodiesel standard EN (14214). Kinematic viscosity (υ) is a measure of the internal friction or resistance of oil to flow. The operation of fuel injection equipment and fluidity of the fuel are highly affected by kinematic viscosity (Sivaramakrishnan and Ravikumar, 2012). In our study, kinematic viscosity of the produced biodiesel was 4.5 mm^2s^{-1}, which meets international biodiesel standard EN 14214 (3.5-5.0 mm^2s^{-1}). Density (ρ) is another important parameter for biodiesel quality (Ng and Gan, 2012). The value of density was 0.87 gcm^{-3}, which is and in a good agreement with results reported by Duarte and Maugeri (2014). Content of energy in the fuel is measured by the Heat of combustion (HHV). HHV value in the present study (40.05 $MJkg^{-1}$), was closed to that reported by Ramirez-Verduzco *et al.* (2012) and also to that of biodiesel blend Jatropha (39.17- 41.52 $MJkg^{-1}$). Ability of biodiesel to react with oxygen at room temperatureis known as the Iodine Index (IV). In the current study, IV value (105.07 g) is within the limits of the standards and in agreement with the results of Duarte and Maugeri (2014). The Degree of Long Chain Saturation Factor (LCSF) and Unsaturation (DU) values recorded 13.96 and 98.87.

Table 3: Properties of biodiesel produced from trans-esterification process of marine *Candida* sp. fatty acids

Conditions	Biodiesel Standard EN (14214)	Experimental values
DU	-	98.87
LCSF	-	13.96
IV ($gI2100g^{-1}$fat)	≤120	105.07
SV ($mgKOHg^{-1}$)	-	190.34
Cetane number (CN)	≥51	57.43
Saturated fatty acid SFAs (%)	-	22.90
Monounsaturated fatty acid MUFA (%)	-	55.33
Polyunsaturated fatty acid PUFA (%)	-	21.77
Kinematic viscosity (υ) (mm^2s^{-1})	3.5–5.0	4.52
Density (ρ) ($g\ cm^{-3}$)	0.86–0.9	0.87
HHV ($MJ\ kg^{-1}$)	NA	40.05

Antimicrobialproperties

Unsaturated fatty acids (C16–C20) are documented as additives to antibacterial componentsand most food additives,due to antibacterial activity against different pathogens (Sun *et al*., 2003). Thus the present experiment looked for testing the naturally produced fatty acids by marine *Candida* sp. for their antibacterial action

against different fish pathogens aiming to improve the fish health and combating the pathogenic invaders. The tested fatty acids showed antibacterial activity with inhibition zone diameters (10-17 mm), while no activity was detected against *E. coli* and *P. aeruginosa* as shown in Table 4. The producedfatty acids from marine *Candida* sp. have a valuable importance in the fish health and aquaculture sanitary.Their antagonistic activity is attributed to the presence of the unsaturated types (Sun *et al*., 2003). Zheng *et al.* (2005) stated that the unsaturated fatty acids such aslinoleic acid were capable to inhibit *E. coli* and *S. aureus*. Lara-Flores *et al.* (2010) concluded that, supplementation of yeast to protein diet of shrimp enhanced the health and performance.

Table 4: Antibacterial activity of fatty acids produced by *Candida* sp. against different bacterial pathogens

Pathogen	**Inhibition zone diameter (mm)**
A. hydrophila	10
E. coli	-
P. aeruginosa	-
Pseudomonas sp.	17
V. cholera	14
V. fluvialis	10
V. parahaemolyticus	15

The potentiality of the produced fatty acids as antifungal agent was tested. Results in Table 5 revealed that complete inhibition of *Helminthosporium* sp. was observed. On the other side, the tested fatty acids inhibited the growth of *R. solani*, *R. oryzae* and *F. solani* with 89, 78, and 50%, respectively. In the present study, the produced fatty acids were known to have potential elimination of fungal growth as was previously reported (Agoramoorthy *et al*., 2007; Pinto *et al*., 2017).

Table 5:Antifungal activity of fatty acids produced by *Candida* sp. against different fungal pathogens.

Fungal pathogens	**Growth inhibition (%)**
*Helminthosporium*sp.	100
R. solani	89
R. oryzae	78
F. solani	50

CONCLUSION

The present study aimed to the accumulation of lipid by marine yeast isolates as natural and ecofriendly source using cheap natural materials that have potential to reduce the price of lipid forsome potential applications. The effect of different factors on yeast dry mass and accumulation of lipid was studied. Among the screened carbon and nitrogen sources, molasses and urea lead to higher lipid production (71%) at pH 5 and 20% as inoculum size at incubation temperature 25^{o}C. Marine *Candida* sp. is a promising than microalgae in the biodiesel production because of their fast and high biomass formation with no need to light or costly requirements. In sight into enhancement of biodiesel properties will be conducted in the future work. Indeed, polyunsaturated fatty acids, especially omegathreedetected in the lipid content of *Candida* sp.exhibited good antimicrobial activities against different bacterial and fungalpathogens.Consequently, marine *Candida* sp. is considered a good natural and cheap source for applications concerning fish health.

Acknowledgement

We thank Mr. Mustafa Gazal (Chemistry Lab., Environmental Division, NIOF; Alexandria, Egypt) for his effort.

REFERENCES

Ageitos, J. M.; Vallejo, J. A. ; Veiga-Crespo, P. and Villa, T. (2011). Oily yeasts as oleaginous cell factories. Appl. Microbiol. Biotechnol., 90(4): 1219-27.

Agoramoorthy, M.; Chandrasekaran, V.; Venkatesalu, M.J. and Hsu, G. (2007). Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz. J. Microbiol., 38: 739-742.

Azad, A. K.; Ferdoush, A. A.; Hasan, M. M, Karim; M. R. and Jahan, A. (2014). Production of microbial lipids from rice straw Hydrolysates by *Lipomyce-sstarkeyi* for biodiesel synthesis. J. Microbial. Biochem. Technol., S8: 1-6.

Barnett, J. A.; Payne, R. W. and Yarrow, D. (1990). Yeast-characteristics and identification. Cambridge, Cambridge University Press. Pp: 18-226.

Carsanba, E.; Papanikolaou, S.; Erten, H. (2018). Production of oils and fats by oleaginous microorganisms with an emphasis given to the potential of the nonconventional yeast *Yarrowia lipolytica.* Crit. Rev. Biotechnol., 38(8): 1230-1243.

Christophe, G.; Kumar, V., Nouaille, R.; Gaudet, G.; Fontanille, P.; Pandey, A.; Soccol, C.R. and Larroche, C. (2012). Recent developments in microbial oils production: a possible alternative to vegetable oils for biodiesel without competition with human food. Braz. Arch. Biol. Technol., 55(1): 29-46.

Dai, C-c.; Tao, J., Xie, F.; Dai, Y-j., and Zhao, M. (2007). Biodiesel generation from oleaginous yeast *Rhodotorula glutinis* with xylose assimilating capacity. Afr. J. Biotechnol., 6 (18): 2130-2134.

Devi, N.M. and Velayutham, P. (2011). Biodiesel production from fungi. Ind. J. Nat. Sci., 11(5): 275-281.

Dias, C.; Silva, C.; Freitas, C.; Reis, A.and da Silva, T.L. (2016). Effect of medium pH on *Rhodosporidium toruloides* NCYC 921 carotenoid and lipid production evaluated by flow cytometry.Appl. Biochem. Biotechnol., 179(5): 776-87.

Duarte, S. H. and Maugeri, F. (2014). Prediction of quality properties for biodiesel production by oleaginous yeast cultivated in pure and raw glycerol.Chemical. Eng. Transactions, 37: 463-468.

El-Fadaly, H.A.; El-Naggar, N.E-A.and Marwan, E-S.M. (2009). Single cell oil production by an oleaginous yeast strain in a low cost cultivation medium. Res. J. Microbiol., 4(8): 301-313·

El-Masry, M. H.; Khalil, A. I.; Hassouna, M.S. and Ibrahim, H. A. (2002). *In situ* and *invitro* suppressive effect of agricultural composts and their water extracts on some phytopathogenic fungi. World. J. Microbiol. Biotechnol., 18(6): 551–558.

Enshaeieh, M.; Abdoli, A.; Nahvi, I. and Madani, M. (2012). Bioconversion of different carbon sources into microbial oil and biodiesel using oleaginous yeasts. J. Biol., 1(2): 42- 47.

Fei, Q.; Chang, H.N; Shang L, et al. (2011). The effect of volatile fatty acids as a sole carbon source on lipid accumulation by *Cryptococcus albidus* for biodiesel production. Biores. Technol., 102(3): 2695-2697.

Gutierrez, L.F.; Ratti, C. and Belkacemi, K. (2008). Effects of drying method on the extraction yields and quality of oils from quebec sea buckthorn (*Hippophaer hamnoides* L.) seeds and pulp. Food Chem., 106: 896-904.

Helwani, Z.; Othman, M.R.; Aziz, N, *et al*. (2009). Technologies for production of biodiesel focusing on green catalytic techniques.Fuel.Process. Technol., 90: 1502–1514.

Huang, C.; Liu, H.Z-J.; Cai, J., *et al*. (2012). Effect of organic acids on the growth and lipid accumulation of oleaginous yeast *Trichosporon fermentans.* Biotechnol. Biofuels, 5: 4-7.

Islam, M. A.; Magnusson, M.; Brown, R. J., *et al*. (2013). Microalgal species selection for biodiesel production based on fuel properties derived from fatty acid profiles. Energies, 6: 5676-5702.

Jiru, T.M.; Groenewald, M.; Pohl, C., *et al*. (2017). Optimization of cultivation conditions for biotechnological production of lipid by *Rhodotorula kratochvilovae* (syn, *Rhodosporidium kratochvilovae*) SY89 for biodiesel preparation. Biotech. 7: 145-152.

Khambhaty, Y.; Upadhyay, D.; Kriplani, Y., *et al*. (2013). Bioethanol from macroalgal biomass: utilization of marine yeast for production of the same. Bioeng. Res., 6: 188-195.

Kolouchová, I.; Maťátková, O.; Sigler, K., *et al*. (2016). Production of palmitoleic and linoleic acid in oleaginous and nonoleaginous yeast biomass. Int. J. Anal. Chem., 9: 1-9.

Kosa, M. and Ragauskas, A. J. (2011). Lipids from heterotrophic microbes: advances in metabolism research. Trends Biotechnol., 29: 53-56.

Kraisintu, P.; Yongmanitchai, W. and Limtong, S. (2010). Selection and optimization for lipid production of newly isolated oleaginous yeast, *Rdodosporidium toruloides* DMKU3-TK16.Kasetsart University, Thailand. 44: 436-445.

Kumar, P.; Dhiman, S.; Bhatt, R.P. and Singh, L. (2011). *In vitro* antifungal activity of *Sapium sebiferum* L. against *Aspergillus niger* and aflatoxigenic *Aspergillus flavus.* J. Appl. Pharm. Sci., 19: 108-110.

Lara-Flores, M.; Olivera-Castillo, L. and Olvera-Novoa, M.A. (2010). Effect of the inclusion of a bacterial mix (*Streptococcus faecium* and *Lactobacillus acidophilus*), and the yeast (*Saccharomyces cerevisiae*) on growth, feed utilization and intestinal enzymatic activity of Nile tilapia (*Oreochromis niloticus*). Int. J. Fisher. Aquat., 2(4): 93-101.

Lewis, T.; Nichols, P.D. and McMeekin, T.A. (2000). Evaluation of extraction methods for recovery of fatty acids from lipid-producing microheterotrophs. J. Microbiol. Meth., 43: 107-116.

Liang, M.H. and Jiang, J.G. (2013).Advancing oleaginous microorganisms to produce lipid *via* metabolic engineering technology. Prog.Lipid. Res., 52: 395-408.

Makri, A.; Fakas, S. and Aggelis G. (2010). Metabolic activities of biotechnological interest in *Yarrowia lipolytica* grown on glycerol in repeated batch cultures.Biores. Technol., 101: 2351–2358.

Park. G.W.; Kim, N. and Chang, H.(2018). Microbial lipid production from volatile fatty acids by oleaginous yeast.Emerging Areas in Bioengineering, pp.203-213

Pinto, M.E.A.; Araújo, S.G.; Morais, M.I., et al. (2017). Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils.Anais da Academia Brasileira de Ciências, 89(3): 1671-1681.

Ng, H.K. and Gan, S. (2012). Characterization of engine-out responses from a light-duty diesel engine fuelled with palm methyl ester (PME). Appl. Energy., 90: 58-67.

Obara, N.; Ishida, M.; Hamada-Sato, N. and Urano, N. (2012). Efficient bioethanol production from scrap paper shredder by a marine *Saccharomyces cerevisiae* derived C-19. Stud. Sci. Technol., 1: 127–132.

Ramírez-Verduzco, L. F.; Rodríguez-Rodríguez, J.E. and Jaramillo-Jacob, Ad.R. (2012). Predicting cetane number, kinematic viscosity, density and higher heating value of biodiesel from its fatty acid methyl ester composition. Fuel, 91: 102–111.

Ratledge, C. (2005). Single Cell Oils, Lipid Research Center, University of Hull, and Department of biological sciences, Pp: 1-20.

Saravanan, S. and Chandrasekar, S. (2013). Predictive equations for viscosity and cetane number biodiesel. Ger. J. Renew. Sust.Ener. Res., 1: 23-28.

Scholza, U.; Diazb, G.G.; Ricqueb, D., *et al*. (1999). Enhancement of vibriosis resistance in juvenile *Penaeusvannamei* by supplementation of diets with different yeast products.Aqua., 176 (3-4): 271-283.

Sitepu, I.R.; Sestric, R.; Ignatia, L., *et al*. (2013). Manipulation of culture conditions alters lipid content and fatty acid profiles of a wide variety of known and new oleaginous yeasts species. Bioresour. Technol., 144: 10-15.

Sivaramakrishnan, K. and Ravikumar, P. (2012). Determination of cetane number of biodiesel and its influence on physical properties. ARPN J. Eng. Appl. Sci., 7: 205-211.

Sun, CQ.; O'Connor, C.J. and Roberton, A.M. (2003). Antibacterial actions of fatty acids and monoglycerides against *Helicobacter pylori*. FEMS Immunol. Med. Microbiol., 36: 9-17.

Taskin, M.; Saghafian, A.; Aydogan, M.N.; Arslan, NP.(2015). Biofuels microbial lipid productionby cold-adapted oleaginou yeast *Yarrowia lipolytica* B9in non-sterile whey medium. Bioprod. Biorefinin., 9(5): 595–605.

Tsakraklides, V.; Kamineni, A.; Consiglio, A.L., et al. (2018). High-oleate yeast oil without polyunsaturated fatty acids.Biotechnol.Biofuels, 11:131- 142.

Vairavan, K.; Thukkaiyannau, P.; Paramathma, M.; Venkatachalam, P. and Sampathrajan, A. (2010). Biofuels Crops: Cultivation and Management, Jatropha, Sweet sorghum and Sugarbeet. Agrobios (India), Jodhpur, India, Pp 3-9: 68-92.

Xue, Z.; Sharpe, P. L.; Hong, S.P. *et al*. (2013). Production of omega-3 eicosapentaenoic acid by metabolic engineering of *Yarrowia lipolytica*.Nat. Biotechnol., 31(8): 734-740.

Yamada, R.; Kashihara, T. and Ogino, H. (2017). Improvement of lipid production by the oleaginous yeast *Rhodosporidium toruloides* through UV mutagenesis. World J. Microbiol.Biotechnol., 33(99): 1-9.

Zaky, A.S.; Tucker, G.A.; Yehia, Z. and Du, D.C. (2014). Marine yeast isolation and industrial application. FEMS Yeast Res., 14(6): 813–825.

Zheng, C.; Yoo, J.S; Lee T.G.; Cho, H.Y.; Kim, Y.H. and Kim, W.G. (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Lett., 579(23): 5157–5162.

Zhu, L.Y.; Zong, M.H. and Wu, H. (2008). Efficient lipid production with *Trichosporon fermentans* and its use for biodiesel preparation. Bioresour. Technol., 99: 7881-7885.

ARABIC SUMMARY

زيادة تراكم الدهون في الخميرة البحرية *Candida* sp. من أجل إنتاج الديزيل الحيوي والخواص المضادة للميكروبات

سحر وفقي مصطفى حسن، خلود محمد بركات، حسن عبد الله حسن إبراهيم
قسم الميكروبيولوجيا البحرية، شعبة البيئة البحرية، المعهد القومي لعلوم البحار والمصايد
قايتباي، الأنفوشي، الإسكندرية

الأحماض الدهنية، سواء كانت حرة أو كجزء من الدهون المعقدة، هي مكونات أساسية لجميع الأغشية الحيوية. وبالتالي، فإن الهدف من هذه الدراسة هو إنتاج الأحماض الدهنية عن طريق الخميرة البحرية باستخدام بعض المصادر الطبيعية منخفضة السعر. عُزلت *Candida* sp. البحرية من سيدي بشر، بالبحر الأبيض المتوسط، مصر. أظهرت أعلى قدرة تراكم الدهون بنسبة ٤٢.٨٪ بكتلة حيوية ١.٩ جرام للتر. تم دراسة ظروف التخمر المثلى لإنتاج الدهون بها باستخدام عامل واحد مع الزمن. أظهر المولاس واليوريا كمصدر للكربون والنيتروجين أعلى نسبة دهون ٧١٪ بكتلة حيوية ٦ جرام في اللتر، على التوالي، وعند درجة حموضة ٥ و ٢٥ درجة مئوية و ٢٠٪ من حجم التلقيح، حيث زاد تراكم الدهون والكتلة الحيوية الجافة لـ*Candida* sp. البحرية بـ ١.٧ و ٣.٢ ضعف، على التوالي. تم إجراء عمليةاسترة للدهون المتراكمة، مما أسفر عن ٣٤ استرات ميثيل حمض دهني (FAMS) تم اكتشافها بواسطة تحليل كروماتوجرافيا الغاز. تم تقدير خصائص الديزل الحيوي الناتج فتبين أن له درجة عدم التشبع (DU: 98.87)، معامل تشبع السلسلة الطويلة (LCSF: 13.96)، قيمة اليود (IV: 105.074)، قيمة التصبن (SV : 190.34)، رقم السيتان (CN: 57.43)، اللزوجة الحركية (υ: 4.52)، الكثافة (ρ: 0.87)، وقيمة التسخين الأعلى (HHV: 40.05). أظهر النشاط المضاد للبكتيريا في الدهون المستخلصة أقطار منطقة التثبيط، تراوحت من ١٠ إلى ١٧ مم. علاوة على ذلك، تراوح النشاط المضاد للفطريات ضد بعض مسببات الأمراض بين ٥٠ و ١٠٠٪.