

## ISOLATION AND CHARACTERIZATION OF OLEAGINOUS MARINE YEAST PRODUCING OF FATTY ACIDS

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### ABSTRACT:

Because of the limitation of lipid sources from animal and plant, there are great interest searches to finding new alternative lipid sources such as microbial cells that can be used as a key player in different applications such as biodiesel production as well as pharmaceutical and food industries. Therefore, ten sediment and seawater samples were collected from different marine environments for oleaginous yeast isolation purposes. A total of 20 yeast isolates were obtained and screened to accumulate intracellular fatty acids using Nile red staining under a fluorescence microscope as well as Triphenyltetrazolium chloride (TTC) dye at  $A_{485nm}$ . Amongst all isolates, two yeast isolates namely C5, and L4 have exhibited the best results for cellular lipid content accumulation. They showed the highest potentiality of lipid accumulation from 14.3 to 15.1g<sup>l</sup><sup>-1</sup>, respectively of dry mass and the percent of lipid content appeared from 25.4 to 28.2%, respectively when cultured on YPD medium at 25°C. Based on phenotypic and genotypic criteria these isolates were identified as *Candida parapsilosis* C5 and *Cryptococcus albidus* L4. The study of fatty acids profile composition of these isolates showed that linoleic acid (C18:2), gamma-Linolenic acid (C18), oleic acid (C18:1), stearic acid (C18:0), and palmitic acid (C16:0) are the main fatty acids produced that similar to those present in plant oils. The main fatty acids obtained by both strains represent  $\approx 76\%$  and 91% for isolate C5 and L4, respectively. Also, these strains displayed 68-70% of unsaturated fatty acids and produced an appropriate amount of saturated fatty acids ranged between 30.5 to 32 % of the total fatty acids make these strains a suitable solution to solve many global problems related to dietary supplements, diseases treatment, and energy renewable sources such as biodiesel production.

**Keywords:** Isolation, marine, oleaginous, yeast, polyunsaturated fatty acids (PUFAs), Lipid production, screening, *Candida parapsilosis*, *Cryptococcus albidus*.

## 1-Introduction

Polyunsaturated fatty acids (PUFAs) are essential components of phospholipid cell membranes, regulating the fluidity of cellular membranes and modulating enzyme activities, carriers, and membrane receptors as well as it may be useful in the prevention and management of many chronic diseases such as problems resulted from auto-immune disorders, cancer, hypertension, diabetes, inflammatory and coronary heart disease, atopic eczema, major depression, schizophrenia, Alzheimer dementia and multiple sclerosis (Yeung *et al.*, 2018; Wang *et al.*, 2019).

Fish is the most important dietary source of PUFAs for humans, as well as a good source of vitamins, proteins, and minerals. The recommended regular PUFAs intake levels differ across the world, depending on the regional type or health organizations recommendations. Nonetheless, most experts believe that the average person can eat at least two portions of fish every week one of them preferred to be oily (Mohanty *et al.*, 2019).

Therefore, as the world's population and demand for seafood grow, and wild catch fisheries exceed over their exploitable limits, because more fish bound for the table market is being farmed. During the past few decades, aquaculture has been the fastest-growing, animal-food-producing industry, outpacing population growth, and now provides roughly half of the world's fish and seafood for human requirements (Sprague *et al.*, 2016).

The growing demand for fish oil from aquaculture to pharmaceutical industries, and other manufactures requirements as well as the permanent natural climate changes, affect oil supply and prices. Also, overfishing has depleted ocean fisheries, making them a non-renewable source of PUFAs to meet rising demand (Harwood, 2019). Furthermore, environmental pollutants have been discovered in fish oils, necessitating the creation of a renewable, land-based source of PUFAs. To overcome these constraints, biotechnological industries began producing PUFAs directly from microorganism's mainly oleaginous microorganisms in large-scale fermentation processes (Kraic *et al.*, 2018).

Oleaginous microorganisms including yeast, filamentous fungi, microalgae, bacteria, and cyanobacteria, have the potential ability to accumulate intracellular lipids up to 20% of their dry mass and hence they are considered desirable next-generation precursors for fatty acids or lipids production (Bruder *et al.*, 2018).

Oleaginous bacteria have received less attention to date because their lipid content is lower than that of other oleaginous microorganisms, and their growth rates are also slower. Oleaginous cyanobacteria and microalgae are desirable hosts for the development and production of fatty acids due to their special photosynthesis capability, which transforms solar energy and CO<sub>2</sub> directly into cellular lipid contents (Nilsson *et al.*, 2020). However, they are also more difficult to manipulate genetically than bacteria, yeast, and fungi, and their cultivation and growth processes are more complicated and costly. These previous obstacles have limited their use in the production of fatty acid-derived chemicals

via metabolic engineering. Similarly, the use of oleaginous filamentous fungi as production candidate hosts is hampered by a lack of effective genetic transformation techniques (**Kumar *et al.*, 2019**).

In contrast to other oleaginous microbial lipid sources, oleaginous yeast has several advantages that make it the most promising cell factory for producing fatty acid-derived chemicals. They can achieve extremely high levels of lipid accumulation of more than 70% of their dry weight in easy and inexpensive culture. They can also use various types of waste residues as substrate nutrients and they are more genetically tractable than other oleaginous microorganisms. Moreover marine yeasts have several distinct and promising advantages over terrestrial yeasts, including greater osmosis tolerance, increased special chemical productivity, and the production of industrial enzymes make them a great deal of potential for use in a variety of industries (**Diwan & Gupta, 2020**). Therefore, this study aimed to isolate, identify, and screen for oleaginous marine yeast strains with high specific growth rates and high cellular lipid content that could be used as the precursor of PUFA production as well as study the characterization of fatty acid profiles obtained by these strains.

## **2. Materials and Methods**

### **2.1. Samples collection**

Ten sediment and seawater samples were collected from the Abu Qir Bay in the Mediterranean Sea at Alexandria governorate (March 2017) and the Red Sea (Suez Canal, January 2017) in Egypt. The collected samples were stored in icebox at 4 °C and transferred under aseptic conditions to the laboratory for yeast isolation procedures.

### **2.2. Isolation and cultivation of lipid producing yeast**

The isolation and cultivation procedure were performed within 24 hours of samples collection according to the method described by **Krieg & Padgett (2011)**.

### **2.3. Screening for lipid production by yeast isolates**

The ability of purified yeast colonies to produce polyunsaturated fatty acids was screened by using two different methods. Firstly, according to (**Kimura *et al.*, 2004**) protocol by measuring the fluorescence of Nile Red (9-diethylamino-5H-benzo[ $\alpha$ ]phenoxazine-5-one) staining under a fluorescence microscope (BX-40, Olympus, Tokyo, Japan) and the isolates that showed the higher light intensity was selected as lipid producing strain. The previously selected isolates were further screened for their internal lipid contents by measuring the absorbance of Triphenyltetrazolium chloride (TTC) at wavelength 485nm using of 0.6% TTC solution prepared according to the method suggested by (**Arora *et al.*, 2015**) and the staining procedures were conducted based on the method reported by (**Vadivelan & Venkateswaran 2014**) using of methanol replaced of

ethyl acetate. Yeast isolates with the higher potential of lipid accumulation were maintained in YPD agar slants to use for further characterization and identification.

## **2.4. Identification of the most promising lipid-producing isolates**

### **2.4.1. Morphological and biochemical characteristics**

Morphological properties of the most promising lipid-producing yeast isolates were investigated after incubation at 25 °C ±1 for 48 h on YPD agar plates. The purity and cell shape of yeast isolates were examined by methylene blue stain (0.1%, w/v) under an oil immersion lens (100X).

The ability of these isolates to grow aerobically after incubation for 48 h on different carbon sources such as citrate, glycerol, sucrose, D-glucose, xylitol, lactose, soluble starch, arabinose, and D-xylose and different nitrogen sources such as nitrate, ammonium sulfate, and urea as the sole sources of carbon and nitrogen was investigated using replica plate method and compared with a negative control without carbon and nitrogen. Also, the abilities of these isolates to grow at different temperatures ranged from 4 to 40°C were determined (Mbagwu, 2017).

### **2.4.2. Genetic characteristics**

Genetic identification of the most efficient lipid-producing strains was accomplished by using 18S ribosomal RNA. Genomic DNA of the most potent strains was extracted by the CTAB method and amplified according to (Van Dijken *et al.*, 2000) with primers ITS5 (5'- GACTCCTGGTCCGTGTT -3') as forward and ITS4 (5' ATTACCGCGGCTGCTGGCACC- 3') as the reverse. The products of polymerase chain reaction (PCR) were tested by gel electrophoresis containing 1% of agarose and 0.1 µg/ml of ethidium bromide dye and carried out at 125 volts. Then, the amplified product was purified using EZ-10 Spin Column PCR Products Purification Kit BS664 (Bio-basic, USA). The sequencing was performed by Applied Bio-systems automated DNA sequencer-Sanger Sequencing Technology (model; ABI 3730XL DNA Analyzer-Applied Bio-systems, USA; service provided by Macrogen Inc., South Korea). The alignments and sequence analyses were performed by the BLAST database of the National Center for Biotechnology Information (NCBI-BLAST programs) and the phylogenetic relationship of studied strains was analyzed and displayed based on distance-based Neighbor-Joining tree.

## 2.5. Poly-unsaturated fatty acids Production conditions

### 2.5.1. Preparation of inoculum

The propagation process for the most potent strains was carried out in YPD broth medium and incubated at  $25\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  for 48 h under shaking conditions (150 rpm). The optical density (OD) of the cultures was adjusted at 1 nm. Two milliliters of cultured broth medium were centrifuged for 5 min at 8000  $xg$ , and washed with sodium chloride saline solution (0.9 %), and centrifuged again and finally the collected pellets of lipid producing strain re-suspended in one milliliter of saline solution and used as standard inoculum (Yuangsaard *et al.*, 2013).

### 2.5.2. Production medium

Cellular lipid accumulation by the most promising oleaginous yeast isolates (C5 and L4) was observed on the productive medium through the cultivation of one milliliter of propagation culture on a nitrogen-limited medium composed of (g/L): 50 glucose, 5 peptone, and 3 yeast extract dissolved in one liter of seawater and the pH was adjusted at 5.8 and incubated at  $25\text{ }^{\circ}\text{C} \pm 2$  under shaking condition (150 rpm) (Pan *et al.*, 2009).

### 2.5.3. Biomass determination by cell dry weight

Biomass dry weight (CDW,  $\text{gL}^{-1}$ ) of most potent strains was evaluated gravimetrically according to (Chang *et al.*, 2015). Biomass of cultured cells was collected by centrifuging at 5,000  $xg$  for 15 min, washed twice with the same volume sterile saline solution, and then dried at  $55\text{ }^{\circ}\text{C}$  to the constant weight of cell biomass. Triplicate samples were determined for dry biomass and total lipid content.

## 2.6. Lipid extraction and purification

Cellular lipid content of yeast isolates was extracted based on the standard methodology described by (Bligh & Dyer 1959) in which 50 ml of cultured cells was centrifuged at 5000  $xg$  for 5 min, and the pellets were rinsed twice with 50 ml of distilled water and followed by addition into 10 ml of 4 M HCl and incubated for two hours at  $60^{\circ}\text{C}$  to lysis the cell wall of yeast strains. The above acid-hydrolyzed solution was constantly stirred at room temperature with 20 ml of solvents (chloroform/methanol mixture, 2:1; v:v) for 3 h. after that, the solution was centrifuged at 2000  $xg$  for 5 min at room temperature to separate the organic lower phases from an aqueous upper phase. The organic lower phase containing the lipids content was filtered through filter paper and recovered then completely evaporated by oven at  $55\text{ }^{\circ}\text{C}$  until constant weight and dry biomass, and then the lipid content was determined gravimetrically as previously mentioned.

## 2.7. Fatty acid composition and GC-MS analysis

The fatty acids of cellular lipid content were subjected to the methanolysis process according to (Amaretti *et al.*, 2010) as methyl esters by mixing crude lipid extract with 2 ml of methanolic sulfuric acid (6% H<sub>2</sub>SO<sub>4</sub>) for 30 sec. After that, two milliliters of petroleum ether and 1 milliliter of distilled water were mixed to collect fatty acid methyl esters (FAMES) for 30 sec and the upper petroleum layer was evaporated at 38°C under nitrogen atmosphere until reached to dryness state. The residual of FAMES were re-dissolved in 200 ml hexane and analyzed by Gas Chromatography (6890N) connected by Agilent 55973 Mass Spectrometer with an HP-5 capillary column (30 mx 0.25 mm id, 0.25 mm film thickness; J&W Scientific, USA). The injection temperature was maintained at 300 °C and the oven was programmed for 2 min at 150 °C, then increased to 300 °C at 4 °C min<sup>-1</sup> and maintained for 20 min at this temperature and the carrier gas was helium at a flow rate of 1.0 ml min<sup>-1</sup>. The Fatty acid methyl esters were quantified and identified by comparing their retention time and mass spectra with the spectral standard of Wiley and NIST (Rubiolo *et al.*, 2010).

## 3. Results and Discussion

### 3.1. Isolation and Screening of oleaginous yeast

A total of 20 yeast isolates were obtained from different marine samples collected from two harbors from the Mediterranean Sea and the Red Sea in Egypt. These isolates exhibited the typical morphology and properties of the yeast cell.

Yeasts are a polyphyletic group of basidiomycetous like *Cryptococcus laurentii* and ascomycetous such as *Pichia kudriavzevii* (Barghoth *et al.*, 2018) fungi with the ability to grow in unicellular manner. Yeasts are isolated from a wide range of aquatic, marine, atmospheric and terrestrial habitats. Yeasts seldom occur in the absence of either molds or bacteria. Consequently, selective techniques are often used for recovery of yeasts, employing media that permit the yeasts to grow, while suppressing the growth of molds and bacteria (Klymiuk, 2018).

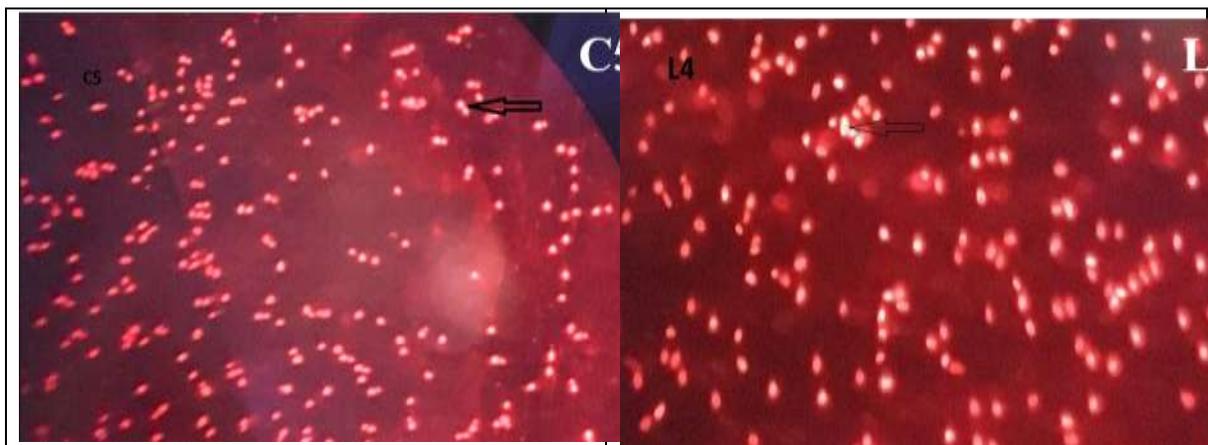
Preliminary screening for cellular lipid accumulation potential of the collected yeast isolates was point out by Nile red stain based on the ability of these isolates to emit the yellow gold fluorescence due to their cellular lipid content. Amongst all isolates, only two yeast strains coded as C5 and L4 revealed a significant content of cellular lipid with lipid accumulation ranged from 25 to higher than 35% of the cell area when stained with the Nile red (Fig. 1). Nile red is one of the faster separation techniques that prefer by many researchers to select oil-producing strains from other non-producing ones through the emitting of gold-like fluorescence when reacting with a hydrophobic compound such as lipids (Govender *et al.*, 2012). The internal lipid concentration inside yeast strains is directly correlated with the fluorescence intensity of cells under UV light in which the

stronger fluorescence intensity the higher accumulation of cellular lipid contents (**Daniel et al., 2011**).

The appearance of different lipid body shapes in oleaginous yeast when stained with Nile red dye was fully consistent with the results previously described by many authors depending on the genus even to species and the culture growth conditions. Moreover, the fluorescence emission observed in **Fig. 1** was completely identical to those present in other oleaginous yeasts (**Kimura et al., 2004; Kraisintu et al., 2010; Enshaeieh et al., 2014; Vinarta et al., 2016**).

The potential ability of isolates C5 and L4 to accumulate lipid droplets was confirmed calorimetrically by measuring the reduction of TTC stain from colorless to pink/red color at 485 nm. The data recorded in **Table 1** demonstrated that the higher degree of staining at  $A_{485\text{ nm}}$  was exhibited by isolate L4 (O.D.  $\approx$  2.51) followed by isolate C5 (O.D.  $\approx$  1.32).

The reduction of TTC dye (colorless) to triphenylformazan (TPF) (pink/red color) indicates that the capabilities of these isolates to form of PUFAs because of the reduction of TTC dye is regulated by numerous enzymes (dehydrogenases) responsible for the PUFAs biosynthesis process (**Ryan et al., 2010; Abd El Razak et al., 2014; Xue et al., 2018**). The potential ability of lipid accumulation inside yeast strains has been noted by many investigators (**Meng et al., 2009; Zhu et al., 2012; Magdouli et al., 2014 ; Ochsenreither et al., 2016; Kosa et al., 2017; Osorio-Gonzalez et al., 2019; Maza et al., 2020; Marika et al., 2021**).



**Fig. 1:** Fluorescence micrographs of lipid droplets of isolates C5 and L4 stained with the Nile Red dye.

### 3.2. Lipid content and biomass determination

The quantitative assessment of dry biomass and lipid content for the most promising yeast isolates C5 and L4 after growing on YPD medium containing a high amount of glucose sugar and limited source of nitrogen at 25°C and 5.8 of pH represented in **Table 1**. The most potent strains C5 and L4 showed the highest potentiality of lipid accumulation from 14.3 to 15.1 gL<sup>-1</sup>, respectively of dry mass and the percent of lipid content appeared from 25.4 to 28.2% respectively (**Ageitos et al. 2011**) reported that microorganisms that accumulate lipid content more than 20% corresponding to their dry mass are known as oleaginous microorganisms therefore isolates C5 and L4 indeed oleaginous yeast because they produce lipid content between 25.4 to 28.2%.

In general, many previous studies reported that oleaginous yeasts can accumulate and store cellular lipids when the cultivation nutrients become exhausted except the source of carbon is still available and hence they assimilating this carbon in the shape of lipids droplets inside their cells (**Ratledge & Wynn, 2002; Czabany et al., 2007; Vinarta et al., 2016**).

Biomass production by these strains (14.3 -15.1 gL<sup>-1</sup>) is too similar to results reported by other oleaginous yeasts such as *Cryptococcus curvatus* NRRLY1511, *Rhodospiridium glacialis*, *Candida freyschussii*, and *Rhodospiridium toruloides* Y4, and some Antarctic *Rhodotorula* spp. (**Li et al., 2007; Li et al., 2008; El-Fadaly et al., 2009; Amaretti et al., 2012; Vinarta et al., 2016**). Also, the lipid bodies that accumulate in oleaginous yeast have different percent, composition and shapes depending on the genus even to species producers and culture conditions such as carbon and nitrogen sources, nutrient availability, incubation time and temperature, inoculum size, pH of medium (**Kraisintu et al., 2010; Ravikumar et al., 2012**).

The ability of oleaginous yeasts such as genera *Cryptococcus*, *Lipomyces*, *Rhodotorula*, *Yarrowia*, and *Trichosporon* to accumulate a high percentage of lipid content inside their cells has reported by many investigators (**Kurtzman et al., 2011; Vinarta et al., 2016; Park et al., 2017; Maza et al., 2020**).

**Table 1: Staining degree, dry biomass, and total lipid content of yeast isolates C5 and L4**

Isolates	degree of Staining (A <sub>485</sub> nm)	Dry biomass (g/L)	Total lipid content (%)
C5	1.32	14.3	25.4
L4	2.51	15.1	28.2

### 3.3. Identification of oleaginous yeast strains

Morphological and microscopical characteristics of the most potent isolates showed that the isolate C5 appear cream white color, faint to shiny and oval to spherical shape with bipolar budding. While the isolate L4 appear mucoid or slimy with yellowish-white color and elongated to oval shape their cells surrounding with sticky layers. **Table 2** showed the biochemical and physiological characterizations of isolates C5 and L4 in which both strains can utilize D-glucose, glycerol, starch, xylitol, D-xylose, arabinose, citrate, and sucrose as the sole source of carbon but the isolate L4 not able to consume lactose sugar. In addition to these isolates can utilize all nitrogen sources except the isolate C5 not able to use organic nitrogen (urea). The lower temperature degree affects the growth of isolate C5 while the higher temperature degree inhibited the growth of isolates L4.

**Table 2: Biochemical properties of the oleaginous yeast isolates C5 and L4.**

Test	Isolate C5	Isolate L4
Glycerol	+ve	+ve
Starch	+ve	+ve
D-Glucose	+ve	+ve
Lactose	-ve	+ve
Xylitol	+ve	+ve
Sucrose	+ve	+ve
L-Arabinose	+ve	+ve
D-Xylose	+ve	+ve
Citrate (Sodium)	+ve	+ve
Urea	-ve	+ve
Nitrate	+ve	+ve
Ammonium Sulfate	+ve	+ve
Growth At 4°C	-ve	+ve
Growth At 25°C	+ve	+ve
Growth At 37°C	+ve	+ve
Growth At 40°C	+ve	-ve

+ve; Positive    -ve; Negative

The selected isolates C5 and L4 that appeared the highest potential capability in lipid production were identified using the genetic taxonomic approach. The result of BLAST analysis of the 18S rRNA gene sequence of the most potent yeast strains C5 and L4 were showed the perfect match of strain C5 with *Candida parapsilosis* strain CBS 604 (Gen-bank access number; [MH545914.1](#)), *Candida metapsilosis* strain CBS 10907 ([MK394127.1](#)) and *Candida parapsilosis* isolate XS2 ([KY118177.1](#)) with similarity of 97.6% for all these strains with more accession identical to *Candida parapsilosis* species and hence it is identified as *Candida parapsilosis* C5 (**Fig. 2**). On the other hand, the evolutionary distance of strain L4 with closely related species revealed a good match with *Cryptococcus albidus* also known as *Naganishia albida* ([AB032617.1](#)) and *Basidiomycete*

yeast sp. BG02-5-23-001-C2 (AY520208.1) with a similarity of 91.98% and 91.88%, respectively and hence it is identified as *Cryptococcus albidus* L4 (Fig. 3).

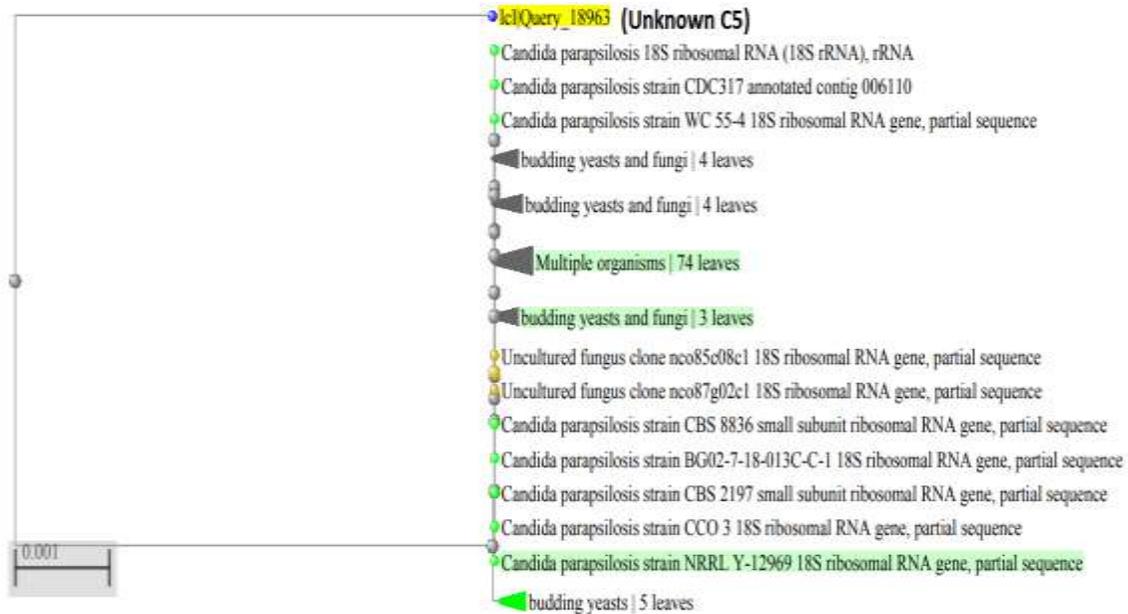


Fig. 2. Phylogenetic tree based on the sequence of 18S rRNA gene showing the similarity of oleaginous yeast isolate C5 with closely related strains.

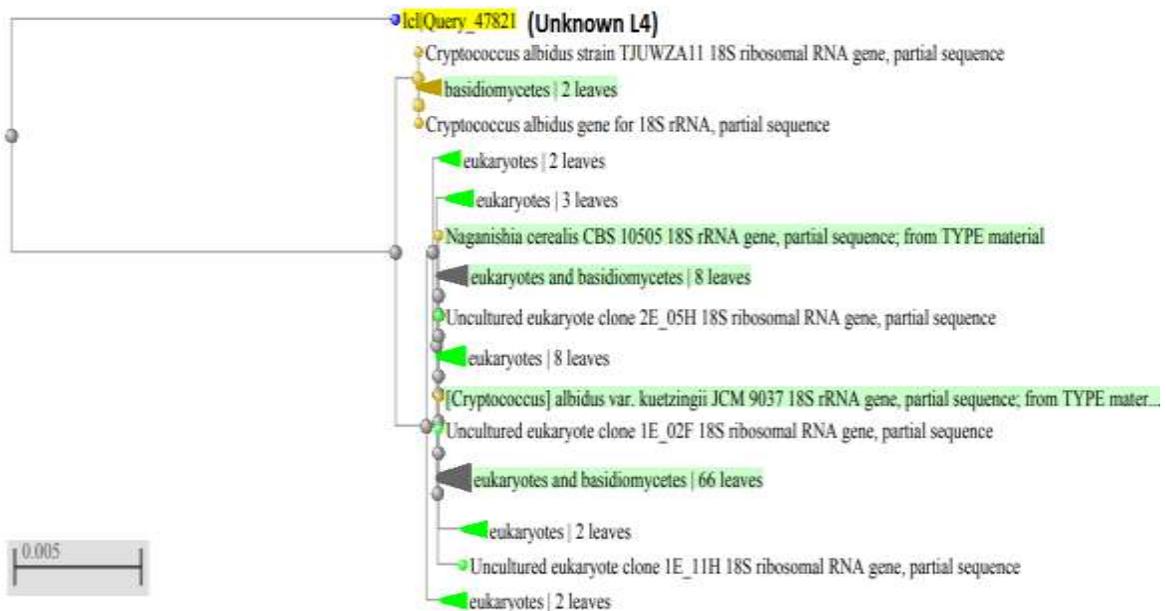


Fig. 3. Phylogenetic tree based on the sequence of 18S rRNA gene showing the similarity of oleaginous yeast isolate L4 with closely related strains.

### 3.4. Fatty acid composition and profile analysis

In this study, the most promising yeast isolates *Candida parapsilosis* C5 and *Cryptococcus albidus* L4 which showed the highest efficiency to accumulate cellular lipid droplets were subjected to detect their fatty acid profile composition. Determination of the fatty acid profile of oils produced by yeast isolates is highly essential because its composition affects the biotechnological application routes such as biodiesel quality or dietary supplements or disease treatment (Maza *et al.*, 2020).

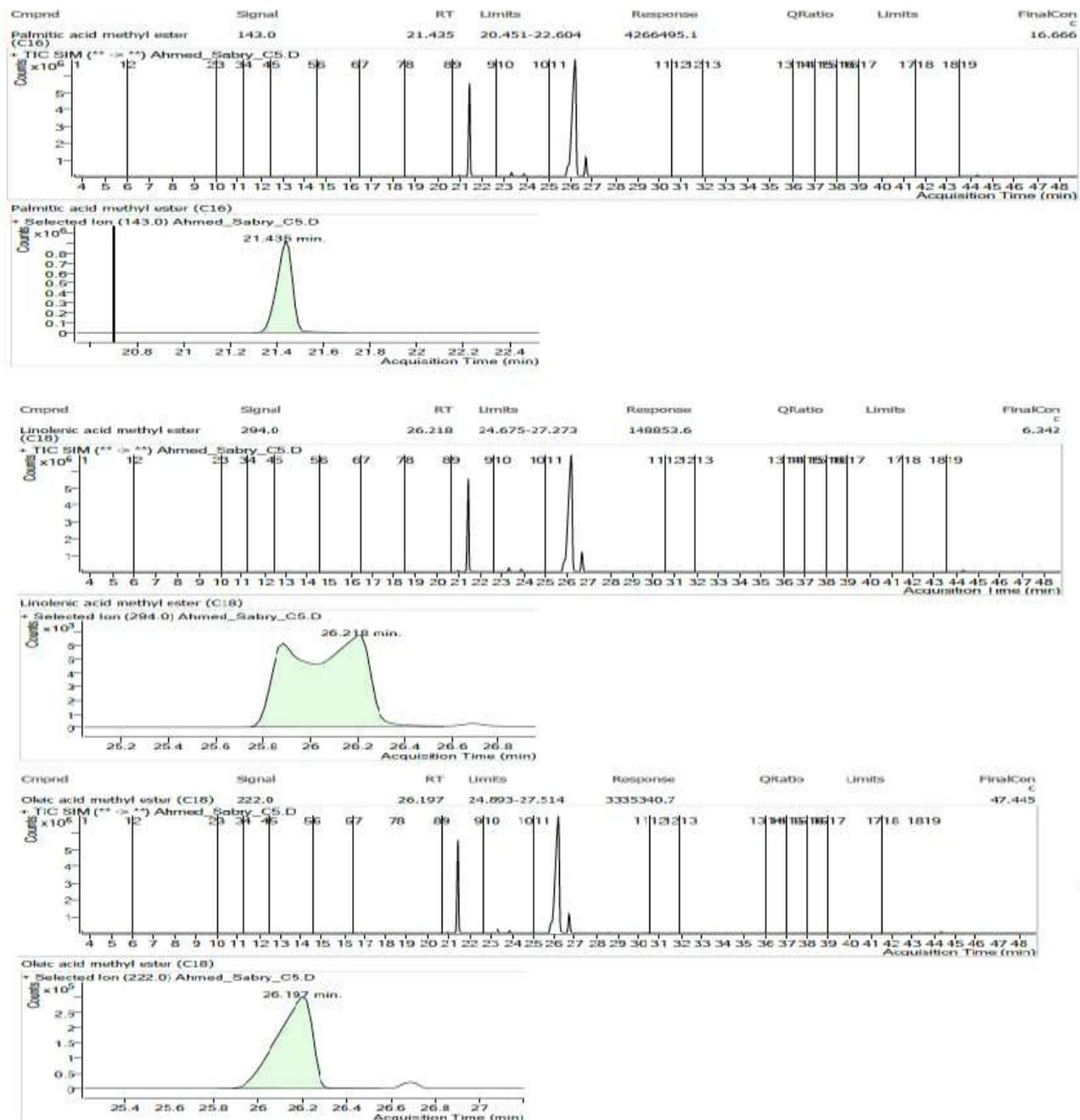
**Table 3** shows that the composition of the fatty acid profile including saturated, polyunsaturated, monounsaturated, and total lipid content accumulating by strains *Candida parapsilosis* C5 and *Cryptococcus albidus* L4. Data observed in this work point out that these strains produced a mixture of fatty acids similar to those present in vegetable oils such as triacylglycerols (TAG), linoleic acid (C18:2), oleic acid (C18:1), stearic acid (C18:0), and palmitic acid (C16:0) (Figs 4, 5). Many investigators reported that oleaginous yeasts have the potential ability to produce microbial oils with a composition similar to vegetable or plant oils and containing predominantly monounsaturated fatty acid (MUFA) or saturated fatty acid (SFA) with a carbon chain length of 16 and 18 (Li *et al.*, 2008; Beopoulos *et al.*, 2009; Meng *et al.*, 2009; Papanikolaou & Aggelis, 2011; Dalia *et al.*, 2014; Kosa *et al.*, 2018; Maza *et al.*, 2020).

Linoleic acid (LA) is commonly produced from plants; in particular, it was enriched and presented in seed oils. Linoleic acid is the only essential omega-6 fatty acid that must be obtained from the daily diet. Many other omega-6 fatty acids can be created from linoleic acid using elongase and desaturase enzymes and therefore linoleic acid serves as a precursor for the production of the essential fatty acid such as arachidonic acid, as well as other n-6 acyl species (Visioli & Poli, 2020).

On the same side, oleic acid is the most common unsaturated fatty acid that can be used as the precursor for the production of most other PUFAs. For instance plants produce both n-3 and n-6 PUFAs from oleic acid while animals can be elongate and de-saturate oleic acid into a variety of n-9 fatty acids (Saini & Keum, 2018)

**Table 3: Fatty acid methyl ester profiles of strains *Candida parapsilosis* C5 and *Cryptococcus albidus* L4.**

Name of fatty acid	Type of fatty acid	Percentage of each fatty acid	
		Isolate C5	Isolate L4
Caproic acid (C6)	Saturated	1.17	0.39
Caprylic acid (C8:0)	Saturated	2.10	0.65
Capric acid (10)	Saturated	0.27	0.08
Undecanoic acid (C11)	Saturated	0.22	0.08
Lauric acid (C12)	Saturated	0.19	0.06
Tridecanoic acid (C13)	Saturated	0.26	0.08
Myristoleic acid (C14)	Unsaturated	0.63	0.23
Myristic acid (C14)	Saturated	0.38	0.26
cis-10-Pentadecenoic acid (C15)	Unsaturated	0.73	0.23
Pentadecanoic cid (C15)	Saturated	0.31	0.12
Palmitoleic acid (C16)	Unsaturated	1.17	0.93
Palmitic acid (C16)	Saturated	15.59	22.18
cis-10-Heptadecenoic acid (C17)	Unsaturated	1.55	0.43
Heptadecanoic acid (C17)	Saturated	0.73	0.27
gama-Linolenic acid (C18)	Unsaturated	2.62	4.52
Linolenic acid (C18)	Unsaturated	5.93	17.94
Oleic acid (C18)	Unsaturated	44.39	38.98
Elaidic acid (C18)	Unsaturated	2.00	2.88
Stearic acid (C18)	Saturated	2.56	2.97
Arachidonic (C20)	Unsaturated	1.56	0
cis-5,8,11,14,17-Eicosapentaenoic acid (C20)	Unsaturated	1.41	0.48
cis-8,11,14-Eicosatrienoic (C20)	Unsaturated	1.54	0.48
cis-11,14-Eicosadienoic acid (C20)	Unsaturated	0	0.46
cis-11-Eicosenoic acid (C20)	Unsaturated	0	0.47
cis-11,14,17-Eicosatrienoic acid (C20)	Unsaturated	1.42	0.45
Arachidic acid (C20)	Saturated	0.79	0.26
Heneicosanoic acid (C21)	Saturated	1.13	0.32
cis-4,7,10,13,16,19-Docosahexaenoic acid (C22)	Unsaturated	1.54	0.52
cis-13,16-Docosadienoic acid(C22)	Unsaturated	0	0
Behenoic acid (C22)	Saturated	1.89	0.63
Tricosanoic acid (C23)	Saturated	1.36	0.55
Nervonic acid (C24)	Unsaturated	1.63	0.50
Lignoceric acid (C24)	Saturated	2.93	1.60
Total fatty acid		100	100
Total Saturated fatty acids		31.88	30.50
Total unsaturated fatty acids		68.12	69.50



**Fig.4.** The GC-MS chromatogram showed the main fatty acids composition produced by yeast isolate *Candida parapsilosis* C5.

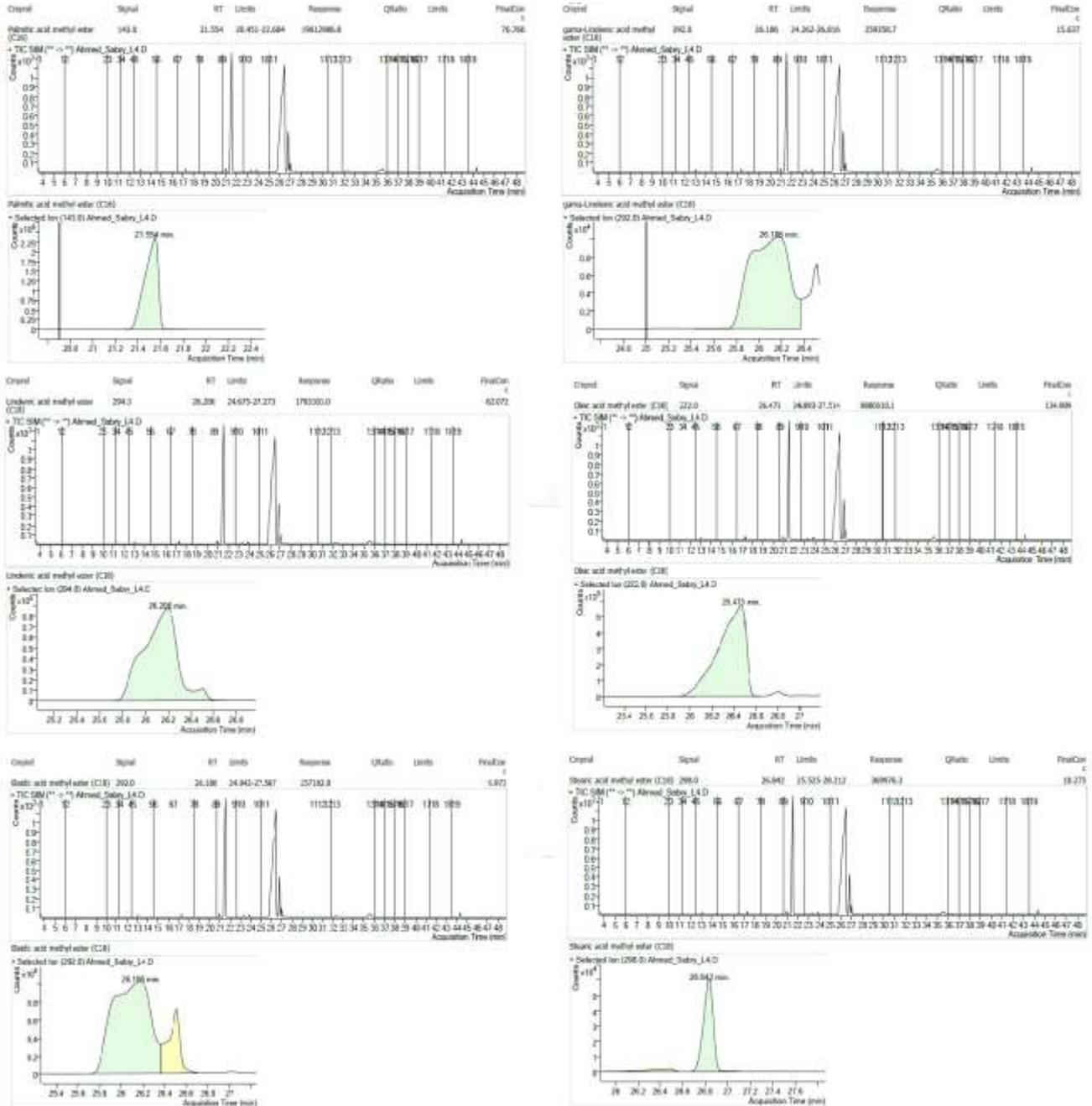


Fig.5. The GC-MS chromatogram showed the main fatty acids composition produced by yeast isolate *Cryptococcus albidus* L4.

An appropriate ratio between the composition of unsaturated and saturated fatty acids makes these strains a good candidate to produce polyunsaturated fatty acids with suitable quality as reported by (Knothe, 2005; Liang & Jiang, 2013) reported that oleaginous yeasts are considered one of the most important tools that preferred to produce fatty acid when compared to plant oils and it is known as oil factories microorganism or single cell oils (SCO). They have numerous advantages such as the scale-up steps of oil production is easier, the shorter life cycle, cost-effective and under control i.e. not affected by climate state or season of cultivation or geographic location like plants.

The comparative study showed that the major fatty acids produced by strains *Candida parapsilosis* C5 were oleic (C18:1) followed by palmitic acid (C16:0) then linoleic (C18:2) and finally lignoceric (C24), gamma-Linolenic acid (C18), Elaidic acid (C18) and stearic (C18:0) acids with 44.39%, 15.59%, 5.93%, 2.93%, 2.62%, 2.00%, and 2.56%, respectively which represent over 76 % of the total fatty acids. On the other hand, the major fatty acids produced by strains *Cryptococcus albidus* L4 were oleic (C18:1) followed by palmitic acid (C16:0) then linoleic (C18:2) and finally gamma-linolenic acid (C18), stearic acid (C18:0), elaidic acid (C18), and lignoceric acid (C24) with 38.98%, 22.18%, 17.94%, 4.52%, 2.97%, 2.88%, and 1.6%, respectively which represent over 91 % of the total fatty acids. Whereas capric (10), undecanoic (C11), lauric (C12), tridecanoic (C13), pentadecanoic (C15) acids were produced in lesser amounts by both strains (**Table 3**) (**Figs 4, 5**).

Also in this study, we observed that the L4 strain not capable to produce arachidonic (C20) fatty acid which may be attributed to the absence of delta-5 desaturase enzyme that responsible for synthesizing arachidonic fatty acid from gamma-linolenic acid (C18) (**Huang & Ziboh, 2001**).

Arachidonic acid is an essential fatty acid obtained from food such as poultry, meat, fish, seafood, and eggs (**Khan, et al., 2017**). It's incorporated in phospholipids in the cells' cytosol, adjacent to the endoplasmic reticulum membrane that is studded with the proteins necessary for phospholipid synthesis and their allocation to the diverse biological membranes. Arachidonic acid is vital to the operation of the prostaglandin system. Prostaglandins are part of a class of substances called eicosanoids. Eicosanoids influence numerous metabolic activities including platelet aggregation (blood clotting), inflammation, hemorrhages, vasoconstriction and vasodilation, blood pressure, and immune function (**James, 2014; Layne, 2018; Thiriet, 2019**). Therefore the presence of ARA in the fatty acids profile of isolate C5 makes this strain a sustainable source for large scale production of ARA.

In contrast, the C5 strain wasn't capable of producing cis-11, 14-eicosadienoic acid (C20), and cis-11-Eicosenoic acid (C20) fatty acids which may be attributed to the absence of delta-9 elongase enzyme which converts linoleic acid to eicosadienoic acid (C20). The cis-11-eicosenoic acid (C20) is a monounsaturated fatty acid called gondoic acid that is contained in a variety of plant oils and nuts. In particular, eicosadienoic acid represents

70% of the total fatty acids in jojoba oil (**Kumar et al., 2020**) Jojoba oil is remarkable for its industrial usage, which is in engine lubricating oil, pharmaceutical compounds, and cosmetics also, used as a raw material for medical supplies and a moisturizing component of cosmetic creams as well as it is used as a precursor of erucic acid (C22) in higher plants (**Pratt et al., 2002; Padgett, 2015**).

Eicosadienoic acid is an omega-6 fatty acid that is found mainly in small amounts in animal tissues that be able to modulate the metabolism of polyunsaturated fatty acids and alter the responsiveness of macrophages to inflammatory stimulations as well as it can inhibit the binding of leukotriene B4 to pig neutrophil membranes, which may account in part for its anti-inflammatory activities (**Schunck et al., 2018; Kimura et al., 2020**). Therefore the presence of cis-11, 14-Eicosadienoic acid (C20) and cis-11-Eicosenoic acid (C20) in the fatty acids profile of isolate L5 makes this strain a sustainable source for large scale production of these acids.

The highest content of total unsaturated fatty acid (USFA) was present in strain *Cryptococcus albidus* L4  $\approx$ 70% followed by strain *Candida parapsilosis* C5  $\approx$ 68%. Whereas the major content of total saturated fatty acids (SFAs) was showed in strain *Candida parapsilosis* C5 with  $\approx$ 32% followed by strain L4 with  $\approx$ 30.5% of total fatty acids. These results are similar and fully consistent with data reported by (**Dalia et al., 2014**) and her co-authors they revealed that *Candida tropicalis* S5 and *Pichia kudriavzevii* D5 strains produced 30.33% and 27.55%, of total saturated fatty acids and 67.57% and 71.43%, of total unsaturated fatty acid, respectively. Also, with data recorded by **Barghoth et al. (2018)** they isolated two strains of *Candida parapsilosis* encoded E2 and D1 have the potentially to produce unsaturated fatty acids and saturated fatty acids content  $\approx$ 70% and  $\approx$ 30%, respectively.

In this work, the fatty acid compositional profiles determined for strains *Candida parapsilosis* C5 and *Cryptococcus albidus* L4 mainly consisted of oleic acid (C18:1), palmitic acid (C16:0), linolenic acid (C18:2), stearic acid (C18:0), and palmitoleic acid (C16:1) in both oleaginous strains. The content of these unsaturated fatty acids comprised up to 70–91% of total fatty acid content and increased in presence of a high amount of carbon and a limited amount of nitrogen sources. Also, the fatty acid profiles of these strains are quite similar to oils extracted from different plant sources such as cottonseed, sunflower, rapeseed, and soybean, and these results correspond with those observed by (**Li et al., 2007; Amaretti et al., 2010; Kraisintu et al., 2010; Dalia et al., 2014; Vinarta et al., 2016**).

Therefore, the results of fatty acids profiles of strains *Candida parapsilosis* C5 and *Cryptococcus albidus* L4, indicated that the microbial lipids produced by these strains are highly rich in a quite fatty acid might be utilized as a promising tool for polyunsaturated fatty acids production similar to that produced from plant oils origins.

Also, (Kennedy *et al.*, 2010) pointed out that unsaturated linoleic acids are used for obesity treatment by lowering the required energy and increasing the fat mobilization process during the fatty acid oxidation step. Furthermore oleaginous yeasts that have the potential ability to produce dietary fatty acids such as linoleic (essential omega-6 fatty acid) and palmitoleic in high percent might be used as an alternative tool for biotechnological application for nutritional utilization as previously described by (Sitepu *et al.*, 2013).

#### 4. Conclusion

Results obtained in this work revealed that the marine environment is a promising source for the isolation of oleaginous yeasts. During this work, 20 yeast isolates were obtained from different marine samples. Amongst these two isolates encoded as C5 and L4 were selected as promising alternative sources for the production of polyunsaturated fatty acids (PUFAs) they storage a valuable content of cellular lipid more than 20 % per cell dry weight. These strains were identified as *Candida parapsilosis* C5 and *Cryptococcus albidus* L4 based on genetic approach taxonomy. Fatty acid profile of these strain composed of a mixture of fatty acids similar to those present in vegetable oils such as triacylglycerols (TAG), linoleic acid (C18:2), oleic acid (C18:1), stearic acid (C18:0), and palmitic acid (C16:0) which represent over 76 % for isolate C5 and 91% for isolate L4 of the total fatty acids. An appropriate content of these fatty acids could be used in different applications such as biodiesel production also, Linolenic acid (omega-3) produced by these strains is very important to be applicable in food and pharmacological applications. Further investigations are recommended to improve and increase lipid production through several optimization processes.

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## عزل وتوصيف الخمائر الزيتية البحرية المنتجة للأحماض الدهنية

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يوجد اهتمام كبير بالبحث عن مصادر دهنية جديدة مثل الخلايا الميكروبية التي يمكن استخدامها كعامل رئيسي في تطبيقات مختلفة مثل إنتاج الوقود الحيوي وكذلك الصناعات الدوائية والغذائية وذلك بسبب عدم توافر مصادر متعددة للدهون غير الحيوانات والنباتات وكذلك زيادة متطلبات الانسان منها كل عام . لذلك تم تجميع عشر عينات من الرواسب والمياه من بيئات بحرية مختلفة بغرض عزل الخمائر الزيتية. تم الحصول على عدد ٢٠ عزلة من الخميرة وفحص قدرتها على تجميع الأحماض الدهنية داخل خلاياها باستخدام صبغة النابيل الاحمر تحت المجهر الفلوريسينسي وصبغة التراي فينايل تترا زوليوم كلوريد عند طول موجي ٤٨٥. أظهرت عزلتان من الخمائر وهما (L4 و C5) من بين جميع العزلات ، أفضل النتائج لتراكم محتوى الدهن الخلوي. حيث أظهرت أعلى احتمالية لتراكم الدهون من ١٤.٣ إلى ١٥.١ جرام لكل لتر على التوالي من الكتلة الجافة وكانت النسبة المئوية للمحتوى الدهني هي ٢٥.٤ و ٢٨.٢٪ على التوالي عند نموها على وسط YPD عند ٢٥ درجة مئوية. تم تعريف هذه العزلات بناءً على الصفات المظهرية والجينية ، على أنها *Candida parapsilosis* C5 و *Cryptococcus albidus* L4 كما أظهرت الدراسة ان تركيب الأحماض الدهنية لهذه العزلات احتوى على حمض اللينوليك وحمض الأوليك وحمض الستيريك وحمض البالمتيك كالأحماض الدهنية الرئيسية منتجة تشبه بذلك الأحماض الموجودة في الزيوت النباتية. وكان مجموع الأحماض الدهنية التي تم الحصول عليها من كلا السلالتين تمثل تقريبا ٧٦٪ و ٩١٪ للعزلة C5 و L4 على التوالي. كما أن هاتين السلالتين كان لها القدرة على إنتاج ٦٨-٧٠٪ من الأحماض الدهنية غير المشبعة بينما كمية الأحماض الدهنية المشبعة تتراوح بين ٣٠.٥ إلى ٣٢٪ من إجمالي الأحماض الدهنية مما يجعل هذه السلالات مصدراً مناسباً لحل العديد من المشاكل العالمية المتعلقة بالمكملات الغذائية والأمراض ومعالجة مشاكل الطاقة المتجددة مثل إنتاج الوقود الحيوي.

**الكلمات المفتاحية :** عزل ، البحرية ، الزيتية ، الأحماض الدهنية المتعددة غير المشبعة ، إنتاج الدهون ، الفحص ، كانديدا بارابسيلوسيز ، كريبتوكوكس البيدس