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ORIGINAL ARTICLE

Biosynthesis of polyunsaturated fatty acids by two newly cold-adapted Egyptian marine yeast

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Abstract

The widespread awareness of polyunsaturated fatty acids (PUFAs) benefits for human health has increased the need for their commercial production. Two oleaginous yeast were isolated from the Mediterranean Sea fish and Red Sea fish *Epinephelus aeneus* and *E. areolatus*, respectively. These marine candidates were identified by MALDI-TOF/MS biotyper® as *Lodderomyces elongisporus* and *Rhodotorula mucilaginosa*. The effect of incubation temperature (7, 15, and 26 °C) and glucose concentration (3% and 8%) on their lipids content were investigated using Sulfo-Phospho-Vanillin (SPV) assay. Their intercellular lipids were visualized by fluorescence microscope using Nile-Red dye. *L. elongisporus* and *R. mucilaginosa* produced 20.04% and 26.79% of Linoleic acid, respectively, on normal Basal-Defatted Medium (BDM). Linoleic acid (21.4–22.7%) and α-Linolenic acid (7.5–10.8%) were produced by *R. mucilaginosa* and *L. elongisporus,* on normal BDM at 15 °C. High-Glucose BDM induced a positive effect on the total lipids production that reached its maximum of 48% and 54% by *R. mucilaginosa* and *L. elongisporus*, respectively, grown at 15 °C. Remarkably, 12.12% of long-chain 15-Docosenoic acid ($C_{22:1}$) and 21.49% of Tricosanoic acid ($C_{23:0}$) were detected in the FAs profile of *L. elongisporus*, when grown on normal BDM at 26 °C. The present study is the first one reporting the FAs profile of the Egyptian Marine *L. elongisporus*, and its capability to accumulate high amounts of lipids under appropriate fermentation conditions; thus, it could be considered for scaling up production.

Keywords PUFAs · Marine · Yeast · *Lodderomyces elongisporus* · *Rhodotorula mucilaginosa* · Optimization

Introduction

Yeasts represent valuable source for the production of various industrial products, such as vitamins, enzymes, mono- and polysaccharides, citric acid, alcohols, carotenoids, and lipids (Satyanarayana and Kunze 2009; El-Baz et al. 2011, 2016; Johansen et al. 2019). Polyunsaturated fatty acids (PUFAs) are components of biological membranes; they are essential for membrane structure and function (Stokes et al. 2020). PUFAs serve as precursors for bioactive molecules, like eicosanoids, in mammals, acting as anti-inflammatory agents, such as leukotrienes, prostaglandins, and thromboxane, to mediate inflammations, blood pressure, neurotransmission, and cholesterol metabolism (Funk 2001; Parolini 2020). PUFAs mainly promote many immune functions; thus, they modulate the risk of various diseases (Sorour et al. 2012a, b; Stokes et al. 2020). Moreover, many in vivo studies have revealed the ability of marine ω-3 PUFAs [eicosapentaenoic (EPA, $C_{20:5}$) and docosahexaenoic acid (DHA, $C_{22:6}$)] to modulate obesity, Alzheimer's disease, and Sclerosis progression (Parolini 2020). As a result, the production of PUFAs had attracted great attention because of their health-related benefits (Connor and Connor 2007). In addition, various scientific reports proposed both ω-3 and ω-6 FAs to be included in a balanced diet (Bellou et al. 2016). Therefore, ω-PUFAs are currently sold as supplements, even added to enrich baby's formulas (Stokes et al. 2020).

On one hand, fish is a source of many bioactive molecules, such as proteins, omega-3 PUFAs, minerals, and vitamins, it is precious, but limited resource facing many problems (Xu et al. 2020). The crucial problem of fish oil is its unsustainability due to the decline of fish stocks, as

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well as the unpleasant odour, low stability, and the presence of contaminants, such as heavy metals (Bellou et al. 2016). Therefore, the ever-growing population and the limited natural PUFA sources increased research toward finding alternative sustainable resources for PUFA production. In this respect, the marine environment has always been attractive, because of its diversity of microorganisms that can be exploited for various valuable compounds (Gupta et al. 2012; El-Baz et al. 2018).

On the other hand, some microorganisms in the cold saline environment can produce PUFAs, to provide fluidity for their cell membrane, and thus help in their protection and overcome the negative effect of cold (Chintalapati et al. 2004). Therefore, the microbial source plays an important role in supplying PUFAs to meet the market demands being environmentally safe, and more sustainable source (Qiu et al. 2020). Generally, psychrophiles and psychrotrophs can adjust their enzymes and cellular membranes to be metabolically active in the cold environments. The optimum temperature for psychrophilic yeasts usually occurs at 15 °C or lower, and up to 25 °C, but still capable of growing at 0 °C or below (Satyanarayana and Kunze 2009). They can decrease their membrane fluidity through increasing the percentage of monounsaturated FAs and PUFAs (Skerratt et al. 2002; Rossi et al. 2009). In addition, oleaginous microorganisms can grow on many cost-effective substrates, with high growth rates, short life cycle, and easy scale-up (Qiu et al. 2020). However, few microbial oils containing PUFAs are commercially available, such as Arachidonic acid (ARA) by *Mortierella alpina* fungus (CABIO), gamma-Linolenic acid by *Mucor circinelloides* fungus (J. and E. Sturge), and EPA by *Yarrowia lipolytica* yeast (E.I. Du Pont) (Bellou et al. 2016; Galán et al. 2020). Commercial production of microbial oils is mainly restricted to filamentous fungi, yeasts, and microalgae. However, oleaginous fungi belonging to genera *Mucor,* and *Mortierella*, can produce some PUFA, such as ɤ-linolenic acid, DHA, EPA, and ARA (Papanikolaou and Aggelis 2019), as well as accumulate high quantities of lipids up to 70% of their weight. However, the major problem lies in the difficulty of their cultivation in submerged cultures where they show rheological problems, and different morphological forms depending on the medium/cultivation conditions, with low lipids accumulations (Troiano et al. 2020). On the other hand, oleaginous yeasts represent fascinating microbial factories, since these heterotrophic microorganisms are able to grow rapidly and accumulate high levels of lipids on variety of raw substrates. Their easy manipulation in fermenters makes them good candidates for bio-refinery practice as compared to other microorganisms. Therefore, studying naturally occurring marine PUFAs-producers can be very promising for new biotechnological applications. In the current study, isolation, screening, and identification of new marine PUFAs-producers were investigated. The effect of incubation temperature, glucose concentration on the biomass, lipids accumulation, and the degree of FAs unsaturation, using the two isolated marine yeast (*R. mucilaginosa* and *L. elongisporus*) were studied. The selection of optimum conditions to maximize the biomass and PUFAs production was also determined.

Materials and methods

Chemicals and media

Yeast extract (Techno Pharmchem, India), dextrose (Alamia company for chemicals, Egypt), peptone, agar, and vanillin (Lobal Chemie, India), Chloroform (Fisher Scientific, UK), Nile-Red (Aldrich Chemicals-Milwaukee, USA), dimethyl sulfoxide (DMSO), methyl alcohol (El-Nasr Pharmaceutical Chemicals Co., Egypt), and phosphoric acid (El-Goumhouria Co., Egypt) were used in this study. All other chemicals and reagents were of analytically reagent grade. Yeast Peptone Dextrose (YPD) was used for yeast isolation, propagation, and maintenance (Yazawa 1996) with the following composition (%): yeast extract 1, peptone 2, glucose 2 (pH 6.0). Basal Broth Medium (BBM) was used for lipids production (Li et al. 2010) with the following composition (%): KH_2PO_4 0.7, Na_2HPO_4 0.25, $MgSO_4.7H_2O$ 0.15, $CaCl_2$ 0.015, $FeCl_3.7H_2O$ 0.015, $ZnSO_4.7H_2O$ 0.002, $(NH_4)_2SO_4$ 0.05, yeast extract 0.05, and glucose 2.0 (pH 6.0). Basal Defatted Medium (BDM) was used as a minimal medium for lipids production (Gupta et al. 2012) with the following composition (g/L): glucose 30, yeast extract 10, NaCl 30, KCl 0.7, $MgCl_2$ 10.8, $MgSO_4$ 5.4, and $CaCl_2$ 1.0 (pH 5.6). Dalmau plate culture on corn meal agar—containing (g/L) corn meal infusion 2, agar 15, and 7 mL of Tween 80—was used for the identification of yeast (Yarrow 1998).

Sampling and isolation of psychrotrophs

Newly killed fish (*Epinephelus areolatus*), *Epinephelus aeneus*, and shrimp samples were collected from the Red and Mediterranean Sea, located at 2° N 38° E/22° N 38° E Coordinates: 22° N 38° E/22° N 38° E, and 35° N 18° E/35° N 18° E Coordinates: 35° N 18° E/35° N 18° E, respectively, Egypt, between the 18th of December and 13th of January, (2014–2015). All Samples were placed in an icebox and used within 24 h (Fig. 1). Psychrophilics were isolated from fresh deep-sea samples, collected in Niskin bottles at a depth of 2 m; the intestine and gills of the samples were aseptically removed, homogenized in 0.9% NaCl, serially diluted, and plated on YPD medium. All plates were incubated in the dark at 5, 15, and 28 ºC for 20–30 days. Pure developed single colonies were obtained by conventional streak plate technique, kept at 4 ºC on slants, and subcultured

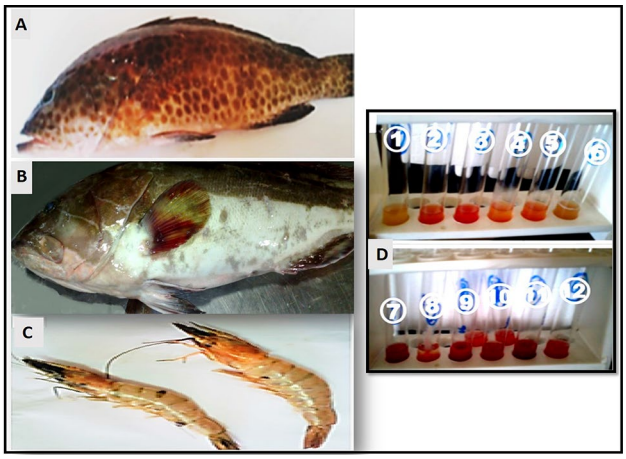

Fig. 1 Red Sea (Spotted grouper) fish (**A**), Mediterranean Sea (White grouper) fish (**B**), Mediterranean Sea shrimps (**C**); TTC test for yeast isolates showing different intensity of red color of formazan (**D**)

twice a month. All yeast cultures were routinely stored in glycerol solution (20% v/v) at − 80 °C.

Macroscopic and microscopic identification

Yeast isolates were identified based on their colony size, edge, color, etc., developed on agar media, and their shape under the light microscope. Yeast isolates were predominately identified to the genus level according to their macroscopical and microscopical morphology (Yarrow 1998).

MALDI-TOF/MS Biotyper® identification of selected yeast isolates

Identification was carried out using matrix-assisted laser desorption/ionization time of flight/mass spectrophotometry (MALDI-TOF/MS) Biotyper® (Bruker) (Wieser et al. 2012). 24 h-old yeast culture was mixed with 1 µL of matrix solution, and placed on the steel target plate to co-crystallize the sample. The loaded target plate was placed into the machine, and samples were hit by short laser pulses of MS spectrometer. The microbial sample and the matrix are vaporized by the laser's energy which ionize the ribosomal proteins of microorganism. The TOF of sample to reach the MS detector is precisely measured, and the ionization degree with the protein's molecular mass generates their distinct TOF. Based on TOF information, a characteristic spectrum is recorded, which represents a fingerprint for each sample, that is specific for a given species. The computer software displays the identification results after comparison of the generated spectrum with the stored database (Wieser et al. 2012).

Rapid screening of Δ5-desaturase activity using TTC assay

YPD broth media were inoculated with pure single colony of each yeast isolate and were incubated at 15 °C and 28 °C for 7 and 5 days, respectively. Triphenyltetrazolium Chloride (TTC) (0.1% w/v) was added to the culture broth (1:1, v/v), and the mixtures were then incubated for an additional 1 h at 15 °C. The formation of red color of the reduced form (formazan) represents a positive result (Ryan et al. 2010).

Production of lipids

Seed cultures were prepared for the two selected yeast isolates and the two control yeast (*Candida lipolytica* as positive PUFAs producer, and *Saccharomyces boulardii* as a negative control). Yeast were inoculated individually into 20 mL of YPD broth and incubated at 15 °C and 28 °C for 72 h. The seed cultures were used to inoculate 180 mL of BBM medium in 500 mL Erlenmeyer flasks, incubated at 15 °C in a rotary shaking incubator (New Brunswick, CA) at 150 rpm for 15 days until the early stage of stationary phase. This medium was used as screening medium and was compared with YPD medium for its ability to stimulate PUFAs production by the two selected yeast isolates. Yeast cells were collected by centrifugation at 5000 × *g* at 4 °C, washed three times with sterile saline solution, and dried at 80 °C until constant weight. Total lipids were extracted based on Axelsson and Gentili (2014) method, and the lipids content was calculated using the following formula:

$$\text{Lipids content (\%)} = \frac{\left[\text{Wt. of tube with lipids after extraction (g)} - \text{Wt. of empty tube (g)}\right]}{\text{Wt. of sample (g)}}$$

Lipids estimation in intact yeast cells using Sulfo-Phospho-Vanillin (SPV) assay

Lipids were measured using the modified SPV assay as described by Cheng et al. (2011) and Mishra et al. (2014). Pre-washed yeast cells (10 mg/mL) was transferred to 96-well microplate at 10, 20, 30, and 40 µL aliquots, further diluted to 50 µL with distilled water. 100 µL of sulfuric acid was added, mixed intensively by re-pipetting, and incubated at 90 °C for 20 min. The reaction mixtures were rapidly cooled in ice, and initial pre-vanillin background absorbance was measured spectrophotometry at λ_{570}. SPV reagent (100 µL of 0.2 mg vanillin/mL, 17% phosphoric acid) was added, incubated at 25 °C for 10 min in the dark, and a post-vanillin absorbance was determined. Final SPV response of samples was defined as the difference between final post-vanillin and initial-vanillin absorbance, measured at λ_{570}. Fish oil gelatin capsules (1000 mg, ω-3: 18%EPA, 12%DHA), commercial Flax seed oil (52.9% LNA), and Coconut oil (85.5% saturated FAs) were used as the standard lipids.

Lipids detection using Nile-Red fluorescent dye

Nile-Red solution was freshly prepared by dissolving 0.1 mg of Nile-Red dye in 1 mL of acetone (Kimura et al. 2004) and stored away from light at 4 °C. 1 mL aliquots of the yeast samples were centrifuged for 5 min at 2000 rpm, and cells were re-suspended in 1 mL of 10 mM PBS buffer (pH 7.4). Yeast suspensions (20 µL) were mixed with 400 µL of DMSO (25%), and smears were prepared by spreading 10 µL aliquots of the final cell suspension on a glass slide, then air-dried to fix the yeast cells. 10 µL of Nile-Red solution was added to each smear and kept for 5 min at room temperature. Finally, the excess of Nile-Red dye was washed out using PBS buffer, then examined using a fluorescence light microscope (Leica DMi8 S-platform, Germany), 100×objective lens at an excitation $\lambda_{450–500}$ nm and emission λ_{528} nm. Lipids were observed as yellow-golden droplets, whose surface area was visually estimated in relation to their cell surface area (Wang et al. 2017).

Extraction of total lipids, FAs methylation, and GLC analysis

Total lipids were extracted as described by Axelsson and Gentili (2014), where 230–300 mg of yeast biomass was extracted by 10 mL solvent (Chloroform:Methanol 2:1, v/v), shaken vigorously for few seconds; then 0.73% solvent:saline solution (Chloroform:Methanol:Saline 2:1:0.8, v/v/v) was added. For FAs methylation, 20 mg of freeze-dried cells were suspended in 2 mL methanolic-HCl (5%) and heated at 70 °C using water bath for 2 h in sealed glass tubes. Tubes were cooled at 25 °C for 30 min; then 1 mL double-distilled water was added and vortexed. To extract the methylated fatty acids (MEFAs), 1 mL hexane was added, vigorously vortexed, and the upper layer was transferred into clean glass vials, dried using nitrogen stream, and stored at − 20 °C. FAs was analyzed at the Regional Center for Food and Feed, Cairo, Egypt, according to Jostensen and Landfald (1997) method. FAs composition was determined using PerkinElmer (Waltham, MA) Clarus-580 GLC equipped with an FID, and an HP88 capillary column (30 m × 0.25 mm i.d, 0.20 µm film thickness). GLC-461 (NuChek Prep, Inc., Elysian, Minnesota, USA) was used as a reference standard to allow the determination of C_{12}–C_{24} FAs. Standard TAG ($C_{17:0}$) was included as internal standard. Helium was used as a carrier gas with a flow rate of 15 mL/min. The program conditions were held at 100 °C for 5 min; temperature was raised from 100 to 220 °C at 10 °C/min increment rate, then held at 220 °C for 15 min. The injection volume was 1 µL (10:1 split ratio). The temperature of the injector and the detector were held at 240 °C and 280 °C, respectively. MEFAs peaks were identified as compared to the reference standards injected under the same conditions.

Effect of temperature on lipids production

For preliminary optimization of the lipids content, selected marine yeasts were inoculated into BDM (1:10 v/v) prepared in three groups, and incubated at 7, 15, and 26 °C in a rotary shaking incubator (New Brunswick, CA) at 150 rpm for 20 days. Lipid production was tested every 5 days' by withdrawing broth samples, washed twice with sterile saline solution after centrifugation at 5000 × *g* and 4 °C, resuspended in 1 mL saline solution, and stored at − 20 °C for lipids analysis using SPV assay. Yeast cell dry weight CDW (g/L) was measured by drying the cell pellets at 80 °C until constant weight. Lipids were extracted and stored at − 20 °C until FAs analysis by GLC-FID.

Effect of glucose concentration on lipids production

200 mL BDM containing 80 g/L glucose (pH 5.6) was prepared in 500 mL Erlenmeyer flasks capped with rubber plugs. The flasks were inoculated, incubated at 7 °C, and aerated using air pump with a flow rate of 15 mL/min for 20 days. Samples were withdrawn every 5 days' centrifuged at 5000 × *g* under cooling at 4 °C and washed twice with sterile saline solution. CDW (g/L) was measured by drying the cell pellets at 80 °C until constant weight. Lipids were extracted and stored at − 20 °C until FAs analysis by GLC-FID.

Statistical analysis

All experiments were conducted in triplicate. Results are expressed as the means ± standard error. The effect of the temperature and time on lipids production were compared by two-way ANOVA analysis using SPSS software (Version 17) at $P \leq 0.05$ to determine significant differences.

Results and discussion

TTC assay for rapid isolation and screening of lipids producers

The colorless salt 2,3,5-triphenyl-tetrazolium chloride (TTC) turns red when it is reduced to triphenyl formazan (TF), by Δ5-desaturase enzyme. Some marine microbes use the same enzyme in their metabolic pathway to produce ω-3 PUFAs, converting eicosatetraenoic acid to EPA (Ratledge 2002). Results in Fig. 1 and (Supplementary material file1) show the reaction of different microbial isolates with TTC giving different color intensities at different time intervals. TTC assay is simple and rapid for screening PUFAs producers, and its main advantage is 0% false negative for tested microorganisms (Abd Elrazak et al. 2013). The two yeast isolates with deep red color and fast reaction with TTC test (≤ 30 min) were selected for further optimization of PUFAs production (Supplementary material file 1). TTC assay was used to compare the ability of YPD and BBM screening media to stimulate PUFAs production by the selected yeast isolates. There was no difference in the color intensity for both selected yeast isolates in their reaction with TTC in both media. In eukaryotes, conventional biosynthetic pathway of PUFA is through an aerobic pathway with sequential addition of double bonds to saturated FAs, mainly $C_{18:0}$ and $C_{16:0}$, via $_{\Delta 9}$ and $_{\Delta}12$ desaturases to produce LA($C_{18:2}$), which is further desaturated by $_{\Delta}15$ desaturase to give $C_{18:3}$ (ALA). A sequence of desaturases inserts double bonds between the Δ9 bond and the carboxyl terminal, while elongases convert ALA to EPA and DHA, and the sequence is $_{\Delta}6$ desaturase → elongase → $_{\Delta}5$ desaturase → elongase → $_{\Delta}4$ desaturase (Guschina and Harwood, 2006; Ryan et al. 2010).

Results (Supplementary material file 1), show that the BBM medium stimulates PUFAs production and increases the enzymes expression necessary for the de-novo pathway of PUFAs production, like Δ5-desaturase enzyme, thus reduced the reaction time of TTC assay (Ratledge 2002). Likewise, Zhu et al. (2004) investigated the relation between the staining intensity of *Mortierella alpina* using TTC and its arachidonic acid (AA) content, reporting that the staining intensity of mycelia was increased when AA content was increased. The enzyme Δ5-desaturase is part of the FA synthase pathway; it represents the dehydrogenase responsible for the reduction of dihomo-γ-linoleic acid to AA (20-carbon ω-6 PUFA), which is probably responsible for reducing TTC to TF red formazan (Ryan et al. 2010).

Macroscopic and microscopic identification

The white yeast isolate showed moderate growth at 28 °C after 3 days as compared to the orange pigmented yeast isolate which appeared small and faint colonies. The microscopic examination (Fig. 2) of the two selected isolates on Corn-Meal Agar incubated at 15 °C shows as follows: (1) Orange pigmented yeast cells after 30 days of incubation by Dalmau plate culture; pseudomycelium and true hyphae

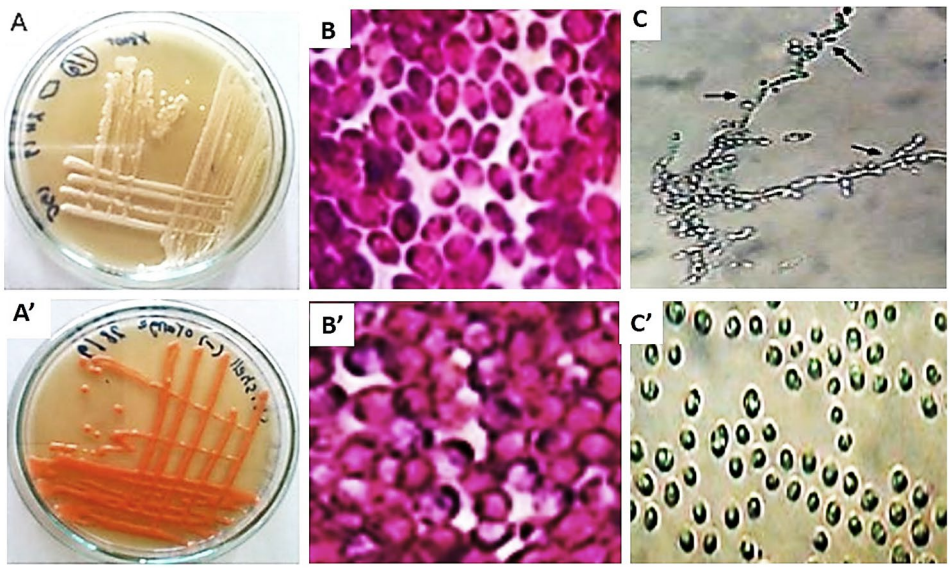

Fig. 2 Colony morphology of *L. elongisporus* on YPD medium (**A**); cells using simple stain (×400) (**B**); branched and curved pseudomycelium under light microscope, simple stained (×400), arrows refer to ascospores in asci (**C**). *R. mucilaginosa* on YPD medium (**A'**); cells under light microscope (×400) (**B', C'**)

were absent, only blastoconidia appear, reproduction by multilateral budding, single cells, or pairs, short chains or clusters. (2) White yeast cells after 30 days of incubation; pseudomycelium was present, but true hyphae were absent, at × 400 magnification; cells were slender, branched with curved pseudohyphae, short-chain elongated blastospore, with few ascospores (Fig. 2). Based on the morphological properties and mode of reproduction of the two selected yeast isolates, and referring to the standard methods with some modifications (Yarrow 1998; Jones et al. 2009), the two yeast isolates were preliminary identified to the genera level, as *Rhodotorula* sp. and *Saccharomyces* sp. belonging to Basidiomycetes and Ascomycetes yeast, respectively.

Identification using MALDI-TOF/MS Biotyper®

MALDI-TOF/MS has been successfully used in research for the determination of molecular mass of peptides and proteins (Marvin et al. 2003). Recently, it has been widely applied and utilized for microorganisms' identification (Wieser et al. 2012; Ahmed et al. 2021). The selected marine isolates were identified as the Basidiomycetes candidate *Rhodotorula mucilaginous* and the Ascomycetes candidate *Lodderomyces elongisporus* (Supplementary material file 2), with score of 2.11 and 2.35, respectively. The high match in the peaks pattern between samples and standards ribosomal proteins verified the species consistency. The software compares the spectra and creates a *numerical score-value*, based on similarities between the obtained and software database; this *score-value* provides information for method validity (Wieser et al. 2012). Above 2.0, the *score-value* is considered valid for identification at the species level. Likewise, Marklein et al. (2009) confirmed that 96% of 250 *Candida* isolates from 15 different spp. were correctly identified by MALDI-TOF/MS Biotyper®. It is faster than conventional identification methods, and highly accurate for the identification of isolated bacteria and yeast (Wieser et al. 2012; Agustini et al. 2014). MALDI-TOF/MS Biotyper® is a powerful tool for the identification of microbial isolates, it is recently used in clinical microbiology, water, and food applications, it is rapid, cost-effective, and efficient identification technology (Ahmed et al. 2021), as well as it is FDA approved.

Biomass and lipids content determination

Cell dry weight (CDW) of the two selected yeast isolates was significantly increased when grown on BBM screening medium for 15 days. *L. elongisporus* biomass was increased 4.4-fold from 13.4 to 58.6 mg/mL, while *R. mucilaginous* biomass was increased 2.5-fold. Also, the biomass was increased 5.6-fold and 2.5-fold for *C. lipolytica* and *S. boulardii*, respectively, under the same growth conditions. Results (Fig. 3) showed that the lipids content of *C. lipolytica* was 57.40%, followed by *R. mucilaginous* (41.20%), then *L. elongisporus* (29.0%). These findings are in accordance with Ageitos et al. (2011) who reported that similar amounts of lipids (20–25%) have been produced by different oily yeast genera, such as *Yarrowia, Rhodotorula, Candida, Rhodosporidium, Cryptococcus,* and *Lipomyces* using different fermentation conditions. Ratledge (1982) reported that among different spp. of *Rhodotorula*, the lipid production by *R. mucilaginosa* reached 28% of its CDW when grown on glucose and sucrose as the carbon source, while *R. glutinis* was able to accumulate up to 66% lipids when glucose was used as carbon source (Beopoulos et al. 2009). Also, Papanikolaou and Aggelis (2002) reported that *Y. lipolytica* produced 44% and 40% lipids, after 5 and 10 days, respectively, when grown at 28 °C on medium containing glycerol.

GLC analysis (Table 1) confirmed the presence of PUFAs, thus verified the positive results of the TTC assay for the two selected marine yeast. Results confirmed the presence of

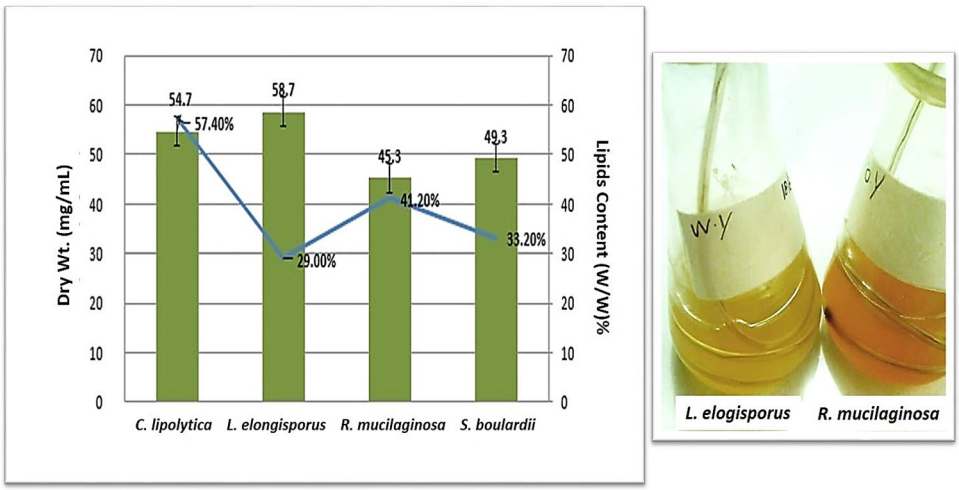

Fig. 3 Dry weight and lipids content of *C. lipolytica*, *L. elongisporus*, *R. mucilaginosa,* and *S. boulardii* after incubation for 15 days at 15 °C using screening Basal Broth Medium

Table 1 Fatty acids content (%) of *C. lipolytica*, *L. elongisporus*, *R. mucilaginosa,* and *S. boulardii* after 15 days incubation on the screening media

Fatty acids	Name	*C. lipolytica*	*L. elongisporus*	*R. mucilaginosa*	*S. boulardii*
$C_{12:0}$	Lauric acid	1.0	2.9	3.7	–
$C_{13:0}$	Tridecanoic acid	–	–	–	4.4
$C_{14:0}$	Myristic acid	3.1	–	3.8	–
$C_{15:0}$	Pentadecanoic acid	0.8	–	–	–
$C_{16:0}$	Palmitic acid	22.1	25.8	27.4	21.5
$C_{16:1}\omega7$	Palmitoleic acid	2.3	–	–	6.7
$C_{16:4}\omega3$	Hexadecatetraenoic acid	0.5	–	–	–
$C_{17:0}$	Heptadecanoic acid	1.2	–	–	20.5
$C_{18:0}$	Stearic acid (SA)	7.9	16.1	12.6	14.7
$C_{18:1}$ ω7	Vaccinic acid	3.2	–	–	–
$C_{18:1}$ ω9	Oleic acid (OA)	33.5	35.2	25.7	14.9
$C_{18:2}$ ω6	Linoleic acid (LA)	19.8	20.0	26.8	17.3
$C_{18:3}$ ω3	α-Linolenic acid (ALA)	1.6	–	–	–
$C_{20:1}$ ω9	Erucic acid	3.1	–	–	–
NA	Non-Identified FA	0.01	–	0.01	–
Total SFAs		36.1	44.8	47.5	61.1
Total MUFAs		42.1	35.2	25.7	21.6
Total PUFAs		21.9	20.0	26.8	17.3
Total UNSFAs		64	55.2	52.5	38.9

Screening medium (KH_2PO_4, 0.7%; Na_2HPO_4, 0.25%; $MgSO_4.7H_2O$, 0.15%; $CaCl_2$, 0.015%; $FeCl_3{\cdot}7H_2O$, 0.015%; $ZnSO_4{\cdot}7H_2O$, 0.002%; $(NH_4)_2SO_4$, 0.05%; yeast extract, 0.05%; glucose, 2.0%, and pH 6)

SFAs saturated fatty acids, *MUFAs* monounsaturated fatty acids, *PUFAs* polyunsaturated fatty acids, *UNSFA* unsaturated fatty acids

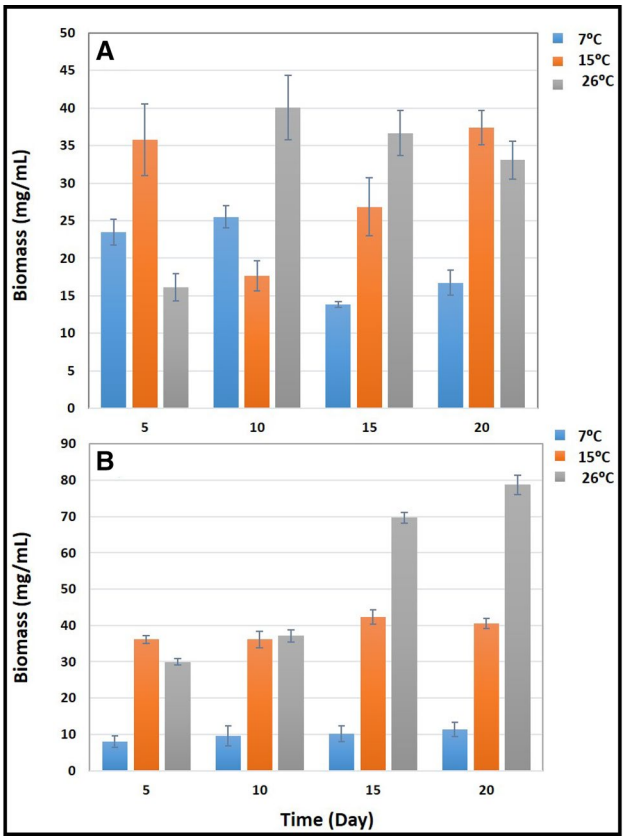

Fig. 4 Biomass concentration (g/mL) of *R. mucilaginosa* (**A**) and *L. elongisporus* (**B**) after incubation at 7, 15, and 26 °C, for 20 days on Basal Defatted medium (BDM)

four major FAs, being oleic acid (OA), palmitic acid (PA), stearic acid (SA), and linoleic acid (LA) produced by the four tested yeast. Similarly, Li et al. (2008) reported that the main FAs detected in oleaginous yeast (OY) were myristic acid (MA), PA, SA, OA, LA, and LNA. Results (Table 1) showed that the most prominent FA was OA representing 35.7% of the total FAs. LA was also present in significant amount in all tested yeast strains, *R. mucilaginosa* produced the highest LA (26.8%) followed by *L. elongisporus* (20%). Li et al. (2008) reported that *C. lipolytica* as an OY has a profile of $C_{16:0}$ (11%), $C_{18:1}$ (28%), $C_{18:2}$ (51%), and $C_{18:3}$ (1%); however, its production of long-chain (LC) PUFAs can only be attained by genetic manipulation (Beopoulos et al. 2009).

In the present study, LA ($C_{18:2}$) which is an important precursor in the *de-novo* pathway of PUFAs production (Ratledge 2002) was detected in relatively high amounts (20% and 26.8%) in the two marine yeast as compared to *C. lipolytica* (19.8%) (Table 1). The FAs profile (Table 1) showed that *R. mucilaginous* have 52.5% unsaturated FAs with 26.8% PUFAs, which was close to the FAs profile of the marine *R. mucilaginosa* AMCQ8A that accumulated 65% lipids with only 6–7% ω-3 content (Gupta et al. 2012). Li et al. (2010) also investigated lipids production by the marine *R. mucilaginosa* TJY15a, and reported that OA was (54.7–63.5%), while LA was (5.7–11.3%) when grown on various carbon and nitrogen sources. For industrial oil production DuPont (USA) has developed genetically modified *Y. lipolytica* as an alternative to microalgae-producing EPA (Galán et al. 2020). However, using naturally occurring OY, such as *Rhodosporidium toruloides, Rhodotorula glutinis,* and *Candida curvata*, DuPont was able to produce EPA, and the EPA-rich oil has received the GRAS status from FDA (Galán et al. 2020)

The present study is the first one reporting the FAs profile of the local marine *L. elongisporus*, where only few scientific papers mentioned its applications in industrial biotechnology. In this regard, Wang et al. (2007) isolated the marine yeast, *L. elongisporus* YF12c, and *R. mucilaginosa* L10-29 for lipase production. Also, Ma et al. (2015) isolated *L. elongisporus* SYB-2A that have petroleum-degrading activity. In addition, You et al. (2017) used *L. elongisporus* to ferment flavor liquor and suggested its usage in the food industry.

Effect of temperature on lipids production and growth

R. mucilaginosa and *L. elongisporus* were incubated at different temperatures (7, 15, and 26 °C) in normal BDM (3% glucose). These cold adapted strains can grow well at 7 °C, but their abundant growth was observed at 15 °C, and up to 26 °C. *R. mucilaginosa* can grow well in temperature ranging from 7 to 30 °C, while *L. elongisporus* shows rapid growth up to 35 °C. Therefore, *R. mucilaginosa* and *L. elongisporus* can be regarded as facultative psychrophilic strains based on their classification (Margesin 2009). The growth temperature ranges for yeast isolated from cold areas can be surprisingly high, some common spp. can be found in cold and warm habitats, such as *Cryptococcus macerans* and *R. mucilaginosa* (De Garcia et al. 2007). Similarly, Butinar et al. (2011) reported that most ascomycetous yeasts isolated from glacier ice can grow well at 25–30 °C in addition to their growth at 4 °C.

A significant increase in the CDW was observed with the increase of temperature at the same day along the whole time course, while no significance was observed at the same temperature throughout the time course (Fig. 4). Although both Egyptian yeast strains had different growth rate over time, an obvious increase in the biomass was achieved through increasing the incubation temperature, where *R. mucilaginosa* and *L. elongisporus* reached their maximum biomass of 40 and 78.75 mg/mL, after 10 and 20 days, respectively, when grown at 26 °C (Fig. 4). In contrast, Amaretti et al. (2010) reported that the temperature did not affect the yield

Table 2 Fatty acids content (%) of *L. elongisporus* and *R. mucilaginosa* after growth in normal BDM and HG-BDM media

Fatty acids	Name	BDM						HG-BDM	
		L elongisporus			*R mucilaginosa*			*L elongisporus*	*R. mucilaginosa*
		7 °C	15 °C	26 °C	7 °C	15 °C	26 °C	7 °C	7 °C
$C_{11:0}$	Undecanoic acid	2.56	–	–	–	–	9.69	4.90	1.82
$C_{12:0}$	Lauric acid	4.84	0.91	22.34	–	1.54	14.98	11.47	4.58
$C_{13:0}$	Tridecanoic acid	3.29	–	9.61	–	–	12.74	8.59	2.52
$C_{14:0}$	Myristic acid	–	4.42	–	–	4.38	–	–	3.83
$C_{15:0}$	Pentadecanoic acid	2.17	–	–	–	–	–	23.74	–
$C_{16:0}$	Palmitic acid	18.94	25.24	–	28.1	22.81	19.11	12.6	23.16
$C_{16:1}\omega7$	Palmitoleic acid	9.0	–	–	–	4.14	–	–	–
$C_{17:0}$	Heptadecanoic acid	–	–	–	–	–	–	4.15	2.71
$C_{18:0}$	Stearic acid	5.44	9.56	–	14.60	7.87	–	8.11	11.92
$C_{18:1}\omega7$	Vaccinic acid	–	–	–	–	–	–	–	–
$C_{18:1}\omega9$	Oleic acid (OA)	38	29.73	34.42	32.9	27.24	25.83	15.50	21.84
$C_{18:2}\omega6$	Linoleic acid (LA)	15.75	22.67	–	24.5	21.35	17.66	10.91	27.63
$C_{18:3}\omega3$	α-Linolenic acid (α -LA)	–	7.47	–	–	10.67	–	–	–
$C_{22:1}\omega7$	15-Docosenoic acid	–	–	12.12	–	–	–	–	–
$C_{23:0}$	Tricosanoic acid	–	–	21.49	–	–	–	–	–
NA	Non-Identified FA	0.01	–	0.02	–	–	–	0.03	–
Total SFA		37.24	40.13	53.44	42.7	36.6	56.52	73.56	50.54
Total MUFA		47	29.73	46.54	32.9	31.38	25.83	15.50	21.84
Total PUFAs		15.75	30.14	–	24.5	32.02	17.66	10.91	27.63
Total UNSFA		62.75	59.87	46.54	57.4	63.4	43.49	26.41	49.47

FA saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFAs* polyunsaturated fatty acids, *UNSFA* unsaturated fatty acid, *BDM* Basal Defatted medium, *HG-BDM* High-Glucose-Basal Defatted medium

of lipids and biomass of *Rhodotorula glacialis DBVPG 4785*, but only had positive effect on its growth rate; however, *R. glacialis* is an obligate psychrophilic that cannot grow at 26 °C as compared to the current isolated facultative psychrophilic marine yeast.

Effect of glucose concentration on FAs composition

The total lipids content can vary greatly within a species; however, the overall FAs profiles have been quite consistent within a sp. if grown under consistent conditions; thus, it was used to identify yeast before ribosomal sequencing became affordable (Botha and Kock 1993). High-Glucose (8%) HG-BDM induced a positive effect on the total lipids production that reached its maximum of 48% and 54% by *R. mucilaginosa* and *L. elongisporus*, respectively, when grown at 15 °C. Relative FAs content (Table 1), showed that the lipid profiles of *R. mucilaginosa* and *L. elongisporus* were mainly composed of LC-FAs with 16 and 18 carbon atoms. The distribution of some FAs, namely, $C_{12:0}$ (lauric acid), $C_{16:0}$ (palmitic acid), $C_{16:1}$ (palmitoleic acid), and $C_{18:3}$ (LNA), were almost constant during their growth time course. The relative content of OA ($C_{18:1}$) was decreased from 38% and 32.9% to 34.4% and 25.8% with the increasing incubation temperature from 7 to 26 °C on normal BDM, for *L. elongisporus* and *R. mucilaginosa,* respectively. While, further decrease in OA from 38% and 32.9% to 15.5% and 21.8% was observed in HG-BDM, respectively. However, LA content was slightly changed from 15.75 to 10.91% and from 24.5 to 27.63% for *L. elongisporus* and *R. mucilaginosa*, respectively, with the increasing glucose concentration to 8%. On the contrary, Gupta et al. (2012) reported the glucose enhancing effect in *R. mucilaginosa AMCQ8A* when glucose was increased from 2 to 10%, where ALA was increased from 1.7 to 6.21%, while OA and LA were highly increased from 7.96 to 35.71% and from 3.68 to 23.69%, respectively. Overall, a trend of increasing the FAs unsaturation degree was observed for both yeast strains with the decreasing incubation temperature. In contrast, the opposite trend was observed with the increasing glucose concentration from 3 to 8%, at 7 °C (Table 2).

At low temperature, in order to regulate the membrane fluidity and functionality, *R. mucilaginosa* and *L. elongisporus* exploited diverse changes in their lipid's composition,

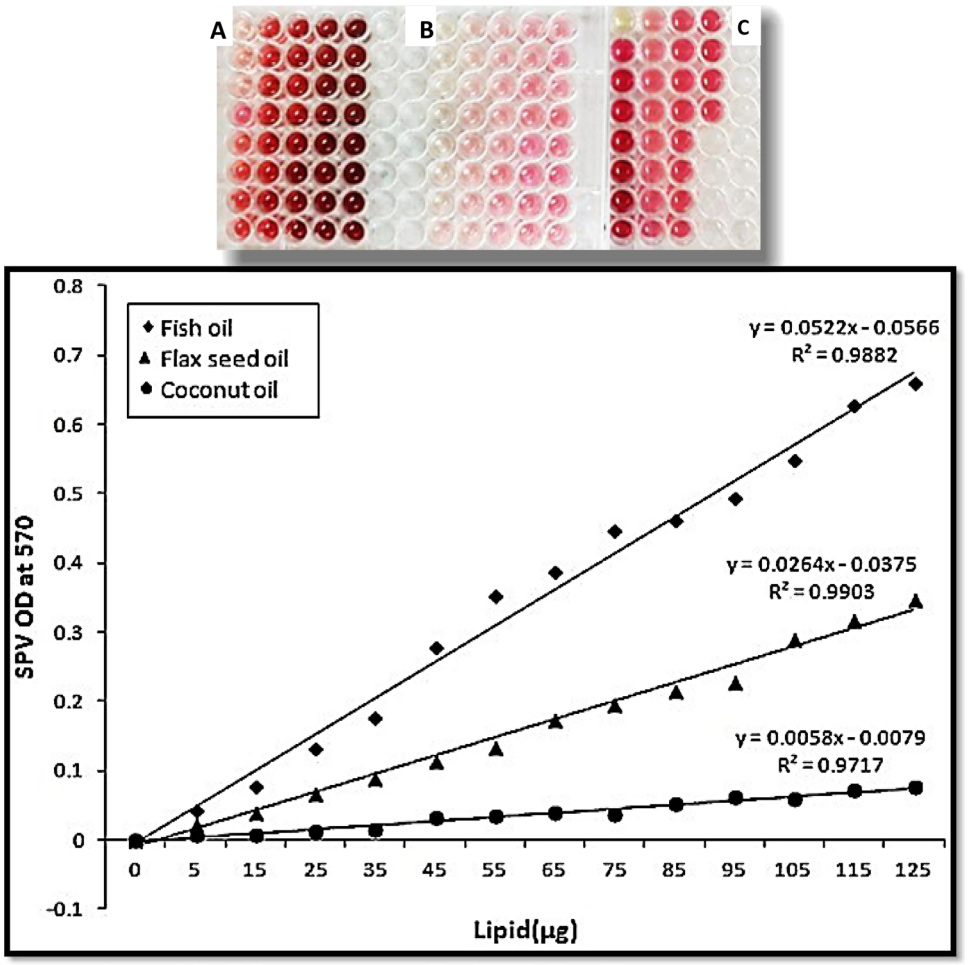

Fig. 5 Absorbance linearity of three different standard oils, Fish (**A**), Flax (**B**), and Coconut (**C**) using SPV assay. Absorbance was measured at λ_{570}, data points represent the mean of two replicates. Representative color intensity in SPV assay with the three standard lipids (5–200 µg)

such as the degree of unsaturation, namely, palmitoleic ($C_{16:1}$, Δ9), OA ($C_{18:1}$, Δ9), LA ($C_{18:2}$, Δ9,12), and α-LNA ($C_{18:3}$, Δ9,12,15), were increased (Table 2). Likewise, the presence of 16–18 carbons LC-FAs was clearly observed as a common trend at low temperature (0–15 °C). Similarly, Amaretti et al. (2010) reported that both the glucose concentration and the growth temperature influenced the FAs composition of *R. mucilaginosa AMCQ8A*, where the degree of FAs unsaturation was decreased when the temperature or glucose was increased. In the present study, at low temperature (7 °C), the increase in glucose concentration from 3 to 8%, decreased the degree of unsaturation, namely, OA ($C_{18:1}$; Δ9) and LA ($C_{18:2}$, Δ9,12) (Table 2). Also, an increase in the ratio of short-chain unsaturated FAs (C_{11}–C_{15}) was observed for both marine yeast strains in HG-BDM. Likewise, Sitepu et al. (2013) suggested that the variation in the medium composition and the incubation time affect the relative quantities of certain FAs, such as LA ($C_{18:2}$).

LA is an essential FA that cannot be synthesized by humans or animals, but must be ingested for good health; therefore, yeasts capable to produce significant amounts of ω-6 FAs can be considered as good candidates for its commercial production. Although some microorganisms produce PUFAs naturally, native microorganisms produce low yields, which are usually far below the level for commercial production. Therefore, more research efforts are needed to explore new candidates and modify them through metabolic engineering to accumulate higher amounts of lipids enriched in PUFAs (Galán et al. 2020). In addition, Xue et al. (2013) reported that wild oleaginous yeast strains are not able to produce very LC-PUFAs. In same regard, PUFAs, such as ɤ-LNA ($C_{18:3}$) and stearidonic acid ($C_{18:4}$), were detected in low amounts (< 0.4%), or α- LNA ($C_{18:3}$) as 6.2% in some marine yeast (Wang et al. 2007; Gupta et al. 2012). Interestingly, results in Table 2 showed the presence of LC-FAs, such as 15-Docosenoic acid ($C_{22:1}$, ω7) and Tricosanoic acid ($C_{23:0}$) in the FAs profile of marine *L. elongisporus* with significant amount of 12.12% and 21.49%, respectively, on normal BDM. These FAs were present in the marine environment, as well as in the marine standard oil used for FAs identification in marine samples (Restek-Corporation 2018; Dikma-Technologies 2021). However, few scientific papers reported the isolation of *L. elongisporus* from marine habitat (Wang et al. 2007; Ma et al. 2015). In

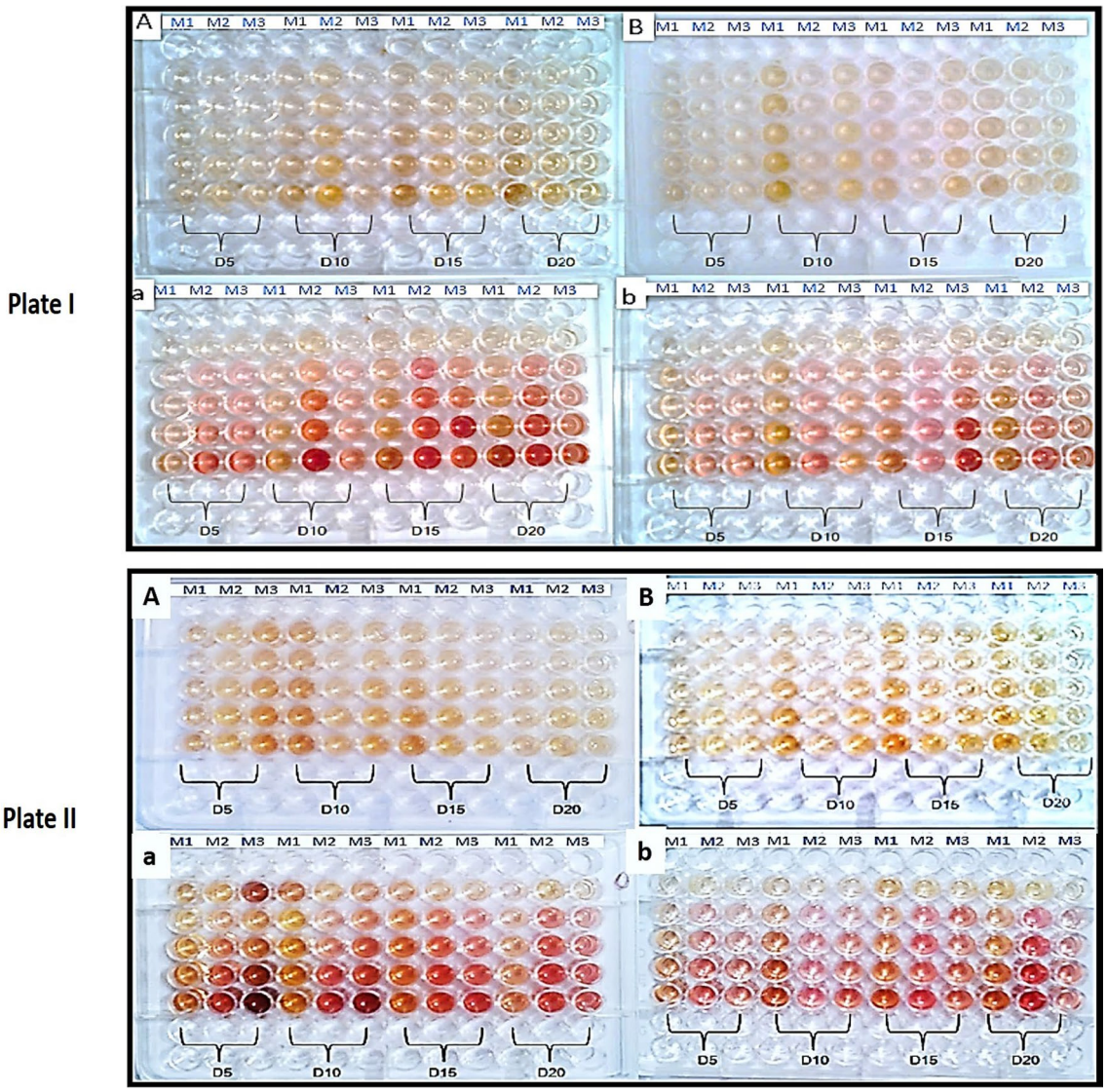

Fig. 6 SPV assay reaction and color development with set of experiments conducted at 15 °C (plate I) and 26 °C (plate II). *R. mucilaginosa* (**A, a**), *L. elongisporus* (**B, b**) were cultured in three media (**M_1, M_2, M_3**) for 20 days. (**A**, **B**: background colors), and (**a**, **b**: SPV assay colors). M_1; Basal medium, M_2; Basal Defatted medium, M_3; High-glucose medium, D; day

the present study, *L. elongisporus* represents an oleaginous Egyptian marine yeast, and this is the first study to report its FAs profile and its capability for lipids accumulation under different cultural conditions.

Lipids quantification using SPV assay

To validate a simple and sensitive micro-scale assay, three different oils were used to design standard calibration curves, including Fish, Flax seed, and Coconut oils. Selection of the standard oils depends mainly on understanding the major lipids content in the marine yeasts taking into consideration the saturated/unsaturated FAs distributions (Rossi et al. 2009). To detect the production of PUFAs in the designed experiment, Fish oil was used as standard oil because of its EPA (18%) and DHA (12%) content, while Flax seed oil was used because of its LNA content (52.9%) and Coconut oil for its saturated FAs content (85.5%). Calibration curves were generated by measuring the absorbance at λ_{570} and were plotted against the lipid's quantity. The three calibration curves showed a very strong linear relationship with R^2 values of 0.9903, 0.9882, and 0.9717 using Flax seed, Fish, and Coconut oils, respectively (Fig. 5). In general, the increase in standard oil concentration was accompanied with the gradual increase in the pink color intensity. Furthermore, responses of the three standard oils were obviously varied, which indicates that they were significantly affected by their lipid's composition. Results (Supplementary material file 3)

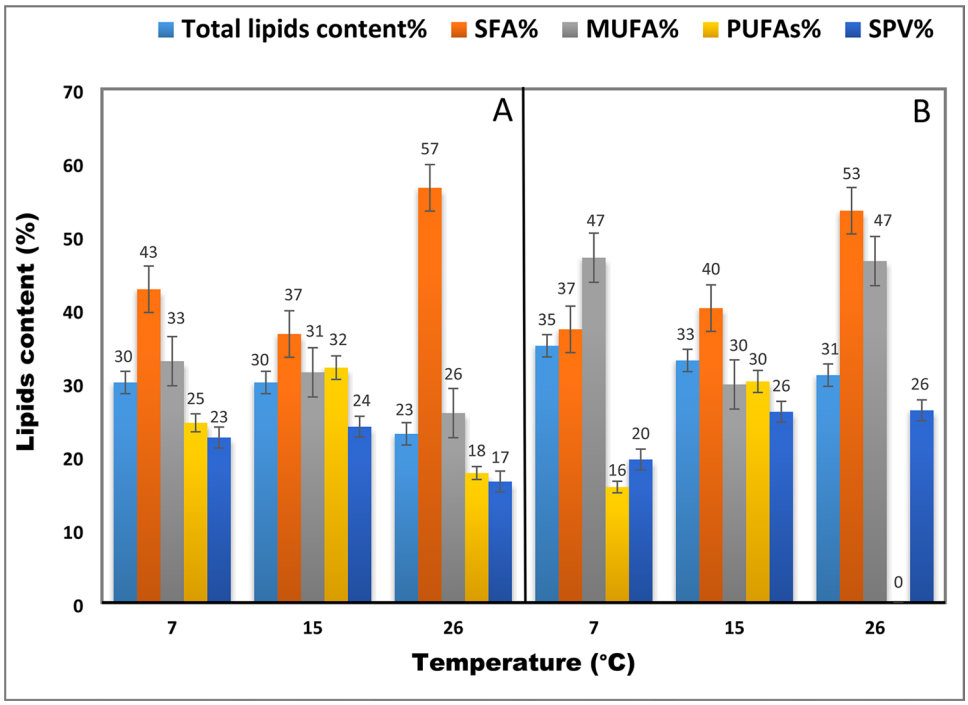

Fig. 7 Lipids content and fatty acids composition of *R. mucilaginosa* (**A**), *L. elongisporus* (**B**) grown on normal BDM medium at 7, 15, 26 °C, and their corresponding SPV results. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; and PUFAs, polyunsaturated fatty acids

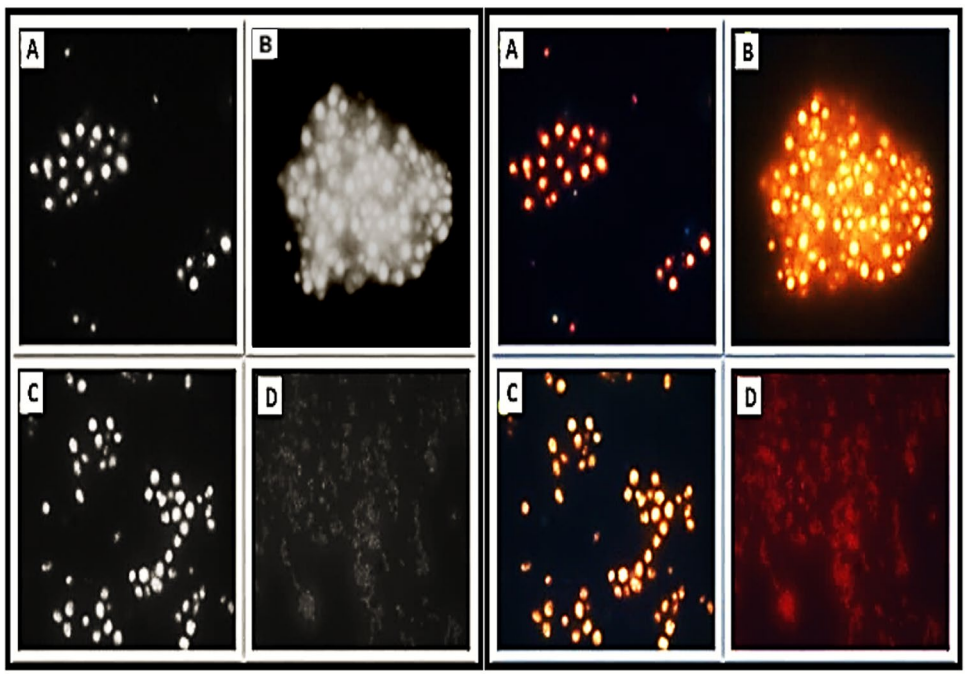

Fig. 8 The four examined yeast strains were classified according to the area covered by lipid droplets (yellow-gold) inside the cell visualized with oil immersion objective lenses (100x). *L. elongisporus* (**A**), *R. mucilaginosa* (**B**), *S. boulardii* (**C**), and *C. lipolytica* (**D**); all photos are shown in fluorescent view (right) and grey background (left)

showed that total lipids content of both marine yeasts had a good coefficient of variation typically ≤ 7% with respect to the SPV results with an exception of *L. elongisporus* at 7 °C, which is probably due to its high amount of MUFAs (47%), as compared to PUFAs (16%) (Table 2). Similarly, other studies reported that the SPV assay was used in the quantification of total intracellular lipids of microalgal, bacterial, and yeast cells (Wang et al. 2009; Cheng et al. 2011; Mishra et al. 2014; Dien et al. 2016).

In the current study, *R. mucilaginosa* and *L. elongisporus* were grown at three incubation temperatures (7, 15, 26 °C). All samples showed an increase in the color intensity with biomass increase (Fig. 6). The lipids content was calculated using the standard oils curves. Assay linearity was tested

for each of the yeast strain by plotting amounts of measured lipids versus amounts of yeast biomass (Supplementary material file 3). The highest lipids production occurred after 15 days of incubation in BDM at 7 °C by *R. mucilaginosa* and *L. elongisporus* using SPV assay.

Results in Fig. 7 showed the relationship between SPV results of both marine yeast, and their PUFAs and MUFA contents, when incubated at different temperatures. In agreement with the chemical reaction, an obvious relationship between FAs composition and SPV results was found, when LA and OA were the only PUFAs and MUFA; the SPV response was closely associated to the LA amount in *R. mucilaginosa* (Table 2 and Fig. 7). Depending on the chemical nature of SPV reaction, only a single carbonium ion is formed per molecule, where multiple ones would not be stable and steric hindrance can occur in case of multiple unsaturated compounds. Therefore, OA shows highly reaction response, and more intense color than LA and LNA (Knight et al. 1972). In case of multiple unsaturated FAs, the steric hindrance was observed in the presence of PUFA (LA and ALA) with OA alone or OA with Palmitoleic acid (MUFAs), which resulted in reaction response of 24–26% as compared to (30–32%) in the presence of PUFAs and MUFAs for both yeast strains grown at 15 °C (Table 2 and Fig. 7). In addition, high presence of OA with other MUFAs (Palmitoleic acid) and 15-Docosenoic acid ($C_{22:1}$) showed a higher reaction response of SPV (20–26%) as compared to LA alone (0–16%) in *L. elongisporus*. Overall, when an appropriate reference oil is used and compared with tested biological samples, the SPV reaction can provide meaningful estimates of the overall lipids content of the tested samples (Dien et al. 2016).

Visualization of lipid droplets using Nile-Red fluorescent dye

Nile-Red fluorescent dye is widely used to determine lipids accumulation in yeast (Wang et al. 2017). Nile-Red dye can be applied to cells, where it dissolves preferentially in lipids, and its fluorescence can be observed only in the stained substances (Greenspan et al. 1985). Staining of *R. mucilaginosa* and *L. elongisporus* cells showed large spherical, fluorescent cytoplasmic structures (Fig. 8). Although these structures varied in size, fluorescence intensity, and distribution, their presence gave a good indicator for high lipid production. In order to test the effect of low temperature on the accumulation of lipids, both *R. mucilaginosa*, *L. elongisporus,* and the two control (*C. lipolytica* and *S. boulardii*) were stained by Nile-Red dye, after incubation for 15 days on normal BDM at 7 °C. Results in Fig. 8 showed yellow-golden fluorescent lipids that can be viewed in the stained cells. The Nile-Red stained cells of *S. boulardii* exhibit numerous small discrete bodies distributed throughout its cytoplasm, although large individual lipid bodies were observed alone. However, stained cells of *C. lipolytica* did not exhibit clear yellow-golden fluorescence, in spite of apparent diffused fluorescence inside and outside its cells (Fig. 8). Similarly, Diniz Rufino et al. (2014) reported that the low intensity of fluorescent lipids and the diffusion of Nile-Red dye outside *Y. lipolytica* cells might be due to its pathogenicity nature that accumulates cell biomass in its thick mucoid secretion of hydrophobic FAs. Thus, the interaction of Nile-Red and the yeast mucoid secretion appears as low intensity fluorescence around it (Fig. 8). Nile-Red staining confirmed the effect of BDM on increasing the lipids accumulation at low temperature in *R. mucilaginosa* and *L. elongisporus* cells by the presence of yellow-golden fluorescent lipid droplets (Fig. 8). Based on the yellow-golden fluorescence (Poli et al. 2013), the four examined yeast strains were classified according to the area covered by lipid droplets (yellow-gold) inside the cell; *R. mucilaginosa* had lipid droplets filling ≥ 50% of its cell area, *L. elongisporus* had lipid droplets filling 30–50%, and *S. boulardii* had lipid droplets filling up to 30% (Fig. 8), as previously described by Poli et al. (2013). Therefore, Nile-Red can be recommended for staining intercellular lipids.

Conclusion

This study explored the production of lipids using two newly isolated marine yeast, *Rhodotorula mucilaginosa* and *Lodderomyces elongisporus,* in terms of growth temperature and C:N ratio. Both strains can accumulate high amounts of lipids (48–54%) when cultured in BDM with high C:N (8:1) at 15 °C. The unsaturation degree of FAs was decreased by raising the temperature from 7 to 26 °C, especially total PUFAs. An obvious increase in the biomass (40–78.75 mg/mL) was achieved through increasing the incubation temperature to 26 °C. Their FAs profile and lipids content suggest their usage as alternative source for high-value edible oil, and could be considered as a promising source for the production of single cell oils, since they can grow and produce lipids over a wide range of temperatures. Significant amounts of LC-15-Docosenoic acid ($C_{22:1}$, ω7) and Tricosanoic acid ($C_{23:0}$) were detected as 12.12% and 21.49%, respectively, in the FAs profile of *L. elongisporus*, when incubated at 26 °C on normal BDM. Normal BDM (3% glucose) and 15 °C were the best conditions to increase the production of PUFAs by both strains. This study is considered promising application for the newly isolated marine oleaginous *L. elongisporus* that can accumulate high amounts of lipids, with considerable amounts of LC-FAs through appropriate fermentation conditions. The outcomes of this study are promising and can serve as basis for the

development of new biotechnological applications using the new marine Egyptian yeast.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13205-021-03010-4.

Declarations

Conflict of interest All the authors declare that there are no financial/commercial conflicts of interest.

References

Abd Elrazak A, Ward AC, Glassey J (2013) Polyunsaturated fatty acid production by marine bacteria. Bioprocess Biosyst Eng 36:1641–1652

Ageitos JM, Vallejo JA, Veiga-Crespo P, Villa TG (2011) Oily yeasts as oleaginous cell factories. Appl Microbiol Biotechnol 90:1219–1227

Agustini BC, Silva LP, Bloch C et al (2014) Evaluation of MALDI-TOF mass spectrometry for identification of environmental yeasts and development of supplementary database. Appl Microbiol Biotechnol 98:5645–5654

Ahmed SO, Nasser AA, Abbas RN et al (2021) Production of bio-Concrete with improved durability properties using Alkaliphilic Egyptian bacteria. 3 Biotech 11:1–15

Amaretti A, Raimondi S, Sala M et al (2010) Single cell oils of the cold-adapted oleaginous yeast *Rhodotorula glacialis* DBVPG 4785. Microb Cell Fact 9:1–6

Axelsson M, Gentili F (2014) A single-step method for rapid extraction of total lipids from green microalgae. PLoS ONE 9:e89643

Bellou S, Triantaphyllidou I-E, Aggeli D et al (2016) Microbial oils as food additives: recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. Curr Opin Biotechnol 37:24–35

Beopoulos A, Cescut J, Haddouche R et al (2009) *Yarrowia lipolytica* as a model for bio-oil production. Prog Lipid Res 48:375–387

Botha A, Kock JL (1993) Application of fatty acid profiles in the identification of yeasts. Int J Food Microbiol 19:39–51

Butinar L, Strmole T, Gunde-Cimerman N (2011) Relative incidence of ascomycetous yeasts in arctic coastal environments. Microb Ecol 61:832–843

Cheng Y-S, Zheng Y, VanderGheynst JS (2011) Rapid quantitative analysis of lipids using a colorimetric method in a microplate format. Lipids 46:95–103

Chintalapati S, Kiran M, Shivaji S (2004) Role of membrane lipid fatty acids in cold adaptation. Cell Mol Biol (noisy-Le-Grand) 50:631–642

Connor WE, Connor SL (2007) The importance of fish and docosahexaenoic acid in Alzheimer disease. Am J Clin Nutr 85(4):929–930

De Garcia V, Brizzio S, Libkind D et al (2007) Biodiversity of cold-adapted yeasts from glacial meltwater rivers in Patagonia, Argentina. FEMS Microbiol Ecol 59:331–341

Dien BS, Slininger PJ, Kurtzman CP et al (2016) Identification of superior lipid producing Lipomyces and Myxozyma yeasts. AIMS Environ Sci 3:1–20

Dikma-Technologies (2021) Fames (marine oil standard). Retrieved from: http://www.dikmatech.com/Application/show/id/186

Diniz Rufino R, Moura de Luna J, de Campos Takaki GM, Asfora Sarubbo L (2014) Characterization and properties of the biosurfactant produced by *Candida lipolytica* UCP 0988. Electron J Biotechnol 17:6–6

El-Baz AF, Shetaia YM, Elkhouli RR (2011) Xylitol production by *Candida tropicalis* under different statistically optimized growth conditions. Afr J Biotech 10:15353–15363

El-Baz AF, Sorour NM, Shetaia YM (2016) *Trichosporon jirovecii*–mediated synthesis of cadmium sulfide nanoparticles. J Basic Microbiol 56:520–553

El-Baz AF, El-Enshasy HA, Shetaia YM et al (2018) Semi-industrial scale production of a new yeast with probiotic traits, *Cryptococcus* sp. YMHS, isolated from the Red Sea. Probiotics Antimicrob Proteins 10:77–88

Funk CD (2001) Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 294:1871–1875

Galán B, Santos-Merino M, Nogales J, et al (2020) Microbial oils as nutraceuticals and animal feeds. In: Health consequences of microbial interactions with hydrocarbons, oils, and lipids. pp 401–445

Greenspan P, Mayer EP, Fowler SD (1985) Nile red: a selective fluorescent stain for intracellular lipid droplets. J Cell Biol 100:965–973

Gupta A, Vongsvivut J, Barrow CJ, Puri M (2012) Molecular identification of marine yeast and its spectroscopic analysis establishes unsaturated fatty acid accumulation. J Biosci Bioeng 114:411–417

Guschina IA, Harwood JL (2006) Lipids and lipid metabolism in eukaryotic algae. Prog Lipid Res 45:160–186

Johansen PG, Owusu-Kwarteng J, Parkouda C et al (2019) Occurrence and importance of yeasts in indigenous fermented food and beverages produced in Sub-Saharan Africa. Front Microbiol 10:1789

Jones E, Sakayaroj J, Suetrong S et al (2009) Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. Fungal Divers 35:187

Jostensen J-P, Landfald B (1997) High prevalence of polyunsaturated-fatty-acid producing bacteria in arctic invertebrates. FEMS Microbiol Lett 151:95–101

Kimura K, Yamaoka M, Kamisaka Y (2004) Rapid estimation of lipids in oleaginous fungi and yeasts using Nile red fluorescence. J Microbiol Methods 56:331–338

Knight JA, Anderson S, Rawle JM (1972) Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. Clin Chem 18:199–202

Li Q, Du W, Liu D (2008) Perspectives of microbial oils for biodiesel production. Appl Microbiol Biotechnol 80:749–756

Li M, Liu G-L, Chi Z, Chi Z-M (2010) Single cell oil production from hydrolysate of cassava starch by marine-derived yeast *Rhodotorula mucilaginosa* TJY15a. Biomass Bioenerg 34:101–107

Ma C, Liu J, Zhou T et al (2015) Identification and phylogenetic analysis of two marine petroleum-degrading yeasts from South China Sea Offshore based on magnetic nanoparticles extraction. J Bionanosci 9:215–221

Margesin R (2009) Effect of temperature on growth parameters of psychrophilic bacteria and yeasts. Extremophiles 13:257–262

Marklein G, Josten M, Klanke U et al (2009) Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J Clin Microbiol 47:2912–2917

Marvin LF, Roberts MA, Fay LB (2003) Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in clinical chemistry. Clin Chim Acta 337:11–21

Mishra SK, Suh WI, Farooq W et al (2014) Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method. Biores Technol 155:330–333

Papanikolaou S, Aggelis G (2002) Lipid production by *Yarrowia lipolytica* growing on industrial glycerol in a single-stage continuous culture. Biores Technol 82:43–49

Papanikolaou S, Aggelis G (2019) Sources of microbial oils with emphasis to *Mortierella (Umbelopsis) isabellina* fungus. World J Microbiol Biotechnol 35:63

Parolini C (2020) Marine n-3 polyunsaturated fatty acids: Efficacy on inflammatory-based disorders. Life Sci 263:118591

Poli JS, Dallé P, Senter L et al (2013) Fatty acid methyl esters produced by oleaginous yeast *Yarrowia lipolytica* QU21: an alternative for vegetable oils. Revista Brasileira de Biociências 11

Qiu X, Xie X, Meesapyodsuk D (2020) Molecular mechanisms for biosynthesis and assembly of nutritionally important very long chain polyunsaturated fatty acids in microorganisms. Progr Lipid Res 79:101047

Ratledge C (1982) Microbial oils and fats: An assessment of their commercial potential [algae, yeasts, fungi]. Progr Ind Microbiol 16:119–206

Ratledge C (2002) Regulation of lipid accumulation in oleaginous microorganisms. Biochem Soc Trans 30:1047–1050

Restek-Corporation (2018) Fames (marine oil standard) on famewax. Retrieved from: http://www.restek.com/chromatogram/view/GC-FF00568

Rossi M, Buzzini P, Cordisco L et al (2009) Growth, lipid accumulation, and fatty acid composition in obligate psychrophilic, facultative psychrophilic, and mesophilic yeasts. FEMS Microbiol Ecol 69:363–372

Ryan J, Farr H, Visnovsky S et al (2010) A rapid method for the isolation of eicosapentaenoic acid-producing marine bacteria. J Microbiol Methods 82:49–53

Satyanarayana T, Kunze G (2009) Yeast biotechnology: diversity and applications. Springer

Sitepu IR, 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 yeast species. Biores Technol 144:360–369

Skerratt JH, Bowman JP, Nichols PD (2002) *Shewanella olleyana* sp. *nov*. A marine species isolated from a temperate estuary which produces high levels of polyunsaturated fatty acids. Int J Syst Evol Microbiol 52:2101–2106

Sorour N, Karboune S, Saint-Louis R, Kermasha S (2012a) Lipase-catalyzed synthesis of structured phenolic lipids in solvent-free system using flaxseed oil and selected phenolic acids as substrates. J Biotechnol 158:128–136

Sorour N, Karboune S, Saint-Louis R, Kermasha S (2012b) Enzymatic synthesis of phenolic lipids in solvent-free medium using flaxseed oil and 3, 4-dihydroxyphenyl acetic acid. Process Biochem 47:1813–1819

Stokes J, Tu R, Peters M et al (2020) Omega-3 fatty acids from algae produced biodiesel. Algal Res 51:102047

Troiano D, Orsat V, Dumont MJ (2020) Status of filamentous fungi in integrated biorefineries. Renew Sustain Energy Rev 117:109472

Wang L, Chi Z, Wang X et al (2007) Diversity of lipase-producing yeasts from marine environments and oil hydrolysis by their crude enzymes. Ann Microbiol 57:495–501

Wang J, Li R, Lu D et al (2009) A quick isolation method for mutants with high lipid yield in oleaginous yeast. World J Microbiol Biotechnol 25:921–925

Wang Q, Cui Y, Sen B et al (2017) Characterization and robust nature of newly isolated oleaginous marine yeast *Rhodosporidium* spp. from coastal water of Northern China. AMB Express 7:1–13

Wieser A, Schneider L, Jung J, Schubert S (2012) MALDI-TOF/MS in microbiological diagnostics-identification of microorganisms and beyond (mini review). Appl Microbiol Biotechnol 93:965–974

Xu H, Turchini GM, Francis DS et al (2020) Are fish what they eat? A fatty acid's perspective. Progr Lipid Res 80:101064

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

Yarrow D (1998) Methods for the isolation, maintenance and identification of yeasts. The yeasts. Elsevier, pp 77–100

Yazawa K (1996) Production of eicosapentaenoic acid from marine bacteria. Lipids 31:S297–S300

You L, Li M, Wang Z et al (2017) Influences of a *Lodderomyces elongisporus* strain on fermentation of strong-flavored liquor. Food Ferment Ind 43:9–13

Zhu M, Yu L, Liu Z, Xu H (2004) Isolating *Mortierella alpina* strains of high yield of arachidonic acid. Lett Appl Microbiol 39:332–335