Egypt. J.Microbiol. **49**, pp. 81- 98 (2014)

Evaluation of Lipid-producing Yeast for Biodiesel Production

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A TOTAL of 76 yeast isolates were isolated from different sources included soil, organic manure, rotted fruits and different pickles based on the typical morphological character of yeast using light microscopy. These isolates were screened on their ability to accumulate intracellular lipids within the cells by culturing on nitrogen-limited medium (productive medium) and using Sudan Black B staining technique. Sixty lipid producing yeast isolates were detected. Estimation of biomass, lipid yield and lipid content were done for 33 oleaginous yeast isolates, based on amount of lipid accumulation. Four isolates (S5, D5, J3 and C9) proved to have high lipid levels. The growth parameters (lipid content, biomass yield, sugar utilization efficiency, conversion coefficient) were determined during the fermentation time under circumstances of N-limitation medium using a shake flask technique. Lipid synthesis was partially associated with both the linear growth phase and during the stationary phase. The fatty acids profile analysis revealed that the lipid extracted from the four promising yeast isolates mainly contained the principal fatty acids (triacylglyceols, TAGs) as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) which is similar to that of vegetable oils. The ratio of saturated and unsaturated fatty acids for the four oleaginous yeasts varied. Isolates S5 and D5 which showed the most potential in lipid production and had similar fatty acid profile to plant oils, were identified as *Candida tropicalis* and *Pichia kudriavzevii* respectively using a molecular genetics technique. Finally, the data show that the identified strains of oleaginous yeast are promising as viable producers of biodiesel

Keywords: Biodiesel, Lipids-producing yeast, Fatty acid profile, 26S rRNA gene.

Due to the energy crisis and environmental concerns, high energy prices and potential depletion of fossil fuel, biodiesel has gained much interest as an alternative renewable energy and a substitute for petroleum diesel fuels. Biodiesel has many advantages, such as inherent lubricity, superior flash point, and biodegradability (Xu *et al*., 2013). It contributes to the reduction of carbon dioxide from fossil fuels and sulfur emissions to the atmosphere so is considered an environmentally friendly solution for global warming (Meng *et al*., 2009). Biodiesel consists of monoalkylesters of long-chain fatty acids with short-chain alcohols, primarily methanol and ethanol, resulting in fatty acid methyl esters

(FAMEs) and/or fatty acid ethyl esters (FAEEs), respectively. In general, biodiesel is produced by transesterification of triacylglycerides (TAGs) with alcohols in the presence of different types of catalysts, such as bases, acids or enzymes (Liu & Zhao, 2007; Ferella *et al*., 2010; Cao *et al*., 2012; Liu *et al*., 2012 and Fedosov *et al*., 2013). Conventionally, vegetable oils such as palm oil, soybean oil, rapeseed oil, and sunflower oil have been explored as the feedstock for biodiesel production in many countries (Escobar *et al.*, 2009). However, vegetable oils are relatively expensive, In addition, being a common food staple, vegetable oils may be subject to shortage if a significant amount of the oils are dedicated to biodiesel production.

Oleaginous microorganisms, such as yeasts, fungi, and microalgae, can accumulate high amounts of neutral storage lipids under appropriate cultivation conditions. There is an increasing interest in microbial lipid compounds, called single cell oils (SCO) due to potential biotechnological applications, such food ingredients, antimicrobial activity, and biodiesel production (Papanikolaou *et al*., 2004; Li *et al*., 2008; Beopoulos *et al*., 2009 and Amaretti *et al*., 2010). Heterotrophic oleaginous microorganisms have gained significant attention : the utilization of this oleaginous biomass has been successfully exploited as a source of triacylglycerides (TAGs) for the production of biodiesel (Liu & Zhao, 2007; Zhu *et al*., 2008; Azócar *et al*., 2010 and Yoo *et al*., 2010). TAGs that exhibit similar fatty acids (FAs) composition and energy value to plant oils or microbial lipids have also many advantages beside a short life cycle. They can be produced in any location, unaffected by season or climate, are easy to scale-up and can be growen on a variety of substances and thus promise to overcome many limitations of plant oils. Oleaginous yeasts are capable to synthesize and accumulate high amounts of neutral lipids which are stored in specialized intracellular compartments known as lipids bodies (Czabany *et al*., 2007). Oleaginous yeasts have been reported as good producers of lipids, mostly consisting of TAGs (Rossi *et al*., 2009). Oleaginous yeast species are highly productive on a per-cell basis, with lipid yields of up to 70% dry weight and grow to high densities with biomass yields of up to 10 to100 g/l within 3 to 7 days (Angerbauer *et al*., 2008 and Liu *et al*., 2008). The most common yeasts so far examined for biofuel production are *Rhodotorula glutinis, Yarrowia lipolytica* and *Lipomyces starkeyii* (Angerbauer *et al*., 2008 and Yen & Yang, 2012). Appropriate process conditions are required by these organisms to induce lipogenesis and to produce large amounts of storage lipids. The most efficient condition described thus far is growth under nitrogen limitation in the presence of an excessive source of carbon (Granger *et al*., 1993 and Ratledge & Wynn, 2002).

The aim of this work was to isolate some oleaginous yeast from different sources and evaluate their ability to produce intracellular triglycerides lipid for biodiesel production.

Materials and Methods

Sample collection

Five soil samples collected from fields of different plants grown in various sites in Giza Governorate, samples of sugar cane juice, rotten fruits and pickles from a local market as well as samples of organic manure were collected. The collected samples were packed in plastic bags and transferred to the laboratory in an ice box for yeast isolation.

Isolation of lipid producing yeasts

One gram from each soil or organic manure sample was added in 50 ml of glycerol-enriched medium consisting of (gl^{-1}): glycerol 100ml, $(NH_4)_2SO_4$ 1.0, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, yeast extract 0.2, pH 5.5 in a 250-ml Erlenmeyer flask, and incubated in an incubator shaker (150 rpm) at 28 °C for 48 h (Pan *et al*., 2009). For isolation of yeasts, 1 ml of the above pre-cultured samples was added to 9 ml of saline solution (0.9 % NaCl) and 10-fold serial dilutions were followed. Portions of 1 ml from each dilution were spread onto plates contained medium (I) consisting of (gl^{-1}): glucose 20.0, $(NH_4)_2SO_4$ 5.0, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, yeast extract 0.5, agar 20.0 in presence of 3.3 ml of streptomycin solution (10 000 U/ml) with an adjusted pH 5.5 (Pan *et al*., 2009). In the case of different rotten fruits and pickles, 1 g sample was added to 9 ml saline solution and was streaked onto plates contained the previous medium. The plates were incubated at 28- 30°C for 2 days, and those containing isolated colonies with the morphology typical of yeasts were used for further study**.**

Screening for oleaginous yeasts

The purified yeast cultures were cultivated on nitrogen limited medium (Dai *et al*., 2007) and after the incubation period, the yeast colonies were further screened for their cellular lipid contents by qualitative analysis with a Sudan Black B Staining technique according to Thakur *et al*. (1988). Black lipid bodies can be observed with an optical microscope. The pattern of lipid content can be observed by the density of the granules of yeast cells. The results were recorded according to the following criteria: +, ++, +++, ++++ corresponding to less than 20%, 20-40%, 50-70% and more than 70% of cells presenting granules, respectively. The potential oleaginous yeast colonies were maintained on slants of yeast peptone glucose medium (Seki *et al.*, 1985). Yeast isolates with high lipid content were collected and screened further for their lipid production ability quantitatively.

Determination of lipid production in isolated yeast

Preparation of inoculum

The propagation was carried out in 250 ml Erlenmeyer flasks containing 100 ml broth of medium (I), inoculated with 1 ml standard inoculum (~ 10^8cfu/ml) of each isolate and incubated at 28-30ºC for 2 days with shaking (150 rpm).

Procedure technique

An aliquot of 5 ml of propagation cultures of each tested isolate was transferred into a flask containing 45 ml of nitrogen-limited medium (productive medium), then incubated at 28-30ºC in a shaking incubator (150 rpm) for 5 days (Pan *et al*., 2009). Duplicate samples were analyzed for dry biomass, lipid weight and lipid content.

Lipid extraction

Total yeast lipids were extracted according to the procedures described by Folch *et al.* (1957). After incubation, the yeast cells were harvested by centrifugation at 3,000 rpm for 15 min. The yeast cells were collected and washed twice with distilled water, to be ready for lipid extraction. Approximate 2 grams (wet weight) of the rinsed cells was extracted with 40 ml of mixture chloroform/methanol (2:1 v/v) at room temperature for 1 hour. The solvent mixture containing the extracted lipids was separated from the residual biomass by centrifugation and all the fractions from each stage were pooled. The extracted lipids in the chloroform phase were separated from the aqueous phase by addition of 8 ml of saline solution (0.9%NaCl) in a separating funnel with stirring vigorously for phase separation. The upper aqueous phase containing water, methanol and non-lipid compounds was discarded and the lower phase (chloroform) was filtered using a filter paper containing 1 g of anhydrous sodium sulfate. The lipid extract in chloroform was collected into weight-measured glass vials, then the solvent evaporated. After drying, the amount of lipid extracted from each yeast isolate was determined.

Fermentation process of growth and lipid synthesis

This experiment was constructed to study the growth behavior, lipid production and sugar consumption of the selected yeast isolates individually, on nitrogen-limited medium (Pan *et al.,* 2009). After the incubation period, 100 ml of inoculum, as mentioned previously, was centrifuged at 5000 rpm for 10 min under aseptic conditions and the sediment cells were washed twice with sterilized distilled water to inoculate the second stage flasks which contained 100 ml of production medium then incubated at 28-30ºC under shaking conditions (150 rpm) for 128 hr. Samples were taken periodically to determine the cell dry weight, lipid weight, lipid content, sugar consumed, sugar utilization efficiency and conversion coefficient during cultivation. Growth curves of the selected isolates were carried out by plotting the relation between time (h) and cell dry weight (gl^{-1}). Specific growth rate (μ), doubling time (t_d), multiplication rate (MR) and number of generation (N) were also calculated. All tests were performed in triplicates.

Analysis of fatty acid profile

The fatty acids of lipid were estimated as methyl esters according to Luddy *et al*. (1960) using gas chromatography:(Perkin Elmer Auto System XL) with Capillary Column containing silica ZB-Wax (60 m x 0.32 mm i.d) and equipped with flame

ionization detector (FID), Oven temperature was maintained initially at 50ºC and programmed from 50 to 220ºC for 2 min, Injector temp. 230ºC, detector temp. 250 ºC and the carrier gas was helium at a flow rate: 1 ml/min. Fatty acid methyl esters were identified and quantified by comparison of their retention time with authentic standards.

Identification of the potential lipid-producing yeast

In order to identify the most efficient yeast isolates, sequence analysis of the variable D1/D2 domain of the large subunit (26S) of ribosomal DNA was performed. The divergent D1/D2 domain of 26S rDNA was amplified with primers NL-1 (5'- GCA TAT CAA TAA GCG GAG GAA AAG-3') and NL4 (5'-GGT CCG TGT TTC AAG ACG G-3') (Kurtzman & Robnett, 1998). The polymerase chain reaction (PCR) product was checked by agarose gel electrophoresis. The amplified product was then purified using Gene JET™ PCR purification Kit (Thermo) and sequenced by GATC Company (Germany). The 26S sequence of the isolated yeasts was used for a BLAST search in the EMBL/Gen Bank database. Identification was based on the Kurtzman & Robnett (1998) statement that yeast strains showing nucleotide substitutions greater than 1% in the D1/D2 domain of the LSU rRNA gene were usually different species.

Phylogenetic relationships

The BLAST database of National Center for Biotechnology Information was used to compare resolved sequences of the most efficient isolates with known 26S rDNA sequences (Altschul *et al.,* 1997). Determination of phylogenetic relationships was analyzed by the program Phylogenetic Analysis.

Statistical analysis

Statistical analysis (standard error "SE") was according to Fisher (1970).

Results and Discussion

Screening and characterization of oleaginous yeast

A total number of 76 yeast cultures with morphology typical of yeasts were isolated from different sources. Lipid accumulation capacity of the isolated yeast was revealed by Sudan Black B staining of the cells grown in nitrogen-limited medium (Table 1& Fig 1). The lipid accumulation process requires the exhaustion of a nutrient, usually nitrogen, to allow excess carbon to be incorporated into lipids (Pan *et al*., 2009). The preliminary screening of all 76 yeast isolates stained by Sudan Black B exhibited that 33 isolates of yeast were lipid producers, showing good growth and lipid accumulation as seen in Table1. The data demonstrated that the isolates S5,D5,J3 and C9 grew extremely well and accumulated the highest lipid content based on the presence of black particles inside the cell as shown by microscopic examination.

TABLE 1. Detection of lipid droplets produced by yeast isolates stained with Sudan Black B using microscopic examination.

Isolate's code	Lipid detection	Isolate's code	Lipid detection
S1,S14,S21,S22,S23,D3, F5, O2	+++	S19, S24, G1, J2, J4, Pe1, B1, A1, Sc1, C2, C3, C7,T1, T2, T4,T5, O3, On1, On2, Cr1, Cr2, L1, mP2, mP3	+
S2,S3,S4,S6,S7,S8,S11,S12, S13, S15, S16, S17, S20, S25, D1, F1, F3, F6, B2, C1, C4, C5, C8, O1, O4, L3	++	S9,S10,S18,D2,D4,J1,M1,M2,F2, F4, C6,T3,L2,mp1	-
S5,D5,J3,C9	++++		

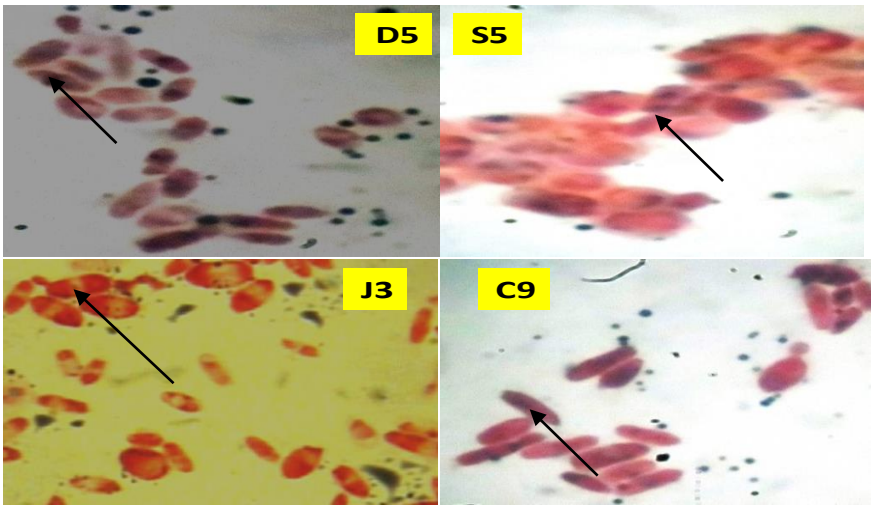

Fig.1. Lipid droplets of yeast isolates D5, S5, J3 and C9 grown on N-limited medium, stained with Sudan Black B as seen under light microscopy (1700 x).

Determination of lipid content

Table 2 shows the quantitative assessment of selected 33 yeast isolates after growth on N-limiting medium by extraction and estimation of cell dry weight, lipid weight and lipid content. Wide variations occurred in all tested parameters between the examined isolates. Among 33 isolates ,four isolates designated S5, D5, J3 and C9 gave the highest potentiality in accumulation of lipids ranging between 0.59- 1.24gl^{-1} .The corresponding figures for lipid content(%) ranged between 18.77- 24.47% respectively. Therefore these isolates were selected for further studies. Many investigators have reported that some yeasts have the ability to accumulate high percentage of lipid globules in their cells such as *Rhodotorula glutinis, Yarrowia lipolitica* and *Lipomyces starkeyii* (Angerbauer *et al*., 2008 and Yen & Yang, 2012).

TABLE 2. Quantitative assessment of lipid content for yeast isolates grown on N-limited medium.

Isolates code	Cell dry weight (g/l)	Lipid weight (g/l)	Lipid content (%)*
S1	6.83±0.056	0.46±0.02	6.73±0.29
S2	3.69±0.29	0.19±0.03	5.15±0.29
S3	11.39±0.29	0.77±0.03	6.76±0.29
S4	3.09±0.29	0.24±0.02	7.76±0.29
S5	2.66±0.29	0.59±0.03	22.18±1.15
S6	6.93±0.29	0.33±0.017	4.76±0.11
S7	4.4±0.23	0.48±0.03	10.90±0.26
S8	5.85±0.26	0.72±0.12	12.30±0.29
S11	11.6±0.29	0.45±0.02	3.88±0.29
S12	5.44±0.26	0.13±0.01	2.39±0.23
S13	4.34±0.29	0.27±0.03	6.22±0.29
S14	5.36±0.17	0.62±0.06	11.56±0.58
S15	15.15±0.58	0.56±0.03	3.69±0.29
S16	4.17±0.29	0.44±0.02	10.55±0.58
S17	9.62±0.29	0.41±0.06	4.26±0.58
S21	5.58±0.29	0.71±0.12	12.72±0.29
S23	3.89±0.29	0.12±0.01	3.08±0.58
D1	6.78±0.29	1.02±0.29	15.04±0.58
D3	1.47±0.29	0.11±0.00	7.48±0.58
D5	5.28±0.44	1.24±0.29	23.48±0.29
F1	6.8±0.29	0.11±0.00	1.61±0.29
F3	3.5±0.29	0.17±0.03	4.85±0.06
F6	6.04±0.29	0.13±0.03	2.15±0.58
J3	6.18±0.58	1.16±0.29	18.77±0.58
J4	4.14±0.29	0.32±0.03	7.72±0.58
B2	9.48±0.29	1.47±0.23	15.50±0.58
C1	11.15±0.58	1.72±0.29	15.42±0.58
C9	2.82±0.29	0.69±0.17	24.47±1.15
C8	8.14±0.29	0.72±0.29	8.84±0.58
L.3	4.25±0.47	0.14±0.02	3.29±0.47
O1	1.92±0.29	0.26±0.03	13.54±0.29
O2	4.9±0.29	0.82±0.29	16.73±0.29
On2	11.21±0.58	0.53±0.02	4.72±0.29

*Lipid content % = lipid weight (gl^{-1}) / cell dry weight (gl^{-1}) x 100

Results are expressed as the means ± standard error of three replicates.

Time course of biomass production and lipid accumulation

Data presented in Fig. 2 show the correlation between growth development of the isolates S5, D5, J3 and C9 grown in nitrogen-limited medium during 128 h of fermentation. Growth development was determined by the dry weight of cell mass (gl^{-1}) in a semi-logarithmic scale. It is obvious that the exponential phase of growth lasted for about 24 h of incubation, whereas the stationary phase occurred during 24-120 hr for isolates D5, J3, C9 and from 24-104 h for isolate S5. High positive correlation coefficient was observed between the cell dry weight(gl^{-1}) and time of incubation period (h) with all tested isolates. Some parameters of growth were calculated during exponential phase presented in Table 3. The highest value of specific growth rate (µ) was recorded by S5 (0.089 h^{-1}) followed by D5 (0.058h^{-1}). These results were reflected in multiplication rates (MR) and doubling times (t_d) to be 0.012 h^{-1}, 7.87 h^{-1} for S5 and 0.08h^{-1}, 11.94 h^{-1} for D5, respectively. The highest generation number was recorded by S5 and the lowest value was recorded by D5. It was observed that lipid production started during the exponential phase of growth then sharply increased to achieve the maximum values of lipid weight (gl^{-1}) and lipid content (%) during stationary phase after 80, 120, 96 and 104 h of fermentation time for S5, D5, J3 and C9 isolates, where the corresponding values of lipid content were 35.08, 30.06, 36.72 and 37.57, respectively. Vijayakumar *et al*. (2010) evaluated lipid production of *Rhodotorulla glutinis* in different carbon sources, between the sources; glucose caused lipid yield and lipid content of 2.43 g/l and 23.78 %. The present data yielded higher values compared to those reported by Dai *et al*. (2007) and Easterling *et al*. (2009) since they found that *Rhodoturula glutinis* and *Rhodosporidium toruloides* produced 25% and 36.6% lipid respectively using glucose as a carbon sources. Consumed sugar increased gradually to record the highest value by S5 and D5 at 72 h being 39.63 and 39.73 gl^{-1} and at 96 h by J3 being 37.88gl^{-1}, respectively, whereas C9 attained the highest value at 104 h (38.86 gl^{-1}). The corresponding figures of sugar utilization efficiency (SUE %) and conversion coefficient (CC%) were 99.09, 3.20% for S5, 99.32, 2.27% for D5, 94.7, 4.67% for J3 and 97.15, 5.26 % for C9, respectively as shown in Fig. 3. On the other hand when xylose used as a source of carbon, Li *et al*. (2005) investigated the lipid coefficients of 10 oleaginous yeasts utilizing xylose and showed that *Rhodosporidium toruloides* had the maximum conversion rate of 10.6 g of lipid per 100 g of consumed xylose. It was found that the biomass yield (%) gradually decreased during the incubation period then slightly increased due to assimilation of lipid content as carbon sources. Lipid synthesis was partially associated with the linear growth phase as well as during stationary phase.

TABLE 3.Growth kinetics of the selected isolates (S5, D5, J3, C9) grown in nitrogen-limited medium (productive medium) at 28-30 ºC during 128 h under shaking condition.

Yeast isolates	Growth kinetics			
	*µ (h^{-1})	*t_d (h^{-1})	*MR (h^{-1})	*(N)
S5	0.089	7.87	0.012	2.04
D5	0.058	11.94	0.08	1.15
J3	0.049	14.14	0.07	1.24
C9	0.056	12.37	0.08	1.29

*Specific growth rate (μ) = $(\ln X - \ln X_0)(t - t_0)^{-1}$ *Doubling time (t_d) = $\ln 2 / \mu$

*Multiplication rate (MR) = $1/t_d$ or $=Nt^{-1}$ * Generation number (N) = t / t_d

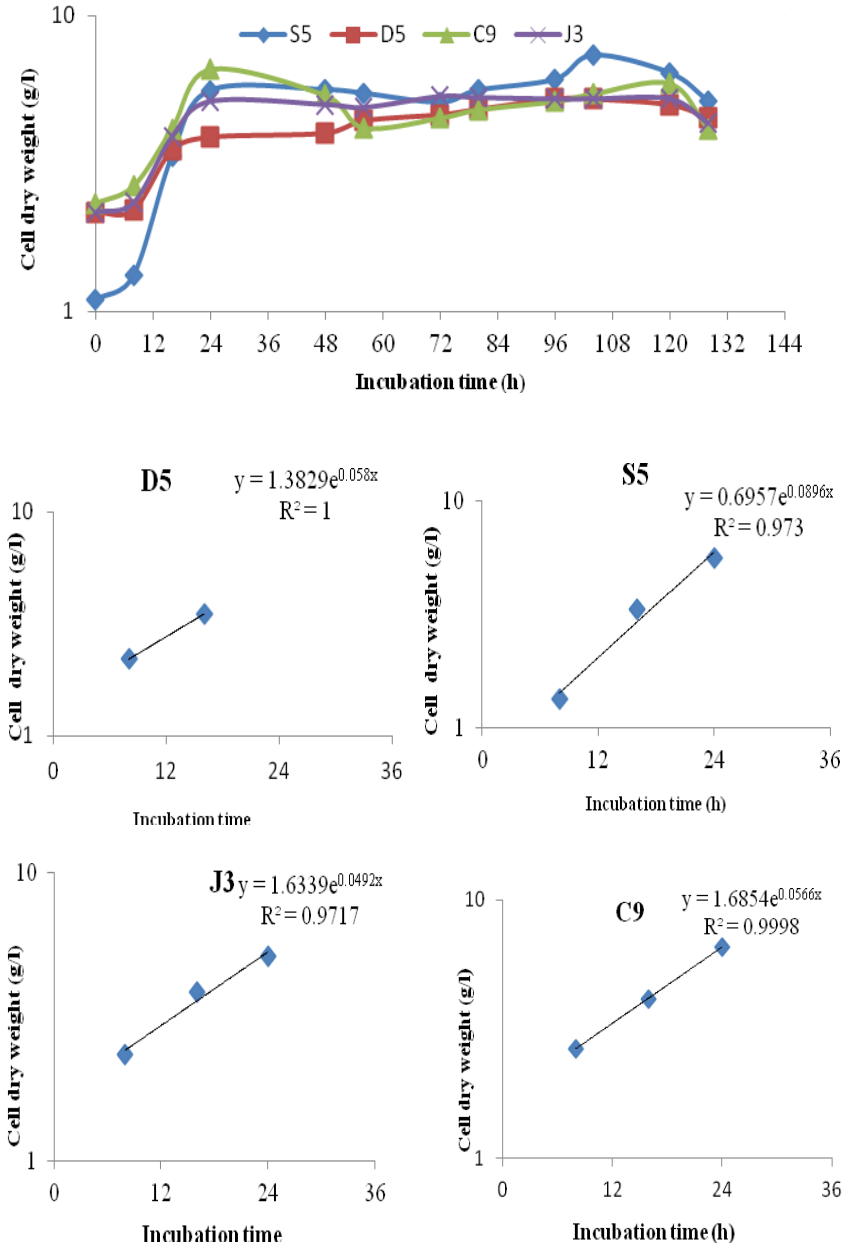

Fig. 2. Growth curve and correlation coefficient between the dry weight of biomass (gl^{-1}) and incubation time of the tested isolates (S5, D5, J3, C9) detected using a semi logarithmic scale.

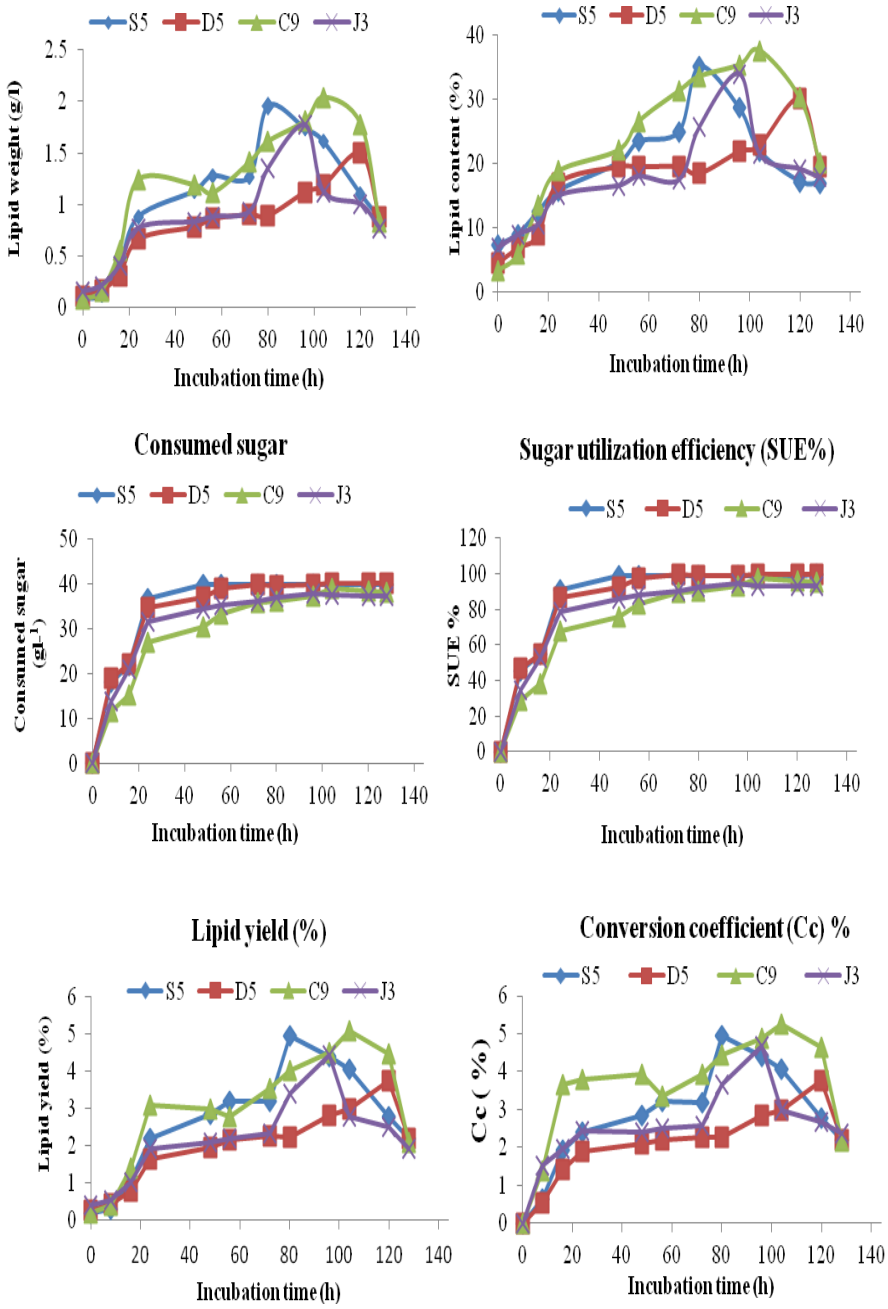

Fig. 3. Growth and lipid production of selected isolates (S5, D5, J3, C9) in production medium (nitrogen-limited medium) during 128 h using shake flask.

Fatty acids profile analysis

In this investigation, the four yeast isolates which showed high efficiency to accumulate lipid droplets, were used to detect fatty acid profile. Table 4 shows that the fatty acids profiles for isolates S5, D5, C9 and J3, including triacylglycerols (TAG) palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) similar to those of vegetable oils. To obtain a biodiesel with appropriate quality characteristics, an appropriate ratio between saturated and unsaturated fatty acids should be maintained (Knothe, 2005). Regarding the saturated and unsaturated fatty acids composition of S5,D5,C9 and J3 ,data revealed that saturated fatty acids were 30.33%, 27.55%,23.95% and 35.83 % while total of un saturated fatty acid were 67.57%, 71.43%, 43.61% and 40.66 % respectively. The total fatty acid methyl esters C16 and C18 were 92.68 % and 94.45% for isolates S5 and D5, but these proportions were sharply decreased to 64.36% and 67.49% for both C9 and J3 isolates, respectively (Table 4 and Fig. 4). It was also observed that the highest dominant fatty acid among the fatty acids profile of the 4 isolates was oleic acid (C18:1) with content ranging between 29.14% and 55.69%, followed by palmitic acid (C16:0) with content ranging between 16.08% to 31.52% among the 4 isolates. In addition, palmitoleic acid (C16:1) which was 13.75%, 15.74% and 8.08%, as one of the main products with S5, D5 and C9 whereas isolate J3 produced negligible amounts of this fatty acid. These data correspond with those obtained by Li *et al.*, (2010) who reported that the fatty acids from *Rhodotorula mucilaginosa* TJY15a were mainly composed of palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1) and linolenic acid (C18:2). Whereas Zhu *et al.* (2008) reported that the lipid of yeast *Trichosporon fermentans* mainly contains palmitic acid, stearic acid, oleic acid and linolenic acid and the unsaturated fatty acids amount to about 64% of the total fatty acids which is similar to those of plant oil. In this respect, Bajpai & Tyagi (2006) found that soybean, cotton seed and rapeseed oil contained stearic acid (C18:0) ranging from 2.32%- 4.67%. Therefore, based on fatty acids profile data, the microbial lipids of D5 and S5 which are highly rich in appropriate fatty acids could be used as a promising alternative feed stock for biodiesel production.

Genetic analysis of oleaginous yeasts

The selected isolates (D5 and S5) that showed the most potential ability in producing lipids and have the similar fatty acid profile to plant oils abundant in low degree unsaturated long chain fatty acid (C18:1) and saturated long chain fatty acids (C16:0)} were identified using a molecular taxonomic approach. BLAST analysis of the 26S rRNA gene sequence of the yeast isolates D5&S5 were revealed to be a perfect match with that of *Pichia kudriavzevii* APKU-5 and *Candida tropicalis* ssm-39 type strains, respectively. Alignment and comparison of the 26S sequence of the D5& S5 isolates to the published 26S rRNA sequences belonging to reference strains of phenotypically close species of *Pichia* and *Candida* confirmed the 99% similarity. Therefore, the isolates D5 and S5 were identified as *Pichia kudriavzevii* and *Candidda tropicalis*, respectively, (See Fig. 5).

TABLE 4. Fatty acids profile of the lipids accumulated in yeast isolates (C9, S5, J3, D5) grown on N-limited medium.

Type of fatty acids	Fatty acid profile	Fatty acid (%,w/w TFA*)			
		S5	D5	C9	J3
Saturated fatty acids	Capric acid (C10:0)	0.11	nd	nd	nd
	Tridecylic acid (C13:0)	0.18	nd	nd	nd
	Mysteric acid (C14:0)	0.38	0.46	1.43	nd
	Pentadecanoic acid (C15:0)	nd	0.03	1.17	nd
	Palmitic acid (C16:0)	21.89	20.70	16.08	31.52
	Margaric acid (C17:0)	4.55	4.04	0.60	nd
	Stearic acid (C18:0)	3.22	2.32	4.67	4.31
	Total saturated fatty acids	30.33	27.55	23.95	35.83
Unsaturated fatty acids	Palmetoleic acid (C16:1)	13.75	15.74	8.08	nd
	Oleic acid (C18:1)	53.82	55.69	27.85	29.14
	Linoleic acid (C18:2)	nd	nd	7.68	11.52
	Total unsaturated fatty acids	67.57	71.43	43.61	40.66
Poly unsaturated fatty acid	Arachidic acid ($C_{20}H_{30}O_2$)	nd	2.09	nd	nd
Total C16 and C18 methyl ester yield %		92.68	94.45	64.36	67.49

TFA *: Total fatty acids
nd: not detected

Conclusion

Data obtained in this study deduced that isolated *Pichia kudriavzevii* D5 and *Candidda tropicalis* S5 could be elected as promising alternative feedstock for biodiesel production. The parameters are depending on total C16 and C18 methylesters yield which were found in high proportion among both S5 and D5 (92.68 % and 94.45 % respectively). Additionally to that, the data of fatty acids profile analysis demonstrated that the ratio of saturated and unsaturated fatty acids in the produced triacylglycerides of yeast isolates were 30.33 %, 27.55 % for saturated fatty acids while unsaturated fatty acid were 67.57 % and 71.43 % for both strains, respectively.

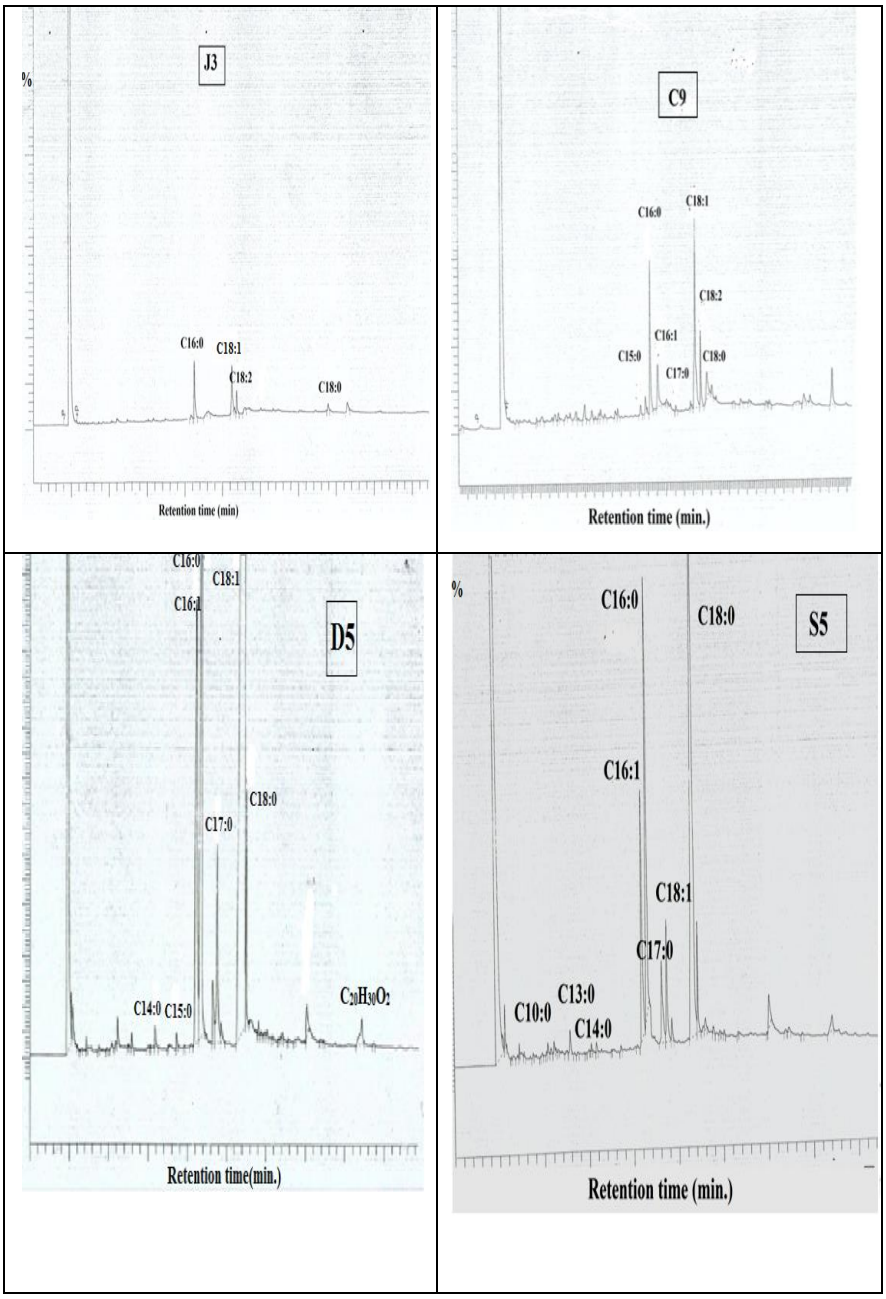

Fig. 4. Fatty acids profile of selected isolates D5, S5, C9, and J3 by Gas chromatography.

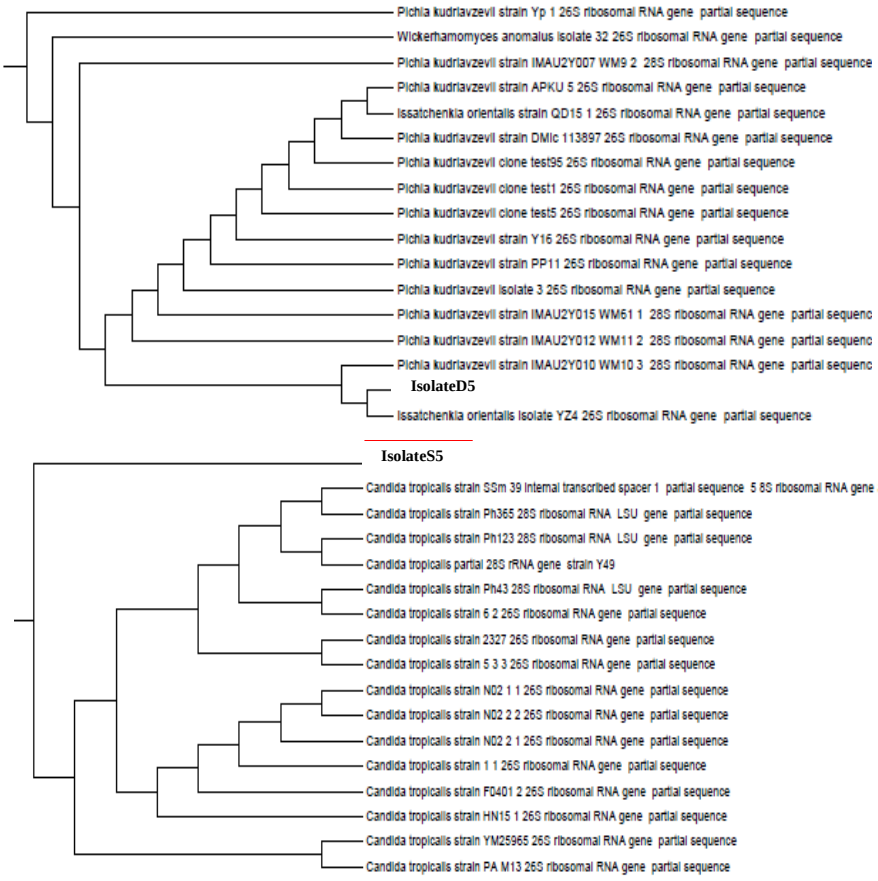

Fig. 5. Phylogenetic trees based on the sequences of the D1/D2 domain of the LSU rRNA gene, showing position of the isolates D5&S5 with closely related species.

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(*Received* 20 / 10/2014;
accepted 23 / 11/ 2014)

تقييم الخمائر المنتجة لليبيدات لإنتاج الديزل الحيوى

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تناولت هذه الدراسة عزل عدد 76 عزلة خميرة من مصادر مختلفة (التربة ، السماد العضوى ، الفواكة المعطوبة ، عصير القصب وبعض المخللات المختلفة) وفقا للصفات المورفولوجية باستخدام الميكروسكوب الضوئى. اختبرت هذه العزلات على قدرتها لتخزين الليبيدات بعد تنميتها على بيئه فقيرة فى محتواها من النيتروجين باستخدام صبغة اسود سودان. وقد تم تقييم محصول الخلايا الحيوى والليبدات ل 33 عزلة من الخمائر المنتجة لليبيدات. تم اختيار افضل 4 عزلات وهما (S5,D5,J3, C9) طبقا للكتلة الحيوية والمحتوى الليبيدى لهذه العزلات. وقد تم تقييم النشاط الحيوى لهذه العزلات فى بيئة فقيرة من النيتروجين باستخدام المزارع المهتزة وتم تقدير بعض معايير النمو الخاصة بهم مثل الوزن الجاف للخلايا وزن الليبيدات والنسبة المئوية لمحتوى الليبيد والسكر المستهلك وكفاءة استخدام السكر وكفاءة انتاج الليبيد خلال فترة التخمير. وكان تخليق الليبد مصاحب للنمو خلال مرحلة النمو التضاعفى ومرحلة الثبات. وقد اثبت تحليل الليبيدات المتكونة لهذه العزلات انها تحتوى على احماض دهنية اساسية مثل البالمتيك ، الاستياريك ، الاوليك ، اللينولينك) والتى تماثل تلك الموجودة فى الزيوت النباتية، كذلك نسبة الاحماض الدهنية المشبعة لغير المشبعة تم تقديرها . وقد اثبتت هذه النتائج ان عزلتين وهما (S5,D5) لهم كفاءة عالية فى انتاج الليبيد كما ان ليبيداتها تماثل الاحماض الدهنية الموجودة فى الزيوت النباتية وتم تعريف هذه العزلات بالتتابع الجينى وهما *Pichia kudriavzevii* و *Candida tropicalis* .لذلك تعتبر هذه السلالات مناسبة لاستخدامها فى انتاج الديزل الحيوى.